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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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REPORT ON THE BIOASSAY OF 4-CHLORO-m-PHENYLENEDIAMINE
FOR POSSIBLE CARCINOGENICITY

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

FOREWORD: This report presents the results of the bioassay of 4-chloro-m-phenylenediamine conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 4-chloro-m-phenylenediamine was conducted by Mason Research Institute, Worcester, Massachusetts, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. E. Smith (3) and Dr. A. Handler (3). Animal treatment and observation were supervised by Mr. G. Wade (3) and Ms. E. Zepp (3). Chemical analysis was performed by Midwest Research Institute (4) and the analytical results were reviewed by Dr. N. Zimmerman (5).

Histopathologic examinations were performed by Dr. A. S. Krishna Murthy (3) and Dr. A. Russfield (3) at the Mason Research Institute, the pathology narratives were written by Dr. A. S. Krishna Murthy (3), and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (6).

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

This report was prepared at METREK, a Division of The MITRE Corporation (5) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (5), task leader Dr. M. R. Kornreich (5), senior biologist Ms. P. Walker (5), biochemist Mr. S. C. Drill (5), and technical editor Ms. P. A. Miller (5). The final report was reviewed by members of the participating organizations.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of the NCI: Dr. J. J. Gart (8), Mr. J. Nam (8), Dr. H. M. Pettigrew (8), and Dr. R. E. Tarone (8).

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

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SUMMARY

A bioassay of 4-chloro-m-phenylenediamine for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F1 mice. 4-Chloro-m-phenylenediamine was administered in the feed, at either of two concentrations, to groups of 49 or 50 male and 50 female animals of each species. The dietary concentrations used in the chronic bioassay for low and high dose rats were 0.2 and 0.4 percent, respectively. The time-weighted average dietary concentrations used for low and high dose mice were 0.7 and 1.4 percent, respectively. After a 78-week period of compound administration, observation of rats continued for an additional 27 weeks and observation of mice continued for an additional 17 weeks. For each species, 50 animals of each sex were placed on test as untreated controls.

In both species, adequate numbers of animals in all groups survived long enough to be at risk from late-developing tumors.

Among male rats, an increased incidence of adrenal pheochromocytomas was statistically associated with dosage of 4-chloro-m-phenylenediamine. The incidence of these tumors was significantly higher in the high dose group than in the control group.

Among female mice, there was a significantly increased incidence of hepatocellular carcinomas in the low dose group and a significantly increased combined incidence of hepatocellular carcinomas and hepatocellular adenomas in both low and high dose groups as compared to controls.

No other neoplasms in either species were considered to be related to compound administration.

Under the conditions of this bioassay, dietary administration of 4-chloro-m-phenylenediamine was carcinogenic to the experimental animals, causing an increased incidence of hepatocellular tumors in female B6C3F1 mice and an increased incidence of adrenal pheochromocytomas in male Fischer 344 rats.

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I. INTRODUCTION

4-Chloro-m-phenylenediamine (NCI No. C03305), an intermediate in the preparation of dyes, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer reported among workers in the dye manufacturing industry (Anthony and Thomas, 1970; Wynder et al., 1963). Occupational exposure to several types of compounds, including aromatic amines, is thought to contribute to the increased cancer risk in this industry (Wynder et al., 1963).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 4-chloro-1,3-benzenediamine.* It is also called 4-chlorophene-1,3-diamine; 4-chloro-1,3-phenylenediamine; and C.I. (Colour Index) 76027.

4-Chloro-m-phenylenediamine can be used as an intermediate in the production of C.I. Mordant Brown 48, C.I. Acid Brown 145, Paranil Brown G., C.I. Direct Black 11, and Diamine Fast Black XCD (Society of Dyers and Colourists, 1956), and in the synthesis of disazo pigments (Mueller, 1975; Papenfuhs, 1975). The compound can also be used as a coupler in hair dye preparations (Corbett, 1973).

Patents have been issued for several applications of 4-chloro-m-phenylenediamine including use as a color forming agent in formulations applied to paper used for microfilm enlargements (Wiswell,

*The CAS registry number is 5131-60-2.

1968), a color intensifier in photographic films (Mattor and Price, 1970), and a cross-linking agent for polyurethane manufacture (Krisnan, 1975).

Specific production figures for 4-chloro-m-phenylenediamine are not available; however, the inclusion of the compound in the 1977 Directory of Chemical Producers, U.S.A. (Stanford Research Institute, 1977) implies an annual production in excess of 1000 pounds or $1000 in value.

The potential for exposure to 4-chloro-m-phenylenediamine may be considerable for workers in dye and chemical production facilities and those engaged in research involving this compound.

II. MATERIALS AND METHODS

A. Chemicals

4-Chloro-m-phenylenediamine (Figure 1) was purchased from Carroll Products, Wood River Junction, Rhode Island and analysis was performed by Midwest Research Institute, Kansas City, Missouri. The narrow range of the experimentally determined melting point (87° to 90°C) and its proximity to the literature value (91°C) suggested a compound of high purity. Elemental analysis yielded results closely approximating the theoretical analyses. Thin-layer chromatography utilizing two solvent systems (benzene:methanol and methanol:acetic acid) and visualized with ultraviolet light and furfural revealed, respectively, one and two impurities. Vapor-phase chromatography showed the presence of one extraneous peak. Nonaqueous titration of the amine function was approximately 97 percent of the theoretical amount. This indicates maximum possible purity only, as other amine compounds might also have been present.

Throughout this report the term 4-chloro-m-phenylenediamine is used to represent this compound.

B. Dietary Preparation

The basal laboratory diet for both treated and control animals consisted of Wayne Lab-Blox® (Allied Mills, Inc., Chicago, Illinois). 4-Chloro-m-phenylenediamine was administered to the treated animals as a component of the diet. The compound was ground in a Quaker City crystal mill, added to an aliquot of ground feed, and hand mixed with

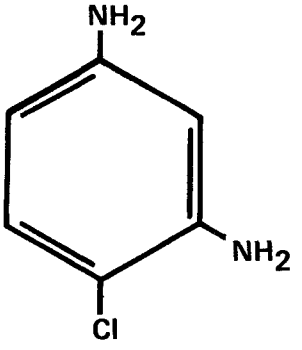

FIGURE 1
CHEMICAL STRUCTURE OF 4-CHLORO-m-PHENYLENEDIAMINE

a mortar and pestle until visual uniformity was attained. This premix was then placed into a 6 kg capacity Patterson-Kelley twin-shell stainless steel V-blender along with the remainder of the meal and blended for 20 minutes. Prepared diets were placed in double plastic bags and stored in the dark at 4°C. Mixtures were prepared weekly and stored for not longer than 2 weeks.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. Fischer 344 rats and B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. The animals were received in several separate shipments from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Rats and mice to be treated were received 3 weeks later than rats and mice to be used as controls.

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

D. Animal Maintenance

All animals were housed by species in rooms having a temperature range of 23° to 34°C. Incoming air was filtered through Tri-Dek® 15/40 denier Dacron® filters (Tri-Dim Filter Corp., Hawthorne, New

Jersey) providing six changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

Rats were housed five per cage by sex. During quarantine and for the first 13 months of study, rats were kept in galvanized- or stainless-steel wire-mesh cages suspended above newspapers. Newspapers under cages were replaced daily and cages and racks washed weekly. For the remainder of the study, rats were held in suspended polycarbonate cages equipped with disposable nonwoven fiber filter sheets. Clean bedding and cages were provided twice weekly. Corncob bedding, SAN-I-CEL® (Paxton Processing Company, Paxton, Illinois), was used for the first 6 months that the rats were housed in polycarbonate cages. Thereafter, a hardwood chip bedding (Aspen bedding, American Excelsior Company, Baltimore, Maryland) was used. Stainless steel cage racks were cleaned every 2 weeks, and disposable filters were replaced at that time.

Mice were housed by sex in polycarbonate shoe box type cages. Cages were fitted with perforated stainless steel lids (Lab Products, Inc., Garfield, New Jersey) and nonwoven fiber filter bonnets. Mice were housed ten per cage for the first 11 months of study and five per cage thereafter. Clean cages, lids, and bedding were provided three times per week when cage populations were ten and twice per week when cage populations were reduced to five. SAN-I-CEL® was used as bedding for the first 12 months of study. A second corncob bedding (Bed-o-Cobs®, The Andersons Cob Division, Maumee, Ohio) was

used for the next 8 months. Aspen bedding was then used until the end of study. Reusable filter bonnets and pipe racks were sanitized every 2 weeks throughout the study.

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

Wayne Lab-Blox® was used throughout the entire bioassay. Wayne Lab-Blox® meal containing the appropriate concentrations of 4-chloro-m-phenylenediamine was supplied to treated animals during the period of compound administration. Control animals had untreated meal available. Meal was dispensed in Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc., Woburn, Massachusetts) with stainless steel baffles for the first 13 months of study. After that time, animals were fed from stainless steel gangstyle hoppers (Scientific Cages, Inc., Bryan, Texas). Food hoppers were changed on the same schedule as were cages. Food was replenished daily in Alpine® feed cups.

4-Chloro-m-phenylenediamine-treated and control rats were housed in a room with other rats receiving diets containing* acetylaminofluorene (53-96-3); p-cresidine (120-71-8); 2,3,5,6-tetrachloro-4-nitroanisole (2438-88-2); 4-chloro-o-phenylenediamine (95-83-0); and 1H-benzotriazole (95-14-7).

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*CAS registry numbers are given in parentheses.

Treated and control mice shared a room with other mice receiving diets containing 2-methyl-1-nitroanthraquinone (129-15-7); p-cresidine (120-71-8); fenaminosulf (140-56-7); and acetylaminofluorene (53-96-3).

E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of 4-chloro-m-phenylenediamine for administration to treated animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among several groups, each consisting of five males and five females. The chemical was incorporated into the basal laboratory diet and supplied ad libitum to four of five rat groups in concentrations of 0.1, 0.3, 1.0, and 3.0 percent and five of six mouse groups in concentrations of 0.03, 0.1, 0.3, 1.0 and 3.0 percent. The fifth rat group and the sixth mouse group served as controls, receiving only the basal laboratory diet. The dosed dietary preparations were administered for a period of 8 weeks. All survivors were sacrificed at the end of the 8-week period.

The highest concentration causing no deaths, no compound-related abnormalities, and no mean group body weight depression in excess of 15 percent was selected as the high concentration utilized for the rat and mouse chronic bioassays.

All rats receiving dietary concentrations of 3.0 percent 4-chloro-m-phenylenediamine and three male rats receiving 1.0 percent died by

the end of the 8-week study. No other deaths were recorded among rats in the subchronic test. At the end of the subchronic study, males treated with 1.0 and 0.3 percent weighed approximately 80 and 97 percent, respectively, the weight of controls and females receiving the same concentrations weighed approximately 84 and 104 percent the weight of controls. A 4-chloro-m-phenylenediamine concentration of 0.4 percent was selected for use as the high dose in the rat chronic bioassay for both males and females.

Two deaths were observed among mice treated with the compound; one, a female fed a concentration of 3.0 percent and the other, a female fed a concentration of 0.3 percent. Mean group body weight depression was approximately 74 and 90 percent in males treated with 3.0 and 1.0 percent 4-chloro-m-phenylenediamine, respectively, and 85 and 88 percent in females receiving the same concentrations. The concentration of the compound selected for use as the high dose for males and females in the mouse chronic bioassay was 2.0 percent.

F. Experimental Design

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

At initiation of the study, the rats were approximately 6 weeks old. Rats received dietary concentrations of 0.4 or 0.2 percent throughout the 78-week period of compound administration. Throughout this report the rat groups receiving the former concentration are

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
4-CHLORO-m-PHENYLENEDIAMINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	4-CHLORO-m-PHENYLENEDIAMINE CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	50	0	0	106
LOW DOSE	50	0.2	78	
		0		26
HIGH DOSE	49	0.4	78	
		0		27
FEMALE				
CONTROL	50	0	0	106
LOW DOSE	50	0.2	78	
		0		26
HIGH DOSE	50	0.4	78	
		0		27

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
4-CHLORO-m-PHENYLENEDIAMINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	4-CHLORO-m-PHENYLENEDIAMINE CONCENTRATION (PERCENT)	OBSERVATION PERIOD		TIME-WEIGHTED AVERAGE CONCENTRATION[a] (PERCENT)
			TREATED (WEEKS)	UNTREATED (WEEKS)	
MALE					
CONTROL	50	0	0	97	0
LOW DOSE	50	1.0	29		0.7
		0.5	49		
		0		17	
HIGH DOSE	50	2.0	29		1.4
		1.0	49		
		0		17	
FEMALE					
CONTROL	50	0	0	97	0
LOW DOSE	50	1.0	29		0.7
		0.5	49		
		0		17	
HIGH DOSE	49	2.0	29		1.4
		1.0	49		
		0		17	

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

referred to as the high dose groups while those receiving the latter are referred to as the low dose groups. Control animals had untreated feed available. After the 78-week dosing period, rats were observed for an additional 26 or 27 weeks.

At initiation of the study the mice were approximately 6 weeks old. Mice initially received dietary concentrations of 2.0 or 1.0 percent. Throughout this report those mice initially receiving the former concentration are referred to as the high dose groups while those receiving the latter concentration are referred to as the low dose groups. After 29 weeks, the dosages were lowered to 1.0 and 0.5 percent for high and low dose mice, respectively. These concentrations were maintained for the remainder of the 78-week dosing period. After the period of chemical administration, mice were observed for an additional 17 weeks.

G. Clinical and Histopathologic Examinations

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

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

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

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, seminal vesicle, brain, uterus, mammary gland, and ovary.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined

microscopically varies and does not necessarily represent the number of animals that were placed on experiment in each group.

H. Data Recording and Statistical Analyses

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

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

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

for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

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

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

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

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

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing

these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

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

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it

can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

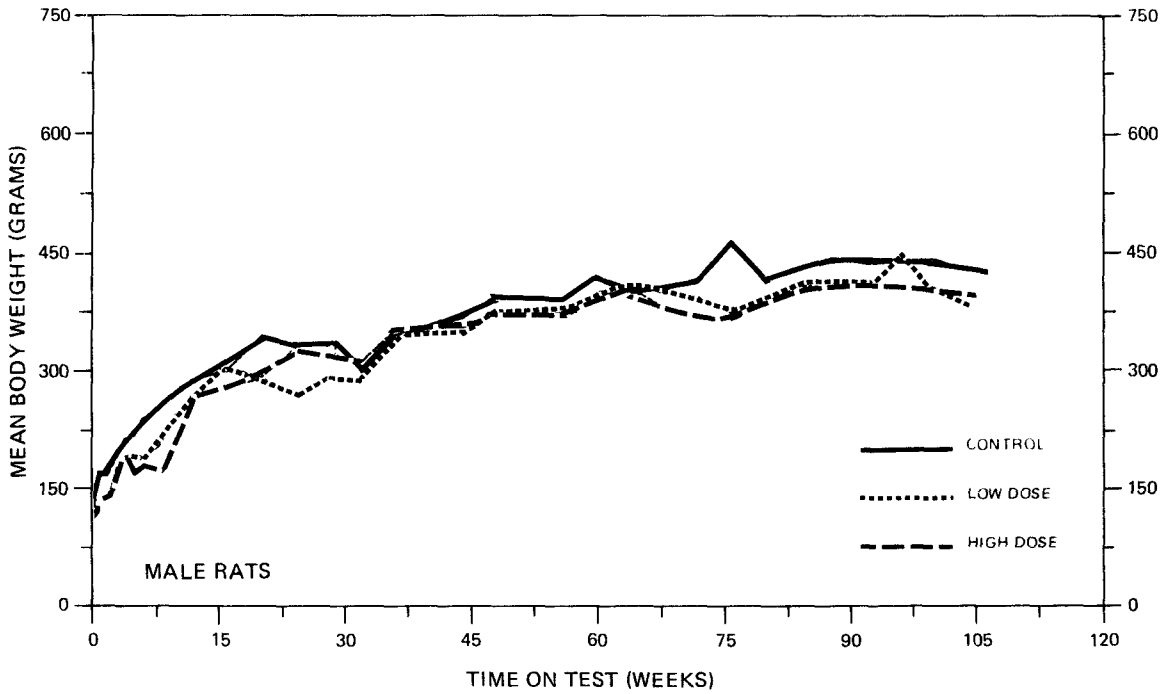
No consistent pattern of mean group body weight depression was observed among rats of either sex (Figure 2).

Subcutaneous masses were detected in two control males, three control females, three low dose males, one low dose female, five high dose males, and four high dose females. Cutaneous lesions/masses were observed in two control males, two low dose females, one high dose male, and one high dose female. Abdominal distention and cyanosis were observed in one low dose male. Two control males exhibited eye discoloration. No other clinical abnormalities were reported for either males or females.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 4-chloro-m-phenylenediamine-dosed groups are shown in Figure 3.

In male rats the Tarone test did not indicate a significant association between increased dosage and accelerated mortality. Five rats from the high dose group and five rats from the control group were sacrificed in week 78. There were adequate numbers of male rats at risk from late-developing tumors with 73 percent (36/49) of the high dose, 86 percent (43/50) of the low dose, and 64 percent (32/50) of the control group surviving until the end of the study.



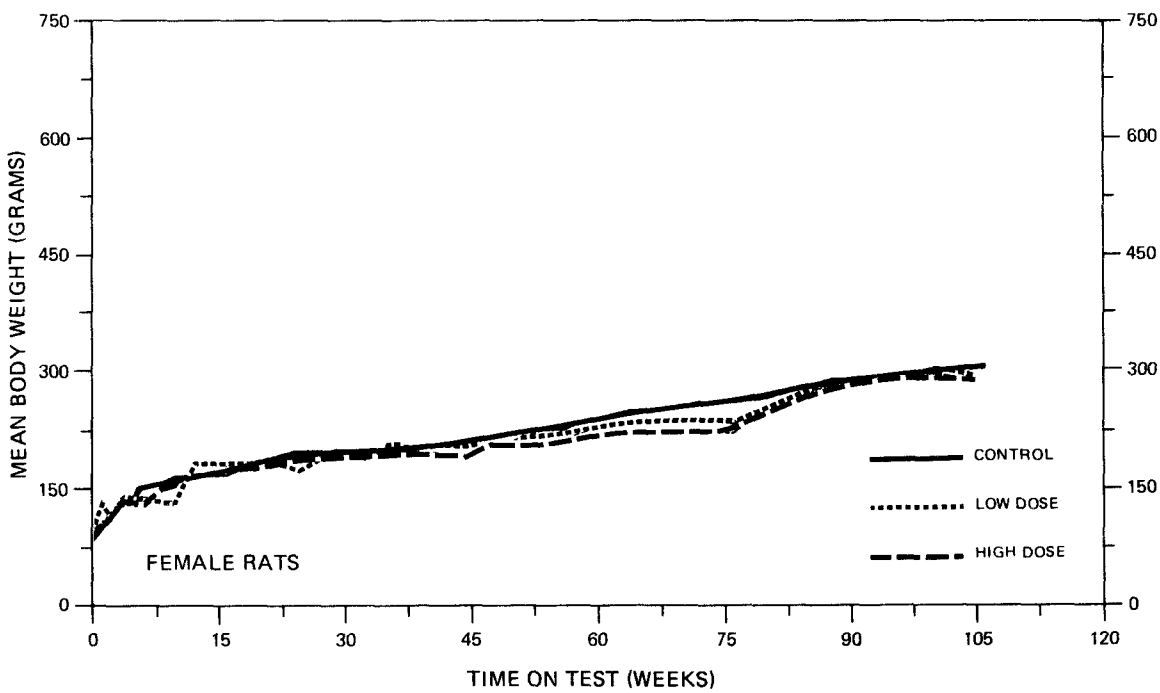
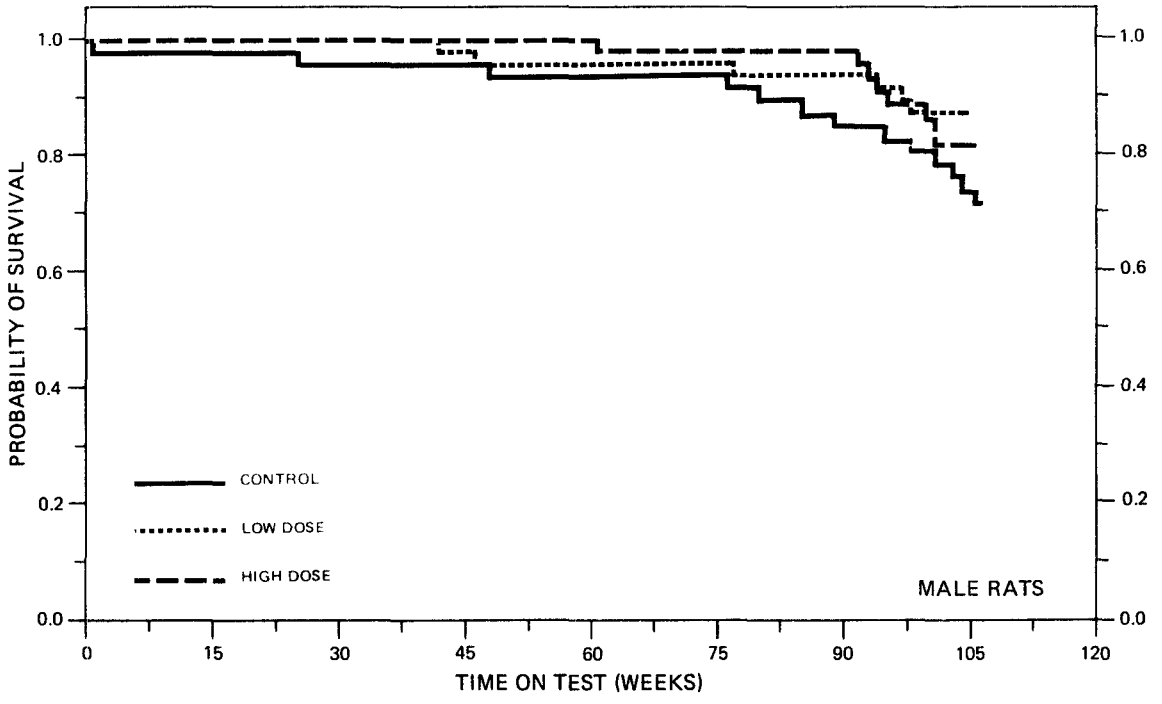


FIGURE 2
GROWTH CURVES FOR 4-CHLORO-m-PHENYLENEDIAMINE CHRONIC STUDY RATS



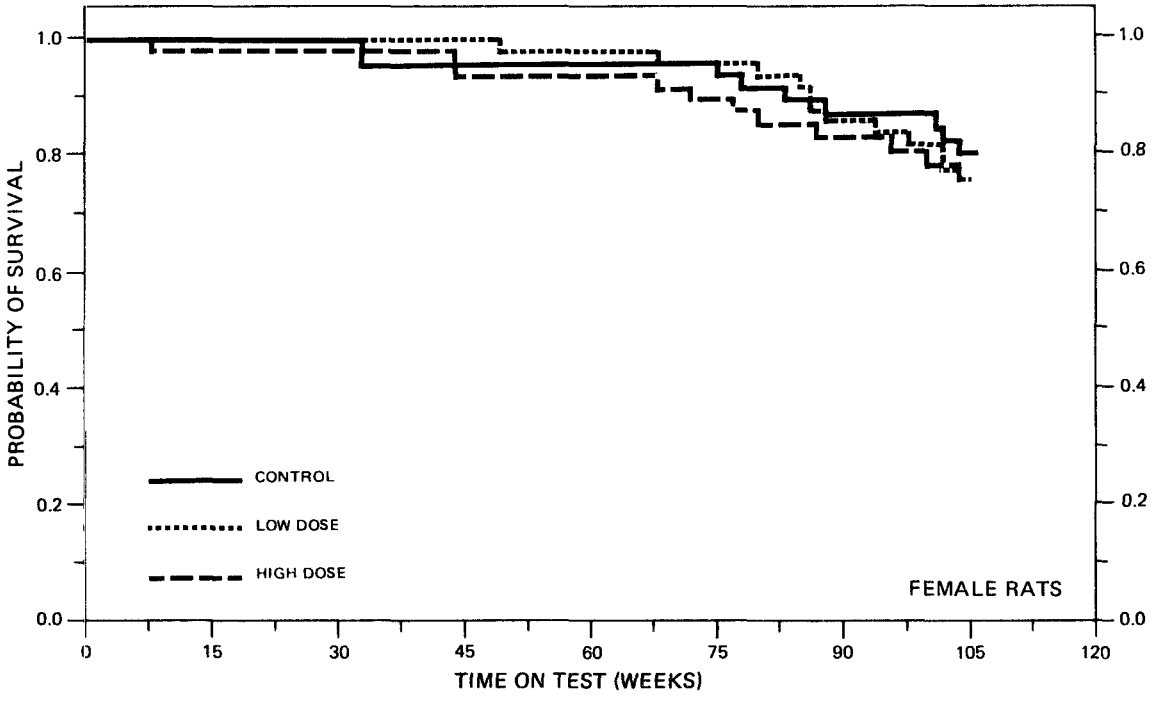


FIGURE 3
SURVIVAL COMPARISONS OF 4-CHLORO-m-PHENYLENEDIAMINE CHRONIC STUDY RATS

In female rats the Tarone test again did not indicate a significant association between increased dosage and accelerated mortality. Five rats from the high dose group and five rats from the control group were sacrificed in week 78. There were adequate numbers of female rats at risk from late-developing tumors with 66 percent (33/50) of the high dose, 78 percent (39/50) of the low dose, and 72 percent (36/50) of the control group surviving until the termination of the study.

C. Pathology

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

A variety of neoplasms were seen in both control and treated rats. There were instances of neoplastic lesions occurring only in treated animals, or with increased frequency in the treated groups when compared to the control group. The nature and incidences of the majority of the lesions were similar to those known to occur spontaneously in aged Fischer 344 rats. However, the incidence of adrenal pheochromocytoma was higher in the treated male rats (14/48 [29 percent] high dose, 7/48 [15 percent] low dose) than in the control (4/46 [9 percent]) male rats in this study. Zymbals' gland tumors occurred only in treated rats (2/49 [4 percent] high dose males and 1/47 [2 percent] high dose females).

A variety of nonneoplastic lesions were observed in this study. All of these lesions are commonly seen in aged Fischer 344 rats and occurred with approximately equal frequency in the treated and control animals.

Based on the results of this histopathologic examination, the administration of 4-chloro-m-phenylenediamine was associated with an increased incidence of adrenal pheochromocytomas in male Fischer 344 rats.

D. Statistical Analyses of Results

The results of the statistical anlayses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of tumor in either sex where at least two such tumors were observed in at least one of the control or 4-chloro-m-phenylenediamine-dosed groups and where such tumors were observed in at least 5 percent of the group.

In male rats the incidence of adrenal pheochromocytomas was increased in the treated groups. The Cochran-Armitage test indicated a significant ($P = 0.007$) positive association between dosage and tumor incidence. The Fisher exact test comparing the high dose treated males to the control supported these findings with significant ($P = 0.011$) results. The historical combined incidence rate for pheochromocytomas and malignant pheochromocytomas was 32/250 (13 percent) in data collected on the untreated male Fischer 344 rats at Mason Research Institute for the NCI Carcinogenesis Testing Program. Based

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 4-CHLORO-m-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	3/48(0.06)	3/49(0.06)	4/49(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.980	1.306
Lower Limit	---	0.138	0.233
Upper Limit	---	6.979	8.495
Weeks to First Observed Tumor	105	104	104
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	3/48(0.06)	1/49(0.02)	0/49(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.327	0.000
Lower Limit	---	0.006	0.000
Upper Limit	---	3.898	1.628
Weeks to First Observed Tumor	105	104	---
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	7/48(0.15)	0/49(0.00)	1/49(0.02)
P Values[c]	P = 0.007(N)	P = 0.006(N)	P = 0.028(N)
Departure from Linear Trend[e]	P = 0.036	---	---
Relative Risk (Control)[d]	---	0.000	0.140
Lower Limit	---	0.000	0.003
Upper Limit	---	0.504	1.029
Weeks to First Observed Tumor	80	---	93

TABLE 3 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma NOS[b]	10/45(0.22)	6/43(0.14)	15/47(0.32)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.628	1.436
Lower Limit	---	0.205	0.679
Upper Limit	---	1.732	3.185
Weeks to First Observed Tumor	103	104	78
Adrenal: Pheochromocytoma[b]	4/46(0.09)	7/48(0.15)	14/48(0.29)
P Values[c]	P = 0.007	N.S.	P = 0.011
Relative Risk (Control)[d]	---	1.677	3.354
Lower Limit	---	0.459	1.153
Upper Limit	---	7.336	12.990
Weeks to First Observed Tumor	78	104	78
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	5/43(0.12)	3/48(0.06)	6/48(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.538	1.075
Lower Limit	---	0.088	0.295
Upper Limit	---	2.595	4.153
Weeks to First Observed Tumor	95	104	95

TABLE 3 (Concluded)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Testis: Interstitial-Cell Tumor[b]	37/48(0.77)	43/49(0.88)	45/49(0.92)
P Values[c]	P = 0.027	N.S.	P = 0.041
Relative Risk (Control)[d]	---	1.138	1.191
Lower Limit	---	0.926	0.981
Upper Limit	---	1.353	1.366
Weeks to First Observed Tumor	78	94	78

[a]Treated groups received time-weighted averaged doses of 0.2 or 0.4 percent in feed.

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

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

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

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 4-CHLORO-m-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma,NOS or Chromophobe Adenoma[b]	17/40(0.43)	15/46(0.33)	17/44(0.39)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.767	0.909
Lower Limit	---	0.417	0.514
Upper Limit	---	1.413	1.621
Weeks to First Observed Tumor	101	68	78
Adrenal: Pheochromocytoma[b]	6/48(0.13)	2/49(0.04)	4/46(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.327	0.696
Lower Limit	---	0.034	0.154
Upper Limit	---	1.720	2.736
Weeks to First Observed Tumor	106	104	78
Mammary Gland: Fibroadenoma[b]	6/50(0.12)	4/50(0.08)	5/47(0.11)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.667	0.887
Lower Limit	---	0.147	0.229
Upper Limit	---	2.635	3.249
Weeks to First Observed Tumor	106	85	68

TABLE 4 (Concluded)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Uterus: Endometrial Stromal Polyp[b]	2/48(0.04)	12/48(0.25)	5/46(0.11)
P Values[c]	N.S.	P = 0.004	N.S.
Departure from Linear Trend[e]	P = 0.004	---	---
Relative Risk (Control)[d]	---	6.000	2.609
Lower Limit	---	1.438	0.453
Upper Limit	---	52.712	26.300
Weeks to First Observed Tumor	106	104	105

[a]Treated groups received time-weighted average doses of 0.2 or 0.4 percent in feed.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when P < 0.05; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when P < 0.05: otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

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

[e]The probability level of the test for departure from linear trend is given beneath the control group when P < 0.05.

on the results of these findings, the administration of 4-chloro-m-phenylenediamine was associated with the incidence of adrenal pheochromocytoma in male rats.

For female rats an increase in the incidence of endometrial stromal polyps was observed. The Fisher exact test comparing the incidence of tumors in the low dose group to that in the control was significant (P = 0.004). The historical incidence of this tumor in data on untreated female Fischer 344 rats at Mason Research Institute was 31/249 (12 percent), which was greater than that observed for either the control or the high dose group. Based upon these results the statistical conclusion was that there was inadequate evidence to conclude that compound administration induced these uterine tumors.

For male rats the Cochran-Armitage test for interstitial-cell tumors of the testis indicated a significant (P = 0.027) positive association between dosage and incidence. The Fisher exact test comparing the high dose treated males to the controls yielded a value of P = 0.041, a marginal result which was not significant under the Bonferroni criterion.

For male rats the possibility of a negative association between chemical administration and the incidence of malignant lymphomas or leukemia was noted.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

A distinct pattern of dose-related mean group body weight depression was apparent in mice of both sexes (Figure 4).

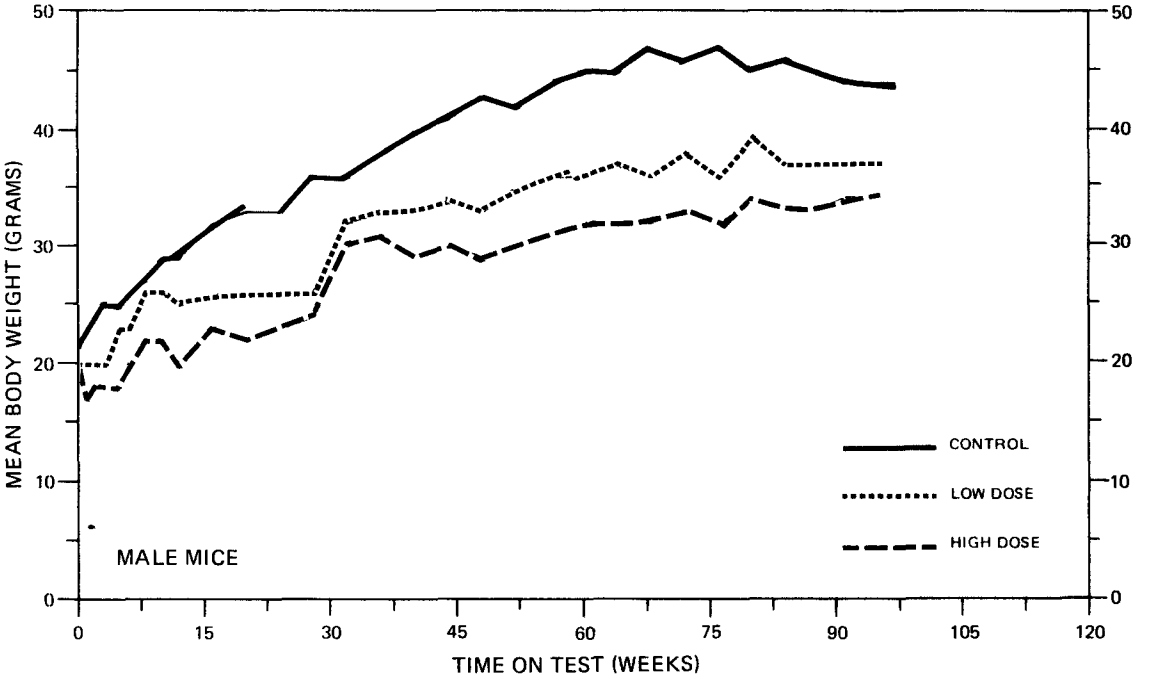
No clinical abnormalities were reported for male or female mice.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 4-chloro-m-phenylenediamine-dosed groups are shown in Figure 5.

In male mice the Tarone test for association between increased dosage and accelerated mortality was not significant. Five high dose and five control males were sacrificed in week 78. Survival was relatively good with 80 percent (40/50) of the high dose, 82 percent (41/50) of the low dose, and 84 percent (42/50) of the controls living until the end of the study. Thus, there were adequate numbers of male mice at risk from late-developing tumors.

In female mice the Tarone test did not indicate a significant association between increased dosage and mortality. Five high dose and five control females were sacrificed in week 78. There were adequate numbers of female mice at risk from late-developing tumors as 78 percent (38/49) of the high dose, 74 percent (37/50) of the low dose, and 72 percent (36/50) of the controls survived until termination of the study.



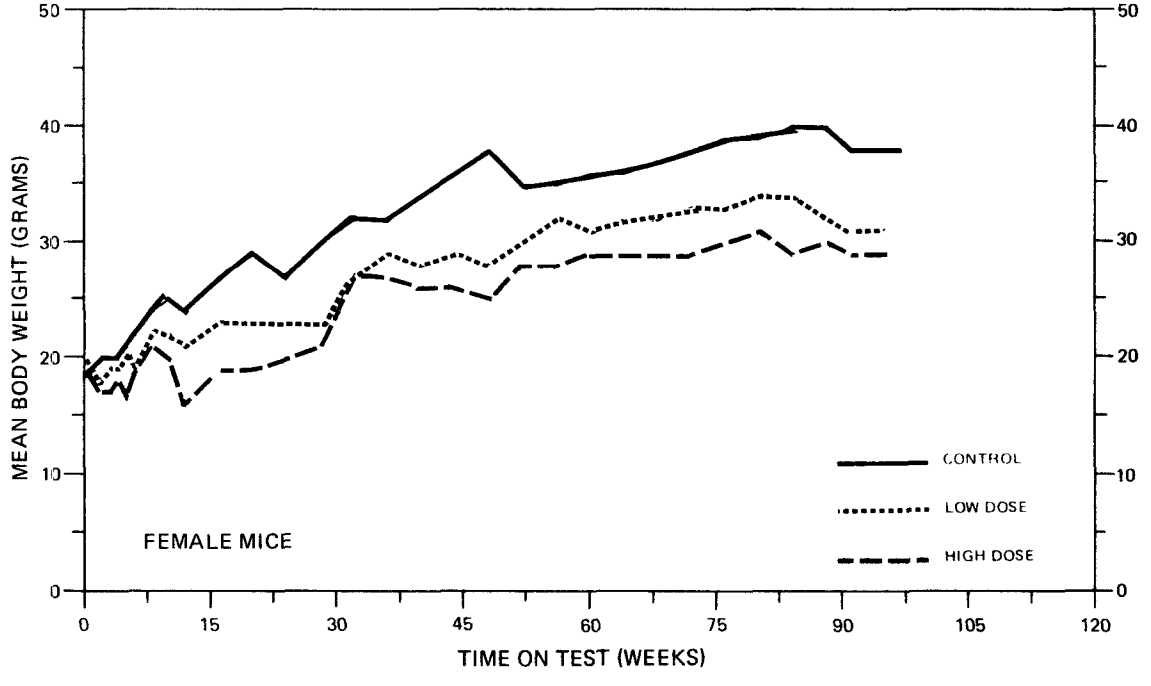
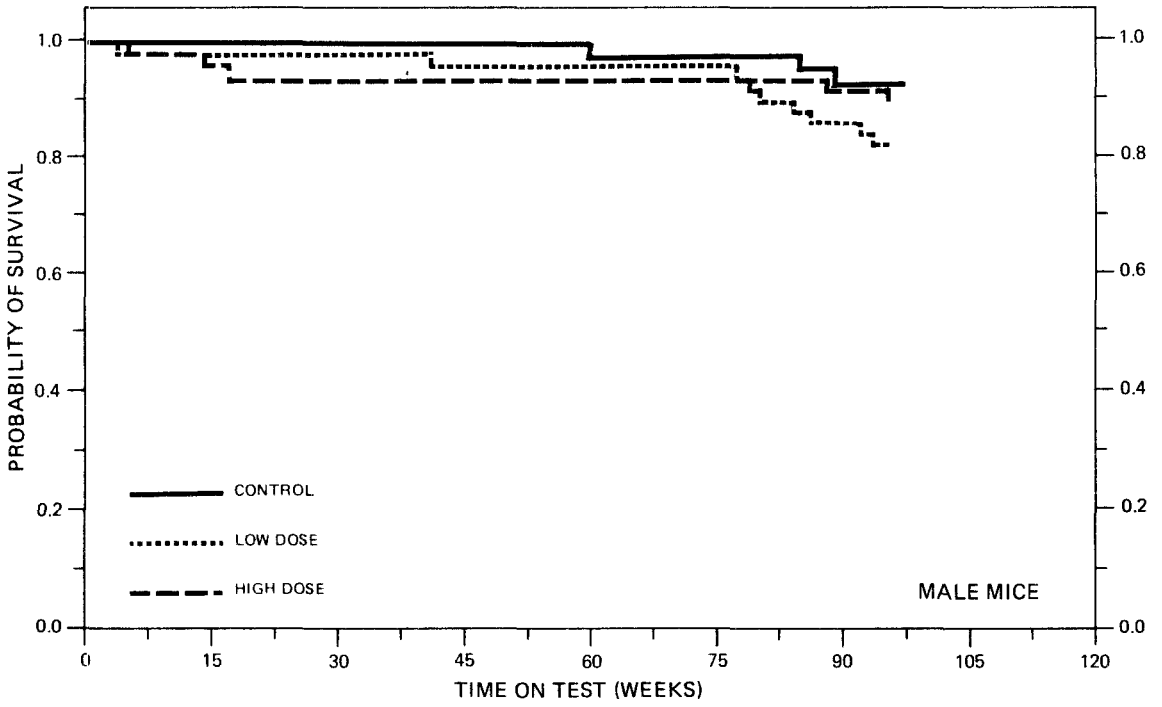


FIGURE 4
GROWTH CURVES FOR 4-CHLORO-m-PHENYLENEDIAMINE CHRONIC STUDY MICE



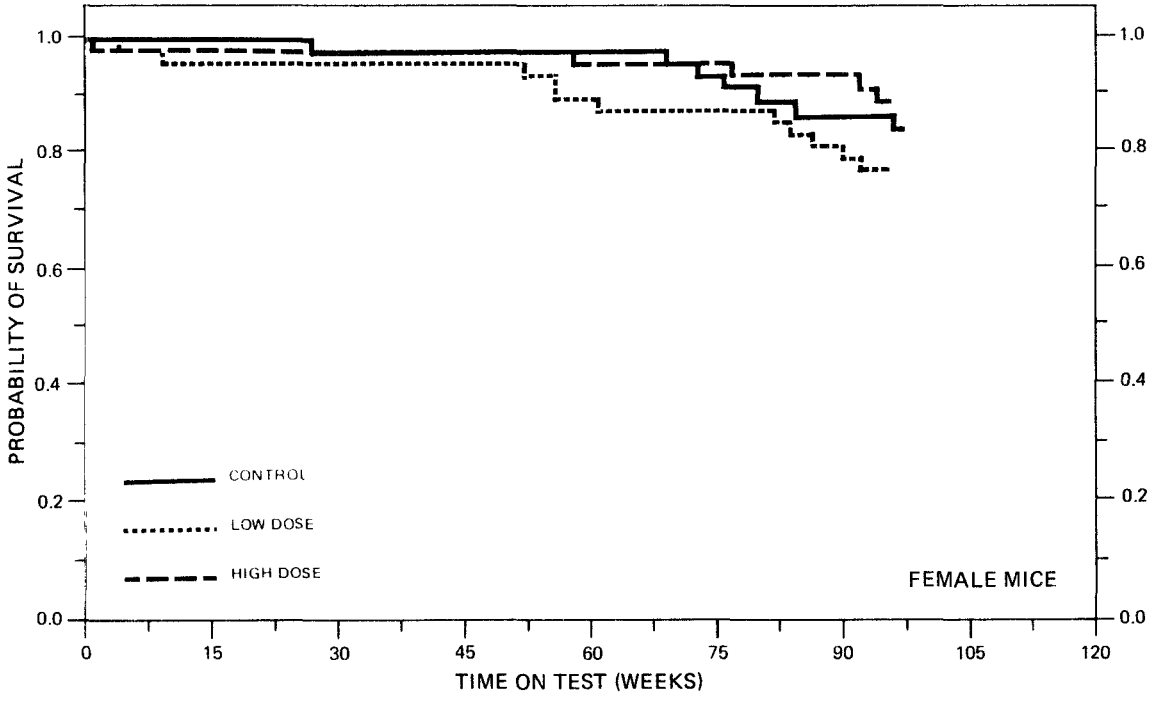


FIGURE 5
SURVIVAL COMPARISONS OF 4-CHLORO-m-PHENYLENEDIAMINE CHRONIC STUDY MICE

C. Pathology

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

A variety of neoplasms occurred with approximately equal frequency in treated and control mice. In addition, there was an increased incidence of hepatocellular neoplasms observed in the treated female mice when compared with the control group. These are summarized in the following table:

	Control	Low Dose	High Dose
Number of Female Mice with Livers Examined Histopathologically	46	44	45
Hepatocellular Adenomas	0	3	3
Hepatocellular Carcinomas	0	8	5

The incidence of hepatocellular neoplasms among male mice was similar in the treated and control groups.

The hepatocellular adenomas consisted of small circumscribed nodules lacking normal lobular architecture, comprised of large hepatocytes with eosinophilic cytoplasm and vesicular nuclei. Hepatocellular carcinomas replaced partial or entire lobes of the liver. The hepatic architecture was distorted and the neoplastic hepatocytes were large in size with eosinophilic cytoplasm and lipid vacuoles in some cells. A pleomorphism in nuclear size was evident and mitotic figures were numerous. Metastasis to the lung occurred in one treated male but in none of the female mice.

The treated and control mice had a variety of nonneoplastic lesions which are commonly seen in aged B6C3F1 mice. The incidence and severity of the lesions were similar in the control and treated mice.

The results of this histopathologic examination indicate that 4-chloro-m-phenylenediamine was carcinogenic in female B6C3F1 mice, as it was associated with an increased incidence of hepatocellular neoplasms under the conditions of this study. There did not appear to be any compound-related neoplastic lesions in the male B6C3F1 mice in this study.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of tumor in either sex where at least two such tumors were observed in at least one of the control or 4-chloro-m-phenylenediamine-dosed groups and where such tumors were observed in at least 5 percent of the group.

For females an increase in the incidence of liver tumors was observed in treated mice. The Fisher exact test indicated a significantly ($P = 0.002$) higher incidence of hepatocellular carcinomas in the low dose group than in the control. For the high dose comparison the probability level was $P = 0.026$, a marginal result that was not significant under the Bonferroni criterion. When incidences were combined so that the numerator represented mice with either a hepatocellular carcinoma or a hepatocellular adenoma, the Cochran-Armitage

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH 4-CHLORO-m-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	7/50(0.14)	4/47(0.09)	3/48(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.608	0.446
Lower Limit	---	0.139	0.078
Upper Limit	---	2.226	1.828
Weeks to First Observed Tumor	97	92	95
Hematopoietic System: Malignant Lymphoma[b]	4/50(0.08)	3/50(0.06)	1/50(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.750	0.250
Lower Limit	---	0.115	0.005
Upper Limit	---	4.206	2.508
Weeks to First Observed Tumor	97	92	95
Liver: Hepatocellular Carcinoma[b]	10/50(0.20)	8/48(0.17)	15/48(0.31)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.833	1.563
Lower Limit	---	0.312	0.732
Upper Limit	---	2.150	3.486
Weeks to First Observed Tumor	60	84	95

TABLE 5 (Concluded)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma[b]	15/50(0.30)	10/48(0.21)	19/48(0.40)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.694	1.319
Lower Limit	---	0.311	0.724
Upper Limit	---	1.481	2.437
Weeks to First Observed Tumor	60	84	95

[a]Treated groups received time-weighted average doses of 0.7 or 1.4 percent in feed.

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

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

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

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 4-CHLORO-m-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	3/46(0.07)	1/42(0.02)	0/45(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.365	0.000
Lower Limit	---	0.007	0.000
Upper Limit	---	4.332	1.694
Weeks to First Observed Tumor	97	95	---
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	4/46(0.09)	1/42(0.02)	1/45(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.274	0.256
Lower Limit	---	0.006	0.005
Upper Limit	---	2.620	2.452
Weeks to First Observed Tumor	97	95	95
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	6/47(0.13)	7/46(0.15)	3/46(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.192	0.511
Lower Limit	---	0.371	0.089
Upper Limit	---	3.971	2.284
Weeks to First Observed Tumor	69	86	77

TABLE 6 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	0/46(0.00)	8/44(0.18)	5/45(0.11)
P Values[c]	N.S.	P = 0.002	P = 0.026
Departure from Linear Trend[e]	P = 0.020	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	2.397	1.293
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	92	95
Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma[b]	0/46(0.00)	11/44(0.25)	8/45(0.18)
P Values[c]	P = 0.011	P < 0.001	P = 0.003
Departure from Linear Trend[e]	P = 0.012	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	3.485	2.343
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	92	95
Pituitary: Adenoma,NOS[b]	1/33(0.03)	2/32(0.06)	0/27(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.063	0.000
Lower Limit	---	0.113	0.000
Upper Limit	---	117.749	22.441
Weeks to First Observed Tumor	80	61	---

TABLE 6 (Concluded)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Ovary: Papillary Adenoma or Papillary Cystadenoma,NOS[b]	1/40(0.03)	2/40(0.05)	0/41(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.000	0.000
Lower Limit	---	0.109	0.000
Upper Limit	---	114.994	18.131
Weeks to First Observed Tumor	97	95	---

[a]Treated groups received time-weighted average doses of 0.7 or 1.4 percent in feed.

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

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

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

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

test showed a significant positive ($P = 0.011$) association between dosage and incidence. The Fisher exact tests also showed significant comparisons between the incidence in the control and that in both the high dose ($P = 0.003$) and low dose ($P < 0.001$) groups. The departure from linear trend was also significant ($P = 0.012$) since the incidence in the low dose group was greater than that in the high dose group. The historical incidence rate in untreated female B6C3F1 control mice at Mason Research Institute was 19/275 (7 percent).

Based upon these results the statistical conclusion is that the administration of 4-chloro-m-phenylenediamine was associated with the combined incidence of hepatocellular adenomas and hepatocellular carcinomas in female B6C3F1 mice.

No statistical tests for tumors of other sites were significant for either male or female mice.

V. DISCUSSION

In both species adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

An increased incidence of adrenal pheochromocytomas in male rats was statistically associated with dosage of 4-chloro-m-phenylenediamine. The incidence in the high dose group (14/48 or 29 percent) was significantly higher than the incidence in the control group. These statistical findings indicate that the development of adrenal pheochromocytomas was associated with administration of the chemical. Six male control groups at this laboratory had a mean incidence of adrenal pheochromocytomas of 33/241 (13 percent), with one group having as high as a 26 percent incidence of this lesion. An increased incidence of interstitial-cell tumors of the testis, although statistically associated with dosage, was not considered to be caused by compound administration. The incidences in the high and low dose groups were not significantly higher than that in controls and these tumors commonly appear at high incidences in untreated Fischer 344 rats.

Among female rats, the incidence of endometrial stromal polyps was significantly higher in the low dose group than in the control group. The incidence was not, however, dose-related and the incidence among historical controls was higher than the incidence observed in either the control or high dose group. It was, therefore,

concluded that the incidence of uterine stromal polyps was not caused by dietary administration of 4-chloro-m-phenylenediamine.

Among female mice, the incidence of hepatocellular carcinoma was significant in the low dose group, but the incidence in the high dose group was not significant under the Bonferroni criterion. When incidences of hepatocellular carcinoma and hepatocellular adenoma were combined, the proportion of female mice in each dosed group having either a carcinoma, an adenoma, or both was significant.

Under the conditions of this bioassay, dietary administration of 4-chloro-m-phenylenediamine was carcinogenic to the experimental animals, causing an increased incidence of hepatocellular tumors in female B6C3F1 mice, and an increased incidence of adrenal pheochromocytomas in male Fischer 344 rats.

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

April 26, 1978

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

The primary reviewer agreed with the conclusion that 4-Chloro-*m*-phenylenediamine was carcinogenic in mice and rats. After a brief description of the experimental design, he noted a significant dose-related increase in the incidence of adrenal pheochromocytomas in treated male rats and an increased incidence of hepatocellular carcinomas and adenomas in treated mice. The primary reviewer concluded that the bioassay was adequate to assess the carcinogenicity of 4-Chloro-*m*-phenylenediamine in rats and mice. He said that 4-Chloro-*m*-phenylenediamine would appear to pose a carcinogenic risk to humans.

The secondary reviewer also agreed with the conclusion given in the report. A discussion followed on the adequacy of the bioassay to provide useful dose-response data. A Subgroup member pointed out that the studies were not

designed for this purpose. Traditionally, the lower dose level was a backup in the event animals at the higher one died from toxicity.

It was moved that the report on the bioassay of 4-Chloro-*m*-phenylenediamine be accepted. The motion was seconded and approved unanimously.

Members present were:

Michael Shimkin (Acting Chairman), University of California at San Diego
Joseph Highland, Environmental Defense Fund
George Roush, Jr., Monsanto Company
Louise Strong, University of Texas Health Sciences Center
John Weisburger, American Health Foundation
(Sidney Wolfe, Health Research Group, submitted a written review)

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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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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 4-CHLORO-m-PHENYLENEDIAMINE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED
WITH 4-CHLORO-*m*-PHENYLENEDIAMINE

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0225	HIGH DOSE 01-0230
ANIMALS INITIALLY IN STUDY	50	50	@50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	48	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	48	49	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(48)	(49)	(49)
FIBROMA	3 (6%)	3 (6%)	4 (8%)
FIBROSARCOMA	2 (4%)	1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(48)	(49)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (6%)		
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
FIBROSARCOMA, METASTATIC	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(48)	(49)	(49)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
UNDIFFERENTIATED LEUKEMIA	2 (4%)		
#SPLEEN	(48)	(49)	(49)
MESOTHELIOMA, METASTATIC		1 (2%)	
MYELOMONOCYTIC LEUKEMIA	4 (8%)		
#LYMPH NODE	(43)	(44)	(47)
C-CELL CARCINOMA, METASTATIC		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(48)	(48)	(49)
NEOPLASTIC NODULE		1 (2%)	1 (2%)

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A FEMALE IN A MALE GROUP.
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0225	HIGH DOSE 01-0230
HEPATOCELLULAR CARCINOMA			1 (2%)
#JEJUNUM	(46)	(48)	(49)
SARCOMA, NOS			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(43)	(47)
ADENOMA, NOS	10 (22%)	6 (14%)	15 (32%)
#ADRENAL	(46)	(48)	(48)
CORTICAL ADENOMA			1 (2%)
PHEOCHROMOCYTOMA	4 (9%)	7 (15%)	14 (29%)
#THYROID	(43)	(48)	(48)
FOLLICULAR-CELL ADENOMA			1 (2%)
FOLLICULAR-CELL CARCINOMA			2 (4%)
C-CELL ADENOMA	3 (7%)	2 (4%)	3 (6%)
C-CELL CARCINOMA	2 (5%)	1 (2%)	3 (6%)
#PARATHYROID	(25)	(27)	(25)
ADENOMA, NOS	1 (4%)	1 (4%)	
#PANCREATIC ISLETS	(44)	(48)	(49)
ISLET-CELL ADENOMA		2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(48)	(49)	(49)
ADENOMA, NOS		1 (2%)	1 (2%)
#PROSTATE	(45)	(47)	(44)
ADENOMA, NOS		1 (2%)	
#TESTIS	(48)	(49)	(49)
INTERSTITIAL-CELL TUMOR	37 (77%)	43 (88%)	45 (92%)
NERVOUS SYSTEM			
#BRAIN	(46)	(49)	(49)
OSTEOSARCOMA, METASTATIC	1 (2%)		

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0225	HIGH DOSE 01-0230
ASTROCYTOMA			1 (2%)
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(48)	(49)	(49)
SEBACEOUS ADENOCARCINOMA			2 (4%)
MUSCULOSKELETAL SYSTEM			
*SKULL	(48)	(49)	(49)
OSTEOSARCOMA	1 (2%)		
BODY CAVITIES			
*BODY CAVITIES	(48)	(49)	(49)
MESOTHELIOMA, NOS	1 (2%)	1 (2%)	2 (4%)
MESOTHELIOMA, MALIGNANT		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	8	4	2
MORIBUND SACRIFICE	5	2	6
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	32	43	36
ANIMAL MISSING		1	
ANIMAL DELETED (WRONG SEX)			1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0225	HIGH DOSE 01-0230
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	42	46	46
TOTAL PRIMARY TUMORS	74	72	99
TOTAL ANIMALS WITH BENIGN TUMORS	40	44	46
TOTAL BENIGN TUMORS	61	66	85
TOTAL ANIMALS WITH MALIGNANT TUMORS	11	4	10
TOTAL MALIGNANT TUMORS	12	4	11
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	2	
TOTAL SECONDARY TUMORS	2	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	2	3
TOTAL UNCERTAIN TUMORS	1	2	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED
WITH 4-CHLORO-m-PHENYLENEDIAMINE

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0225	HIGH DOSE 02-0230
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	50	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	50	47
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(47)
SARCOMA, NOS	1 (2%)		
FIBROMA		1 (2%)	
FIBROSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(47)
ADENOCARCINOMA, NOS, METASTATIC		2 (4%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(47)
MALIGNANT LYMPHOMA, NOS	2 (4%)		
#SPLEEN	(50)	(50)	(47)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
HEMANGIOMA		1 (2%)	1 (2%)
MYELOMONOCYTIC LEUKEMIA	2 (4%)		
#LYMPH NODE	(43)	(50)	(45)
ADENOCARCINOMA, NOS, METASTATIC		2 (4%)	
#THYMUS	(31)	(31)	(35)
THYMOMA			1 (3%)
CIRCULATORY SYSTEM			
#HEART	(50)	(50)	(47)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0225	HIGH DOSE 02-0230
DIGESTIVE SYSTEM			
*INTESTINAL TRACT	(50)	(50)	(47)
ADENOCARCINOMA, NOS		1 (2%)	
#LIVER	(50)	(50)	(47)
NEOPLASTIC NODULE			2 (4%)
#PANCREAS	(46)	(48)	(46)
ISLET-CELL ADENOMA			1 (2%)
#STOMACH	(49)	(49)	(46)
ADENOCARCINOMA, NOS, METASTATIC		2 (4%)	
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(47)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(40)	(46)	(44)
CARCINOMA,NOS		1 (2%)	
ADENOMA, NOS	16 (40%)	15 (33%)	17 (39%)
CHROMOPHOBE ADENOMA	1 (3%)		
#ADRENAL	(48)	(49)	(46)
PHEOCHROMOCYTOMA	6 (13%)	2 (4%)	4 (9%)
#THYROID	(43)	(45)	(44)
FOLLICULAR-CELL ADENOMA		1 (2%)	
FOLLICULAR-CELL CARCINOMA		1 (2%)	1 (2%)
C-CELL ADENOMA		2 (4%)	
C-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(47)
ADENOMA, NOS		1 (2%)	
ADENOCARCINOMA, NOS	1 (2%)		1 (2%)
FIBROADENOMA	6 (12%)	4 (8%)	5 (11%)

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0225	HIGH DOSE 02-0230
*CLITORAL GLAND	(50)	(50)	(47)
CARCINOMA, NOS			1 (2%)
SQUAMOUS CELL CARCINOMA		1 (2%)	
ADENOMA, NOS		1 (2%)	
#UTERUS	(48)	(48)	(46)
ADENOCARCINOMA, NOS	1 (2%)	2 (4%)	
FIBROMA	2 (4%)		
ENDOMETRIAL STROMAL POLYP	2 (4%)	12 (25%)	5 (11%)
ENDOMETRIAL STROMAL SARCOMA	2 (4%)		
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(47)
ASTROCYTOMA			1 (2%)
*SPINAL CORD	(50)	(50)	(47)
ASTROCYTOMA		1 (2%)	
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(50)	(50)	(47)
SEBACEOUS ADENOCARCINOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0225	HIGH DOSE 02-0230
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	7	4	6
MORIBUND SACRIFICE	2	7	5
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	39	33
ANIMAL MISSING			1
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	27	33	30
TOTAL PRIMARY TUMORS	42	49	42
TOTAL ANIMALS WITH BENIGN TUMORS	23	31	27
TOTAL BENIGN TUMORS	33	40	34
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	9	6
TOTAL MALIGNANT TUMORS	9	9	6
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	
TOTAL SECONDARY TUMORS		9	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			2
TOTAL UNCERTAIN TUMORS			2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 4-CHLORO-m-PHENYLENEDIAMINE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH 4-CHLORO-m-PHENYLENEDIAMINE

	CONTROL (UNTR) 05-0220	LOW DOSE 05-0235	HIGH DOSE 05-0240
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	50	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(50)	(47)	(48)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	5 (10%)	2 (4%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (4%)	2 (4%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	
#SPLEEN	(50)	(48)	(47)
HEMANGIOMA	1 (2%)		1 (2%)
HEMANGIOSARCOMA	2 (4%)		
#MESENTERIC L. NODE	(44)	(41)	(43)
HEMANGIOSARCOMA		1 (2%)	
MALIGNANT LYMPHOMA, NOS	1 (2%)	1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
#LIVER	(50)	(48)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
#KIDNEY	(50)	(48)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 05-0220	LOW DOSE 05-0235	HIGH DOSE 05-0240
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(48)
NEOPLASM, NOS		1 (2%)	
HEPATOCELLULAR ADENOMA	5 (10%)	2 (4%)	4 (8%)
HEPATOCELLULAR CARCINOMA	10 (20%)	8 (17%)	15 (31%)
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(48)
TUBULAR-CELL ADENOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(34)	(25)	(28)
ADENOMA, NOS		1 (4%)	
#ADRENAL/CAPSULE	(42)	(47)	(45)
ADENOMA, NOS			1 (2%)
#THYROID	(39)	(43)	(42)
FOLLICULAR-CELL ADENOMA	1 (3%)		1 (2%)
FOLLICULAR-CELL CARCINOMA	1 (3%)		
REPRODUCTIVE SYSTEM			
#TESTIS	(50)	(48)	(48)
EMBRYONAL CARCINOMA	1 (2%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(50)
CYSTADENOMA, NOS	1 (2%)		2 (4%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 05-0220	LOW DOSE 05-0235	HIGH DOSE 05-0240
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
SITE UNKNOWN			
HEMANGIOMA			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	3	6	5
MORIBUND SACRIFICE		3	
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	42	41	40
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	22	19	25
TOTAL PRIMARY TUMORS	33	23	29
TOTAL ANIMALS WITH BENIGN TUMORS	12	7	12
TOTAL BENIGN TUMORS	13	7	12
TOTAL ANIMALS WITH MALIGNANT TUMORS	16	14	17
TOTAL MALIGNANT TUMORS	20	15	17
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

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED
WITH 4-CHLORO-m-PHENYLENEDIAMINE

	CONTROL (UNTR) 06-0220	LOW DOSE 06-0235	HIGH DOSE 06-0240
ANIMALS INITIALLY IN STUDY	50	50	@50
ANIMALS MISSING	2	2	1
ANIMALS NECROPSIED	47	46	46
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	47	44	46
INTEGUMENTARY SYSTEM			
*SKIN	(47)	(46)	(46)
KERATOACANTHOMA	1 (2%)		
*SUBCUT TISSUE	(47)	(46)	(46)
SQUAMOUS CELL CARCINOMA		1 (2%)	
SARCOMA, NOS			1 (2%)
HEMANGIOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(46)	(42)	(45)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)		1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (7%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(47)	(46)	(46)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	3 (6%)	2 (4%)	
UNDIFFERENTIATED LEUKEMIA		1 (2%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	
#SPLEEN	(45)	(41)	(45)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (4%)		
#MESENTERIC L. NODE	(38)	(39)	(41)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#LIVER	(46)	(44)	(45)
UNDIFFERENTIATED LEUKEMIA			1 (2%)
#PEYERS PATCH	(44)	(42)	(45)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	1 (2%)

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

@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A MALE IN A FEMALE GROUP.

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL(UNTR) 06-0220	LOW DOSE 06-0235	HIGH DOSE 06-0240
#KIDNEY	(46)	(44)	(45)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#THYMUS	(30)	(20)	(29)
MALIGNANT LYMPHOMA, NOS		1 (5%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(42)	(40)	(44)
TRANSITIONAL-CELL CARCINOMA, INV			1 (2%)
#LIVER	(46)	(44)	(45)
HEPATOCELLULAR ADENOMA		3 (7%)	3 (7%)
HEPATOCELLULAR CARCINOMA		8 (18%)	5 (11%)
URINARY SYSTEM			
#URINARY BLADDER	(45)	(41)	(45)
TRANSITIONAL-CELL CARCINOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(33)	(32)	(27)
ADENOMA, NOS	1 (3%)	2 (6%)	
#ADRENAL/CAPSULE	(40)	(41)	(44)
ADENOMA, NOS	1 (3%)		
#THYROID	(29)	(40)	(32)
FOLLICULAR-CELL ADENOMA	1 (3%)	1 (3%)	1 (3%)
REPRODUCTIVE SYSTEM			
#UTERUS	(45)	(41)	(44)
LEIOMYOSARCOMA		1 (2%)	

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0220	LOW DOSE 06-0235	HIGH DOSE 06-0240
ENDOMETRIAL STROMAL POLYP		1 (2%)	
*OVARY	(40)	(40)	(41)
PAPILLARY ADENOMA	1 (3%)		
PAPILLARY CYSTADENOMA, NOS		2 (5%)	
MUCINOUS CYSTADENOCA, INVASIVE		1 (3%)	
TUBULAR ADENOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	4	9	4
MORIBUND SACRIFICE	3	2	1
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	37	38
ANIMAL MISSING	2	2	1
ANIMAL DELETED (WRONG SEX)			1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 06-0220	LOW DOSE 06-0235	HIGH DOSE 06-0240
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	20	16
TOTAL PRIMARY TUMORS	15	27	17
TOTAL ANIMALS WITH BENIGN TUMORS	5	8	7
TOTAL BENIGN TUMORS	6	9	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	16	10
TOTAL MALIGNANT TUMORS	9	18	10
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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 4-CHLORO-m-PHENYLENEDIAMINE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH 4-CHLORO-m-PHENYLENEDIAMINE

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0225	HIGH DOSE 01-0230
ANIMALS INITIALLY IN STUDY	50	50	@50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	48	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	48	49	49
INTEGUMENTARY SYSTEM			
*SKIN	(48)	(49)	(49)
EPIDERMAL INCLUSION CYST	1 (2%)		
INFLAMMATION, NOS			1 (2%)
*SUBCUT TISSUE	(48)	(49)	(49)
ABSCESS, NOS	3 (6%)		
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(48)	(49)	(49)
INFLAMMATION, SUPPURATIVE	1 (2%)		
#LUNG/BRONCHUS	(48)	(49)	(49)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, FOCAL			1 (2%)
#LUNG	(48)	(49)	(49)
PNEUMONIA, CHRONIC MURINE		1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(48)	(49)	(49)
INFARCT, NOS	1 (2%)		
HEMATOPOIESIS		5 (10%)	4 (8%)
#MEDIASTINAL L.NODE	(43)	(44)	(47)
PLASMACYTOSIS			1 (2%)
#THYMUS	(32)	(30)	(32)
HYPERPLASIA, NOS	1 (3%)		

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

@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A FEMALE IN A MALE GROUP.

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0225	HIGH DOSE 01-0230
CIRCULATORY SYSTEM			
#MYOCARDIUM	(48)	(49)	(49)
INFLAMMATION, NOS			1 (2%)
FIBROSIS		9 (18%)	6 (12%)
DEGENERATION, NOS		9 (18%)	5 (10%)
*AORTA	(48)	(49)	(49)
INFLAMMATION, NOS		2 (4%)	
DIGESTIVE SYSTEM			
#LIVER	(48)	(48)	(49)
NECROSIS, FOCAL		7 (15%)	1 (2%)
METAMORPHOSIS FATTY	2 (4%)	4 (8%)	4 (8%)
BASOPHILIC CYTO CHANGE		7 (15%)	9 (18%)
HYPERPLASIA, FOCAL	3 (6%)	2 (4%)	
ANGIECTASIS		1 (2%)	1 (2%)
HEMATOPOIESIS			1 (2%)
#PANCREAS	(44)	(48)	(49)
INFLAMMATION, NOS	2 (5%)		
INFLAMMATION, FOCAL		1 (2%)	
#PANCREATIC ACINUS	(44)	(48)	(49)
ATROPHY, NOS		5 (10%)	3 (6%)
#COLON	(42)	(44)	(48)
PARASITISM		2 (5%)	
URINARY SYSTEM			
#KIDNEY	(48)	(49)	(49)
NEPHROPATHY	35 (73%)	47 (96%)	48 (98%)
CALCIFICATION, NOS		1 (2%)	
#RENAL PAPILLA	(48)	(49)	(49)
MINERALIZATION		3 (6%)	
#KIDNEY/TUBULE	(48)	(49)	(49)
MINERALIZATION		1 (2%)	

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0225	HIGH DOSE 01-0230
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(43)	(47)
INFLAMMATION, ACUTE		1 (2%)	
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL	1 (2%)		1 (2%)
#ADRENAL MEDULLA	(46)	(48)	(48)
HYPERPLASIA, NODULAR		1 (2%)	
HYPERPLASIA, NOS	1 (2%)		1 (2%)
HYPERPLASIA, FOCAL	3 (7%)	1 (2%)	
#THYROID	(43)	(48)	(48)
HYPERPLASIA, C-CELL		3 (6%)	1 (2%)
#PARATHYROID	(25)	(27)	(25)
HYPERPLASIA, NOS			1 (4%)
#PANCREATIC ISLETS	(44)	(48)	(49)
HYPERPLASIA, NOS	1 (2%)	2 (4%)	
REPRODUCTIVE SYSTEM			
#PROSTATE	(45)	(47)	(44)
INFLAMMATION, NOS		1 (2%)	
#TESTIS	(48)	(49)	(49)
MINERALIZATION	1 (2%)	3 (6%)	3 (6%)
ATROPHY, NOS	4 (8%)	7 (14%)	7 (14%)
HYPERPLASIA, INTERSTITIAL CELL	3 (6%)	3 (6%)	3 (6%)
*EPIDIDYMIS	(48)	(49)	(49)
ABSCESS, NOS	1 (2%)		
GRANULOMA, SPERMATIC			2 (4%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(48)	(49)	(49)
CATARACT	1 (2%)		

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0225	HIGH DOSE 01-0230
*EYE/RETINA	(48)	(49)	(49)
ATROPHY, NOS	2 (4%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, NOS			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	
ANIMAL MISSING/NO NECROPSY		1	
AUTO/NECROPSY/HISTO PERF	1		
AUTOLYSIS/NO NECROPSY	2		

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

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
TREATED WITH 4-CHLORO-m-PHENYLENEDIAMINE

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0225	HIGH DOSE 02-0230
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	50	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	50	47
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(47)
EPIDERMAL INCLUSION CYST		1 (2%)	
*SUBCUT TISSUE	(50)	(50)	(47)
MINERALIZATION			1 (2%)
NECROSIS, NOS			1 (2%)
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(50)	(50)	(47)
INFLAMMATION, NOS	1 (2%)		
#LUNG/BRONCHUS	(50)	(50)	(47)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, NECROTIZING		1 (2%)	
#LUNG	(50)	(50)	(47)
INFLAMMATION, NOS	1 (2%)	3 (6%)	2 (4%)
INFLAMMATION, INTERSTITIAL	1 (2%)		
ABSCESS, NOS	2 (4%)		
PNEUMONIA, CHRONIC MURINE	1 (2%)		1 (2%)
GRANULOMA, NOS	1 (2%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(50)	(47)
HEMATOPOIESIS	7 (14%)	22 (44%)	9 (19%)
#LYMPH NODE	(43)	(50)	(45)
HYPERPLASIA, LYMPHOID		1 (2%)	

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0225	HIGH DOSE 02-0230
CIRCULATORY SYSTEM			
#MYOCARDIUM	(50)	(50)	(47)
FIBROSIS		2 (4%)	2 (4%)
DEGENERATION, NOS		4 (8%)	7 (15%)
*AORTA	(50)	(50)	(47)
INFLAMMATION, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(47)
NECROSIS, FOCAL		4 (8%)	1 (2%)
METAMORPHOSIS FATTY	4 (8%)		2 (4%)
BASOPHILIC CYTO CHANGE		27 (54%)	18 (38%)
HYPERPLASIA, FOCAL	9 (18%)		
HEMATOPOIESIS		1 (2%)	
#PANCREAS	(46)	(48)	(46)
INFLAMMATION, NOS		1 (2%)	
#PANCREATIC ACINUS	(46)	(48)	(46)
ATROPHY, NOS		6 (13%)	
#STOMACH	(49)	(49)	(46)
HYPERPLASIA, PAPILLARY	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(47)
MINERALIZATION			1 (2%)
HYDRONEPHROSIS		1 (2%)	
GLOMERULONEPHRITIS, NOS	1 (2%)		
NEPHROPATHY	18 (37%)	43 (86%)	39 (83%)
#KIDNEY/TUBULE	(49)	(50)	(47)
MINERALIZATION		5 (10%)	
ENDOCRINE SYSTEM			
#PITUITARY	(40)	(46)	(44)
MINERALIZATION	1 (3%)		

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0225	HIGH DOSE 02-0230
#ADRENAL	(48)	(49)	(46)
HYPERPLASIA, NOS		1 (2%)	
#ADRENAL CORTEX	(48)	(49)	(46)
HYPERPLASIA, NOS		1 (2%)	
#THYROID	(43)	(45)	(44)
HYPERPLASIA, C-CELL	1 (2%)	2 (4%)	1 (2%)
#PARATHYROID	(31)	(19)	(25)
HYPERPLASIA, NODULAR	1 (3%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(47)
GALACTOCELE	2 (4%)	1 (2%)	3 (6%)
HYPERPLASIA, NOS	1 (2%)		
*CLITORAL GLAND	(50)	(50)	(47)
ABSCESS, NOS			1 (2%)
NECROSIS, NOS			1 (2%)
#UTERUS	(48)	(48)	(46)
HYDROMETRA		2 (4%)	
INFLAMMATION, NOS		6 (13%)	4 (9%)
PYOMETRA		1 (2%)	4 (9%)
ABSCESS, NOS	1 (2%)		
NECROSIS, NOS	1 (2%)		
METAPLASIA, SQUAMOUS		1 (2%)	
#UTERUS/ENDOMETRIUM	(48)	(48)	(46)
INFLAMMATION, NOS	1 (2%)		
HYPERPLASIA, NOS		1 (2%)	2 (4%)
#OVARY	(49)	(46)	(46)
CYST, NOS			2 (4%)
FOLLICULAR CYST, NOS		1 (2%)	
INFLAMMATION, NOS	1 (2%)	2 (4%)	
DEGENERATION, CYSTIC	2 (4%)		
NERVOUS SYSTEM			
NONE			

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

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0225	HIGH DOSE 02-0230
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(47)
INFLAMMATION, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, FAT		1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	11	2	4
ANIMAL MISSING/NO NECROPSY			1
AUTO/NECROPSY/HISTO PERF	1		
AUTOLYSIS/NO NECROPSY			2

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 4-CHLORO-m-PHENYLENEDIAMINE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH 4-CHLORO-m-PHENYLENEDIAMINE

	CONTROL (UNTR) 05-0220	LOW DOSE 05-0235	HIGH DOSE 05-0240
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	50	49	48
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
ABSCESS, NOS	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(50)	(47)	(48)
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, FOCAL		1 (2%)	
#LUNG	(50)	(47)	(48)
INFLAMMATION, INTERSTITIAL		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(48)	(47)
HYPERPLASIA, LYMPHOID			1 (2%)
HEMATOPOIESIS		1 (2%)	2 (4%)
#MESENTERIC L. NODE	(44)	(41)	(43)
HEMATOPOIESIS	1 (2%)	3 (7%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(50)	(48)	(48)
DEGENERATION, NOS		1 (2%)	1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(48)
NECROSIS, FOCAL		3 (6%)	

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0220	LOW DOSE 05-0235	HIGH DOSE 05-0240
NECROSIS, COAGULATIVE	1 (2%)		
HYPERPLASTIC NODULE		3 (6%)	
HYPERPLASIA, FOCAL		1 (2%)	
HYPERPLASIA, DIFFUSE			2 (4%)
HEMATOPOIESIS			1 (2%)
*GALLBLADDER	(50)	(50)	(50)
INFLAMMATION, NOS		3 (6%)	
#PANCREAS	(50)	(43)	(46)
DEGENERATION, CYSTIC		1 (2%)	
#PANCREATIC ACINUS	(50)	(43)	(46)
ATROPHY, NOS	1 (2%)		
HYPERTROPHY, FOCAL	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(48)
HYDRONEPHROSIS	1 (2%)		
CYST, NOS			1 (2%)
GLOMERULONEPHRITIS, NOS		3 (6%)	23 (48%)
INFLAMMATION, NOS		1 (2%)	12 (25%)
INFLAMMATION, INTERSTITIAL		7 (15%)	
#KIDNEY/TUBULE	(50)	(48)	(48)
MINERALIZATION			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(34)	(25)	(28)
HYPERPLASIA, NOS			1 (4%)
#ADRENAL/CAPSULE	(42)	(47)	(45)
HYPERPLASIA, NOS		2 (4%)	1 (2%)
#ADRENAL CORTEX	(42)	(47)	(45)
HYPERTROPHY, FOCAL		1 (2%)	
#PANCREATIC ISLETS	(50)	(43)	(46)
INFLAMMATION, NOS	3 (6%)		
HYPERPLASIA, ADENOMATOUS	1 (2%)		

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

TABLE D1 (CONTINUED)

	CONTROL(UNTR) 05-0220	LOW DOSE 05-0235	HIGH DOSE 05-0240
REPRODUCTIVE SYSTEM			
#TESTIS	(50)	(48)	(48)
ATROPHY, NOS	1 (2%)		
#TESTIS/TUBULE	(50)	(48)	(48)
MINERALIZATION	2 (4%)		1 (2%)
NERVOUS SYSTEM			
#BRAIN	(50)	(48)	(47)
CHOLESTEATOMA		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(50)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
*HARDERIAN GLAND	(50)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
HYPERPLASIA, PAPILLARY		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	22	12	3

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

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 05-0220	LOW DOSE 05-0235	HIGH DOSE 05-0240
NECROPSY PERF/NO HISTO PERFORMED		1	1
AUTO/NECROPSY/HISTO PERF		1	1
AUTO/NECROPSY/NO HISTO			1

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

TABLE D2

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH 4-CHLORO-m-PHENYLENEDIAMINE

	CONTROL (UNTR) 06-0220	LOW DOSE 06-0235	HIGH DOSE 06-0240
ANIMALS INITIALLY IN STUDY	50	50	@50
ANIMALS MISSING	2	2	1
ANIMALS NECROPSIED	47	46	46
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	47	44	46
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(47)	(46)	(46)
HEMORRHAGE			1 (2%)
INFLAMMATION, NOS	1 (2%)		
RESPIRATORY SYSTEM			
*LUNG	(46)	(42)	(45)
INFLAMMATION, NOS			1 (2%)
HEMATOPOIETIC SYSTEM			
*BONE MARROW	(39)	(42)	(43)
MYELOFIBROSIS		8 (19%)	1 (2%)
*SPLEEN	(45)	(41)	(45)
HYPERPLASIA, LYMPHOID		6 (15%)	2 (4%)
HEMATOPOIESIS	1 (2%)	1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*LIVER	(46)	(44)	(45)
NECROSIS, FOCAL		1 (2%)	3 (7%)
METAMORPHOSIS FATTY	1 (2%)		
HYPERPLASTIC NODULE		2 (5%)	1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	3 (7%)

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

@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A MALE IN A FEMALE GROUP.

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0220	LOW DOSE 06-0235	HIGH DOSE 06-0240
*GALLBLADDER	(47)	(46)	(46)
HYPERPLASIA, PAPILLARY			2 (4%)
#PANCREATIC ACINUS	(45)	(42)	(43)
DEGENERATION, NOS		1 (2%)	
HYPERTROPHY, FOCAL			1 (2%)
URINARY SYSTEM			
#KIDNEY	(46)	(44)	(45)
HYDRONEPHROSIS			1 (2%)
GLOMERULONEPHRITIS, NOS	1 (2%)	2 (5%)	4 (9%)
INFLAMMATION, NOS		2 (5%)	6 (13%)
INFLAMMATION, CHRONIC			1 (2%)
#KIDNEY/TUBULE	(46)	(44)	(45)
MINERALIZATION		2 (5%)	
#URINARY BLADDER	(45)	(41)	(45)
INFLAMMATION, NOS			4 (9%)
HYPERPLASIA, EPITHELIAL			3 (7%)
ENDOCRINE SYSTEM			
#PITUITARY	(33)	(32)	(27)
HYPERPLASIA, NOS		2 (6%)	
#ADRENAL/CAPSULE	(40)	(41)	(44)
HYPERPLASIA, NOS		3 (7%)	4 (9%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(47)	(46)	(46)
HYPERPLASIA, NOS	1 (2%)		
#UTERUS/ENDOMETRIUM	(45)	(41)	(44)
INFLAMMATION, NOS			1 (2%)
HYPERPLASIA, NOS			9 (20%)
HYPERPLASIA, CYSTIC		1 (2%)	
#OVARY/OVIDUCT	(45)	(41)	(44)
DEGENERATION, NOS	1 (2%)		

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

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 06-0220	LOW DOSE 06-0235	HIGH DOSE 06-0240
*OVARY	(40)	(40)	(41)
CYST, NOS		1 (3%)	2 (5%)
DEGENERATION, CYSTIC			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(47)	(46)	(46)
HYPERPLASIA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	30	10	15
ANIMAL MISSING/NO NECROPSY	2	2	1
NECROPSY PERF/NO HISTO PERFORMED		1	
AUTO/NECROPSY/HISTO PERF	1		
AUTO/NECROPSY/NO HISTO		1	
AUTOLYSIS/NO NECROPSY	1	2	2

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

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