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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 o-toluidine hydrochloride 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 found to be carcinogenic in animals requires a wider analysis.

CONTRIBUTORS: This bioassay of o-toluidine hydrochloride 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, and Mr. L. Callahan for management of the facilities. Mr. A. Butler performed the computer services. Histopathologic evaluations for rats were performed by Dr. J. F. Hardisty (3), and the histopathologic evaluations for mice were performed by Dr. C. E. Gilmore (3). The diagnoses included in this report represent the interpretations of Drs. Hardisty and Gilmore.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (4). Statistical analyses were performed by Dr. J. R. Joiner (5) and Ms. P. L. Yong (5), using methods selected for the bioassay program by Dr. J. J. Gart (6).

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.

This report was prepared at Tracor Jitco (5) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. L. A. Campbell, 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. Waitz, Ms. M. S. King, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley and Ms. P. J. Graboske.

The following scientists at NCI were responsible for evaluating the bioassay experiment, 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 o-toluidine hydrochloride for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F1 mice.

Groups of 50 rats of each sex and 50 mice of each sex were administered o-toluidine hydrochloride at one of several doses, either 3,000 or 6,000 ppm for the rats and either 1,000 or 3,000 ppm for the mice, for 101 to 104 weeks. Matched controls consisted of 20 untreated rats of each sex and 20 untreated mice of each sex. All surviving rats and mice were killed at the end of administration of the test chemical.

Mean body weights of dosed male and female rats and mice were lower than those of corresponding matched controls and were dose related. Mortalities of the male and female rats were dose related and were relatively high at the end of the bioassay. Mortalities of the male and female mice were not, however, significantly affected by administration of the test chemical.

In rats, the administration of the test chemical induced several types of sarcomas of the spleen and other organs in both males and females, mesotheliomas of the abdominal cavity or scrotum in males, and transitional-cell carcinomas of the urinary bladder in females. Administration of the o-toluidine hydrochloride also resulted in increased incidences of fibromas of the subcutaneous tissue in the males and fibroadenomas or adenomas of the mammary gland in the females.

In mice, hemangiosarcomas were induced at various sites in males, and hepatocellular carcinomas or adenomas were induced in females.

Under the conditions of this bioassay, o-toluidine hydrochloride was carcinogenic in both male and female F344 rats and B6C3F1 mice, producing a significant increased incidence of one or more types of neoplasms.

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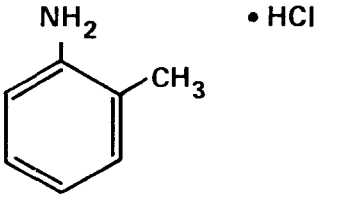
I. INTRODUCTION

o-Toluidine (CAS 636-21-5; NCI C02335) and its hydrochloride salt are dye intermediates used in the manufacture of a large number of textile dyes which include some of the azo, triarylmethane, sulfur, and indigoid compounds (Society of Dyers and Colourists, 1971). In addition, there are numerous substituted o-toluidines that are used as dye intermediates (Society of Dyers and Colourists, 1971). o-Toluidine also functions as a photographic dye (Stanford Research Institute, 1976), as a reagent in a clinical assay for glucose (Indriksons, 1975) and hemoglobin (Ferretti et al., 1971), and as an antioxidant in the manufacture of rubber (Stanford Research Institute, 1976).



o-Toluidine hydrochloride

This chemical has been in commercial use in the United States for over 50 years (International Agency for Research on Cancer, 1974). The annual U. S. production volume is not reported; however, 24,720 pounds were imported to this country in 1976 (United States International Trade Commission, 1977).

The oral LD_{50} of o-toluidine hydrochloride has been reported as 2,951 mg/kg body weight when administered in water by stomach tube to male Osborne-Mendel rats (Lindstrom et al., 1969); that of o-toluidine has been reported as 900 mg/kg body weight when the undiluted chemical was administered by intubation to Sprague-Dawley rats (Jacobson, 1972) and as 515 mg/kg body weight when the chemical was administered in oil to mice of unspecified strain (Lunkin, 1967). Toxic properties of o-toluidine, in common with those of the analog aniline, include the ability to induce the formation of methemoglobin in man (Hamblin, 1963), rats (Lunkin, 1967), and mice (Nomura, 1977) and to cause hematuria in man (Hamblin, 1963).

The primary urinary metabolic products identified when o-toluidine hydrochloride was administered subcutaneously to male CDF rats were the sulfate and glucuronide conjugates of 4-amino-m-cresol and N-acetyl-4-amino-m-cresol (Son et al., 1977).

Preliminary investigations of o-toluidine sponsored by the National Cancer Institute indicated that this chemical was carcinogenic (Homburger et al., 1972; Russfield et al., 1973a). o-Toluidine hydrochloride was selected for testing in the Carcinogenesis Testing Program to confirm these earlier results under the conditions of a standard bioassay.

II. MATERIALS AND METHODS

A. Chemical

o-Toluidine hydrochloride (2-aminotoluene hydrochloride; $C_7H_{10}NCl$) was obtained as technical-grade nonformulated material from American Cyanamid Co. as a fine, grayish powder. Its purity was estimated by reversed-phase high-pressure liquid chromatography as greater than 99%, with the presence of one minor contaminant of less than 1%. Thin layer chromatography showed only one spot, with no impurities. Mass spectral analysis showed a molecular ion for the free amine at m/e 107, and a base peak at m/e 106. The melting point of the test material was 215°C (literature: 215°C). Elemental analysis showed 57.8% carbon, 7.2% hydrogen, and 9.5% nitrogen (theoretical: 58.5% carbon, 7.0% hydrogen, and 9.7% nitrogen).

B. Dietary Preparation

Test diets containing o-toluidine hydrochloride were prepared fresh 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 (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 stored at 5°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 Frederick Cancer Research Center animal farm (Frederick, Md.) monitored by the Division of Cancer Treatment, NCI. The animals were housed within the test facility for 2 weeks and then were assigned four rats to a cage and five mice to a cage on a weight basis for each cage of animals of a given species and sex. For use in the chronic study, male rats were required to weigh 90 to 105 g, averaging at least 100 g; female rats, 80 to 95 g, averaging at least 90 g; male mice, 18 to 22 g, averaging at least 19.5 g; and 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 with 4% fat, provided ad libitum in suspended stainless steel hoppers and replenished at least three times per week. Water, acidified to pH 2.5, was supplied ad libitum from glass bottles. Sipper tubes (Lab Products, Inc.) were 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 Corp., Mataway, N. J.), using the detergents, Clout® (Pharmacal Research Laboratories, Greenwich, Conn.) or Oxford D'Chlor (Oxford Chemicals, Atlanta, Ga.). The glass bottles 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 Supply Co.) once per month, using the Calgen Commercial Division detergent, and the filter paper was changed at the same time.

The air in the animal rooms was maintained at a temperature of 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 o-toluidine hydrochloride and their controls were housed in the same room as rats on feeding studies of the following chemicals:

(CAS 85-44-9) phthalic anhydride
(CAS 95-80-7) 2,4-diaminotoluene

Mice administered o-toluidine hydrochloride and their controls were housed in the same room as mice on feeding studies of the following chemicals:

(CAS 3165-93-3) 4-chloro-o-toluidine hydrochloride
(CAS 97-77-8) ethyl tuads
(CAS 128-04-1) sodium diethyldithiocarbamate
(CAS 128-37-0) butylated hydroxytoluene (BHT)

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses (MTD's) of o-toluidine hydrochloride, 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 one of several doses of o-toluidine hydrochloride for 7 weeks, followed by 1 week on basal diet. Groups of five control animals of each species and sex received basal diet only. Each animal was weighed twice per week. Table 1 shows the doses fed and the mean body weights of dosed animals at week 7, expressed as percentages of mean body weights of the controls.

Table 1. o-Toluidine Hydrochloride Subchronic Feeding Studies in Rats and Mice

Dose (ppm)	Mean Weight at Week 7 as % of Control	
	Male	Female
RATS		
1,000	93	106
2,000	87	104
3,000	88	98
4,000	88	99
6,000	88	92
6,200	91	93
12,500	74	88
25,000	45	60
50,000	51	42
MICE		
3,100	89	88
6,200	86	83
8,000	85	85
10,000	83	81
12,500	79	81
20,000	74	81
25,000	74	77
50,000	63	69

At the end of the subchronic studies, all animals were killed using CO_2 and necropsied. Small amounts of renal and splenic pigmentation were observed in male and female rats receiving 12,500 ppm. The red pulp of the spleens in male and female mice at 50,000 ppm had pigment deposition, apparently lipofuscin. Similar pigmentation was observed in trace to small amounts in the tubular epithelium of the kidneys, and there were trace amounts of pigment seen in Kupffer's cells of the hepatic sinusoids.

All dosed and control animals survived to the end of the subchronic studies except male and female rats administered 50,000 ppm. In these groups four males and three females died by week 3 on study.

Ten percent depression in body weight was taken as the major criterion 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.

Based on the data thus obtained, the low and high doses for rats were set at 3,000 and 6,000 ppm; and for mice at 1,000 and 3,000 ppm.

F. Chronic Studies

The test groups, doses administered, and durations of the chronic feeding studies are shown in tables 2 and 3.

G. Clinical and Pathologic Examinations

All animals were checked twice daily for deaths. 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 animals that survived to the end of the bioassay were killed using CO_2 and necropsied.

The pathologic evaluation consisted of gross and microscopic

Table 2. o-Toluidine Hydrochloride Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals (a)	o-Toluidine Hydrochloride Doses (b) (ppm)	Time on Study
Male			
Matched-Control	20	0	104
Low-Dose	50	3,000	104
High-Dose	50	6,000	101 (c)
Female			
Matched-Control	20	0	104
Low-Dose	50	3,000	104
High-Dose	50	6,000	104

(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.

(c) Administration of the test chemical was terminated at the time indicated due to deaths of all animals.

Table 3. o-Toluidine Hydrochloride Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals (a)	o-Toluidine Hydrochloride Doses (b) (ppm)	Time on Study
Male			
Matched-Control	20	0	103
Low-Dose	50	1,000	103
High-Dose	50	3,000	102
Female			
Matched-Control	20	0	103
Low-Dose	50	1,000	103
High-Dose	50	3,000	103

(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.

examination of major tissues, major organs, and all gross lesions. The tissues were preserved in 10% 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, pancreatic islets, 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 sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox

(1972) for testing two groups for equality and Tarone's (1975) extensions of Cox 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 as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for 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 relationship. This method also provides a two-tailed test of departure from linear trend.

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

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

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which 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 a 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 both dosed male and dosed female rats were lower than those of corresponding matched controls and were dose related throughout the bioassay (figure 1). No other clinical signs were reported which could clearly be related to administration of the test chemical. Some fluctuations in the growth curves may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered o-toluidine hydrochloride in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2. In each sex, the result of the Tarone test for positive dose-related trend in mortality is significant (P less than 0.001). An indicated departure from linear trend is observed in

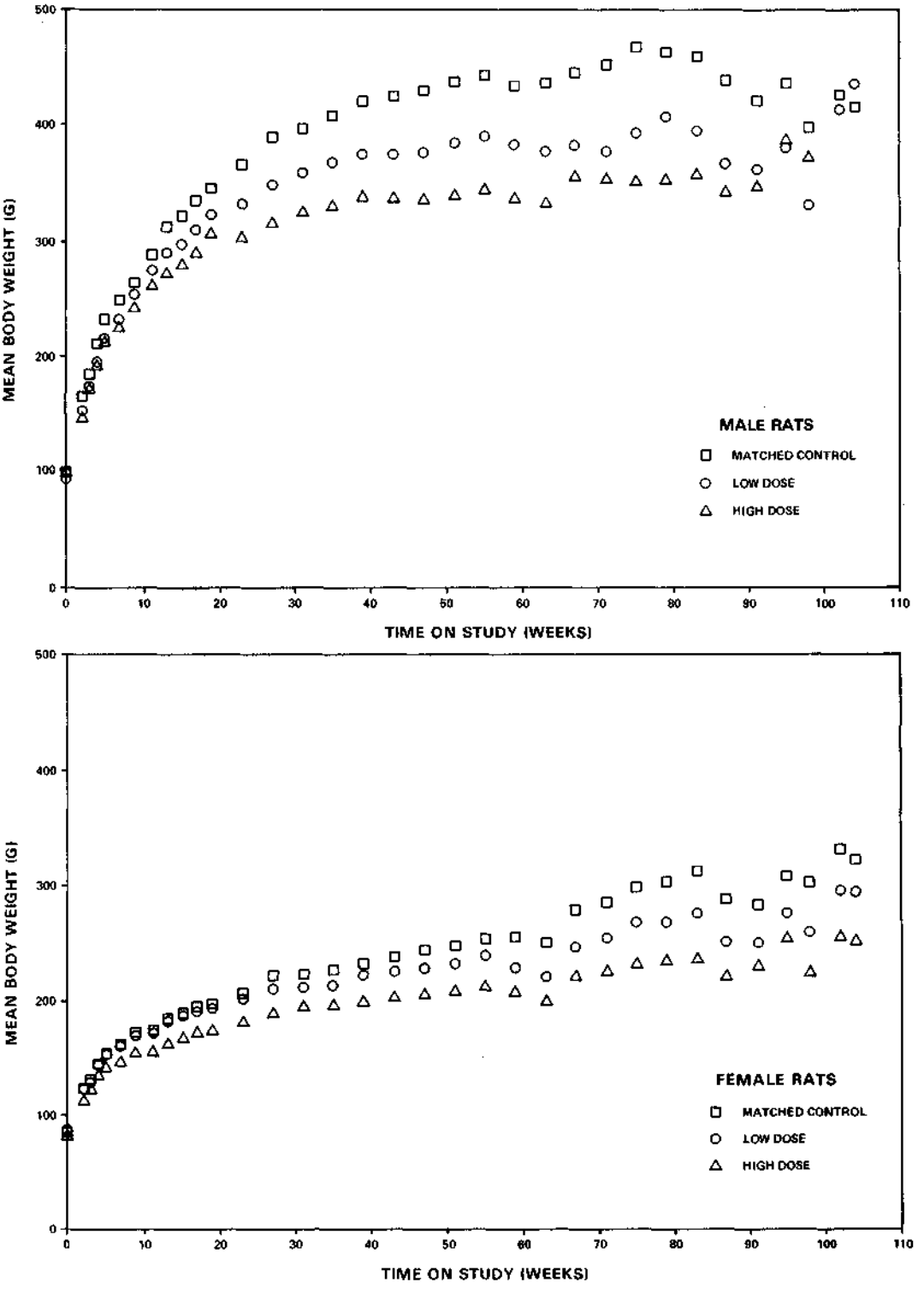


Figure 1. Growth Curves for Rats Administered o-Toluidine Hydrochloride in the Diet

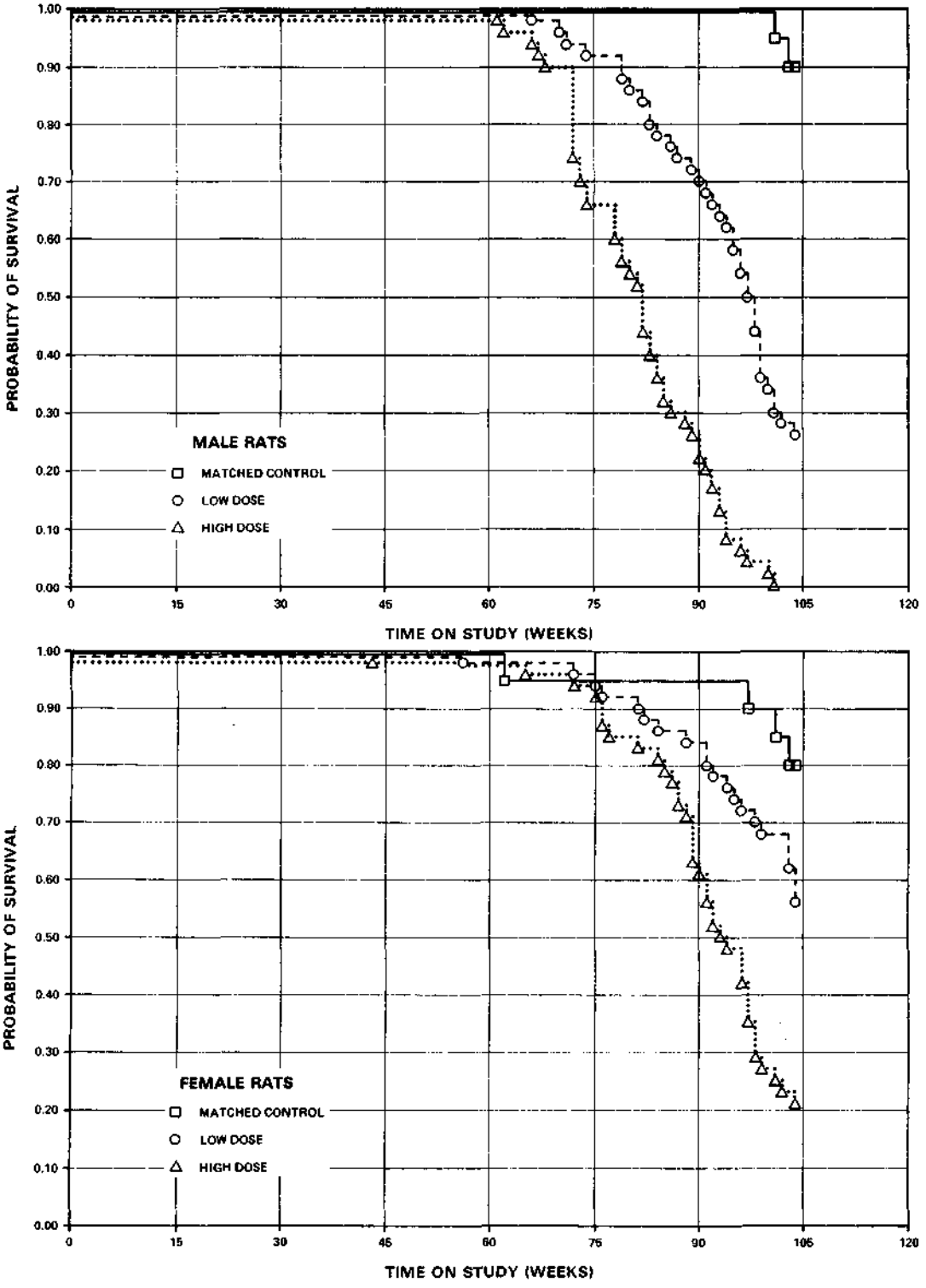


Figure 2. Survival Curves for Rats Administered o-Toluidine Hydrochloride in the Diet

male rats (P = 0.019) due to the steep decrease in survival observed in the dosed groups.

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.

Proliferative lesions and neoplasms involving mesenchymal tissue in the spleen, other visceral organs, and the peritoneal lining of the abdominal cavity and scrotum were observed only in the dosed rats. Proliferative lesions involving the transitional-cell epithelium of the urinary bladder and renal pelvis also were observed only in the dosed rats. Other neoplasms which were observed with increased frequency in the dosed rats when compared with control rats were fibromas of the subcutaneous tissue and fibroadenomas of the mammary gland.

Many of the dosed rats had benign neoplasms involving the fibrous connective tissue of the subcutaneous tissue and mammary gland. In the male rats these neoplasms were predominately fibrous and were classified as fibromas. These neoplasms were characterized

by whorls and interlacing bundles of fibrous connective tissue. Most of the neoplasms were relatively acellular and contained abundant mature collagen. In the female rats, most were classified as fibroadenomas. These neoplasms were characterized by focal proliferations of well-differentiated fibrous connective tissue surrounding proliferating mammary acinar and ductular epithelium. There was much structural variation present in these neoplasms. In some of the neoplasms, the connective tissue stroma was predominant; in others, there was a marked epithelial overgrowth. Several of the fibroadenomas in the female rats and fibromas in the male rats occurred at multiple sites within the subcutaneous tissue, and there were also a variety of malignant mesenchymal neoplasms involving only the subcutaneous tissue of the dosed male rats. The incidence of these neoplasms may be summarized as follows:

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
No. Tissues Examined Histologically	20	50	49	20	50	49
SUBCUTANEOUS TISSUE						
Osteosarcoma	0	0	1	0	0	0
Myxosarcoma	0	2	0	0	0	0
Fibrosarcoma	0	1	2	0	0	0
Sarcoma, NOS	0	1	0	0	0	0
Lipoma	0	4	2	0	0	0
Fibroma	0	28	27	0	4	2
MAMMARY GLAND						
Adenocarcinoma	0	0	0	0	0	1
Adenoma, NOS	0	0	0	1	0	0
Fibroadenoma	0	7	1	6	20	35

Proliferative lesions of the transitional-cell epithelium of the urinary bladder occurred only in dosed rats. These lesions were more frequent in the female rats than in the males. The incidence of these lesions may be summarized as follows:

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
No. Tissues Examined Histologically	20	50	44	20	45	47
URINARY BLADDER						
Squamous-cell Carcinoma	0	1	0	0	0	0
Transitional-cell Carcinoma	0	3	0	0	9	22
Transitional-cell Papilloma	0	0	1	0	1	0
Epithelial Hyperplasia	0	9	7	0	21	13

Hyperplasia of the transitional-cell epithelium of the urinary bladder was characterized by a diffuse thickening of the transitional-cell epithelium of the bladder. In these lesions there was no evidence of invasion into the submucosa or wall of the urinary bladder. There appeared to be a dose-related progression, particularly in the female rats, from transitional-cell epithelial hyperplasia to transitional-cell carcinomas of the urinary bladder. The transitional-cell carcinomas varied considerably in appearance, but they were usually large, papillary neoplasms protruding into the lumen of

the urinary bladder. Invasion of malignant epithelium into the underlying stroma was frequently present. A few of the smaller carcinomas were not papillary and were characterized by a focal mucosal thickening of the atypical epithelial cells with nests of malignant epithelium present in the submucosa. A few of the dosed rats also had hyperplastic lesions involving the transitional epithelium lining the renal pelvis. One low-dose male rat and one high-dose female rat had a transitional-cell carcinoma in the renal pelvis.

Neoplasms involving the spleen, other abdominal viscera, and the peritoneal lining of the abdominal cavity and scrotum occurred only in dosed rats. The spleen was the most frequent organ involved and appears to be a specific target for the carcinogenic activity of o-toluidine hydrochloride. The histologic appearance of each type of neoplasm was similar regardless of the organ involved. All of these neoplasms were of mesenchymal origin and appeared to be closely related. All of the neoplasms were poorly differentiated and difficult to classify. The specific classification of these sarcomas refers to the most differentiated cell type observed histologically. These mesenchymal neoplasms appeared to be highly malignant as evidenced by a high incidence of invasion into adjacent organs and soft tissues and/or metastasis to other sites.

The neoplasms diagnosed as sarcoma, NOS, were too undifferentiated to classify. They were composed of pleomorphic cells with large, vesicular nuclei and abundant eosinophilic cytoplasm. Bizarre nuclear shapes and multinucleated cells were not uncommon. Mitotic figures were scattered throughout these neoplasms. The incidences of these lesions may be summarized as follows:

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
No. Tissues Examined Histologically	20	49	42	20	49	49
SPLEEN						
Sarcoma, NOS	0	1	3	0	1	3
Osteosarcoma	0	0	1	0	1	1
Angiosarcoma	0	7	0	0	7	9
Fibroma	0	10	2	0	4	6
Angioma	0	1	0	0	0	0
Lipoma	0	0	1	0	0	0
No. Animals Necropsied	20	50	49	20	50	49
MULTIPLE ORGANS						
Osteosarcoma	0	3	5	0	0	18
Fibrosarcoma	0	8	20	0	1	0
Angiosarcoma	0	1	1	0	1	1
Mesothelioma, NOS	0	1	0	0	0	0
Mesothelioma, Malignant	0	4	3	0	0	0
Sarcoma, NOS	0	3	11	0	1	2
Fibroma	0	1	0	0	0	0

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
No. Animals Necropsied	20	50	49	20	50	49
BODY CAVITIES						
Tunica Vaginalis						
Mesothelioma, Malignant	0	10	6	0	0	0
Mesentery						
Mesothelioma, NOS	0	2	0	0	0	0
Sarcoma, NOS	0	0	0	0	0	1
Abdominal Cavity						
Sarcoma, NOS	0	0	0	0	0	1

The fibrosarcomas were composed primarily of pleomorphic spindle-shaped cells. The histologic appearance of these neoplasms was quite variable. In some areas, these neoplasms were highly cellular with little evidence of collagen being produced. In these areas, the cells had large nuclei with distinct nuleoli and a high mitotic activity. Occasional multinucleated cells were observed. In other areas, the neoplasm was relatively acellular and contained abundant mature collagen. These neoplasms were highly invasive and completely destroyed the spleen in several animals, with invasion into the liver and other visceral organs. In a few animals, fibrous neoplasms occurred that were acellular and composed of interlacing bundles and whorls of mature collagen. These neoplasms were growing primarily by expansion on the serosal surfaces of the abdominal viscera and were classified as fibromas. Within the neoplasms diagnosed as sarcoma, NOS, fibrosarcoma, and fibroma, areas of osseous metaplasia were

sometimes observed. In these areas, the osseous tissue was present as mature cancellous bone and did not appear to be part of the proliferating tissue.

The osteosarcomas were composed of pleomorphic spindle-shaped cells which were differentiating into osteoblasts that were producing varying amounts of osteoid. In most areas, the individual neoplastic cells were surrounded by acellular eosinophilic material with little progression to bone. In other areas of these neoplasms, the osteoid was differentiating into mature cancellous bone. These neoplasms appeared to be forming only in soft tissues, and no skeletal involvement was observed.

The angiosarcomas of the spleen usually replaced much of the normal splenic architecture. There was great variation in the histologic appearances of these neoplasms. In some areas, the neoplasms were highly vascular and consisted of obvious vascular spaces lined by pleomorphic endothelial cells. In other areas, the stroma of the neoplasm was prominent and vascular spaces were difficult to observe. Many of these vascular neoplasms contained large blood-filled cysts that were associated with areas of hemorrhage and necrosis.

Mesotheliomas were characterized as papillary proliferations of

fibrous connective tissue covered by plump mesothelial cells. Foci of osseous metaplasia were sometimes observed in the fibrous stroma of the malignant mesotheliomas.

A variety of other neoplastic and nonneoplastic lesions were observed with approximately equal frequency in the control and dosed rats. The nature, distribution, and incidence of these neoplasms are similar to those known to occur in aged F344 rats.

Under the conditions of this study, o-toluidine hydrochloride is carcinogenic when administered in the diet to F344 male and female rats, inducing fibromas of the subcutaneous connective tissue, transitional-cell carcinomas of the urinary bladder, and several types of sarcomas involving the spleen and peritoneum. There was also an increased incidence of fibroadenomas of the mammary gland in dosed rats when compared with control rats.

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 positive dose-related trend in the incidence of fibromas of the subcutaneous tissue in the integumentary system is significant (P = 0.001). An indicated departure from linear trend is observed due to the steep increase of incidences of tumors in the dosed groups. The Fisher exact test shows that the incidence in each dosed group is significantly higher than that in the matched controls (P less than 0.001). The statistical conclusion is that the incidence of fibromas of the subcutaneous tissue in the integumentary system of male rats is associated with the administration of o-toluidine hydrochloride. The results of the statistical tests for the incidence of this tumor in female rats are not significant.

In male rats, the results of the Cochran-Armitage test for the incidences of sarcomas of multiple organs and of fibrosarcomas of multiple organs are significant (P = 0.003 and P less than 0.001, respectively). The results of the Fisher exact test show that the incidences in the high-dose group are significantly higher (sarcomas: P = 0.016; fibrosarcomas: P less than 0.001) than those in the controls. When the incidences of male rats with sarcomas, fibrosarcomas, angiosarcomas, or osteosarcomas of multiple organs are combined, the results of the Cochran-Armitage test and those of the Fisher exact test are significant (P less

than or equal to 0.003). The statistical conclusion is that the incidences of these tumors of multiple organs in male rats are associated with the administration of o-toluidine hydrochloride.

In female rats, the results of the Cochran-Armitage test of the incidence of animals with osteosarcomas of multiple organs and also the incidence of animals with sarcomas, fibrosarcomas, angiosarcomas, and osteosarcomas of multiple organs are significant (P less than 0.001). Departures from linear trend are observed (osteosarcoma: P = 0.008; combined incidence: P = 0.042) due to the steep increases in incidences of tumors in the high-dose groups. The results of the Fisher exact test show that the incidences of either of these tumors in the high-dose groups are significantly higher (P less than or equal to 0.001) than those of the controls. The statistical conclusion is that these tumors of multiple organs in female rats are associated with o-toluidine hydrochloride.

In male rats, the incidence of fibromas of the spleen in the low-dose group is significantly higher (P = 0.024) than that in the control group; the incidence in the high-dose group is not significant. The result of the Cochran-Armitage for the incidence of this tumor is not significant.

In females, the result of the Cochran-Armitage test for the incidence of angiosarcomas of the spleen is significant (P = 0.045). The Fisher exact test comparing the incidences of this tumor in the high-dose group with the control group indicates a P value of 0.036, which is above the 0.025 level required for significance when the Bonferroni inequality criterion is used for multiple comparison. However, when incidences of female rats with sarcomas, angiosarcomas, or osteosarcomas in the spleen are combined for analysis, the results of the Cochran-Armitage test are significant (P = 0.018). The results of the Fisher exact test comparing the combined incidence in the low-dose and control groups indicate a P value of 0.036, which is above the 0.025 level required for significance when the Bonferroni inequality criterion is used for multiple comparison, but the Fisher exact test comparing the combined incidence in the high-dose and control groups is significant (P = 0.010). The data in this paragraph indicate that the incidence of those tumors at multiple sites is significant.

In female rats, the results of the Cochran-Armitage test for the incidence of transitional-cell carcinomas of the urinary bladder or the combined incidence of transitional-cell carcinomas and papillomas of the urinary bladder are significant (P less than 0.001). The results of the Fisher exact test comparing the

incidences of the dosed groups with that of the control group are all significant (P less than 0.001 in the high-dose group for both cases, P = 0.028 in the low-dose group for the incidence of carcinomas, and P = 0.018 in the low-dose group for the combined incidence). The statistical conclusion is that the incidence of transitional-cell tumors of the urinary bladder in female rats is dose-related to the administration of o-toluidine hydrochloride. The results of the statistical tests for transitional-cell tumors of the urinary bladder in male rats are not significant.

In female rats, the results of the Cochran-Armitage test for the incidence of fibroadenomas of the mammary gland and the combined incidence of fibroadenomas and adenomas of the mammary gland are significant (P less than 0.001 and P = 0.001, respectively). The Fisher exact test shows that the incidences in the high-dose groups are significantly higher than those in their respective controls (fibroadenomas: P = 0.002; adenomas and fibroadenomas: P = 0.006). The statistical conclusion is that the incidence of these mammary gland tumors in female rats is associated with the administration of o-toluidine hydrochloride.

In male rats, the results of the Fisher exact test on the incidence of mesotheliomas of multiple organs or of the tunica vaginalis show a P value of 0.001 when the incidence in the

low-dose group is compared with that in the control group and a P value of 0.036 when the incidence in the high-dose group is compared with that in the control group. The latter is above the 0.025 level required for significance when the Bonferroni inequality criterion is used for multiple comparison. The historical records of this laboratory show an incidence of mesotheliomas in male F344 rats of only 1/285 (0.4%). Using this rate as a parameter (Fears, 1977) and assuming a binomial distribution, the probability of the chance occurrence of nine or more animals bearing these tumors in the high-dose group of 49 animals is less than 0.001.

Several significant incidences in the negative direction are observed in each sex. This may have been because the dosed animals did not survive as long as the control animals, particulary after week 60 in male rats and after week 75 in female rats.

In summary, the following tumors were related to the administration of o-toluidine hydrochloride:

- Sarcomas of multiple organs in each sex,
- Fibromas of the subcutaneous tissue in male rats,

- Sarcomas of the spleen in female rats,
- Transitional-cell carcinomas of the urinary bladder in female rats,
- Fibroadenomas or adenomas of the mammary gland in female rats, and
- Mesotheliomas in multiple organs or the tunica vaginalis in male rats.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of both dosed male and dosed female mice were lower than those of corresponding controls, particularly after week 18, and were dose related (figure 3). No other clinical signs could clearly be related to administration of the test chemical. Some fluctuations in the growth curves may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation.

B. Survival (Mice)

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

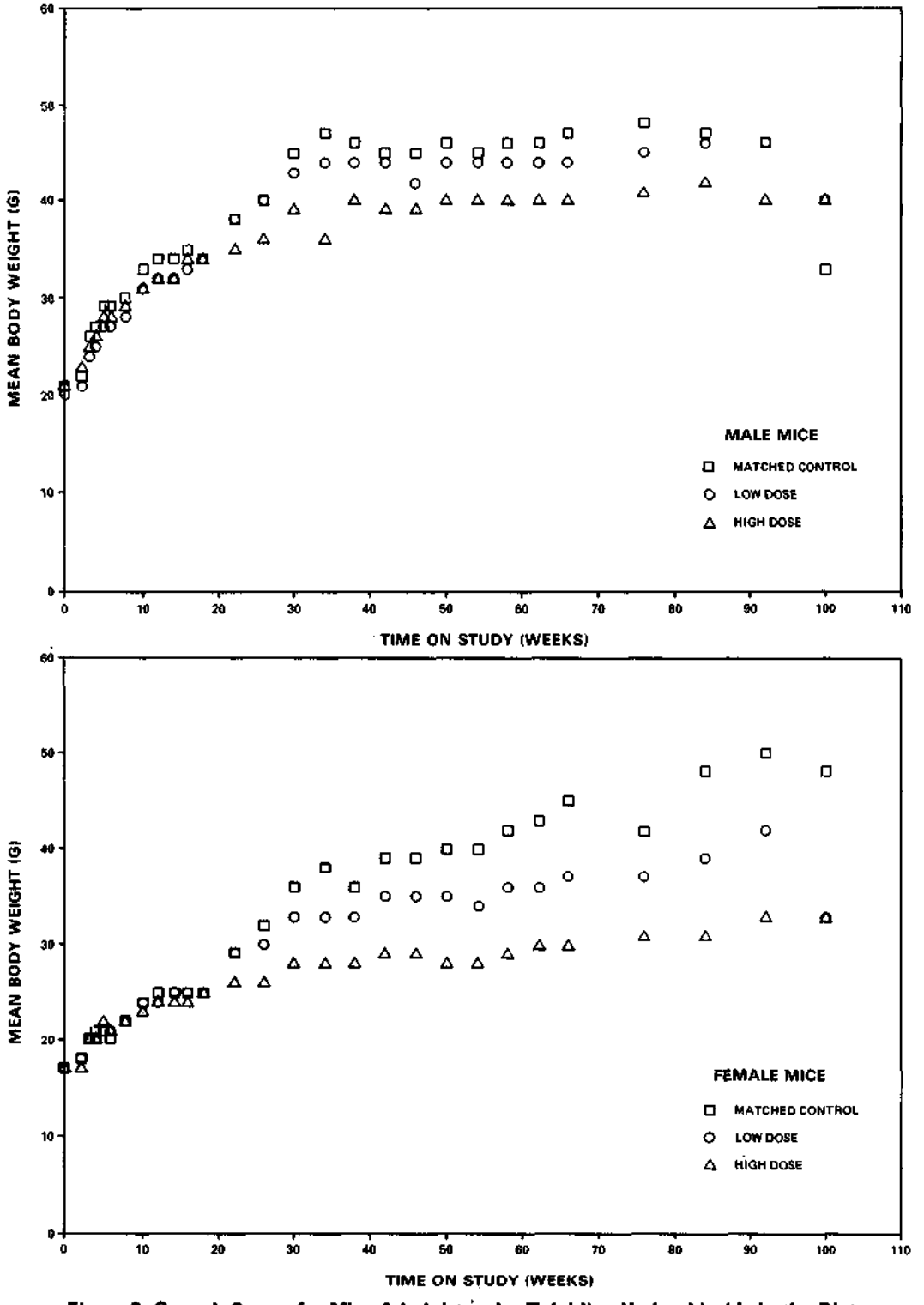


Figure 3. Growth Curves for Mice Administered o-Toluidine Hydrochloride in the Diet

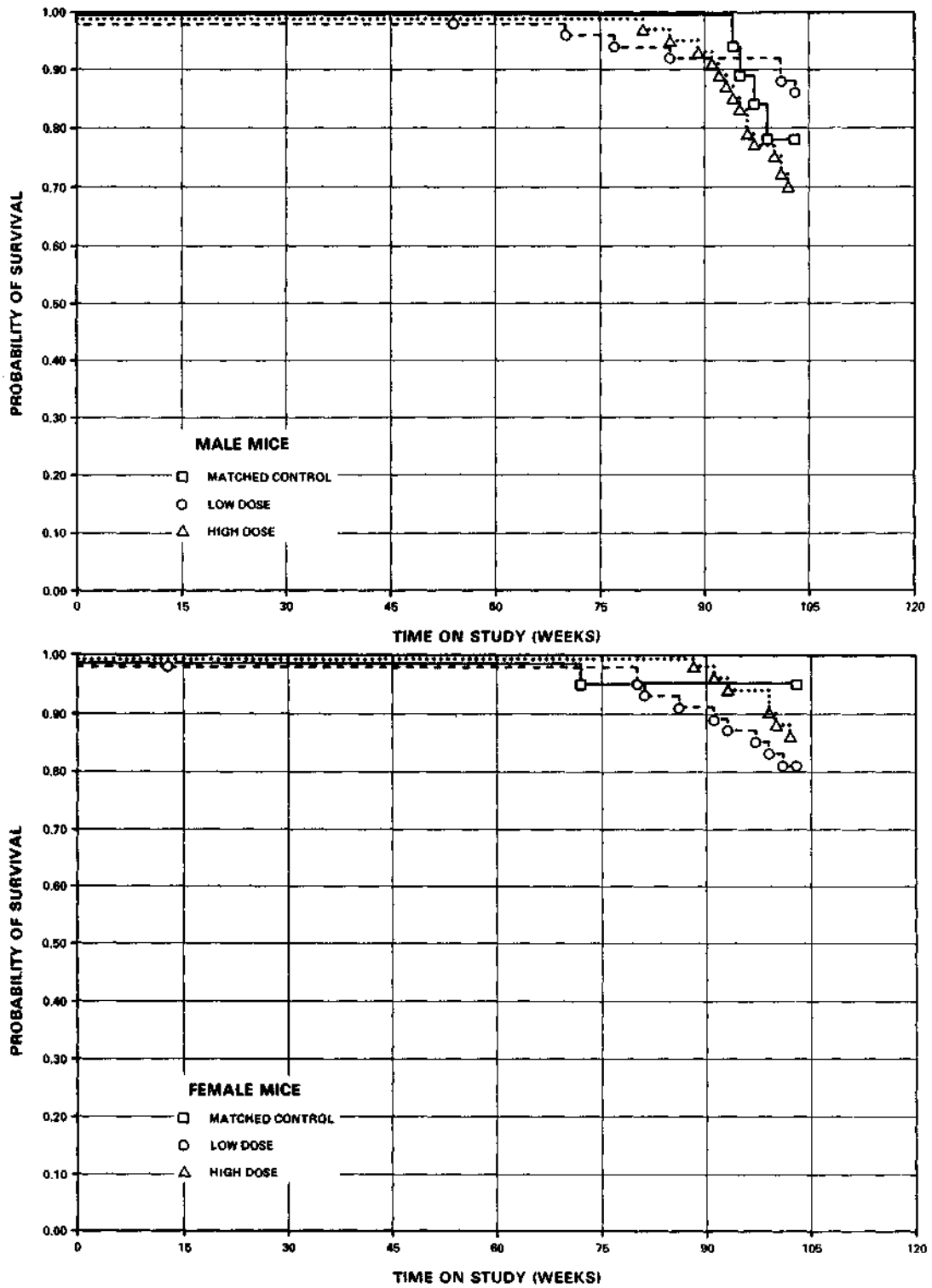


Figure 4. Survival Curves for Mice Administered o-Toluidine Hydrochloride in the Diet

In male mice, 34/50 (68%) of the high-dose group, 43/50 (86%) of the low-dose group, and 15/20 (75%) of the control group lived to the end of the study. In females, 43/50 (86%) of the high-dose group, 39/50 (78%) of the low-dose group, and 19/20 (95%) of the control group lived to the end of the study.

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.

The most common neoplasms encountered were those involving the liver (hepatocellular carcinoma and hepatocellular adenoma). Incidence was slightly higher in the low-dose males than in the control males and considerably higher in the dosed females than in the control females. Distribution was as follows:

	Control		Low-Dose		High-Dose	
	Male	Female	Male	Female	Male	Female
Hepatocellular Carcinoma	4(21%)	0	16(32%)	2(4%)	11(22%)	7(14%)
Hepatocellular Adenoma	1(5%)	0	3(6%)	2(4%)	3(6%)	6(12%)

One hepatocellular carcinoma from a control male mouse and another from a low-dose male mouse metastasized to the lungs.

The hepatocellular carcinomas had a wide morphologic spectrum varying from relatively well to very poorly differentiated. Some were small, unencapsulated, invasive nodules usually with increased basophilic staining; others were large masses involving large portions of the liver. Some appeared as solid masses while others formed cords several cell layers thick and were separated by spaces that, in sections, contained only a small amount of blood. All the tumors had increased mitotic figures.

The tumors classified as hepatocellular adenomas were single nodules of normal to slightly basophilic-staining hepatic cells; there was slight to moderate compression of the surrounding hepatic parenchyma, and the hepatic architecture within the tumors was distorted.

Some mice of each sex in both the dosed and control groups had malignant lymphoma. In some instances, the tumor was confined to one organ or location; in others, the tumor was widespread, involving most organs and tissues in both the thoracic and abdominal cavities. The incidence did not appear unusual for this strain of mouse, and there was no apparent dose relation to the incidence of the tumor.

There was an unusually high incidence of vascular tumors (hemangiosarcoma, hemangioma) in high-dose male mice. Distribution was as follows:

	Control		Low-Dose		High-Dose	
	Male	Female	Male	Female	Male	Female
Hemangiosarcoma	1	1	1	1	10	2
Hemangioma	1	0	1	0	2	1

A single control male had a hemangioma in the spleen and a hemangiosarcoma in the liver. The hemangiosarcoma in the one control female was located in the inguinal subcutis. One of the two tumors in the low-dose males was in a mesenteric lymph node; the other, in the spleen. The single tumor in a low-dose female was in the periuterine fat.

All vascular tumors that occurred in high-dose male mice were in the area of the abdominal genital organs; most were described grossly as involving the periepididymal fat. Two of those in high-dose females were in periuterine fat; the third was in the liver.

Exact location of some of these tumors could not be determined either by gross description or histologic examination. They rarely involved the genital organs directly, but appeared to be localized in the perigenital fat. No evidence of metastases was found.

Hemangiosarcomas were characterized by poorly encapsulated or nonencapsulated masses of basophilic spindle-shaped cells with no particular histologic pattern. There were variably sized vascular spaces separating the neoplastic cells within the tumor mass. Mitotic figures were common in the tumor cells.

Hemangiomas were moderately well encapsulated masses usually composed of a number of nearly equal-sized vascular spaces that were lined with plump endothelial cells. Some of the spaces were separated by sclerotic connective tissue.

Some of the vascular tumors contained large blood-filled cysts. Two high-dose male mice had lesions classified as hematocysts in the periepididymal fat. These were single blood-filled cysts surrounded by connective and granulation tissue with no evidence of neoplasia. The hematocysts may be related to the vascular tumors, but evidence for such a relation is not evident on the tissue sections.

In addition to the proliferative and neoplastic lesions just described, there was a scattering of other tumors that were of single occurrence or very low incidence. All were tumors that may be expected in this strain of mouse and, therefore, are not considered to be related to administration of the test chemical.

In addition to the proliferative lesions, there were a few inflammatory and degenerative changes in some mice in each of the groups. Incidences of these lesions appeared to be approximately equal in control and dosed groups; none were considered to be related to the test chemical.

In conclusion, o-toluidine hydrochloride is considered to be carcinogenic in male and female B6C3F1 mice. This is based on the increased incidences of hepatocellular neoplasms in female mice and hemangiosarcomas in the periepididymal fat in high-dose

males and the unusual, although low, incidence of hemangiosarcomas in the periuterine fat of females exposed to the compound.

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 results of the Cochran-Armitage test for the incidence of hemangiosarcomas of all sites or the combined incidence of hemangiomas and hemangiosarcomas of all sites are significant ($P = 0.004$ and $P = 0.002$, respectively), but the results of the Fisher exact test are not significant. The historical records at this laboratory show an incidence of hemangiosarcomas of 13/323 (4%) in the male control mice. Using this rate as a parameter (Fears, 1977) and assuming a binomial distribution, the probability level of obtaining 10 or more such tumors, given a total of 50 animals, is less than 0.001. The incidence of hemangioma and hemangiosarcoma combined is 17/323 (5%). Using this rate as a parameter and assuming a binomial

distribution, the probability level of obtaining 12 or more such tumors, given a total of 50 animals, is less than 0.001

In female mice, when the animals with either hepatocellular carcinomas or adenomas are combined for analysis, the result of the Cochran-Armitage test for positive dose-related trend is significant (P = 0.001). The Fisher exact test shows that the incidence in the high-dose group is significantly higher (P = 0.007) than that in the control group. The statistical conclusion is that the incidence of tumors of the liver in female mice is associated with the administration of o-tuluidine hydrochloride. The current historical-control groups of female mice at this laboratory have incidences of hepatocellular tumors ranging from 0/20 to 2/19 (11%) with an overall incidence of 14/324 (4.3%). The results of the statistical tests on the incidences of hepatocellular tumors in male mice are not significant.

Several significant results in the negative direction are observed in each sex in which the incidence in the control group exceeds those in the dosed groups. They are: alveolar/bronchiolar tumors in male mice, lipoma of the mesentery in male mice, and follicular-cell adenoma in female mice.

V. DISCUSSION

For rats and mice at the doses used in this bioassay there were dose-related depressions in the amount of body weight gained in both species and a dose-related increase in mortality in the rats. Mortality in the male and female rats was dose related and was relatively high at the end of the bioassay. Mortality in the mice was unaffected by the administration of the test chemical. Other clinical signs occurred only at low frequencies and were common to dosed and control groups of animals.

In preliminary investigations on the carcinogenicity of o-toluidine hydrochloride administered in the diet in 2-year studies, urinary bladder cancers and subcutaneous fibromas or fibrosarcomas were induced at significant incidences in male Sprague-Dawley rats, and vascular tumors were induced at significant incidences in male and female Ha/ICR mice (Homburger et al., 1972; Russfield et al., 1973a; Russfield et al., 1973b). A report in press specifies the doses used in the preceding studies as 8,000 and 16,000 ppm for 3 months and 4,000 and 8,000 ppm for the subsequent 15 months for the rats, 16,000 and 32,000 ppm for 3 months and 8,000 and 16,000 ppm for the subsequent 15 months for the mice (Weisburger et al., in press). In concurrent

studies, the m-toluidine and p-toluidine isomers only induced liver tumors in the mice. The induction of bladder tumors in rats administered aromatic azo compounds in the diet has been attributed to toluidines generated as primary decompositon products of the test compounds (Ekman and Strombeck, 1947, 1949). o-Toluidine has been reported to be nonmutagenic in the Salmonella/microsome test (McCann et al., 1975; Ferretti et al., 1977).

In the present study, the administration of the test chemical to rats induced several types of sarcomas of the spleen and other organs in both males and females, mesotheliomas of the abdominal cavity or scrotum in males, and transitional-cell carcinomas of the urinary bladder in females. Administration of the o-toluidine hydrochloride also resulted in increased incidences of fibromas of the subcutaneous tissue in the males and fibroadenomas or adenomas of the mammary gland in the females.

In mice, hemangiosarcomas were induced at various sites in males, and hepatocellular carcinomas or adenomas were induced in females.

The findings are consistent with those previously published but showed, in addition, the induction of mesotheliomas in male rats, fibroadenomas and adenomas of the mammary gland in female rats,

several types of sarcomas in both male and female rats, and hepatocellular carcinomas or adenomas in female mice.

Under the conditions of this bioassay, o-toluidine hydrochloride was carcinogenic in both male and female F344 rats and B6C3F1 mice, producing a significant increased incidence of one or more types of neoplasms.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED o-TOLUIDINE HYDROCHLORIDE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED o-TOLUIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(49)
SQUAMOUS CELL CARCINOMA		2 (4%)	
*SUBCUT TISSUE	(20)	(50)	(49)
SARCOMA, NOS		1 (2%)	
FIBROMA		28 (56%)	27 (55%)
FIBROSARCOMA		1 (2%)	2 (4%)
MYXOSARCOMA		2 (4%)	
LIPOMA		4 (8%)	2 (4%)
OSTEOSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(48)
ALVEOLAR/BRONCHIOLAR CARCINOMA		2 (4%)	2 (4%)
SARCOMA, NOS, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
MALIG.LYMPHOMA, UNDIFFER-TYPE	3 (15%)	2 (4%)	
#SPLEEN	(20)	(49)	(42)
SARCOMA, NOS		1 (2%)	3 (7%)
FIBROMA		10 (20%)	2 (5%)
LIPOMA			1 (2%)
ANGIOMA		1 (2%)	
ANGIOSARCOMA		7 (14%)	
OSTEOSARCOMA			1 (2%)
#RENAL LYMPH NODE	(20)	(50)	(47)
INTERSTITIAL-CELL TUMOR, METASTA	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
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(49)
NEOPLASTIC NODULE	1 (5%)		2 (4%)
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)
FIBROSARCOMA		1 (2%)	
#PANCREAS	(20)	(47)	(41)
ACINAR-CELL CARCINOMA			1 (2%)
#LARGE INTESTINE	(20)	(47)	(46)
ADENOMATOUS POLYP, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(49)
TUBULAR-CELL ADENOCARCINOMA		1 (2%)	
#KIDNEY/PELVIS	(20)	(50)	(49)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	
#URINARY BLADDER	(20)	(50)	(44)
SQUAMOUS CELL CARCINOMA		1 (2%)	
TRANSITIONAL-CELL PAPILLOMA			1 (2%)
TRANSITIONAL-CELL CARCINOMA		3 (6%)	
ANGIOMA			1 (2%)
ANGIOSARCOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(50)	(44)
ADENOMA, NOS	4 (20%)	7 (14%)	3 (7%)
#ADRENAL	(20)	(50)	(49)
PHEOCHROMOCYTOMA		3 (6%)	2 (4%)
#THYROID	(20)	(50)	(45)
FOLLICULAR-CELL ADENOMA		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
C-CELL ADENOMA	1 (5%)	2 (4%)	2 (4%)
C-CELL CARCINOMA	2 (10%)		
#PANCREATIC ISLETS	(20)	(47)	(41)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
FIBROADENOMA		7 (14%)	1 (2%)
*PREPUTIAL GLAND	(20)	(50)	(49)
ADENOMA, NOS		1 (2%)	
#TESTIS	(20)	(50)	(49)
INTERSTITIAL-CELL TUMOR	16 (80%)	41 (82%)	33 (67%)
INTERSTITIAL-CELL TUMOR, MALIGNA	1 (5%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*TUNICA VAGINALIS	(20)	(50)	(49)
MESOTHELIOMA, NOS		2 (4%)	
MESOTHELIOMA, MALIGNANT		10 (20%)	6 (12%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(49)
SARCOMA, NOS		3 (6%)	11 (22%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FIBROMA		1 (2%)	
FIBROSARCOMA		8 (16%)	20 (41%)
MESOTHELIOMA, NOS		1 (2%)	
MESOTHELIOMA, MALIGNANT		4 (8%)	3 (6%)
ANGIOSARCOMA		1 (2%)	1 (2%)
OSTEOSARCOMA		3 (6%)	5 (10%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	32	45
MORIBUND SACRIFICE		5	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	13	
ANIMAL MISSING			1
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	50	48
TOTAL PRIMARY TUMORS	29	167	134
TOTAL ANIMALS WITH BENIGN TUMORS	18	43	42
TOTAL BENIGN TUMORS	21	108	75
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	42	47
TOTAL MALIGNANT TUMORS	7	56	57
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	
TOTAL SECONDARY TUMORS	1	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	1	3	2
TOTAL UNCERTAIN TUMORS	1	3	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED o-TOLUIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(49)
FIBROMA		4 (8%)	2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(49)
CARCINOMA, NOS, METASTATIC		1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)		
C-CELL CARCINOMA, METASTATIC	1 (5%)		
PHEOCHROMOCYTOMA, METASTATIC			1 (2%)
ANGIOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
MALIG.LYMPHOMA, UNDIFFER-TYPE		3 (6%)	
#SPLEEN	(20)	(49)	(49)
SARCOMA, NOS		1 (2%)	3 (6%)
FIBROMA		4 (8%) 13/49	6 (12%)
ANGIOSARCOMA		7 (14%)	9 (18%)
OSTEOSARCOMA		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(49)
NEOPLASTIC NODULE		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
#STOMACH	(20)	(48)	(49)
SQUAMOUS CELL PAPILLOMA		1 (2%)	1 (2%)
#LARGE INTESTINE	(19)	(44)	(42)
ANGIOSARCOMA		1 (2%)	
URINARY SYSTEM			
#KIDNEY/PELVIS	(20)	(49)	(49)
TRANSITIONAL-CELL CARCINOMA			1 (2%)
#URINARY BLADDER	(20)	(45)	(47)
TRANSITIONAL-CELL PAPILLOMA		1 (2%)	
TRANSITIONAL-CELL CARCINOMA		9 (20%)	22 (47%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(47)	(46)
CARCINOMA,NOS		1 (2%)	
ADENOMA, NOS	5 (25%)	12 (26%)	2 (4%)
#ADRENAL	(20)	(49)	(49)
PHEOCHROMOCYTOMA		4 (8%)	1 (2%)
PHEOCHROMOCYTOMA, MALIGNANT			1 (2%)
#THYROID	(20)	(47)	(46)
FOLLICULAR-CELL ADENOMA		1 (2%)	
C-CELL ADENOMA		3 (6%)	1 (2%)
C-CELL CARCINOMA	1 (5%)	3 (6%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
ADENOMA, NOS	1 (5%)		
ADENOCARCINOMA, NOS			1 (2%)
FIBROADENOMA	6 (30%)	20 (40%)	35 (71%)
*CLITORAL GLAND	(20)	(50)	(49)
ADENOMA, NOS		1 (2%)	1 (2%)
#UTERUS	(20)	(49)	(48)
ENDOMETRIAL STROMAL POLYP	5 (25%)	4 (8%)	1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANGIOSARCOMA		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(20)	(48)	(49)
CARCINOMA, NOS, INVASIVE		1 (2%)	
GLIOMA, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(20)	(50)	(49)
SQUAMOUS CELL CARCINOMA		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(49)
SARCOMA, NOS			1 (2%)
*MESENTERY	(20)	(50)	(49)
SARCOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(49)
SARCOMA, NOS		1 (2%)	2 (4%)
FIBROSARCOMA		1 (2%)	
ANGIOSARCOMA		1 (2%)	1 (2%)
OSTEOSARCOMA			18 (37%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATHə	2	18	35
MORIBUND SACRIFICE	2	4	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	16	28	10
ANIMAL MISSING			1
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	43	48
TOTAL PRIMARY TUMORS	20	89	111
TOTAL ANIMALS WITH BENIGN TUMORS	13	35	38
TOTAL BENIGN TUMORS	17	55	50
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	26	45
TOTAL MALIGNANT TUMORS	3	32	61
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	3	1
TOTAL SECONDARY TUMORS	1	3	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		2	
TOTAL UNCERTAIN TUMORS		2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

ə INCLUDES AUTOLYZED ANIMALS

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADIMINISTERED o-TOLUIDINE HYDROCHLORIDE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED o-TOLUIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	19	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	19	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(19)	(50)	(50)
SARCOMA, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(19)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (5%)	1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (11%)	1 (2%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (16%)	4 (8%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(19)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)	6 (12%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#SPLEEN	(19)	(49)	(50)
HEMANGIOMA	1 (5%)		
HEMANGIOSARCOMA		1 (2%)	
MALIGNANT LYMPHOMA, NOS			1 (2%)
#ABDOMINAL LYMPH NODE	(18)	(48)	(48)
HEMANGIOSARCOMA			1 (2%)
#MESENTERIC L. NODE	(18)	(48)	(48)
HEMANGIOMA		1 (2%)	
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#SMALL INTESTINE	(17)	(47)	(48)
MALIGNANT LYMPHOMA, NOS		1 (2%)	2 (4%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(19)	(50)	(50)
ADENOMA, NOS	1 (5%)		
HEPATOCELLULAR ADENOMA	1 (5%)	3 (6%)	3 (6%)
HEPATOCELLULAR CARCINOMA	4 (21%)	16 (32%)	11 (22%)
HEMANGIOSARCOMA	1 (5%)		
#ESOPHAGUS	(17)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
#SMALL INTESTINE	(17)	(47)	(48)
ADENOCARCINOMA, NOS	1 (6%)		
#CECUM	(15)	(49)	(45)
SARCOMA, NOS			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(46)	(46)
ADENOCARCINOMA, NOS			1 (2%)
#ADRENAL	(19)	(50)	(49)
CORTICAL ADENOMA			2 (4%)
#THYROID	(19)	(49)	(48)
FOLLICULAR-CELL ADENOMA	1 (5%)	1 (2%)	1 (2%)
FOLLICULAR-CELL CARCINOMA	1 (5%)	1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(19)	(50)	(50)
ADENOMA, NOS	1 (5%)		2 (4%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(19)	(50)	(50)
SARCOMA, NOS	1 (5%)		
LIPOMA			2 (4%)
HEMANGIOMA			2 (4%)
HEMANGIOSARCOMA			9 (18%)
*MESENTERY	(19)	(50)	(50)
LIPOMA	2 (11%)		
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	7	13
MORIBUND SACRIFICE			1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			2
TERMINAL SACRIFICE	15	43	34
ANIMAL MISSING	1		

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	17	28	30
TOTAL PRIMARY TUMORS	21	38	44
TOTAL ANIMALS WITH BENIGN TUMORS	8	6	13
TOTAL BENIGN TUMORS	9	6	14
TOTAL ANIMALS WITH MALIGNANT TUMORS	11	24	24
TOTAL MALIGNANT TUMORS	12	32	30
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	
TOTAL SECONDARY TUMORS	1	1	
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 o-TOLUIDINE HYDROCHLORIDE 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)
SARCOMA, NOS			1 (2%)
*SUBCUT TISSUE	(20)	(49)	(50)
LIPOMA	1 (5%)		
HEMANGIOSARCOMA	1 (5%)		
NEUROFIBROSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)	1 (2%)	2 (4%)
NEUROFIBROSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)	7 (14%)	4 (8%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#SPLEEN	(20)	(49)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#LIVER	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#SMALL INTESTINE	(20)	(47)	(50)
MALIGNANT LYMPHOMA, NOS	2 (10%)	2 (4%)	3 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		
CIRCULATORY SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
HEPATOCELLULAR ADENOMA		2 (4%)	6 (12%)
HEPATOCELLULAR CARCINOMA		2 (4%)	7 (14%)
HEMANGIOSARCOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(20)	(44)	(47)
FOLLICULAR-CELL ADENOMA	3 (15%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(50)
ADENOCARCINOMA, NOS		1 (2%)	1 (2%)
#UTERUS	(20)	(46)	(48)
LEIOMYOSARCOMA		2 (4%)	
NERVOUS SYSTEM			
#BRAIN	(20)	(47)	(48)
GLIOMA, NOS	1 (5%)		
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(49)	(50)
ADENOMA, NOS		1 (2%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(49)	(50)
NEOPLASM, NOS			1 (2%)
HEMANGIOMA			1 (2%)
HEMANGIOSARCOMA		1 (2%)	1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(49)	(50)
NEUROFIBROSARCOMA		1 (2%)	
ADIPOSE TISSUE			
LIPOMA			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	8	7
MORIBUND SACRIFICE		1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	19	39	43
ANIMAL MISSING		1	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	19	26
TOTAL PRIMARY TUMORS	11	23	32
TOTAL ANIMALS WITH BENIGN TUMORS	3	4	9
TOTAL BENIGN TUMORS	4	4	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	17	17
TOTAL MALIGNANT TUMORS	7	19	22
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS ADMINISTERED o-TOLUIDINE HYDROCHLORIDE IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED o-TOLUIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(49)
EPIDERMAL INCLUSION CYST		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#TRACHEA	(20)	(50)	(48)
INFLAMMATION, ACUTE		1 (2%)	
#LUNG	(20)	(50)	(48)
HEMORRHAGE			1 (2%)
INFLAMMATION, INTERSTITIAL	1 (5%)		
BRONCHOPNEUMONIA, ACUTE			1 (2%)
INFLAMMATION, CHRONIC	4 (20%)	1 (2%)	
GRANULOMA, NOS		2 (4%)	
PERIVASCULITIS		4 (8%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM	3 (15%)	1 (2%)	2 (4%)
#LUNG/ALVEOLI	(20)	(50)	(48)
HISTIOCYTOSIS	1 (5%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(49)	(42)
FIBROSIS		13 (27%)	5 (12%)
METAMORPHOSIS FATTY		6 (12%)	2 (5%)
LYMPHOID DEPLETION		2 (4%)	7 (17%)
HYPERPLASIA, MESOTHELIAL		1 (2%)	
METAPLASIA, OSSEOUS		2 (4%)	
HEMATOPOIESIS	1 (5%)	3 (6%)	
#SPLENIC CAPSULE	(20)	(49)	(42)
HEMORRHAGIC CYST			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, MESOTHELIAL		18 (37%)	5 (12%)
METAPLASIA, OSSEOUS		1 (2%)	
#MANDIBULAR L. NODE	(20)	(50)	(47)
LYMPHANGIECTASIS		2 (4%)	
#MESENTERIC L. NODE	(20)	(50)	(47)
LYMPHANGIECTASIS		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(48)
PERIARTERITIS		1 (2%)	
#MYOCARDIUM	(20)	(50)	(48)
INFLAMMATION, CHRONIC	3 (15%)		
FIBROSIS		17 (34%)	8 (17%)
*CORONARY ARTERY	(20)	(50)	(49)
MEDIAL CALCIFICATION	1 (5%)	1 (2%)	
*PULMONARY ARTERY	(20)	(50)	(49)
MEDIAL CALCIFICATION	6 (30%)	5 (10%)	1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(49)
FIBROSIS		1 (2%)	
CHOLANGIOFIBROSIS		9 (18%)	
NECROSIS, NOS	2 (10%)	12 (24%)	17 (35%)
METAMORPHOSIS FATTY	1 (5%)	2 (4%)	2 (4%)
CYTOPLASMIC VACUOLIZATION	7 (35%)		
FOCAL CELLULAR CHANGE		2 (4%)	3 (6%)
HEPATOCYTOMEGALY	1 (5%)		1 (2%)
ANGIECTASIS		1 (2%)	
HEMATOPOIESIS			2 (4%)
#BILE DUCT	(20)	(50)	(49)
HYPERPLASIA, NOS	19 (95%)	11 (22%)	6 (12%)
#PANCREAS	(20)	(47)	(41)
STEATITIS		1 (2%)	
PERIARTERITIS	1 (5%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC ACINUS	(20)	(47)	(41)
ATROPHY, NOS	7 (35%)	2 (4%)	1 (2%)
#STOMACH	(20)	(50)	(47)
ULCER, NOS		1 (2%)	1 (2%)
INFLAMMATION, ACUTE			2 (4%)
INFLAMMATION, CHRONIC			1 (2%)
#SMALL INTESTINE	(20)	(50)	(45)
HYPERPLASIA, LYMPHOID	1 (5%)		
#LARGE INTESTINE	(20)	(47)	(46)
NEMATODIASIS	1 (5%)	2 (4%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(49)
HYDRONEPHROSIS		1 (2%)	
PYELONEPHRITIS, NOS		1 (2%)	
INFLAMMATION, CHRONIC	18 (90%)	35 (70%)	34 (69%)
NEPHROSIS, NOS			1 (2%)
HEMOSIDEROSIS			1 (2%)
#KIDNEY/MEDULLA	(20)	(50)	(49)
HYPERPLASIA, NOS		1 (2%)	
#KIDNEY/TUBULE	(20)	(50)	(49)
HEMOSIDEROSIS			3 (6%)
#KIDNEY/PELVIS	(20)	(50)	(49)
HYPERPLASIA, EPITHELIAL		1 (2%)	1 (2%)
#URINARY BLADDER	(20)	(50)	(44)
HYPERPLASIA, EPITHELIAL		9 (18%)	7 (16%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(50)	(44)
CYST, NOS	1 (5%)	1 (2%)	1 (2%)
ANGIECTASIS		2 (4%)	1 (2%)
#ADRENAL	(20)	(50)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#ADRENAL CORTEX	(20)	(50)	(49)
NECROSIS, NOS		1 (2%)	
LIPOIDOSIS		1 (2%)	
HYPERPLASIA, NODULAR		1 (2%)	1 (2%)
#ADRENAL MEDULLA	(20)	(50)	(49)
HYPERPLASIA, NOS		2 (4%)	1 (2%)
#THYROID	(20)	(50)	(45)
CYSTIC FOLLICLES			2 (4%)
HYPERPLASIA, C-CELL	5 (25%)		
HYPERPLASIA, FOLLICULAR-CELL	1 (5%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
DILATATION/DUCTS		1 (2%)	
#PROSTATE	(19)	(49)	(45)
INFLAMMATION, ACUTE	1 (5%)	2 (4%)	1 (2%)
#TESTIS	(20)	(50)	(49)
ATROPHY, NOS	1 (5%)		
HYPERPLASIA, INTERSTITIAL CELL			3 (6%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(48)
HEMORRHAGE			1 (2%)
INFLAMMATION, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(49)
ABSCESS, NOS		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
PERIARTERITIS		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(49)
HEMOSIDEROSIS		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY			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 o-TOLUIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(49)
PNEUMONIA, ASPIRATION			1 (2%)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, CHRONIC		2 (4%)	2 (4%)
BRONCHOPNEUMONIA, CHRONIC	2 (10%)		
PERIVASCULITIS	3 (15%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM		4 (8%)	
#LUNG/ALVEOLI	(20)	(48)	(49)
HISTIOCYTOSIS	1 (5%)	1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(49)	(49)
FIBROSIS		6 (12%)	3 (6%)
METAMORPHOSIS FATTY		1 (2%)	3 (6%)
HEMOSIDEROSIS	4 (20%)	7 (14%)	
HEMATOPOIESIS	1 (5%)	2 (4%)	1 (2%)
#SPLENIC CAPSULE	(20)	(49)	(49)
FIBROSIS		5 (10%)	
HYPERPLASIA, MESOTHELIAL		32 (65%)	12 (24%)
#LYMPH NODE	(20)	(49)	(48)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#MANDIBULAR L. NODE	(20)	(49)	(48)
LYMPHANGIECTASIS		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
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
#HEART	(20)	(48)	(49)
PERIARTERITIS			1 (2%)
#MYOCARDIUM	(20)	(48)	(49)
FIBROSIS		1 (2%)	1 (2%)
*CORONARY ARTERY	(20)	(50)	(49)
PERIVASCULITIS	3 (15%)		
*PULMONARY ARTERY	(20)	(50)	(49)
MEDIAL CALCIFICATION		2 (4%)	1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(49)
NECROSIS, NOS		1 (2%)	15 (31%)
INFARCT, NOS		1 (2%)	
METAMORPHOSIS FATTY			2 (4%)
CYTOPLASMIC VACUOLIZATION		3 (6%)	
FOCAL CELLULAR CHANGE	4 (20%)	22 (45%)	5 (10%)
HEPATOCYTOMEGALY	1 (5%)	3 (6%)	1 (2%)
HEMATOPOIESIS		1 (2%)	
#LIVER/CENTRILOBULAR	(20)	(49)	(49)
CYTOPLASMIC VACUOLIZATION			1 (2%)
#BILE DUCT	(20)	(49)	(49)
HYPERPLASIA, NOS		1 (2%)	5 (10%)
#PANCREATIC ACINUS	(20)	(48)	(46)
ATROPHY, NOS	2 (10%)	2 (4%)	
#STOMACH	(20)	(48)	(49)
ULCER, NOS		1 (2%)	
HYPERKERATOSIS		1 (2%)	
#LARGE INTESTINE	(19)	(44)	(42)
NEMATODIASIS			1 (2%)
HYPERPLASIA, LYMPHOID	1 (5%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(49)
MINERALIZATION		2 (4%)	
HYDRONEPHROSIS			1 (2%)
PYELONEPHRITIS, NOS		1 (2%)	
INFLAMMATION, CHRONIC	8 (40%)	7 (14%)	4 (8%)
NEPHROSIS, NOS			1 (2%)
#KIDNEY/TUBULE	(20)	(49)	(49)
NECROSIS, NOS			2 (4%)
#KIDNEY/PELVIS	(20)	(49)	(49)
MINERALIZATION	1 (5%)		
HYPERPLASIA, EPITHELIAL		3 (6%)	4 (8%)
#URINARY BLADDER	(20)	(45)	(47)
HYPERPLASIA, EPITHELIAL		21 (47%)	13 (28%)
METAPLASIA, SQUAMOUS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(47)	(46)
CYST, NOS		6 (13%)	1 (2%)
ANGIECTASIS		4 (9%)	1 (2%)
#ADRENAL CORTEX	(20)	(49)	(49)
LIPOIDOSIS	1 (5%)		
HYPERPLASIA, NODULAR	3 (15%)	9 (18%)	3 (6%)
ANGIECTASIS	1 (5%)		
#THYROID	(20)	(47)	(46)
HYPERPLASIA, C-CELL	7 (35%)	7 (15%)	2 (4%)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
DILATATION/DUCTS	2 (10%)	2 (4%)	2 (4%)
*VAGINA	(20)	(50)	(49)
POLYP		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#UTERUS/ENDOMETRIUM	(20)	(49)	(48)
CYST, NOS	1 (5%)		
#OVARY	(20)	(49)	(49)
CYST, NOS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(20)	(48)	(49)
HEMORRHAGE	1 (5%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(49)
HEMORRHAGIC CYST		1 (2%)	
*MESENTERY	(20)	(50)	(49)
HEMORRHAGIC CYST			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(49)
HYPERPLASIA, LYMPHOID	1 (5%)		
HEMATOPOIESIS			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY			1
AUTO/NECROPSY/NO HISTO		1	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED o-TOLUIDINE HYDROCHLORIDE IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED o-TOLUIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	19	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	19	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(49)	(50)
HYPERPLASIA, LYMPHOID	1 (5%)	1 (2%)	
HEMATOPOIESIS	1 (5%)	6 (12%)	11 (22%)
#MESENTERIC L. NODE	(18)	(48)	(48)
HYPERPLASIA, LYMPHOID	1 (6%)	1 (2%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(19)	(50)	(50)
INFLAMMATION, FOCAL			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(19)	(50)	(50)
INFLAMMATION, FOCAL		1 (2%)	
NECROSIS, FOCAL		1 (2%)	
MEGALOCYTOSIS			1 (2%)
HYPERPLASIA, NODULAR		1 (2%)	
ANGIECTASIS		1 (2%)	
#LIVER/HEPATOCYTES	(19)	(50)	(50)
CYTOPLASMIC VACUOLIZATION	2 (11%)	2 (4%)	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
#PANCREAS	(18)	(47)	(46)
INFLAMMATION, FOCAL		1 (2%)	
PERIARTERITIS			1 (2%)
ATROPHY, NOS		1 (2%)	
#SMALL INTESTINE	(17)	(47)	(48)
DIVERTICULUM		1 (2%)	
#LARGE INTESTINE	(15)	(49)	(45)
NEMATODIASIS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(18)	(50)	(50)
INFLAMMATION, CHRONIC	1 (6%)		1 (2%)
HYPERPLASIA, TUBULAR CELL	1 (6%)		
ENDOCRINE SYSTEM			
#PANCREATIC ISLETS	(18)	(47)	(46)
HYPERPLASIA, NOS	3 (17%)		
REPRODUCTIVE SYSTEM			
#PROSTATE	(17)	(45)	(42)
INFLAMMATION, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(19)	(48)	(50)
MINERALIZATION	6 (32%)	10 (21%)	11 (22%)
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			
*ABDOMINAL CAVITY	(19)	(50)	(50)
HEMATOMA, NOS			1 (2%)
HEMORRHAGIC CYST			2 (4%)
*MESENTERY	(19)	(50)	(50)
ABSCESS, NOS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	12	11
ANIMAL MISSING/NO NECROPSY	1		
AUTO/NECROPSY/HISTO PERF		1	1

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED o-TOLUIDINE HYDROCHLORIDE 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			
#LUNG	(20)	(48)	(48)
INFLAMMATION PROLIFERATIVE			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(49)	(48)
HYPERPLASIA, LYMPHOID		2 (4%)	1 (2%)
HEMATOPOIESIS	9 (45%)	4 (8%)	6 (13%)
#LYMPH NODE	(20)	(49)	(48)
HYPERPLASIA, LYMPHOID			1 (2%)
#MESENTERIC L. NODE	(20)	(49)	(48)
CYST, NOS	1 (5%)		
HYPERPLASIA, LYMPHOID			2 (4%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
CYST, NOS		1 (2%)	
NECROSIS, FOCAL			1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BASOPHILIC CYTO CHANGE		1 (2%)	
ANGIECTASIS			3 (6%)
HEMATOPOIESIS			1 (2%)
#SMALL INTESTINE	(20)	(47)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(44)	(45)
ANGIECTASIS	1 (5%)		
#THYROID	(20)	(44)	(47)
CYSTIC FOLLICLES		1 (2%)	2 (4%)
REPRODUCTIVE SYSTEM			
#UTERUS/ENDOMETRIUM	(20)	(46)	(48)
CYST, NOS	10 (50%)	14 (30%)	18 (38%)
#OVARY	(20)	(46)	(47)
CYST, NOS	1 (5%)	7 (15%)	6 (13%)
NERVOUS SYSTEM			
#BRAIN	(20)	(47)	(48)
MINERALIZATION	5 (25%)	12 (26%)	11 (23%)
#CEREBELLUM	(20)	(47)	(48)
HEMORRHAGE		1 (2%)	
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			
*ABDOMINAL CAVITY	(20)	(49)	(50)
ABSCESS, NOS			1 (2%)
*MESENTERY	(20)	(49)	(50)
CYST, NOS			1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	8	9
ANIMAL MISSING/NO NECROPSY		1	
AUTO/NECROPSY/HISTO PERF		2	

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED o-TOLUIDINE HYDROCHLORIDE IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered o-Toluidine Hydrochloride in the Diet (a)

Topography: Morphology	Control	Low Dose	High Dose
Integumentary System: Fibroma of the Subcutaneous Tissue (b)	0/20 (0)	28/50 (56)	27/49 (55)
P Values (c,d)	P = 0.001	P less than 0.001	P less than 0.001
Departure from Linear Trend (e)	P = 0.004		
Relative Risk (f)		Infinite	Infinite
Lower Limit		3.873	3.803
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	82	62
Integumentary System: Lipoma of the Subcutaneous Tissue (b)	0/20 (0)	4/50 (8)	2/49 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.386	0.125
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	80	82

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Integumentary System: Sarcoma, NOS, Fibrosarcoma, Myxosarcoma, or Osteosarcoma (b)	0/20 (0)	4/50 (8)	3/49 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.386	0.255
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	66	72
Hematopoietic System:			
Lymphoma (b)	4/20 (20)	2/50 (4)	0/49 (0)
P Values (c,d)	P = 0.002 (N)	N.S.	P = 0.006 (N)
Relative Risk (f)		0.200	0.000
Lower Limit		0.020	0.000
Upper Limit		1.297	0.435
Weeks to First Observed Tumor	101	71	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Spleen: Sarcoma, NOS (b)	0/20 (0)	1/49 (2)	3/42 (7)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.023	0.298
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	104	72
Spleen: Fibroma (b)	0/20 (0)	10/49 (20)	2/42 (5)
P Values (c,d)	N.S.	P = 0.024	N.S.
Departure from Linear Trend (e)	P = 0.004		
Relative Risk (f)		Infinite	Infinite
Lower Limit		1.266	0.146
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	96	82

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Spleen: Angiosarcoma (b)	0/20 (0)	7/49 (14)	0/42 (0)
P Values (c,d)	N.S.	N.S.	--
Departure from Linear Trend (e)	P = 0.003		
Relative Risk (f)		Infinite	--
Lower Limit		0.826	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	83	--
Spleen: Sarcoma, NOS, Angiosarcoma, or Osteosarcoma (b)	0/20 (0)	8/49 (16)	4/42 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.972	0.460
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	83	72

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Neoplastic Nodule or Hepatocellular Carcinoma (b)	1/20 (5)	1/50 (2)	3/49 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.400	1.224
Lower Limit		0.005	0.108
Upper Limit		30.802	62.958
Weeks to First Observed Tumor	104	99	72
Urinary Bladder: Transitional-cell Carcinoma (b)	0/20 (0)	3/50 (6)	0/44 (0)
P Values (c,d)	N.S.	N.S.	--
Relative Risk (f)		Infinite	--
Lower Limit		0.250	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	99	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Urinary Bladder: Transitional-cell Carcinoma or Papilloma (b)	0/20 (0)	3/50 (6)	1/44 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.250	0.025
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	—	99	72
Adrenal: Pheochromocytoma (b)	0/20 (0)	3/50 (6)	2/49 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.250	0.125
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	80	82

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Adenoma, NOS (b)	4/20 (20)	7/50 (14)	3/44 (7)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.700	0.341
Lower Limit		0.207	0.056
Upper Limit		2.994	1.857
Weeks to First Observed Tumor	104	79	79
Thyroid: C-cell Carcinoma (b)	2/20 (10)	0/50 (0)	0/45 (0)
P Values (c,d)	P = 0.028 (N)	N.S.	N.S.
Departure from Linear Trend (e)	P = 0.048		
Relative Risk (f)		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.345	1.491
Weeks to First Observed Tumor	104	--	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma or Adenoma (b)	3/20 (15)	2/50 (4)	2/45 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.267	0.296
Lower Limit		0.024	0.027
Upper Limit		2.190	2.424
Weeks to First Observed Tumor	104	99	78
Mammary Gland: Fibroadenoma (b)	0/20 (0)	7/50 (14)	1/49 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Departure from Linear Trend (e)	P = 0.008		
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.809	0.023
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	74	93

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Testis: Interstitial-Cell Tumor (b)	17/20 (85)	41/50 (82)	33/49 (67)
P Values (c,d)	P = 0.045(N)	N.S.	N.S.
Relative Risk (f)		0.965	0.792
Lower Limit		0.802	0.649
Upper Limit		1.310	1.139
Weeks to First Observed Tumor	104	70	72
Multiple Organs or Tunica Vaginalis: Mesothelioma (b)	0/20 (0)	17/50 (34)	9/49 (18)
P Values	N.S.	P = 0.001	P = 0.036
Departure from Linear Trend (e)	P = 0.002		
Relative Risk (f)		Infinite	Infinite
Lower Limit		2.256	1.119
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	66	72

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Multiple Organs: Sarcoma, NOS (b)	0/20 (0)	3/50 (6)	11/49 (22)
P Values (c,d)	P = 0.003	N.S.	P = 0.016
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.250	1.413
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	84	61
Multiple Organs: Fibrosarcoma (b)	0/20 (0)	8/50 (16)	20/49 (41)
P Values (c,d)	P less than 0.001	N.S.	P less than 0.001
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.952	2.750
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	70	67

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Multiple Organs: Osteosarcoma (b)	0/20 (0)	3/50 (6)	5/49 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.250	0.536
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	92	82
Multiple Organs: Sarcoma, NOS, Fibrosarcoma, Angiosarcoma, or Osteosarcoma (b)	0/20 (0)	15/50 (30)	37/49 (76)
P Values (c,d)	P less than 0.001	P = 0.003	P less than 0.001
Relative Risk (f)		Infinite	Infinite
Lower Limit		1.964	5.361
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	70	61

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

(a) Dosed groups received 3,000 or 6,000 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P 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 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 a control group.

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

(f) The 95% 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 o-Toluidine Hydrochloride in the Diet (a)

Topography: Morphology	Control	Low Dose	High Dose
Integumentary System: Fibroma of the Subcutaneous Tissue (b)	0/20 (0)	4/50 (8)	2/49 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.386	0.125
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	99	87
Hematopoietic System: Lymphoma (b)	1/20 (5)	3/50 (6)	0/49 (0)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.200	0.000
Lower Limit		0.106	0.000
Upper Limit		61.724	7.624
Weeks to First Observed Tumor	101	96	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Spleen: Sarcoma, NOS (b)	0/20 (0)	1/49 (2)	3/49 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.023	0.255
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	104	81
Spleen: Fibroma (b)	0/20 (0)	4/49 (8)	6/49 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.394	0.680
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	104	87

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Spleen: Angiosarcoma (b)	0/20 (0)	7/49 (14)	9/49 (18)
P Values (c,d)	P = 0.045	N.S.	P = 0.036
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.826	1.119
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	103	65
Spleen: Sarcoma, NOS, Angiosarcoma, or Osteosarcoma (b)	0/20 (0)	9/49 (18)	12/49 (24)
P Values (c,d)	P = 0.018	P = 0.036	P = 0.010
Relative Risk (f)		Infinite	Infinite
Lower Limit		1.119	1.561
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	92	65

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Urinary Bladder: Transitional-cell Carcinoma (b)	0/20 (0)	9/45 (20)	22/47 (47)
P Values (c,d)	P less than 0.001	P = 0.028	P less than 0.001
Relative Risk (f)		Infinite	Infinite
Lower Limit		1.219	3.182
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	103	65
Urinary Bladder: Transitional-cell Carcinoma or Papilloma (b)	0/20 (0)	10/45 (22)	22/47 (47)
P Values (c,d)	P less than 0.001	P = 0.018	P less than 0.001
Relative Risk (f)		Infinite	Infinite
Lower Limit		1.379	3.182
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	103	65

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Adenoma, NOS (b)	5/20 (25)	12/47 (26)	2/46 (4)
P Values (c,d)	P = 0.009 (N)	N.S.	P = 0.023 (N)
Relative Risk (f)		1.021	0.174
Lower Limit		0.400	0.018
Upper Limit		3.310	0.975
Weeks to First Observed Tumor	104	88	89
Adrenal: Pheochromocytoma (b)	0/20 (0)	4/49 (8)	2/49 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.394	0.125
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	104	104

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma (b)	1/20 (5)	3/47 (6)	0/46 (0)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.277	0.000
Lower Limit		0.112	0.000
Upper Limit		65.563	8.111
Weeks to First Observed Tumor	104	104	--
Thyroid: C-cell Carcinoma or Adenoma (b)	1/20 (5)	6/47 (13)	1/46 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.553	0.435
Lower Limit		0.346	0.006
Upper Limit		114.692	33.420
Weeks to First Observed Tumor	104	75	104

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma (b)	6/20 (30)	20/50 (40)	35/49 (71)
P Values (c,d)	P less than 0.001	N.S.	P = 0.002
Relative Risk (f)		1.333	2.381
Lower Limit		0.631	1.240
Upper Limit		3.541	5.608
Weeks to First Observed Tumor	97	94	72
Mammary Gland: Fibroadenoma or Adenoma, NOS (b)	7/20 (35)	20/50 (40)	35/49 (71)
P Values (c,d)	P = 0.001	N.S.	P = 0.006
Relative Risk (f)		1.143	2.041
Lower Limit		0.575	1.130
Upper Limit		2.760	4.361
Weeks to First Observed Tumor	97	94	72

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp (b)	5/20 (25)	4/49 (8)	1/48 (2)
P Values (c,d)	P = 0.004 (N)	N.S.	P = 0.007 (N)
Relative Risk (f)		0.327	0.083
Lower Limit		0.074	0.002
Upper Limit		1.385	0.690
Weeks to First Observed Tumor	104	88	104
Multiple Organs: Osteosarcoma (b)	0/20 (0)	0/50 (0)	18/49 (37)
P Values (c,d)	P less than 0.001	--	P = 0.001
Departure from Linear Trend (e)	P = 0.008		
Relative Risk (f)		--	Infinite
Lower Limit		--	2.451
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	84

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Multiple Organs: Sarcoma, NOS, Fibrosarcoma, Angiosarcoma, or Osteosarcoma (b)	0/20 (0)	3/50 (6)	21/49 (43)
P Values (c,d)	P less than 0.001	N.S.	P less than 0.001
Departure from Linear Trend (e)	P = 0.042		
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.250	2.899
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	76	76

(a) Dosed groups received 3,000 or 6,000 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P 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 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 a control group.

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

(f) The 95% 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 o-TOLUIDINE HYDROCHLORIDE IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered o-Toluidine Hydrochloride in the Diet (a)

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	3/19 (16)	4/50 (8)	1/50 (2)
P Values (c,d)	P = 0.032 (N)	N.S.	N.S.
Relative Risk (f)		0.507	0.127
Lower Limit		0.097	0.003
Upper Limit		3.234	1.487
Weeks to First Observed Tumor	103	103	102
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	5/19 (26)	5/50 (10)	3/50 (6)
P Values (c,d)	P = 0.033 (N)	N.S.	P = 0.032 (N)
Relative Risk (f)		0.380	0.228
Lower Limit		0.102	0.040
Upper Limit		1.500	1.071
Weeks to First Observed Tumor	103	54	102

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: Lymphoma (b)	1/19 (5)	9/50 (18)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		3.420	1.520
Lower Limit		0.536	0.167
Upper Limit		146.437	73.309
Weeks to First Observed Tumor	101	101	50
All Sites: Hemangiosarcoma (b)	1/19 (5)	1/50 (2)	10/50 (20)
P Values (c,d)	P = 0.004	N.S.	N.S.
Relative Risk (f)		0.380	3.800
Lower Limit		0.005	0.613
Upper Limit		29.260	160.949
Weeks to First Observed Tumor	99	103	94

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
All Sites: Hemangioma or Hemangiosarcoma (b)	1/19 (5)	2/50 (4)	12/50 (24)
P Values (c,d)	P = 0.002	N.S.	N.S.
Relative Risk (f)		0.760	4.560
Lower Limit		0.043	0.767
Upper Limit		43.961	190.018
Weeks to First Observed Tumor	99	103	102
Liver: Hepatocellular Carcinoma (b)	4/19 (21)	16/50 (32)	11/50 (22)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.520	1.045
Lower Limit		0.587	0.367
Upper Limit		5.639	4.095
Weeks to First Observed Tumor	94	70	92

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Adenoma (b)	5/19 (26)	19/50 (38)	14/50 (28)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.444	1.064
Lower Limit		0.635	0.438
Upper Limit		4.371	3.368
Weeks to First Observed Tumor	94	70	92
Liver: Hepatocellular Adenoma or Adenoma, NOS (b)	2/19 (11)	3/50 (6)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.570	0.570
Lower Limit		0.073	0.073
Upper Limit		6.511	6.511
Weeks to First Observed Tumor	103	103	94

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: Follicular-cell Adenoma or Carcinoma (b)	2/19 (11)	2/49 (4)	2/49 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.388	0.388
Lower Limit		0.031	0.031
Upper Limit		5.108	5.108
Weeks to First Observed Tumor	103	103	102
Mesentery: Lipoma (b)	2/19 (11)	0/50 (0)	0/50 (0)
P Values (c,d)	P = 0.041 (N)	N.S.	N.S.
Departure from Linear Trend (e)	P = 0.011		
Relative Risk (f)		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.278	1.278
Weeks to First Observed Tumor	103	--	--

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

(a) Dosed groups received 1,000 or 3,000 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P 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 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 a control group.

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

(f) The 95% 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 o-Toluidine Hydrochloride in the Diet (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: Lymphoma (b)	4/20 (20)	10/49 (20)	9/50 (18)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.020	0.900
Lower Limit		0.346	0.294
Upper Limit		4.068	3.660
Weeks to First Observed Tumor	103	80	88
All Sites: Hemangiosarcoma or Hemangioma (b)	1/20 (5)	1/49 (2)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.408	1.200
Lower Limit		0.005	0.106
Upper Limit		31.413	61.724
Weeks to First Observed Tumor	103	103	102

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	0/20 (0)	2/49 (4)	7/50 (14)
P Values (c,d)	P = 0.015	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.125	0.809
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	103	102
Liver: Hepatocellular Carcinoma or Adenoma (b)	0/20 (0)	4/49 (8)	13/50 (26)
P Values (c,d)	P = 0.001	N.S.	P = 0.007
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.394	1.674
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	103	102

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: Follicular-cell Adenoma (b)	3/20 (15)	0/44 (0)	0/47 (0)
P Values (c,d)	P = 0.014 (N)	P = 0.027 (N)	P = 0.024 (N)
Departure from Linear Trend (e)	P = 0.005		
Relative Risk (f)		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		0.747	0.700
Weeks to First Observed Tumor	103	--	--

(a) Dosed groups received 1,000 or 3,000 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P 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 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 a control group.

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

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

Review of the Bioassay of *o*-Toluidine Hydrochloride* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

October 25, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, 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 *o*-Toluidine Hydrochloride for carcinogenicity.

The primary reviewer for the report of the bioassay of *o*-Toluidine hydrochloride said that, under the conditions of test, the compound was carcinogenic in both sexes of treated rats and mice. After briefly describing the experimental design, he noted an increased incidence of urinary bladder epithelial and spleen capsule mesothelial hyperplasia in both sexes of treated rats. He suggested that this finding was worthy of special mention in the report. The primary reviewer pointed out that it was necessary to use historical control mice for the statistical analysis of hemangiosarcomas due to the small number of matched controls. Based on the results of the bioassay, he said that *o*-Toluidine hydrochloride would have to be considered to pose some human cancer risk.

The secondary reviewer said that the bioassay of *o*-Toluidine hydrochloride was acceptable and that the results clearly indicated that the compound was carcinogenic. He also concluded that the compound must be considered to be a potential human carcinogen.

It was moved that the report on the bioassay of *o*-Toluidine hydrochloride be accepted as written. The motion was seconded and approved unanimously.

Clearinghouse Members present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
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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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