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BIOASSAY OF 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE FOR POSSIBLE CARCINOGENICITY

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

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FOREWORD: This report presents the results of the bioassay of 4-chloro-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 a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. A positive result demonstrates that a test chemical is carcinogenic for animals under the conditions of the test and indicates that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic in animals requires a wider analysis.

CONTRIBUTORS: This bioassay of 4-chloro-o-toluidine hydrochloride was conducted at the NCI Frederick Cancer Research Center (FCRC) (1), Frederick, Maryland, operated for NCI (2) by Litton Bionetics, Inc.

The manager of the bioassay at FCRC was Dr. B. Ulland, the toxicologist was Dr. E. Gordon, and Drs. R. Cardy and D. Creasia compiled the data. Ms. S. Toms was responsible for management of data, Mr. D. Cameron for management of histopathology, Mr. L. Callahan for management of the computer branch, and Mr. R. Cypher for management of the facilities. Mr. A. Butler performed the computer services. Histopathologic evaluations for rats were performed by Dr. B. C. Zook and histopathologic evaluations for mice were performed by Dr. H. R. Seibold. The diagnoses included in this report represent their interpretations.

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

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

The following scientists at NCI were responsible for evaluating the bioassay 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 4-chloro-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 were administered 4-chloro-o-toluidine in the diet at one of two doses, either 1,250 or 5,000 ppm, for 107 weeks. Groups of 50 mice of each sex were administered the test chemical in the diet at one of two doses, either 3,750 or 15,000 ppm for the males and either 1,250 or 5,000 ppm for the females, for 99 weeks, except for the high-dose females (92 weeks). Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the end of administration of the test chemical.

Mean body weights of the high-dose rats and the low- and high-dose mice of each sex were lower than those of corresponding controls, and those of the mice were dose related. Mortality was not significantly affected by administration of the test chemical to rats of either sex and survival was 75% or greater at the end of the study in dosed and control groups. Sufficient numbers of rats were at risk for the development of late-appearing tumors. In mice, mortality was dose related for each sex.

In rats no tumors occurred at incidences which could clearly be related to administration of the test chemical.

In both male and female mice, hemangiosarcomas occurred at incidences that were dose related (P less than or equal to 0.001), and in direct comparisons the incidences in the high-dose males and the low- and high-dose females were significantly higher (P less than 0.001) than those in the corresponding controls (males: controls 0/20, low-dose 3/50, high-dose 37/50; females: controls 0/18, low-dose 40/49, high-dose 39/50). The combined incidences of hemangiosarcomas and hemangiomas also were dose related and were significantly higher in the dosed groups of male and female mice than in corresponding controls. There was a high incidence of hemosiderin deposit in the renal tubular epithelium, particularly in mice with hemangiosarcomas.

It is concluded that under the conditions of this bioassay, 4-chloro-o-toluidine hydrochloride was not carcinogenic for F344 rats but was carcinogenic for B6C3F1 mice, inducing hemangiosarcomas and hemangiomas in both males and females.

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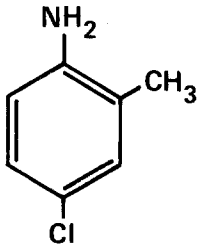
I. INTRODUCTION



• HCl

4-Chloro-o-toluidine hydrochloride

4-Chloro-o-toluidine hydrochloride (CAS 3165-93-3: NCI C02368) is used commercially as an intermediate for dyestuffs intended for textiles, e.g., pigment yellow 49 and pigment red 7, as well as C.I. 12800, azoic coupling component 8, and azoic diazo component 11 (Society of Dyers and Colourists, 1971). These latter two components are used in the synthesis of some azoic dyes, which are made by a two-stage process involving diazotization of a primary amine component and coupling of the diazotized amine with a naphthol-derived coupling component (Society of Dyers and Colourists, 1971). The discovery of the greater number of these colorants derived from 4-chloro-o-toluidine was made in 1921 (Society of Dyers and Colourists, 1971). 4-Chloro-o-toluidine is currently produced in the United States by at least one manufacturer (USITC, 1977a); imports during 1976 amounted to 25,000 pounds (USITC, 1977b).

Studies were initiated by the NCI in the 1960's which were designed to assess the carcinogenic effects of monocyclic aromatic amines (Homburger, 1972; Weisburger et al., in press). During these studies, LD_{50}'s of 4-chloro-o-toluidine by intraperitoneal administration were determined to be as follows: male and female Charles River CD-1 mice, 720 and 680 mg/kg, respectively, and male and female Charles River CD rats, 560 and 700 mg/kg, respectively (Weisburger et al., in press). The chronic studies showed 4-chloro-o-toluidine to be carcinogenic in mice but not in rats. Because prior studies were conducted with relatively small numbers of animals, 4-chloro-o-toluidine was selected for study in the Carcinogenesis Testing Program, using an expanded bioassay protocol.

II. MATERIALS AND METHODS

A. Chemical

4-Chloro-o-toluidine hydrochloride (2-amino-4-chlorotoluene hydrochloride) was obtained from American Aniline Products as a fine, light-pink powder. The infrared spectrum was consistent with its chemical structure. The eluate from high-pressure liquid chromatography (reversed-pack packing; mobile phase of 50% methanol in 0.01M KH_2PO_4; with absorption determined at 280 nm) contained two components, one of which was greater than 99% of the total amount of material eluated. Thin-layer chromatography showed only one spot.

B. Dietary Preparation

Test diets containing 4-chloro-o-toluidine hydrochloride were prepared weekly in 6-kg batches at appropriate doses. A known weight of the chemical was first mixed with an equal weight of autoclaved Wayne® Sterilizable Lab Meal containing 4% fat (Allied Mills, Inc., Chicago, Ill.), using a mortar and pestle. The mixing was continued with second and third additions of feed,

and final mixing was performed with the remaining quantity of feed for a minimum of 15 minutes in a Patterson-Kelly twin-shell blender with an intensifier bar. The diets were stored at 7°C until used.

C. Animals

Male and female F344 (Fischer) rats and B6C3F1 mice were obtained as 4-week-old weanlings, all within 3 days of the same age, from the NCI Frederick Cancer Research Center (Frederick, Md.). 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. The initial weight for male rats was 90 to 105 g, averaging at least 100 g; for female rats, 80 to 95 g, averaging at least 90 g; for male mice, 18 to 22 g, averaging at least 19.5 g; and for female mice, 17 to 21 g, averaging at least 18.5 g. Individual animals were identified by ear punch.

D. Animal Maintenance

The animals were housed in polycarbonate cages (Lab Products,

Inc., Garfield, N.J.), 19 x 10-1/2 x 8 inches for the rats and 11-1/2 x 7-1/2 x 5 inches for the mice. The cages were suspended from aluminum racks (Scientific Cages, Inc., Bryan, Tex.) and were covered by nonwoven, polyester-fiber, 12-mil-thick filter paper (Hoeltge, Inc., Cincinnati, Ohio). The bedding used was Absorb-dri® hardwood chips (Northeastern Products, Inc., Warrenburg, N.Y.). The feed supplied was presterilized Wayne® Sterilizable Lab Meal containing 4% fat, provided ad libitum in suspended stainless steel hoppers and replenished at least three times per week. Water, acidified to pH 2.5, was supplied ad libitum from glass bottles with sipper tubes (Lab Products, Inc.) 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 and sipper tubes were sanitized at 82 to 88°C in a tunnel-type bottle washer (Consolidated Equipment Supply Co., Mercersburg, Pa.) three times per week, using a Calgen Commercial Division

detergent (St. Louis, Mo.). The racks for the cages were sanitized at or above 82°C in a rack washer (Consolidated Equipment Supply Co.) once per month, using the Calgen Commercial Division detergent, and the filter paper was changed at the same time.

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

Rats administered 4-chloro-o-toluidine hydrochloride and their corresponding controls were housed in the same room as rats on feeding studies of the following chemicals:

(CAS 156-62-7) calcium cyanamide
(CAS 86-30-6) N-nitrosodiphenylamine

Mice administered 4-chloro-o-toluidine hydrochloride and their

corresponding controls were housed in the same room as mice on feeding studies of the following chemicals:

(CAS 128-37-0) butylated hydroxytoluene (BHT)
(CAS 97-77-8) tetraethylthiuram disulfide
(CAS 148-18-5) sodium diethyldithiocarbamate
(CAS 95-53-4) o-toluidine hydrochloride

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses (MTD's) of 4-chloro-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 mice of each sex were fed diets containing 4-chloro-o-toluidine hydrochloride at one of several doses for 7 weeks, followed by 1 week of additional observation, and groups of five control animals of each species and sex were administered basal diet only. In rats, two separate tests were conducted for males and three for females. Each animal was weighed twice per week. Table 1 shows the doses fed, the survival of animals in each dosed group at the end of the study, and the mean body weights of dosed animals at week 7 expressed as percentages of mean body weights of controls.

At the end of the subchronic studies, all animals were killed

Table 1. 4-Chloro-o-Toluidine Hydrochloride Subchronic Feeding Studies in Rats and Mice

	Male			Female		
	Dose (ppm)	Survival(a)	Mean Weight at Week 7 as % of Control	Dose (ppm)	Survival	Mean Weight at Week 7 as % of Control
RATS						
First Study						
	0	5/5	100			
	250	5/5	93			
	500	5/5	94			
	1,000	5/5	95			
	2,000	5/5	94			
	4,000	5/5	92			
Second Study				First Study		
	0	5/5	100	0	5/5	100
	6,000	5/5	91	6,000	5/5	92
	6,200	5/5	98	6,200	5/5	90
	6,500	5/5	99	6,500	5/5	93
	7,000	5/5	92	7,000	5/5	89
	8,000	5/5	89	8,000	5/5	91
	10,000	5/5	92	10,000	5/5	90
				Second Study		
				0	5/5	100
				1,000	5/5	103
				2,500	5/5	101
				3,000	5/5	98
				4,000	5/5	101
				Third Study		
				0	5/5	100
				6,200	5/5	81
				12,500	5/5	67
				25,000	5/5	55
				50,000	0/5	--
MICE						
	0	5/5	100			
	2,000	5/5	103			
	4,000	5/5	96			
	5,000	5/5	99			
	7,500	5/5	97			
	10,000	5/5	98			
	15,000	5/5	89	0	5/5	100
				15,000	5/5	90
				17,500	5/5	90
				20,000	5/5	78

(a) Number surviving/number in group.

using CO_2 and necropsied. During clinical and histopathologic examination of rats, very slight to moderate splenomegaly was noted in both sexes at 10,000 ppm. The enlargement was due to increased hematopoiesis and hyperemia. Very slight to moderate increase in marrow cellularity involving all cell types occurred in both sexes. At 6,500 ppm splenic enlargement was observed only in male rats and was not seen in either sex at lower doses. In male and female mice there were no gross or microscopic compound-related lesions at the highest doses.

Ten percent depression in body weight was a major criterion for estimation of MTD's. The doses that were 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 dosed groups at day 49 relative to weights of corresponding control groups were plotted against the logarithms of the doses, and least squares regressions fitted to the data were used to estimate the doses required to induce 10% depression in weight.

The low and high doses for the chronic studies were set at 1,250 and 5,000 ppm for the male and female rats, 3,750 and 15,000 ppm for the male mice, and 1,250 and 5,000 ppm for the female mice.

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 observed twice daily. Observations for sick, tumor-bearing, and moribund animals were recorded daily. Clinical examination and palpation for masses were performed each month, and the animals were generally weighed at least once per month except for the periods of weeks 50 to 80 and weeks 96 to 104, when weights were not recorded for rats. Moribund animals and animals that survived to the end of the bioassay were killed using CO_2 and necropsied.

Gross and microscopic examination was performed on major tissues, major organs, and all gross lesions. The tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone marrow (femur), spleen, lymph nodes (mesenteric and submandibular), thymus, heart, salivary glands (parotid,

Table 2. 4-Chloro-o-Toluidine Hydrochloride
Chronic Studies in Rats

Sex and Test Group	Initial No. of Animals (a)	4-Chloro-o-Toluidine Hydrochloride in in Diet (b) (ppm)	Time on Study (weeks)
Male			
Matched-Control	20	0	107
Low-Dose	50	1,250	107
High-Dose	50	5,000	107
Female			
Matched-Control	20	0	107
Low-Dose	50	1,250	107
High-Dose	50	5,000	107

(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.

Table 3. 4-Chloro-o-Toluidine Hydrochloride
Chronic Studies in Mice

Sex and Test Group	Initial No. of Animals(a)	4-Chloro-o-Toluidine Hydrochloride in Diet (b) (ppm)	Time on Study weeks)
Male			
Matched-Control	20	0	99
Low-Dose	50	3,750	99
High-Dose	50	15,000	99
Female			
Matched-Control	20	0	99
Low-Dose	50	1,250	99
High-Dose	50	5,000	92(c)

(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 chemical to high-dose females was terminated at week 92, due to death of all animals.

sublingual, and submaxillary), liver, pancreas, esophagus, stomach (glandular and nonglandular), small and large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, mammary gland, uterus, ovary, brain (cerebrum and cerebellum), and all tissue masses. Peripheral blood smears also were made for all animals, whenever possible.

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

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the

International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

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

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

The incidence of neoplastic or nonneoplastic lesions has been

given 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 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)

The recorded mean body weights of the high-dose male and female rats were lower than those of the corresponding controls (figure 1). No other compound-related clinical signs were recorded.

B. Survival (Rats)

Estimates of the probabilities of survival for male and female rats administered 4-chloro-o- toluidine hydrochloride in the diet at the doses of this bioassay, together with those of the matched controls, are shown in the Kaplan and Meier curves in figure 2. The result of the Tarone test indicates no significant dose-related trend in mortality in either sex.

In male rats, 41/50 (82%) of the high-dose group, 38/50 (76%) of the low-dose group, and 11/20 (55%) of the control group lived to the end of the bioassay. In females, 45/50 (90%) of the high-dose group, 42/50 (84%) of the low-dose group, and 15/20 (75%) of the control group lived to the end of the bioassay.

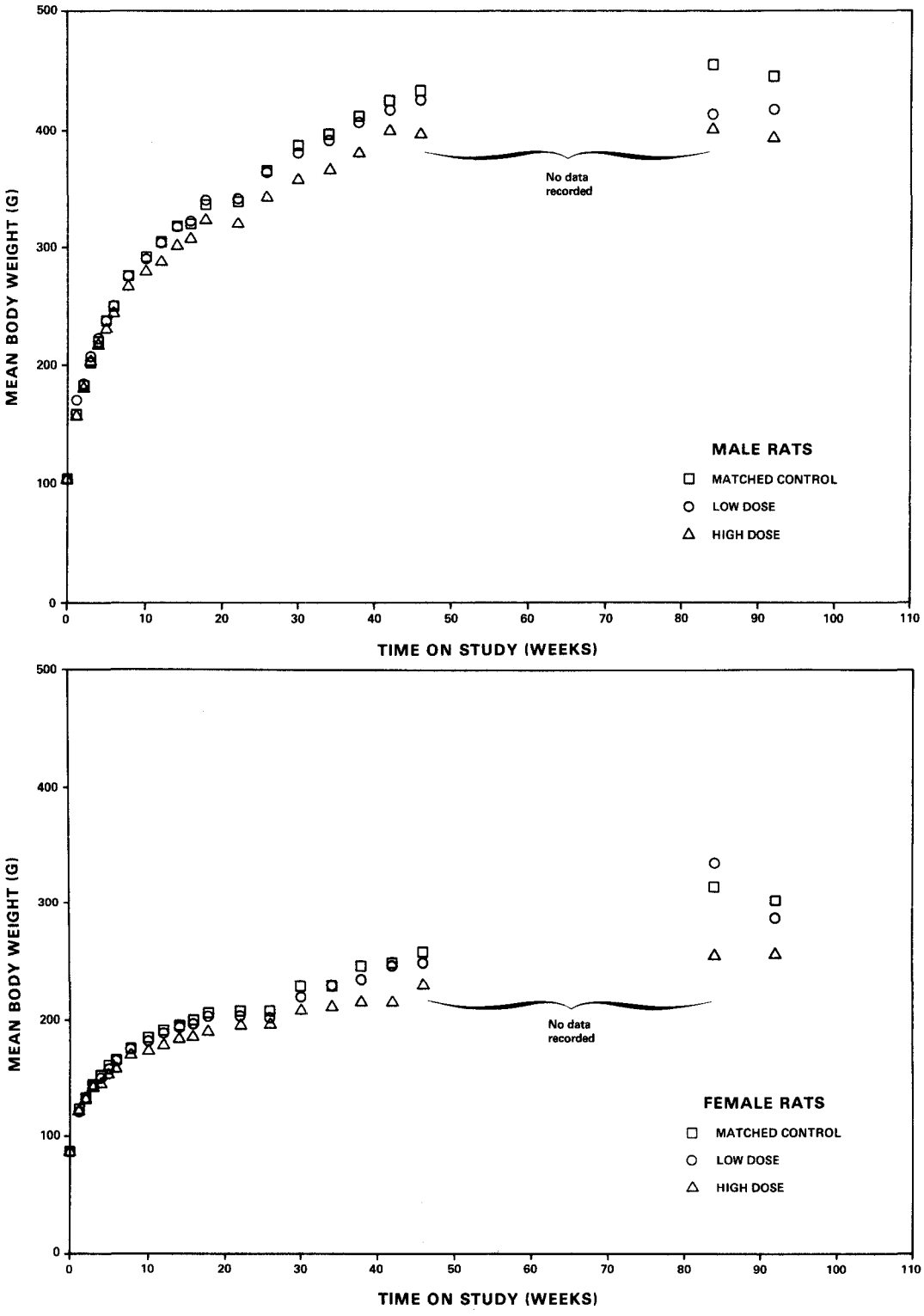


Figure 1. Growth Curves for Rats Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet

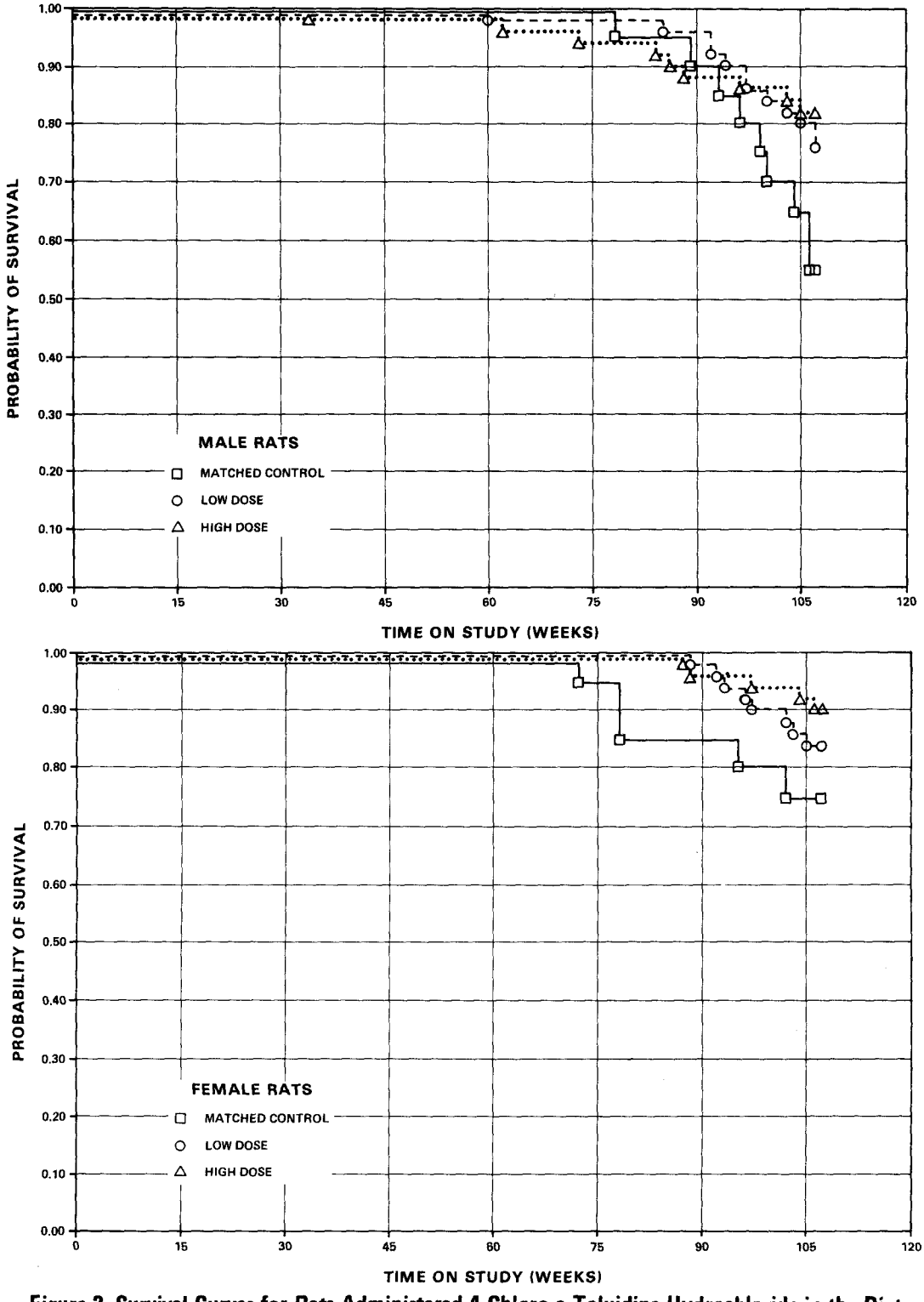


Figure 2. Survival Curves for Rats Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet

Sufficient numbers of rats of each sex were at risk for the development of late-appearing tumors.

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in Appendix A, tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix B, tables B1 and B2.

A variety of neoplastic and nonneoplastic changes were noted in the control and dosed rats. A few neoplasms occurred only, or with greater frequency, in rats in dosed groups as compared with controls. All the types of neoplasms that were observed have, however, been reported to occur in control F344 rats.

Adenomas of chromophobe cells of the pituitary gland were more numerous in both male and female dosed rats than in corresponding controls. As may be seen in the following tabulation, this occurrence appears to be dose related.

	Male			Female		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Number of Animals with Tissue Examined	19	48	47	19	48	48
Chromophobe Adenoma	2(11%)	6(13%)	15(32%)	1(5%)	13(27%)	15(31%)
Chromophobe Hyperplasia	0(0%)	0(0%)	2(4%)	0(0%)	3(6%)	1(2%)

All such tumors were benign, although in nine of the rats the expanding masses compressed the hypothalamus. Hyperplasias of chromophobe cells also were seen at higher incidences in dosed than control animals.

The benign pituitary adenomas may be considered to be compound related on the basis of this study; however, it should be noted that this tumor is common in this strain of rat and has occurred in 21% of previous control female rats at this laboratory. The tumors were generally well-circumscribed and were composed of uniform polygonal cells. The cytoplasm of the tumor cells was generally free of granules. Pigment deposits and telangiectasis were associated with many tumors. No anaplastic cells or bizarre nuclei were observed, and mitotic activity was rare.

A number of inflammatory and degenerative lesions were

encountered in both control and dosed rats. The lesions were all well recognized as occurring in older rats of this strain and no nonneoplastic lesions were considered to be compound related. It was judged that most degenerative and inflammatory lesions were of a relatively mild nature, and few caused illness or death of dosed or control rats.

Based on the histopathologic examination, it was concluded that 4-chloro-o-toluidine hydrochloride was not carcinogenic in F344 rats under the conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

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

In male rats, the result of the Cochran-Armitage test for dose-related trend in the incidence of chromophobe adenomas of the pituitary is significant ($P = 0.006$), but the results of the Fisher exact test are not significant. The historical records of this laboratory show an incidence of tumors of 52/285 (18%) in

control F344 male rats, which is higher than that in the control group (2/19, or 11%) or the low-dose group (6/48, or 13%) of this study. In females, the result of the Cochran-Armitage test on the incidence of this tumor is not significant. However, the Fisher exact comparison between the incidences in the low-dose and control groups and between the incidences in the high-dose and control groups show P values of 0.039 and 0.020, respectively. The former 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 tumors of 60/285 (21%) in control F344 female rats, which is higher than that in the control group (1/19, or 5%) of the present study.

In male rats, the result of the Cochran-Armitage test for the incidence of pheochromocytomas of the adrenal is significant (P = 0.014), but the results of the Fisher exact test are not significant.

Significant results in the negative direction are observed in the incidence of hematopoietic tumors in each sex.

In each of the 95% confidence intervals for relative risk shown in the tables, except for the incidence of chromphobe adenomas of

the pituitary in high-dose female rats, the value of one or less than one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals, except that for the incidence of hematopoietic tumors in male rats, has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by 4-chloro-o-toluidine hydrochloride, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the dosed groups of male and female mice were lower than those of the corresponding controls, especially in the females, and were dose related throughout the bioassay (figure 3). Tissue masses occurred more frequently in dosed groups than in control groups.

B. Survival (Mice)

Estimates of the probabilities of survival for male and female mice administered 4-chloro-o-toludine hydrochloride in the diet at the doses of this bioassay, together with those of the matched controls, are shown in the Kaplan and Meier curves in figure 4. In each sex, the result of the Tarone test for dose-related trend in mortality is significant (P less than 0.001). In female mice, an indicated departure from linear trend is observed (P = 0.003), due to the relatively steep decrease in survival in the dosed groups.

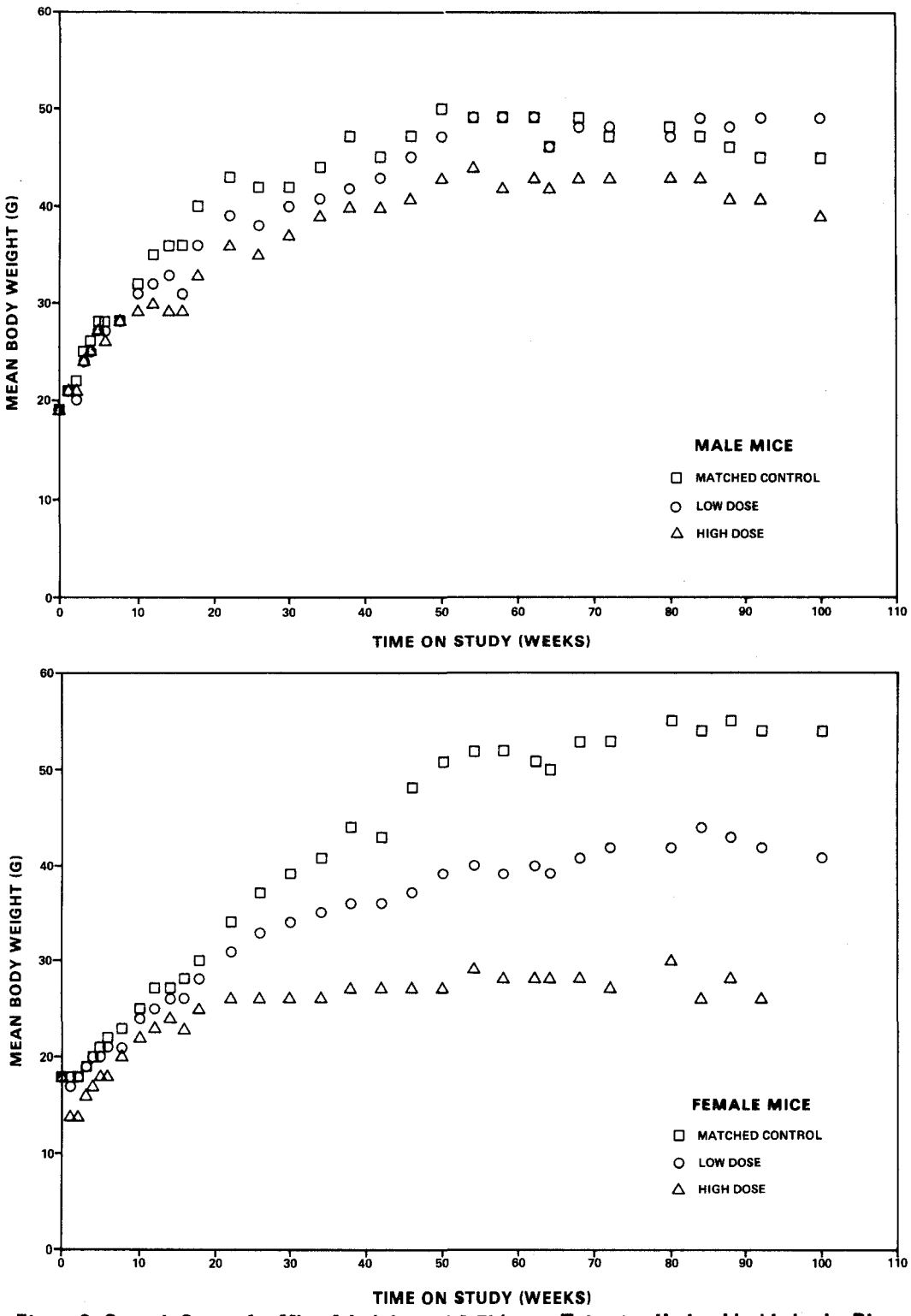


Figure 3. Growth Curves for Mice Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet

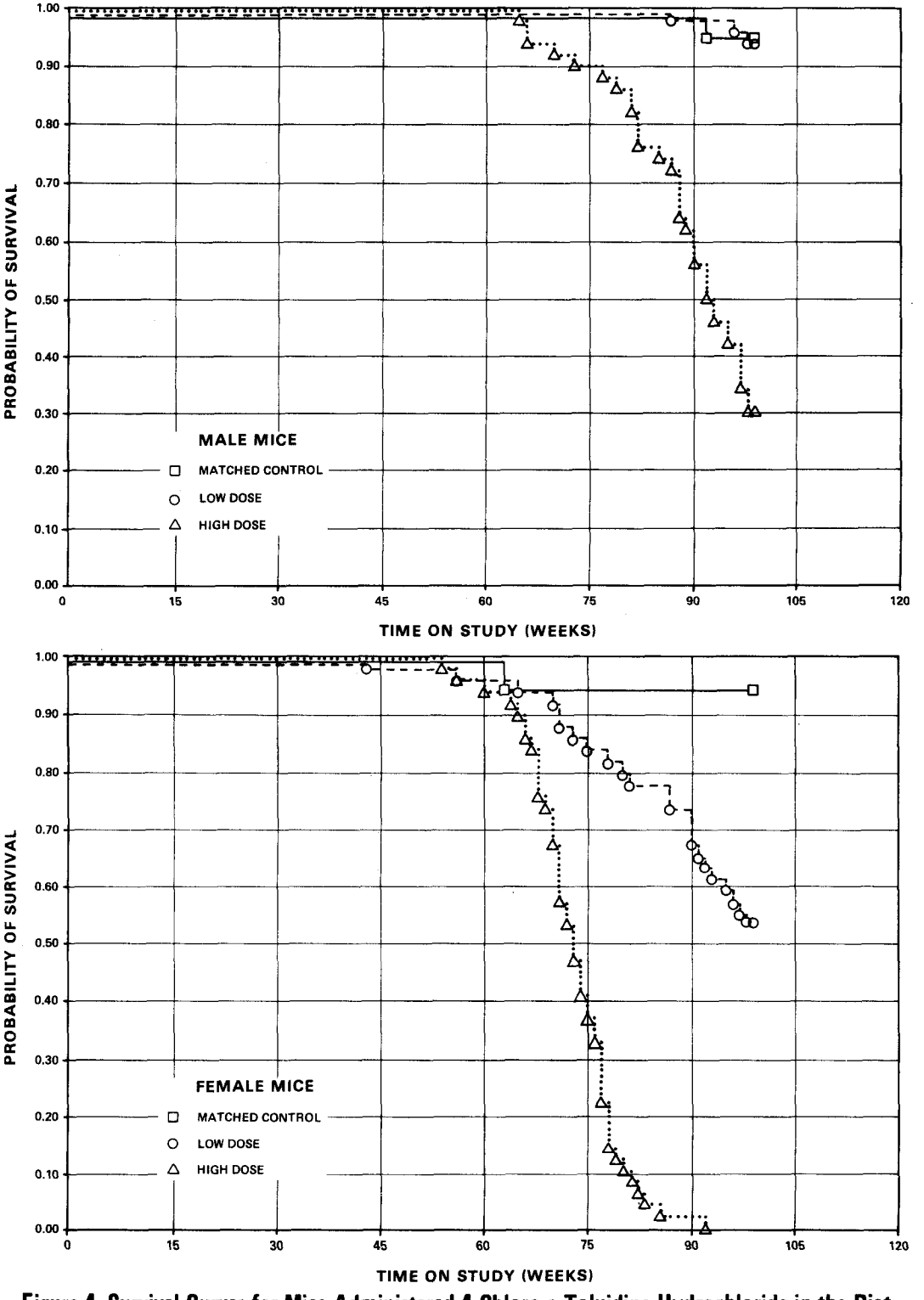


Figure 4. Survival Curves for Mice Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet

In male mice, all 50 animals from each dosed group and all 20 control animals lived beyond week 52 on study. In females, 49/50 (98%) of the high-dose group, 48/50 (96%) of the low-dose group, and 19/20 (95%) of the control group lived beyond week 52 on study.

Sufficient numbers of mice of each sex were at risk for the development of 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.

There was a dose-related incidence of hemangiosarcomas in the dosed groups of mice, as indicated below.

	Male			Female		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Number of Animals Necropsied	20	50	50	18	49	50
Hemangio-sarcoma	0(0%)	3(6%)	37(74%)	0(0%)	40(82%)	39(78%)
Hemangioma	0(0%)	3(6%)	5(10%)	1(6%)	6(12%)	0(0%)

The hemangiosarcomas observed in the male and female mice apparently originated in fatty tissue adjacent to the genital organs, and not in a particular organ. In some instances, they were observed to infiltrate the abdominal muscles, uterus, ovary, prostate, or urinary bladder.

There was considerable variation in the size and shape of the tumor cells, but they characteristically had plump, hyperchromatic nuclei and enlongated, somewhat basophilic cytoplasm. The tumor cells proliferated in an angiomatous pattern, producing an irregular complex of blood-filled spaces lined by tumor cells and ranging in size from capillary diameter to large, cavernous spaces. The cellularity, anaplasia, mitotic index, and amount of stroma were variable among the tumors.

Pulmonary metastasis was observed in only 5 (4%) of the 119 dosed animals bearing hemangiosarcomas, but the tumor was lethal to 89 (75%) of the affected mice, due to hemorrhage in the peritoneal cavity and the space-consuming character of the lesions.

In many instances large hematomatous masses of necrotizing extravasated blood comprised the bulk of hemangiosarcomas, and only wisps of tumor tissue were found at the periphery. These lesions and the tumors in some of the mice that died could not be

studied in detail, but there was enough material in a satisfactory state of preservation to establish the pattern of the lesions. Associated pathological alterations that were recorded at necropsy were hemorrhage in the peritoneal cavity and variable enlargement of the spleen. The splenic enlargement appeared on microscopic examination to have resulted from increased extramedullary hemopoiesis in consequence of continuing hemorrhage from the tumors.

Benign hemangiomas in the genital fat were found in one male and one female mouse in the low-dose groups. There was also a low incidence of hemangiomas in other organs and tissues, as indicated above. Various other benign and malignant neoplasms were found in dosed and control mice. These included hepatocellular carcinomas and adenomas, bronchiolar/alveolar carcinomas and adenomas, and malignant lymphomas.

There was a high incidence of hemosiderin deposition in the renal tubular epithelium (43 among 119 mice with hemangiosarcomas). Ten of the 119 mice with hemangiosarcomas also had some degree of hydronephrosis, presumably from compression of ureters by the tumor.

There was a very low incidence and a limited variety of

inflammatory and degenerative lesions. A majority of the lesions seen appeared to be complications of anemia, debility, and tissue displacement caused by the hemangiosarcomas. There were no indications that endemic disease was present in the dosed and control mice.

Based on the histopathologic examination, it is concluded that 4-chloro-o-toluidine hydrochloride was carcinogenic in B6C3F1 mice, inducing hemangiosarcomas in each sex.

D. Statistical Analyses of Results (Mice)

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

In male mice, the result of the Cochran-Armitage test on the incidence of hemangiosarcomas is significant (P less than 0.001), and the Fisher exact test shows that the incidence in the high-dose group is significantly higher (P less than 0.001) than that in the control group. When the combined incidence of hemangiomas and hemangiosarcomas in male mice is analyzed, similar

significant results are obtained, which are mainly due to the incidence of hemangiosarcomas. In female mice, the result of the Cochran-Armitage test on the incidence of hemangiosarcomas is significant (P = 0.001). An indicated departure from linear trend is observed (P less than 0.001), due to the relatively steep increase in the incidence of this tumor in the dosed groups. The results of the Fisher exact test show that the incidence in each dosed group is significantly higher (P less than 0.001) than that in the control group. When the combined incidence of hemangiomas and hemangiosarcomas in female mice is analyzed, similar significant results are observed, which are mainly due to the incidence of hemangiosarcomas. The statistical conclusion is that the incidences of hemangiosarcomas in both male and female groups of mice are associated with the administration of 4-chloro-o-toluidine hydrochloride.

Significant trends in the negative direction are observed in the incidence of lung tumors in male mice and in the incidences of hemangiomas and liver tumors in female mice. This significance in the negative direction may be accounted for by the lower survival of the high-dose males and the high- and low-dose females than of the corresponding controls.

V. DISCUSSION

Mean body weights of the high-dose rats and both low- and high-dose mice of each sex were lower than those of corresponding controls, and those of the mice were dose related. Mortality was not significantly affected by administration of the test chemical to the rats of either sex, and survival was 75% or greater at the end of the study in all dosed and control groups except the high-dose male and female mice. Sufficient numbers of rats were at risk for the development of late-appearing tumors. In mice, mortality was dose related for each sex.

In the male rats, chromophobe adenomas of the pituitary occurred at incidences that were dose related ($P = 0.006$); however, in direct comparisons the incidences in the individual dosed groups were not significantly higher than those in the corresponding control group (controls 2/19, low-dose 6/48, high-dose 15/47). In the female rats, chromophobe adenomas of the pituitary occurred at incidences that were not dose related; however, in a direct comparison the incidence in the high-dose group was significantly higher ($P = 0.020$) than that in the corresponding control group (controls 1/19, low-dose 13/47, high-dose 15/48). The incidences of the chromophobe adenomas of the pituitary in

the controls of this bioassay (males, 10%; females, 5%) were abnormally low compared with those of historical male (52/285, or 18%) or female (60/285, or 21%) controls. Because of these data, the occurrence of pituitary tumors in the dosed rats cannot clearly be related to administration of the test chemical.

In both the male and female mice, hemangiosarcomas occurred at incidences that were dose related (P less than or equal to 0.001), and in direct comparisons the incidences in the high-dose male and female groups and the low-dose female group were significantly higher (P less than 0.001) than those in the corresponding control groups (males: controls 0/20, low-dose 3/50, high-dose 37/50; females: controls 0/18, low-dose 40/49, high-dose 39/50). The combined incidences of hemangiosarcomas and hemangiomas also were dose related and were significantly higher in the dosed groups of mice of each sex than in the corresponding controls. There was a high incidence of hemosiderin deposit in the renal tubular epithelium, particularly in mice with hemangiosarcomas.

In preliminary investigations on the carcinogenicity of 4-chloro-o-toluidine administered in the diet in 2-year studies, hemangiosarcomas and hemangiomas were induced at significant incidences in male and female CD-1 (HaM/ICR derived) mice, but no tumors were induced at significant incidences in male or female

CD (Sprague-Dawley derived) rats (Homburger et al., 1972, Weisburger et al., in press). The low and high doses of test chemical used in these studies were 2,000 and 4,000 ppm for 3 months and 500 and 1,000 ppm for the subsequent 15 months for the rats, 750 and 1,500 ppm for 18 months for the male mice, and 2,000 and 4,000 ppm for 18 months for the female mice. These results are consistent with those of the present study.

It is concluded that under the conditions of this bioassay, 4-chloro-o-toluidine hydrochloride was not carcinogenic for F344 rats but was carcinogenic for B6C3F1 mice, inducing hemangiosarcomas and hemangiomas in both males and females.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
PAPILLOMA, NOS			1 (2%)
SQUAMOUS CELL CARCINOMA		1 (2%)	
TRICHOEPITHELIOMA		2 (4%)	1 (2%)
SEBACEOUS ADENOMA		2 (4%)	
KERATOACANTHOMA		1 (2%)	1 (2%)
FIBROMA	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(50)
SEBACEOUS ADENOCARCINOMA			1 (2%)
FIBROMA		4 (8%)	2 (4%)
HEMANGIOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	5 (10%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
LEUKEMIA,NOS	4 (20%)		
UNDIFFERENTIATED LEUKEMIA	1 (5%)		
GRANULOCYTIC LEUKEMIA	1 (5%)		
#SPLEEN	(20)	(49)	(49)
MALIGNANT LYMPHOMA, NOS			1 (2%)
*LYMPH NODE	(20)	(50)	(49)
SQUAMOUS CELL CARCINOMA, METASTA		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
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
ADENOCARCINOMA, NOS			1 (2%)
HEPATOCELLULAR ADENOMA		5 (10%)	3 (6%)
HEPATOCELLULAR CARCINOMA			1 (2%)
#STOMACH	(20)	(49)	(49)
PAPILLOMA, NOS		1 (2%)	
#COLON	(20)	(49)	(49)
ADENOCARCINOMA, NOS		1 (2%)	
URINARY SYSTEM			
#URINARY BLADDER	(20)	(46)	(48)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(48)	(47)
NEOPLASM, NOS		1 (2%)	
ADENOMA, NOS		1 (2%)	
CHROMOPHOBE ADENOMA	2 (11%)	6 (13%)	15 (32%)
#ADRENAL	(20)	(49)	(49)
PHEOCHROMOCYTOMA			4 (8%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (5%)		
#THYROID	(19)	(49)	(49)
FOLLICULAR-CELL ADENOMA			4 (8%)
FOLLICULAR-CELL CARCINOMA	1 (5%)		
C-CELL ADENOMA		2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOMA, NOS			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
FIBROMA		1 (2%)	2 (4%)
FIBROADENOMA		1 (2%)	1 (2%)
#TESTIS	(20)	(48)	(50)
INTERSTITIAL-CELL TUMOR	16 (80%)	39 (81%)	42 (84%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*MANDIBLE	(20)	(50)	(50)
OSTEOSARCOMA			1 (2%)
*PELVIC BONES	(20)	(50)	(50)
OSTEOSARCOMA		1 (2%)	
*SKELETAL MUSCLE	(20)	(50)	(50)
LIPOMA			1 (2%)
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
LIPOMA			1 (2%)
*TUNICA VAGINALIS	(20)	(50)	(50)
MESOTHELIOMA, NOS	2 (10%)		
ALL OTHER SYSTEMS			
THORACIC CAVITY			
MESOTHELIOMA, NOS	1		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	9	10	6
MORIBUND SACRIFICE		2	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	11	38	41
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	19	44	49
TOTAL PRIMARY TUMORS	31	78	88
TOTAL ANIMALS WITH BENIGN TUMORS	17	42	48
TOTAL BENIGN TUMORS	20	71	82
TOTAL ANIMALS WITH MALIGNANT TUMORS	8	6	6
TOTAL MALIGNANT TUMORS	8	6	6
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	3	1	
TOTAL UNCERTAIN TUMORS	3	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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA		2 (4%)	1 (2%)
BASAL-CELL TUMOR	1 (5%)		
TRICHOEPITHELIOMA	1 (5%)	2 (4%)	1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROADENOMA			1 (2%)
RESPIRATORY SYSTEM			
*NASAL CAVITY	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA, INVASIV		1 (2%)	
*LUNG	(19)	(49)	(50)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)		2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
LEUKEMIA,NOS	1 (5%)	2 (4%)	
UNDIFFERENTIATED LEUKEMIA	2 (10%)	2 (4%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*LIVER	(19)	(49)	(49)
HEPATOCELLULAR ADENOMA	1 (5%)		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
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(47)	(48)
NEOPLASM, NOS			1 (2%)
ADENOMA, NOS		1 (2%)	1 (2%)
CHROMOPHOBE ADENOMA	1 (5%)	13 (28%)	15 (31%)
#ADRENAL	(19)	(49)	(50)
PHEOCHROMOCYTOMA		1 (2%)	
PHEOCHROMOCYTOMA, MALIGNANT		1 (2%)	
#THYROID	(19)	(49)	(50)
FOLLICULAR-CELL CARCINOMA	1 (5%)	1 (2%)	
C-CELL ADENOMA	1 (5%)	2 (4%)	
#PANCREATIC ISLETS	(19)	(46)	(50)
ISLET-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOMA, NOS		6 (12%)	1 (2%)
ADENOCARCINOMA, NOS		1 (2%)	
CYSTADENOMA, NOS		1 (2%)	1 (2%)
FIBROMA		2 (4%)	1 (2%)
FIBROADENOMA	4 (20%)	10 (20%)	6 (12%)
#UTERUS	(19)	(49)	(49)
LEIOMYOMA		1 (2%)	
ENDOMETRIAL STROMAL POLYP	5 (26%)	5 (10%)	8 (16%)
NERVOUS SYSTEM			
#BRAIN	(19)	(49)	(49)
SQUAMOUS CELL CARCINOMA, INVASIV		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
LIPOMA		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	5	4
MORIBUND SACRIFICE	3	3	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	15	42	45
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	34	32
TOTAL PRIMARY TUMORS	19	54	42
TOTAL ANIMALS WITH BENIGN TUMORS	10	28	31
TOTAL BENIGN TUMORS	15	45	39
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	8	2
TOTAL MALIGNANT TUMORS	4	9	2
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	
TOTAL SECONDARY TUMORS		3	
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 B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
FIBROSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(46)	(48)
HEPATOCELLULAR CARCINOMA, METAST	1 (5%)		1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)	7 (15%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (10%)	7 (15%)	1 (2%)
HEMANGIOSARCOMA, METASTATIC			2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	2 (4%)	
#SPLEEN	(20)	(50)	(50)
HEMANGIOMA		1 (2%)	2 (4%)
#THYMUS	(12)	(39)	(27)
MALIGNANT LYMPHOMA, NOS		1 (3%)	
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(50)
HEMANGIOMA			2 (4%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
HEPATOCELLULAR ADENOMA		2 (4%)	4 (8%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA	4 (20%)	5 (10%)	7 (14%)
SARCOMA, NOS, METASTATIC	1 (5%)		
HEMANGIOMA			1 (2%)
#PANCREAS	(20)	(50)	(39)
HEMANGIOSARCOMA, METASTATIC			1 (3%)
#SMALL INTESTINE	(20)	(46)	(43)
ADENOMATOUS POLYP, NOS	1 (5%)		
#SMALL INTEST./SEROSA	(20)	(46)	(43)
SARCOMA, NOS	1 (5%)		
#JEJUNUM	(20)	(46)	(43)
ADENOCARCINOMA, NOS		1 (2%)	
#COLON	(20)	(49)	(48)
HEMANGIOMA		1 (2%)	
URINARY SYSTEM			
*GENITOURINARY TRACT	(20)	(50)	(50)
HEMANGIOSARCOMA		2 (4%)	4 (8%)
#KIDNEY	(20)	(50)	(50)
TUBULAR-CELL ADENOMA		1 (2%)	1 (2%)
HEMANGIOMA			1 (2%)
#URINARY BLADDER	(19)	(48)	(47)
HEMANGIOSARCOMA			2 (4%)
ENDOCRINE SYSTEM			
#ADRENAL	(20)	(49)	(49)
PHEOCHROMOCYTOMA			1 (2%)
#PANCREATIC ISLETS	(20)	(50)	(39)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MALE GENITAL SYSTEM	(20)	(50)	(50)
HEMANGIOSARCOMA			6 (12%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#PROSTATE	(19)	(47)	(35)
HEMANGIOSARCOMA			1 (3%)
#TESTIS	(20)	(50)	(50)
HEMANGIOSARCOMA			3 (6%)
*EPIDIDYMIS	(20)	(50)	(50)
HEMANGIOMA		1 (2%)	1 (2%)
HEMANGIOSARCOMA			12 (24%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(50)	(50)
PAPILLARY CYSTADENOMA, NOS	1 (5%)	1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)
*PERITONEUM	(20)	(50)	(50)
HEMANGIOSARCOMA			2 (4%)
*PELVIS	(20)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)
*INGUINAL REGION	(20)	(50)	(50)
HEMANGIOSARCOMA			2 (4%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
SARCOMA, NOS			1 (2%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
OSTEOSARCOMA, METASTATIC	1 (5%)		
SITE UNKNOWN			
HEMANGIOSARCOMA		1	3
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	3	32
MORIBUND SACRIFICE			3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	19	47	15
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	10	28	45
TOTAL PRIMARY TUMORS	12	35	62
TOTAL ANIMALS WITH BENIGN TUMORS	3	12	12
TOTAL BENIGN TUMORS	4	15	15
TOTAL ANIMALS WITH MALIGNANT TUMORS	8	19	41
TOTAL MALIGNANT TUMORS	8	20	47
TOTAL ANIMALS WITH SECONDARY TUMORS#	3		4
TOTAL SECONDARY TUMORS	3		4
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 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	2	1	
ANIMALS NECROPSIED	18	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	18	49	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(18)	(49)	(50)
HEMANGIOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(18)	(47)	(48)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	3 (6%)
HEMANGIOSARCOMA, METASTATIC		1 (2%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(18)	(49)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (6%)		
GRANULOCYTIC LEUKEMIA		1 (2%)	
#SPLEEN	(18)	(48)	(46)
HEMANGIOMA	1 (6%)	2 (4%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	
#MESENTERIC L. NODE	(18)	(44)	(38)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (5%)	
#AXILLARY LYMPH NODE	(18)	(44)	(38)
HEMANGIOMA		1 (2%)	
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	(18)	(49)	(49)
HEPATOCELLULAR ADENOMA	1 (6%)	2 (4%)	
HEPATOCELLULAR CARCINOMA		2 (4%)	
#PANCREAS	(16)	(47)	(36)
HEMANGIOSARCOMA, METASTATIC			1 (3%)
#SMALL INTESTINE	(17)	(45)	(39)
ADENOMATOUS POLYP, NOS		1 (2%)	
URINARY SYSTEM			
*GENITOURINARY TRACT	(18)	(49)	(50)
HEMANGIOSARCOMA		12 (24%)	13 (26%)
#URINARY BLADDER	(16)	(39)	(34)
HEMANGIOSARCOMA		6 (15%)	6 (18%)
ENDOCRINE SYSTEM			
#THYROID	(18)	(45)	(40)
FOLLICULAR-CELL ADENOMA	1 (6%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(18)	(49)	(50)
ADENOCARCINOMA, NOS	1 (6%)		
HEMANGIOMA		1 (2%)	
*FEMALE GENITAL SYSTE	(18)	(49)	(50)
HEMANGIOSARCOMA			1 (2%)
#UTERUS	(18)	(46)	(43)
ENDOMETRIAL STROMAL SARCOMA		1 (2%)	
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA		8 (17%)	9 (21%)
#CERVIX UTERI	(18)	(46)	(43)
HEMANGIOSARCOMA		1 (2%)	3 (7%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#UTERUS/MYOMETRIUM	(18)	(46)	(43)
HEMANGIOSARCOMA		1 (2%)	
#OVARY	(16)	(45)	(44)
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(18)	(49)	(50)
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA		8 (16%)	
*PERITONEUM	(18)	(49)	(50)
HEMANGIOSARCOMA			7 (14%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(18)	(49)	(50)
HEMANGIOSARCOMA		1 (2%)	
SITE UNKNOWN			
HEMANGIOSARCOMA		1	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	29	48
MORIBUND SACRIFICE		1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	17	19	
ANIMAL MISSING	2	1	
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	5	45	42
TOTAL PRIMARY TUMORS	5	61	43
TOTAL ANIMALS WITH BENIGN TUMORS	3	10	3
TOTAL BENIGN TUMORS	3	12	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	41	40
TOTAL MALIGNANT TUMORS	2	49	40
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	2
TOTAL SECONDARY TUMORS		2	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS ADMINISTERED 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
PNEUMONIA, ASPIRATION		2 (4%)	1 (2%)
INFLAMMATION, ACUTE		1 (2%)	
ABSCESS, NOS		1 (2%)	
PNEUMONIA, CHRONIC MURINE			4 (8%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(49)	(49)
HEMOSIDEROSIS			1 (2%)
#LYMPH NODE	(20)	(50)	(49)
HYPERPLASIA, RETICULUM CELL		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(49)
PERIARTERITIS			1 (2%)
#HEART/ATRIUM	(20)	(50)	(49)
THROMBOSIS, NOS	1 (5%)		
#MYOCARDIUM	(20)	(50)	(49)
FIBROSIS	5 (25%)	7 (14%)	3 (6%)
FIBROSIS, DIFFUSE			5 (10%)

\# 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%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(50)	(45)
METAPLASIA, SQUAMOUS			2 (4%)
#LIVER	(20)	(50)	(50)
NECROSIS, FOCAL		1 (2%)	
METAMORPHOSIS FATTY	2 (10%)	7 (14%)	9 (18%)
HYPERPLASIA, NODULAR		1 (2%)	
HYPERPLASIA, RETICULUM CELL		1 (2%)	
#BILE DUCT	(20)	(50)	(50)
CYST, NOS	1 (5%)		
HYPERPLASIA, NOS	1 (5%)	2 (4%)	1 (2%)
#PANCREAS	(20)	(47)	(49)
FIBROSIS	1 (5%)		2 (4%)
FIBROSIS, FOCAL			4 (8%)
PERIARTERITIS			1 (2%)
#SMALL INTESTINE	(20)	(49)	(49)
ULCER, NOS		1 (2%)	
#DUODENUM	(20)	(49)	(49)
POLYP		1 (2%)	
#COLON	(20)	(49)	(49)
NEMATODIASIS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
HYDRONEPHROSIS		1 (2%)	
CYST, NOS		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC	12 (60%)	30 (60%)	36 (72%)
NECROSIS, MEDULLARY	1 (5%)		
#URINARY BLADDER	(20)	(46)	(48)
INFLAMMATION, ACUTE		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(48)	(47)
CYST, 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
HYPERPLASIA, CHROMOPHOBE-CELL		1 (2%)	2 (4%)
#ADRENAL	(20)	(49)	(49)
LIPOIDOSIS			2 (4%)
#ADRENAL MEDULLA	(20)	(49)	(49)
SCAR		1 (2%)	
#THYROID	(19)	(49)	(49)
COLLOID CYST			1 (2%)
HYPERPLASIA, C-CELL		2 (4%)	4 (8%)
#PARATHYROID	(17)	(33)	(35)
HYPERPLASIA, NOS			1 (3%)
#PANCREATIC ISLETS	(20)	(47)	(49)
HYPERPLASIA, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
CYSTIC DUCTS	1 (5%)		
LACTATION	2 (10%)	2 (4%)	
*SEMINAL VESICLE	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
#TESTIS	(20)	(48)	(50)
ATROPHY, NOS		3 (6%)	1 (2%)
NERVOUS SYSTEM			
#CEREBRAL VENTRICLE	(19)	(50)	(49)
DILATATION, NOS			1 (2%)
#CEREBRUM	(19)	(50)	(49)
CYST, NOS			1 (2%)
#BRAIN	(19)	(50)	(49)
COMPRESSION		3 (6%)	
ATROPHY, PRESSURE			2 (4%)
#CEREBELLUM	(19)	(50)	(49)
DEMYELINIZATION			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
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(20)	(50)	(50)
OPACITIES		5 (10%)	3 (6%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
NECROSIS, FAT		1 (2%)	
*PERITONEUM	(20)	(50)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
*MESENTERY	(20)	(50)	(50)
PERIARTERITIS		7 (14%)	1 (2%)
NECROSIS, FAT		2 (4%)	
ALL OTHER SYSTEMS			
DIAPHRAGM			
HERNIA, NOS	3	3	1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	
AUTO/NECROPSY/HISTO PERF		1	

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, EPITHELIAL			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(50)
CONGESTION, NOS			1 (2%)
EDEMA, NOS		1 (2%)	
HEMORRHAGE			1 (2%)
PNEUMONIA, ASPIRATION			2 (4%)
INFLAMMATION, ACUTE SUPPURATIVE	1 (5%)		
PNEUMONIA, CHRONIC MURINE		2 (4%)	
HEMATOPOIETIC SYSTEM			
#MESENTERIC L. NODE	(19)	(49)	(50)
HYPERPLASIA, NOS			1 (2%)
CIRCULATORY SYSTEM			
#HEART/ATRIUM	(19)	(49)	(50)
THROMBOSIS, NOS			1 (2%)
#MYOCARDIUM	(19)	(49)	(50)
FIBROSIS		3 (6%)	4 (8%)
FIBROSIS, FOCAL			2 (4%)
#ENDOCARDIUM	(19)	(49)	(50)
FIBROSIS			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
*CEREBRAL ARTERY	(20)	(50)	(50)
INFLAMMATION PROLIFERATIVE		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(19)	(49)	(49)
DIVERTICULUM		1 (2%)	
FIBROSIS, FOCAL			1 (2%)
SCAR			1 (2%)
NECROSIS, FOCAL	1 (5%)	1 (2%)	1 (2%)
METAMORPHOSIS FATTY		2 (4%)	3 (6%)
HYPERPLASIA, NODULAR			1 (2%)
HYPERPLASTIC NODULE		1 (2%)	
#LIVER/HEPATOCYTES	(19)	(49)	(49)
NECROSIS, NOS		1 (2%)	
#PANCREAS	(19)	(46)	(50)
FIBROSIS			1 (2%)
FIBROSIS, FOCAL	1 (5%)		1 (2%)
ATROPHY, FOCAL			1 (2%)
#ESOPHAGUS	(19)	(48)	(49)
NECROSIS, FAT	1 (5%)		
#STOMACH	(19)	(49)	(50)
ULCER, NOS		1 (2%)	
#PEYERS PATCH	(19)	(48)	(49)
HYPERPLASIA, NOS			1 (2%)
#COLON	(19)	(48)	(50)
NEMATODIASIS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(19)	(49)	(50)
INFLAMMATION, CHRONIC	3 (16%)	4 (8%)	7 (14%)
CALCINOSIS, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(47)	(48)
CYST, NOS	2 (11%)	1 (2%)	1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, CHROMOPHOBE-CELL		3 (6%)	1 (2%)
#ADRENAL	(19)	(49)	(50)
INFARCT, NOS			1 (2%)
#ADRENAL CORTEX	(19)	(49)	(50)
METAMORPHOSIS FATTY		1 (2%)	
#THYROID	(19)	(49)	(50)
THYROGLOSSAL DUCT CYST		1 (2%)	
HYPERPLASIA, C-CELL	1 (5%)	4 (8%)	3 (6%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
CYSTIC DUCTS			1 (2%)
HYPERPLASIA, NODULAR	1 (5%)		
HYPERPLASIA, NOS			1 (2%)
LACTATION	5 (25%)	12 (24%)	8 (16%)
#UTERUS	(19)	(49)	(49)
ATROPHY, NOS			1 (2%)
POLYP		1 (2%)	
POLYP, INFLAMMATORY		2 (4%)	
#CERVIX UTERI	(19)	(49)	(49)
HAMARTOMA			1 (2%)
#UTERUS/ENDOMETRIUM	(19)	(49)	(49)
HYPERPLASIA, CYSTIC		2 (4%)	
NERVOUS SYSTEM			
#BRAIN	(19)	(49)	(49)
COMPRESSION		3 (6%)	
HEMORRHAGE		1 (2%)	
ATROPHY, PRESSURE			2 (4%)
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(20)	(50)	(50)
INFLAMMATION, ACUTE		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
OPACITIES	1 (5%)	5 (10%)	11 (22%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
NECROSIS, FAT		1 (2%)	
*MESENTERY	(20)	(50)	(50)
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
DIAPHRAGM			
HERNIA, NOS	1	4	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	7	5

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
ABSCESS, NOS	1 (5%)		
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
INFLAMMATION, NECRO GRAN			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
GRANULOMA, PYOGENIC		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(46)	(48)
HEMORRHAGE			1 (2%)
PNEUMONIA, ASPIRATION			1 (2%)
HEMOSIDEROSIS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(47)	(48)
GRANULOMA, NOS			1 (2%)
HYPERPLASIA, GRANULOCYTIC	1 (5%)		
#SPLEEN	(20)	(50)	(50)
NECROSIS, NOS			1 (2%)
HYPERPLASIA, NOS			1 (2%)
HEMATOPOIESIS	1 (5%)		3 (6%)
#MESENTERIC L. NODE	(20)	(50)	(48)
HEMORRHAGE	11 (55%)	29 (58%)	14 (29%)
HEMATOMA, NOS			1 (2%)
HYPERPLASIA, NOS			1 (2%)
#THYMUS	(12)	(39)	(27)
KARYORRHEXIS			1 (4%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NOS		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
PELIOSIS HEPATIS		1 (2%)	
NECROSIS, FOCAL			2 (4%)
NECROSIS, CENTRAL		1 (2%)	2 (4%)
INFARCT, NOS		1 (2%)	
METAMORPHOSIS FATTY		1 (2%)	2 (4%)
HYPERPLASIA, NODULAR			1 (2%)
HYPERPLASTIC NODULE	2 (10%)	4 (8%)	4 (8%)
#LIVER/CENTRILOBULAR	(20)	(50)	(50)
LIPOIDOSIS			1 (2%)
#STOMACH	(20)	(49)	(50)
ULCER, CHRONIC		1 (2%)	
#PEYERS PATCH	(20)	(46)	(43)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
#COLON	(20)	(49)	(48)
NEMATODIASIS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
HYDRONEPHROSIS			7 (14%)
GLOMERULONEPHRITIS, SUBACUTE		1 (2%)	
PYELONEPHRITIS, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL	2 (10%)		
NEPHROSIS, NOS			2 (4%)
INFARCT, FOCAL			1 (2%)
METAMORPHOSIS FATTY			1 (2%)
HEMOSIDEROSIS		1 (2%)	7 (14%)
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(50)	(34)
CYST, NOS	1 (6%)		

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#THYROID	(20)	(50)	(49)
CYSTIC FOLLICLES	17 (85%)	37 (74%)	33 (67%)
REPRODUCTIVE SYSTEM			
#TESTIS	(20)	(50)	(50)
NECROSIS, DIFFUSE			1 (2%)
INFARCT, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(50)
CALCIFICATION, FOCAL	13 (65%)	30 (60%)	32 (64%)
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(50)	(50)
CYST, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
NECROSIS, FAT		2 (4%)	1 (2%)
*PERITONEUM	(20)	(50)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
*MESENTERY	(20)	(50)	(50)
HEMORRHAGE	1 (5%)		
NECROSIS, FAT	1 (5%)	1 (2%)	
EOSINOPHILIC GRANULOMA		1 (2%)	
ALL OTHER SYSTEMS			
ORBITAL REGION			
ABSCESS, NOS			1

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	2	1	
ANIMALS NECROPSIED	18	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	18	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(18)	(49)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(18)	(47)	(48)
HYPEREMIA			1 (2%)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, INTERSTITIAL			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(16)	(48)	(49)
HYPERPLASIA, HEMATOPOIETIC			1 (2%)
#SPLEEN	(18)	(48)	(46)
HEMATOPOIESIS	1 (6%)		5 (11%)
#MESENTERIC L. NODE	(18)	(44)	(38)
HEMORRHAGE	2 (11%)	11 (25%)	2 (5%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(18)	(47)	(46)
MINERALIZATION			1 (2%)
*BLOOD VESSEL	(18)	(49)	(50)
INFLAMMATION, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(18)	(49)	(49)
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
NECROSIS, CENTRAL		2 (4%)	
METAMORPHOSIS FATTY		1 (2%)	
LIPOIDOSIS		1 (2%)	3 (6%)
HEMOSIDEROSIS		1 (2%)	
HYPERPLASIA, NODULAR	2 (11%)	1 (2%)	1 (2%)
HYPERPLASTIC NODULE	2 (11%)		1 (2%)
HEMATOPOIESIS			1 (2%)
#LIVER/CENTRILOBULAR	(18)	(49)	(49)
NECROSIS, NOS			1 (2%)
LIPOIDOSIS			1 (2%)
*GALLBLADDER	(18)	(49)	(50)
EOSINOPHILIC CYTO CHANGE	1 (6%)		
#PANCREAS	(16)	(47)	(36)
CYST, NOS		1 (2%)	
#STOMACH	(18)	(47)	(48)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
#PEYERS PATCH	(17)	(45)	(39)
HYPERPLASIA, DIFFUSE	2 (12%)		
#DUODENAL GLAND	(17)	(45)	(39)
HYPERPLASIA, NOS			1 (3%)
URINARY SYSTEM			
#KIDNEY	(18)	(49)	(48)
HYDRONEPHROSIS		2 (4%)	1 (2%)
GLOMERULONEPHRITIS, CHRONIC	2 (11%)		
NEPHROSIS, NOS		1 (2%)	1 (2%)
HEMOSIDEROSIS		18 (37%)	16 (33%)
#URINARY BLADDER	(16)	(39)	(34)
INFLAMMATION, CHRONIC DIFFUSE			1 (3%)
#U.BLADDER/SUBMUCOSA	(16)	(39)	(34)
FIBROSIS		1 (3%)	
ENDOCRINE SYSTEM			
#THYROID	(18)	(45)	(40)
CYSTIC FOLLICLES	17 (94%)	29 (64%)	12 (30%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(18)	(49)	(50)
METAPLASIA, SQUAMOUS	1 (6%)		
#UTERUS	(18)	(46)	(43)
PYOMETRA		1 (2%)	
NECROSIS, FAT	1 (6%)		
#UTERUS/ENDOMETRIUM	(18)	(46)	(43)
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, VESICULAR			1 (2%)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, CYSTIC	4 (22%)	12 (26%)	2 (5%)
#OVARY	(16)	(45)	(44)
CYST, NOS	6 (38%)	7 (16%)	3 (7%)
#MESOVARIUM	(16)	(45)	(44)
NECROSIS, FAT	2 (13%)		
NERVOUS SYSTEM			
#BRAIN	(18)	(49)	(48)
CALCIFICATION, FOCAL	6 (33%)	18 (37%)	18 (38%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*BONE	(18)	(49)	(50)
FIBROUS OSTEODYSTROPHY			1 (2%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
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
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1		2
ANIMAL MISSING/NO NECROPSY	2	1	
AUTO/NECROPSY/HISTO PERF		1	2

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Fibroma (b)	1/20 (5)	4/50 (8)	2/50 (4)
P Values (c,d)	N S.	N.S.	N.S.
Relative Risk (f)		1.600	0.800
Lower Limit		0.175	0.045
Upper Limit		77.169	46.273
Weeks to First Observed Tumor (g)	107	107	107
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	1/20 (5)	6/50 (12)	2/49 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.400	0.816
Lower Limit		0.325	0.046
Upper Limit		108.021	47.195
Weeks to First Observed Tumor	107	107	107

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)
(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma or Leukemia (b)	6/20 (30)	1/50 (2)	1/50 (2)
P Values (c,d)	P = 0.007 (N)	P = 0.002 (N)	P = 0.002 (N)
Departure from Linear Trend (e)	P less than 0.001		
Relative Risk (f)		0.067	0.067
Lower Limit		0.002	0.002
Upper Limit		0.506	0.506
Weeks to First Observed Tumor	89	92	73
Liver: Hepatocellular Carcinoma or Adenoma (b)	0/20 (0)	5/50 (10)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.525	0.386
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	100	107

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma (b)	2/19 (11)	6/48 (13)	15/47 (32)
P Values (c,d)	P = 0.006	N.S.	N.S.
Relative Risk (f)		1.188	3.032
Lower Limit		0.242	0.820
Upper Limit		11.426	25.659
Weeks to First Observed Tumor	107	92	84
Adrenal: Pheochromocytoma (b)	0/20 (0)	0/49 (0)	4/49 (8)
P Values (c,d)	P = 0.014	--	N.S.
Relative Risk (f)		--	Infinite
Lower Limit		--	0.394
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	107

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Carcinoma or Adenoma (b)	1/19 (5)	0/49 (0)	4/49 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.000	1.551
Lower Limit		0.000	0.171
Upper Limit		7.244	74.767
Weeks to First Observed Tumor	107	--	62
Testis: Interstitial-cell Tumor (b)	16/20 (80)	39/48 (81)	42/50 (84)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.016	1.050
Lower Limit		0.808	0.840
Upper Limit		1.415	1.439
Weeks to First Observed Tumor	93	92	62

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Tunica Vaginalis:			
Mesothelioma, NOS (b)	2/20 (10)	0/50 (0)	0/50 (0)
P Values (c,d)	N.S.	N.S.	N.S.
Departure From Linear Trend (e)	P = 0.009		
Relative Risk (f)		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.345	1.345
Weeks to First Observed Tumor	107	--	--

(a) Dosed groups received 1,250 or 5,000 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 is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

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

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

(g) Weeks to first observed tumor is based on time of death with tumor.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma or Leukemia (b)	3/20 (15)	4/50 (8)	1/50 (2)
P Values (c,d)	P = 0.036 (N)	N.S.	N.S.
Relative Risk (f)		0.533	0.133
Lower Limit		0.102	0.003
Upper Limit		3.410	1.568
Weeks to First Observed Tumor	72	88	107
Pituitary: Chromophobe			
Adenoma (b)	1/19 (5)	13/47 (28)	15/48 (31)
P Values (c,d)	N.S.	P = 0.039	P = 0.020
Relative Risk (f)		5.255	5.938
Lower Limit		0.900	1.043
Upper Limit		217.071	242.667
Weeks to First Observed Tumor	107	92	88

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland:			
Adenoma, NOS (b)	0/20 (0)	6/50 (12)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Departure From Linear Trend (e)	P = 0.027		
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.667	0.022
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	103	107
Mammary Gland:			
Fibroadenoma (b)	4/20 (20)	10/50 (20)	6/50(12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.000	0.600
Lower Limit		0.339	0.164
Upper Limit		3.991	2.659
Weeks to First Observed Tumor	102	107	107

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp (b)	5/19 (26)	5/49 (10)	8/49 (16)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.388	0.620
Lower Limit		0.104	0.213
Upper Limit		1.529	2.172
Weeks to First Observed Tumor	107	107	107

(a) Dosed groups received 1,250 or 5,000 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 is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

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

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED 4-CHLORO-o-TOLUIDINE HYDROCHLORIDE IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	2/20 (10)	7/46 (15)	1/48 (2)
P Values (c,d)	P = 0.032 (N)	N.S.	N.S.
Relative Risk (f)		1.522	0.208
Lower Limit		0.330	0.004
Upper Limit		14.233	3.830
Weeks to First Observed Tumor	99	96	95
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	4/20 (20)	14/46 (30)	3/48 (6)
P Values (c,d)	P = 0.006 (N)	N.S.	N.S.
Relative Risk (f)		1.522	0.313
Lower Limit		0.567	0.051
Upper Limit		5.728	1.708
Weeks to First Observed Tumor	99	96	95

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)
(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma (b)	1/20 (5)	3/50 (6)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.200	0.400
Lower Limit		0.106	0.005
Upper Limit		61.724	30.802
Weeks to First Observed Tumor	99	99	99
All Sites: Hemangioma (b)	0/20 (0)	3/50 (6)	5/50 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.250	0.525
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	99	65

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangiosarcoma (b)	0/20 (0)	3/50 (6)	37/50 (74)
P Values (c,d)	P less than 0.001	N.S.	P less than 0.001
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.250	5.243
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	87	66
All Sites: Hemangioma or Hemangiosarcoma (b)	0/20 (0)	6/50 (12)	41/50 (82)
P Values (c,d)	P less than 0.001	N.S.	P less than 0.001
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.667	5.895
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	87	65

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	4/20 (20)	5/50 (10)	7/50 (14)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.500	0.700
Lower Limit		0.124	0.207
Upper Limit		2.322	2.994
Weeks to First Observed Tumor	99	99	77
Liver: Hepatocellular Carcinoma or Adenoma (b)	4/20 (20)	7/50 (14)	10/50 (20)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.700	1.000
Lower Limit		0.207	0.339
Upper Limit		2.994	3.991
Weeks to First Observed Tumor	99	99	77

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)

(continued)

(a) Dosed groups received 3,750 or 15,000 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 is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

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

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

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma (b)	0/18 (0)	2/47 (4)	3/48 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.118	0.236
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	99	64
Hematopoietic System: Lymphoma or Leukemia (b)	1/18 (6)	6/49 (12)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.204	0.360
Lower Limit		0.302	0.005
Upper Limit		99.144	27.724
Weeks to First Observed Tumor	99	72	82

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)
(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangioma (b)	1/18 (6)	6/49 (12)	0/50 (0)
P Values (c,d)	P = 0.024 (N)	N.S.	N.S.
Relative Risk (f)		2.204	0.000
Lower Limit		0.302	0.000
Upper Limit		99.144	6.729
Weeks to First Observed Tumor	99	65	--
All Sites: Hemangiosarcoma (b)	0/18 (0)	40/49 (82)	39/50 (78)
P Values (c,d)	P = 0.001	P less than 0.001	P less than 0.001
Departure From Linear Trend (e)	P less than 0.001		
Relative Risk (f)		Infinite	Infinite
Lower Limit		5.314	5.043
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	43	66

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangioma or Hemangiosarcoma (b)	1/18 (6)	44/49 (90)	39/50 (78)
P Values (c,d)	P = 0.005	P less than 0.001	P less than 0.001
Departure From Linear Trend (e)	P less than 0.001		
Relative Risk (f)		16.163	14.040
Lower Limit		3.420	2.836
Upper Limit		525.757	518.295
Weeks to First Observed Tumor	99	43	66
Liver: Hepatocellular Carcinoma or Adenoma (b)	1/18 (6)	4/49 (8)	0/49 (0)
P Values (c,d)	P = 0.047 (N)	N.S.	N.S.
Relative Risk (f)		1.469	0.000
Lower Limit		0.163	0.000
Upper Limit		70.853	6.864
Weeks to First Observed Tumor	99	96	--

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 4-Chloro-o-Toluidine Hydrochloride in the Diet (a)

(continued)

(a) Dosed groups received 1,250 or 5,000 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 is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

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

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

Review of the Bioassay of 4-Chloro-*o*-toluidine HCL* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

December 13, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute on the Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, and State health officials. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 4-Chloro-*o*-toluidine HCL.

The primary reviewer for the report on the bioassay of 4-Chloro-*o*-toluidine HCL said that the compound was not carcinogenic in treated rats but was sarcomagenic in treated mice. After a brief description of the experimental design and conditions of test, the reviewer commented on the high incidence of hemosiderosis in the kidneys of treated mice and on the earlier mortality among high-dose treated mice. He raised the question as to whether the sarcomagenic effect might be a secondary response to the deposition of iron in the reticulo-endothelial system of the treated mice. In comparison to other aromatic amines, the reviewer said 4-Chloro-*o*-toluidine HCL appeared to be a relatively weak carcinogen.

The secondary reviewer noted the reduced incidence of lymphomas in treated rats and lung tumors in treated male mice. Since the relationship of the hemagiosarcomas and hemangiomas to humans is nebulous, he recommended that the conclusion simply state the fact that the compound induced the neoplasms and that the term "carcinogenic" be dropped.

There was no objection to the primary reviewer's motion that the report on the bioassay of 4-Chloro-*o*-toluidine HCL be approved as written.

Clearinghouse Members Present:

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

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

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