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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
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Bethesda, Maryland 20014

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FOREWORD: This report presents the results of the bioassay of 2,4,5-trimethylaniline conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. A positive result demonstrates that the 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 that are carcinogenic in animals requires a wider analysis.

CONTRIBUTORS: This bioassay of 2,4,5-trimethylaniline was conducted by The NCI Frederick Cancer Research Center (FCRC) (1), Frederick, Maryland, operated for NCI (2) by Litton Bionetics, Inc.

The manager of the bioassay at FCRC was Dr. B. Ulland, the toxicologist was Dr. E. Gordon, and Drs. R. Cardy and D. Creasia compiled the data. Ms. S. Toms was responsible for management of data, Mr. D. Cameron for management of histopathology, Mr. L. Callahan for management of the computer branch, and Mr. R. Cypher for management of the facilities. Mr. A. Butler performed the computer services. Necropsies were performed by Drs. B. Ulland, R. Schueler, R. Ball, and R. Cardy. Histopathologic evaluations were performed by Drs. M. D. Reuber and R. N. Empson, Jr., and the diagnoses included in this report represent their interpretation.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (3). The statistical analyses were performed by Dr. J. R. Joiner (4) and Ms. P. L. Yong (4), using methods selected for the bioassay program by Dr. J. J. Gart (5). The chemicals used in this bioassay were analyzed at FCRC (1) by Dr. W. Zielinsky. The chemical analyses and narrative were reviewed and approved by Dr. W. Lijinsky (1).

This report was prepared at Tracor Jitco (4) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. C. R. Angel, Acting Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. J. F. Robens, toxicologist; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Owen, Ms. M. S. King, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The following scientists at NCI were responsible for evaluating the bioassay, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. Morton H. Levitt, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. A. R. Patel, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

A bioassay of 2,4,5-trimethylaniline for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F1 mice.

Groups of 50 rats and 50 mice of each sex were administered 2,4,5-trimethylaniline at one of two doses, either 200 or 800 ppm for the rats and either 50 or 100 ppm for the mice, for 101 weeks. Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the end of administration of the test chemical.

Mean body weights of the dosed male and female rats were generally lower than those of corresponding controls; mean body weights of the dosed mice were only slightly lower in the males than in the corresponding controls and were unaffected or affected irregularly in the females. Survival was not affected significantly when the rats or mice were administered the test chemical and was 70% or greater in all dosed or control groups. Sufficient numbers of animals were at risk for the development of late-appearing tumors.

In the rats, hepatocellular carcinomas or neoplastic nodules occurred at incidences that were dose related in both males and females (P less than or equal to 0.001), and in direct comparisons the incidences were significantly higher in the high-dose males, high-dose females, and low-dose females (P less than or equal to 0.004) than in corresponding controls (males: controls 1/19, low-dose 6/50, high-dose 20/50; females: controls 0/20, low-dose 12/49, high-dose 27/50). In addition, alveolar/bronchiolar carcinomas or adenomas occurred in the female rats at incidences that were dose related (P = 0.003), and in a direct comparison the incidence was significantly higher in the high-dose group (P = 0.017) than in the corresponding control group (controls 0/20, low-dose 3/43, high-dose 11/50).

In the female mice, hepatocellular carcinomas occurred at incidences that were dose related (P less than or equal to 0.001), and in direct comparisons the incidences were significantly higher (P less than or equal to 0.001) in the low- and high-dose animals than in corresponding controls (controls 0/20, low-dose 18/49, high-dose 40/50). Because historical records of this laboratory for control B6C3F1 male mice show a relatively high incidence of hepatocellular carcinomas, an increased incidence of these tumors in 2,4,5-trimethylaniline

dosed male mice as compared with matched controls could not be clearly associated with administration of the test compound.

It is concluded that under the conditions of this bioassay, 2,4,5-trimethylaniline was carcinogenic for male and female F344 rats and female B6C3F1 mice, inducing hepatocellular carcinomas or neoplastic nodules in the rats of each sex, alveolar/bronchiolar carcinomas in the female rats, and hepatocellular carcinomas in the female mice.

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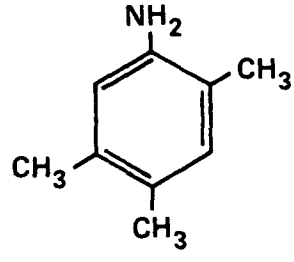
I. INTRODUCTION



2, 4, 5-Trimethylaniline

2,4,5-Trimethylaniline (CAS 137-17-7; NCI C02299), or pseudocumidine, is a component of a mixture of aromatic amines used in the synthesis of the red dye Ponceau 3R. This dye is produced by diazotizing a mixture of amine intermediates, some of which have been identified as methyl-, dimethyl-, or trimethylanilines, and coupling them with 2-naphthol-3,6-disulfonic acid. Ponceau 3R is therefore a complex mixture containing some 1-(2,4,5-trimethylphenylazo)-2-naphthol-3,6-disulfonic acid (Grice et al., 1961; Hansen et al., 1963).

Ponceau 3R has been used as a color additive in foods since 1907. It was certified as FD&C (Food Drug and Cosmetic) Red No. 1 from 1940 until 1960, at which time it was withdrawn from general use. Provisional recertification as Ext. D&C (External Drug and Cosmetic) Red No. 15 was granted shortly thereafter in 1961, but revoked in 1968 (Hansen et al., 1963; Code of Federal Regulations, 1977).

The acute oral LD_{50} of 2,4,5-trimethylaniline in male Osborne-Mendel rats when administered in an aqueous solution by stomach tube was 1,585 mg/kg, which was intermediate in relation to six anilines derived from Ponceau 3R (Lindstrom et al., 1969). Administration of 2,4,5-trimethylaniline in the diet at 375 to 5,000 ppm, for 90 days resulted in increased liver and kidney weights; at 750 to 5,000 ppm slight focal bile duct proliferation was found (Lindstrom et al., 1969).

Ponceau 3R was found to produce liver tumors of the hepatocytes and bile duct in rats (Hansen et al., 1963; Grice et al., 1961). The azo linkage of some components of Ponceau 3R are cleaved in vivo to give 1-amino-2-naphthol-3,6-disulfonic acid and the various aniline moieties, including 2,4,5-trimethylaniline; however, since the naphthol derivative is a component of other nontoxic food dyes, these carcinogenic effects have been attributed to the anilines (Lindstrom et al., 1969). Earlier 2-year studies conducted by the National Cancer Institute in male Charles River rats and in male and female HaM/ICR mice suggested that 2,4,5trimethylaniline was carcinogenic in the mice and possibly also in the rats (Homburger et al., 1972; Weisburger et al., in press). 2,4,5-Trimethylaniline was selected for study in the Carcinogenesis Testing Program, using an expanded bioassay protocol.

II. MATERIALS AND METHODS

A. Chemical

2,4,5-Trimethylaniline was obtained from Research Organic/ Inorganic Chemical as a fine, gray-white powder. Its melting point was 64°C. Elemental analysis showed 78.6% carbon, 9,9% hydrogen, and 10.2% nitrogen (theoretical: 80.0% C, 9.6% H, and 10.4% N). Analysis by gas-liquid chromatography indicated two components, one of which was greater than 99.9%. Its infrared spectrum was consistent with its chemical structure, and mass spectral analysis gave a molecular ion at m/e 135 and a base peak at m/e 120.

The test material was stored at 5°C until used.

B. Dietary Preparation

Test diets containing 2,4,5-trimethylaniline were prepared at Frederick Cancer Research Center (FCRC) every 1 to 1-1/2 weeks in 6- to 12-kg batches at the appropriate doses. A known weight of the chemical was first mixed with an equal weight of autoclaved

Wayne® Sterilizable Lab Meal containing 4% fat (Allied Mills, Inc., Chicago, Ill.), using a mortar and pestle. The mixing was continued with second and third additions of feed, and final mixing was performed with the remaining quantity of feed for a minimum of 15 minutes in a Patterson-Kelly® twin-shell blender. The diets were routinely stored at 7°C until used.

C. Animals

Male and female F344 (Fischer) rats and B6C3F1 mice were obtained as 4-week-old weanlings, all within 3 days of the same age, from the NCI FCRC (Frederick, Md), which is monitored by the Division of Cancer Treatment, NCI. The animals were housed within the test facility for 2 weeks and were then assigned four rats of the same sex to a cage and five mice of the same sex to a cage. The initial weights for male rats were 90 to 105 g, averaging at least 100 g; for female rats, 80 to 95 g, averaging at least 90 g; for male mice, 18 to 22 g, averaging at least 19.5 g; and for female mice, 17 to 21 g, averaging at least 18.5 g. Individual animals were identified by ear punch.

D. Animal Maintenance

The animals were housed in polycarbonate cages (Lab Products Inc., Garfield, N. J.), 19 x 10-1/2 x 8 inches for the rats and 11-1/2 x 7-1/2 x 5 inches for the mice. The cages were suspended from aluminum racks (Scientific Cages, Inc., Bryan, Tex.) and were covered by nonwoven polyester-fiber 12-mil-thick filter paper (Hoeltge, Inc., Cincinnati, Ohio). The bedding used was Absorb-dri® hardwood chips (Northeastern Products, Inc., Warrenburg, N. Y). The feed supplied was presterilized Wayne® Sterilizable Lab Meal containing 4% fat, provided ad libitum in suspended stainless steel hoppers and replenished as required, at least three times per week. Water, acidified to pH 2.5, was supplied ad libitum from glass bottles with sipper tubes suspended through the tops of the cages.

The contaminated bedding was disposed of through an enclosed vacuum line that led to a holding tank from which the bedding was fed periodically into an incinerator. The cages were sanitized twice per week and the feed hoppers twice per month at 82 to 88°C in a tunnel-type cagewasher (Industrial Washing Machine Corp., Mataway, N. J.), using the detergents, Clout® (Pharmacal Research Laboratories, Greenwich, Conn.) or Oxford D'Chlor (Oxford Chemicals, Atlanta, Ga.). The glass bottles and sipper

tubes were sanitized at 82 to 88°C in a tunnel-type bottle washer (Consolidated Equipment Supply Co., Mercersburg, Pa.) three times per week, using a Calgen Commercial Division detergent (St. Louis, Mo.). The racks for the cages were sanitized at or above 82°C in a rack washer (Consolidated Equipment Co.) once per month, using the Calgen Commercial Division detergent, and the filter paper was changed at the same time.

The animal rooms were maintained at 22 to 24°C and 45 to 55% relative humidity. Incoming air was passed through a filter of 65% efficiency and a bag filter of 95% efficiency at the intake and expelled without recirculation through a "Z"-type roughing filter of 30% efficiency and a bag system of 90 to 95% efficiency at the exhaust (American Air Filters, Louisville, Ky.; Mine Safety Appliances, Pittsburgh, Pa.). Room air was changed 15 times per hour. The air pressure was maintained negative to a clean hallway and positive to a return hallway. Fluorescent lighting was provided automatically on a 12-hour-per-day cycle.

Rats administered 2,4,5-trimethylaniline and their corresponding controls were housed in the same room as rats on feeding studies of the following chemicals:

(CAS 128-37-0) butylated hydroxytoluene (BHT)
(CAS 88-96-0) phthalamide

Mice administered 2,4,5-trimethylaniline and their corresponding controls were housed in the same room as mice on feeding studies of the following chemicals:

(CAS 156-62-7) calcium cyanamide
(CAS 999-81-5) (2-chloroethyl) trimethylammonium chloride (CCC)
(CAS 95-80-7) 2,4-diaminotoluene
(CAS 19010-66-3) lead dimethyldithiocarbamate
(CAS 86-30-6) N-nitrosodiphenylamine
(CAS 88-96-0) phthalamide
(CAS 120-62-7) piperonyl sulfoxide

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses (MTD's) of 2,4,5-trimethylaniline, on the basis of which two concentrations (referred to in this report as "low" and "high" doses) were selected for administration in the chronic studies. Groups of five rats and five mice of each sex were fed diets containing 2,4,5-trimethylaniline at one of several doses for 7 weeks, followed by 1 week of observation, and groups of five control animals of each species and sex were administered basal diet only. Each animal was weighed twice per week. Tables 1 and 2 show doses fed, the survival of animals in each dosed group at the end of the study, and the mean body weights of each

Table 1. 2,4,5-Trimethylaniline Subchronic Feeding Studies in Rats

Dose (ppm)	Male		Female	
	Survival (a)	Mean Weight at Week 7 as % of Control	Survival (a)	Mean Weight at Week 7 as % of Control
RATS				
0	5/5	100	5/5	100
1,000	5/5	93	5/5	91
1,500	5/5	92	5/5	83
2,000	5/5	86	5/5	84
3,200(b)	5/5	67	5/5	73
4,600	5/5	56	3/5	64

(a) Number surviving/number in group.

(b) Small amounts of bile-duct hyperplasia and periportal edema were observed in livers. Focal hyperplasia and hepatocellular hypertrophy were occasionally noted.

Table 2. 2,4,5-Trimethylaniline Subchronic Feeding Studies in Mice

Dose (ppm)	Male		Female	
	Survival (a)	Mean Weight at Week 7 as % of Control	Survival (a)	Mean Weight at Week 7 as % of Control
MICE				
First Study				
0	4/5	100	5/5	100
1,000	5/5	86	5/5	87
1,500	5/5	83	5/5	82
2,000	5/5	80	2/5	90
3,200	5/5	71	5/5	79
4,600	5/5	72	5/5	74
Second Study				
0	5/5	100	5/5	100
25	5/5	107	5/5	100
50	5/5	100	5/5	102
100	5/5	101	5/5	82
200	5/5	105	5/5	95
500	5/5	97	5/5	92
1,000(b)	5/5	82	5/5	84

(a) Number surviving/number in group.

(b) Very slight to moderate increase in splenic hematopoiesis in one female and three males. A trace of Kupffer-cell pigmentation in livers of four males and five females.

dosed group at week 7, expressed as percentages of mean body weights of controls. At the end of the subchronic studies, all animals were killed using CO_2 and necropsied. Histopathologic findings are included as footnotes to tables 1 and 2.

Ten percent depression in body weight was one of the main criteria used for the estimation of MTD's. The doses required to produce this response were determined by the following procedure: first, least squares regressions of mean body weights versus days on study were used to estimate mean body weights of each of the dosed groups at day 49. Next, probits of the percent weights of the dosed groups at day 49 relative to weights of corresponding control groups were plotted against the logarithms of the doses, and least squares regressions fitted to the data were used to estimate the doses required to induce 10% depression in weight.

The low and high doses were set at 200 and 800 ppm for rats and at 50 and 100 ppm for mice.

F. Chronic Studies

The test groups, doses administered, and durations of the chronic feeding studies are shown in table 3.

Table 3. 2,4,5-Trimethylaniline Chronic Feeding Studies in Rats and Mice

Species, Sex, and Test Group	Initial No. Animals (a)	2,4,5-Trimethyl-aniline in Diet (b) (ppm)	Time on Study (weeks)
RATS			
Male			
Matched-Control	20	0	101
Low-Dose	50	200	101
High-Dose	50	800	101
Female			
Matched-Control	20	0	101
Low-Dose	50	200	101
High-Dose	50	800	101
MICE			
Male			
Matched-Control	20	0	101
Low-Dose	50	50	101
High-Dose	50	100	101
Female			
Matched-Control	20	0	101
Low-Dose	50	50	101
High-Dose	50	100	101

(a) All animals were 6 weeks of age when placed on study.

(b) Test and control diets were provided ad libitum 7 days per week.

G. Clinical and Pathologic Examinations

All animals were observed twice daily. Observations for sick, tumor-bearing, and moribund animals were recorded daily. Clinical examination and palpation for masses were performed each month, and the animals were weighed at least once per month. Moribund animals and those that survived to the end of the bioassay were killed using CO_2 and necropsied.

Gross and microscopic examinations of major tissues, major organs, and all gross lesions were performed. The 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 marrow (femur), spleen, lymph nodes (mesenteric and submandibular), thymus, heart, salivary glands (parotid, sublingual, and submaxillary), liver, pancreas, esophagus, stomach (glandular and nonglandular), small and large intestines, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, mammary gland, uterus, ovary, brain (cerebrum and cerebellum), and all tissue masses. Peripheral blood smears also were made for all animals, whenever possible.

Necropsies were also 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.

H. Data Recording and Statistical Analyses

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

These data were analyzed using the appropriate statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of

carcinogenicity are discussed in the statistical narrative section.

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 only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given 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 a number of dosed groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), 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 relation-

ship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which

used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity (P less than 0.05, two-tailed test) were also noted.

The approximate 95 percent 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 relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a dosed group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a dosed group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the dosed group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% 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 (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) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of low- and high-dose males and high-dose females were lower than those of corresponding controls throughout the bioassay (figure 1); mean body weights of low-dose females were lower than those of corresponding controls only after week 46. Other clinical signs were common to both control and dosed groups of rats.

B. Survival (Rats)

Estimates of the probabilities of survival for male and female rats administered 2,4,5-trimethylaniline in the diet at the doses of this bioassay, together with those of the matched controls, are shown in the Kaplan and Meier curves in figure 2. The result of the Tarone test for dose-related trend in mortality is not significant in either sex.

In male rats, 43/50 (86%) of the high-dose group, 37/50 (74%) of the low-dose group, and 16/20 (80%) of the matched-control group

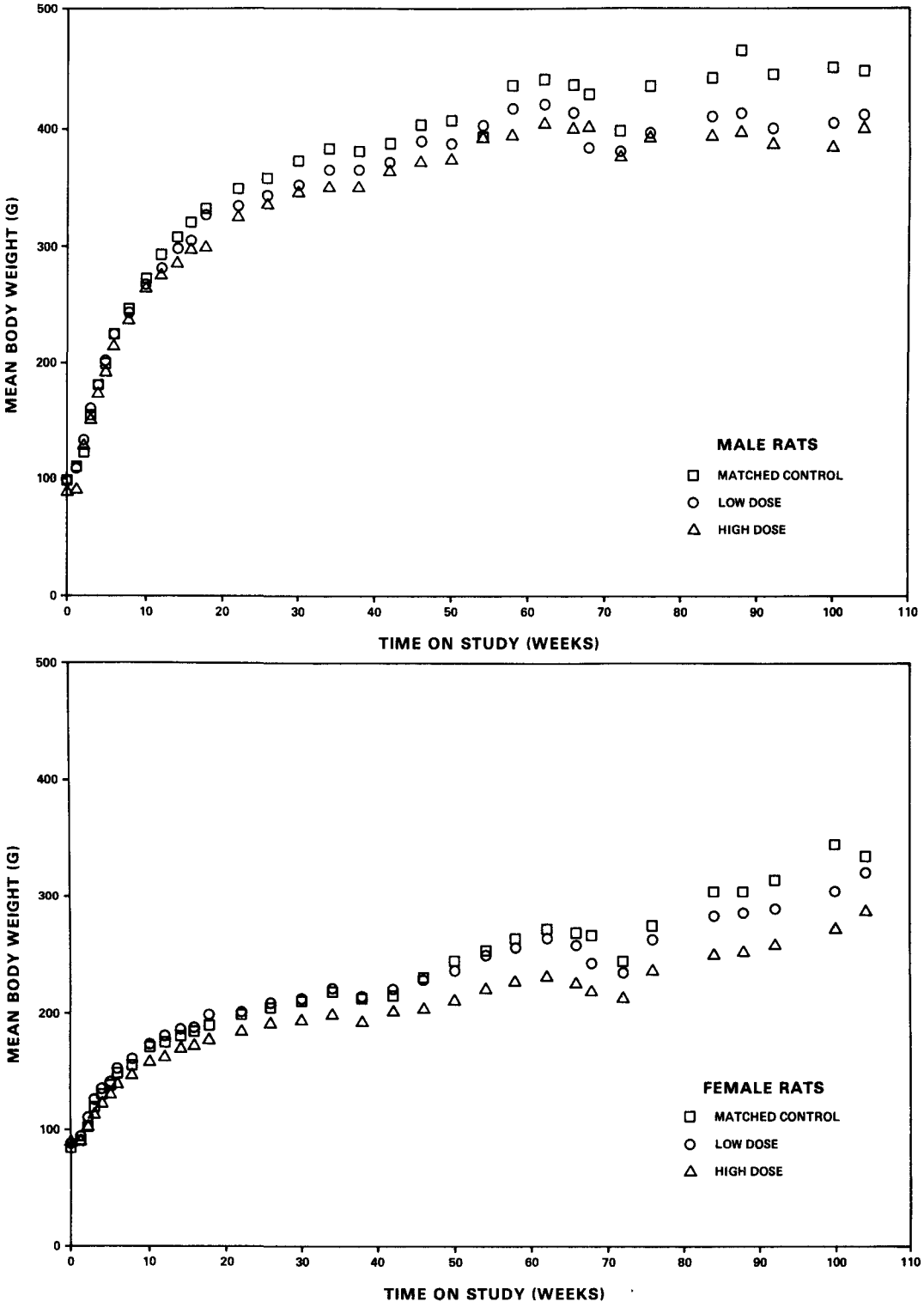


Figure 1. Growth Curves for Rats Administered 2,4,5-Trimethylaniline in the Diet

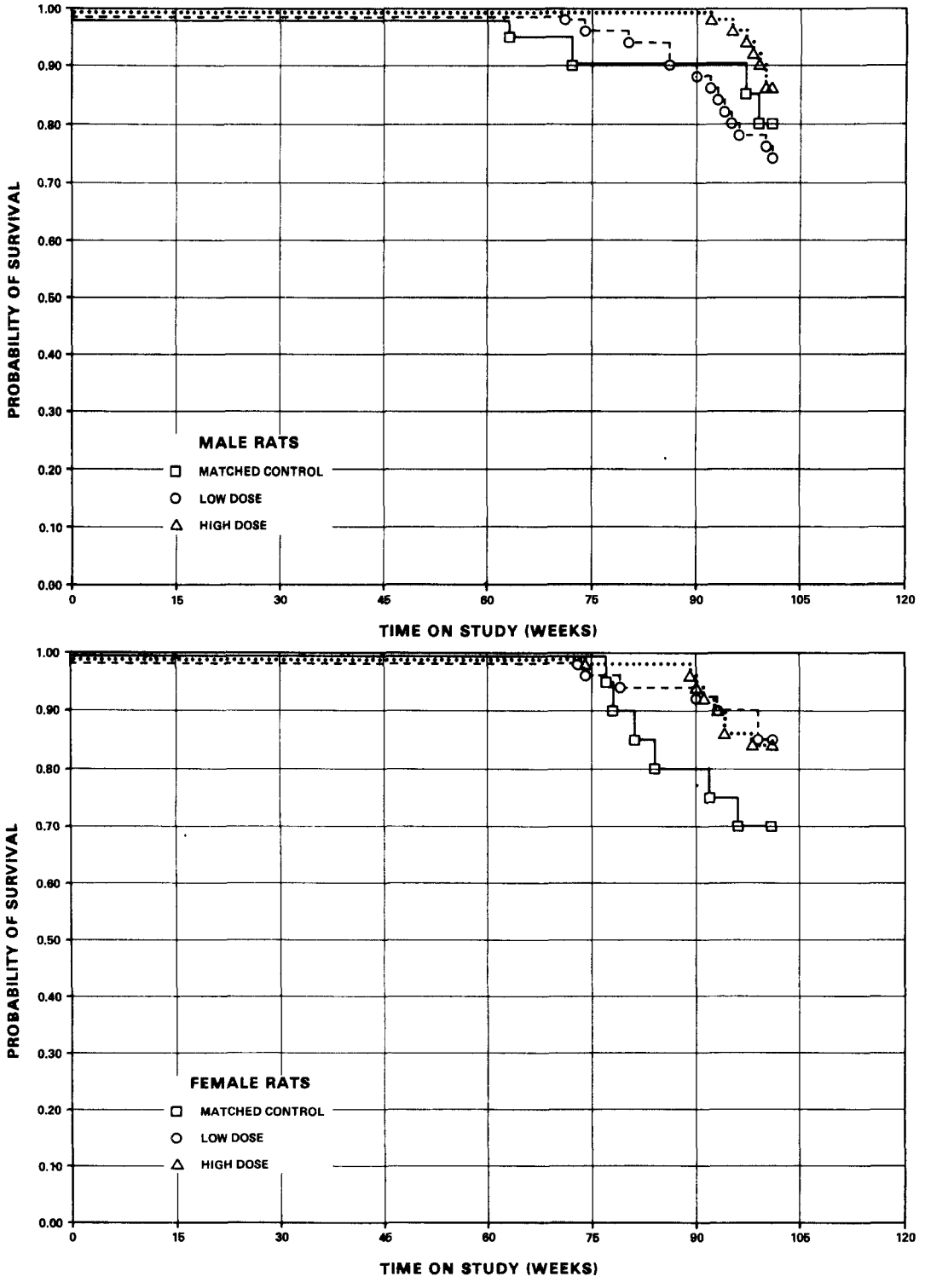


Figure 2. Survival Curves for Rats Administered 2,4,5-Trimethylaniline in the Diet

lived to the end of the bioassay. In females, 42/50 (84%) of each dosed group and 14/20 (70%) of the matched-control group lived to the end of the bioassay.

Sufficient numbers 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.

Neoplasms were observed with greatest frequency in the livers and lungs of dosed male and female rats.

Hepatocellular carcinomas generally were observed on gross examination; histologically they were well-differentiated or poorly differentiated, and a few were undifferentiated, carcinomas. The cells in well-differentiated carcinomas had vesicular nuclei and eosinophilic cytoplasm and grew in cords two or several cells in thickness. Cells in poorly differentiated carcinomas had basophilic or eosinophilic cytoplasms and grew in

sheets. Undifferentiated carcinoma cells varied greatly in size and shape and were quite anaplastic. Bile-duct carcinomas were well differentiated. Cells varied in size but usually tended to be columnar and formed ducts. Neoplastic nodules were distinct, small, early lesions in which the cells were clearly demarcated from, and compressed the adjacent parenchymal cells, at least focally. Cells had lightly staining eosinophilic cytoplasm and occasional double nuclei. The incidences of the different neoplasms of the liver in the rats were as follows:

	Male			Female		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Number of Tissues Examined	19	50	50	20	49	50
Hepatocellular Carcinoma	0	3(6%)	11(22%)	0	0	9(18%)
Neoplastic Nodule	1(5%)	3(6%)	11(22%)	0	12(24%)	20(40%)
Bile-Duct Carcinoma	0	0	4(8%)	0	0	1(2%)
Number of Animals with Tumors	1(5%)	6(12%)	20(40%)	0	12(24%)	27(54%)

Alveolar/bronchiolar carcinomas of the lung were well-differentiated papillary adenocarcinomas or poorly differentiated carcinomas. Cells in the well-differentiated carcinomas were

columnar and formed papillary growths as well as glands. Cells in poorly differentiated carcinomas were small cells growing in sheets. The incidences of alveolar/bronchiolar carcinomas and adenomas in the rats were as follows:

	Male			Female		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Number of Tissues Examined	20	49	50	20	43	50
Alveolar/Bronchiolar Carcinoma	1(5%)	0	2(4%)	0	2(5%)	2(4%)
Alveolar/Bronchiolar Adenoma	0	0	5(10%)	0	1(2%)	9(18%)
Number of Animals with Tumors	1(5%)	0	7(14%)	0	3(7%)	11(22%)

There also were neoplasms of the hematopoietic system and vascular system in some groups of rats; however, these occurred in both control and dosed groups.

A variety of nonneoplastic lesions and disorders were encountered with regularity in both control and dosed rats. Such lesions were considered to be common in aged F344 rats.

Based on the histopathologic examination, the incidence of

neoplasms of the liver and the lung was increased in the male and female rats administered 2,4,5-trimethylaniline.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In male rats, the result of the Cochran-Armitage test for dose-related trend in the incidence of hepatocellular carcinomas is significant (P = 0.002). The result of the Fisher exact test shows that the incidence of the tumors in the high-dose group is significantly higher (P = 0.020) than that in the matched-control group. When the incidence of either hepatocellular carcinomas or neoplastic nodules in male rats is analyzed, increased significance is observed in the Cochran-Armitage test for linear trend (P less than 0.001) and in the Fisher exact test (P = 0.004) between the high-dose and control groups. In females, the result of the Cochran-Armitage test on the incidence of hepatocellular carcinomas is significant (P less than 0.001). The Fisher exact comparison of the incidences of tumors in the high-dose and matched-control

groups shows a P value of 0.039, which is above the 0.025 level required for significance when the Bonferroni inequality criterion is used for multiple comparison. However, when the incidence of either hepatocellular carcinomas or neoplastic nodules in female rats is analyzed, significant results of the Cochran-Armitage test (P less than 0.001) and Fisher exact test (P less than or equal to 0.010) between each dosed group and the control group are observed. The statistical conclusion is that the incidence of liver tumors in rats of each sex is associated with the administration of 2,4,5-trimethylaniline.

In female rats, the result of the Cochran-Armitage test for the incidence of lung tumors is significant (P = 0.003), and the result of the Fisher exact test shows that the incidence of tumors in the high-dose group is significantly higher (P = 0.017) than that in the matched-control group. The statistical conclusion is that the incidence of lung tumors in female rats is associated with the administration of the test chemical.

In male rats, the results of the Cochran-Armitage test on the incidence of lung tumors, the incidence of bile-duct carcinoma of the liver, and the incidence of C-cell carcinoma of the thyroid are significant, but the results of the Fisher exact test are not significant.

Significant dose-related trends in the negative direction are observed in the incidence of hematopoietic tumors and in the incidence of mesotheliomas of the tunica vaginalis in male rats. In females, the incidence of endometrial stromal polyps is higher in the matched-control group than in the low-dose group.

In summary, the incidences of lung tumors in female rats and hepatic tumors in rats of each sex were associated with the administration of 2,4,5-trimethylaniline.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the dosed male mice were slightly lower than those of the corresponding controls throughout the bioassay for the high-dose group and after week 16 for the low-dose group (figure 3). Mean body weights of the high-dose females were essentially the same as those of the corresponding controls throughout the bioassay; mean body weights of the low-dose females were slightly lower than those of the controls for the first 30 weeks and slightly higher thereafter. Clinical signs occurred at comparable incidences in control and dosed groups of both male and female mice.

B. Survival (Mice)

Estimates of the probabilities of survival for male and female mice administered 2,4,5-trimethylaniline in the diet at the doses of this bioassay, together with those of the matched controls, are shown in the Kaplan and Meier curves in figure 4. The result

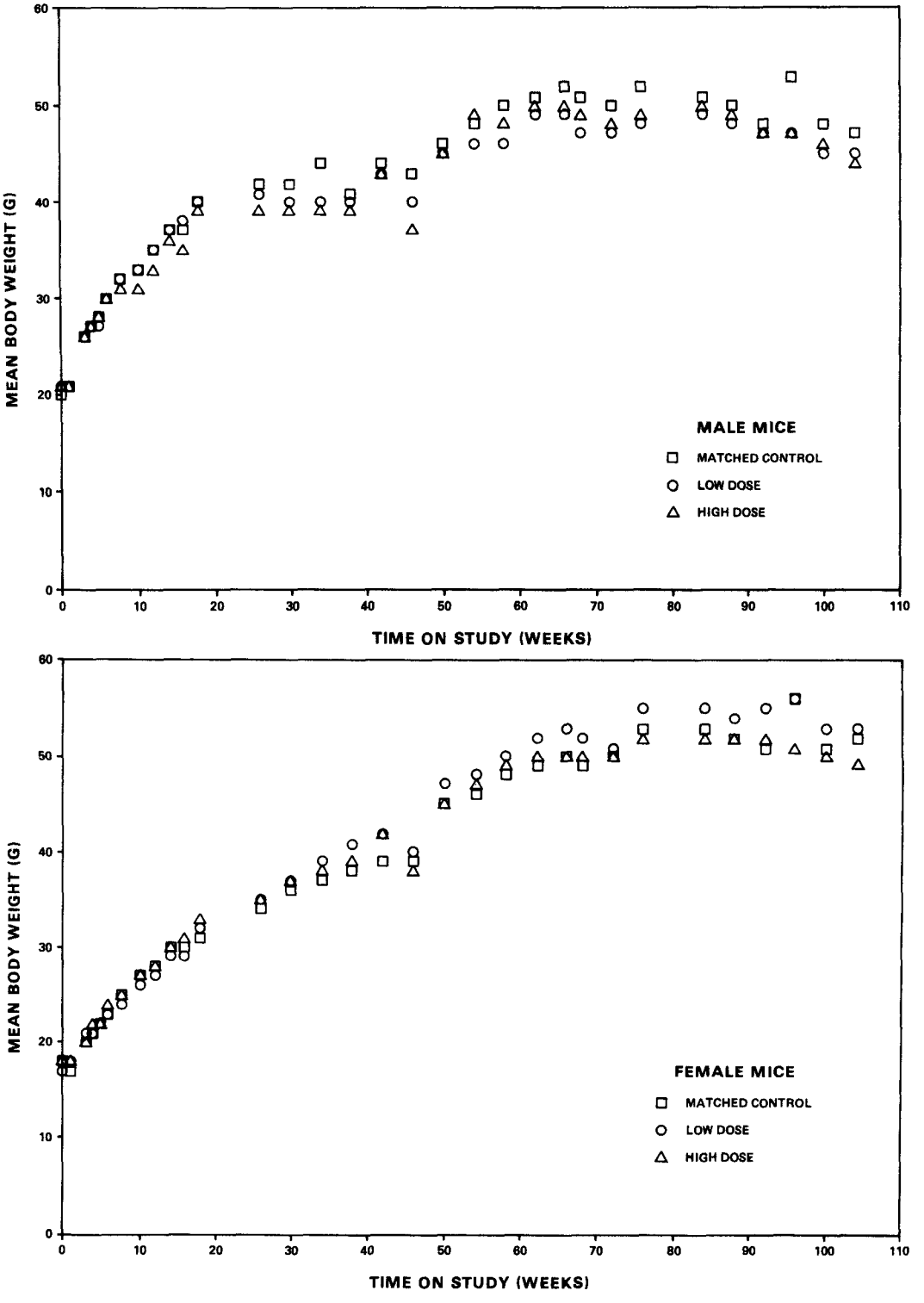


Figure 3. Growth Curves for Mice Administered 2,4,5-Trimethylaniline in the Diet

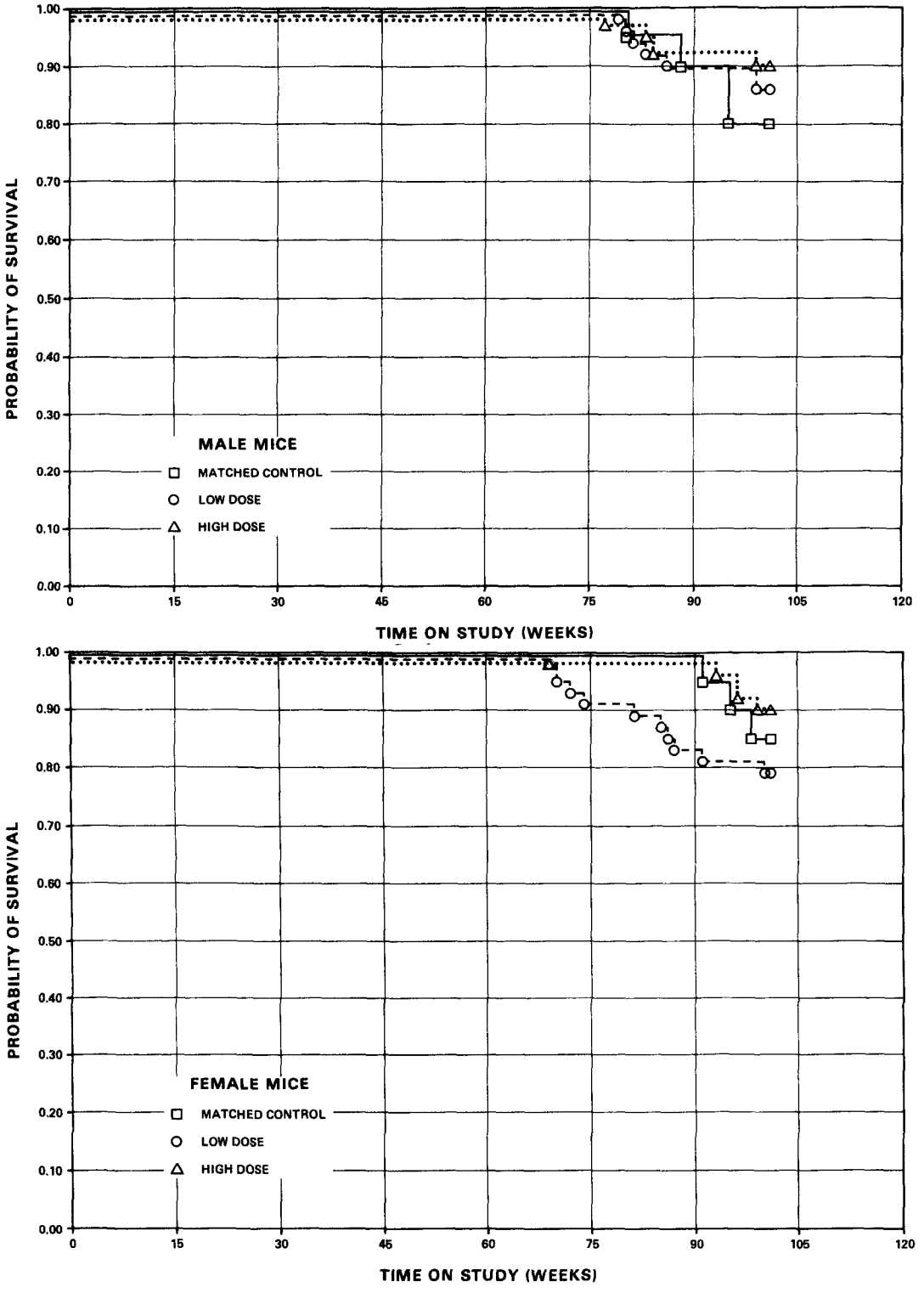


Figure 4. Survival Curves for Mice Administered 2,4,5-Trimethylaniline in the Diet

of the Tarone test for dose-related trend in mortality is not significant in either sex.

In male mice, 38/50 (76%) of the high-dose group, 43/50 (86%) of the low-dose group, and 16/20 (80%) of the matched-control group lived to the end of the bioassay. In females, 45/50 (90%) of the high-dose group, 39/50 (78%) of the low-dose group, and 17/20 (85%) of the matched-control group lived to the end of the bioassay.

Sufficient numbers 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 were observed with greatest frequency in the liver, hematopoietic system, and vascular system in mice administered 2,4,5-trimethylaniline.

The incidence of hepatocellular carcinomas was increased in dosed

male and female mice when compared with their respective controls. These tumors generally were observed on gross examination; histologically they were well-differentiated or poorly differentiated, and a few were undifferentiated, carcinomas. The cells in well-differentiated carcinomas had vesicular nuclei and eosinophilic cytoplasm and grew in cords two or several cells in thickness. Cells in poorly differentiated carcinomas had basophilic or eosinophilic cytoplasms and grew in sheets. Undifferentiated carcinoma cells varied greatly in size and shape and were quite anaplastic. Carcinomas of the liver metastasized to the lungs in one control male, two low-dose males, and one high-dose male. The incidences of neoplasms of the liver and gallbladder in the mice were as follows:

	Male			Female		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Number of Tissues Examined	20	50	50	20	49	50
Hepatocellular Carcinoma	5(25%)	26(52%)	27(54%)	0	18(36%)	40(80%)
Bile-Duct Carcinoma	0	2(4%)	2(4%)	0	0	0
Hyperplastic Nodule	1(5%)	3(6%)	7(14%)	0	4(8%)	13(26%)
Gallbladder Carcinoma	0	1(2%)	0	0	0	0
Number of Animals with Tumors	5(25%)	27(54%)	27(54%)	0	18(36%)	40(80%)

Neoplasms of the hematopoietic system were all diagnosed as lymphomas. The incidences of these hematopoietic neoplasms were not, however, higher in dosed groups than in corresponding controls.

Incidences of neoplasms of the lung and of the vascular system were slightly increased in female mice administered 2,4,5-trimethylaniline when compared with controls. Carcinomas of the lung were well-differentiated papillary adenocarcinomas or poorly differentiated carcinomas. Cells in the well-differentiated carcinomas were columnar and formed papillary growths as well as glands. Cells in poorly differentiated carcinomas were small cells growing in sheets. The incidences of alveolar/bronchiolar adenomas and carcinomas were as follows:

	Male			Female		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Number of Tissues Examined	20	50	50	19	49	48
Alveolar/Bronchiolar Carcinoma	4(20%)	7(14%)	1(2%)	0	4(8%)	6(12%)
Alveolar/Bronchiolar Adenoma	0	2(4%)	0	0	1(2%)	0
Number of Animals with Tumors	4(20%)	9(18%)	1(2%)	0	5(10%)	6(12%)

Hemangiomas and hemangiosarcomas in female mice receiving 2,4,5-trimethylaniline most often were seen in lymph nodes, but they also were present in the bone, skeletal muscle, liver, testis, and adipose tissue. Hemangiosarcomas were made up of proliferating endothelial cells that formed blood-filled vascular channels and generally were invasive. The incidences of the different neoplasms of the vascular system were as follows:

	Male			Female		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Number of Tissues Examined	20	50	50	20	49	50
Hemangiosarcoma	2(10%)	3(6%)	6(12%)	1(5%)	11(22%)	7(14%)
Hemangioma	0	0	0	0	1(2%)	0
Number of Animals with Tumors	2(10%)	3(6%)	6(12%)	1(5%)	11(22%)	7(14%)

A variety of nonneoplastic lesions and disorders were encountered with regularity in both control and dosed mice. Such lesions were considered common in aged B6C3F1 mice.

Based on the histopathologic examination, incidences of neoplasms of the liver were increased in male and female B6C3F1 mice administered 2,4,5-trimethylaniline, and incidences of neoplasms of the lung and of the vascular system were increased in females.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In male mice, the result of the Cochran-Armitage test for the incidence of hepatocellular carcinomas is significant (P = 0.039). The Fisher exact comparison of the incidences of tumors in the matched-control group and the low-dose group shows a P value of 0.035, which is above the 0.025 level required for significance when the Bonferroni inequality criterion is used for multiple comparison. The comparison between the control and high-dose groups shows a P value of 0.025, which is at the critical point when the Bonferroni inequality criterion is used for multiple comparison. The historical records of this laboratory for control B6C3F1 male mice having hepatocellular carcinomas show an incidence of 137/422 (32%), with incidences in individual control groups as high as 11/19 (58%) and 10/20 (50%), as compared with 4/20 (20%) in the control group in this bioassay, 26/50 (52%) in the low-dose group, and 27/50 (54%) in the high-dose group. In females, the result of the Cochran-Armitage test for the incidence of hepatocellular carcinomas is

significant (P less than 0.001), and the results of the Fisher exact test show that the incidence of these tumors in each dosed group is significantly higher (P less than or equal to 0.001) than that in the matched-control group. The statistical conclusion is that the incidence of hepatocellular carcinomas in female mice is associated with the administration of 2,4,5-trimethylaniline.

In female mice, the result of the Cochran-Armitage test for the incidence of carcinomas of the pituitary is significant (P = 0.043), but the results of the Fisher exact test are not significant. When the incidence of the combination of carcinomas or adenomas of the pituitary in female mice is analyzed, the results of the statistical test are not significant.

In male mice, significant results in the negative direction are observed in the incidence of lung tumors. In females, a significant trend in the negative direction is observed in the incidence of lymphomas.

V. DISCUSSION

Mean body weights of the dosed male and female rats were generally lower than those of corresponding controls; mean body weights of the dosed mice were only slightly lower in the males than in the corresponding controls and were unaffected or affected irregularly in the females. Survival was not affected significantly when the rats or mice were administered the test chemical and was 70% or greater in all dosed or control groups. Sufficient numbers of animals were at risk for the development of late-appearing tumors.

In the rats, hepatocellular carcinomas or neoplastic nodules occurred at incidences that were dose related in both males and females (P less than or equal to 0.001), and in direct comparisons the incidences were significantly higher in the high-dose males, high-dose females, and low-dose females (P less than or equal to 0.004) than in corresponding controls (males: controls 1/19, low-dose 6/50, high-dose 20/50; females: controls 0/20, low-dose 12/49, high-dose 27/50). In addition, alveolar/bronchiolar carcinomas or adenomas occurred in the female rats at incidences that were dose related (P = 0.003), and in a direct

comparison the incidence was significantly higher in the high-dose group (P = 0.017) than in the corresponding control group (controls 0/20, low-dose 3/43, high-dose 11/50).

In the mice, hepatocellular carcinomas occurred at incidences that were dose related in both males and females (P less than or equal to 0.039), and in direct comparisons the incidences were significantly higher (P less than or equal to 0.025) in the high-dose males, high-dose females, and low-dose females than in corresponding controls (males: controls 5/20, low-dose 26/50, high-dose 27/50; females: controls 0/20, low-dose 18/49, high-dose 40/50). However, the comparison between the controls and the high-dose males shows a P value of 0.025, which is at the critical point when the Bonferroni inequality criterion is used for multiple comparison. The historical records of this laboratory for control B6C3F1 male mice having hepatocellular carcinomas show an incidence of 137/422 (32%), with incidences of individual control groups as high as 11/19 (58%) and 10/20 (50%). Thus, in male mice these tumors cannot be clearly associated with administration of 2,4,5-trimethylaniline.

In prior investigations of the carcinogenicity of 2,4,5-trimethylaniline administered in the diet in 2-year studies, hepatic and pulmonary tumors occurred in increased incidences in male and

female HaM/ICR mice, but marginal results were obtained using male Sprague-Dawley rats (Homburger et al., 1972; Weisburger et al., in press). The low and high doses of test chemical used in these studies were 3,000 and 6,000 ppm for 5 months and 1,500 and 3,000 ppm for the subsequent 13 months for the rats and 6,000 and 12,000 ppm for 18 months for the mice. The occurrence of hepatic tumors in B6C3F1 mice administered 2,4,5-trimethylaniline in the present bioassay is consistent with the occurrence of these tumors in the HaM/ICR mice of the earlier studies. The occurrence of hepatic tumors in male and female F344 rats and pulmonary tumors in female F344 rats administered 2,4,5-trimethylaniline in the present bioassay differs, however, from the marginal results obtained in the earlier studies using male Sprague-Dawley rats. Although the doses of test chemical used in the earlier studies were higher than those used in the present bioassay, especially for the mice, the duration of administration was shorter in the earlier studies. The induction of liver adenomas or carcinomas in rats of various strains administered Ponceau 3R in the feed (Grice et al., 1961; Hansen et al., 1963) may be attributable to the in vivo cleavage of the dye to yield its aniline moieties, among which 2,4,5-trimethylaniline is a predominant component (Lindstrom et al., 1969).

It is concluded that under the conditions of this bioassay,

2,4,5-trimethylaniline was carcinogenic for male and female F344 rats and female B6C3F1 mice, inducing hepatocellular carcinomas or neoplastic nodules in the rats of each sex, alveolar/bronchiolar carcinomas in the female rats, and hepatocellular carcinomas in female mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED 2,4,5-TRIMETHYLANILINE

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED 2,4,5-TRIMETHYLANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	1 (5%)		
SQUAMOUS CELL CARCINOMA			1 (2%)
FIBROMA	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
CARCINOMA, NOS, METASTATIC		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA			5 (10%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)		2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		2 (4%)	
LEUKEMIA,NOS	4 (20%)	10 (20%)	3 (6%)
MONOCYTIC LEUKEMIA		1 (2%)	
#LYMPH NODE	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)	
#SPLEEN	(20)	(49)	(49)
HEMANGIOSARCOMA		1 (2%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(19)	(50)	(50)
LYMPHANGIOMA			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(50)	(49)
ADENOCARCINOMA, NOS	1 (5%)		
#LIVER	(19)	(50)	(50)
BILE DUCT CARCINOMA			4 (8%)
NEOPLASTIC NODULE	1 (5%)	3 (6%)	11 (22%)
HEPATOCELLULAR CARCINOMA		3 (6%)	11 (22%)
#PANCREAS	(19)	(50)	(48)
ACINAR-CELL ADENOMA			1 (2%)
#STOMACH	(19)	(50)	(50)
SQUAMOUS CELL PAPILLOMA			1 (2%)
#PEYERS PATCH	(20)	(50)	(50)
LEIOMYOSARCOMA		1 (2%)	
#COLON	(19)	(50)	(50)
ADENOMATOUS POLYP, NOS	1 (5%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(50)	(50)
CARCINOMA,NOS	5 (25%)	6 (12%)	10 (20%)
ADENOMA, NOS	3 (15%)	11 (22%)	15 (30%)
#ADRENAL	(20)	(50)	(50)
CORTICAL CARCINOMA			2 (4%)
PHEOCHROMOCYTOMA	2 (10%)	5 (10%)	5 (10%)
GANGLIONEUROMA			1 (2%)
#THYROID	(20)	(50)	(50)
FOLLICULAR-CELL ADENOMA	1 (5%)		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FOLLICULAR-CELL CARCINOMA		1 (2%)	
C-CELL ADENOMA		1 (2%)	2 (4%)
C-CELL CARCINOMA		1 (2%)	4 (8%)
#PANCREATIC ISLETS	(19)	(50)	(48)
ISLET-CELL ADENOMA		2 (4%)	1 (2%)
ISLET-CELL CARCINOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
FIBROMA		1 (2%)	1 (2%)
LEIOMYOSARCOMA		1 (2%)	
FIBROADENOMA		1 (2%)	
#TESTIS	(20)	(50)	(49)
INTERSTITIAL-CELL TUMOR	6 (30%)	10 (20%)	13 (27%)
INTERSTITIAL-CELL TUMOR, MALIGNA	8 (40%)	32 (64%)	32 (65%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(50)
ASTROCYTOMA	1 (5%)	1 (2%)	
OLIGODENDROGLIOMA	1 (5%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(50)
MESOTHELIOMA, METASTATIC		1 (2%)	
*TUNICA VAGINALIS	(20)	(50)	(50)
MESOTHELIOMA, NOS	2 (10%)		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MESOTHELIOMA, MALIGNANT		1 (2%)	
ALL OTHER SYSTEMS			
HEAD			
ADENOCARCINOMA, NOS, METASTATIC	1		
ORBITAL REGION			
CARCINOMA,NOS		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	10	3
MORIBUND SACRIFICE	1	3	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	37	43
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	19	48	50
TOTAL PRIMARY TUMORS	39	99	127
TOTAL ANIMALS WITH BENIGN TUMORS	10	25	32
TOTAL BENIGN TUMORS	15	31	46
TOTAL ANIMALS WITH MALIGNANT TUMORS	15	44	45
TOTAL MALIGNANT TUMORS	21	65	70
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	2	
TOTAL SECONDARY TUMORS	1	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	3	3	11
TOTAL UNCERTAIN TUMORS	3	3	11
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, 4, 5-TRIMETHYLANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(49)	(50)
SQUAMOUS CELL CARCINOMA			2 (4%)
*SUBCUT TISSUE	(20)	(49)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(43)	(50)
CARCINOMA,NOS		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	9 (18%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		2 (5%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS		4 (8%)	5 (10%)
LEUKEMIA,NOS	1 (5%)		1 (2%)
#SPLEEN	(20)	(49)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
*INTESTINAL TRACT	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#LIVER	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(49)	(49)
CARCINOSARCOMA		1 (2%)	
#LIVER	(20)	(49)	(50)
BILE DUCT CARCINOMA			1 (2%)
NEOPLASTIC NODULE		12 (24%)	20 (40%)
HEPATOCELLULAR CARCINOMA			9 (18%)
URINARY SYSTEM			
#URINARY BLADDER	(19)	(49)	(49)
LEIOMYOSARCOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(48)	(49)
CARCINOMA,NOS	3 (15%)	12 (25%)	5 (10%)
ADENOMA, NOS	5 (25%)	10 (21%)	17 (35%)
#ADRENAL	(20)	(49)	(49)
CORTICAL ADENOMA	2 (10%)	2 (4%)	1 (2%)
PHEOCHROMOCYTOMA		2 (4%)	
#THYROID	(20)	(46)	(49)
FOLLICULAR-CELL CARCINOMA			1 (2%)
C-CELL ADENOMA		6 (13%)	5 (10%)
C-CELL CARCINOMA	1 (5%)	1 (2%)	2 (4%)
#PANCREATIC ISLETS	(20)	(49)	(49)
ISLET-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(50)
CARCINOMA,NOS		1 (2%)	
SQUAMOUS CELL CARCINOMA			1 (2%)
ADENOCARCINOMA, NOS		6 (12%)	4 (8%)
CYSTADENOMA, NOS			1 (2%)
CYSTADENOCARCINOMA, NOS		2 (4%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FIBROMA	2 (10%)	2 (4%)	1 (2%)
FIBROADENOMA	5 (25%)	8 (16%)	7 (14%)
#UTERUS	(20)	(49)	(49)
ENDOMETRIAL STROMAL POLYP	6 (30%)	1 (2%)	7 (14%)
#OVARY	(20)	(49)	(49)
GRANULOSA-CELL CARCINOMA			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(48)	(48)
ASTROCYTOMA			1 (2%)
SPECIAL SENSE ORGANS			
*MIDDLE EAR	(20)	(49)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	4	3
MORIBUND SACRIFICE	3	3	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	14	42	42
ANIMAL MISSING		1	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	17	39	45
TOTAL PRIMARY TUMORS	25	77	107
TOTAL ANIMALS WITH BENIGN TUMORS	16	25	31
TOTAL BENIGN TUMORS	20	32	48
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	24	29
TOTAL MALIGNANT TUMORS	5	33	39
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		12	20
TOTAL UNCERTAIN TUMORS		12	20
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED 2,4,5-TRIMETHYLANILINE

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED 2,4,5-TRIMETHYLANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
BILE DUCT CARCINOMA, METASTATIC		1 (2%)	1 (2%)
HEPATOCELLULAR CARCINOMA, METAST	1 (5%)	2 (4%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	4 (20%)	7 (14%)	1 (2%)
TUBULAR-CELL ADENOCARCINOMA, MET			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	2 (10%)	4 (8%)	5 (10%)
#SPLEEN	(19)	(50)	(49)
MALIGNANT LYMPHOMA, NOS		2 (4%)	
#LYMPH NODE	(20)	(50)	(50)
TUBULAR-CELL ADENOCARCINOMA, MET			1 (2%)
MALIGNANT LYMPHOMA, NOS	1 (5%)	1 (2%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#MESENTERIC L. NODE	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#PEYERS PATCH	(19)	(45)	(45)
MALIGNANT LYMPHOMA, NOS		2 (4%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
HEMANGIOSARCOMA		2 (4%)	1 (2%)
#BONE MARROW	(20)	(50)	(50)
HEMANGIOSARCOMA			2 (4%)
#SPLEEN	(19)	(50)	(49)
HEMANGIOSARCOMA	2 (11%)		
*ADIPOSE TISSUE	(20)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)
#LIVER	(20)	(50)	(50)
HEMANGIOSARCOMA			2 (4%)
#TESTIS	(20)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)	
DIGESTIVE SYSTEM			
*INTESTINAL TRACT	(20)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
#LIVER	(20)	(50)	(50)
BILE DUCT CARCINOMA		2 (4%)	2 (4%)
HEPATOCELLULAR CARCINOMA	5 (25%)	26 (52%)	27 (54%)
*GALLBLADDER	(20)	(50)	(50)
CARCINOMA,NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
TUBULAR-CELL ADENOCARCINOMA			1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(19)	(50)	(48)
FOLLICULAR-CELL CARCINOMA			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
#PANCREATIC ISLETS	(19)	(49)	(49)
ISLET-CELL ADENOMA	1 (5%)		
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(20)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
PAPILLARY CYSTADENOCARCINOMA,NOS		2 (4%)	
MUSCULOSKELETAL SYSTEM			
*INTERCOSTAL MUSCLE	(20)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
BODY CAVITIES			
*MEDIASTINUM	(20)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALL OTHER SYSTEMS			
DIAPHRAGM			
HEPATOCELLULAR CARCINOMA, METAST		1	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	7	4
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			8
TERMINAL SACRIFICE	16	43	38
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	34	32
TOTAL PRIMARY TUMORS	15	56	45
TOTAL ANIMALS WITH BENIGN TUMORS	1	2	
TOTAL BENIGN TUMORS	1	2	
TOTAL ANIMALS WITH MALIGNANT TUMORS	11	33	32
TOTAL MALIGNANT TUMORS	14	54	45
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	2	2
TOTAL SECONDARY TUMORS	1	6	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED 2,4,5-TRIMETHYLANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(49)	(50)
LIPOMA		1 (2%)	
NEUROFIBROSARCOMA	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		4 (8%)	6 (13%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	3 (15%)	13 (27%)	3 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (10%)		2 (4%)
#SPLEEN	(19)	(49)	(49)
MALIGNANT LYMPHOMA, NOS	1 (5%)	2 (4%)	2 (4%)
#LYMPH NODE	(20)	(49)	(49)
MALIGNANT LYMPHOMA, NOS			3 (6%)
#LIVER	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
#KIDNEY	(20)	(49)	(49)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
#THYMUS	(18)	(46)	(44)
MALIGNANT LYMPHOMA, NOS	1 (6%)	1 (2%)	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			
*MULTIPLE ORGANS	(20)	(49)	(50)
HEMANGIOSARCOMA		1 (2%)	
*SKIN	(20)	(49)	(50)
HEMANGIOSARCOMA			1 (2%)
#BONE MARROW	(20)	(49)	(49)
HEMANGIOSARCOMA		7 (14%)	
#SPLEEN	(19)	(49)	(49)
HEMANGIOSARCOMA		1 (2%)	
#LYMPH NODE	(20)	(49)	(49)
HEMANGIOSARCOMA			1 (2%)
#MESENTERIC L. NODE	(20)	(49)	(49)
HEMANGIOSARCOMA			1 (2%)
*ADIPOSE TISSUE	(20)	(49)	(50)
HEMANGIOSARCOMA		1 (2%)	
*BONE	(20)	(49)	(50)
HEMANGIOSARCOMA	1 (5%)		3 (6%)
#LIVER	(20)	(49)	(50)
HEMANGIOSARCOMA		1 (2%)	1 (2%)
#OVARY	(19)	(47)	(49)
HEMANGIOMA		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
HEPATOCELLULAR CARCINOMA		18 (37%)	40 (80%)
#CECUM	(17)	(48)	(47)
LEIOMYOSARCOMA		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(49)
CARCINOMA,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
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(48)	(50)
CARCINOMA,NOS			4 (8%)
ADENOMA, NOS	1 (5%)	1 (2%)	1 (2%)
#ADRENAL	(20)	(49)	(49)
CORTICAL CARCINOMA		1 (2%)	1 (2%)
#THYROID	(20)	(45)	(49)
FOLLICULAR-CELL ADENOMA	1 (5%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(50)
CARCINOMA,NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(20)	(49)	(50)
ADENOCARCINOMA, NOS			1 (2%)
PAPILLARY ADENOCARCINOMA		2 (4%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(49)	(50)
LEIOMYOSARCOMA	1 (5%)		

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SITE UNKNOWN			
LEIOMYOSARCOMA	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	10	5
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	39	45
ANIMAL MISSING		1	
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	41	45
TOTAL PRIMARY TUMORS	15	59	71
TOTAL ANIMALS WITH BENIGN TUMORS	2	4	1
TOTAL BENIGN TUMORS	2	4	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	12	41	45
TOTAL MALIGNANT TUMORS	13	55	70
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
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 2,4,5-TRIMETHYLANILINE

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED 2,4,5-TRIMETHYLANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
ABSCESS, NOS	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (5%)		
RESPIRATORY SYSTEM			
#TRACHEA	(20)	(50)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		2 (4%)
#LUNG	(20)	(49)	(50)
INFLAMMATION, SUPPURATIVE	1 (5%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM		4 (8%)	11 (22%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
#SPLEEN	(20)	(49)	(49)
CONGESTION, ACUTE	1 (5%)		
HEMOSIDEROSIS			2 (4%)
HYPERPLASIA, NOS		1 (2%)	
HEMATOPOIESIS		1 (2%)	
#SPLENIC SINUSOIDS	(20)	(49)	(49)
HYPERPLASIA, NOS			1 (2%)
#LYMPH NODE	(20)	(50)	(50)
HYPERPLASIA, NOS		14 (28%)	25 (50%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#SPLEEN	(20)	(49)	(49)
THROMBOSIS, NOS	1 (5%)		
#LYMPH NODE	(20)	(50)	(50)
LYMPHANGIECTASIS			1 (2%)
#MANDIBULAR L. NODE	(20)	(50)	(50)
LYMPHANGIECTASIS			1 (2%)
#HEART	(20)	(50)	(48)
FIBROSIS, FOCAL		1 (2%)	
PERIARTERITIS	1 (5%)	2 (4%)	1 (2%)
#HEART/ATRIUM	(20)	(50)	(48)
THROMBOSIS, NOS		1 (2%)	
#HEART/VENTRICLE	(20)	(50)	(48)
THROMBOSIS, NOS		1 (2%)	
#MYOCARDIUM	(20)	(50)	(48)
FIBROSIS	1 (5%)	7 (14%)	9 (19%)
FIBROSIS, FOCAL	13 (65%)	10 (20%)	25 (52%)
#ENDOCARDIUM OF LEFT	(20)	(50)	(48)
HYPERPLASIA, NOS	1 (5%)		
#PANCREAS	(19)	(50)	(48)
PERIARTERITIS	1 (5%)		3 (6%)
*MESENTERY	(20)	(50)	(50)
PERIARTERITIS	1 (5%)	1 (2%)	5 (10%)
DIGESTIVE SYSTEM			
#LIVER	(19)	(50)	(50)
METAMORPHOSIS FATTY		2 (4%)	4 (8%)
HEMOSIDEROSIS			1 (2%)
HYPERPLASIA, NOS		1 (2%)	20 (40%)
HYPERPLASIA, FOCAL	1 (5%)	1 (2%)	5 (10%)
HYPERPLASIA, DIFFUSE		1 (2%)	1 (2%)
#LIVER/CENTRILOBULAR	(19)	(50)	(50)
NECROSIS, NOS		2 (4%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
METAMORPHOSIS FATTY		1 (2%)	
#PANCREAS	(19)	(50)	(48)
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION, CHRONIC FOCAL	1 (5%)	1 (2%)	3 (6%)
#STOMACH	(19)	(50)	(50)
INFLAMMATION, FOCAL			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
INFLAMMATION, INTERSTITIAL			2 (4%)
INFLAMMATION, CHRONIC FOCAL	1 (5%)	15 (30%)	15 (30%)
INFLAMMATION, CHRONIC DIFFUSE			2 (4%)
HYPERPLASIA, TUBULAR CELL		1 (2%)	
HYPERPLASIA, DIFFUSE			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(50)	(50)
CYST, NOS	2 (10%)		
HYPERPLASIA, FOCAL			1 (2%)
#ADRENAL CORTEX	(20)	(50)	(50)
NODULE		1 (2%)	
HYPERPLASIA, NOS			1 (2%)
#ADRENAL MEDULLA	(20)	(50)	(50)
HYPERPLASIA, NOS			5 (10%)
HYPERPLASIA, FOCAL	1 (5%)		1 (2%)
#THYROID	(20)	(50)	(50)
HYPERPLASIA, C-CELL		8 (16%)	18 (36%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
DILATATION/DUCTS	1 (5%)		5 (10%)
ATROPHY, NOS		1 (2%)	
HYPERTROPHY, NOS	1 (5%)	1 (2%)	
HYPERPLASIA, NOS		18 (36%)	2 (4%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, DIFFUSE		1 (2%)	
#PROSTATE	(11)	(49)	(48)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, SUPPURATIVE	1 (9%)	3 (6%)	1 (2%)
INFLAMMATION, ACUTE FOCAL			1 (2%)
INFLAMMATION ACTIVE CHRONIC			1 (2%)
FIBROSIS			1 (2%)
ATROPHY, NOS		18 (37%)	21 (44%)
HYPERPLASIA, NOS		1 (2%)	5 (10%)
HYPERPLASIA, FOCAL		6 (12%)	
HYPERPLASIA, PAPILLARY			1 (2%)
HYPERPLASIA, CYSTIC	1 (9%)		
*SEMINAL VESICLE	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#TESTIS	(20)	(50)	(49)
ATROPHY, NOS		41 (82%)	37 (76%)
HYPERPLASIA, INTERSTITIAL CELL	1 (5%)	9 (18%)	2 (4%)
*EPIDIDYMIS	(20)	(50)	(50)
INFLAMMATION, CHRONIC			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(50)
HYDROCEPHALUS, NOS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
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
ALL OTHER SYSTEMS			
SITE UNKNOWN			
ABSCESS, NOS			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	1	

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED 2,4,5-TRIMETHYLANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(20)	(49)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		
#LUNG/BRONCHUS	(20)	(43)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
#LUNG/BRONCHIOLE	(20)	(43)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
#LUNG	(20)	(43)	(50)
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	7 (14%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(49)	(50)
HYPERPLASIA, NOS	1 (5%)	1 (2%)	2 (4%)
HYPERPLASIA, HEMATOPOIETIC	1 (5%)		
#SPLEEN	(20)	(49)	(49)
HEMOSIDEROSIS	3 (15%)	27 (55%)	12 (24%)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, HEMATOPOIETIC	1 (5%)		
HYPERPLASIA, LYMPHOID	1 (5%)		
HEMATOPOIESIS		2 (4%)	
#LYMPH NODE	(20)	(49)	(50)
HYPERPLASIA, NOS	1 (5%)	29 (59%)	29 (58%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#MANDIBULAR L. NODE	(20)	(49)	(50)
INFLAMMATION, NECROTIZING	1 (5%)		
HYPERPLASIA, LYMPHOID	2 (10%)		
CIRCULATORY SYSTEM			
#MANDIBULAR L. NODE	(20)	(49)	(50)
LYMPHANGIECTASIS	1 (5%)		
#MESENTERIC L. NODE	(20)	(49)	(50)
LYMPHANGIECTASIS	1 (5%)		
#MYOCARDIUM	(20)	(49)	(48)
FIBROSIS	5 (25%)	1 (2%)	
FIBROSIS, FOCAL	4 (20%)		6 (13%)
*PULMONARY ARTERY	(20)	(49)	(50)
CALCIFICATION, FOCAL		1 (2%)	
*MESENTERY	(20)	(49)	(50)
PERIARTERITIS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
METAMORPHOSIS FATTY		2 (4%)	3 (6%)
HEPATOCYTOMEGALY		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	3 (6%)
HYPERPLASIA, FOCAL	14 (70%)	24 (49%)	24 (48%)
HYPERPLASIA, DIFFUSE		1 (2%)	
#BILE DUCT	(20)	(49)	(50)
CYST, NOS			1 (2%)
#PANCREAS	(20)	(49)	(49)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	2 (4%)
#GASTRIC MUCOSA	(20)	(49)	(50)
HYPERPLASIA, NOS		1 (2%)	
#GASTRIC SUBMUCOSA	(20)	(49)	(50)
INFLAMMATION, NOS		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
#COLON	(19)	(48)	(49)
NEMATODIASIS		2 (4%)	
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(49)
HYDRONEPHROSIS	1 (5%)		
INFLAMMATION, INTERSTITIAL	1 (5%)		
PYELONEPHRITIS SUPPURATIVE	1 (5%)		
INFLAMMATION, CHRONIC FOCAL		1 (2%)	6 (12%)
#URINARY BLADDER	(19)	(49)	(49)
ULCER, NOS	1 (5%)		
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(48)	(49)
CYST, NOS	1 (5%)		1 (2%)
HEMORRHAGIC CYST		1 (2%)	
HYPERPLASIA, NOS		3 (6%)	13 (27%)
HYPERPLASIA, FOCAL		1 (2%)	
#ADRENAL CORTEX	(20)	(49)	(49)
NODULE		2 (4%)	
#ADRENAL MEDULLA	(20)	(49)	(49)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
#THYROID	(20)	(46)	(49)
HYPERPLASIA, C-CELL	2 (10%)	9 (20%)	4 (8%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(50)
DILATATION, NOS			1 (2%)
DILATATION/DUCTS	7 (35%)		1 (2%)
CYST, NOS		1 (2%)	
HYPERPLASIA, NOS	1 (5%)	19 (39%)	8 (16%)
HYPERPLASIA, DIFFUSE			1 (2%)
HYPERPLASIA, CYSTIC		1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(49)	(49)
CYST, NOS		1 (2%)	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
INFLAMMATION, SUPPURATIVE		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
#OVARY	(20)	(49)	(49)
FOLLICULAR CYST, NOS	1 (5%)		
HYPERPLASIA, STROMAL		3 (6%)	3 (6%)
NERVOUS SYSTEM			
#BRAIN	(20)	(48)	(48)
HEMORRHAGE			1 (2%)
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, CHRONIC	1 (5%)		
INFLAMMATION, CHRONIC FOCAL	1 (5%)		
#MEDULLA OBLONGATA	(20)	(48)	(48)
ABSCESS, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*MUSCLE HIP/THIGH	(20)	(49)	(50)
INFLAMMATION, NECROTIZING			1 (2%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY		1	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED 2,4,5-TRIMETHYLANILINE

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED 2,4,5-TRIMETHYLANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(50)
ABSCESS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
HEMOSIDEROSIS		1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (5%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
#SPLEEN	(19)	(50)	(49)
HYPERPLASIA, NOS	1 (5%)	3 (6%)	16 (33%)
#LYMPH NODE	(20)	(50)	(50)
HYPERPLASIA, NOS	2 (10%)	7 (14%)	14 (28%)
#THYMUS	(20)	(41)	(43)
HYPERPLASIA, NOS			1 (2%)
CIRCULATORY SYSTEM			
*CARDIOVASCULAR SYSTE	(20)	(50)	(50)
PERIARTERITIS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
NECROSIS, FOCAL			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
INFARCT, NOS			1 (2%)
METAMORPHOSIS FATTY	1 (5%)		
HYPERPLASTIC NODULE	1 (5%)	3 (6%)	7 (14%)
HYPERPLASIA, NOS	1 (5%)		5 (10%)
HYPERPLASIA, FOCAL		1 (2%)	1 (2%)
#LIVER/CENTRILOBULAR	(20)	(50)	(50)
NECROSIS, DIFFUSE			1 (2%)
HYPERPLASIA, NOS		11 (22%)	8 (16%)
#BILE DUCT	(20)	(50)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
#PANCREAS	(19)	(49)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
#GASTRIC MUCOSA	(20)	(50)	(50)
NECROSIS, FOCAL			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFARCT, NOS	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	2	2
AUTO/NECROPSY/HISTO PERF		1	

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED 2,4,5-TRIMETHYLANILINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(49)	(50)
INFLAMMATION VESICULAR GRANULOMA	1 (5%)		
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(49)	(49)
ATROPHY, NOS			1 (2%)
HYPERPLASIA, NOS	2 (11%)	8 (16%)	14 (29%)
HYPERPLASIA, LYMPHOID		2 (4%)	
#LYMPH NODE	(20)	(49)	(49)
HYPERPLASIA, NOS	3 (15%)	4 (8%)	12 (24%)
#THYMUS	(18)	(46)	(44)
HYPERPLASIA, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
INFLAMMATION, NECROTIZING			1 (2%)
NECROSIS, FOCAL	1 (5%)		3 (6%)

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		2 (4%)	
HYPERPLASTIC NODULE		4 (8%)	13 (26%)
#LIVER/CENTRILOBULAR	(20)	(49)	(50)
HYPERPLASIA, NOS		1 (2%)	
#PANCREAS	(20)	(49)	(50)
DILATATION/DUCTS			1 (2%)
HYPERPLASIA, NOS			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(20)	(45)	(49)
HYPERPLASIA, C-CELL			1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS/ENDOMETRIUM	(20)	(48)	(49)
INFLAMMATION, FOCAL			1 (2%)
HYPERPLASIA, NOS	12 (60%)	23 (48%)	36 (73%)
HYPERPLASIA, CYSTIC	2 (10%)		1 (2%)
#OVARY	(19)	(47)	(49)
CYST, NOS	2 (11%)	4 (9%)	3 (6%)
CORPUS LUTEUM CYST			1 (2%)
MULTIPLE CYSTS		1 (2%)	4 (8%)
HEMORRHAGIC CYST		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		5	
ANIMAL MISSING/NO NECROPSY		1	

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED 2,4,5-TRIMETHYLANILINE

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4,5-Trimethylaniline in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	1/20 (5)	0/49 (0)	7/50 (14)
P Values (c,d)	P = 0.009	N.S.	N.S.
Relative Risk (f)		0.000	2.800
Lower Limit		0.000	0.403
Upper Limit		7.624	123.407
Weeks to First Observed Tumor	101	--	98
Hematopoietic System: Lymphoma or Leukemia (b)	4/20 (20)	14/50 (28)	3/50 (6)
P Values (c,d)	P = 0.006 (N)	N.S.	N.S.
Relative Risk (f)		1.400	0.300
Lower Limit		0.520	0.049
Upper Limit		5.303	1.642
Weeks to First Observed Tumor	72	74	95

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4,5-Trimethylaniline in the Diet (a)
(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Bile-Duct Carcinoma (b)	0/19 (0)	0/50 (0)	4/50 (8)
P Values (c,d)	P = 0.015	—	N.S.
Relative Risk (f)		—	Infinite
Lower Limit		—	0.368
Upper Limit		—	Infinite
Weeks to First Observed Tumor	—	—	100
Liver: Hepatocellular Carcinoma (b)	0/19 (0)	3/50 (6)	11/50 (22)
P Values (c,d)	P = 0.002	N.S.	P = 0.020
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.238	1.320
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	—	101	97

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4,5-Trimethylaniline in the Diet (a)
(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Neoplastic Nodule (b)	1/19 (5)	6/50 (12)	20/50 (40)
P Values (c,d)	P less than 0.001	N.S.	P = 0.004
Relative Risk (f)		2.280	7.600
Lower Limit		0.311	1.394
Upper Limit		102.629	304.933
Weeks to First Observed Tumor	101	92	97
Pituitary: Carcinoma, NOS (b)	5/20 (25)	6/50 (12)	10/50 (20)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.480	0.800
Lower Limit		0.143	0.296
Upper Limit		1.807	2.689
Weeks to First Observed Tumor	101	101	98

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4,5-Trimethylaniline in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Carcinoma, NOS, or Adenoma, NOS (b)	8/20 (40)	17/50 (34)	25/50 (50)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.850	1.250
Lower Limit		0.435	0.689
Upper Limit		1.957	2.700
Weeks to First Observed Tumor	101	74	101
Adrenal: Pheochromocytoma (b)	2/20 (10)	5/50 (10)	5/50 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.000	1.000
Lower Limit		0.184	0.184
Upper Limit		10.007	10.007
Weeks to First Observed Tumor	101	92	92

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4,5-Trimethylaniline in the Diet (a)
(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma (b)	0/20 (0)	1/50 (2)	4/50 (8)
P Values (c,d)	P = 0.047	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.022	0.386
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	—	101	101
Thyroid: C-cell Carcinoma or Adenoma (b)	0/20 (0)	2/50 (4)	5/50 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.123	0.525
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	101	101	101

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4,5-Trimethylaniline in the Diet (a)
(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pancreatic Islets: Islet-cell Carcinoma or Adenoma (b)	0/19 (0)	3/50 (6)	1/48 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.238	0.022
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	—	101	101
Testis: Interstitial-cell Tumor (b)	14/20 (70)	41/50 (82)	40/49 (82)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.171	1.166
Lower Limit		0.871	0.865
Upper Limit		1.704	1.699
Weeks to First Observed Tumor	97	86	92

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4,5-Trimethylaniline in the Diet (a)
(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Tunica Vaginalis: Mesothelioma (b)	2/20 (10)	1/50 (2)	0/50 (0)
P Values (c,d)	P = 0.047(N)	N.S.	N.S.
Relative Risk (f)		0.200	0.000
Lower Limit		0.004	0.000
Upper Limit		3.681	1.345
Weeks to First Observed Tumor	101	95	--

(a) Dosed groups received 200 or 800 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in a dosed 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 dosed group than in the control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4,5-Trimethylaniline in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	0/20 (0)	2/43 (5)	2/50 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.143	0.123
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	101	101
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	0/20 (0)	3/43 (7)	11/50 (22)
P Values (c,d)	P = 0.003	N.S.	P = 0.017
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.291	1.384
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	101	93

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4,5-Trimethylaniline in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia (b)	1/20 (5)	5/49 (10)	8/50 (16)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.041	3.200
Lower Limit		0.254	0.482
Upper Limit		94.440	138.771
Weeks to First Observed Tumor	77	79	89
Liver: Hepatocellular Carcinoma (b)	0/20 (0)	0/49 (0)	9/50 (18)
P Values (c,d)	P less than 0.001	--	P = 0.039
Relative Risk (f)		--	Infinite
Lower Limit		--	1.096
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	89

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4,5-Trimethylaniline in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Neoplastic Nodule (b)	0/20 (0)	12/49 (24)	27/50 (54)
P Values (c,d)	P less than 0.001	P =.0.010	P less than 0.001
Relative Risk (f)		Infinite	Infinite
Lower Limit		1.561	3.725
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	101	89
Pituitary: Carcinoma, NOS (b)	3/20 (15)	12/48 (25)	5/49 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.667	0.680
Lower Limit		0.524	0.150
Upper Limit		8.505	4.092
Weeks to First Observed Tumor	81	90	101

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4,5-Trimethylaniline in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Carcinoma, NOS, or Adenoma, NOS (b)	8/20 (40)	22/48 (46)	22/49 (45)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.146	1.122
Lower Limit		0.618	0.605
Upper Limit		2.516	2.471
Weeks to First Observed Tumor	81	90	90
Adrenal: Cortical Adenoma (b)	2/20 (10)	2/49 (4)	1/49 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.408	0.204
Lower Limit		0.032	0.004
Upper Limit		5.381	3.754
Weeks to First Observed Tumor	101	101	101

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4,5-Trimethylaniline in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma or Adenoma (b)	1/20 (5)	7/46 (15)	7/49 (14)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		3.043	2.857
Lower Limit		0.439	0.411
Upper Limit		133.816	125.833
Weeks to First Observed Tumor	101	74	74
Mammary Gland: Fibroma (b)	2/20 (10)	2/49 (4)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.408	0.200
Lower Limit		0.032	0.004
Upper Limit		5.381	3.681
Weeks to First Observed Tumor	92	90	101

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4,5-Trimethylaniline in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Adenocarcinoma, NOS (b)	0/20 (0)	6/49 (12)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.680	0.386
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	93	101
Mammary Gland: Fibroadenoma (b)	5/20 (25)	8/49 (16)	7/50 (14)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.653	0.560
Lower Limit		0.222	0.180
Upper Limit		2.293	2.029
Weeks to First Observed Tumor	96	90	89

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4,5-Trimethylaniline in the Diet (a)
(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp (b)	7/49 (14)	6/20 (30)	1/49 (2)
P Values (c,d)	N.S.	P = 0.002(N)	N.S.
Departure from Linear Trend (e)	P = 0.001		
Relative Risk (f)		0.068	0.476
Lower Limit		0.002	0.163
Upper Limit		0.516	1.537
Weeks to First Observed Tumor	77	101	94

(a) Dosed groups received 200 or 800 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in a dosed 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 dosed group than in the control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED 2,4,5-TRIMETHYLANILINE

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 2,4,5-Trimethylaniline in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	4/20 (20)	7/50 (14)	1/50 (2)
P Values (c,d)	P = 0.010(N)	N.S.	P = 0.021(N)
Relative Risk (f)		0.700	0.100
Lower Limit		0.207	0.002
Upper Limit		2.994	0.944
Weeks to First Observed Tumor	95	79	83
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	4/20 (20)	9/50 (18)	1/50 (2)
P Values (c,d)	P = 0.009(N)	N.S.	P = 0.021(N)
Relative Risk (f)		0.900	0.100
Lower Limit		0.294	0.002
Upper Limit		3.660	0.944
Weeks to First Observed Tumor	95	79	83

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 2,4,5-Trimethylaniline in the Diet (a)
(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma (b)	3/20 (15)	11/50 (22)	7/50 (14)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.467	0.933
Lower Limit		0.450	0.245
Upper Limit		7.594	5.215
Weeks to First Observed Tumor	80	81	101
All Sites: Hemangiosarcoma (b)	2/20 (10)	3/50 (6)	6/50 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.600	1.200
Lower Limit		0.076	0.243
Upper Limit		6.860	11.574
Weeks to First Observed Tumor	88	101	101

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 2,4,5-Trimethylaniline in the Diet (a) (continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	5/20 (25)	26/50 (52)	27/50 (54)
P Values (c,d)	P = 0.039	P = 0.035	P = 0.025
Relative Risk (f)		2.080	2.160
Lower Limit		0.956	0.999
Upper Limit		6.030	6.222
Weeks to First Observed Tumor	101	81	84

(a) Dosed groups received 50 or 100 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in a dosed 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 dosed group than in the control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 2,4,5-Trimethylaniline in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	0/19 (0)	4/49 (8)	6/48 (13)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.375	0.662
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	85	96
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	0/19 (0)	5/49 (10)	6/48 (13)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.511	0.662
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	85	96

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 2,4,5-Trimethylaniline in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma (b)	9/20 (45)	16/49 (33)	11/50 (22)
P Values (c,d)	P = 0.035(N)	N.S.	N.S.
Relative Risk (f)		0.726	0.489
Lower Limit		0.383	0.232
Upper Limit		1.588	1.152
Weeks to First Observed Tumor	91	69	69
All Sites: Hemangiosarcoma or Hemangioma (b)	1/20 (5)	11/49 (22)	7/50 (14)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		4.490	2.800
Lower Limit		0.737	0.403
Upper Limit		188.359	123.407
Weeks to First Observed Tumor	101	70	101

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 2,4,5-Trimethylaniline in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	0/20 (0)	18/49 (37)	40/50 (80)
P Values (c,d)	P less than 0.001	P = 0.001	P less than 0.001
Relative Risk (f)		Infinite	Infinite
Lower Limit		2.451	5.727
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	101	96
Pituitary: Carcinoma, NOS (b)	0/19 (0)	0/48 (0)	4/50 (8)
P Values (c,d)	P = 0.043	--	N.S.
Relative Risk (f)		--	Infinite
Lower Limit		--	0.368
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	101

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 2,4,5-Trimethylaniline in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Carcinoma, NOS, or Adenoma, NOS (b)	1/19(5)	1/48(2)	5/50(10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.396	1.900
Lower Limit		0.005	0.238
Upper Limit		30.454	87.985
Weeks to First Observed Tumor	95	101	101

(a) Dosed groups received 50 or 100 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in a dosed 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 grou 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 dosed group than in the control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

Review of the Bioassay of 2,4,5-Trimethylaniline* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

December 13, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute on the Institute's bioassay program to identify and 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, and State health officials. 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 2,4,5-Trimethylaniline for carcinogenicity.

The primary reviewer for the report on the bioassay of 2,4,5-Trimethylaniline said that the compound was carcinogenic in both sexes of treated rats and in treated female mice. Since the study was well designed and conducted, he concluded that 2,4,5-Trimethylaniline may pose a carcinogenic risk to humans.

The secondary reviewer said that the results were sufficiently significant as to obviate the experimental shortcomings. It was moved that the report on the bioassay of 2,4,5-Trimethylaniline be accepted as written. The motion was seconded and approved without objection.

Clearinghouse Members Present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center
Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Verald K. Rowe, Dow Chemical USA
Michael Shimkin, University of California at San Diego

Louise Strong, University of Texas Health Sciences Center
Kenneth Wilcox, Michigan State Health Department

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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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