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# BIOASSAY OF 2, 6-TOLUENEDIAMINE DIHYDROCHLORIDE FOR POSSIBLE CARCINOGENICITY

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

# BIOASSAY OF

# 2,6-TOLUENEDIAMINE DIHYDROCHLORIDE

# FOR POSSIBLE CARCINOGENICITY

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2,6-TOLUENEDIAMINE DIHYDROCHLORIDE

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National Cancer Institute/National Toxicology Program

## FOREWORD

This report presents the results of the bioassay of 2,6-toluenediamine dihydrochloride conducted for the Carcinogenesis Testing Program, National Cancer Institute (NCI)/National Toxicology Program (NTP). This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. A positive result demonstrates that a test chemical is carcinogenic for animals under the conditions of the test and indicates that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic in animals requires a wider analysis.

## CONTRIBUTORS

The bioassay of 2,6-toluenediamine dihydrochloride was conducted at EG&G Mason Research Institute, Worcester, Massachusetts, under a subcontract to Tracor Jitco, Inc., the prime contractor for the NCI Carcinogenesis Testing Program.

The bioassay was conducted under the supervision of Drs. A. Handler (1,2), H. Lilja (1), E. Massaro (1,3), and E. Smith (1,4), principal investigators, and Mr. G. Wade (1). The program manager was Ms. R. Monson (1). Ms. A. Good (1) supervised the technicians in charge of animal care, and Ms. E. Zepp (1) supervised the preparation of the feed mixtures and collected samples of the diets for analysis. Ms. D. Bouthot (1) kept all daily records of the test. Dr. A. S. Krishna Murthy (1), pathologist, directed the necropsies and performed the histopathologic examinations. The pathology report and selected slides were evaluated by the NCI Pathology Working Group as described in Ward et al. (1978).

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute, Rockville, Maryland (5). The statistical analyses were performed by Dr. J. R. Joiner (6) and Ms. S. Vatsan (6), using methods selected for the bioassay program by Dr. J. J. Gart (7).

Chemicals used in this bioassay were analyzed at Midwest Research Institute (8), and dosed feed mixtures were analyzed by Dr. M. Hagopian (1).

This report was prepared at Tracor Jitco (6) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. C. R. Angel, Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. R. L. Schueler, pathologist; Dr. A. C. Jacobs, bioscience writer; and Dr. W. D. Theriault and Ms. M. W. Glasser, technical editors.

The following scientists at NCI (9) were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Michael P. Dieter, Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Charles K. Grieshaber, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. Y. Jack Lee, Dr. Harry Mahar, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Marcelina B. Powers, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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## SUMMARY

2,6-Toluenediamine is used as an intermediate in the production of dyes for furs and textiles, and of flexible polyurethane foams and elastomers. A bioassay of 2,6-toluenediamine dihydrochloride for possible carcinogenicity was conducted by feeding diets containing the test chemical to F344 rats and B6C3F1 mice.

Groups of 50 rats of each sex were fed the test chemical at two doses, 250 or 500 ppm, for 103 weeks and observed for 1 additional week. Groups of 50 mice of each sex were fed the test chemical at two doses, 50 or 100 ppm, for 103 weeks and then observed for 1 additional week. Groups of 50 untreated rats and 50 untreated mice of each sex were used as matched controls. All surviving animals were killed and necropsied at 104 weeks.

Weight gain depression was less than 10% for dosed groups of male rats and male and female mice, when compared with controls. Mean body weight gain was depressed 17% in low-dose female rats and 27% in high-dose female rats. Mortality was not increased in rats or mice of either sex by the test chemical. No clinical evidence indicated that mice of either sex received a maximum tolerated dose of the compound.

In male rats, islet-cell adenomas of the pancreas and neoplastic nodules or carcinomas of the liver occurred in dose-related trends that were significant using the Cochran-Armitage test (P=0.025 and P=0.037, respectively). The results of the Fisher exact test were not significant for either lesion. The occurrences of tumors in dosed female rats were not significantly different from those in control rats.

Significant results in the negative direction were observed in the incidences of C-cell tumors of the thyroid in male rats and of fibroadenomas of the mammary gland in female rats.

In male mice, in the low-dose group, lymphomas occurred at an incidence significantly higher (P=0.046) than that of the corresponding control group; however, the incidence was not significant when the Bonferroni criterion for multiple comparison was used.

The occurrence of hepatocellular carcinomas in female mice was dose related, but the result of the Fisher exact test comparing the incidence in the high-dose group with that in the controls was not significant.

It was concluded that, under conditions of the bioassay, 2,6-toluenediamine dihydrochloride was not carcinogenic for male and female F344 rats or for male and female B6C3F1 mice.

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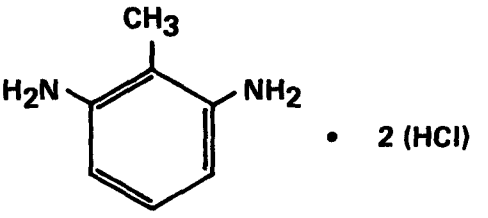
# I. INTRODUCTION




## 2, 6-TOLUENEDIAMINE DIHYDROCHLORIDE

2,6-Toluenediamine (CAS 823-40-5) is a by-product of the synthesis of 2,4-toluenediamine (CAS 95-80-7).

Mixtures of 2,6-toluenediamine and 2,4-toluenediamine are used in the synthesis of toluene diisocyanate, which is the predominant isocyanate used in the production of flexible polyurethane foams and elastomers (Milligan, 1968; Layer, 1964). Commercial mixtures of 2,4- and 2,6-toluenediamine normally contain 80% of the 2,4 isomer and 20% of the 2,6 isomer. A commercial mixture containing 65% of the 2,4 isomer and 35% of the 2,6 is also available (Buist, 1970). Annual production of the 80/20 mixture of 2,4- and 2,6-toluene diisocyanate in 1977 was 583 million pounds (United States International Trade Commission, 1978). Based on this figure, the annual production of 2,6-toluenediamine in 1977 is estimated to have been greater than 116 million pounds. 2,6-Toluenediamine (or its dihydrochloride salt) is also an intermediate used in the synthesis of several dyes used for textiles and furs (Society of Dyers and Colourists, 1971).

During production of urethane foam, significant quantities of volatile toluene diisocyanate are carried into the air above the foam and may reach concentrations as high as 10 ppm (Buist, 1970). These isocyanates are unstable in an aqueous environment and are readily converted to toluenediamine (Lowe, 1970).

2,6-Toluenediamine, activated by rat or mouse liver microsomal fractions, is weakly mutagenic in Salmonella typhimurium TA 1538, with a reversion rate similar to 2,4-toluenediamine (Dybing and Thorgeirsson, 1977).

2,6-Toluenediamine was selected for testing by the Carcinogenesis Testing Program because of its close structural relationship to 2,4-toluenediamine, a reported carcinogen (Ito et al., 1969). (The carcinogenic effects of 2,4-toluenediamine were recently confirmed -- NCI, 1979.) The dosed feed route was chosen for its convenience and comparability with results from several previous studies using structural analogs which had been administered by this route. The dihydrochloride of 2,6-toluenediamine (CAS 15481-70-6) was found to be more stable than the free amine in feed and was used in the bioassay.

## II. MATERIALS AND METHODS

### A. Chemical

2,6-Toluenediamine (Lot No. 012157) containing a trace impurity (unidentified) was obtained from Aldrich Chemical Company, Metuchen, New Jersey, and converted to the dihydrochloride salt (Lot No. 090857-MRI) at Midwest Research Institute. Analyses of both 2,6-toluenediamine and 2,6-toluenediamine dihydrochloride at Midwest Research Institute (elemental analysis; melting point, thin-layer and vapor-phase chromatography; and spectral analyses including infrared, ultraviolet, and nuclear magnetic resonance) were consistent with the respective structures (Appendixes E and F). Vapor-phase chromatography revealed a single homogeneous peak at two alternate settings. Thin-layer chromatography showed only a trace impurity (less than 1%) at the origin. 2,6-Toluenediamine dihydrochloride was stored at 4°C in its original container and transferred to amber stock bottles as needed.

### B. Dietary Preparation

Test diets were prepared by first mixing the chemical and an aliquot of powdered Wayne® Lab Blox animal feed (Allied Mills, Chicago, Illinois) with a mortar and pestle, placing this mixture in a Patterson-Kelly® twin-shell blender with the remainder of the feed, and mixing for 20 minutes. Test diets were sealed in labelled plastic bags and stored at 4°C for no longer than 14 days.

The stability of 2,6-toluenediamine dihydrochloride in feed was determined at Midwest Research Institute by assaying sample diet mixtures containing 10% 2,6-toluenediamine dihydrochloride that had been stored at -20°, 5°, 25°, or 45°C for 2 weeks. Amounts of the test chemical present were determined by gas chromatography as described in Appendix G. The compound was stable in feed for 2 weeks at temperatures as high as 45°C.

The amounts of 2,6-toluenediamine dihydrochloride in selected batches of feed were measured at 2- to 3-month intervals. In preliminary tests, 2-gram samples were extracted with 50 ml of 95% ethanol and analyzed by gas chromatography on a Tenax column at 235°C. Subsequent samples were extracted and analyzed as described in Appendix H.

The mean concentration of 10 samples, measured in duplicate and containing a theoretical level of 500 ppm, was 450±140 ppm. The mean concentration of 11 samples, measured in duplicate and containing a theoretical level of 250, was 240±50 ppm. Variation in the concentration of the compound among samples from the same mixture indicated some lack of homogeneity in the dosed-feed preparations.

## C. Animals

Three-week old F344 rats and 4- to 5-week old B6C3F1 mice were obtained from the NCI Frederick Cancer Research Center, Frederick, Maryland. The animals were acclimated for 2 weeks and then assigned to control or dosed groups in such a manner that average cage weights were approximately equal for all animals of the same sex and species.

## D. Animal Maintenance

Rats were housed four per cage in suspended polycarbonate cages (Lab Products, Inc., Garfield, N.J.) equipped with disposable nonwoven fiber filter sheets (Webrex). Mice were housed five per cage in polycarbonate shoe-box type cages covered with spunbonded Filtek filter bonnets (Lab Products). Aspen-bed® hardwood chips (American Excelsior, Summerville, Mass.) were used as bedding. Clean bedding and cages were provided twice weekly. Cage racks were changed every 2 weeks and disposable filters and filter bonnets were replaced at the same time. Filter bonnets were cleaned for reuse.

Water was available *ad libitum* for both species. Clean glass water bottles were provided twice weekly. Powdered Wayne® Lab Blox diet was available *ad libitum* in stainless-steel, gang-style hoppers (Scientific Cages, Inc., Bryan, Texas) which were changed once per week.

The temperature in the animal rooms was 19°-28°C and the relative humidity was 8%-74%. Incoming air was filtered through Tri-Dek 15/40 denier Dacron filters, with 10 to 12 air changes per hour. Fluorescent lighting was provided 12 hours per day.

Rats and mice were housed by species in rooms in which chronic tests were also being conducted on 4,4'-oxydianiline (CAS 101-80-4).

## E. Range-Finding and 14-Day Repeated Dose Studies

Range-finding and 14-day repeated dose feed studies were conducted using F344 rats and B6C3F1 mice to determine the concentrations of 2,6-toluenediamine dihydrochloride to be used in the subchronic studies.

In the range-finding study, groups of two males and two females of each species were tested at each of five doses by administering a single dose of the test substance in corn oil by gavage (as shown in Table 1). All surviving animals were killed after 14 days. At the three highest doses, 2,6-toluenediamine dihydrochloride induced hemorrhage of the fundic portion of the stomach and the intestinal tract of rats. 2,6-Toluenediamine dihydrochloride was lethal for male rats at doses greater than 1 g/kg and at 0.3 g/kg for females. Deaths occurred in mice at all doses.

In the repeated dose study, groups of five males and five females of each species were tested for 2 weeks with five dose levels of the test substance in the feed. Groups of five males and five females of each species were maintained as untreated controls. All surviving animals were killed after 2 weeks. Survival of dosed groups at 2 weeks is shown in Table 2. No deaths occurred in rats at any of the doses tested. Weight gain was depressed 10% or more in both male and female rats at doses of 1,000 ppm or higher. Weight gain depression was 12.5% for male rats fed diets containing 1,000 ppm and 47% for male rats at 3,000 ppm. Weight gain depression was 52% for female rats fed diets containing 1,000 ppm and 88% at 3,000 ppm. All of the male mice and 3/5 female mice that received 3,000 ppm 2,6-toluenediamine dihydrochloride in their feed died. Hemorrhage of the stomach, digestive tract, and renal medullae were detected in the mice that died.

Table 1. Dosage and Survival of Rats and Mice Administered A Single Dose of 2,6-Toluenediamine Dihydrochloride by Gavage

| Dose (g/kg) | Survival | | | |
|---|---|---|---|---|
| | Rats | | Mice | |
| | Male | Female | Male | Female |
| 0.1 | 2/2 | 2/2 | 1/2 | 1/2 |
| 0.3 | 2/2 | 2/2 | 0/2 | 0/2 |
| 1.0 | 2/2 | 0/2 | 0/2 | 1/2 |
| 3.0 | 0/2 | 0/2 | 0/2 | 0/2 |
| 10.0 | 0/2 | 0/2 | 0/2 | 0/2 |

Table 2. Dosage and Survival of Rats and Mice Administered Repeated Doses of 2,6-Toluenediamine Dihydrochloride in the Feed for 14 Days

| Dose (ppm) | Survival | | | |
|---|---|---|---|---|
| | Rats | | Mice | |
| | Male | Female | Male | Female |
| 0 | 5/5 | 5/5 | 5/5 | 5/5 |
| 100 | 5/5 | 5/5 | 5/5 | 5/5 |
| 300 | 5/5 | 5/5 | 5/5 | 5/5 |
| 1,000 | 5/5 | 5/5 | 5/5 | 5/5 |
| 3,000 | 5/5 | 5/5 | 0/5 | 2/5 |

## F. Subchronic Studies

Subchronic studies were conducted to determine the two concentrations (referred to in this report as "low" and "high" doses) to be used in the chronic studies. Diets containing 0, 100, 300, 1,000, 3,000, or 10,000 ppm 2,6-toluenediamine dihydrochloride were fed for 13 weeks to groups of 10 male and 10 female rats (Table 3), and groups of 10 male and 10 female mice received diets with 0, 10, 30, 100, 300, or 1,000 ppm (Table 4).

Clinical observations were made twice daily and animals were weighed weekly. At the end of the 91-day study, survivors were killed. Necropsies were performed on all animals and tissues were taken for histopathologic analysis.

Two out of 10 male rats and 7/10 female rats that received 10,000 ppm died before the end of the study. One of the males and four of the females died during week 1 of the study. No deaths occurred at any of the other dose levels.

Weight gains were depressed in male rats at all doses and in female rats at the three highest doses (1,000, 3,000, and 10,000 ppm).

Slight to moderate thyroid enlargement and darkening of the spleen, numerous lymph nodes, liver, kidney, adrenals, and nasal turbinates were observed in the eight surviving male rats that received the 10,000-ppm dose. Darkening of the same organs as in the males was observed in the three surviving female rats receiving the 10,000-ppm dose, but only one of these females had an enlarged thyroid. Among the rats examined, diffuse bilateral adenomatous hyperplasia of the thyroid with or without macroscopic enlargement was seen in 7/10 male rats fed 3,000 and 8/8 fed 10,000 ppm, and in 3/7 female rats fed 10,000 ppm. The only thyroid lesion observed in controls was seen in one male with papillary cyst hyperplasia. Bone marrow hyperplasia was seen in 8/8 male and 7/7 female rats at 10,000 ppm. Nephrosis was seen in 5/8 males and in 1/7 female rats fed the 10,000-ppm dose. Darkening of the nasal turbinates occurred in both male and female rats that received 2,000 or 3,000 ppm. No significant gross abnormalities were observed in the rats at the 300- and 1,000-ppm dose levels or in the controls.

Table 3. Dosage, Survival, and Mean Body Weights of Rats Fed 2,6-Toluenediamine Dihydrochloride in Diets for the 13-Week Subchronic Study

| Dose (ppm) | Survival(a) | Mean Body Weights (grams) Initial(b) | Final | Gain | Weight Change Relative to Controls(c) (Percent) |
|---|---|---|---|---|---|
| MALE | | | | | |
| 0 | 10/10 | 110 | 298 | 188 | |
| 100 | 10/10 | 110 | 271 | 161 | -14 |
| 300 | 10/10 | 110 | 269 | 159 | -15 |
| 1,000 | 10/10 | 110 | 258 | 148 | -19 |
| 3,000 | 10/10 | 110 | 229 | 119 | -36 |
| 10,000 | 8/10 | 110 | 158 | 48 | -74 |
| FEMALE | | | | | |
| 0 | 10/10 | 98 | 180 | 82 | |
| 100 | 10/10 | 98 | 177 | 79 | -3 |
| 300 | 10/10 | 98 | 176 | 78 | -5 |
| 1,000 | 10/10 | 98 | 156 | 58 | -29 |
| 3,000 | 10/10 | 98 | 123 | 25 | -70 |
| 10,000 | 3/10 | 98 | 103 | 5 | -91 |

(a) Number surviving/number per group.
(b) The initial weights were obtained from graphs submitted by the test laboratory.
(c) Weight Change Relative to Controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

Table 4. Dosage, Survival, and Mean Body Weights of Mice Fed 2,6-Toluenediamine Dihydrochloride in Diets for the 13-Week Subchronic Study

| Dose (ppm) | Survival(a) | Mean Body Weights(grams) Initial(b) | Final | Gain | Weight Change Relative to Controls(c) (Percent) |
|---|---|---|---|---|---|
| MALE | | | | | |
| 0 | 10/10 | 20.9 | 35.0 | 14.1 | |
| 100 | 10/10 | 20.9 | 34.9 | 14.0 | 0.7 |
| 300 | 10/10 | 20.9 | 33.0 | 12.1 | -14 |
| 1,000 | 10/10 | 20.9 | 32.9 | 12.0 | -15 |
| 3,000 | 10/10 | 20.9 | 31.5 | 10.6 | -25 |
| 10,000 | 10/10 | 20.9 | 30.0 | 9.1 | -35 |
| FEMALE | | | | | |
| 0 | 10/10 | 17.2 | 24.3 | 7.1 | |
| 100 | 10/10 | 17.2 | 24.2 | 7.0 | -1.4 |
| 300 | 10/10 | 17.2 | 25.3 | 8.1 | 14 |
| 1,000 | 10/10 | 17.2 | 23.6 | 6.4 | -10 |
| 3,000 | 10/10 | 17.2 | 23.6 | 6.4 | -10 |
| 10,000 | 10/10 | 17.2 | 21.3 | 4.1 | -42 |

(a) Number surviving/number per group.
(b) The initial weights were obtained from graphs submitted by the test laboratory.
(c) Weight Change Relative to Controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

No deaths occurred among the mice. Male mice receiving 300 ppm and females receiving a 1,000-ppm or higher dose had weight gain depressions of 10% or more.

Among the mice fed 1,000 ppm, squamous papilloma of the forestomach was detected in 1/10 males, and renal hyperpigmentation similar to that seen in the rats occurred in a second male and 2/10 females.

The low and high doses for the chronic studies were established at 250 and 500 ppm for the rats and at 50 and 100 ppm for the mice.

## G. Chronic Studies

The test groups, doses administered, and durations of the chronic studies are shown in Table 5.

## H. Clinical Examinations and Pathology

Animals were inspected twice daily. Body weights were recorded every 4 weeks. Animals that were moribund and those that survived to the termination of the study were killed and necropsied following anesthetization using $CO_2$ inhalation.

Gross and microscopic examinations were performed on major tissues, major organs, and all gross lesions from killed animals and from animals found dead. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, pancreas, stomach, small intestine, large intestine, kidneys, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate and seminal vesicles or uterus, testis or ovary, brain, thymus, larynx, and esophagus.

Necropsies were performed on all animals found dead unless precluded in whole or in part by autolysis or cannibalization. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group.

Table 5. Experimental Design of Chronic Feeding Studies with 2,6-Toluenediamine Dihydrochloride in Rats and Mice

| Sex, Species and Test Group | Initial No. of Animals | 2,6-Toluene-diamine 2(HCl) in Diet (a) (ppm) | Time on Study | |
|---|---|---|---|---|
| | | | Dosed (weeks) | Observed (weeks) |
| MALE RATS | | | | |
| Matched-Control | 50 | 0 | 0 | 104 |
| Low-Dose | 50 | 250 | 103 | 1 |
| High-Dose | 50 | 500 | 103 | 1 |
| FEMALE RATS | | | | |
| Matched-Control | 50 | 0 | 0 | 104 |
| Low-Dose | 50 | 250 | 103 | 1 |
| High-Dose | 50 | 500 | 103 | 1 |
| MALE MICE | | | | |
| Matched-Control | 50 | 0 | 0 | 104 |
| Low-Dose | 50 | 50 | 103 | 1 |
| High-Dose | 50 | 100 | 103 | 1 |
| FEMALE MICE | | | | |
| Matched-Control | 50 | 0 | 0 | 104 |
| Low-Dose | 50 | 50 | 103 | 1 |
| High-Dose | 50 | 100 | 103 | 1 |

(a) Diets were available ad libitum.

## I. Data Recording and Statistical Analyses

Data on this experiment were recorded in 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).

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) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is reported only 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 is 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) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for two dosed groups are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 is made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or

equal to 0.025. When 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), was also used. Under the assumption of a linear trend, this test determines 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 is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

The approximate 95% confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971). 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% of a large number of identical experiments, the true ratio of the risk in a dosed 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 has occurred (P less than 0.025 one-tailed test when the control incidence is not zero, P less than 0.050 when the control incidence is zero). 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. RESULTS - RATS

### A. Body Weights and Clinical Signs (Rats)

Mean body weight gain was depressed 17% in low-dose female rats and 27% in high-dose female rats when compared with controls. Weight gain depression was less than 10% for dosed male rats (Figure 1). No other clinical signs were observed that could be related to administration of the test chemical.

### B. Survival (Rats)

Estimates of the probabilities of survival for male and female rats administered 2,6-toluenediamine dihydrochloride in the diet at the doses of this bioassay, together with those of the matched controls, are shown by the Kaplan and Meier curves in Figure 2. The result of the Tarone test for dose-related trend in the proportions surviving is not significant in either sex.

In male rats, 33/50 (66%) of the high-dose group, 34/50 (68%) of the low-dose group, and 25/50 (50%) of the matched-control group lived to the end of the bioassay. In females, 39/50 (78%) of the high-dose group, 35/50 (70%) of the low-dose group, and 35/50 (70%) of the matched-control group lived to the end of the study.

A sufficient number of rats of each sex were at risk for the development of late-appearing tumors.

### C. Pathology (Rats)

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.

The various types of neoplasms occurring in both control and dosed rats did not appear to be compound related and were the types commonly found in aging F344 rats.

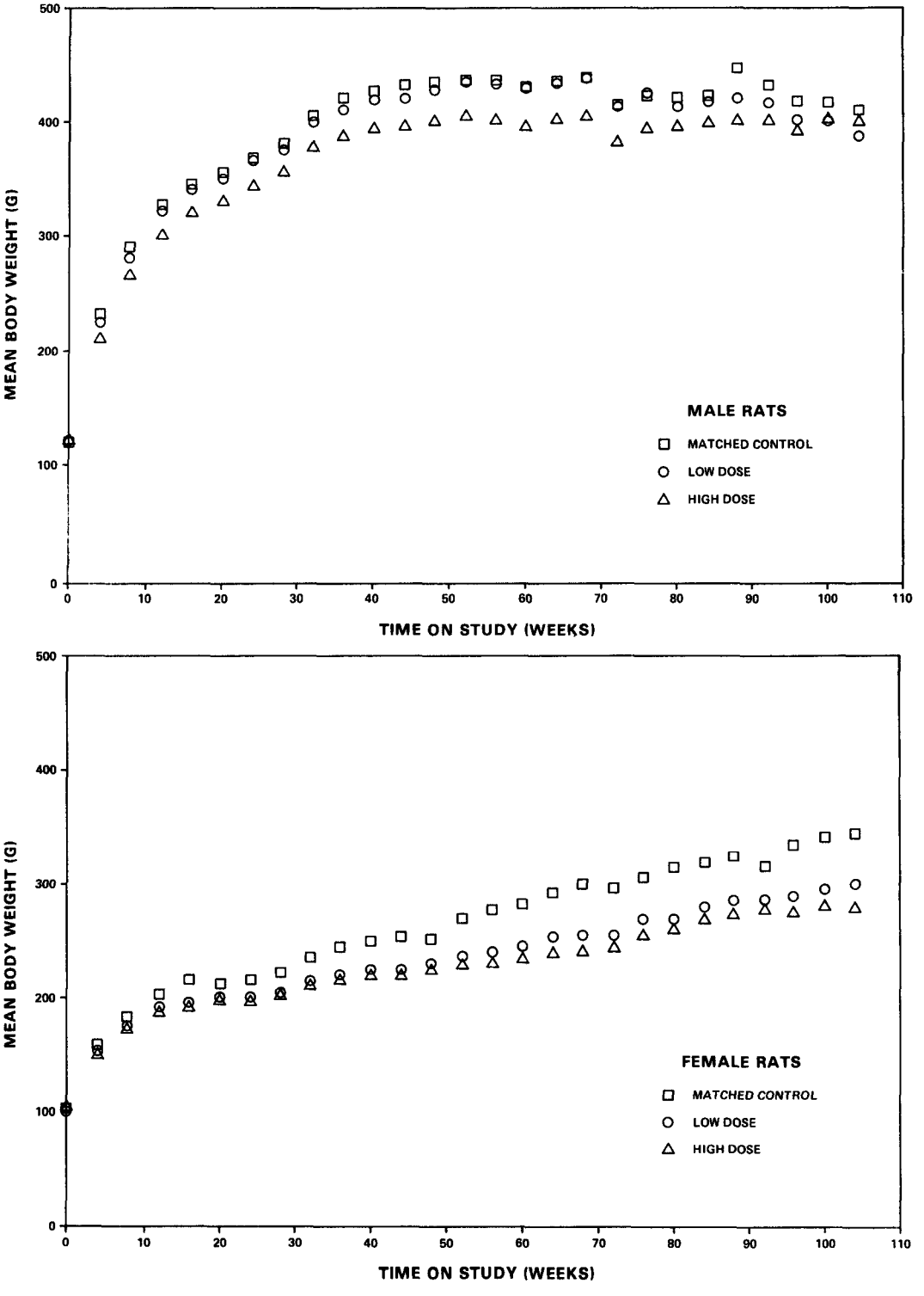



**Figure 1. Growth Curves for Rats Administered 2,6-Toluenediamine dihydrochloride in the Diet**

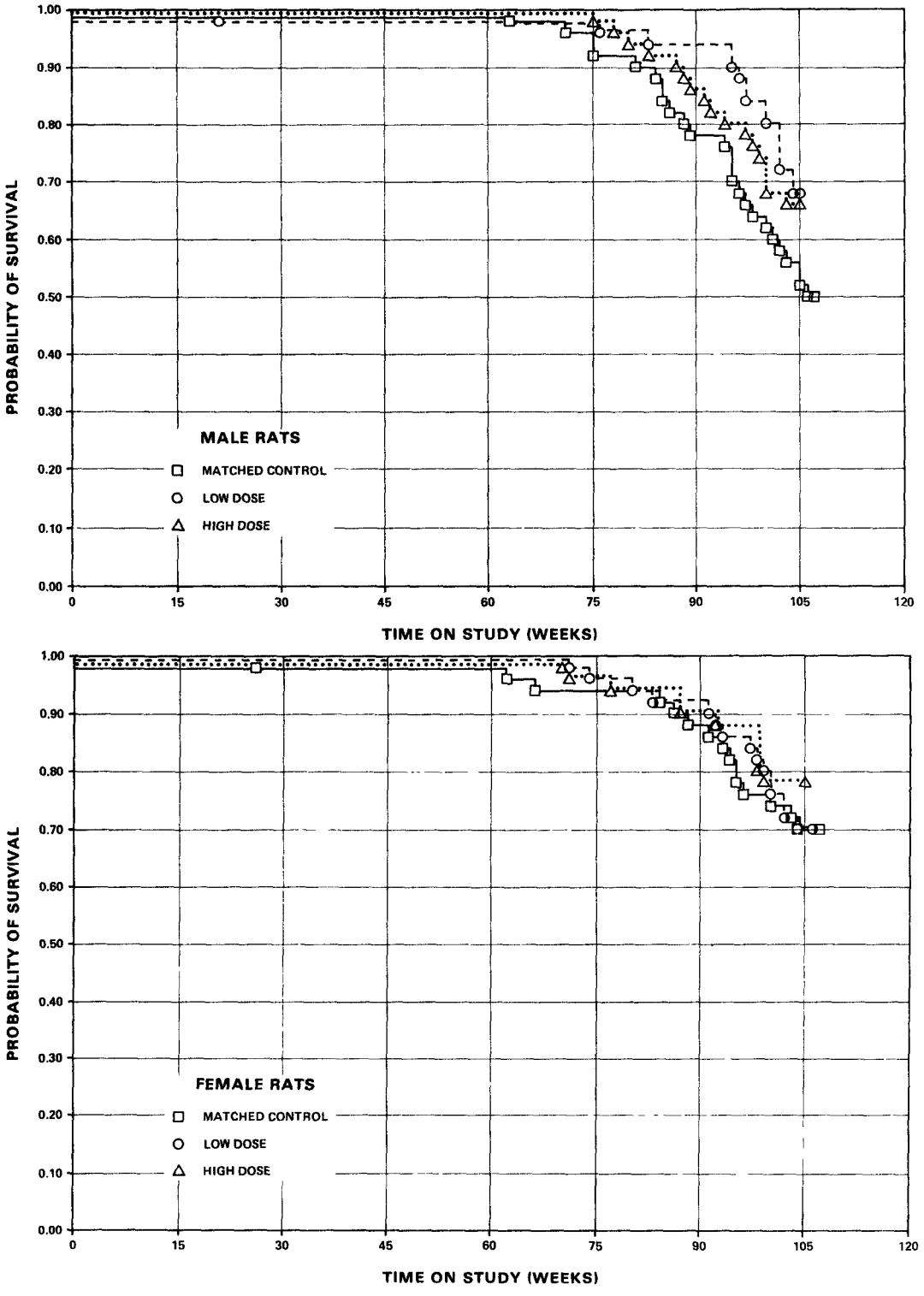



**Figure 2. Survival Curves for Rats Administered 2,6-Toluenediamine dihydrochloride in the Diet**

A variety of nonneoplastic lesions were observed in both control and dosed rats, none of which appeared to be compound related.

According to the histopathologic examination, 2,6-toluenediamine dihydrochloride was not carcinogenic to F344 rats under the conditions of this bioassay.

## D. Statistical Analyses of Results (Rats)

Tables 6 and 7 contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and at an incidence of at least 5% in one or more groups.

In male rats, a significant positive dose-related trend is observed in the incidences of animals with neoplastic nodules or hepatocellular carcinomas (P=0.037). The historical incidence at this laboratory of untreated male rats with neoplastic nodules or hepatocellular adenomas or carcinomas is 2/334 (0.6%), which is lower than the 4/50 (8%) observed in the high dose on this study. The historical incidence across all laboratories of these tumors in male rats is 36/2,230 (1.6%). The 4/50 (8%) incidence in the high-dose when compared with 0/50 in the control groups by the Fisher exact test has a significance level of 0.059 which is above the 0.025 level required for statistical significance when the Bonferroni inequality is applied; therefore, the evidence associating the administration of the chemical and the development of these liver tumors is not statistically conclusive.

In male rats, a significant positive dose-related trend is observed in the incidences of animals with islet-cell adenomas of the pancreas (P=0.025). The historical incidence at this laboratory for this tumor in control male rats is 0/334. One group of vehicle control male rats had a 2/35 (5.7%) incidence of this tumor. The Fisher exact test comparing the high-dose incidence with that of the controls has a significance level of 0.058 which is above the P=0.025 level required for statistical significance in this study; therefore, the evidence associating the administration of the chemical and the development of these pancreatic tumors is not statistically conclusive.

Significant trends in the negative direction are observed in the incidences of C-cell tumors of the thyroid in male rats and of the fibroadenomas of the mammary gland in female rats.

In each of the 95% confidence intervals for relative risk shown in the tables, the value of one or less than one is included: this indicates the absence of significant positive results. It should also be noted that each of the intervals, except for incidences of fibroadenomas of the mammary gland in high-dose female rats and C-cell adenomas of the thyroid in dosed groups of male rats, has an upper limit greater than one indicating that 2,6-toluenediamine dihydrochloride might induce tumors that could not be detected under the conditions of this test.

Table 6. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,6-Toluenediamine dihydrochloride in the Diet (a)

| Topography: Morphology | Matched Dose | Low Dose | High Dose |
|---|---|---|---|
| Lung: Alveolar/Bronchiolar Adenoma (b) | 3/49 (6) | 1/50 (2) | 2/50 (4) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 0.327 | 0.653 |
| Lower Limit | | 0.006 | 0.057 |
| Upper Limit | | 3.903 | 5.457 |
| Weeks to First Observed Tumor | 98 | 105 | 94 |
| Hematopoietic System: All Lymphomas (b) | 2/50 (4) | 2/50 (4) | 4/50 (8) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.000 | 2.000 |
| Lower Limit | | 0.075 | 0.301 |
| Upper Limit | | 13.326 | 21.316 |
| Weeks to First Observed Tumor | 85 | 95 | 100 |
| Hematopoietic System: All Leukemias (b) | 9/50 (18) | 10/50 (20) | 7/50 (14) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.111 | 0.778 |
| Lower Limit | | 0.445 | 0.267 |
| Upper Limit | | 2.823 | 2.159 |
| Weeks to First Observed Tumor | 84 | 83 | 97 |
| Hematopoietic System: Lymphoma or Leukemia (b) | 11/50 (22) | 12/50 (24) | 11/50 (22) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.091 | 1.000 |
| Lower Limit | | 0.488 | 0.434 |
| Upper Limit | | 2.465 | 2.303 |
| Weeks to First Observed Tumor | 84 | 83 | 97 |

Table 6. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,6-Toluenediamine dihydrochloride in the Diet (a) (continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Hematopoietic System: | | | |
| Neoplasm, NOS (b) | 2/50 (4) | 6/50 (12) | 1/50 (2) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Departure from Linear Trend (f) | P=0.029 | | |
| Relative Risk (e) | | 3.000 | 0.500 |
| Lower Limit | | 0.569 | 0.009 |
| Upper Limit | | 29.254 | 9.290 |
| Weeks to First Observed Tumor | 88 | 76 | 99 |
| Liver: Neoplastic Nodule (b) | 0/50 (0) | 0/50 (0) | 3/50 (6) |
| P Values (c,d) | P=0.037 | N.S. | N.S. |
| Relative Risk (e) | | — | Infinite |
| Lower Limit | | — | 0.601 |
| Upper Limit | | — | Infinite |
| Weeks to First Observed Tumor | — | — | 104 |
| Liver: Neoplastic Nodule or Hepatocellular Carcinoma (b) | 0/50 (0) | 2/50 (4) | 4/50 (8) |
| P Values (c,d) | P=0.037 | N.S. | N.S. |
| Relative Risk (e) | | Infinite | Infinite |
| Lower Limit | | 0.296 | 0.927 |
| Upper Limit | | Infinite | Infinite |
| Weeks to First Observed Tumor | — | 102 | 97 |
| Pituitary: Adenoma, NOS (b) | 10/45 (22) | 11/49 (22) | 15/44 (34) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.010 | 1.534 |
| Lower Limit | | 0.433 | 0.727 |
| Upper Limit | | 2.397 | 3.378 |
| Weeks to First Observed Tumor | 84 | 100 | 88 |

Table 6. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,6-Toluenediamine dihydrochloride in the Diet (a) (continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Adrenal: Pheochromocytoma (b) | 10/50 (20) | 6/50 (12) | 8/48 (17) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 0.600 | 0.833 |
| Lower Limit | | 0.194 | 0.312 |
| Upper Limit | | 1.676 | 2.140 |
| Weeks to First Observed Tumor | 96 | 97 | 75 |
| Thyroid: C-cell Adenoma (b) | 5/44 (11) | 0/48 (0) | 0/47 (0) |
| P Values (c,d) | P=0.005(N) | P=0.022(N) | P=0.023(N) |
| Relative Risk (e) | | 0.000 | 0.000 |
| Lower Limit | | 0.000 | 0.000 |
| Upper Limit | | 0.725 | 0.740 |
| Weeks to First Observed Tumor | 98 | -- | -- |
| Thyroid: C-cel l Carcinoma (b) | 2/44 (5) | 2/48 (4) | 2/47 (4) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 0.917 | 0.936 |
| Lower Limit | | 0.069 | 0.071 |
| Upper Limit | | 12.189 | 12.441 |
| Weeks to First Observed Tumor | 101 | 105 | 105 |
| Thyroid: C-cell Adenoma or Carcinoma(b) | 7/44 (16) | 2/48 (4) | 2/47 (4) |
| P Values (c,d) | P=0.033 (N) | N.S. | N.S. |
| Relative Risk (e) | | 0.262 | 0.267 |
| Lower Limit | | 0.028 | 0.028 |
| Upper Limit | | 1.288 | 1.314 |
| Weeks to First Observed Tumor | 98 | 105 | 105 |

Table 6. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,6-Toluenediamine dihydrochloride in the Diet (a) (continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Pancreatic Islets: Islet-cell Adenoma (b) | 0/45 (0) | 1/46 (2) | 4/45 (9) |
| P Values (c,d) | P=0.025 | N.S. | N.S. |
| Relative Risk (e) | | Infinite | Infinite |
| Lower Limit | | 0.053 | 0.930 |
| Upper Limit | | Infinite | Infinite |
| Weeks to First Observed Tumor | — | 105 | 94 |
| Preputial Gland: Adenoma, NOS (b) | 2/50 (4) | 3/50 (6) | 4/50 (8) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.500 | 2.000 |
| Lower Limit | | 0.180 | 0.301 |
| Upper Limit | | 17.329 | 21.316 |
| Weeks to First Observed Tumor | 107 | 105 | 88 |
| Testis: Interstitial-cell Tumor (b) | 42/49 (86) | 47/49 (96) | 44/49 (90) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.119 | 1.048 |
| Lower Limit | | 0.966 | 0.890 |
| Upper Limit | | 1.210 | 1.209 |
| Weeks to First Observed Tumor | 63 | 76 | 78 |

Table 6. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,6-Toluenediamine dihydrochloride in the Diet (a) (continued)

(a) Dosed groups received doses of 250 or 500 ppm.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dose group than in a control group.
(e) The 95% confidence interval of the relative risk between each dosed group and the control group.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

Table 7. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,6-Toluenediamine dihydrochloride in the Diet (a)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Hematopoietic System: All Leukemias (b) | 4/50 (8) | 4/50 (8) | 5/50 (10) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.000 | 1.250 |
| Lower Limit | | 0.197 | 0.286 |
| Upper Limit | | 5.083 | 5.954 |
| Weeks to First Observed Tumor | 26 | 97 | 104 |
| Hematopoietic System: Leukemia or Lymphoma (b) | 5/50 (10) | 5/50 (10) | 5/50 (10) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.000 | 1.000 |
| Lower Limit | | 0.245 | 0.245 |
| Upper Limit | | 4.082 | 4.082 |
| Weeks to First Observed Tumor | 26 | 97 | 104 |
| Pituitary: Adenoma, NOS (b) | 19/48 (40) | 23/43 (53) | 25/48 (52) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.351 | 1.316 |
| Lower Limit | | 0.827 | 0.814 |
| Upper Limit | | 2.195 | 2.139 |
| Weeks to First Observed Tumor | 95 | 74 | 70 |
| Thyroid: C-cell Adenoma or Carcinoma (b) | 3/49 (6) | 3/49 (6) | 1/48 (2) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.000 | 0.340 |
| Lower Limit | | 0.140 | 0.007 |
| Upper Limit | | 7.126 | 4.060 |
| Weeks to First Observed Tumor | 62 | 106 | 105 |

Table 7. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,6-Toluenediamine dihydrochloride in the Diet (a) (continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Mammary Gland: Fibroadenoma (b) | 11/50 (22) | 6/50 (12) | 2/50 (4) |
| P Values (c,d) | P=0.006 (N) | N.S. | P = 0.007 (N) |
| Relative Risk (e) | | 0.545 | 0.182 |
| Lower Limit | | 0.179 | 0.020 |
| Upper Limit | | 1.477 | 0.777 |
| Weeks to First Observed Tumor | 96 | 105 | 98 |
| Uterus: Endometrial Stromal Polyp (b) | 9/48 (19) | 10/49 (20) | 11/50 (22) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.088 | 1.173 |
| Lower Limit | | 0.437 | 0.487 |
| Upper Limit | | 2.758 | 2.916 |
| Weeks to First Observed Tumor | 62 | 80 | 92 |

(a) Dosed groups received doses of 250 or 500 ppm.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dose group than in a control group.
(e) The 95% confidence interval of the relative risk between each dosed group and the control group.

# IV. RESULTS - MICE

## A. Body Weights and Clinical Signs (Mice)

Weight gain depression when compared with controls was less than 10% for dosed male and female mice (Figure 3). No other clinical signs were observed that could be related to the administration of the test chemical.

## B. Survival (Mice)

Estimates of the probabilities of survival for male and female mice administered 2,6-toluenediamine dihydrochloride in the diet at the doses of this bioassay, together with those of the matched controls, are shown by the Kaplan and Meier curves in Figure 4. The result of the Tarone test for dose-related trend in the proportions surviving is not significant in either sex.

In male mice, 32/50 (64%) of the high-dose group, 31/50 (62%) of the low-dose group, and 31/50 (62%) of the matched-control group lived to the end of the bioassay. In females, 39/50 (78%) of the high-dose group, 40/50 (80%) of the low-dose group, and 39/50 (78%) of the matched-control group lived to the end of the study.

A sufficient number of mice of each sex were at risk for the development of late-appearing tumors.

## C. Pathology (Mice)

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.

Neoplasms occurred in both matched-control and dosed mice. A slight increase over that in controls occurred in the incidence of vascular neoplasms (hemangioma/hemangiosarcoma) of the spleen and liver in dosed male mice. Vascular neoplasms were noted in 1/50 control male mice, 5/50 (10%) male mice of the low-dose group, and 3/49 (6%) of the high-dose group.

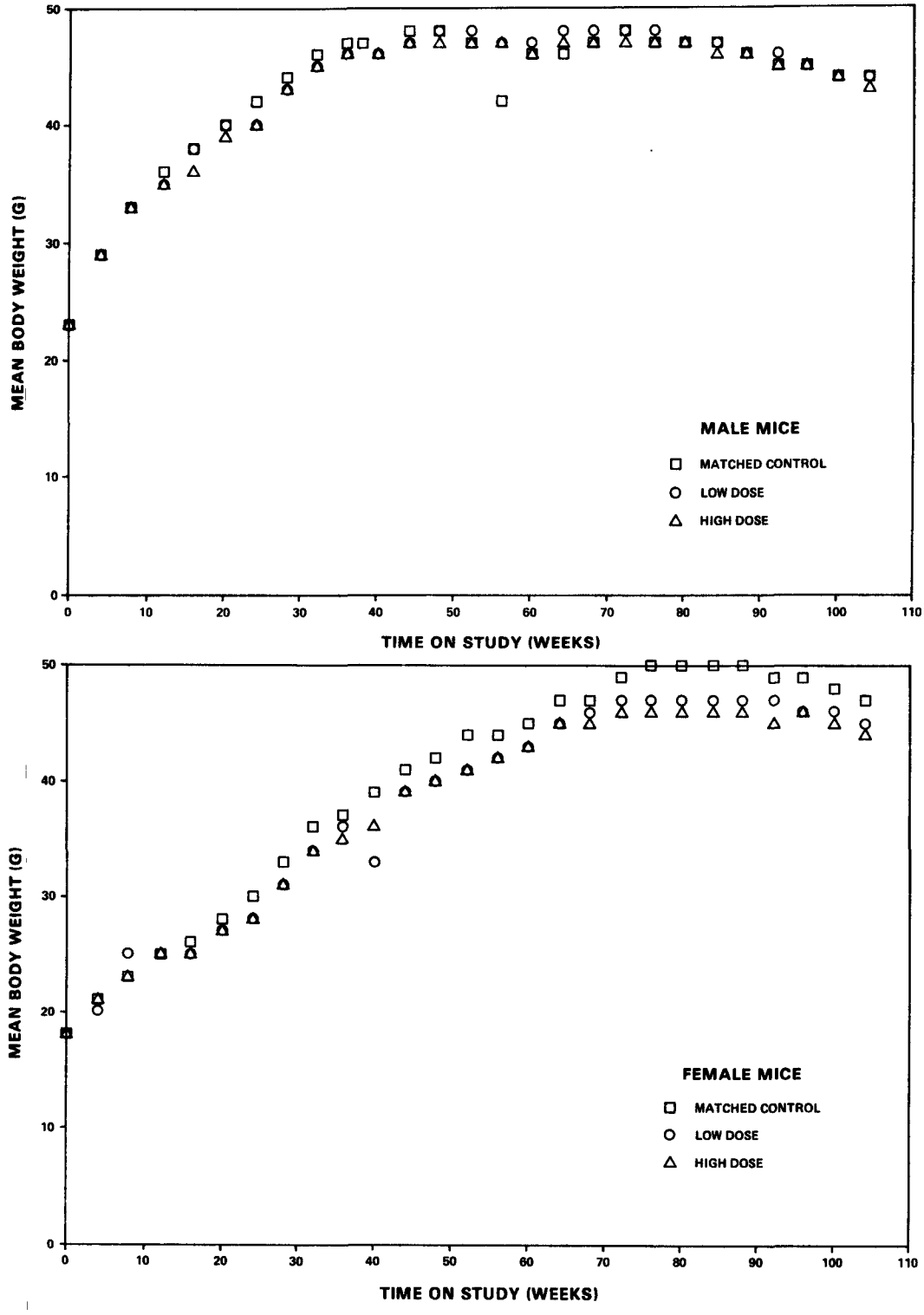



**Figure 3. Growth Curves for Mice Administered 2,6-Toluenediamine dihydrochloride in the Diet**

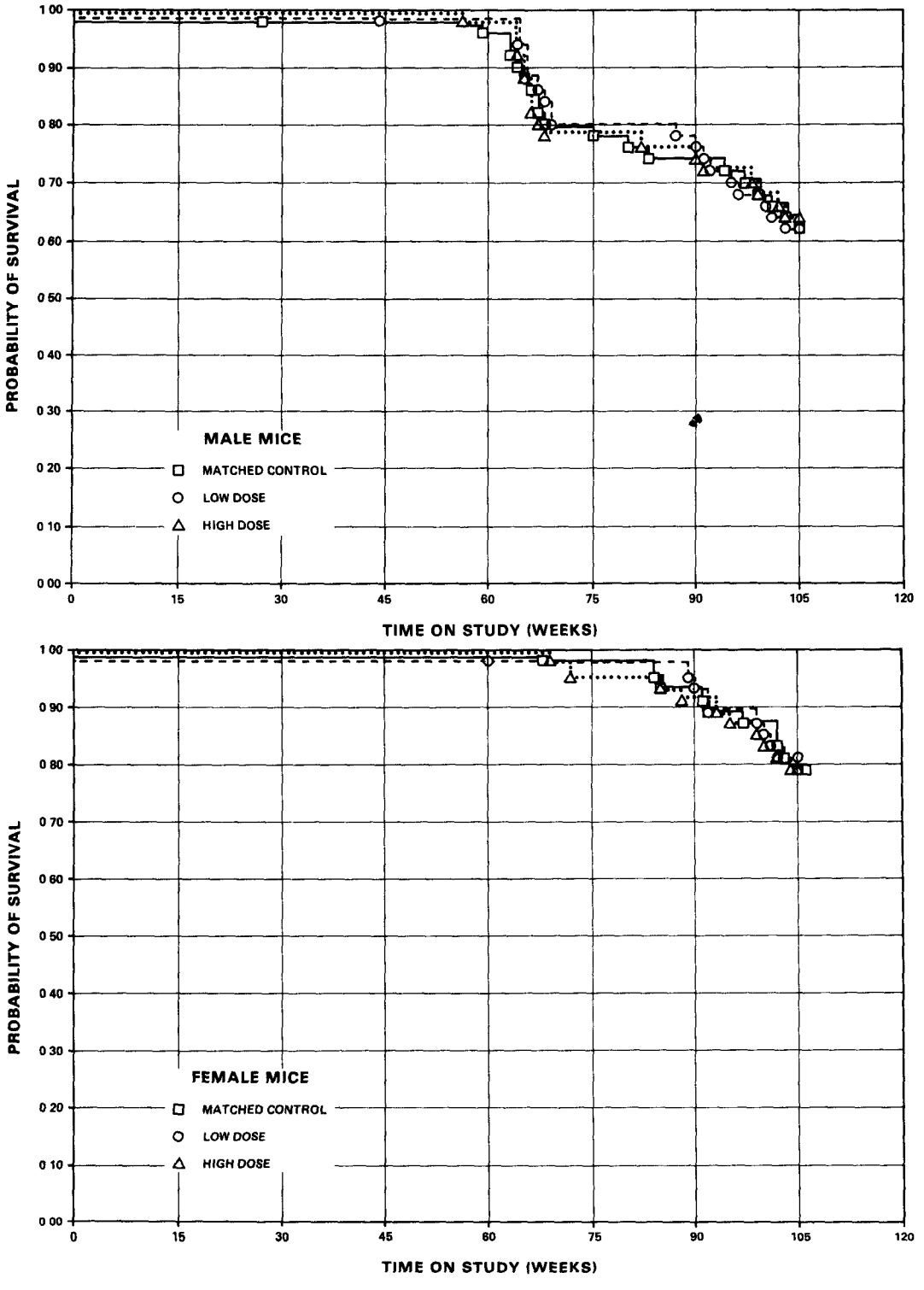



**Figure 4. Survival Curves for Mice Administered 2,6-Toluenediamine dihydrochloride in the Diet**

Hemangiomas were small lesions with distended capillaries or sinusoids and anastomosing vascular cysts lined by transformed endothelial cells which were fusiform. Cytoplasm of the cells was eosinophilic and nuclei were hyperchromatic. A marked variation in the size of cavernous vascular spaces was characteristic of hemangiosarcoma. The cells were pleomorphic and crowded in areas. Nuclei were large and vesicular. Mitotic figures were numerous. In the smaller tumors in the liver, some of the hepatocytes encircled by the transformed endothelial cells were hypertrophic and hyperplastic. Hepatocytes were obliterated in the large tumors. In two male mice of the low-dose group, hemangiosarcoma was found in association with hepatocellular carcinoma.

A variety of nonneoplastic lesions were seen in matched-control and dosed mice. None of these lesions appeared to be related to chemical administration.

The histopathologic examination provided no evidence that 2,6-toluenediamine dihydrochloride was carcinogenic to B6C3F1 mice, under the conditions of this bioassay.

## D. Statistical Analyses of Results (Mice)

Tables 8 and 9 contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and at an incidence of at least 5% in one or more groups.

In male mice, the Cochran-Armitage test indicates a departure from linear trend in the incidence of lymphomas in animals due to the higher incidence in the low-dose group (8/50, 16%) when compared with the high-dose group (2/50, 4%). The result of the Fisher exact test indicates an increased incidence in the low-dose group, but the P=0.046 observed in this group is above the level required for significance when the Bonferroni inequality criterion is used in the comparison of two dosed groups with a single control group (P=0.025). In females, a significant (P=0.036) positive linear trend was observed in the incidence of animals with hepatocellular carcinomas of the liver, but the results of the Fisher exact test are not significant.

In each of the 95% confidence intervals for relative risk shown in the tables, one is included: this indicates the absence of significant positive results. It should also be noted that each of the intervals has an upper limit greater than one indicating the theoretical possibility of tumor induction by 2,6-toluenediamine dihydrochloride, which could not be detected under the conditions of this test.

Table 8. Analyses of the Incidence of Primary Tumors in Male Mice Administered 2,6-Toluenediamine dihydrochloride in the Diet (a)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Lung: Alveolar/Bronchiolar Carcinoma (b) | 4/50 (8) | 5/50 (10) | 1/50 (2) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.250 | 0.250 |
| Lower Limit | | 0.286 | 0.005 |
| Upper Limit | | 5.954 | 2.411 |
| Weeks to First Observed Tumor | 75 | 105 | 104 |
| Lung: Alveolar/Bronchiolar Adenoma (b) | 7/50 (14) | 8/50 (16) | 6/50 (12) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.143 | 0.857 |
| Lower Limit | | 0.392 | 0.256 |
| Upper Limit | | 3.423 | 2.766 |
| Weeks to First Observed Tumor | 101 | 69 | 104 |
| Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b) | 11/50 (22) | 13/50 (26) | 7/50 (14) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.182 | 0.636 |
| Lower Limit | | 0.542 | 0.228 |
| Upper Limit | | 2.626 | 1.645 |
| Weeks to First Observed Tumor | 75 | 69 | 104 |
| Hematopoietic System: All Lymphomas (b) | 2/50 (4) | 8/50 (16) | 2/50 (4) |
| P Values (c,d) | N.S. | P=0.046 | N.S. |
| Departure from Linear Trend (f) | P=0.011 | | |
| Relative Risk (e) | | 4.000 | 1.000 |
| Lower Limit | | 0.851 | 0.075 |
| Upper Limit | | 37.147 | 13.326 |
| Weeks to First Observed Tumor | 94 | 90 | 91 |

Table 8. Analyses of the Incidence of Primary Tumors in Male Mice Administered 2,6-Toluenediamine dihydrochloride in the Diet (a) (continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Circulatory System: Hemangioma, Hemangiosarcoma, or Angiosarcoma (b) | 1/50 (2) | 5/50 (10) | 3/50 (6) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 4.000 | 3.000 |
| Lower Limit | | 0.415 | 0.251 |
| Upper Limit | | 192.805 | 154.270 |
| Weeks to First Observed Tumor | 83 | 96 | 56 |
| Liver: Hepatocellular Carcinoma (b) | 14/50 (28) | 12/50 (24) | 13/49 (27) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 0.857 | 0.948 |
| Lower Limit | | 0.404 | 0.459 |
| Upper Limit | | 1.790 | 1.940 |
| Weeks to First Observed Tumor | 67 | 64 | 90 |
| Liver: Hepatocellular Adenoma (b) | 7/50 (14) | 5/50 (10) | 5/49 (10) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 0.714 | 0.729 |
| Lower Limit | | 0.191 | 0.195 |
| Upper Limit | | 2.434 | 2.481 |
| Weeks to First Observed Tumor | 63 | 105 | 104 |
| Liver: Hepatocellular Carcinoma or Adenoma (b) | 21/50 (42) | 17/50 (34) | 18/49 (37) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 0.810 | 0.875 |
| Lower Limit | | 0.462 | 0.507 |
| Upper Limit | | 1.405 | 1.496 |
| Weeks to First Observed Tumor | 63 | 64 | 90 |

Table 8. Analyses of the Incidence of Primary Tumors in Male Mice Administered 2,6-Toluenediamine dihydrochloride in the Diet (a) (continued)

(a) Dosed groups received doses of 50 or 100 ppm.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dose group than in a control group.
(e) The 95% confidence interval of the relative risk between each dosed group and the control group.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

Table 9. Analyses of the Incidence of Primary Tumors in Female Mice Administered 2,6-Toluenediamine dihydrochloride in the Diet (a)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Lung: Alveolar/Bronchiolar Adenoma (b) | 4/50 (8) | 7/50 (14) | 3/49 (6) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.750 | 0.765 |
| Lower Limit | | 0.476 | 0.118 |
| Upper Limit | | 7.682 | 4.288 |
| Weeks to First Observed Tumor | 92 | 105 | 105 |
| Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b) | 4/50 (8) | 8/50 (16) | 3/49 (6) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 2.000 | 0.765 |
| Lower Limit | | 0.576 | 0.118 |
| Upper Limit | | 8.539 | 4.288 |
| Weeks to First Observed Tumor | 92 | 105 | 105 |
| Hematopoietic System: All Lymphomas (b) | 4/50 (8) | 10/50 (20) | 6/50 (12) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 2.500 | 1.500 |
| Lower Limit | | 0.779 | 0.380 |
| Upper Limit | | 10.246 | 6.820 |
| Weeks to First Observed Tumor | 91 | 89 | 88 |
| Hematopoietic System: Neoplasm, NOS (b) | 4/50 (8) | 1/50 (2) | 1/50 (2) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 0.250 | 0.250 |
| Lower Limit | | 0.005 | 0.005 |
| Upper Limit | | 2.411 | 2.411 |
| Weeks to First Observed Tumor | 84 | 90 | 85 |

Table 9. Analyses of the Incidence of Primary Tumors in Female Mice Administered 2,6-Toluenediamine dihydrochloride in the Diet (a) (continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Liver: Hepatocellular Carcinoma (b) | 0/50 (0) | 0/49 (0) | 3/49 (6) |
| P Values (c,d) | P=0.036 | — | N.S. |
| Relative Risk (e) | | — | Infinite |
| Lower Limit | | — | 0.614 |
| Upper Limit | | — | Infinite |
| Weeks to First Observed Tumor | — | — | 93 |
| Liver: Hepatocellular Adenoma (b) | 4/50 (8) | 3/49 (6) | 4/49 (8) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 0.765 | 1.020 |
| Lower Limit | | 0.118 | 0.201 |
| Upper Limit | | 4.288 | 5.183 |
| Weeks to First Observed Tumor | 106 | 105 | 105 |
| Liver: Hepatocellular Carcinoma or Adenoma (b) | 4/50 (8) | 3/49 (6) | 7/49 (14) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 0.765 | 1.786 |
| Lower Limit | | 0.118 | 0.486 |
| Upper Limit | | 4.288 | 7.830 |
| Weeks to First Observed Tumor | 106 | 105 | 93 |
| Pituitary: Adenoma, NOS (b) | 3/37 (8) | 5/38 (13) | 6/38 (16) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (e) | | 1.623 | 1.947 |
| Lower Limit | | 0.342 | 0.452 |
| Upper Limit | | 9.783 | 11.243 |
| Weeks to First Observed Tumor | 106 | 105 | 105 |

Table 9. Analyses of the Incidence of Primary Tumors in Female Mice Administered 2,6-Toluenediamine dihydrochloride in the Diet (a) (continued)

(a) Dosed groups received doses of 50 or 100 ppm.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dose group than in a control group.
(e) The 95% confidence interval of the relative risk between each dosed group and the control group.

# V. DISCUSSION

Mean body weight gain was depressed 17% in low-dose female rats and 27% in high-dose female rats. Weight gain depression was less than 10% for dosed groups of male rats and of male and female mice when compared with the controls.

The slight depression in weight gain among male and female mice receiving the test chemical as well as the lack of other observable clinical signs suggest that the maximum tolerated dose may not have been given to the mice.

In dosed male and female rats, no tumors occurred in statistically significant numbers when compared with those in control rats.

Diffuse bilateral adenomatous hyperplasia and enlargement of the thyroid occurred in the subchronic studies in male rats administered doses 6- to 20-fold higher than those used in the chronic studies. In contrast, in the chronic studies significant results in the negative direction were observed in the incidence of C-cell tumors of the thyroid in male rats.

In male rats, neoplastic nodules or hepatocellular carcinomas occurred with a dose-related trend that was significant ($P=0.037$), but the result of the Fisher exact test was not significant. Islet-cell adenomas of the pancreas occurred with a dose-related trend that was significant ($P=0.025$), but the result of the Fisher exact test was not significant.

In female rats, significant results in the negative direction were observed in the incidences of fibroadenoma of the mammary gland.

In male mice, lymphomas occurred in the low-dose group at an incidence that was significantly higher ($P=0.046$) than that of the corresponding matched-control group; however, this level of significance is below the level required when the Bonferroni criterion is used for multiple comparison.

In female mice, hepatocellular carcinomas occurred with a dose-related trend that was significant ($P=0.036$), but the result of the Fisher exact test comparing the incidence in the high-dose group with that in the matched controls was not significant.

In a previous study conducted independently but under the protocols of the Carcinogenesis Testing Program (NCI, 1979), the structurally related 2,4-isomer of toluenediamine was found to be carcinogenic for F344 rats, inducing

hepatocellular or neoplastic nodules in both males and females and carcinomas or adenomas of the mammary gland in females. The 2,4-isomer was also carcinogenic for B6C3F1 mice, inducing hepatocellular carcinomas. In those studies, rats received approximately 33% of the dose that animals received daily in the present studies, and the period of compound administration was 40 to 60 weeks shorter. The mice in the 2,4-toluenediamine study received a dose 2-fold higher than mice in the present study.

Primary occupational exposure is to the 80/20 or 65/35 mixtures of 2,4-toluenediamine and 2,6-toluenediamine, rather than to the 2,6-toluenediamine alone (Buist, 1970). The 2,4-isomer has been shown to be carcinogenic (NCI, 1979; Ito et al., 1969), but in the present study the 2,6-isomer has not been demonstrated to be carcinogenic.

Some aromatic amines have previously been found to be carcinogenic while others have been found to be without carcinogenic effects; for example, m-phenylenediamine has not yet been found to be carcinogenic in rats, but the addition of an ortho methyl group as in 2,4-toluenediamine enhances carcinogenic effects; 2,4,6-trimethylaniline is clearly carcinogenic for rats, but 2,4,5-trimethylaniline has an equivocal effect in rats; o-toluidine is carcinogenic, yet addition of a second amine substituent ortho to the methyl group as in 2,6-toluenediamine eliminates carcinogenic effects (Weisburger et al., 1978). Thus, it appears that a monocyclic aromatic compound that has two methyl groups ortho to an amine substituent is carcinogenic, whereas a monocyclic aromatic compound that has two amine groups ortho to a methyl group is not. While the role of steric effects in this group of compounds is not yet clear, the carcinogenicity of toluenediamines appears to be determined by the exact ring positions of the methyl and amino groups.

# VI. CONCLUSIONS

Under conditions of this bioassay, 2,6-toluenediamine dihydrochloride was not carcinogenic for either F344 rats or for B6C3F1 mice.

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# APPENDIX A

## Summary of the Incidence of Neoplasms in Rats Administered 2,6-Toluenediamine dihydrochloride in the Diet

## TABLE A1.

## SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED 2,6-TOLUENEDIAMINE DIHYDROCHLORIDE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (50) | (50) | (50) |
| BASAL-CELL CARCINOMA | | | 1 (2%) |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| CARCINOMA,NOS | 1 (2%) | | |
| SQUAMOUS CELL CARCINOMA | 1 (2%) | | |
| SARCOMA, NOS | | 2 (4%) | |
| FIBROMA | | 2 (4%) | 1 (2%) |
| NEURILEMOMA, MALIGNANT | | | 1 (2%) |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (49) | (50) | (50) |
| ALVEOLAR/BRONCHIOLAR ADENOMA | 3 (6%) | 1 (2%) | 2 (4%) |
| SARCOMA, NOS, METASTATIC | | 1 (2%) | |
| NEURILEMOMA, METASTATIC | | | 1 (2%) |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| MALIGNANT LYMPHOMA, NOS | 2 (4%) | 2 (4%) | 4 (8%) |
| LEUKEMIA,NOS | 9 (18%) | 10 (20%) | 7 (14%) |
| *HEMATOPOIETIC SYSTEM | (50) | (50) | (50) |
| NEOPLASM, NOS | 2 (4%) | 6 (12%) | 1 (2%) |
| #SPLEEN | (50) | (50) | (49) |
| SARCOMA, NOS | | | 1 (2%) |
| CIRCULATORY SYSTEM | | | |
| NONE | | | |

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

**TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)**

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| DIGESTIVE SYSTEM | | | |
| *LIP | (50) | (50) | (50) |
| SQUAMOUS CELL CARCINOMA | | 1 (2%) | |
| #LIVER | (50) | (50) | (50) |
| NEOPLASTIC NODULE | | | 3 (6%) |
| HEPATOCELLULAR CARCINOMA | | 2 (4%) | 1 (2%) |
| URINARY SYSTEM | | | |
| #KIDNEY | (50) | (50) | (50) |
| TUBULAR-CELL ADENOCARCINOMA | | 1 (2%) | |
| LEIOMYOSARCOMA | 1 (2%) | | |
| #KIDNEY/PELVIS | (50) | (50) | (50) |
| TRANSITIONAL-CELL PAPILLOMA | | | 1 (2%) |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (45) | (49) | (44) |
| NEOPLASM, NOS | 1 (2%) | | |
| ADENOMA, NOS | 10 (22%) | 11 (22%) | 15 (34%) |
| #ADRENAL | (50) | (50) | (48) |
| CORTICAL ADENOMA | | | 1 (2%) |
| PHEOCHROMOCYTOMA | 10 (20%) | 6 (12%) | 8 (17%) |
| #THYROID | (44) | (48) | (47) |
| FOLLICULAR-CELL CARCINOMA | | | 1 (2%) |
| C-CELL ADENOMA | 5 (11%) | | |
| C-CELL CARCINOMA | 2 (5%) | 2 (4%) | 2 (4%) |
| #PANCREATIC ISLETS | (45) | (46) | (45) |
| ISLET-CELL ADENOMA | | 1 (2%) | 4 (9%) |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (50) | (50) | (50) |
| ADENOCARCINOMA, NOS | 1 (2%) | | |
| *PREPUTIAL GLAND | (50) | (50) | (50) |
| ADENOMA, NOS | 2 (4%) | 3 (6%) | 4 (8%) |

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

## TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| #TESTIS | (49) | (49) | (49) |
| INTERSTITIAL-CELL TUMOR | 42 (86%) | 47 (96%) | 44 (90%) |
| NERVOUS SYSTEM | | | |
| #BRAIN | (50) | (50) | (50) |
| ASTROCYTOMA | | 1 (2%) | |
| SPECIAL SENSE ORGANS | | | |
| *ZYMBAL'S GLAND | (50) | (50) | (50) |
| CARCINOMA,NOS | | 1 (2%) | 1 (2%) |
| MUSCULOSKELETAL SYSTEM | | | |
| *SKULL | (50) | (50) | (50) |
| OSTEOSARCOMA | | | 1 (2%) |
| BODY CAVITIES | | | |
| *TUNICA VAGINALIS | (50) | (50) | (50) |
| MESOTHELIOMA, NOS | 2 (4%) | 1 (2%) | |
| ALL OTHER SYSTEMS | | | |
| SITE UNKNOWN | | | |
| CARCINOMA,NOS | 1 | | |
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| NATURAL DEATH@ | 17 | 12 | 11 |
| MORIBUND SACRIFICE | 8 | 4 | 6 |
| SCHEDULED SACRIFICE | | | |
| ACCIDENTALLY KILLED | | | |
| TERMINAL SACRIFICE | 25 | 34 | 33 |
| ANIMAL MISSING | | | |

@ INCLUDES AUTOLYZED ANIMALS

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

**TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)**

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 47 | 49 | 49 |
| TOTAL PRIMARY TUMORS | 95 | 100 | 104 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 46 | 48 | 49 |
| TOTAL BENIGN TUMORS | 72 | 71 | 80 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 16 | 20 | 16 |
| TOTAL MALIGNANT TUMORS | 18 | 22 | 20 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | | 1 | 1 |
| TOTAL SECONDARY TUMORS | | 1 | 1 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT | 5 | 7 | 4 |
| TOTAL UNCERTAIN TUMORS | 5 | 7 | 4 |
| 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 ADMINISTERED 2,6-TOLUENEDIAMINE DIHYDROCHLORIDE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (50) | (50) | (50) |
| BASAL-CELL CARCINOMA | | 1 (2%) | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| NEOPLASM, NOS | | | 1 (2%) |
| SQUAMOUS CELL CARCINOMA | 1 (2%) | | 1 (2%) |
| SARCOMA, NOS | | 2 (4%) | |
| FIBROMA | 1 (2%) | | |
| FIBROUS HISTIOCYTOMA | | | 1 (2%) |
| LEIOMYOSARCOMA | | | 1 (2%) |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (50) | (50) | (50) |
| NEOPLASM, NOS, METASTATIC | | 1 (2%) | |
| UNDIFFERENTIATED CARCINOMA | | | 1 (2%) |
| SQUAMOUS CELL CARCINOMA, METASTA | 1 (2%) | | |
| ALVEOLAR/BRONCHIOLAR ADENOMA | 1 (2%) | 2 (4%) | 1 (2%) |
| C-CELL CARCINOMA, METASTATIC | 1 (2%) | 1 (2%) | |
| SARCOMA, NOS, METASTATIC | | 1 (2%) | |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| MALIGNANT LYMPHOMA, NOS | | 1 (2%) | |
| LEUKEMIA,NOS | 4 (8%) | 4 (8%) | 5 (10%) |
| *HEMATOPOIETIC SYSTEM | (50) | (50) | (50) |
| NEOPLASM, NOS | 1 (2%) | 2 (4%) | |
| #SPLEEN | (49) | (50) | (50) |
| FIBROSARCOMA, INVASIVE | 1 (2%) | | |

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

## TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| MALIG.LYMPHOMA, HISTIOCYTIC TYPE | 1 (2%) | | |
| #LYMPH NODE | (47) | (44) | (47) |
| UNDIFFERENTIATED CARCINOMA METAS | | | 1 (2%) |
| C-CELL CARCINOMA, METASTATIC | 1 (2%) | | |
| #THYMUS | (35) | (30) | (32) |
| THYMOMA | | | 1 (3%) |
| CIRCULATORY SYSTEM | | | |
| NONE | | | |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (50) | (50) | (50) |
| NEOPLASTIC NODULE | | | 1 (2%) |
| HEPATOCELLULAR CARCINOMA | | | 1 (2%) |
| ENDOMETRIAL STROMAL SARCOMA, MET | | 1 (2%) | |
| #STOMACH | (50) | (48) | (50) |
| FIBROSARCOMA | 1 (2%) | | |
| #JEJUNUM | (49) | (48) | (48) |
| SARCOMA, NOS | | 1 (2%) | |
| URINARY SYSTEM | | | |
| #KIDNEY | (50) | (50) | (50) |
| UNDIFFERENTIATED CARCINOMA METAS | | | 1 (2%) |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (48) | (43) | (48) |
| ADENOMA, NOS | 19 (40%) | 23 (53%) | 25 (52%) |
| #ADRENAL | (50) | (50) | (50) |
| PHEOCHROMOCYTOMA | 2 (4%) | 2 (4%) | 1 (2%) |
| #THYROID | (49) | (49) | (48) |
| C-CELL ADENOMA | 2 (4%) | 1 (2%) | 1 (2%) |

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

## TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| C-CELL CARCINOMA | 1 (2%) | 2 (4%) | |
| #PANCREATIC ISLETS | (48) | (47) | (46) |
| ISLET-CELL ADENOMA | 1 (2%) | | |
| **REPRODUCTIVE SYSTEM** | | | |
| *MAMMARY GLAND | (50) | (50) | (50) |
| ADENOMA, NOS | 1 (2%) | 1 (2%) | 2 (4%) |
| FIBROADENOMA | 11 (22%) | 6 (12%) | 2 (4%) |
| *CLITORAL GLAND | (50) | (50) | (50) |
| ADENOMA, NOS | 1 (2%) | | 1 (2%) |
| #UTERUS | (48) | (49) | (50) |
| ENDOMETRIAL STROMAL POLYP | 9 (19%) | 10 (20%) | 11 (22%) |
| ENDOMETRIAL STROMAL SARCOMA | 1 (2%) | 1 (2%) | |
| #OVARY | (47) | (47) | (50) |
| UNDIFFERENTIATED CARCINOMA METAS | | | 1 (2%) |
| **NERVOUS SYSTEM** | | | |
| #BRAIN | (49) | (49) | (50) |
| NEOPLASM, NOS | | 1 (2%) | |
| ASTROCYTOMA | 1 (2%) | 1 (2%) | |
| **SPECIAL SENSE ORGANS** | | | |
| *ZYMBAL'S GLAND | (50) | (50) | (50) |
| CARCINOMA,NOS | 1 (2%) | | |
| **MUSCULOSKELETAL SYSTEM** | | | |
| NONE | | | |
| **BODY CAVITIES** | | | |
| *ABDOMINAL CAVITY | (50) | (50) | (50) |
| MESOTHELIOMA, NOS | | 1 (2%) | |
| **ALL OTHER SYSTEMS** | | | |
| NONE | | | |

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

## TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| NATURAL DEATHə | 9 | 10 | 7 |
| MORIBUND SACRIFICE | 6 | 5 | 4 |
| SCHEDULED SACRIFICE | | | |
| ACCIDENTALLY KILLED | | | |
| TERMINAL SACRIFICE | 35 | 35 | 39 |
| ANIMAL MISSING | | | |
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 42 | 37 | 39 |
| TOTAL PRIMARY TUMORS | 60 | 62 | 57 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 34 | 31 | 33 |
| TOTAL BENIGN TUMORS | 48 | 45 | 46 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 11 | 12 | 9 |
| TOTAL MALIGNANT TUMORS | 11 | 13 | 9 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | 3 | 4 | 1 |
| TOTAL SECONDARY TUMORS | 4 | 4 | 3 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT | 1 | 3 | 2 |
| TOTAL UNCERTAIN TUMORS | 1 | 4 | 2 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC | | | |
| TOTAL UNCERTAIN TUMORS | | | |

ə INCLUDES AUTOLYZED ANIMALS

* 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 Administered 2,6-Toluenediamine dihydrochloride in the Diet

## TABLE B1.

## SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED 2,6-TOLUENEDIAMINE DIHYDROCHLORIDE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| **INTEGUMENTARY SYSTEM** | | | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| FIBROMA | | 1 (2%) | |
| FIBROSARCOMA | 1 (2%) | 1 (2%) | |
| **RESPIRATORY SYSTEM** | | | |
| #LUNG | (50) | (50) | (50) |
| HEPATOCELLULAR CARCINOMA, METAST | 4 (8%) | 2 (4%) | 4 (8%) |
| ALVEOLAR/BRONCHIOLAR ADENOMA | 7 (14%) | 8 (16%) | 6 (12%) |
| ALVEOLAR/BRONCHIOLAR CARCINOMA | 4 (8%) | 5 (10%) | 1 (2%) |
| FIBROSARCOMA, METASTATIC | | 1 (2%) | |
| **HEMATOPOIETIC SYSTEM** | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| MALIGNANT LYMPHOMA, NOS | 2 (4%) | 5 (10%) | 1 (2%) |
| *HEMATOPOIETIC SYSTEM | (50) | (50) | (50) |
| NEOPLASM, NOS | | | 2 (4%) |
| #SPLEEN | (49) | (50) | (50) |
| MALIGNANT LYMPHOMA, NOS | | 1 (2%) | |
| #LYMPH NODE | (44) | (38) | (41) |
| HEPATOCELLULAR CARCINOMA, METAST | | 1 (3%) | |
| MALIGNANT LYMPHOMA, NOS | | 1 (3%) | 1 (2%) |
| #PEYER'S PATCH | (47) | (48) | (45) |
| MALIGNANT LYMPHOMA, NOS | | 1 (2%) | |
| **CIRCULATORY SYSTEM** | | | |
| #SPLEEN | (49) | (50) | (50) |
| HEMANGIOMA | | 1 (2%) | 1 (2%) |

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

**TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)**

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| HEMANGIOSARCOMA | | 1 (2%) | |
| #LIVER | (50) | (50) | (49) |
| HEMANGIOMA | 1 (2%) | 1 (2%) | 1 (2%) |
| HEMANGIOSARCOMA | | 2 (4%) | 1 (2%) |
| ANGIOSARCOMA | | 1 (2%) | |
| **DIGESTIVE SYSTEM** | | | |
| #LIVER | (50) | (50) | (49) |
| NEOPLASM, NOS | 3 (6%) | | |
| HEPATOCELLULAR ADENOMA | 7 (14%) | 5 (10%) | 5 (10%) |
| HEPATOCELLULAR CARCINOMA | 14 (28%) | 12 (24%) | 13 (27%) |
| FIBROSARCOMA, METASTATIC | | 1 (2%) | |
| #STOMACH | (48) | (49) | (47) |
| HEPATOCELLULAR CARCINOMA, METAST | | 1 (2%) | |
| **URINARY SYSTEM** | | | |
| NONE | | | |
| **ENDOCRINE SYSTEM** | | | |
| #PITUITARY | (42) | (37) | (42) |
| ADENOMA, NOS | 1 (2%) | 1 (3%) | |
| #ADRENAL | (47) | (43) | (47) |
| CORTICAL ADENOMA | | 1 (2%) | |
| #ADRENAL/CAPSULE | (47) | (43) | (47) |
| ADENOMA, NOS | | | 1 (2%) |
| **REPRODUCTIVE SYSTEM** | | | |
| NONE | | | |
| **NERVOUS SYSTEM** | | | |
| NONE | | | |

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

## TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| SPECIAL SENSE ORGANS | | | |
| *HARDERIAN GLAND | (50) | (50) | (50) |
| ADENOMA, NOS | | 2 (4%) | 1 (2%) |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| NONE | | | |
| ALL OTHER SYSTEMS | | | |
| SITE UNKNOWN | | | |
| CARCINOMA,NOS | | 1 | |
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| NATURAL DEATH@ | 17 | 17 | 15 |
| MORIBUND SACRIFICE | 2 | 2 | 3 |
| SCHEDULED SACRIFICE | | | |
| ACCIDENTALLY KILLED | | | |
| TERMINAL SACRIFICE | 31 | 31 | 32 |
| ANIMAL MISSING | | | |

@ INCLUDES AUTOLYZED ANIMALS

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

**TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)**

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 31 | 36 | 26 |
| TOTAL PRIMARY TUMORS | 40 | 51 | 34 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 15 | 17 | 14 |
| TOTAL BENIGN TUMORS | 16 | 20 | 15 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 19 | 26 | 16 |
| TOTAL MALIGNANT TUMORS | 21 | 31 | 17 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | 4 | 3 | 4 |
| TOTAL SECONDARY TUMORS | 4 | 6 | 4 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT | 3 | | 2 |
| TOTAL UNCERTAIN TUMORS | 3 | | 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 ADMINISTERED 2,6-TOLUENEDIAMINE DIHYDROCHLORIDE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (50) | (50) | (50) |
| SQUAMOUS CELL PAPILLOMA | | 1 (2%) | |
| SQUAMOUS CELL CARCINOMA | | 1 (2%) | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| SQUAMOUS CELL CARCINOMA | 1 (2%) | | |
| FIBROSARCOMA | | | 2 (4%) |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (50) | (50) | (49) |
| ALVEOLAR/BRONCHIOLAR ADENOMA | 4 (8%) | 7 (14%) | 3 (6%) |
| ALVEOLAR/BRONCHIOLAR CARCINOMA | | 1 (2%) | |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| MALIGNANT LYMPHOMA, NOS | 3 (6%) | 9 (18%) | 5 (10%) |
| MALIG.LYMPHOMA, HISTIOCYTIC TYPE | 1 (2%) | | |
| *HEMATOPOIETIC SYSTEM | (50) | (50) | (50) |
| NEOPLASM, NOS | 4 (8%) | 1 (2%) | 1 (2%) |
| #SPLEEN | (49) | (49) | (49) |
| MALIGNANT LYMPHOMA, NOS | | | 1 (2%) |
| #LYMPH NODE | (40) | (44) | (39) |
| ENDOMETRIAL STROMAL SARCOMA, MET | | | 1 (3%) |
| #PEYER'S PATCH | (47) | (47) | (44) |
| MALIGNANT LYMPHOMA, NOS | | 1 (2%) | |

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

**TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)**

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| CIRCULATORY SYSTEM | | | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| HEMANGIOMA | 1 (2%) | | |
| HEMANGIOSARCOMA | | 1 (2%) | |
| #SPLEEN | (49) | (49) | (49) |
| HEMANGIOSARCOMA | | 1 (2%) | |
| #LIVER | (50) | (49) | (49) |
| HEMANGIOMA | 1 (2%) | | 1 (2%) |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (50) | (49) | (49) |
| HEPATOCELLULAR ADENOMA | 4 (8%) | 3 (6%) | 4 (8%) |
| HEPATOCELLULAR CARCINOMA | | | 3 (6%) |
| ENDOMETRIAL STROMAL SARCOMA, MET | | | 1 (2%) |
| #DUODENUM | (47) | (47) | (44) |
| ADENOMATOUS POLYP, NOS | | 1 (2%) | |
| URINARY SYSTEM | | | |
| NONE | | | |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (37) | (38) | (38) |
| ADENOMA, NOS | 3 (8%) | 5 (13%) | 6 (16%) |
| #ADRENAL | (45) | (47) | (42) |
| CORTICAL ADENOMA | | | 1 (2%) |
| PHEOCHROMOCYTOMA | | 1 (2%) | |
| #ADRENAL/CAPSULE | (45) | (47) | (42) |
| ADENOMA, NOS | | 1 (2%) | 1 (2%) |
| #THYROID | (38) | (49) | (46) |
| FOLLICULAR-CELL ADENOMA | | 1 (2%) | |

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

**TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)**

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| REPRODUCTIVE SYSTEM | | | |
| #UTERUS | (48) | (48) | (50) |
| ENDOMETRIAL STROMAL SARCOMA | | | 1 (2%) |
| #OVARY | (35) | (47) | (39) |
| TUBULAR ADENOMA | | 1 (2%) | 1 (3%) |
| NERVOUS SYSTEM | | | |
| NONE | | | |
| SPECIAL SENSE ORGANS | | | |
| *HARDERIAN GLAND | (50) | (50) | (50) |
| ADENOMA, NOS | | 1 (2%) | |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| NONE | | | |
| ALL OTHER SYSTEMS | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| SARCOMA, NOS | | 1 (2%) | |
| LOWER LEG | | | |
| OSTEOSARCOMA | | | 1 |
| OMENTUM | | | |
| SARCOMA, NOS | | 1 | |

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

## TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| NATURAL DEATH@ | 10 | 9 | 10 |
| MORIBUND SACRIFICE | | | |
| SCHEDULED SACRIFICE | | | |
| ACCIDENTALLY KILLED | 1 | 1 | 1 |
| TERMINAL SACRIFICE | 39 | 40 | 39 |
| ANIMAL MISSING | | | |
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 21 | 30 | 24 |
| TOTAL PRIMARY TUMORS | 22 | 39 | 31 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 13 | 16 | 16 |
| TOTAL BENIGN TUMORS | 13 | 22 | 17 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 5 | 15 | 12 |
| TOTAL MALIGNANT TUMORS | 5 | 16 | 13 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | | | 1 |
| TOTAL SECONDARY TUMORS | | | 2 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT | 4 | 1 | 1 |
| TOTAL UNCERTAIN TUMORS | 4 | 1 | 1 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC | | | |
| TOTAL UNCERTAIN TUMORS | | | |

@ INCLUDES AUTOLYZED ANIMALS

* 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 Administered 2,6-Toluenediamine dihydrochloride in the Diet

## TABLE C1.

## SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED 2,6-TOLUENEDIAMINE DIHYDROCHLORIDE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (50) | (50) | (50) |
| EPIDERMAL INCLUSION CYST | | 1 (2%) | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| NECROSIS, NOS | 1 (2%) | | |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (49) | (50) | (50) |
| FIBROSIS | 1 (2%) | | |
| HEMATOPOIETIC SYSTEM | | | |
| #SPLEEN | (50) | (50) | (49) |
| FIBROSIS | | 1 (2%) | |
| INFARCT, NOS | | 1 (2%) | |
| HEMATOPOIESIS | 2 (4%) | 2 (4%) | 3 (6%) |
| #SPLENIC FOLLICLES | (50) | (50) | (49) |
| ATROPHY, NOS | | 2 (4%) | |
| CIRCULATORY SYSTEM | | | |
| #HEART | (48) | (50) | (50) |
| MINERALIZATION | | | 1 (2%) |
| THROMBOSIS, NOS | 1 (2%) | | |
| CALCIFICATION, NOS | | 1 (2%) | |
| #AURICULAR APPENDAGE | (48) | (50) | (50) |
| THROMBOSIS, NOS | 1 (2%) | | 3 (6%) |

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

## TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| #MYOCARDIUM | (48) | (50) | (50) |
| INFLAMMATION, NOS | | 1 (2%) | |
| DEGENERATION, NOS | 2 (4%) | 4 (8%) | 7 (14%) |
| *MESENTERY | (50) | (50) | (50) |
| PERIVASCULITIS | 2 (4%) | | |
| #URINARY BLADDER | (45) | (45) | (44) |
| PERIVASCULITIS | 1 (2%) | | |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (50) | (50) | (50) |
| NECROSIS, FOCAL | 5 (10%) | 1 (2%) | 3 (6%) |
| METAMORPHOSIS FATTY | 8 (16%) | 5 (10%) | 1 (2%) |
| BASOPHILIC CYTO CHANGE | 3 (6%) | 5 (10%) | 10 (20%) |
| FOCAL CELLULAR CHANGE | 1 (2%) | 1 (2%) | 5 (10%) |
| CLEAR-CELL CHANGE | | | 1 (2%) |
| #BILE DUCT | (50) | (50) | (50) |
| HYPERPLASIA, NOS | 1 (2%) | 2 (4%) | |
| #STOMACH | (49) | (49) | (48) |
| MINERALIZATION | 2 (4%) | | |
| CALCIFICATION, NOS | | 1 (2%) | |
| HYPERPLASIA, BASAL CELL | 1 (2%) | 1 (2%) | |
| #PEYER'S PATCH | (47) | (48) | (48) |
| HYPERPLASIA, NOS | 1 (2%) | | |
| URINARY SYSTEM | | | |
| #KIDNEY | (50) | (50) | (50) |
| MINERALIZATION | | | 1 (2%) |
| HYDRONEPHROSIS | 1 (2%) | | |
| NEPHROPATHY | 32 (64%) | 39 (78%) | 41 (82%) |
| NECROSIS, NOS | | 1 (2%) | |
| CALCIFICATION, NOS | | 1 (2%) | |
| HYPERPLASIA, EPITHELIAL | 1 (2%) | | |
| #KIDNEY/PELVIS | (50) | (50) | (50) |
| HYPERPLASIA, EPITHELIAL | | 1 (2%) | 1 (2%) |

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

## TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (45) | (49) | (44) |
| ANGIECTASIS | | 1 (2%) | |
| #ADRENAL MEDULLA | (50) | (50) | (48) |
| HYPERPLASIA, NODULAR | 1 (2%) | | |
| #THYROID | (44) | (48) | (47) |
| CALCIFICATION, FOCAL | | 1 (2%) | |
| HYPERPLASIA, C-CELL | | 2 (4%) | 2 (4%) |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (50) | (50) | (50) |
| GALACTOCELE | 1 (2%) | | 1 (2%) |
| *PREPUTIAL GLAND | (50) | (50) | (50) |
| INFLAMMATION, NOS | 1 (2%) | 1 (2%) | 1 (2%) |
| #PROSTATE | (42) | (44) | (42) |
| INFLAMMATION, NOS | | 1 (2%) | |
| INFLAMMATION, ACUTE | 1 (2%) | | |
| *SEMINAL VESICLE | (50) | (50) | (50) |
| CALCULUS, NOS | | 1 (2%) | |
| #TESTIS | (49) | (49) | (49) |
| MINERALIZATION | 1 (2%) | 2 (4%) | |
| ATROPHY, NOS | 1 (2%) | 4 (8%) | |
| HYPERPLASIA, INTERSTITIAL CELL | 2 (4%) | | 1 (2%) |
| NERVOUS SYSTEM | | | |
| #BRAIN | (50) | (50) | (50) |
| HEMORRHAGE | 1 (2%) | | |
| NECROSIS, NOS | | 1 (2%) | |
| NECROSIS, HEMORRHAGIC | | 1 (2%) | |
| SPECIAL SENSE ORGANS | | | |
| NONE | | | |

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

## TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| *MEDIASTINUM | (50) | (50) | (50) |
| MINERALIZATION | 1 (2%) | | |
| NECROSIS, NOS | 1 (2%) | | |
| *ABDOMINAL CAVITY | (50) | (50) | (50) |
| HEMORRHAGE | | | 1 (2%) |
| *MESENTERY | (50) | (50) | (50) |
| NECROSIS, FAT | 1 (2%) | | |
| ALL OTHER SYSTEMS | | | |
| OMENTUM | | | |
| NECROSIS, FAT | 2 | 6 | 7 |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NO LESION REPORTED | | | 1 |
| AUTO/NECROPSY/HISTO PERF | | 1 | |

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

## TABLE C2.

## SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED 2,6-TOLUENEDIAMINE DIHYDROCHLORIDE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| NONE | | | |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (50) | (50) | (50) |
| INFLAMMATION, NOS | 1 (2%) | 1 (2%) | |
| INFLAMMATION, INTERSTITIAL | | 1 (2%) | |
| GRANULOMA, FOREIGN BODY | | 3 (6%) | |
| HEMATOPOIETIC SYSTEM | | | |
| #SPLEEN | (49) | (50) | (50) |
| CONGESTION, NOS | 1 (2%) | | |
| HEMATOPOIESIS | 7 (14%) | 10 (20%) | 11 (22%) |
| CIRCULATORY SYSTEM | | | |
| #HEART | (50) | (49) | (50) |
| MINERALIZATION | 1 (2%) | | |
| #AURICULAR APPENDAGE | (50) | (49) | (50) |
| THROMBOSIS, NOS | | | 1 (2%) |
| #MYOCARDIUM | (50) | (49) | (50) |
| DEGENERATION, NOS | 1 (2%) | | 2 (4%) |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (50) | (50) | (50) |
| NECROSIS, FOCAL | 1 (2%) | 3 (6%) | |

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

**TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)**

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| NECROSIS, HEMORRHAGIC | 1 (2%) | | |
| METAMORPHOSIS FATTY | 7 (14%) | 4 (8%) | |
| BASOPHILIC CYTO CHANGE | 21 (42%) | 29 (58%) | 41 (82%) |
| FOCAL CELLULAR CHANGE | | 2 (4%) | |
| CLEAR-CELL CHANGE | | | 2 (4%) |
| #BILE DUCT | (50) | (50) | (50) |
| HYPERPLASIA, NOS | | 1 (2%) | |
| #STOMACH | (50) | (48) | (50) |
| MINERALIZATION | 1 (2%) | | |
| HYPERPLASIA, BASAL CELL | | 3 (6%) | |
| ACANTHOSIS | | 1 (2%) | |
| #FORESTOMACH | (50) | (48) | (50) |
| HYPERPLASIA, BASAL CELL | 1 (2%) | | |
| #JEJUNAL SUBMUCOSA | (49) | (48) | (48) |
| INFLAMMATION, NOS | | | 1 (2%) |
| URINARY SYSTEM | | | |
| #KIDNEY | (50) | (50) | (50) |
| MINERALIZATION | 3 (6%) | 3 (6%) | 4 (8%) |
| NEPHROPATHY | 14 (28%) | 10 (20%) | 7 (14%) |
| #KIDNEY/PELVIS | (50) | (50) | (50) |
| HYPERPLASIA, EPITHELIAL | | 1 (2%) | |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (48) | (43) | (48) |
| HEMORRHAGIC CYST | 1 (2%) | | |
| #THYROID | (49) | (49) | (48) |
| HYPERPLASIA, C-CELL | 1 (2%) | | 1 (2%) |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (50) | (50) | (50) |
| GALACTOCELE | 4 (8%) | 4 (8%) | 6 (12%) |
| *VAGINA | (50) | (50) | (50) |
| POLYP | | | 1 (2%) |

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

## TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| #UTERUS | (48) | (49) | (50) |
| HYDROMETRA | 1 (2%) | | |
| #UTERUS/ENDOMETRIUM | (48) | (49) | (50) |
| HYPERPLASIA, NOS | | | 1 (2%) |
| NERVOUS SYSTEM | | | |
| #BRAIN | (49) | (49) | (50) |
| HEMORRHAGE | | 1 (2%) | |
| SPECIAL SENSE ORGANS | | | |
| NONE | | | |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| NONE | | | |
| ALL OTHER SYSTEMS | | | |
| FOOT | | | |
| INFLAMMATION, NOS | 1 | | |
| NECROSIS, NOS | 1 | | |
| OMENTUM | | | |
| NECROSIS, FAT | 2 | 3 | 1 |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NO LESION REPORTED | 2 | 1 | 2 |

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

# APPENDIX D

## Summary of the Incidence of Nonneoplastic Lesions in Mice Administered 2,6-Toluenediamine dihydrochloride in the Diet

## TABLE D1.

## SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED 2,6-TOLUENEDIAMINE DIHYDROCHLORIDE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| INFLAMMATION, ACUTE/CHRONIC | | 1 (2%) | |
| NECROSIS, NOS | 3 (6%) | | 4 (8%) |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (50) | (50) | (50) |
| INFLAMMATION, NOS | | | 1 (2%) |
| GRANULOMA, FOREIGN BODY | | 1 (2%) | |
| HEMATOPOIETIC SYSTEM | | | |
| #BONE MARROW | (47) | (43) | (49) |
| HYPERPLASIA, HEMATOPOIETIC | 2 (4%) | | |
| #SPLEEN | (49) | (50) | (50) |
| HYPERPLASIA, NOS | | 1 (2%) | 1 (2%) |
| HYPERPLASIA, HEMATOPOIETIC | 1 (2%) | | |
| HEMATOPOIESIS | 6 (12%) | 9 (18%) | 3 (6%) |
| #LYMPH NODE | (44) | (38) | (41) |
| HYPERPLASIA, NOS | | | 1 (2%) |
| HEMATOPOIESIS | 4 (9%) | 2 (5%) | 6 (15%) |
| #MESENTERIC L. NODE | (44) | (38) | (41) |
| HYPERPLASIA, NOS | 1 (2%) | | |
| CIRCULATORY SYSTEM | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| PERIARTERITIS | | 1 (2%) | |

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

**TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)**

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| PERIVASCULITIS | | 1 (2%) | |
| #HEART | (50) | (49) | (50) |
| MINERALIZATION | | | 1 (2%) |
| FIBROSIS | 2 (4%) | 1 (2%) | |
| CALCIFICATION, FOCAL | | | 1 (2%) |
| #MYOCARDIUM | (50) | (49) | (50) |
| INFLAMMATION, NOS | 2 (4%) | 3 (6%) | 8 (16%) |
| DEGENERATION, NOS | 1 (2%) | | |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (50) | (50) | (49) |
| MINERALIZATION | | | 1 (2%) |
| HEMORRHAGE | | 1 (2%) | |
| FIBROSIS | | 1 (2%) | |
| NECROSIS, FOCAL | 2 (4%) | | 4 (8%) |
| METAMORPHOSIS FATTY | 1 (2%) | 4 (8%) | 7 (14%) |
| CLEAR-CELL CHANGE | | | 1 (2%) |
| HEPATOCYTOMEGALY | | 1 (2%) | |
| *GALLBLADDER | (50) | (50) | (50) |
| HYPERPLASIA, PAPILLARY | | 1 (2%) | |
| #PANCREAS | (43) | (48) | (43) |
| NECROSIS, FOCAL | 1 (2%) | | |
| #PANCREATIC ACINUS | (43) | (48) | (43) |
| ATROPHY, NOS | 1 (2%) | | |
| ATROPHY, FOCAL | | | 1 (2%) |
| #STOMACH | (48) | (49) | (47) |
| MINERALIZATION | 1 (2%) | | |
| INFLAMMATION, NOS | 1 (2%) | 2 (4%) | 1 (2%) |
| HYPERKERATOSIS | 1 (2%) | | |
| ACANTHOSIS | 1 (2%) | | 1 (2%) |
| #SMALL INTESTINE | (47) | (48) | (45) |
| INFLAMMATION, NOS | | | 1 (2%) |
| URINARY SYSTEM | | | |
| #KIDNEY | (49) | (50) | (49) |
| MINERALIZATION | | 10 (20%) | 6 (12%) |

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

**TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)**

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| HYDRONEPHROSIS | | 1 (2%) | |
| INFLAMMATION, INTERSTITIAL | | | 1 (2%) |
| DEGENERATION, CYSTIC | 1 (2%) | | |
| NEPHROSIS, NOS | 1 (2%) | | |
| NECROSIS, NOS | | 1 (2%) | |
| #KIDNEY/TUBULE | (49) | (50) | (49) |
| DEGENERATION, NOS | 1 (2%) | | |
| NECROSIS, FOCAL | 1 (2%) | | |
| REGENERATION, NOS | | | 1 (2%) |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (42) | (37) | (42) |
| CYST, NOS | | | 1 (2%) |
| #ADRENAL/CAPSULE | (47) | (43) | (47) |
| HYPERPLASIA, NOS | 1 (2%) | | |
| #THYROID | (45) | (47) | (48) |
| FOLLICULAR CYST, NOS | | 1 (2%) | |
| HYPERPLASIA, FOLLICULAR-CELL | | | 3 (6%) |
| REPRODUCTIVE SYSTEM | | | |
| *PREPUTIAL GLAND | (50) | (50) | (50) |
| ABSCESS, NOS | | 4 (8%) | |
| NECROSIS, NOS | 1 (2%) | | 1 (2%) |
| #PROSTATE | (41) | (41) | (49) |
| ABSCESS, NOS | | 1 (2%) | |
| #TESTIS | (50) | (49) | (50) |
| MINERALIZATION | 1 (2%) | | |
| NERVOUS SYSTEM | | | |
| NONE | | | |
| SPECIAL SENSE ORGANS | | | |
| *HARDERIAN GLAND | (50) | (50) | (50) |
| MINERALIZATION | | 1 (2%) | |

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

## TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| INFLAMMATION, NOS | | 1 (2%) | |
| HYPERPLASIA, NOS | | 1 (2%) | |
| *EAR | (50) | (50) | (50) |
| GRANULOMA, FOREIGN BODY | 1 (2%) | | |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| NONE | | | |
| ALL OTHER SYSTEMS | | | |
| OMENTUM | | | |
| NECROSIS, FAT | 7 | 4 | 3 |
| CALCIFICATION, NOS | 1 | | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NO LESION REPORTED | 6 | 5 | 3 |

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

## TABLE D2.

## SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED 2,6-TOLUENEDIAMINE DIHYDROCHLORIDE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| NECROSIS, NOS | | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (50) | (50) | (49) |
| CONGESTION, NOS | | 1 (2%) | 1 (2%) |
| HEMORRHAGE | | | 2 (4%) |
| HEMATOPOIETIC SYSTEM | | | |
| #SPLEEN | (49) | (49) | (49) |
| HYPERPLASIA, NOS | 5 (10%) | 1 (2%) | 1 (2%) |
| HEMATOPOIESIS | 7 (14%) | 6 (12%) | 7 (14%) |
| #LYMPH NODE | (40) | (44) | (39) |
| HEMATOPOIESIS | 1 (3%) | 1 (2%) | 1 (3%) |
| #MESENTERIC L. NODE | (40) | (44) | (39) |
| CONGESTION, NOS | 1 (3%) | | |
| #LIVER | (50) | (49) | (49) |
| HEMATOPOIESIS | 2 (4%) | 2 (4%) | |
| CIRCULATORY SYSTEM | | | |
| #HEART | (49) | (50) | (49) |
| MINERALIZATION | | | 1 (2%) |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (50) | (49) | (49) |
| NECROSIS, FOCAL | 3 (6%) | 1 (2%) | |

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

## TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| METAMORPHOSIS FATTY | 1 (2%) | | |
| #PANCREATIC ACINUS | (45) | (47) | (44) |
| DEGENERATION, NOS | 1 (2%) | | |
| #STOMACH | (48) | (47) | (48) |
| INFLAMMATION, NOS | | 1 (2%) | 2 (4%) |
| ACANTHOSIS | | | 1 (2%) |
| #DUODENUM | (47) | (47) | (44) |
| CONGESTION, NOS | 1 (2%) | | |
| #JEJUNUM | (47) | (47) | (44) |
| CONGESTION, NOS | 1 (2%) | | |
| #ILEAL MUCOUS MEMBRAN | (47) | (47) | (44) |
| HEMORRHAGE | 1 (2%) | | |
| **URINARY SYSTEM** | | | |
| #KIDNEY | (50) | (50) | (49) |
| MINERALIZATION | | 1 (2%) | |
| CONGESTION, NOS | | 1 (2%) | |
| INFLAMMATION, INTERSTITIAL | | | 1 (2%) |
| GLOMERULOSCLEROSIS, NOS | | | 1 (2%) |
| #URINARY BLADDER | (48) | (48) | (46) |
| HYPERPLASIA, EPITHELIAL | | 1 (2%) | |
| **ENDOCRINE SYSTEM** | | | |
| #ADRENAL/CAPSULE | (45) | (47) | (42) |
| HYPERPLASIA, NOS | 3 (7%) | 1 (2%) | 1 (2%) |
| #THYROID | (38) | (49) | (46) |
| HYPERPLASIA, FOLLICULAR-CELL | 1 (3%) | | |
| **REPRODUCTIVE SYSTEM** | | | |
| *MAMMARY GLAND | (50) | (50) | (50) |
| HYPERPLASIA, NOS | 1 (2%) | | |
| *VAGINA | (50) | (50) | (50) |
| NECROSIS, NOS | 1 (2%) | | |

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

## TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| #UTERUS | (48) | (48) | (50) |
| HYDROMETRA | 12 (25%) | 4 (8%) | 6 (12%) |
| INFLAMMATION, NOS | 4 (8%) | 2 (4%) | |
| PYOMETRA | 1 (2%) | 1 (2%) | 2 (4%) |
| METAPLASIA, SQUAMOUS | 1 (2%) | | |
| #UTERUS/ENDOMETRIUM | (48) | (48) | (50) |
| HYPERPLASIA, NOS | 1 (2%) | 3 (6%) | 2 (4%) |
| HYPERPLASIA, CYSTIC | 8 (17%) | 23 (48%) | 25 (50%) |
| #OVARY | (35) | (47) | (39) |
| MINERALIZATION | | | 1 (3%) |
| CYST, NOS | 2 (6%) | 1 (2%) | 4 (10%) |
| FOLLICULAR CYST, NOS | | 1 (2%) | |
| CONGESTION, NOS | | 1 (2%) | |
| INFLAMMATION, NOS | | | 1 (3%) |
| DEGENERATION, CYSTIC | | 2 (4%) | 3 (8%) |
| NERVOUS SYSTEM | | | |
| NONE | | | |
| SPECIAL SENSE ORGANS | | | |
| NONE | | | |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| *ABDOMINAL CAVITY | (50) | (50) | (50) |
| ABSCESS, NOS | | 1 (2%) | |
| ALL OTHER SYSTEMS | | | |
| OMENTUM | | | |
| MINERALIZATION | | | 1 |

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

## TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| NECROSIS, FAT | 4 | 5 | 1 |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NO LESION REPORTED | 6 | 2 | 3 |
| AUTO/NECROPSY/HISTO PERF | 1 | | 1 |

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

# APPENDIX E

## Analysis of 2,6-Toluenediamine
## Midwest Research Institute

Appendix E

Analysis of 2,6-Toluenediamine (Lot 012157)

Midwest Research Institute

## A. ELEMENTAL ANALYSIS

| Element | C | H | N |
|---|---|---|---|
| Theory | 68.81 | 8.25 | 22.94 |
| Determined | 68.75 | 8.11 | 22.73 |
| | 68.94 | 8.09 | 22.81 |

## B. MELTING POINT

| Determined | Literature Values |
|---|---|
| 105°C (Dupont 900 DTA) | 105°C (Sadtler Standard Spectra) |
| 104°-105.5°C (visual, capillary) | |

## C. THIN-LAYER CHROMATOGRAPHY

| | |
|---|---|
| Plates: Silica gel, F-254 | Ref. Standard: Aniline |
| Amount Spotted: 100 and 300 μg | Visualization: Ultraviolet 254 and 366nm, and furfural |
| System 1: Ethyl acetate, 100% | System 2: Tetrahydrofuran, 100% |
| $R_f$: 0.37, origin (trace visualized at 254 nm only) | $R_f$: 0.61, origin (trace visualized at 254 nm only) |
| $R_{st}$: 0.58, origin | $R_{st}$: 0.87, origin |

## D. VAPOR-PHASE CHROMATOGRAPHY

Instrument: Bendix 2500
Column: 3% OV-1 on 80/100 Supelcoport, 1.8 m x 4 mm I.D.
Inlet temperature: 280°C
Detector: Flame Ionization
Oven Temperature Program: 5 min at 50°C, then
50°-250°C at
10°C/min
Results: One homogeneous peak, retention time 11.8 min

## E. SPECTRAL DATA

a. Infrared

Instrument: Beckman IR-12
Cell: 0.8% KBr pellet
Results: See Figure 5

Consistent with literature spectrum (Finkleshtein and Boitsov, 1960).

b. Ultraviolet/Visible

Instrument: Cary 118

No literature reference found.

$\epsilon$max 289.5 = (1.48±0.01 ($\delta$)) x $10^3$
Another maximum near the solvent cut-off of 210 nm.
No absorption in the visible region, 350 - 800 nm at 1 mg/ml
Solvent: Methanol

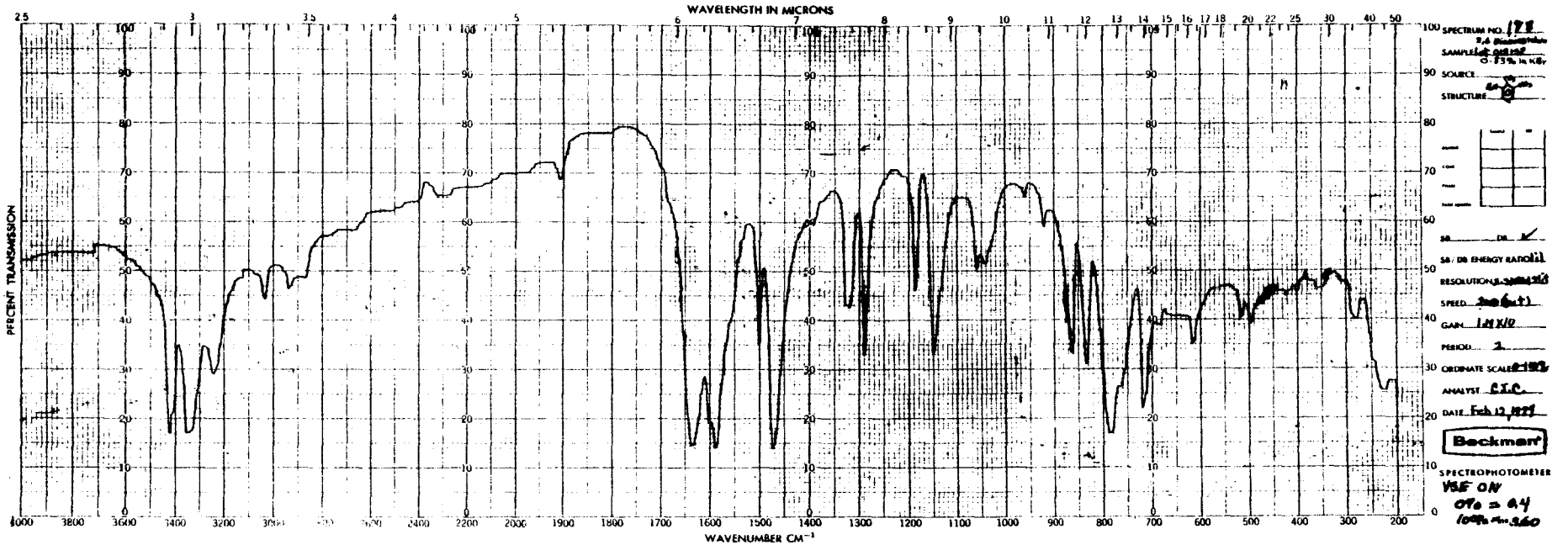


**Figure 5. Infrared Absorption Spectrum of 2, 6-Toluenediamine**

c. Nuclear Magnetic Resonance

Instrument: Varian HA-100
Solvent: $CDCl_3$ with internal TMS
Assignments: (Refer to figure 6)
(a) 1.87$\delta$ (b) 3.42$\delta$ (c) 6.08$\delta$
(d) 6.74$\delta$ , $J_{cd}$ = 8 cps
Integration Ratios: (a)3.06 (b) 3.83 (c) 2.02 (d) 0.93

Consistent with literature spectrum (Sadtler, Standard Spectra)

## CONCLUSIONS

Elemental analysis agrees with theory. Thin-layer chromatography indicates only a trace impurity at the origin. Vapor-phase chromatography gives one homogeneous peak. The infrared and nuclear magnetic resonance spectra are consistent with the structure.

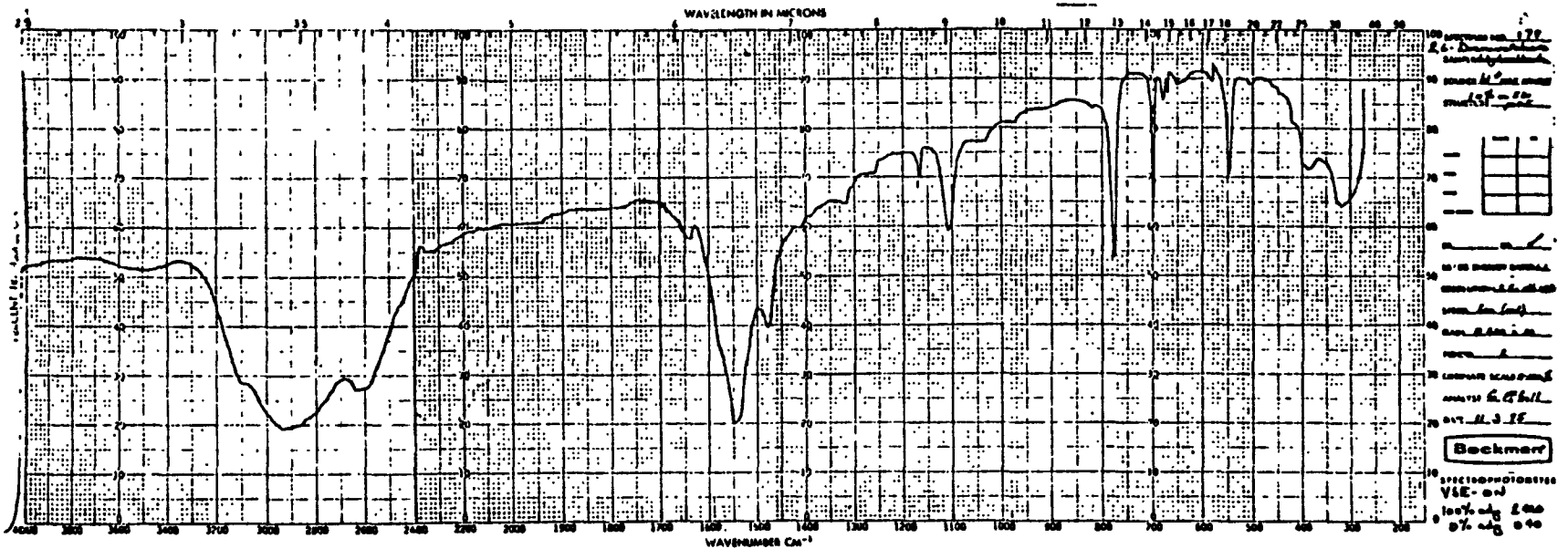


**Figure 6. Infrared Absorption Spectrum of 2, 6-Toluenediamine dihydrochloride**

# APPENDIX F

## Analysis of 2,6-Toluenediamine dihydrochloride
## Midwest Research Institute

## Appendix F

## Analysis of 2,6-Toluenediamine dihydrochloride

## Midwest Research Institute

### A. SYNTHESIS OF 2,6-TOLUENEDIAMINE DIHYDROCHLORIDE

2,6-Toluenediamine (1,921 g, 15.72 eq.) was dissolved in hydrochloric acid (2,880 ml of a 1:1 mixture of concentrated HCl:water). The solution was filtered and an additional 100 ml of concentrated hydrochloric acid was added to the filtrate. The filtrate was cooled in an ice-salt bath. The colorless crystals were collected and dried in a vacuum oven overnight. The yield was 1,863 g. The filtrate was concentrated and a second crop of crystals collected and dried (993 g).

### B. ELEMENTAL ANALYSIS

| Element | C | H | N | Cl |
|---|---|---|---|---|
| Theory | 43.09 | 6.20 | 14.36 | 36.35 |
| Theory with 5.8% $H_2O$ | 42.86 | 6.23 | 14.28 | 36.14 |
| Determined | 43.13 | 6.13 | 14.33 | 35.8 |
| | 43.21 | 6.17 | 14.40 | 36.0 |

### C. MELTING POINT

| Determined | Literature Values |
|---|---|
| 174° to 250°C dec. (visual; sealed, evacuated capillary) | No literature found. |
| 186.5° to 232°C (Dupont 900DTA) | |

D. THIN-LAYER CHROMATOGRAPHY

Plates: Silica gel G, F 254
Amount Spotted: 100 and 300µg
Ref. Standard: Aniline
Visualization: Ultraviolet (254 nm) and furfuraldehyde

System 1: n-Butanol:conc. aqueous ammonium hydroxide (99:1)
$R_f$: 0.54
$R_{st}$: 0.65

System 2: Tetrahydrofuran: conc. aqueous ammonium hydroxide (99:1)
$R_f$: 0.75
$R_{st}$: 0.91

E. VAPOR-PHASE CHROMATOGRAPHY

(a) System 1

Instrument: Tracor MT-220
Detection: Flame ionization
Column: 3% OV-225 on Chromosorb W (HP) 80/100, 1.8 M x 4 mm I.D.
Inlet temperature: 280°C
Detector temperature: 280°C
Oven temperature program: 5 min at 100°C, then 100°-200°C at 10°C/min
Results: One homogeneous peak, retention time 11.8 min

(b) System 2

Instrument: Tracor MT-220
Detection: Flame ionization
Column: 3% OV-1 on Supelcoport, 80/100, 1.8 m x 4 mm I.D.
Inlet temperature: 270°C
Detector temperature: 280°C
Oven temperature program: 5 min at 75°C, then 75°-200°C at 10°C/min
Results: One homogeneous peak, retention time 10.8 min

F. SPECTRAL DATA

(a) Infrared

Instrument: Beckman IR-12
Cell: 1.0% Potassium bromide pellet.
Results: See Figure 6.

Consistent with the spectrum of amine (Finkleshtein and Boitsov, 1960).

(b) Ultraviolet/Visible

Literature Values

Instrument: Cary 118

No literature reference found.

| $\lambda max$ (nm) | $\epsilon \times 10^{-3}$ |
|---|---|
| 282 | 1.928±0.003 ($\delta$) |
| 232.5 | 8.66±0.03 ($\delta$) |

No absorbance between 350 and 800 nm (visible range) at a concentration of 0.3 mg/ml.

Solvent: water

(c) Nuclear Magnetic Resonance

Consistent with literature spectrum of amine (Sadtler, Standard Spectra)

Instrument: Varian HA-100
Solvent: Dimethylsulfoxide-d6 with internal tetramethylsilane
Assignments: (refer to Figure 7)

(a) s, $\delta$ 2.39 ppm (b) m, $\delta$ 7.32-7.74 ppm
(c) s, $\delta$ 9.67 ppm

Integration Ratios:

(a) 2.76 (b) 3.24 (c) $NH_2$ and HCl

G. NUCLEAR MAGNETIC RESONANCE: (undried sample)

Instrument: Varian HA100
Solvent: DMSO-$d_6$ with internal TMS
Assignments: (refer to Figure 8)
(a) 2.33 $\delta$
(b) 7.17-7.60 $\delta$
(c) 10.10 $\delta$
Integration Ratios:
(a) 2.89
(b) 3.11
(c) 6.33

Consistent with literature spectrum of amine (Sadtler Standard Spectra).

CONCLUSIONS

The elemental analysis for chloride is slightly low; carbon, hydrogen, and nitrogen analyses agree with the theoretical values. Thin-layer chromatography shows a trace impurity at the origin. Vapor-phase chromatography indicates one homogeneous peak. The infrared, ultraviolet, visible, and nuclear magnetic resonance spectra conform to the structure.

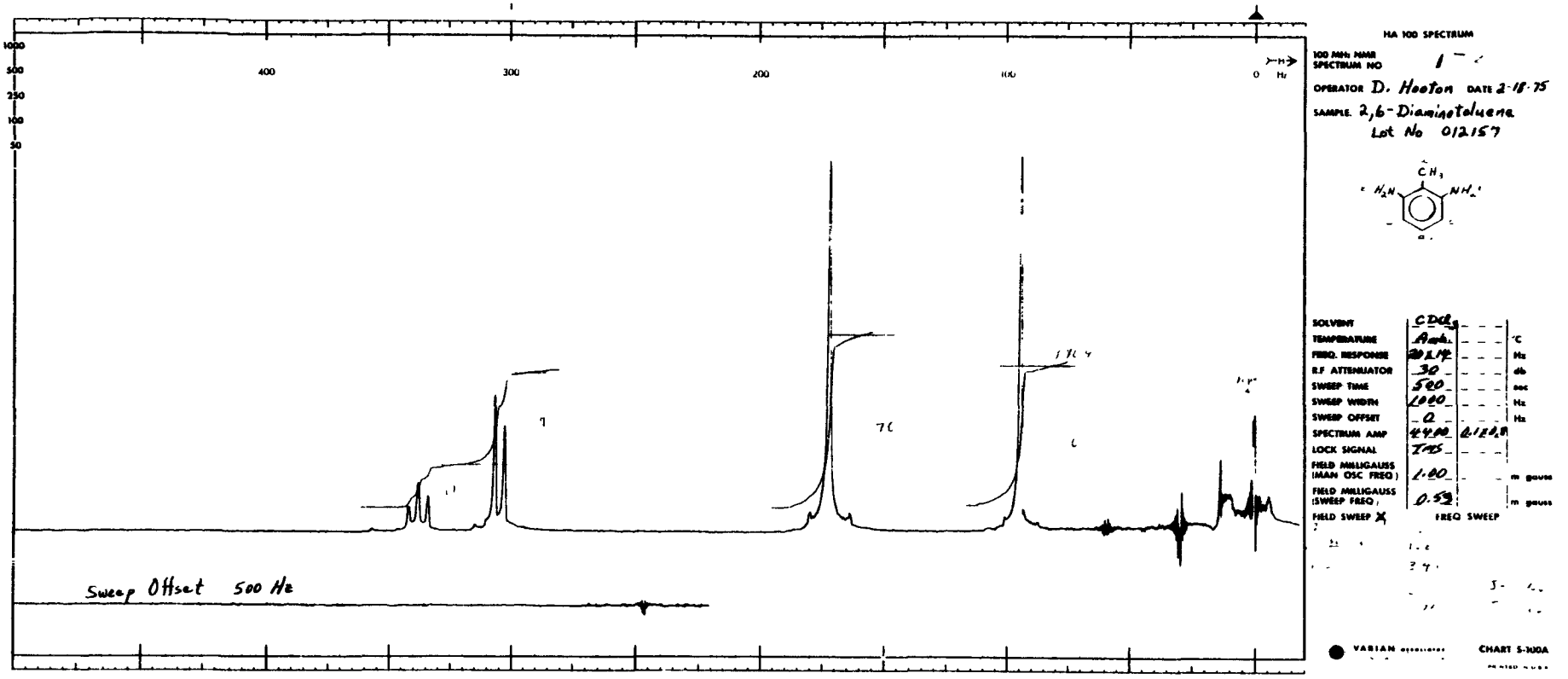


**Figure 7. Nuclear Magnetic Resonance Spectrum of 2, 6-Toluenediamine**

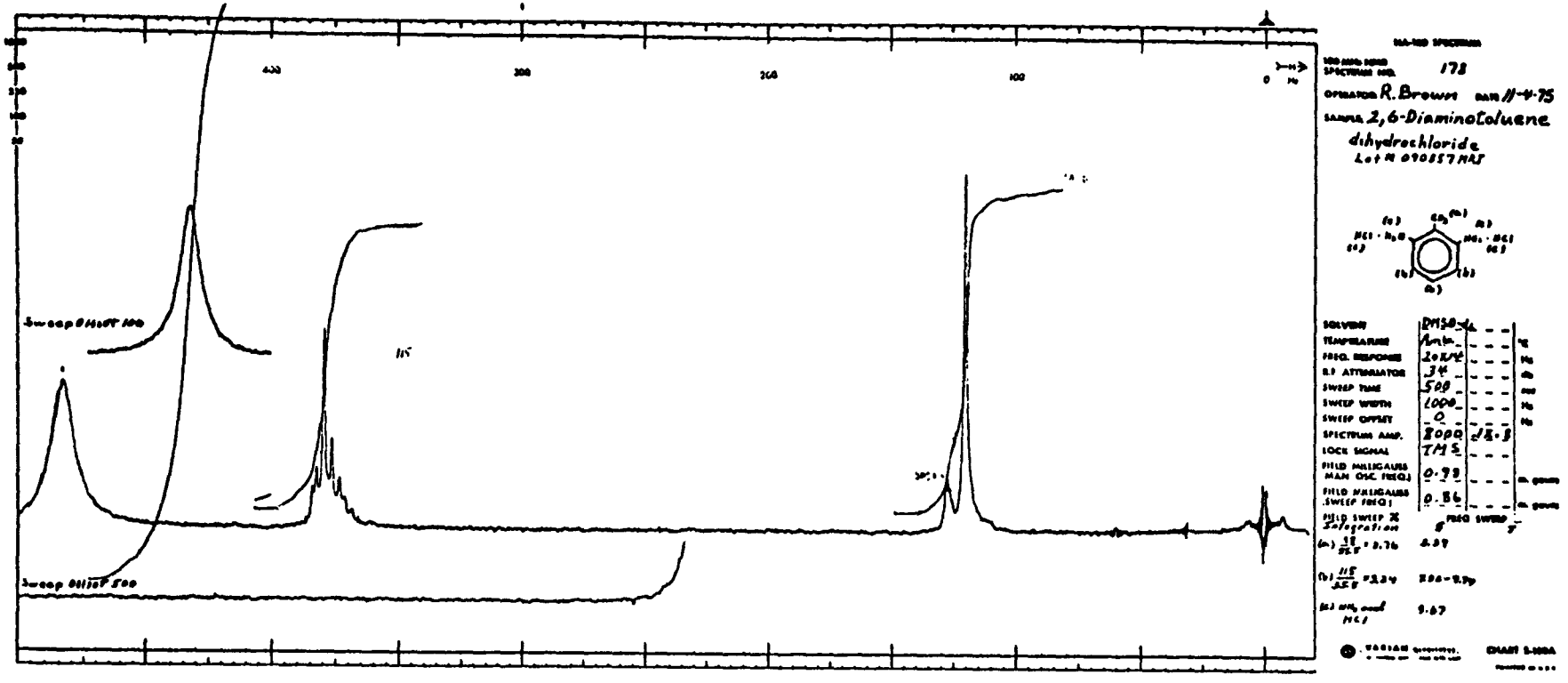


**Figure 8. Nuclear Magnetic Resonance Spectrum of 2, 6-Toluenediamine dihydrochloride**

# APPENDIX G

## Stability Analysis of 2,6-Toluenediamine dihydrochloride
## Midwest Research Institute

APPENDIX G

Stability Analysis of 2,6-Toluenediamine dihydrochloride

Midwest Research Institute

STABILITY TESTING

A. BULK CHEMICAL

1. METHOD: Samples of bulk chemical were stored at -20°, 5°, 25°, and 60°C for 2 weeks and then analyzed by vapor-phase chromatography using the following conditions:

Instrument: Bendix 2500
Detection: Flame Ionization
Column: 3% OV-225 on chromosorb W (HP) 80/10 mesh; glass, 1.8 M x 2 mm
Oven Temperature: 150°C isothermal

2. RESULTS:

| Temperature (°C) | Response (nm/μg) |
|---|---|
| -20 | 16.7±0.2 |
| 5 | 16.6±0.2 |
| 25 | 16.4±0.3 |
| 60 | 16.7±0.2 |

3. CONCLUSION: 2,6-Toluenediamine dihydrochloride is stable in bulk for 2 weeks at 60°C.

B. CHEMICAL/FEED MIXTURE

1. METHOD: A 10.03% 2,6-toluenediamine hydrochloride rat-feed mixture was prepared and stored at -20°, 5°, 25°, and 45°C for 2 weeks. One-gram samples of this mixture were blended with 50 ml of 95% ethanol for 1 minute on a Brinkman Polytron mixer. These blended samples were centrifuged for 10 minutes and the supernatants decanted into 100-ml volumetric flasks. The centrifugates were blended with another 50 ml of 95% ethanol for 1 minute on a Polytron mixer. These blended samples were centrifuged and the supernatants combined with those in the 100-ml volumetric flasks from the previous centrifugations. The resulting solutions were diluted to volume with 95% ethanol and injected on a gas chromatograph under the following conditions:

Instrument: Bendix 2500
Column: 3% OV-225 on chromosorb W (HP); 1.8 m x 2 mm, glass
Temperature: 145°C, isothermal
Detection: Flame ionization
$R_f$ of Test Compound: 2.0 minutes

2. RESULTS:

| Temperature (°C) | Percent 2,6-Toluenediamine dihydrochloride in Feed |
|---|---|
| -20 | 11.36±1.80 |
| 5 | 10.37±1.69 |
| 25 | 11.59±1.90 |
| 45 | 10.25±1.66 |

3. CONCLUSION: 2,6-Toluenediamine dihydrochloride is stable in feed for 2 weeks at temperatures up to 45°C.

# APPENDIX H

## Analytical Procedure for Analysis of 2,6-Toluenediamine dihydrochloride in Feed

## APPENDIX H

### Analytical Procedure for Analysis of 2,6-Toluenediamine dihydrochloride in Feed

Samples of 5 g were extracted twice with 25 ml of 0.6N hydrochloric acid in 100-ml ground glass stoppered graduated cylinders. The supernatant solutions were adjusted to pH $\geq$ 12 by addition of approximately 10 ml of 10% sodium hydroxide solution. After addition of 5 g of sodium chloride, the aqueous phases were extracted with 50 ml of chloroform in separatory funnels. The emulsified organic phases were centrifuged for 12 minutes and 10-ml aliquots were treated with anhydrous sodium sulfate and then reduced in volume to 1 ml by evaporation under nitrogen and analyzed by vapor-phase chromatography at 150$^{o}$ oven temperature on a 3% OV-17, 100/200 Supelcoport glass column. The retention time of the 2,6-toluenediamine dihydrochloride was 6.2 minutes.

# Review of the Bioassay of 2,6-Toluenediamine Dihydrochloride (2,6-TDA)* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

February 15, 1980

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 2,6-Toluenediamine Dihydrochloride (2,6-TDA) for carcinogenicity.

The primary reviewer for the report on the bioassay of 2,6-TDA said that the selection and testing of 2,6-TDA was justified by its broad human exposure; its structural relationship to 2,4-TDA, a known carcinogen; and its mutagenicity in bacteria. After a brief review of the results of the bioassay, he indicated that a dose-related incidence of liver tumors occurred in treated male rats and female mice. He opined that the maximum tolerated dose was not reached. The reviewer pointed out that the mice receiving 2,4-TDA were given a dose about two times the one used in this study. Based on these findings and certain inconsistencies in the language of the write-up, he recommended that the report on the bioassay of 2,6-TDA not be accepted. He said that acceptance of the report would indicate that the study was adequate and the chemical need not be further tested.

The secondary reviewer agreed with some of the reservations expressed by the primary reviewer. Despite the reservations, he said the conclusion that the chemical was not carcinogenic, under the conditions of test, was valid.

A Clearinghouse member suggested that a statement be added to the report indicating the need for further testing to resolve questions raised by deficiencies in the study. Another member agreed that such a statement should be included, especially since there is a wide variety of species differences to aromatic amines. He suggested that the hamster might be an appropriate test species.

The secondary reviewer moved that the part of the report dealing with the study in rats be approved. He further moved that the part dealing with the mice was inadequate because of the use of too low dosages and that additional animal studies should be conducted. The motion was seconded and approved unanimously.

Members present were:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
David B. Clayson, Eppley Institute for Research in Cancer
Joseph Highland, Environmental Defense Fund
William Lijinsky, Federick Cancer Research Center
Henry C. Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
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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