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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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REPORT ON THE BIOASSAY OF 2,5-TOLUENEDIAMINE SULFATE FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of 2,5-toluenediamine sulfate conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 2,5-toluenediamine sulfate was conducted by Mason Research Institute, Worcester, Massachusetts, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. E. Smith (3) and Dr. A. Handler (3). Animal treatment and observation were supervised by Mr. G. Wade (3) and Ms. E. Zepp (3).

Histopathologic examinations were performed by Dr. D. W. Hayden (3), Dr. A. S. Krishna Murthy (3), Dr. A. Russfield (3), and Dr. D. S. Wyand (3) at the Mason Research Institute, and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (4).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (5); the statistical analysis was performed by Mr. W. W. Belew (6,7), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (8).

This report was prepared at METREK, a Division of The MITRE Corporation (6) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (6), task leader Dr. M. R. Kornreich (6,9), senior biologist Ms. P. Walker (6), biochemist Dr. B. Fuller (6), chemist Dr. N. Zimmerman (6), and technical editor Ms. P. A. Miller (6). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1,9), Dr. R. A. Griesemer (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,10), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay for possible carcinogenicity of 2,5-toluenediamine sulfate was conducted using Fischer 344 rats and B6C3F1 mice. 2,5-Toluenediamine sulfate was administered in the feed, at either of two concentrations, to groups of 50 males and 50 females of each species. The high and low time-weighted average concentrations of the compound were, respectively, 0.2 and 0.06 percent for rats and 0.1 and 0.06 percent for mice. Because compound administration to the high and low dose groups of each species was not begun simultaneously, each dosed group was assigned a control group. All control groups consisted of 50 animals, except for the high dose male and female rat control groups which were composed of 25 animals. The dosing period was for 78 weeks, followed by an additional 28 to 31 weeks of observation in rats and an additional 16 to 19 weeks in mice.

There was no significant association between compound administration and accelerated mortality in either sex of either species. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

A statistically significant incidence of interstitial-cell neoplasms of the testis in dosed male rats was not considered attributable to administration of the compound since the spontaneous incidence of these neoplasms in male Fischer 344 rats is both high and variable. No neoplasms were observed in female rats at statistically significant incidences.

A statistically significant increase in lung tumors in high dose female mice was not considered convincing evidence of a compound-related carcinogenic effect because high dose mice were received in separate shipments from their controls and housed in separate rooms from their controls.

Under the conditions of this bioassay, sufficient evidence was not obtained to demonstrate the carcinogenicity of 2,5-toluenediamine sulfate in either Fischer 344 rats or B6C3F1 mice.

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I. INTRODUCTION

2,5-Toluenediamine sulfate (Figure 1) (NCI No. C01832), a salt of 2,5-toluenediamine and sulfuric acid, was selected for bioassay by the National Cancer Institute in an attempt to determine those dye intermediates which may be responsible for the increased incidence of bladder cancer observed among workers in the dye manufacturing industry (Wynder et al., 1963; Anthony and Thomas, 1970). Aromatic amines are one of several classes of chemicals thought to contribute to the increased cancer risk in this industry (Wynder et al., 1963).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 2-methyl-1,4-benzenediamine sulfate.* It is also called p-tolylenediamine sulfate; p-diaminotoluene sulfate; fouramine standard; and Colour Index (C.I.) oxidation base 4 (C.I. No. 76043).

2,5-Toluenediamine is used in the synthesis of safranine, a family of dyes, some of which are useful as biological stains (Hawley, 1971), and as an oxidation base to dye furs a deep brown (Society of Dyers and Colourists, 1971). In addition, 2,5-toluenediamine is a common component of the "permanent" or oxidative-type hair dye formulations (Ames et al., 1975). It may also be contained in indelible ink, antifreeze, and nail polish. Production statistics for 2,5-toluenediamine or its sulfate salt are not available; however, U.S.

*The CAS registry number is 6369-59-1.

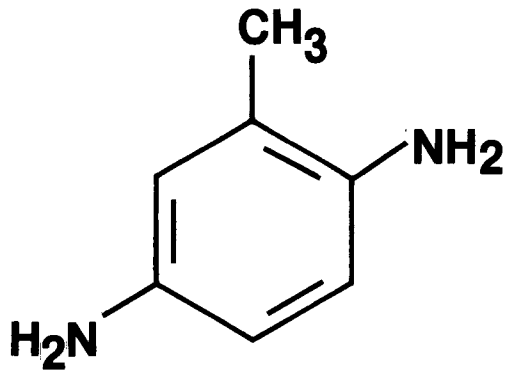


FIGURE 1
CHEMICAL STRUCTURE OF 2,5-TOLUENEDIAMINE (SULFATE)

imports of this chemical through principal U.S. customs districts amounted to 64,680 pounds in 1974 (U.S. International Trade Commission, 1976).

The potential for exposure to 2,5-toluenediamine is greatest among workers in the dye manufacturing industry, although fur dyers and manufacturers of inks as well as printers, engravers and lithographers may also experience significant daily contact with the chemical. Epidemiological studies suggest a relationship between occupational exposure to printing ink and increased incidence of cancer of both the bladder and the liver (Hoover and Fraumeni, 1975).

Exposure to 2,5-toluenediamine is widespread among the general population due to the increasingly common practice of hair dyeing. It has been estimated that approximately 33 million women in the United States dye their hair, often monthly over a period of many years (Staats, 1977).

2,5-Toluenediamine is toxic following both ingestion and inhalation (Sax, 1975). It can also be absorbed through the skin. Three hours after application of 2,5-toluenediamine to the abdomen of dogs, skin penetration was indicated by blood and urine measurements (Kiese et al., 1968). When applied to the scalp of human volunteers in conjunction with detergents, resorcinol and hydrogen peroxide, the diacetyl derivative was excreted in the urine for two days following application, indicating absorption and metabolism through this route as well (Kiese and Rauscher, 1968). The compound has a toxic action

upon the liver and can cause fatty degeneration of that organ. In addition, it is toxic to the central nervous system and produces anemia by destruction of red blood cells. The compound is considered to be highly irritating, producing irritation and blisters on the fingers of sensitive individuals; permanent injury to an eye was reported following use of 2,5-toluenediamine as an eyelash dye (Sax, 1975).

In studies by Ames et al. (1975), 2,5-toluenediamine was found to cause frameshift mutations in a tester strain (TA 1538) of Salmonella typhimurium in the presence of rat liver microsomes. Oxidation of 2,5-toluenediamine by hydrogen peroxide prior to testing resulted in a fortyfold increase in mutagenic activity.

II. MATERIALS AND METHODS

A. Chemicals

2,5-Toluenediamine sulfate was purchased from the Wayland Chemical Division of the Philip A. Hunt Chemical Corporation, Lincoln, Rhode Island. Analysis by the manufacturer suggested a purity of 99 percent with 25 ppm iron, 0.6 percent volatiles, and a maximum of 0.1 percent moisture. Analysis was also performed by Mason Research Institute, Worcester, Massachusetts. The melting point determination showed decomposition above 275°C. No literature value was found for comparison. Thin-layer chromatography utilizing two solvent systems, hexane:benzene:methanol and hexane:ethyl acetate, did not indicate the presence of any impurities. The evidence suggested a compound of high purity.

Throughout this report the term 2,5-toluenediamine sulfate is used to represent this material.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® (Allied Mills, Inc., Chicago, Illinois). 2,5-Toluenediamine sulfate was administered to the dosed animals as a component of the diet. The chemical was mixed with the feed in a 6 kg capacity Patterson-Kelley standard model stainless steel twin-shell V-blender. After 20 minutes of blending, the mixtures were placed in double plastic bags and stored in the dark at 4°C. Mixtures were prepared weekly and stored for not longer than 2 weeks.

C. Animals

Two animal species, rats and mice, were used in the chronic carcinogenicity bioassay. Fischer 344 rats and B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. All mice and the high dose rats and their controls were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. The low dose rats and their controls were supplied by Laboratory Supply Co., Inc., Indianapolis, Indiana. Except for the low dose rats and their controls, all the other dosed animals were received in separate shipments from their respective controls.

Upon arrival, a sample of animals was examined for parasites and other signs of disease. The remaining animals were quarantined by species for 2 weeks prior to initiation of test. Animals were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given sex and species.

D. Animal Maintenance

All animals were housed by species in rooms having a temperature range of 23° to 34°C. Incoming air was filtered through Tri-Dek® 15/40 denier Dacron® filters (Tri-Dim Filter Corp., Hawthorne, New Jersey) providing six changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

Rats were housed five per cage by sex. During quarantine and for the first 13 months of study, low dose rats and their controls

were kept in galvanized- or stainless-steel wire-mesh cages suspended above newspapers. High dose rats and their controls were housed in galvanized-steel wire-mesh cages during quarantine and for the first 11 months of study. Newspapers under cages were replaced daily and cages and racks washed weekly. For the remainder of the study, all rats were housed in suspended polycarbonate cages equipped with disposable nonwoven filter sheets. Clean bedding and cages were provided twice weekly. Low dose rats and their controls received Bed-o-Cobs® corncob bedding (The Andersons Cob Division, Maumee, Ohio) for the first 8 months that they were housed in polycarbonate cages. Thereafter, SAN-I-CEL® corncob bedding (Paxton Processing Company, Paxton, Illinois) was used. High dose rats and their controls were provided with SAN-I-CEL® for the first 11 months that they were housed in polycarbonate cages, and then Aspen hardwood chip bedding (American Excelsior Company, Baltimore, Maryland) was provided for the remainder of the study. Stainless steel cage racks were cleaned once every 2 weeks and disposable filters were replaced at that time.

Mice were housed by sex in polycarbonate cages. During quarantine and periods of chemical administration, cages were fitted with perforated stainless steel lids. Stainless steel wire bar lids were used during the final observation period. Both types of lids were supplied by Lab Products, Inc., Garfield, New Jersey. Nonwoven fiber filter bonnets were used over cage lids. Low dose mice and their

controls were housed ten per cage for the first 18 months of study and five per cage thereafter. The number of high dose mice per cage was reduced from ten to five after 13 months. The number of high dose controls per cage was reduced to five after 12 months. Clean cages, lids, filters, and bedding were provided three times per week when cage populations were ten and twice per week when cage populations were reduced to five. Bedding was the same as that provided to rats. Ab-sorb-dri® (Wilner Wood Products Company, Norway, Maine) hardwood chips were supplied for 2 months to the high dose mice and their controls and for 8 months to the low dose mice and their controls. Both groups received SAN-I-CEL® for the next 12 months. Bed-o-Cobs® was used until the end of the study. Reusable filter bonnets and pipe racks were sanitized every 2 weeks throughout the study.

Water was available to both species from 250 ml water bottles equipped with rubber stoppers and stainless steel sipper tubes. Bottles were replaced twice weekly and, for rats only, refilled as needed between changes.

Pelleted Wayne Lab-Blox® was supplied to all animals during the initial quarantine and final observation periods. During the dosing period, all animals were fed Wayne Lab-Blox® meal containing the appropriate concentration of 2,5-toluenediamine sulfate. Control animals had untreated meal available *ad libitum*. Meal was supplied throughout the study to all mice and to low dose rats and their controls in Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc.,

Woburn, Massachusetts) containing stainless steel baffles. High dose rats and their controls were fed from Alpine® feed cups for the first 14 months of study and from stainless steel gangstyle hoppers (Scientific Cages, Inc., Bryan, Texas) for the remainder of the study. During the final observation period, mice were fed pellets from a wire bar hopper incorporated into the cage lid, and rats were fed pellets on the cage floor. Food hoppers were changed on the same schedule as were cages. Food was replenished daily in Alpine® feed cups.

Dosed rats were housed in a room with other rats receiving diets containing* acetylaminofluorene (53-96-3); a mixture of dulcin (150-69-6) and L-arginine glutamate (4320-30-3); sodium nitrite (7632-00-0); L-arginine glutamate (4320-30-3); N-butylurea (592-31-4); 2-chloro-p-phenylenediamine sulfate (61702-44-1); N,N-dimethyl-p-nitrosoaniline (138-89-6); 2,4-dinitrotoluene (121-14-2); 4-nitroanthranilic acid (619-17-0); 1,5-naphthalenediamine (2243-62-1); N-(1-naphthyl)ethylene-diamine dihydrochloride (1465-25-4); and aniline hydrochloride (142-04-1). Control rats were housed in a room with other rats receiving diets containing 1-nitronaphthalene (86-57-7); 5-nitro-o-toluidine (99-55-8); hydrazobenzene (530-50-7); 2-aminoanthraquinone (117-79-3); 6-nitrobenzimidazole (94-52-0); 3-amino-9-ethylcarbazole hydrochloride; 2,4-diaminoanisole sulfate (615-05-4); and APC (8003-03-0).

*CAS registry numbers are given in parentheses.

High dose mice shared a room with other mice receiving diets containing 5-nitro-o-toluidine (99-55-8); hydrazobenzene (530-50-7); 3-amino-9-ethylcarbazole hydrochloride; 1-nitronaphthalene (86-57-7); 6-nitrobenzimidazole (94-52-0); 5-nitro-o-anisidine (99-59-2); and 2,4-diaminoanisole sulfate (615-05-4). High dose control mice were housed in a room with other mice receiving diets containing 2-methyl-1-nitroanthraquinone (129-15-7); 4-chloro-m-phenylenediamine (5131-60-2); acetylaminofluorene (53-96-3); p-cresidine (120-71-8); and fenaminosulf (140-56-7). Low dose mice and their controls were in a room with other mice receiving diets containing amitrole (61-82-5); APC (8003-03-0); N,N-dimethyl-p-nitrosoaniline (138-89-6); 2,4-dinitrotoluene (121-14-2); 4-nitroanthranilic acid (619-17-0); 2-aminoanthraquinone (117-79-3); 3-amino-4-ethoxyacetanilide (17026-81-2); 3-amino-9-ethylcarbazole hydrochloride; 1-amino-2-methylanthraquinone (82-28-0); 5-nitro-o-anisidine (99-59-2); 1-nitronaphthalene (86-57-7); 5-nitroacenaphthene (602-87-9); 3-nitro-p-acetophenetide (1777-84-0); and 2,4-diaminoanisole sulfate (615-05-4).

E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of 2,5-toluenediamine sulfate for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both Fischer 344 rats and C57BL/6 mice.* Rats and mice were distributed among five groups, each consisting of five males and five

*This strain was used due to the unavailability at that time of B6C3F1 mice.

females. 2,5-Toluenediamine sulfate was incorporated into the basal laboratory diet and fed ad libitum to four of the five rat groups and four of the five mouse groups in concentrations of 0.02, 0.05, 0.08, and 0.11 percent. The remaining group of each species served as control groups, receiving only the basal laboratory diet. The dosed dietary preparations were administered for a period of 4 weeks, followed by 2 weeks of observation.

All animals survived until necropsy. Mean body weight depression relative to controls was observed in all dosed rat groups and in dosed female mice; however, the depression was not dose-related. No mean body weight depression was observed in male mice.

The high concentrations selected for administration in the chronic study were 0.05 percent for rats and 0.06 percent for mice.

F. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, actual concentrations administered, duration of treated and untreated observation periods, and time-weighted average concentrations) are summarized in Tables 1 and 2.

At initiation of the study, all animals were approximately 6 weeks old. High dose rats and their controls were placed on test 11 months after low dose rats and their controls. Each dosed rat group was placed on test during the same week as its respective control group. Rats initially received 2,5-toluenediamine sulfate at dietary concentrations of 0.05 and 0.03 percent. Because of a lack

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
2,5-TOLUENEDIAMINE SULFATE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2,5-TOLUENEDIAMINE SULFATE CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION[a]
MALE					
LOW DOSE CONTROL	50	0	0	107	0
HIGH DOSE CONTROL	25	0	0	109	0
LOW DOSE	50	0.05 0.06 0	14 64	 28	0.06
HIGH DOSE	50	0.2 0	78	 30	0.2
FEMALE					
LOW DOSE CONTROL	50	0	0	108	0
HIGH DOSE CONTROL	25	0	0	109	0
LOW DOSE	50	0.05 0.06 0	14 64	 29	0.06
HIGH DOSE	50	0.2 0	78	 31	0.2

[a] Time-weighted average concentration = $\frac{\Sigma(\text{concentration X weeks received})}{\Sigma(\text{weeks receiving chemical})}$

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
2,5-TOLUENEDIAMINE SULFATE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2,5-TOLUENEDIAMINE SULFATE CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
LOW DOSE CONTROL	50	0	0	96
HIGH DOSE CONTROL	50	0	0	98
LOW DOSE	50	0.06 0	78	16
HIGH DOSE	50	0.1 0	78	19
FEMALE				
LOW DOSE CONTROL	50	0	0	97
HIGH DOSE CONTROL	50	0	0	98
LOW DOSE	50	0.06 0	78	16
HIGH DOSE	50	0.1 0	78	19

of observed mean body weight depression or other clinical signs at a level of 0.05 percent, the groups receiving 0.03 percent were terminated 2 months after initiation, and a new group was initiated at a concentration of 0.2 percent along with a new control group. Throughout this report those rats receiving a concentration of 0.2 percent and their controls are referred to as the high dose and high dose control groups, respectively, while those rats initially receiving a concentration of 0.05 percent and their controls are referred to as the low dose and low dose control groups, respectively. From week 15, the concentration received by the low dose group was increased from 0.05 to 0.06 percent.

Low dose mice were placed on test 6 months before high dose mice. Low dose mice were placed on test 2 weeks before their controls. High dose mice were placed on test 2 months after their controls. The initial dietary concentrations utilized for mice were 0.06 and 0.03 percent. Again, because of a lack of observed mean body weight depression or other clinical signs, the group receiving 0.03 percent was discontinued 6 months after initiation and a new group was initiated at a concentration of 0.1 percent, accompanied by a new control group. Throughout this report those mice receiving a concentration of 0.1 percent and their controls are referred to as the high dose and high dose control groups, respectively, while those mice receiving a concentration of 0.06 percent and their controls are referred to as the low dose and low dose control groups, respectively.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. Body weights were recorded twice weekly for the first 12 weeks of the study and at monthly intervals thereafter. Food consumption, for two cages from each group, was monitored for seven consecutive days once a month for the first nine months of the bioassay and for three consecutive days each month thereafter. The presence of tissue masses and lesions was determined by monthly observation and palpation of each animal. From the first day, all animals were inspected twice daily for mortality.

A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized by carbon dioxide inhalation, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in 10 percent buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination. An occasional section was subjected to special staining techniques for more definitive diagnosis.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph

nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, seminal vesicle, brain, tunica vaginalis, muscle, ear, uterus, mammary gland, and ovary.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were placed on experiment in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals.

As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test determined if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early

tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group

and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

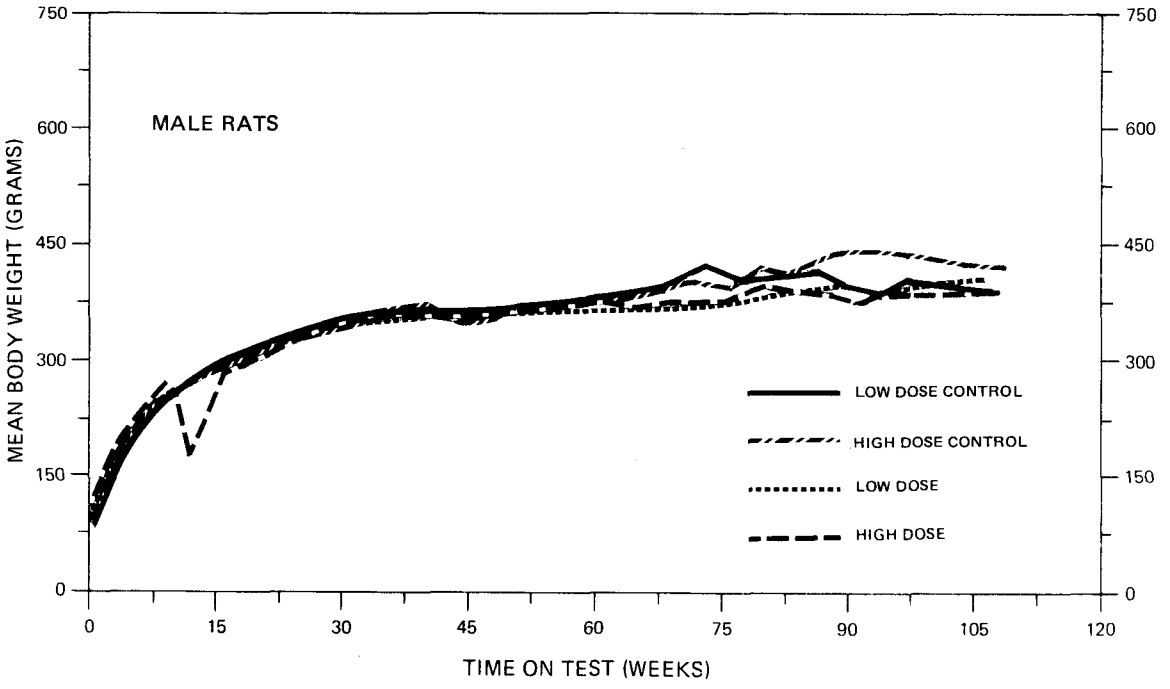
When compared to their respective controls, the high dose female rats exhibited consistent mean body weight depression. This trend was not as evident in the other groups of dosed rats (Figure 2).

Only isolated clinical observations were reported. These included crusted lesions on the dorsolateral surface in one low dose control male and one low dose male and on the side of the head in one low dose female; tissue masses on the ear in two low dose males and on the foreleg in one low dose male; a subcutaneous mass under the base of the tail in one high dose male; and growths on the foreleg in three low dose females and on the ear in another low dose female.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 2,5-toluenediamine sulfate-dosed groups are shown in Figure 3. For both male and female rats there was no significant association between dosage and mortality.

There were adequate numbers of male rats at risk from late-developing tumors despite the sacrifice in week 78 of five rats from the low dose and each control group, and the sacrifice in week 29 of ten additional rats from the low dose control group. Five males from the high dose group died in week 78. Ninety percent (45/50) of the high dose, 84 percent (42/50) of the low dose, 72 percent (18/25) of



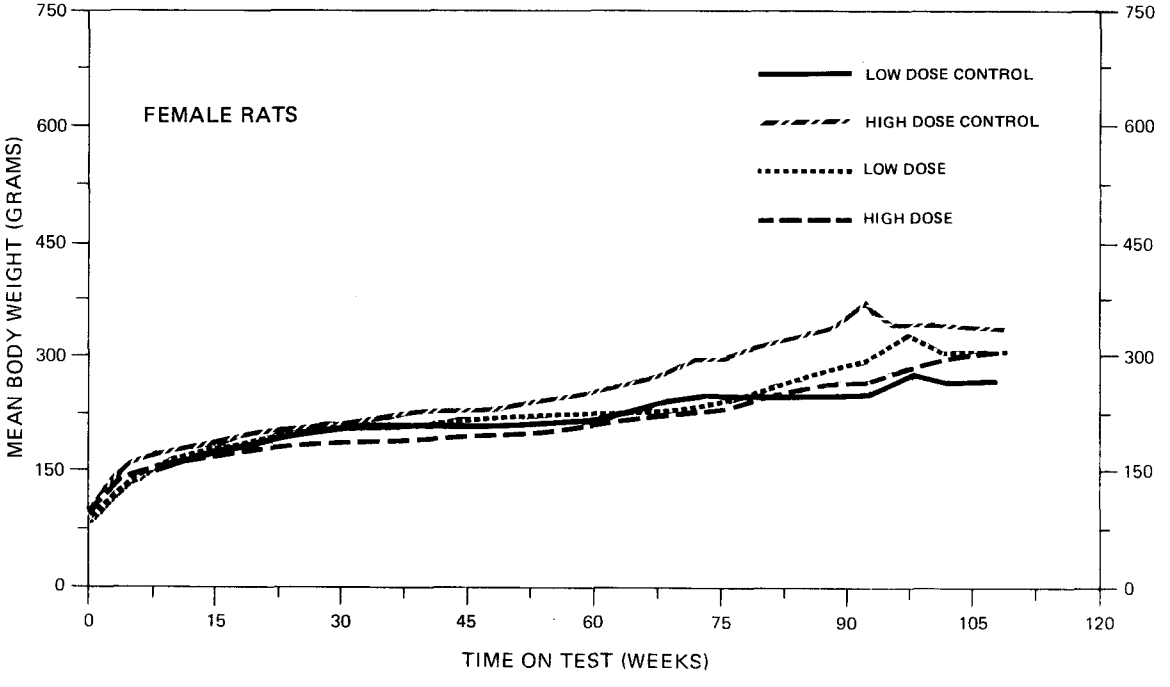
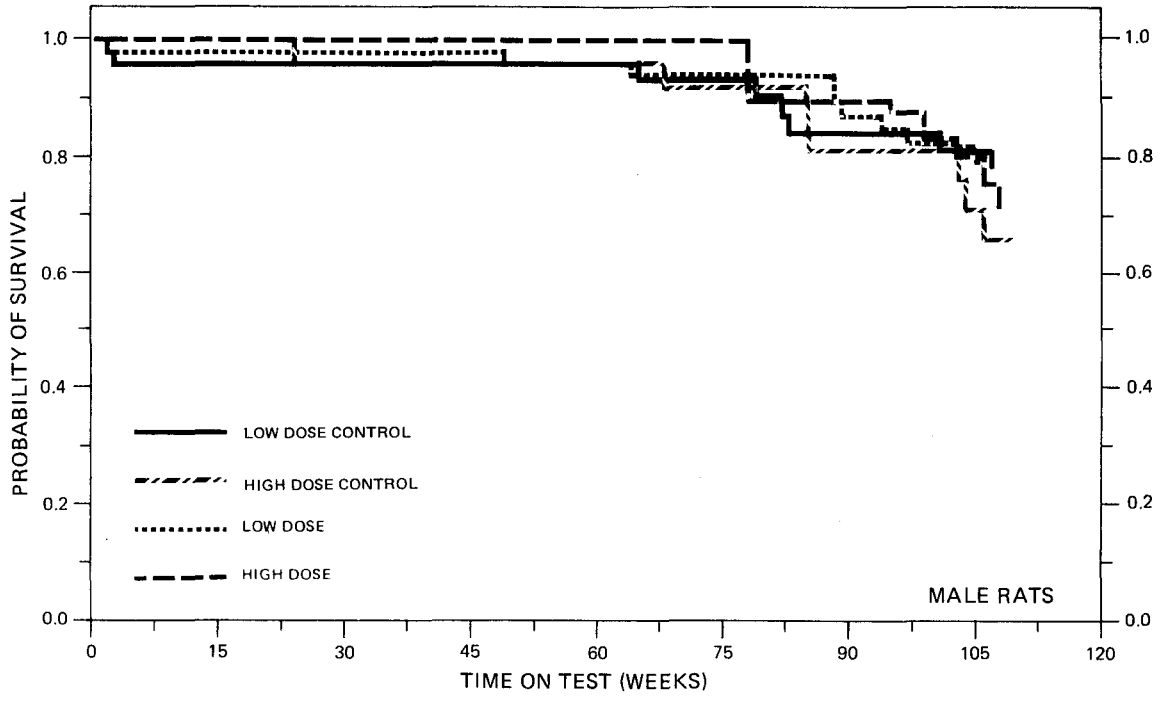


FIGURE 2
GROWTH CURVES FOR 2,5-TOLUENEDIAMINE SULFATE CHRONIC STUDY RATS



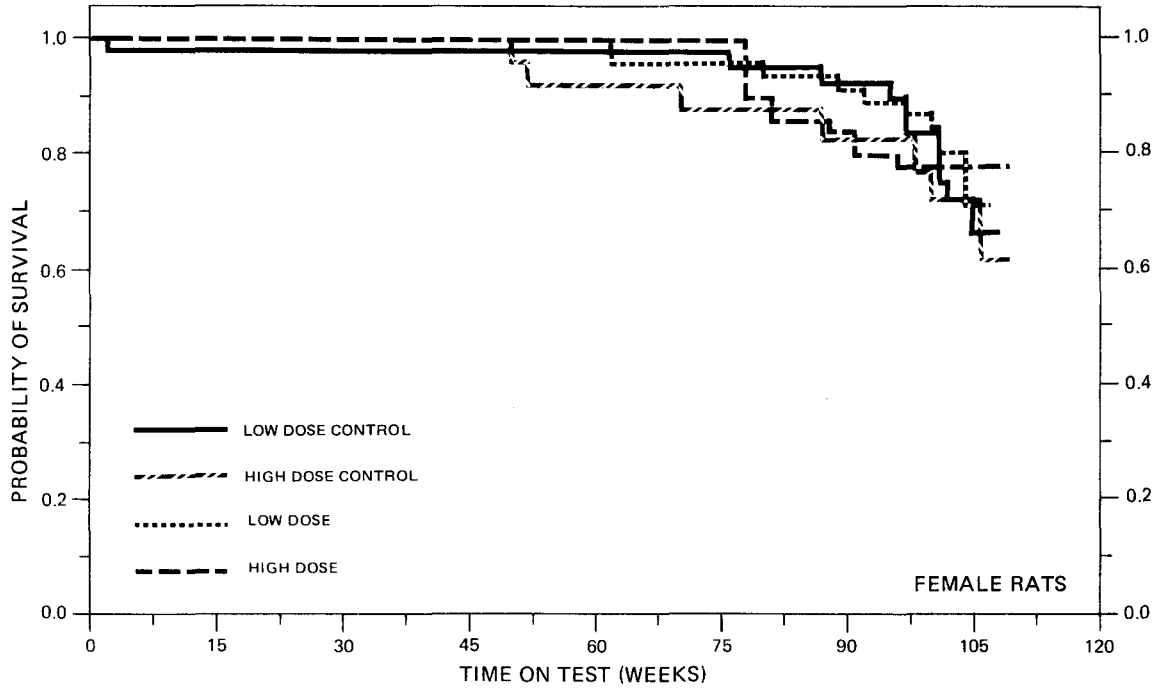


FIGURE 3
SURVIVAL COMPARISONS OF 2,5-TOLUENEDIAMINE SULFATE CHRONIC STUDY RATS

the high dose control, and 58 percent (29/50) of the low dose control group survived on test at least 85 weeks.

There were adequate numbers of female rats at risk from late-developing tumors despite the sacrifice in week 78 of five rats from the low dose and each control group and the sacrifice in week 29 of ten additional rats from the low dose control group. Five of the high dose females died in week 78. Eighty-six percent (43/50) of the high dose, 84 percent (42/50) of the low dose, 68 percent (17/25) of the high dose control, and 66 percent (33/50) of the low dose control group survived on test at least 85 weeks.

C. Pathology

Histopathologic findings on neoplasms in rats are summarized in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are summarized in Appendix C (Tables C1 and C2).

A variety of neoplasms which are routinely seen in this strain of rat was recorded. The incidences of these tumors in dosed rats were not judged to be attributable to the administration of 2,5-toluenediamine sulfate.

Nonneoplastic lesions which commonly occur in aging Fischer 344 rats and which were unrelated to the administration of the test compound were seen.

This pathologic examination provided no evidence for the carcinogenicity of 2,5-toluenediamine sulfate in Fischer 344 rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis for every type of tumor that was observed in more than 5 percent of any of the 2,5-toluenediamine sulfate-dosed groups of either sex is included. In addition to these analyses, time-adjusted analyses were performed based upon animals surviving at least 52 weeks; the time-adjusted analyses did not produce different conclusions from the analyses presented here.

In male rats, the Fisher exact test indicated a significant incidence of interstitial-cell tumors of the testis when the high dose group was compared to the high dose control ($P = 0.014$). The comparison of low dose to low dose control had a probability level of $P = 0.039$, which was not significant under the Bonferroni criterion. These results were discounted, however, due to the well-known high variation in the spontaneous incidence of this tumor (Cockrell and Garner, 1976).

For females the Fisher exact test indicated a significantly ($P = 0.006$) lower incidence of pituitary adenomas in the low dose group than in the low dose control group. The high dose comparison was not significant.

No other test for any site in either male or female rats was significant under the Bonferroni criterion. Based upon these statistical results there was no convincing evidence of the carcinogenicity of 2,5-toluenediamine sulfate in rats.

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 2,5-TOLUENEDIAMINE SULFATE[a]

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	0/46(0.00)	3/25(0.12)	1/48(0.02)	0/49(0.00)
P Values[c]	---	---	N.S.	P = 0.035(N)
Relative Risk (Control)[d]	---	---	Infinite	0.000
Lower Limit	---	---	0.051	0.000
Upper Limit	---	---	Infinite	0.843
Weeks to First Observed Tumor	---	78	97	---
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	2/46(0.04)	4/25(0.16)	5/49(0.10)	8/49(0.16)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	2.347	1.020
Lower Limit	---	---	0.407	0.310
Upper Limit	---	---	23.709	4.278
Weeks to First Observed Tumor	79	85	89	99
Pituitary: Adenoma NOS, Basophil Adenoma or Chromophobe Adenoma[b]	12/41(0.29)	3/21(0.14)	3/45(0.07)	3/40(0.08)
P Values[c]	---	---	P = 0.006(N)	N.S.
Relative Risk (Control)[d]	---	---	0.228	0.525
Lower Limit	---	---	0.044	0.078
Upper Limit	---	---	0.772	3.650
Weeks to First Observed Tumor	101	78	106	108

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Adrenal: Pheochromocytoma or Pheochromocytoma Malignant[b]	6/43(0.14)	4/25(0.16)	2/48(0.04)	1/48(0.02)
P Values[c]	---	---	N.S.	P = 0.044(N)
Relative Risk (Control)[d]	---	---	0.299	0.130
Lower Limit	---	---	0.031	0.003
Upper Limit	---	---	1.568	1.237
Weeks to First Observed Tumor	107	68	103	108
Thyroid: Papillary Adenocarcinoma[b]	0/45(0.00)	0/23(0.00)	3/46(0.07)	0/47(0.00)
P Values[c]	---	---	N.S.	---
Relative Risk (Control)[d]	---	---	Infinite	---
Lower Limit	---	---	0.590	---
Upper Limit	---	---	Infinite	---
Weeks to First Observed Tumor	---	---	106	---
Thyroid: Adenocarcinoma NOS, Papillary Adenocarcinoma, Cystadenocarcinoma NOS, or Papillary Cystadenocarcinoma NOS[b]	2/45(0.04)	0/23(0.00)	3/46(0.07)	3/47(0.06)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	1.467	Infinite
Lower Limit	---	---	0.176	0.303
Upper Limit	---	---	16.894	Infinite
Weeks to First Observed Tumor	107	---	106	105

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Pancreatic Islets: Islet-Cell Adenoma[b]	2/42(0.05)	2/25(0.08)	4/48(0.08)	1/48(0.02)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	1.750	0.260
Lower Limit	---	---	0.266	0.005
Upper Limit	---	---	18.600	4.803
Weeks to First Observed Tumor	107	109	106	108
Preputial Gland: Adenoma NOS or Carcinoma NOS[b]	0/46(0.00)	2/25(0.08)	0/49(0.00)	2/49(0.04)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	---	0.510
Lower Limit	---	---	---	0.040
Upper Limit	---	---	---	6.750
Weeks to First Observed Tumor	---	85	---	108
Testis: Interstitial-Cell Tumor[b]	33/45(0.73)	19/24(0.79)	43/48(0.90)	47/48(0.98)
P Values[c]	---	---	P = 0.039	P = 0.014
Relative Risk (Control)[d]	---	---	1.222	1.237
Lower Limit	---	---	0.981	1.017
Upper Limit	---	---	1.446	1.331
Weeks to First Observed Tumor	78	78	78	78

TABLE 3 (CONCLUDED)

[a]Treated groups received time-weighted average doses of 0.06 or 0.20 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Fisher exact test for the comparison of a treated group with its control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. A negative designation (N) indicates a lower incidence in the treated group than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 2,5-TOLUENEDIAMINE SULFATE[a]

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	4/49(0.08)	2/23(0.09)	2/50(0.04)	10/50(0.20)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.490	2.300
Lower Limit	---	---	0.046	0.553
Upper Limit	---	---	3.251	20.530
Weeks to First Observed Tumor	101	106	107	81
Pituitary: Adenoma NOS or Chromophobe Adenoma[b]	18/43(0.42)	8/21(0.38)	17/48(0.35)	10/43(0.23)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.846	0.611
Lower Limit	---	---	0.477	0.266
Upper Limit	---	---	1.507	1.546
Weeks to First Observed Tumor	76	78	80	108
Adrenal: Pheochromocytoma or Pheochromocytoma Malignant[b]	2/46(0.04)	3/23(0.13)	1/50(0.02)	1/48(0.02)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.460	0.160
Lower Limit	---	---	0.008	0.003
Upper Limit	---	---	8.542	1.882
Weeks to First Observed Tumor	108	109	107	109

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	1/47(0.02)	3/21(0.14)	3/48(0.06)	0/49(0.00)
P Values[c]	---	---	N.S.	P = 0.025(N)
Relative Risk (Control)[d]	---	---	2.938	0.000
Lower Limit	---	---	0.246	0.000
Upper Limit	---	---	150.900	0.707
Weeks to First Observed Tumor	107	109	107	---
Mammary Gland: Adenocarcinoma NOS, Papillary Adenocarcinoma, Papillary Cystadenocarcinoma NOS or Infiltrating Duct Carcinoma[b]	2/49(0.04)	3/23(0.13)	1/50(0.02)	1/50(0.02)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.490	0.153
Lower Limit	---	---	0.008	0.003
Upper Limit	---	---	9.103	1.810
Weeks to First Observed Tumor	101	52	107	108
Mammary Gland: Fibroadenoma[b]	4/49(0.08)	4/23(0.17)	6/50(0.12)	9/50(0.18)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	1.470	1.035
Lower Limit	---	---	0.372	0.332
Upper Limit	---	---	6.681	4.234
Weeks to First Observed Tumor	101	109	100	108

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Uterus: Endometrial Stromal Polyp[b]	10/48(0.21)	6/23(0.26)	7/49(0.14)	7/45(0.16)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.686	0.596
Lower Limit	---	---	0.241	0.199
Upper Limit	---	---	1.826	1.932
Weeks to First Observed Tumor	78	87	101	108
Uterus: Adenocarcinoma NOS[b]	4/48(0.08)	0/23(0.00)	3/49(0.06)	1/45(0.02)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.735	Infinite
Lower Limit	---	---	0.113	0.028
Upper Limit	---	---	4.114	Infinite
Weeks to First Observed Tumor	95	---	92	109

[a]Treated groups received time-weighted average doses of 0.06 or 0.20 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Fisher exact test for the comparison of a treated group with its control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. A negative designation (N) indicates a lower incidence in the treated group than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by 2,5-toluenediamine sulfate that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

The only group exhibiting distinct compound-related mean body weight depression when compared to its control group was the high dose female group (Figure 4). It should be noted, however, that the growth pattern of the female high dose control group was unusual (i.e., it did not level off as the animals approached maturity).

No clinical abnormalities were recorded for dosed or control mice of either sex.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 2,5-toluenediamine sulfate-dosed groups are shown in Figure 5. There was no significant association between dosage and mortality for male or female mice.

Five male and five female mice were sacrificed in week 78 from each group except the low dose group. There were adequate numbers of male mice at risk from late-developing tumors as 74 percent (37/50) of the high dose, 94 percent (47/50) of the low dose, 74 percent (37/50) of the high dose control, and 84 percent (42/50) of the low dose control mice survived on test until the end of the study. There were also adequate numbers of female mice at risk from late-developing tumors as 66 percent (33/50) of the high dose, 78 percent (39/50) of the low dose, 70 percent (35/50) of the high dose control, and 74

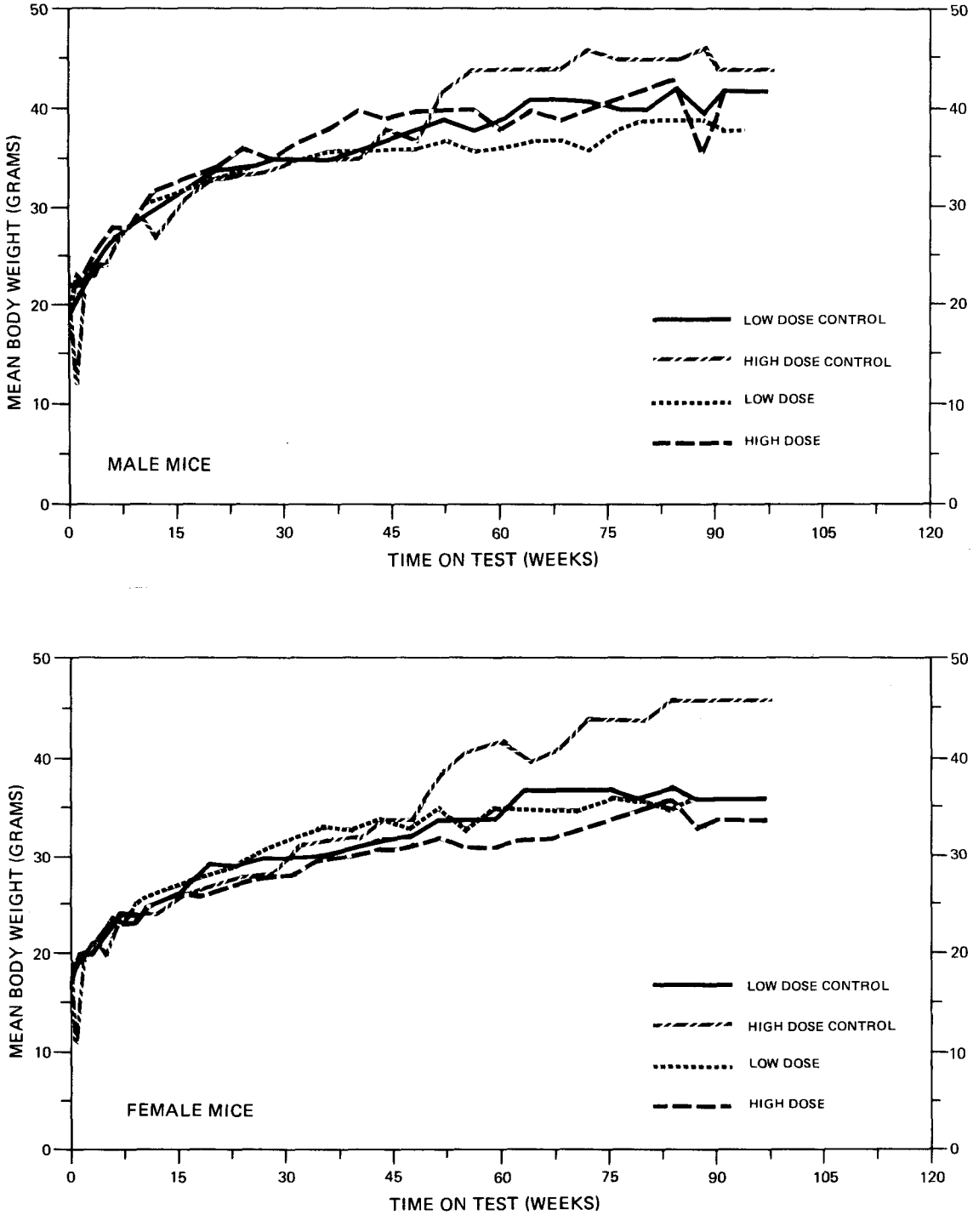
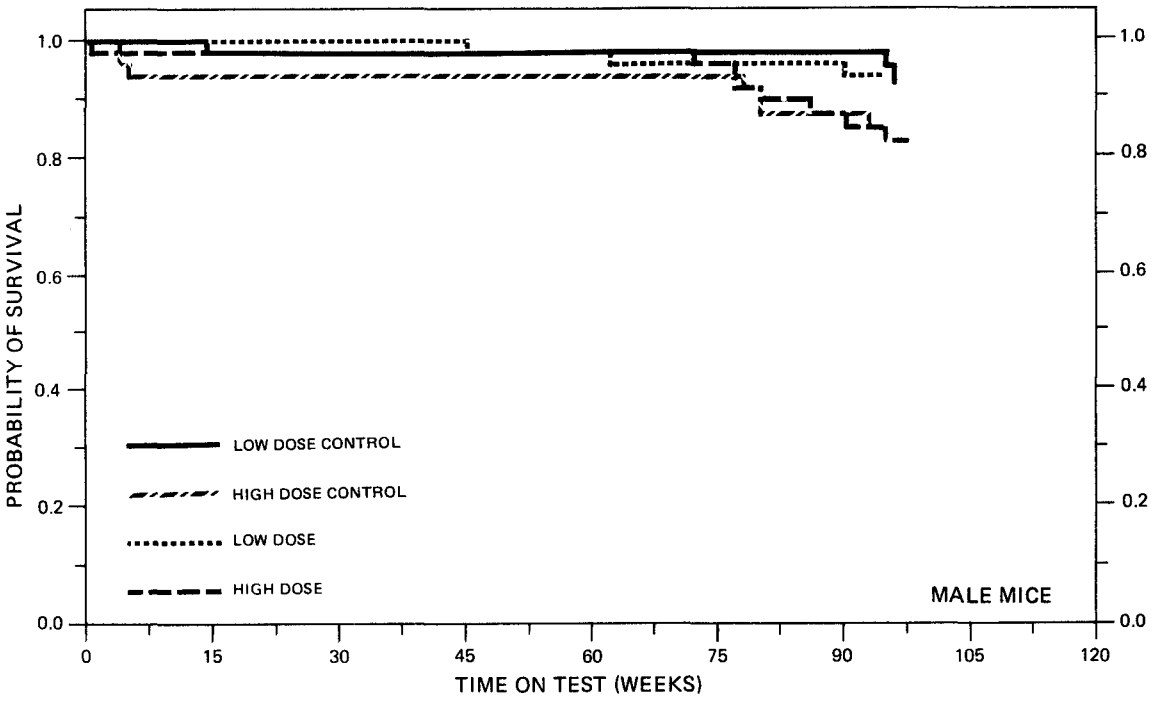


FIGURE 4
GROWTH CURVES FOR 2,5-TOLUENEDIAMINE SULFATE CHRONIC STUDY MICE



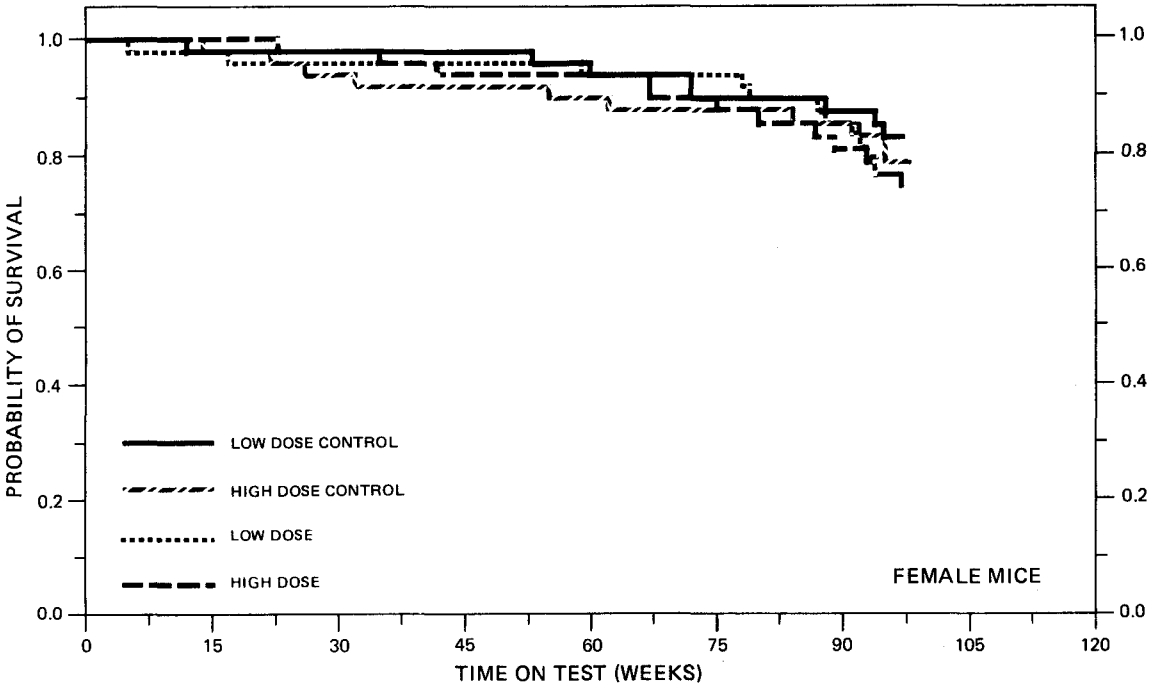


FIGURE 5
SURVIVAL COMPARISONS OF 2,5-TOLUENEDIAMINE SULFATE CHRONIC STUDY MICE

percent (37/50) of the low dose control mice survived on test until the end of the study.

C. Pathology

Histopathologic findings on neoplasms in mice are summarized in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are summarized in Appendix D (Tables D1 and D2).

Hepatocellular carcinoma occurred in 7/48 (15 percent) of the low dose control males, 10/45 (22 percent) of the high dose control males, 8/48 (17 percent) of the low dose males, and 16/49 (33 percent) of the high dose males. None of the tumors was judged to be due to the administration of the test chemical.

A variety of inflammatory and degenerative lesions which commonly occur in aging mice of this strain was seen. These nonneoplastic lesions were not considered to be compound-related.

This pathologic examination provided no evidence for the carcinogenicity of 2,5-toluenediamine sulfate in B6C3F1 mice.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis for every type of tumor that was observed in more than 5 percent of any of the 2,5-toluenediamine sulfate-dosed groups of either sex is included.

Elevated incidences of alveolar/bronchiolar adenomas and alveolar/bronchiolar carcinomas were observed among dosed female mice. For

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH 2,5-TOLUENEDIAMINE SULFATE[a]

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	6/48(0.13)	4/45(0.09)	1/47(0.02)	5/49(0.10)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.170	1.148
Lower Limit	---	---	0.004	0.264
Upper Limit	---	---	1.326	5.453
Weeks to First Observed Tumor	96	97	94	96
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	6/48(0.13)	11/45(0.24)	6/47(0.13)	10/49(0.20)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	1.021	0.835
Lower Limit	---	---	0.294	0.353
Upper Limit	---	---	3.548	1.955
Weeks to First Observed Tumor	96	78	94	96
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	4/48(0.08)	2/46(0.04)	5/49(0.10)	4/49(0.08)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	1.224	1.878
Lower Limit	---	---	0.281	0.284
Upper Limit	---	---	5.823	19.990
Weeks to First Observed Tumor	96	97	94	72

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	7/48(0.15)	10/45(0.22)	8/48(0.17)	16/49(0.33)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	1.143	1.469
Lower Limit	---	---	0.394	0.705
Upper Limit	---	---	3.411	3.233
Weeks to First Observed Tumor	78	93	94	77
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	7/48(0.15)	10/45(0.22)	8/48(0.17)	18/49(0.37)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	1.143	1.653
Lower Limit	---	---	0.394	0.817
Upper Limit	---	---	3.411	3.558
Weeks to First Observed Tumor	78	93	94	77

[a]Treated groups received doses of 0.06 or 0.10 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Fisher exact test for the comparison of a treated group with its control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. A negative designation (N) indicates a lower incidence in the treated group than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 2,5-TOLUENEDIAMINE SULFATE[a]

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	4/46(0.09)	1/45(0.02)	6/42(0.14)	8/45(0.17)
P Values[c]	---	---	N.S.	P = 0.016
Relative Risk (Control)[d]	---	---	1.643	7.826
Lower Limit	---	---	0.419	1.118
Upper Limit	---	---	7.390	338.408
Weeks to First Observed Tumor	96	98	94	78
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	5/48(0.10)	12/46(0.26)	4/45(0.09)	8/47(0.17)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.853	0.653
Lower Limit	---	---	0.180	0.256
Upper Limit	---	---	3.711	1.568
Weeks to First Observed Tumor	96	95	94	78
Liver: Hepatocellular Carcinoma[b]	1/47(0.02)	4/45(0.09)	2/42(0.05)	4/46(0.09)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	2.238	1.000
Lower Limit	---	---	0.121	0.198
Upper Limit	---	---	128.900	5.050
Weeks to First Observed Tumor	96	78	93	94

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma NOS or Carcinoma NOS[b]	3/42(0.07)	6/37(0.16)	1/38(0.03)	0/38(0.00)
P Values[c]	---	---	N.S.	P = 0.012(N)
Relative Risk (Control)[d]	---	---	0.368	0.000
Lower Limit	---	---	0.007	0.000
Upper Limit	---	---	4.349	0.602
Weeks to First Observed Tumor	96	98	94	---
Ovary: Papillary Cystadenoma NOS or Tubular Adenoma[b]	1/45(0.02)	0/41(0.00)	0/36(0.00)	2/39(0.05)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.000	Infinite
Lower Limit	---	---	0.000	0.313
Upper Limit	---	---	23.152	Infinite
Weeks to First Observed Tumor	78	---	---	97

[a]Treated groups received doses of 0.06 or 0.10 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Fisher exact test for the comparison of a treated group with its control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. A negative designation (N) indicates a lower incidence in the treated group than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

the combined incidence in females, the Fisher exact test comparing high dose to high dose control was significant (P = 0.016). In historical data collected by this laboratory for the NCI Carcinogenesis Testing Program 12/350 (3 percent) of the untreated B6C3F1 female mice had an alveolar/bronchiolar adenoma, alveolar/bronchiolar carcinoma, alveolar-cell adenocarcinoma, or an adenoma NOS of the lung/alveoli--compared to the 4/46 (9 percent), 1/45 (2 percent), 6/42 (14 percent), and 8/45 (17 percent) observed in the low dose control, high dose control, low dose, and high dose groups, respectively, in this bioassay.

Based upon these results the statistical conclusion is that the administration of 2,5-toluenediamine sulfate was associated with the incidence of alveolar/bronchiolar neoplasms in female B6C3F1 mice.

For females the Fisher exact test indicated a significantly (P = 0.012) lower combined incidence of pituitary adenomas NOS or pituitary carcinomas NOS in the high dose group than in the high dose control. The low dose comparison was not significant.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than

one, indicating the theoretical possibility of tumor induction in mice by 2,5-toluenediamine sulfate that could not be established under the conditions of this test.

V. DISCUSSION

There was no significant association between compound administration and accelerated mortality in either sex of either species. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

Although the incidence of interstitial-cell neoplasms of the testis was statistically significant in each dosed male rat group, development of these tumors was not considered attributable to compound administration since spontaneous incidence of these neoplasms in male Fischer 344 rats is both high and variable. It should also be noted that control rats were housed in a separate room from dosed rats. There were no other neoplasms occurring in male rats at statistically significant incidences, and none of the incidences of neoplasms observed in female rats were statistically significant.

The only site of significantly increased tumor incidence among dosed female mice was the lungs. The combined incidence of alveolar/bronchiolar adenomas and alveolar/bronchiolar carcinomas was statistically significant for the high dose group. The combined incidences of these tumors in both high and low dose female mouse groups were elevated relative to historical controls. However, it should be noted that high dose control mice were housed in a separate room from dosed mice and received in separate shipments from dosed mice. Because of these factors, this increased incidence does not provide

sufficient evidence of a compound-related effect. No significant increase in tumor incidence was observed among dosed male mice.

Under the conditions of this bioassay, sufficient evidence was not provided to conclusively demonstrate the carcinogenicity of 2,5-toluenediamine sulfate in either Fischer 344 rats or B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 2,5-TOLUENEDIAMINE SULFATE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS
TREATED WITH 2,5-TOLUENEDIAMINE SULFATE

	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE CONTROL (UNTR) 01-0037	LOW DOSE 01-0039	HIGH DOSE 01-0090
ANIMALS INITIALLY IN STUDY	25	50	50	50
ANIMALS NECROPSIED	25	46	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	25	46	48	49
INTEGUMENTARY SYSTEM				
*SKIN	(25)	(46)	(49)	(49)
SQUAMOUS CELL CARCINOMA			1 (2%)	
*SUBCUT TISSUE	(25)	(46)	(49)	(49)
FIBROMA			1 (2%)	1 (2%)
LIPOMA			1 (2%)	
HEMANGIOSARCOMA				1 (2%)
NEUROFIBROMA				1 (2%)
RESPIRATORY SYSTEM				
#TRACHEA	(11)	(45)	(46)	(47)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)		
#LUNG	(25)	(46)	(48)	(49)
UNDIFFERENTIATED CARCINOMA METAS				1 (2%)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (8%)		1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (4%)			
PAPILLARY ADENOCARCINOMA, METAST			1 (2%)	
PHEOCHROMOCYTOMA, METASTATIC	1 (4%)			
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(25)	(46)	(49)	(49)
MALIGNANT LYMPHOMA, NOS			1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)	
UNDIFFERENTIATED LEUKEMIA	2 (8%)	1 (2%)		
MYELOMONOCYTIC LEUKEMIA			2 (4%)	5 (10%)
LYMPHOCYTIC LEUKEMIA	2 (8%)		1 (2%)	1 (2%)
MONOCYTIC LEUKEMIA		1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE CONTROL (UNTR) 01-0037	LOW DOSE 01-0039	HIGH DOSE 01-0090
#LYMPH NODE	(24)	(38)	(39)	(43)
ADENOCARCINOMA, NOS, METASTATIC		1 (3%)		
#MEDIASTINAL L.NODE	(24)	(38)	(39)	(43)
UNDIFFERENTIATED CARCINOMA METAS				1 (2%)
#ABDOMINAL LYMPH NODE	(24)	(38)	(39)	(43)
UNDIFFERENTIATED CARCINOMA METAS				1 (2%)
#LIVER	(25)	(46)	(48)	(49)
MYELOMONOCYTIC LEUKEMIA				1 (2%)
GRANULOCYTIC LEUKEMIA				1 (2%)
#THYMUS	(22)	(38)	(39)	(28)
THYMOMA			1 (3%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(25)	(46)	(48)	(49)
UNDIFFERENTIATED CARCINOMA METAS				1 (2%)
NEOPLASTIC NODULE			1 (2%)	
HEPATOCELLULAR CARCINOMA			1 (2%)	
#PANCREAS	(25)	(42)	(48)	(48)
UNDIFFERENTIATED CARCINOMA				1 (2%)
ADENOMA, NOS				1 (2%)
#STOMACH	(24)	(45)	(47)	(49)
SQUAMOUS CELL PAPILLOMA	1 (4%)			
BASAL-CELL CARCINOMA	1 (4%)			
#JEJUNUM	(24)	(43)	(48)	(48)
CYSTADENOCARCINOMA, NOS				1 (2%)
#COLON	(24)	(43)	(47)	(48)
ADENOCARCINOMA, NOS				1 (2%)
URINARY SYSTEM				
#KIDNEY	(24)	(46)	(48)	(49)
TUBULAR-CELL ADENOMA				1 (2%)

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

TABLE A1 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE CONTROL (UNTR) 01-0037	LOW DOSE 01-0039	HIGH DOSE 01-0090
#URINARY BLADDER	(23)	(42)	(48)	(45)
PAPILLOMA, NOS			1 (2%)	1 (2%)
TRANSITIONAL-CELL CARCINOMA			1 (2%)	
ENDOCRINE SYSTEM				
#PITUITARY	(21)	(41)	(45)	(40)
ADENOMA, NOS	1 (5%)	2 (5%)	3 (7%)	3 (8%)
CHROMOPHOBE ADENOMA		10 (24%)		
BASOPHIL ADENOMA	2 (10%)			
#ADRENAL	(25)	(43)	(48)	(48)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)		
PHECCHROMOCYTOMA	2 (8%)	6 (14%)	2 (4%)	1 (2%)
PHECCHROMOCYTOMA, MALIGNANT	2 (8%)			
#THYROID	(23)	(45)	(46)	(47)
ADENOMA, NOS		1 (2%)		
ADENOCARCINOMA, NOS		2 (4%)		1 (2%)
PAPILLARY ADENOCARCINOMA			3 (7%)	
C-CELL ADENOMA		1 (2%)		
C-CELL CARCINOMA			1 (2%)	1 (2%)
CYSTADENOCARCINOMA, NOS				1 (2%)
PAPILLARY CYSTADENOCARCINOMA,NOS				1 (2%)
#PANCREATIC ISLETS	(25)	(42)	(48)	(48)
ISLET-CELL ADENOMA	2 (8%)	2 (5%)	4 (8%)	1 (2%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(25)	(46)	(49)	(49)
FIBROADENOMA	1 (4%)		1 (2%)	
*PREPUTIAL GLAND	(25)	(46)	(49)	(49)
CARCINOMA,NOS	1 (4%)			2 (4%)
ADENOMA, NOS	1 (4%)			
#PROSTATE	(23)	(45)	(47)	(46)
PARAGANGLIOMA, NOS		1 (2%)		
#TESTIS	(24)	(45)	(48)	(48)
INTERSTITIAL-CELL TUMOR	19 (79%)	33 (73%)	43 (90%)	47 (98%)

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

TABLE A1 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE CONTROL (UNTR) 01-0037	LOW DOSE 01-0039	HIGH DOSE 01-0090
*SCROTUM	(25)	(46)	(49)	(49)
LEIOMYOMA				1 (2%)
NERVOUS SYSTEM				
#BRAIN	(25)	(44)	(48)	(48)
ASTROCYTOMA		1 (2%)		
SPECIAL SENSE ORGANS				
*EAR	(25)	(46)	(49)	(49)
FIBROMA				1 (2%)
*EAR CANAL	(25)	(46)	(49)	(49)
SQUAMOUS CELL CARCINOMA	1 (4%)			
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*BODY CAVITIES	(25)	(46)	(49)	(49)
MESOTHELIOMA, NOS			1 (2%)	
*MEDIASTINUM	(25)	(46)	(49)	(49)
ALVEOLAR/BRONCHIOLAR CA, METASTA	1 (4%)			
*PLEURA	(25)	(46)	(49)	(49)
ALVEOLAR/BRONCHIOLAR CA, METASTA	1 (4%)			
ALL OTHER SYSTEMS				
NONE				

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

TABLE A1 (CONCLUDED)

	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE CONTROL (UNTR) 01-0037	LOW DOSE 01-0039	HIGH DOSE 01-0090
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	25	50	50	50
NATURAL DEATH@	3	6	5	3
MORIBUND SACRIFICE	4	2	5	11
SCHEDULED SACRIFICE	5	15	5	
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	13	27	35	36
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	22	34	45	48
TOTAL PRIMARY TUMORS	41	61	72	77
TOTAL ANIMALS WITH BENIGN TUMORS	20	33	44	47
TOTAL BENIGN TUMORS	31	55	58	59
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	5	9	17
TOTAL MALIGNANT TUMORS	10	5	12	18
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	1	1	1
TOTAL SECONDARY TUMORS	3	4	1	4
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	2	
TOTAL UNCERTAIN TUMORS		1	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS				
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN				

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS
TREATED WITH 2,5-TOLUENEDIAMINE SULFATE

	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE CONTROL (UNTR) 02-0037	LOW DOSE 02-0039	HIGH DOSE 02-0090
ANIMALS INITIALLY IN STUDY	25	50	50	50
ANIMALS NECROPSIED	23	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	23	49	50	50
INTEGUMENTARY SYSTEM				
*SKIN	(23)	(49)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)	
SEBACEOUS ADENOCARCINOMA	1 (4%)			
FIBROSARCOMA				1 (2%)
*SUBCUT TISSUE	(23)	(49)	(50)	(50)
SQUAMOUS CELL CARCINOMA				1 (2%)
RESPIRATORY SYSTEM				
#LUNG	(23)	(49)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	1 (2%)	
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (4%)	1 (2%)		1 (2%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(23)	(49)	(50)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		2 (4%)		
LEUKEMIA,NOS				1 (2%)
UNDIFFERENTIATED LEUKEMIA	2 (9%)			
MYELOMONOCYTIC LEUKEMIA			2 (4%)	8 (16%)
MONOCYTIC LEUKEMIA		2 (4%)		
#PENAL LYMPH NODE	(21)	(41)	(47)	(49)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)		
#THYMUS	(20)	(42)	(43)	(44)
MALIGNANT LYMPHOMA, NOS				1 (2%)
CIRCULATORY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE CONTROL (UNTR) 02-0037	LOW DOSE 02-0039	HIGH DOSE 02-0090
DIGESTIVE SYSTEM				
#LIVER	(23)	(49)	(50)	(49)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)		
NEOPLASTIC NODULE	2 (9%)			
HEPATOCELLULAR CARCINOMA		2 (4%)	1 (2%)	1 (2%)
#STOMACH	(23)	(48)	(50)	(49)
SQUAMOUS CELL PAPILLOMA				1 (2%)
#ILEUM	(23)	(47)	(50)	(49)
LEIOMYOSARCOMA				1 (2%)
URINARY SYSTEM				
#URINARY BLADDER	(22)	(41)	(47)	(50)
PAPILLOMA, NOS				1 (2%)
ENDOCRINE SYSTEM				
#PITUITARY	(21)	(43)	(48)	(43)
ADENOMA, NOS	1 (5%)	3 (7%)	17 (35%)	10 (23%)
ADENOCARCINOMA, NOS		2 (5%)		
CHROMOPHOBE ADENOMA	7 (33%)	15 (35%)		
#ADRENAL	(23)	(46)	(50)	(48)
PHEOCHROMOCYTOMA	2 (9%)	2 (4%)	1 (2%)	1 (2%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (4%)			
#THYROID	(21)	(47)	(48)	(49)
ADENOMA, NOS		1 (2%)		
ADENOCARCINOMA, NOS		2 (4%)		1 (2%)
C-CELL ADENOMA	2 (10%)	1 (2%)		
C-CELL CARCINOMA	1 (5%)		3 (6%)	
#THYROID FOLLICLE	(21)	(47)	(48)	(49)
PAPILLARY CYSTADENOCARCINOMA,NOS	1 (5%)			
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(23)	(49)	(50)	(50)
ADENOMA, NOS		1 (2%)		

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

TABLE A2 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE CONTROL (UNTR) 02-0037	LOW DOSE 02-0039	HIGH DOSE 02-0090
ADENOCARCINOMA, NOS	2 (9%)	1 (2%)		1 (2%)
PAPILLARY ADENOCARCINOMA			1 (2%)	
PAPILLARY CYSTADENOMA, NOS				1 (2%)
PAPILLARY CYSTADENOCARCINOMA,NOS		1 (2%)		
INFILTRATING DUCT CARCINOMA	1 (4%)			
FIBROADENOMA	4 (17%)	4 (8%)	6 (12%)	9 (18%)
*CLITORAL GLAND	(23)	(49)	(50)	(50)
CARCINOMA,NOS				1 (2%)
#UTERUS	(23)	(48)	(49)	(45)
ADENOCARCINOMA, NOS		4 (8%)	1 (2%)	1 (2%)
ENDOMETRIAL STROMAL POLYP	6 (26%)	10 (21%)	7 (14%)	7 (16%)
HEMANGIOMA			1 (2%)	
#UTERUS/ENDOMETRIUM	(23)	(48)	(49)	(45)
ADENOCARCINOMA, NOS			2 (4%)	
#OVARY	(22)	(47)	(50)	(46)
TUBULAR ADENOMA				1 (2%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EAR	(23)	(49)	(50)	(50)
SQUAMOUS CELL CARCINOMA			2 (4%)	
*EAR CANAL	(23)	(49)	(50)	(50)
FIBROMA		1 (2%)		
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*BODY CAVITIES	(23)	(49)	(50)	(50)
MESOTHELIOMA, MALIGNANT		1 (2%)		

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

TABLE A2 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE CONTROL (UNTR) 02-0037	LOW DOSE 02-0039	HIGH DOSE 02-0090
*ABDOMINAL CAVITY	(23)	(49)	(50)	(50)
SARCOMA, NOS			1 (2%)	1 (2%)
LEIOMYOSARCOMA			1 (2%)	
*PERITONEUM	(23)	(49)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)	
LIPOMA				1 (2%)
ALL OTHER SYSTEMS				
DIAPHRAGM				
RHABDOMYOSARCOMA				1
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	25	50	50	50
NATURAL DEATH@	3	5	2	
MORIBUND SACRIFICE	5	7	11	11
SCHEDULED SACRIFICE	5	15	5	
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	12	23	32	39
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE CONTROL (UNTR) 02-0037	LOW DOSE 02-0039	HIGH DOSE 02-0090
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	19	32	34	33
TOTAL PRIMARY TUMORS	34	56	47	53
TOTAL ANIMALS WITH BENIGN TUMORS	18	27	28	23
TOTAL BENIGN TUMORS	23	39	32	33
TOTAL ANIMALS WITH MALIGNANT TUMORS	8	15	14	17
TOTAL MALIGNANT TUMORS	9	17	15	20
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	1	
TOTAL SECONDARY TUMORS		4	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	2			
TOTAL UNCERTAIN TUMORS	2			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 2,5-TOLUENEDIAMINE SULFATE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE
TREATED WITH 2,5-TOLUENEDIAMINE SULFATE

	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE CONTROL (UNTR) 05-0037	LOW DOSE 05-0039	HIGH DOSE 05-0089
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS NECROPSIED	46	48	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	45	48	48	49
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(46)	(48)	(49)	(49)
OSTEOMA			1 (2%)	
RESPIRATORY SYSTEM				
#LUNG	(45)	(48)	(47)	(49)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)			2 (4%)
ALVEOLAR/BRONCHIOLAR ADENOMA	7 (16%)		5 (11%)	5 (10%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	4 (9%)	6 (13%)	1 (2%)	5 (10%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(46)	(48)	(49)	(49)
MALIGNANT LYMPHOMA, NOS		2 (4%)	4 (8%)	2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (4%)		
#SPLEEN	(45)	(47)	(48)	(43)
HEMANGIOMA		1 (2%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		1 (2%)	
#MANDIBULAR L. NODE	(35)	(44)	(44)	(36)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (3%)			
#MESENTERIC L. NODE	(35)	(44)	(44)	(36)
MALIGNANT LYMPHOMA, NOS				1 (3%)
#LIVER	(45)	(48)	(48)	(49)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				1 (2%)
CIRCULATORY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE CONTROL (UNTR) 05-0037	LOW DOSE 05-0039	HIGH DOSE 05-0089
DIGESTIVE SYSTEM				
*LIVER	(45)	(48)	(48)	(49)
NEOPLASTIC NODULE				2 (4%)
HEPATOCELLULAR CARCINOMA	10 (22%)	7 (15%)	8 (17%)	16 (33%)
HEMANGIOMA		1 (2%)		
*STOMACH	(42)	(47)	(46)	(48)
SQUAMOUS CELL PAPILLOMA	1 (2%)			
SQUAMOUS CELL CARCINOMA		1 (2%)		
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#ADRENAL	(43)	(45)	(45)	(44)
PHEOCHROMOCYTOMA				1 (2%)
#THYROID	(40)	(47)	(46)	(46)
FOLLICULAR-CELL ADENOMA		1 (2%)		
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(46)	(48)	(49)	(49)
ADENOMA, NOS				1 (2%)
PAPILLARY CYSTADENOMA, NOS			1 (2%)	
*EAR CANAL	(46)	(48)	(49)	(49)
SQUAMOUS CELL CARCINOMA	1 (2%)			
MUSCULOSKELETAL SYSTEM				
NONE				

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

TABLE B1 (CONCLUDED)

	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE CONTROL (UNTR) 05-0037	LOW DOSE 05-0039	HIGH DOSE 05-0089
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	50	50	50
NATURAL DEATH@	7	3	3	7
MORIBUND SACRIFICE	1			1
SCHEDULED SACRIFICE	5	5		5
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	37	42	47	37
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	21	17	19	27
TOTAL PRIMARY TUMORS	25	21	21	34
TOTAL ANIMALS WITH BENIGN TUMORS	8	2	7	7
TOTAL BENIGN TUMORS	8	3	7	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	15	15	13	23
TOTAL MALIGNANT TUMORS	17	18	14	25
TOTAL ANIMALS WITH SECONDARY TUMORS#	1			2
TOTAL SECONDARY TUMORS	1			2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				2
TOTAL UNCERTAIN TUMORS				2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE
TREATED WITH 2,5-TOLUENEDIAMINE SULFATE

	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE CONTROL (UNTR) 06-0037	LOW DOSE 06-0039	HIGH DOSE 06-0089
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS MISSING			1	
ANIMALS NECROPSIED	46	48	45	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	46	47	43	46
INTEGUMENTARY SYSTEM				
*SKIN	(46)	(48)	(45)	(47)
FIBROSARCOMA	2 (4%)			
*SUBCUT TISSUE	(46)	(48)	(45)	(47)
SARCOMA, NOS			1 (2%)	
LEIOMYOSARCOMA		1 (2%)		
RESPIRATORY SYSTEM				
#LUNG	(45)	(46)	(42)	(46)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)	3 (7%)	6 (14%)	7 (15%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)		1 (2%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(46)	(48)	(45)	(47)
MALIGNANT LYMPHOMA, NOS	3 (7%)	1 (2%)	1 (2%)	3 (6%)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (2%)			
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	6 (13%)	2 (4%)	3 (7%)	1 (2%)
LYMPHOCYTIC LEUKEMIA	1 (2%)			
#SPLEEN	(43)	(46)	(42)	(42)
HEMANGIOSARCOMA		1 (2%)	1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)		2 (5%)
#LYMPH NODE	(41)	(39)	(37)	(32)
MALIGNANT LYMPHOMA, NOS				1 (3%)
#PEYERS PATCH	(43)	(44)	(42)	(42)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE CONTROL (UNTR) 06-0037	LOW DOSE 06-0039	HIGH DOSE 06-0089
#DUODENUM	(43)	(44)	(42)	(42)
MALIGNANT LYMPHOMA, NOS				1 (2%)
#THYMUS	(27)	(31)	(29)	(29)
MALIGNANT LYMPHOMA, NOS		1 (3%)		
CIRCULATORY SYSTEM				
*PULMONARY ARTERY	(46)	(48)	(45)	(47)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)	
DIGESTIVE SYSTEM				
#LIVER	(45)	(47)	(42)	(46)
HEPATOCELLULAR CARCINOMA	4 (9%)	1 (2%)	2 (5%)	4 (9%)
FIBROSARCOMA		1 (2%)		
*BILE DUCT	(46)	(48)	(45)	(47)
BILE DUCT CARCINOMA				1 (2%)
#STOMACH	(42)	(44)	(42)	(43)
SQUAMOUS CELL PAPILLOMA	3 (7%)	1 (2%)		
#JEJUNUM	(43)	(44)	(42)	(42)
ADENOCARCINOMA, NOS			1 (2%)	
#COLON	(41)	(40)	(41)	(43)
LEIOMYOSARCOMA		1 (3%)		
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(37)	(42)	(38)	(38)
CARCINOMA,NOS		1 (2%)		
ADENOMA, NOS	6 (16%)	2 (5%)	1 (3%)	
#ADRENAL	(43)	(45)	(38)	(41)
CORTICAL ADENOMA	1 (2%)			

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

TABLE B2 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE CONTROL (UNTR) 06-0037	LOW DOSE 06-0039	HIGH DOSE 06-0089
PHECCHROMOCYTOMA		1 (2%)		
#THYROID	(30)	(43)	(36)	(34)
PAPILLARY ADENOCARCINOMA			1 (3%)	
#PANCREATIC ISLETS	(41)	(44)	(42)	(42)
ISLET-CELL ADENOMA	1 (2%)			
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(46)	(48)	(45)	(47)
ADENOCARCINOMA, NOS	1 (2%)			
#UTERUS	(43)	(45)	(41)	(42)
LEIOMYOSARCOMA		1 (2%)		
ENDOMETRIAL STROMAL POLYP		3 (7%)		
#OVARY	(41)	(45)	(36)	(39)
PAPILLARY CYSTADENOMA, NOS				1 (3%)
LUTECMA	1 (2%)			
TUBULAR ADENOMA		1 (2%)		1 (3%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(46)	(48)	(45)	(47)
PAPILLARY CYSTADENOMA, NOS				1 (2%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				

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

TABLE B2 (CONCLUDED)

	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE CONTROL (UNTR) 06-0037	LOW DOSE 06-0039	HIGH DOSE 06-0089
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	50	50	50
NATURAL DEATH@	8	6	9	10
MORIBUND SACRIFICE	2	2	1	2
SCHEDULED SACRIFICE	5	5		5
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	35	37	39	33
ANIMAL MISSING			1	
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	22	20	15	22
TOTAL PRIMARY TUMORS	32	24	17	24
TOTAL ANIMALS WITH BENIGN TUMORS	12	11	7	10
TOTAL BENIGN TUMORS	13	11	7	10
TOTAL ANIMALS WITH MALIGNANT TUMORS	18	11	9	12
TOTAL MALIGNANT TUMORS	19	13	10	14
TOTAL ANIMALS WITH SECONDARY TUMORS#			2	
TOTAL SECONDARY TUMORS			2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 2,5-TOLUENEDIAMINE SULFATE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS
IN MALE RATS TREATED WITH 2,5-TOLUENEDIAMINE SULFATE

	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE CONTROL (UNTR) 01-0037	LOW DOSE 01-0039	HIGH DOSE 01-0090
ANIMALS INITIALLY IN STUDY	25	50	50	50
ANIMALS NECROPSIED	25	46	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	25	46	48	49
INTEGUMENTARY SYSTEM				
*SKIN	(25)	(46)	(49)	(49)
EPIDERMAL INCLUSION CYST				1 (2%)
ABSCESS, NOS			1 (2%)	
NECROSIS, NOS	1 (4%)			
*SUBCUT TISSUE	(25)	(46)	(49)	(49)
GRANULATION, TISSUE			1 (2%)	
SCAR				2 (4%)
NECROSIS, NOS			1 (2%)	
RESPIRATORY SYSTEM				
*LARYNX	(25)	(46)	(49)	(49)
INFLAMMATION ACUTE AND CHRONIC	1 (4%)			
INFLAMMATION, CHRONIC	7 (28%)			
#TRACHEA	(11)	(45)	(46)	(47)
INFLAMMATION, NOS	1 (9%)	9 (20%)	1 (2%)	
INFLAMMATION, CHRONIC		10 (22%)		
#LUNG/BRONCHUS	(25)	(46)	(48)	(49)
BRONCHIECTASIS	2 (8%)			
INFLAMMATION, FOCAL	1 (4%)			
ABSCESS, NOS			1 (2%)	
INFLAMMATION, CHRONIC		8 (17%)		
#BRONCHIAL MUCOUS GLA	(25)	(46)	(48)	(49)
ABSCESS, NOS		1 (2%)		
NECROSIS, NOS		1 (2%)		
HYPERPLASIA, ADENOMATOUS		1 (2%)		
#LUNG/BRONCHIOLE	(25)	(46)	(48)	(49)
INFLAMMATION, NOS		1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE CONTROL (UNTR) 01-0037	LOW DOSE 01-0039	HIGH DOSE 01-0090
INFLAMMATION, FOCAL		1 (2%)		
#LUNG	(25)	(46)	(48)	(49)
EMBRYONAL DUCT CYST			1 (2%)	
ATELECTASIS		1 (2%)		
CONGESTION, NOS		1 (2%)		
EDEMA, NOS		1 (2%)		
INFLAMMATION, NOS		1 (2%)		
INFLAMMATION, FOCAL		3 (7%)		
INFLAMMATION, INTERSTITIAL	2 (8%)	1 (2%)		
INFLAMMATION, SUPPURATIVE		1 (2%)		
BRONCHOPNEUMONIA, ACUTE	1 (4%)			
ABSCESS, NOS	1 (4%)			
PNEUMONIA, CHRONIC MURINE	11 (44%)	1 (2%)		
INFLAMMATION, CHRONIC		1 (2%)		
GRANULOMA, NOS	1 (4%)			
PERIVASCULITIS		5 (11%)		
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(25)	(44)	(48)	(46)
HYPERPLASIA, HEMATOPOIETIC	2 (8%)			
#SPLEEN	(25)	(46)	(48)	(47)
THROMBOSIS, NOS		1 (2%)		
FIBROSIS		1 (2%)		
INFARCT, HEALED		1 (2%)		
HEMOSIDEROSIS	1 (4%)			
RETICULOCYTOSIS		1 (2%)		
HYPERPLASIA, HEMATOPOIETIC	1 (4%)			
HYPERPLASIA, ERYTHROID	1 (4%)	12 (26%)		
HYPERPLASIA, RETICULUM CELL		8 (17%)		
HEMATOPOIESIS			2 (4%)	
ERYTHROPOIESIS			1 (2%)	
#LYMPH NODE	(24)	(38)	(39)	(43)
INFLAMMATION, NOS		1 (3%)		
HYPERPLASIA, NOS		1 (3%)		
PLASMACYTOSIS	1 (4%)			
HYPERPLASIA, RETICULUM CELL		3 (8%)		
#MEDIASTINAL L.NODE	(24)	(38)	(39)	(43)
PLASMACYTOSIS		1 (3%)		
#RENAL LYMPH NODE	(24)	(38)	(39)	(43)
HEMOSIDEROSIS				1 (2%)

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

TABLE C1 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE CONTROL (UNTR) 01-0037	LOW DOSE 01-0039	HIGH DOSE 01-0090
CIRCULATORY SYSTEM				
*LYMPHATIC VESSELS	(25)	(46)	(49)	(49)
INFLAMMATION, NOS		1 (2%)		
*HEART	(25)	(46)	(48)	(48)
PERIARTERITIS	1 (4%)			
*HEART/VENTRICLE	(25)	(46)	(48)	(48)
FIBROSIS			1 (2%)	
*MYOCARDIUM	(25)	(46)	(48)	(48)
INFLAMMATION, NOS		1 (2%)		
INFLAMMATION, INTERSTITIAL		22 (48%)	1 (2%)	
INFLAMMATION, CHRONIC FOCAL		3 (7%)		
FIBROSIS		7 (15%)		1 (2%)
FIBROSIS, FOCAL	1 (4%)			
DEGENERATION, NOS	10 (40%)			
*AORTA	(25)	(46)	(49)	(49)
INFLAMMATION, CHRONIC FOCAL		1 (2%)		
CALCIFICATION, FOCAL	1 (4%)			
*PULMONARY ARTERY	(25)	(46)	(49)	(49)
HYPERTROPHY, NOS		1 (2%)		
DIGESTIVE SYSTEM				
*SALIVARY GLAND	(24)	(38)	(48)	(48)
CYST, NOS			1 (2%)	
*LIVER	(25)	(46)	(48)	(49)
CONGESTION, CHRONIC PASSIVE	1 (4%)			
INFLAMMATION, CHRONIC			1 (2%)	
CHOLANGIOFIBROSIS	1 (4%)			
NECROSIS, FOCAL	1 (4%)	3 (7%)		
NECROSIS, COAGULATIVE		1 (2%)		1 (2%)
METAMORPHOSIS FATTY	4 (16%)	1 (2%)	1 (2%)	1 (2%)
HYPERPLASIA, FOCAL		23 (50%)		
*LIVER/PERIPORTAL	(25)	(46)	(48)	(49)
FIBROSIS		1 (2%)		
*LIVER/HEPATOCYTES	(25)	(46)	(48)	(49)
DEGENERATION, NOS			1 (2%)	1 (2%)

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

TABLE C1 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE CONTROL (UNTR) 01-0037	LOW DOSE 01-0039	HIGH DOSE 01-0090
HYPERPLASIA, FOCAL			1 (2%)	1 (2%)
*BILE DUCT	(25)	(46)	(49)	(49)
INFLAMMATION, NOS		6 (13%)		
INFLAMMATION, CHRONIC DIFFUSE			1 (2%)	
HYPERPLASIA, NOS	6 (24%)	32 (70%)	6 (12%)	3 (6%)
HYPERPLASIA, FOCAL		1 (2%)		
*PANCREAS	(25)	(42)	(48)	(48)
INFLAMMATION, NOS	1 (4%)	10 (24%)		
HYPERPLASIA, INTRADUCTAL		1 (2%)		
#PANCREATIC DUCT	(25)	(42)	(48)	(48)
HYPERPLASIA, NOS	1 (4%)			
#PANCREATIC ACINUS	(25)	(42)	(48)	(48)
INFLAMMATION, NOS			1 (2%)	
DEGENERATION, NOS				1 (2%)
ATROPHY, NOS		4 (10%)	1 (2%)	
#STOMACH	(24)	(45)	(47)	(49)
EPIDERMAL INCLUSION CYST	1 (4%)	1 (2%)		
ULCER, NOS		2 (4%)		
HYPERPLASIA, NOS		6 (13%)		
HYPERKERATOSIS		1 (2%)		
ACANTHOSIS		1 (2%)		
#GASTRIC MUCOSA	(24)	(45)	(47)	(49)
ULCER, FOCAL				1 (2%)
#PEYERS PATCH	(24)	(43)	(48)	(48)
HYPERPLASIA, NOS	2 (8%)	7 (16%)		
#COLON	(24)	(43)	(47)	(48)
NEMATODIASIS		3 (7%)		
URINARY SYSTEM				
#KIDNEY	(24)	(46)	(48)	(49)
GLOMERULONEPHRITIS, NOS	5 (21%)	33 (72%)		
INFLAMMATION, INTERSTITIAL		1 (2%)	1 (2%)	
NEPHROPATHY	1 (4%)			
NEPHROSIS, NOS	16 (67%)		42 (88%)	46 (94%)
#KIDNEY/CORTEX	(24)	(46)	(48)	(49)
CYST, NOS				1 (2%)

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

TABLE C1 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE CONTROL (UNTR) 01-0037	LOW DOSE 01-0039	HIGH DOSE 01-0090
HEMORRHAGE				1 (2%)
METAMORPHOSIS FATTY				1 (2%)
#KIDNEY/TUBULE	(24)	(46)	(48)	(49)
NECROSIS, NOS			1 (2%)	
#URINARY BLADDER	(23)	(42)	(48)	(45)
CALCULUS, NOS	3 (13%)			
INFLAMMATION, NOS		1 (2%)		
HYPERPLASIA, EPITHELIAL		3 (7%)	1 (2%)	2 (4%)
ENDOCRINE SYSTEM				
#PITUITARY	(21)	(41)	(45)	(40)
HYPERPLASIA, NOS		3 (7%)		
HYPERPLASIA, CHROMOPHOBE-CELL		2 (5%)		
#PITUITARY/BASOPHIL	(21)	(41)	(45)	(40)
NODULE	1 (5%)			
#ADRENAL	(25)	(43)	(48)	(48)
LIPOIDOSIS				1 (2%)
#ADRENAL CORTEX	(25)	(43)	(48)	(48)
HYPERTROPHY, FOCAL	1 (4%)	1 (2%)		
HYPERPLASIA, NOS		1 (2%)		1 (2%)
#ADRENAL MEDULLA	(25)	(43)	(48)	(48)
NECROSIS, NOS		1 (2%)		
CALCIFICATION, NOS		1 (2%)		
HYPERPLASIA, NODULAR		1 (2%)		
HYPERPLASIA, NOS		6 (14%)		
#THYROID	(23)	(45)	(46)	(47)
HYPERPLASIA, ADENOMATOUS		1 (2%)		
HYPERPLASIA, C-CELL		1 (2%)		1 (2%)
#PANCREATIC ISLETS	(25)	(42)	(48)	(48)
HYPERPLASIA, NOS		2 (5%)		
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(25)	(46)	(49)	(49)
HYPERPLASIA, NOS	3 (12%)	5 (11%)	1 (2%)	

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

TABLE C1 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE CONTROL (UNTR) 01-0037	LOW DOSE 01-0039	HIGH DOSE 01-0090
LACTATION	7 (28%)			
*PREPUTIAL GLAND	(25)	(46)	(49)	(49)
DILATATION, NOS			1 (2%)	
ABSCESS, NOS		1 (2%)		
HYPERPLASIA, NOS		1 (2%)	1 (2%)	
#PROSTATE	(23)	(45)	(47)	(46)
INFLAMMATION, NOS	1 (4%)	21 (47%)		
INFLAMMATION, FOCAL		3 (7%)		
INFLAMMATION, SUPPURATIVE			1 (2%)	
INFLAMMATION, ACUTE			1 (2%)	
ATROPHY, NOS	4 (17%)			
HYPERPLASIA, FOCAL		5 (11%)		
HYPERPLASIA, PAPILLARY		2 (4%)		
METAPLASIA, SQUAMOUS		5 (11%)		
*SEMINAL VESICLE	(25)	(46)	(49)	(49)
ATROPHY, NOS	1 (4%)			2 (4%)
#TESTIS	(24)	(45)	(48)	(48)
CALCIFICATION, FOCAL	4 (17%)			
ATROPHY, NOS	12 (50%)	2 (4%)		1 (2%)
ASPERMATOGENESIS		1 (2%)		
HYPERPLASIA, INTERSTITIAL CELL	2 (8%)	19 (42%)		
#TESTIS/TUBULE	(24)	(45)	(48)	(48)
DEGENERATION, NOS		6 (13%)		1 (2%)
NERVOUS SYSTEM				
#BRAIN	(25)	(44)	(48)	(48)
HEMORRHAGE	2 (8%)			
CALCIFICATION, FOCAL	1 (4%)			
SPECIAL SENSE ORGANS				
*EYE	(25)	(46)	(49)	(49)
INFLAMMATION, NOS			1 (2%)	
*EYE/CORNEA	(25)	(46)	(49)	(49)
ULCER, NOS			1 (2%)	

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

TABLE C1 (CONCLUDED)

	HIGH DOSE CONTROL (UNTR) 01-0084	LOW DOSE CONTROL (UNTR) 01-0037	LOW DOSE 01-0039	HIGH DOSE 01-0090
MUSCULOSKELETAL SYSTEM				
*BONE	(25)	(46)	(49)	(49)
OSTEOSCLEROSIS				1 (2%)
*SKELETAL MUSCLE	(25)	(46)	(49)	(49)
CALCIFICATION, FOCAL	1 (4%)			
*CARTILAGE,NOS	(25)	(46)	(49)	(49)
CYST, NOS		1 (2%)		
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED			1	
AUTO/NECROPSY/HISTO PERF		1		
AUTO/NECROPSY/NO HISTO			1	
AUTOLYSIS/NO NECROPSY		4	1	1

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

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS
IN FEMALE RATS TREATED WITH 2,5-TOLUENEDIAMINE SULFATE

	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE CONTROL (UNTR) 02-0037	LOW DOSE 02-0039	HIGH DOSE 02-0090
ANIMALS INITIALLY IN STUDY	25	50	50	50
ANIMALS NECROPSIED	23	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	23	49	50	50
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
*NASAL TURBINATE	(23)	(49)	(50)	(50)
INFLAMMATION, SUPPURATIVE				1 (2%)
*LARYNX	(23)	(49)	(50)	(50)
INFLAMMATION ACUTE AND CHRONIC	1 (4%)			
INFLAMMATION, CHRONIC	3 (13%)			
#TRACHEA	(5)	(48)	(49)	(49)
HEMORRHAGE			1 (2%)	
INFLAMMATION, NOS		9 (19%)	1 (2%)	
INFLAMMATION, CHRONIC		10 (21%)		
POLYP, INFLAMMATORY		1 (2%)		
#LUNG/BRONCHUS	(23)	(49)	(50)	(50)
BRONCHIECTASIS		1 (2%)	1 (2%)	
INFLAMMATION, NOS		1 (2%)		
INFLAMMATION, CHRONIC		9 (18%)		
#LUNG/BRONCHIOLE	(23)	(49)	(50)	(50)
INFLAMMATION, NOS		1 (2%)		
#LUNG	(23)	(49)	(50)	(50)
CYST, NOS			1 (2%)	
INFLAMMATION, NOS		1 (2%)		
INFLAMMATION, FOCAL		7 (14%)		
INFLAMMATION, INTERSTITIAL	3 (13%)	2 (4%)		
PNEUMONIA, CHRONIC MURINE	8 (35%)			
GRANULOMA, FOREIGN BODY	1 (4%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE CONTROL (UNTR) 02-0037	LOW DOSE 02-0039	HIGH DOSE 02-0090
PERIVASCULITIS		6 (12%)		
CALCIFICATION, FOCAL	1 (4%)			
HYPERPLASIA, EPITHELIAL	1 (4%)			
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(22)	(48)	(49)	(45)
HYPERPLASIA, HEMATOPOIETIC	1 (5%)			2 (4%)
#SPLEEN	(23)	(49)	(50)	(49)
HEMATOMA, NOS	1 (4%)			
HEMOSIDEROSIS	2 (9%)			
HYPERPLASIA, NOS		1 (2%)		
HYPERPLASIA, HEMATOPOIETIC	3 (13%)	3 (6%)		
HYPERPLASIA, ERYTHROID	4 (17%)	17 (35%)		
HYPERPLASIA, PLASMA CELL		1 (2%)		
HYPERPLASIA, RETICULUM CELL		11 (22%)		
HEMATOPOIESIS	3 (13%)		1 (2%)	
ERYTHROPOIESIS			3 (6%)	1 (2%)
#LYMPH NODE	(21)	(41)	(47)	(49)
INFLAMMATION, NOS		3 (7%)		
HYPERPLASIA, NOS		2 (5%)		
PLASMACYTOSIS		3 (7%)		
HYPERPLASIA, PLASMA CELL		1 (2%)		
#MEDIASTINAL L.NODE	(21)	(41)	(47)	(49)
HYPERPLASIA, PLASMA CELL				1 (2%)
CIRCULATORY SYSTEM				
#MYOCARDIUM	(23)	(49)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	1 (2%)	
INFLAMMATION, INTERSTITIAL	1 (4%)	24 (49%)	1 (2%)	
INFLAMMATION, CHRONIC			1 (2%)	
FIBROSIS		5 (10%)		
DEGENERATION, NOS	4 (17%)			
*PORTAL VEIN	(23)	(49)	(50)	(50)
THROMBUS, MURAL		1 (2%)		
DIGESTIVE SYSTEM				
#LIVER	(23)	(49)	(50)	(49)
CONGESTION, CHRONIC PASSIVE	1 (4%)			

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

TABLE C2 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE CONTROL (UNTR) 02-0037	LOW DOSE 02-0039	HIGH DOSE 02-0090
INFLAMMATION, CHRONIC			1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)	1 (2%)
FIBROSIS		1 (2%)		
CHOLANGIOFIBROSIS	1 (4%)			
PERIVASCULITIS		1 (2%)		
NECROSIS, NOS			1 (2%)	
NECROSIS, FOCAL		4 (8%)	1 (2%)	1 (2%)
NECROSIS, COAGULATIVE		2 (4%)		
METAMORPHOSIS FATTY	2 (9%)	1 (2%)	2 (4%)	1 (2%)
BASOPHILIC CYTO CHANGE	4 (17%)			
HYPERPLASIA, NODULAR		1 (2%)	1 (2%)	
HYPERPLASIA, FOCAL	3 (13%)	22 (45%)	1 (2%)	
ANGIECTASIS		1 (2%)	1 (2%)	
HEMATOPOIESIS			1 (2%)	
#LIVER/CENTRILOBULAR	(23)	(49)	(50)	(49)
NECROSIS, DIFFUSE			1 (2%)	
#LIVER/PERIPORTAL	(23)	(49)	(50)	(49)
METAMORPHOSIS FATTY				1 (2%)
*BILE DUCT	(23)	(49)	(50)	(50)
INFLAMMATION, NOS		5 (10%)		
INFLAMMATION, CHRONIC				1 (2%)
HYPERPLASIA, NOS	2 (9%)	27 (55%)	2 (4%)	1 (2%)
#PANCREAS	(22)	(46)	(50)	(49)
INFLAMMATION, NOS		7 (15%)		
INFLAMMATION, INTERSTITIAL				1 (2%)
PERIARTERITIS			1 (2%)	
#PANCREATIC DUCT	(22)	(46)	(50)	(49)
HYPERPLASIA, NOS		1 (2%)		
#PANCREATIC ACINUS	(22)	(46)	(50)	(49)
ATROPHY, NOS		2 (4%)		
#STOMACH	(23)	(48)	(50)	(49)
INFLAMMATION, NOS		2 (4%)		
INFLAMMATION, FOCAL		2 (4%)		
PERIARTERITIS			1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)		
#GASTRIC MUCOSA	(23)	(48)	(50)	(49)
HYPERPLASIA, NOS		1 (2%)		

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

TABLE C2 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE CONTROL (UNTR) 02-0037	LOW DOSE 02-0039	HIGH DOSE 02-0090
#PEYERS PATCH	(23)	(47)	(50)	(49)
HYPERPLASIA, NOS	4 (17%)	6 (13%)		
#COLON	(22)	(43)	(48)	(48)
NEMATODIASIS		3 (7%)		
PARASITISM	2 (9%)			
URINARY SYSTEM				
#KIDNEY	(23)	(49)	(50)	(49)
HYDRONEPHROSIS		1 (2%)		
GLOMERULONEPHRITIS, NOS	4 (17%)	33 (67%)		
INFLAMMATION, INTERSTITIAL		1 (2%)	2 (4%)	
GLOMERULONEPHRITIS, MEMBRANOUS		1 (2%)		
PYELONEPHRITIS, ACUTE	1 (4%)			
INFLAMMATION, CHRONIC		1 (2%)		
PYELONEPHRITIS, CHRONIC	1 (4%)			
PERIARTERITIS			1 (2%)	
NEPHROSIS, NOS	10 (43%)		32 (64%)	22 (45%)
GLOMERULOSCLEROSIS, NOS			1 (2%)	
METAMORPHOSIS FATTY			1 (2%)	
CALCIFICATION, FOCAL	1 (4%)			
#KIDNEY/CORTEX	(23)	(49)	(50)	(49)
CYST, NOS			1 (2%)	
#KIDNEY/TUBULE	(23)	(49)	(50)	(49)
NECROSIS, NOS	1 (4%)			
PIGMENTATION, NOS			1 (2%)	
#URINARY BLADDER	(22)	(41)	(47)	(50)
INFLAMMATION, NOS		1 (2%)		
HYPERPLASIA, EPITHELIAL			1 (2%)	
ENDOCRINE SYSTEM				
#PITUITARY	(21)	(43)	(48)	(43)
HEMORRHAGIC CYST	1 (5%)			
HYPERPLASIA, NOS		2 (5%)		
HYPERPLASIA, FOCAL	1 (5%)			
HYPERPLASIA, CHROMOPHOBE-CELL		1 (2%)		
#ADRENAL	(23)	(46)	(50)	(48)
LIPOIDOSIS			1 (2%)	

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

TABLE C2 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE CONTROL (UNTR) 02-0037	LOW DOSE 02-0039	HIGH DOSE 02-0090
HYPERTROPHY, NOS			1 (2%)	
#ADRENAL CORTEX	(23)	(46)	(50)	(48)
NODULE		1 (2%)		
HYPERPLASIA, NOS		7 (15%)		
#ADRENAL MEDULLA	(23)	(46)	(50)	(48)
HYPERPLASIA, NOS		4 (9%)		
#THYROID	(21)	(47)	(48)	(49)
HYPERPLASIA, C-CELL	3 (14%)			
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)		
#PANCREATIC ISLETS	(22)	(46)	(50)	(49)
HYPERPLASIA, NOS		1 (2%)		
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(23)	(49)	(50)	(50)
GALACTOCELE	1 (4%)	5 (10%)		
CYSTIC DUCTS				1 (2%)
HYPERPLASIA, NOS	1 (4%)	17 (35%)		
HYPERPLASIA, FOCAL				1 (2%)
HYPERPLASIA, PAPILLARY		1 (2%)		
HYPERPLASIA, CYSTIC			1 (2%)	
FIBROCYSTIC DISEASE			1 (2%)	
LACTATION	9 (39%)			
*VAGINA	(23)	(49)	(50)	(50)
HYPERTROPHY, NOS			1 (2%)	
#UTERUS	(23)	(48)	(49)	(45)
DILATATION, NOS				2 (4%)
HYDROMETRA		3 (6%)		
HEMATOMA, NOS			1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	1 (2%)	
PYOMETRA	3 (13%)			
ABSCESS, NOS		2 (4%)	2 (4%)	
HYPERPLASIA, ADENOMATOUS		5 (10%)		
#CERVIX UTERI	(23)	(48)	(49)	(45)
INFLAMMATION, SUPPURATIVE				1 (2%)
#UTERUS/ENDOMETRIUM	(23)	(48)	(49)	(45)
INFLAMMATION, NOS	1 (4%)	14 (29%)		

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

TABLE C2 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE CONTROL (UNTR) 02-0037	LOW DOSE 02-0039	HIGH DOSE 02-0090
INFLAMMATION, FOCAL		1 (2%)		
INFLAMMATION, SUPPURATIVE		2 (4%)	8 (16%)	7 (16%)
INFLAMMATION, CHRONIC	1 (4%)			
HYPERPLASIA, NOS	1 (4%)	1 (2%)	1 (2%)	
HYPERPLASIA, CYSTIC	1 (4%)	2 (4%)	4 (8%)	6 (13%)
HYPERPLASIA, ADENOMATOUS		1 (2%)		
#OVARY/OVIDUCT	(23)	(48)	(49)	(45)
INFLAMMATION, NOS		1 (2%)		
INFLAMMATION, ACUTE	1 (4%)			
ABSCESS, NOS	1 (4%)			
#OVARY	(22)	(47)	(50)	(46)
CYST, NOS	3 (14%)	4 (9%)	6 (12%)	
ABSCESS, NOS			1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)		
FIBROSIS			2 (4%)	
HYPERPLASIA, INTERSTITIAL CELL		1 (2%)		
NERVOUS SYSTEM				
#BRAIN	(23)	(49)	(50)	(48)
HYDROCEPHALUS, NOS	1 (4%)			
HEMORRHAGE	1 (4%)			
CALCIFICATION, FOCAL	1 (4%)			
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*MEDIASTINUM	(23)	(49)	(50)	(50)
PERIARTERITIS			1 (2%)	
*MESENTERY	(23)	(49)	(50)	(50)
PERIARTERITIS			1 (2%)	
ALL OTHER SYSTEMS				
NONE				

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

TABLE C2 (CONCLUDED)

	HIGH DOSE CONTROL (UNTR) 02-0084	LOW DOSE CONTROL (UNTR) 02-0037	LOW DOSE 02-0039	HIGH DOSE 02-0090
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED			7	7
AUTOLYSIS/NO NECROPSY	2	1		

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 2,5-TOLUENEDIAMINE SULFATE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH 2,5-TOLUENEDIAMINE SULFATE

	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE CONTROL (UNTR) 05-0037	LOW DOSE 05-0039	HIGH DOSE 05-0089
IMALS INITIALLY IN STUDY	50	50	50	50
IMALS NECROPSIED	46	48	49	49
IMALS EXAMINED HISTOPATHOLOGICALLY**	45	48	48	49
TEGUMENTARY SYSTEM				
SKIN	(46)	(48)	(49)	(49)
EPIDERMAL INCLUSION CYST				1 (2%)
ULCER, FOCAL				1 (2%)
INFLAMMATION, ACUTE/CHRONIC				1 (2%)
INFLAMMATION, CHRONIC FOCAL				1 (2%)
FIBROSIS		1 (2%)		
ALOPECIA		1 (2%)		
HYPERKERATOSIS				1 (2%)
ACANTHOSIS				1 (2%)
SUBCUT TISSUE	(46)	(48)	(49)	(49)
NECROSIS, NOS		1 (2%)		
SPIRATORY SYSTEM				
LUNG/BRONCHUS	(45)	(48)	(47)	(49)
INFLAMMATION, NOS		1 (2%)		
INFLAMMATION, FOCAL		1 (2%)		
LUNG	(45)	(48)	(47)	(49)
BRONCHOPNEUMONIA, NOS			1 (2%)	
INFLAMMATION, NOS		1 (2%)		
INFLAMMATION, INTERSTITIAL		14 (29%)	1 (2%)	
INFLAMMATION, ACUTE FOCAL				1 (2%)
ARTERIOSCLEROSIS, NOS	1 (2%)			
HYPERPLASIA, EPITHELIAL		2 (4%)		
LUNG/ALVEOLI	(45)	(48)	(47)	(49)
HEMORRHAGE				1 (2%)
INFLAMMATION, FOCAL		2 (4%)		
FIBROSIS, FOCAL		1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
NUMBER OF ANIMALS NECROPSIED
EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE CONTROL (UNTR) 05-0037	LOW DOSE 05-0039	HIGH DOSE 05-0089
HEMATOPOIETIC SYSTEM				
#SPLEEN	(45)	(47)	(48)	(43)
INFLAMMATION, NOS		1 (2%)		
FIBROSIS	1 (2%)			
HYPERPLASIA, NOS		2 (4%)		
HYPERPLASIA, HEMATOPOIETIC		2 (4%)		
HYPERPLASIA, ERYTHROID		2 (4%)		
HYPERPLASIA, RETICULUM CELL	3 (7%)			
HYPERPLASIA, LYMPHOID		2 (4%)		
HEMATOPOIESIS	1 (2%)			
ERYTHROPOIESIS				2 (5%)
#LYMPH NODE	(35)	(44)	(44)	(36)
HEMORRHAGIC CYST		1 (2%)		
INFLAMMATION, NOS		13 (30%)		
DEGENERATION, CYSTIC		1 (2%)		
HYPERPLASIA, NOS		2 (5%)		
HYPERPLASIA, HEMATOPOIETIC		1 (2%)		
HYPERPLASIA, LYMPHOID		2 (5%)		
MYELOID METAPLASIA		2 (5%)		
#SUBMANDIBULAR L.NODE	(35)	(44)	(44)	(36)
HYPERPLASIA, PLASMA CELL				2 (6%)
#MEDIASTINAL L.NODE	(35)	(44)	(44)	(36)
NECROSIS, NOS		1 (2%)		
#PANCREATIC L.NODE	(35)	(44)	(44)	(36)
INFLAMMATION, NOS		1 (2%)		
HYPERPLASIA, NOS			1 (2%)	
#MESENTERIC L. NODE	(35)	(44)	(44)	(36)
CONGESTION, NOS			3 (7%)	
HEMORRHAGE		1 (2%)		
INFLAMMATION, NOS		9 (20%)		
HYPERPLASIA, NOS			5 (11%)	2 (6%)
#THYMUS	(19)	(34)	(32)	(33)
NECROSIS, NOS		1 (3%)		
CIRCULATORY SYSTEM				
#HEART/VENTRICLE	(44)	(48)	(47)	(49)
MELANIN		2 (4%)		

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

TABLE D1 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE CONTROL (UNTR) 05-0037	LOW DOSE 05-0039	HIGH DOSE 05-0089
#MYOCARDIUM	(44)	(48)	(47)	(49)
INFLAMMATION, INTERSTITIAL		2 (4%)		
FIBROSIS		5 (10%)		
*BLOOD VESSEL	(46)	(48)	(49)	(49)
INFLAMMATION, NOS		2 (4%)		
*PULMONARY ARTERY	(46)	(48)	(49)	(49)
MINERALIZATION		2 (4%)		
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(43)	(47)	(47)	(45)
INFLAMMATION, NOS		2 (4%)		
PERIVASCULAR CUFFING		1 (2%)		
#LIVER	(45)	(48)	(48)	(49)
HEMATOMA, NOS			1 (2%)	
INFLAMMATION, FOCAL	2 (4%)			
DEGENERATION, NOS	1 (2%)			
NECROSIS, FOCAL		13 (27%)	1 (2%)	
METAMORPHOSIS FATTY	3 (7%)	3 (6%)		
HYPERPLASIA, NODULAR		2 (4%)		
HYPERPLASTIC NODULE				1 (2%)
HYPERPLASIA, FOCAL		1 (2%)		
ANGIECTASIS		1 (2%)		
HEMATOPOIESIS				1 (2%)
MYELOID METAPLASIA		1 (2%)		
#LIVER/PERIPORTAL	(45)	(48)	(48)	(49)
INFLAMMATION, NOS	1 (2%)			
#LIVER/HEPATOCYTES	(45)	(48)	(48)	(49)
DEGENERATION, NOS		1 (2%)		
*GALLBLADDER	(46)	(48)	(49)	(49)
INFLAMMATION, FOCAL		1 (2%)		
*BILE DUCT	(46)	(48)	(49)	(49)
INFLAMMATION, NOS	1 (2%)			
#PANCREAS	(44)	(48)	(45)	(43)
INFLAMMATION, NOS		7 (15%)		

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

TABLE D1 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE CONTROL (UNTR) 05-0037	LOW DOSE 05-0039	HIGH DOSE 05-0089
INFLAMMATION, FOCAL		1 (2%)		
DEGENERATION, CYSTIC		1 (2%)		
METAMORPHOSIS FATTY		1 (2%)		
#PANCREATIC DUCT	(44)	(48)	(45)	(43)
HYPERPLASIA, NOS		1 (2%)		
#PANCREATIC ACINUS	(44)	(48)	(45)	(43)
HYPERTROPHY, FOCAL		1 (2%)		
HYPERPLASIA, FOCAL		1 (2%)		
#STOMACH	(42)	(47)	(46)	(48)
INFLAMMATION, NOS		13 (28%)		
ULCER, NOS		1 (2%)		
INFLAMMATION, FOCAL		1 (2%)		
INFLAMMATION, INTERSTITIAL		1 (2%)		
PERIARTERITIS				1 (2%)
HYPERPLASIA, NOS		1 (2%)		
HYPERPLASIA, FOCAL	1 (2%)	1 (2%)		
HYPERKERATOSIS		3 (6%)		
ACANTHOSIS		3 (6%)		
#GASTRIC MUCOSA	(42)	(47)	(46)	(48)
HYPERPLASIA, FOCAL		1 (2%)		
#PEYERS PATCH	(43)	(48)	(47)	(48)
HYPERPLASIA, NOS		2 (4%)		
#ILEUM	(43)	(48)	(47)	(48)
HEMORRHAGE		1 (2%)		
INFLAMMATION, NOS		2 (4%)		
#COLON	(38)	(45)	(43)	(46)
PARASITISM		1 (2%)		
URINARY SYSTEM				
#KIDNEY	(45)	(47)	(48)	(48)
CALCULUS, NOS	20 (44%)			
GLOMERULONEPHRITIS, NOS		6 (13%)		
INFLAMMATION, NOS		1 (2%)		
INFLAMMATION, INTERSTITIAL	5 (11%)	23 (49%)		
INFLAMMATION, CHRONIC	1 (2%)			1 (2%)
PERIVASCULITIS	2 (4%)			

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

TABLE D-1 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE CONTROL (UNTR) 05-0037	LOW DOSE 05-0039	HIGH DOSE 05-0089
ARTERIOSCLEROSIS, NOS	1 (2%)			
NEPHROSIS, NOS	1 (2%)			
GLOMERULOSCLEROSIS, NOS				1 (2%)
HYPERPLASIA, TUBULAR CELL	2 (4%)			
#KIDNEY/TUBULE	(45)	(47)	(48)	(48)
DEGENERATION, NOS	1 (2%)			
NECROSIS, FOCAL		1 (2%)		
METAMORPHOSIS FATTY	9 (20%)			
#URINARY BLADDER	(44)	(48)	(47)	(47)
INFLAMMATION, NOS		4 (8%)		
HYPERPLASIA, EPITHELIAL		9 (19%)		
ENDOCRINE SYSTEM				
#PITUITARY	(36)	(42)	(41)	(36)
HYPERPLASIA, NOS		3 (7%)		
HYPERPLASIA, FOCAL		3 (7%)		
#ADRENAL CORTEX	(43)	(45)	(45)	(44)
NODULE		1 (2%)		
HYPERTROPHY, FOCAL		1 (2%)		
HYPERPLASIA, NOS		1 (2%)		
#ADRENAL MEDULLA	(43)	(45)	(45)	(44)
DEGENERATION, NOS		1 (2%)		
#THYROID	(40)	(47)	(46)	(46)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)		
HYPERPLASIA, PAPILLARY		1 (2%)		
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)		
#PANCREATIC ISLETS	(44)	(48)	(45)	(43)
HYPERPLASIA, NOS		2 (4%)		
REPRODUCTIVE SYSTEM				
*PREPUTIAL GLAND	(46)	(48)	(49)	(49)
DILATATION/DUCTS				1 (2%)
INFLAMMATION, SUPPURATIVE			1 (2%)	
ABSCESS, NOS		2 (4%)		
#TESTIS/TUBULE	(45)	(47)	(48)	(48)
DEGENERATION, NOS	2 (4%)	4 (9%)		

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

TABLE D1 (CONCLUDED)

	HIGH DOSE CONTROL (UNTR) 05-0077	LOW DOSE CONTROL (UNTR) 05-0037	LOW DOSE 05-0039	HIGH DOSE 05-0089
CALCIFICATION, FOCAL			1 (2%)	
NERVOUS SYSTEM				
#CEREBRAL CORTEX	(45)	(48)	(47)	(44)
MINERALIZATION		3 (6%)		
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(46)	(48)	(49)	(49)
STEATITIS	1 (2%)			
NECROSIS, FAT				1 (2%)
*MESENTERY	(46)	(48)	(49)	(49)
PERIARTERITIS				2 (4%)
ALL OTHER SYSTEMS				
ADIPOSE TISSUE				
NECROSIS, NOS				1
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	8		20	17
AUTO/NECROPSY/NO HISTO	1		1	
AUTOLYSIS/NO NECROPSY	4	2	1	1

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

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
TREATED WITH 2,5-TOLUENEDIAMINE SULFATE

	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE CONTROL (UNTR) 06-0037	LOW DOSE 06-0039	HIGH DOSE 06-0089
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS MISSING			1	
ANIMALS NECROPSIED	46	48	45	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	46	47	43	46
INTEGUMENTARY SYSTEM				
*SKIN	(46)	(48)	(45)	(47)
FIBROSIS	1 (2%)			
FIBROSIS, FOCAL	1 (2%)			
*SUBCUT TISSUE	(46)	(48)	(45)	(47)
MINERALIZATION		1 (2%)		
FIBROSIS		1 (2%)		
RESPIRATORY SYSTEM				
#LUNG/BRONCHUS	(45)	(46)	(42)	(46)
INFLAMMATION, FOCAL		1 (2%)		
#LUNG	(45)	(46)	(42)	(46)
CONGESTION, NOS			1 (2%)	
INFLAMMATION, INTERSTITIAL	2 (4%)	10 (22%)		
PERIARTERITIS	1 (2%)			
HYPERPLASIA, EPITHELIAL		3 (7%)		
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(44)	(45)	(39)	(41)
MYELOFIBROSIS		1 (2%)		
HYPERPLASIA, HEMATOPOIETIC			1 (3%)	
#SPLEEN	(43)	(46)	(42)	(42)
PLASMA-CELL INFILTRATE			1 (2%)	
HYPERPLASIA, HEMATOPOIETIC		16 (35%)		
HYPERPLASIA, ERYTHROID		6 (13%)		
HYPERPLASIA, RETICULUM CELL	2 (5%)			
HYPERPLASIA, LYMPHOID	4 (9%)	10 (22%)	1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE CONTROL (UNTR) 06-0037	LOW DOSE 06-0039	HIGH DOSE 06-0089
HEMATOPOIESIS	1 (2%)	1 (2%)	3 (7%)	
ERYTHROPOIESIS			1 (2%)	
MYELOPOIESIS		1 (2%)		
#LYMPH NODE	(41)	(39)	(37)	(32)
CYST, NOS		1 (3%)		
INFLAMMATION, NOS		15 (38%)		
HYPERPLASIA, NOS		1 (3%)		
RETICULOCYTOSIS		1 (3%)		
HYPERPLASIA, HEMATOPOIETIC		2 (5%)		
MYELOID METAPLASIA		1 (3%)		
CIRCULATORY SYSTEM				
#HEART/VENTRICLE	(45)	(46)	(42)	(46)
MELANIN		4 (9%)		
#MYOCARDIUM	(45)	(46)	(42)	(46)
CALCIFICATION, FOCAL	1 (2%)			
*PULMONARY ARTERY	(46)	(48)	(45)	(47)
HYPERPLASIA, NOS	1 (2%)			
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(43)	(45)	(40)	(43)
INFLAMMATION, NOS		2 (4%)		
PERIARTERITIS				1 (2%)
PERIVASCULAR CUFFING		4 (9%)		
#LIVER	(45)	(47)	(42)	(46)
INFLAMMATION, NOS		1 (2%)		
INFLAMMATION, FOCAL	1 (2%)			
NECROSIS, FOCAL		22 (47%)		
CYTOPLASMIC CHANGE, NOS	1 (2%)			
HYPERPLASTIC NODULE		1 (2%)		1 (2%)
HYPERPLASIA, DIFFUSE	1 (2%)			
ANGIECTASIS		1 (2%)		
HEMATOPOIESIS		3 (6%)	2 (5%)	
#LIVER/PERIPORTAL	(45)	(47)	(42)	(46)
INFLAMMATION, NOS	1 (2%)			
*GALLBLADDER	(46)	(48)	(45)	(47)
INFLAMMATION, NOS		3 (6%)		

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

TABLE D-2 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE CONTROL (UNTR) 06-0037	LOW DOSE 06-0039	HIGH DOSE 06-0089
BILE DUCT	(46)	(48)	(45)	(47)
INFLAMMATION, NOS	1 (2%)	1 (2%)		
PANCREAS	(41)	(44)	(42)	(42)
INFLAMMATION, NOS		5 (11%)		
INFLAMMATION, INTERSTITIAL			1 (2%)	
PERIARTERITIS		1 (2%)		
PANCREATIC DUCT	(41)	(44)	(42)	(42)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)		
STOMACH	(42)	(44)	(42)	(43)
INFLAMMATION, NOS		7 (16%)		
ULCER, NOS		1 (2%)		
INFLAMMATION, FOCAL		1 (2%)		
HYPERPLASIA, NOS		1 (2%)		
HYPERPLASIA, EPITHELIAL		1 (2%)		
HYPERPLASIA, ADENOMATOUS		1 (2%)		
HYPERKERATOSIS		1 (2%)		
ACANTHOSIS		1 (2%)		
GASTRIC MUCOSA	(42)	(44)	(42)	(43)
HYPERPLASIA, FOCAL		1 (2%)		
PEYERS PATCH	(43)	(44)	(42)	(42)
HYPERPLASIA, NOS		1 (2%)		
INARY SYSTEM				
KIDNEY	(43)	(46)	(43)	(45)
CONGESTION, NOS			1 (2%)	
GLOMERULONEPHRITIS, NOS		14 (30%)		
INFLAMMATION, INTERSTITIAL	3 (7%)	16 (35%)		
GLOMERULONEPHRITIS, CHRONIC				1 (2%)
PERIVASCULITIS	4 (9%)			
KIDNEY/GLOMERULUS	(43)	(46)	(43)	(45)
AMYLCIDOSIS	1 (2%)			
KIDNEY/PELVIS	(43)	(46)	(43)	(45)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)			
URINARY BLADDER	(41)	(46)	(34)	(41)
INFLAMMATION, NOS		4 (9%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE CONTROL (UNTR) 06-0037	LOW DOSE 06-0039	HIGH DOSE 06-0089
HYPERPLASIA, EPITHELIAL		10 (22%)		
#U.BLADDER/SUBMUCOSA	(41)	(46)	(34)	(41)
INFLAMMATION, CHRONIC				1 (2%)
ENDOCRINE SYSTEM				
#PITUITARY	(37)	(42)	(38)	(38)
HYPERPLASIA, FOCAL		6 (14%)		
#ADRENAL CORTEX	(43)	(45)	(38)	(41)
NODULE		3 (7%)		
#THYROID	(30)	(43)	(36)	(34)
FOLLICULAR CYST, NOS		1 (2%)		
INFLAMMATION, NOS		1 (2%)		
INFLAMMATION, CHRONIC FOCAL				1 (3%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(46)	(48)	(45)	(47)
GALACTOCELE		1 (2%)		
HYPERPLASIA, NOS		4 (8%)		
#UTERUS	(43)	(45)	(41)	(42)
HYDROMETRA	4 (9%)	1 (2%)	2 (5%)	2 (5%)
INFLAMMATION, ACUTE			1 (2%)	1 (2%)
ABSCESS, NOS		3 (7%)		
FIBROSIS		1 (2%)		
#UTERUS/ENDOMETRIUM	(43)	(45)	(41)	(42)
DILATATION, NOS			5 (12%)	
CYST, NOS	2 (5%)			
INFLAMMATION, NOS		10 (22%)		
INFLAMMATION, SUPPURATIVE		4 (9%)	1 (2%)	4 (10%)
INFLAMMATION, ACUTE			1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE			4 (10%)	
HYPERPLASIA, NOS	1 (2%)	4 (9%)		
HYPERPLASIA, CYSTIC	35 (81%)	18 (40%)	18 (44%)	28 (67%)
HYPERPLASIA, ADENOMATOUS		1 (2%)		
#OVARY/OVIDUCT	(43)	(45)	(41)	(42)
INFLAMMATION, NOS		5 (11%)		

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

TABLE D2 (CONTINUED)

	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE CONTROL (UNTR) 06-0037	LOW DOSE 06-0039	HIGH DOSE 06-0089
INFLAMMATION, SUPPURATIVE			2 (5%)	
OVARY	(41)	(45)	(36)	(39)
CYST, NOS	1 (2%)	3 (7%)		4 (10%)
INFLAMMATION, NOS		4 (9%)		
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)		
INFLAMMATION, SUPPURATIVE		10 (22%)	2 (6%)	6 (15%)
INFLAMMATION, ACUTE			1 (3%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)	
ABSCESS, NOS		4 (9%)		
INFLAMMATION, CHRONIC			4 (11%)	
DEGENERATION, CYSTIC		1 (2%)		
ATROPHY, NOS			1 (3%)	
OVARY/FOLLICLE	(41)	(45)	(36)	(39)
MULTIPLE CYSTS			1 (3%)	
RVOUS SYSTEM				
NONE				
ECIAL SENSE ORGANS				
NONE				
SCULOSKELETAL SYSTEM				
BONE	(46)	(48)	(45)	(47)
RESORPTION		3 (6%)		
VERTEBRA	(46)	(48)	(45)	(47)
OSTEOSCLEROSIS	1 (2%)			
DY CAVITIES				
NONE				
L OTHER SYSTEMS				
OMENTUM				
NECROSIS, FAT		1		
NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY				
NUMBER OF ANIMALS NECROPSIED				

TABLE D-2 (CONCLUDED)

	HIGH DOSE CONTROL (UNTR) 06-0077	LOW DOSE CONTROL (UNTR) 06-0037	LOW DOSE 06-0039	HIGH DOSE 06-0089
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	1	1	4	5
ANIMAL MISSING/NO NECROPSY			1	
AUTO/NECROPSY/HISTO PERF	2	2		1
AUTO/NECROPSY/NO HISTO		1	2	1
AUTOLYSIS/NO NECROPSY	4	2	4	3

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

Review of the Bioassay of 2,5-Toluenediamine Sulfate*
for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

June 29, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 2,5-Toluenediamine Sulfate for carcinogenicity.

Although a carcinogenic response was not demonstrated, the reviewer said that the evidence was suggestive that the compound may have a carcinogenic potential. He recommended that it be considered for retest. In his critique, he noted several experimental flaws, including the use of animals from different shipments, the conduct of the subchronic study in a different mouse strain than used in the chronic phase, and the start of the high dose rats on test some months after the initiation of the low dose animal group. The reviewer said the compound warranted further testing because of the experimental design and study conduct deficiencies, as well as the fact that 2,5-Toluenediamine Sulfate

had been shown to be positive in the Ames assay. The reviewer moved that the report on the bioassay of 2,5-Toluenediamine Sulfate be accepted as written but that the compound be considered for retest. The motion was approved without objection.

Clearinghouse Members present:

Arnold L. Brown (Chairman), Mayo Clinic
Paul Nettesheim, National Institute of Environmental Health Sciences
Verne Ray, Pfizer Medical Research Laboratory
Verald K. Rowe, Dow Chemical U.S.A.
Michael B. Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

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