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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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p-CHLOROANILINE

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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 p-CHLOROANILINE
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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 p-chloroaniline 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 p-chloroaniline was conducted by Litton Bionetics, Inc., Kensington, Maryland, 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. N. P. Page (1,2), Dr. E. K. Weisburger (1) and Dr. J. H. Weisburger (1,3). The principal investigators for the contract were Dr. F. M. Garner (4) and Dr. B. M. Ulland (4,5). Mr. S. Johnson (4) was the coprincipal investigator for the contract. Animal treatment and observation were supervised by Mr. R. Cypher (4), Mr. D. S. Howard (4) and Mr. H. D. Thornett (4); Mr. H. Paulin (4) analyzed dosed feed mixtures. Ms. J. Blalock (4) was responsible for data collection and assembly.

Histopathologic examinations were performed by Dr. F. M. Garner (4), Dr. B. Cockrell (4), Dr. E. Gorgacz (4) and Dr. M. Valerio (4) at Litton Bionetics, Inc., the pathology narratives were written by Dr. F. M. Garner (4), and Dr. J. M. Ward (1) reviewed the slides. The diagnoses included in this report represent the interpretation of these pathologists.

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (6); the statistical analysis was performed by Mr. R. M. Helfand (7) and Dr. J. P. Dirkse, III (8) using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (9).

This report was prepared at METREK, a Division of The MITRE Corporation (7) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (7), task leader Ms. P. Walker (7), senior biologist Mr. M. Morse (7), biochemist Mr. S. C. Drill (7), chemist Dr. N. Zimmerman (7), and technical editor Ms. P. A. Miller (7). The final report was reviewed by members of the participating organizations.

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. R. A. Griesemer (1), Dr. T. E. Hamm (1), Dr. W. V. Hartwell (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. A. R. Patel (1), Dr. R. A. Squire (1,10), Dr. S. F. Stinson (1), and Dr. C. E. Whitmire (1).

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1. Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

2. Now with the U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, D.C.

3. Now with the Naylor Dana Institute for Disease Prevention, American Health Foundation, Hammon House Road, Valhalla, New York.

4. Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland.

5. Now with Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike, Vienna, Virginia.

6. EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

7. The MITRE Corporation, METREK Division, 1820 Dolley Madison Boulevard, McLean, Virginia.

8. Consultant to The MITRE Corporation, currently a professor in the Department of Statistics at The George Washington University, 2100 Eye Street, N.W., Washington, D.C.

9. Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

10. Now with the Division of Comparative Medicine, Johns Hopkins University, School of Medicine, Traylor Building, Baltimore, Maryland.

SUMMARY

A bioassay for the possible carcinogenicity of p-chloroaniline was conducted using Fischer 344 rats and B6C3F1 mice. p-Chloroaniline was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of p-chloroaniline were, respectively, 500 and 250 ppm for rats and 5000 and 2500 ppm for mice. The compound was administered in the diet for 78 weeks, followed by an observation period of 24 weeks for rats and 13 weeks for mice.

There were no significant positive associations between the dietary concentrations of p-chloroaniline administered and mortality in female rats or in mice of either sex; however, there was a significant positive association between concentration and mortality in male rats. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Mean body weight depression, in relation to controls, was observed in high dose female rats and dosed mice of both sexes, indicating that the concentrations of p-chloroaniline administered to these animals may have approximated the maximum tolerated concentrations. Although splenic lesions were observed in male rats, no mean body weight depression relative to controls was associated with administration of p-chloroaniline to these animals. Therefore, it is possible that these animals may have been able to tolerate a higher dietary concentration of the compound.

The only neoplastic lesions found that might be related to administration of the compound were mesenchymal tumors in the spleens of male rats and hemangiomatous tumors in mice. In male rats, there was a significant positive association between compound administration and the incidences of fibroma or fibrosarcoma of the spleen. The incidences of these tumors were not significantly elevated when compared to those in control rats, but the rarity of these tumors in male Fischer 344 rats (0/360 in historical male control rats in this laboratory) strongly suggests a chemically related effect. In addition, three sarcomas of other types were found in high dose male rats. In mice of both sexes, hemangiomas and hemangiosarcomas were found at elevated incidences, when compared to control mice, in the spleen, liver, kidney, and multiple body sites. The increased incidences in dosed mice were statistically related to dose but were not statistically significant when compared directly to matched control animals. In comparison to historical control data, the incidences of hemangiomatous tumors in the dosed mice were elevated, but not greatly. The evidence was considered insufficient to conclusively relate the hemangiomatous tumors in mice to compound administration.

Nonneoplastic proliferative and chronic inflammatory lesions were also found in the spleens of dosed rats and mice.

The finding of small numbers of fibromas and sarcomas in the spleens of male rats was considered strongly suggestive of carcinogenicity because of the rarity of these tumors in the spleens of control rats. Hemangiomatous tumors in dosed mice may also have been associated with administration of p-chloroaniline. However, it is concluded that, under the conditions of this bioassay, sufficient evidence was not found to establish the carcinogenicity of p-chloroaniline for Fischer 344 rats or B6C3F1 mice.

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I. INTRODUCTION

p-Chloroaniline (Figure 1) (NCI No. C02039), a dye and chemical intermediate, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer observed among dye manufacturing industry workers (Anthony and Thomas, 1970; Wynder et al., 1963). Aromatic amines are among several classes of chemicals thought to be responsible for the increased cancer risk in this industry (Clayson and Garner, 1976).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 4-chlorobenzenamine.* It is also called 4-chlorophenylamine; 4-chloroaniline; 4-CA; and 1-amino-4-chlorobenzene.

p-Chloroaniline is used in the manufacture of at least 10 dyes and pigments; C.I. (Colour Index) Pigment Green 10; C.I. Acid Orange 31; C.I. Acid Yellow 2, C.I. Vat Yellow 5; C.I. Vat Green 22; C.I. Vat Brown 21; Antinolo Brown G; C.I. Vat Red 33; C.I. Vat Red 30; and C.I. Vat Red 32 (Society of Dyers and Colourists, 1956). It is also used in the manufacture of at least one azoic dye component, C.I. Azoic Coupling Component 10 (Society of Dyers and Colourists, 1956).

p-Chloroaniline is also used as an intermediate in the manufacture of pharmaceutical and agricultural chemicals (Hawley, 1977).

*The CAS registry number is 106-47-8.

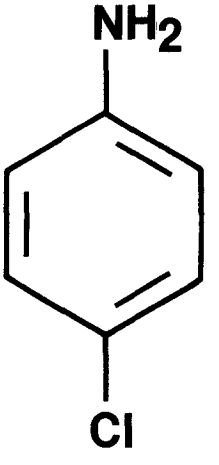

FIGURE 1
CHEMICAL STRUCTURE OF p-CHLOROANILINE

Specific production data for p-chloroaniline are not available; however, this compound is produced in commercial quantities (in excess of 1000 pounds or $1000 in value annually) in the United States. In addition, C.I. Pigment Green 10 and C.I. Vat Red 32, for which p-chloroaniline is used as an intermediate, are also produced in commercial quantities (U.S. International Trade Commission, 1977). Imports of p-chloroaniline through principal U.S. customs districts amounted to 244,896 pounds in 1974 (U.S. International Trade Commission, 1976).

The potential for exposure to p-chloroaniline is greatest for workers in the dye, chemical, and pharmaceutical manufacturing industries. In mammals, p-chloroaniline is a metabolite of the urea herbicides, monuron and aresin (Ernst, 1969), and in avian and aquatic organisms it is a metabolite of difluobenzuron (Dimilin®) (U.S. Department of Agriculture, 1978). Consequently, exposure to this compound may occur upon ingestion of either of these herbicides on contaminated foodstuffs.

The acute toxic effects of p-chloroaniline are similar to those of aniline, but the former compound is somewhat more toxic. The most prominent symptom of acute toxicity is methemeglobinemia. Like aniline, p-chloroaniline can be absorbed through the intact skin (Gosselin et al., 1976).

p-Chloroaniline substantially increased the back mutation frequency of a methionine-requiring auxotroph of Aspergillus nidulans (Prasad, 1970).

II. MATERIALS AND METHODS

A. Chemicals

Technical-grade p-chloroaniline was purchased from E. I. duPont de Nemours & Company, Wilmington, Delaware. Chemical analysis was performed by Litton Bionetics, Inc., Kensington, Maryland. The experimentally determined range in melting point (i.e., 68° to 71°C) closely approximated the value found in the literature (i.e., 72.5°C) (Weast, 1977). Thin-layer chromatography was performed utilizing one solvent system (i.e., diethyl ether:acetic acid:hexane) in two proportions (i.e., 100:5:20 and 100:5:40). Visualization of each plate with visible and ultraviolet light, iodine vapor, and $FeCl_3$-$K_3[Fe(CN)_6]$ spray revealed the presence of only one spot. The results of gas chromatographic analysis showed only one peak. Infrared analysis provided results that were consistent with those reported in the literature (Pouchort, 1975).

Throughout this report, the term p-chloroaniline is used to represent this technical-grade material.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® meal (Allied Mills, Inc., Chicago, Illinois). p-Chloroaniline was administered to the dosed animals as a component of the diet.

An aliquot of the test chemical was blended with a small amount of the feed using a mortar and pestle. Once visual homogeneity was

attained, the mixture was placed in a 6 kg capacity Patterson-Kelley standard model twin-shell stainless steel V-blender along with the remainder of the feed to be prepared. After 20 minutes of blending, the mixtures were placed in double plastic bags and stored in the dark at 4°C. The mixture was prepared once weekly.

Dosed feed preparations containing 250 and 500 ppm of p-chloroaniline were analyzed spectrophotometrically. The mean result immediately after preparation was 83 percent of theoretical (ranging from 80 to 85 percent).

C. Animals

The two animal species, Fischer 344 rats and B6C3F1 mice, used in the carcinogenicity bioassay were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Rats were supplied by A. R. Schmidt, Madison, Wisconsin, and Laboratory Supply Company, Inc., Indianapolis, Indiana. Mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

Rats and mice, approximately 4 weeks old when received, were examined and any obviously ill or runted animals were killed. The remaining animals were quarantined for 2 weeks prior to initiation of test. Animals which did not manifest clinical signs of disease were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenance

Animals were housed by species in rooms maintained at 22° to 26°C and 45 to 55 percent relative humidity. Incoming air was filtered through HEPA filters (Flanders Filters, McLean, Virginia) at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided 8 hours per day (9:00 a.m. to 5:00 p.m.).

Rats were housed four per cage by sex and mice were housed five per cage by sex. Throughout the study dosed and control animals of both species were housed in polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) suspended from aluminum racks. Racks were fitted with a continuous piece of stainless steel mesh over which a sheet of filter paper was firmly secured. Filter paper was changed at 2-week intervals, when the racks were sanitized. Clean cages and bedding (Ab-sorb-dri® hardwood chip bedding [Wilner Wood Products Company, Norway, Maine]) were provided twice weekly.

Acidulated water (pH 2.5) was supplied to animals in water bottles which were changed and washed twice weekly. Sipper tubes were washed at weekly intervals. During the period of chemical administration, dosed and control animals received treated or untreated Wayne Lab-Blox® meal as appropriate. The feed was supplied in hanging stainless steel hoppers which were refilled three times per week and sanitized weekly. Food and water were available ad libitum for both species.

All dosed and control rats were housed in a room with other rats receiving diets containing* Michler's ketone (90-94-8); trimethylthiourea (2489-77-2); and p-nitrosodiphenylamine (156-10-5).

All dosed and control mice were housed in a room with mice receiving diets containing Michler's ketone (90-94-8); 4,4'-methylenebis(N,N-dimethyl)benzenamine (101-61-1); trimethylthiourea (2489-77-2); 5-chloro-o-toluidine (95-79-4); 3-chloro-p-toluidine (95-74-9); 1-phenyl-2-thiourea (103-85-5); dibutyltin diacetate (1067-33-0); N-phenyl-p-phenylenediamine hydrochloride (2198-59-6); and 2-nitro-p-phenylenediamine (5307-14-2).

E. Selection of Initial Concentrations

To establish the concentrations of p-chloroaniline for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Rats were distributed among six groups, each consisting of five males and five females. p-Chloroaniline was incorporated into the basal laboratory diet and supplied ad libitum to five of the six rat groups in concentrations of 70, 145, 315, 680 and 1465 ppm. The remaining rat group served as a control group, receiving only the basal laboratory diet.

Mice were distributed among nine groups, each consisting of five males and five females. p-Chloroaniline was incorporated into the basal laboratory diet and supplied ad libitum to eight of the nine mouse groups in concentrations of 255, 550, 1180, 2550, 5500, 8080,

*CAS registry numbers are given in parentheses.

11,830 and 17,380 ppm. The remaining mouse group served as a control group, receiving only the basal laboratory diet.

The dosed dietary preparations were administered for a period of 4 weeks, followed by a 2-week observation period during which all animals were fed the basal laboratory diet. Individual body weights and food consumption data were recorded twice weekly throughout the study. Upon termination of the study all survivors were euthanized and necropsied.

The following table indicates the mean body weight gain, relative to controls, survival and incidence of enlarged spleens with plaque formation observed in each of the rat groups at the end of the subchronic test.

RAT SUBCHRONIC STUDY RESULTS

	Mean Body Weight Gain (%)[a]		Survival[b]		Observation of Enlarged Spleens with Plaque Formation[b]	
ppm	Males	Females	Males	Females	Males	Females
0	--	--	5/5	5/5	0/5	0/5
70	+42	+ 6	5/5	5/5	0/5	0/5
145	+ 1	+ 8	5/5	5/5	0/5	0/5
315	+32	+20	5/5	5/5	0/5	0/5
680	+28	-11	5/5	5/5	5/5	5/5
1465	+ 3	+ 5	5/5	5/5	5/5	5/5

The high concentration selected for administration to dosed rats in the chronic bioassay, based upon the observation of splenic lesions, was 500 ppm.

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[a] + is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.

[b] Number of animals observed/number of animals originally in group.

The following table indicates the mean body weight gain, relative to controls, survival and incidence of enlarged spleens observed in each of the mouse groups at the end of the subchronic test.

MOUSE SUBCHRONIC STUDY RESULTS

	Mean Body Weight Gain (%)[a]		Survival[b]		Observation of Enlarged Spleens[b]	
ppm	Males	Females	Males	Females	Males	Females
0	--	--	5/5	5/5	0/5	0/5
255	+16	+ 2	5/5	5/5	0/5	0/5
550	+ 4	- 3	5/5	5/5	0/5	0/5
1,180	+16	+ 5	5/5	5/5	0/5	0/5
2,550	+ 3	+ 4	5/5	5/5	0/5	0/5
5,500	+ 4	+ 8	5/5	5/5	0/5	0/5
8,080	--	--	0/5	0/5	0/5	0/5
11,830	+ 3	+ 2	5/5	5/5	5/5	0/5
17,380	--	+10	1/5	5/5	0/5	5/5

No reason for the deaths among the male and female mice receiving 8080 ppm was recorded. The high concentration selected for administration to dosed mice in the chronic bioassay was 5000 ppm.

F. Experimental Design

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

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed rats were

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[a] + is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.

[b] Number of animals observed/number of animals originally in group.

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
p-CHLOROANILINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	p-CHLOROANILINE CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	102
LOW DOSE	50	250	78	
		0		24
HIGH DOSE	50	500	78	
		0		24
FEMALE				
CONTROL	20	0	0	102
LOW DOSE	50	250	78	
		0		24
HIGH DOSE	50	500	78	
		0		24

[a] Concentrations given in parts per million.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
p-CHLOROANILINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	p-CHLOROANILINE CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	91
LOW DOSE	50	2500	78	
		0		13
HIGH DOSE	50	5000	78	
		0		13
FEMALE				
CONTROL	20	0	0	91
LOW DOSE	50	2500	78	
		0		13
HIGH DOSE	50	5000	78	
		0		13

[a]Concentrations given in parts per million.

supplied with diets containing 500 and 250 ppm p-chloroaniline for 78 weeks followed by a 24-week observation period. Throughout this report those rats receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed mice were supplied with diets containing 5000 and 2500 ppm p-chloroaniline for 78 weeks followed by a 13-week observation period. Throughout this report those mice receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment and body weights were recorded once a week for the first 6 weeks, every 2 weeks for the next 12 weeks, and at monthly intervals thereafter. All animals were inspected twice daily. Food consumption data were collected at monthly intervals from 20 percent of the animals in each group.

All moribund animals, animals that developed large, palpable masses that jeopardized their health, or animals that survived until the end of the bioassay were euthanized using carbon dioxide inhalation. Necropsies were performed immediately on these animals and on all animals found dead during the bioassay. Gross and microscopic

examinations were performed on all major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in a 10 percent neutral buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination.

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, 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 recorded in each group at the time that the test was initiated.

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 evidence of dose-related mean body weight depression was apparent in male rats. Slight mean body weight depression, relative to the controls, was observed in high dose females after the 40th week of the bioassay (Figure 2).

No other clinical signs were recorded.

B. Survival

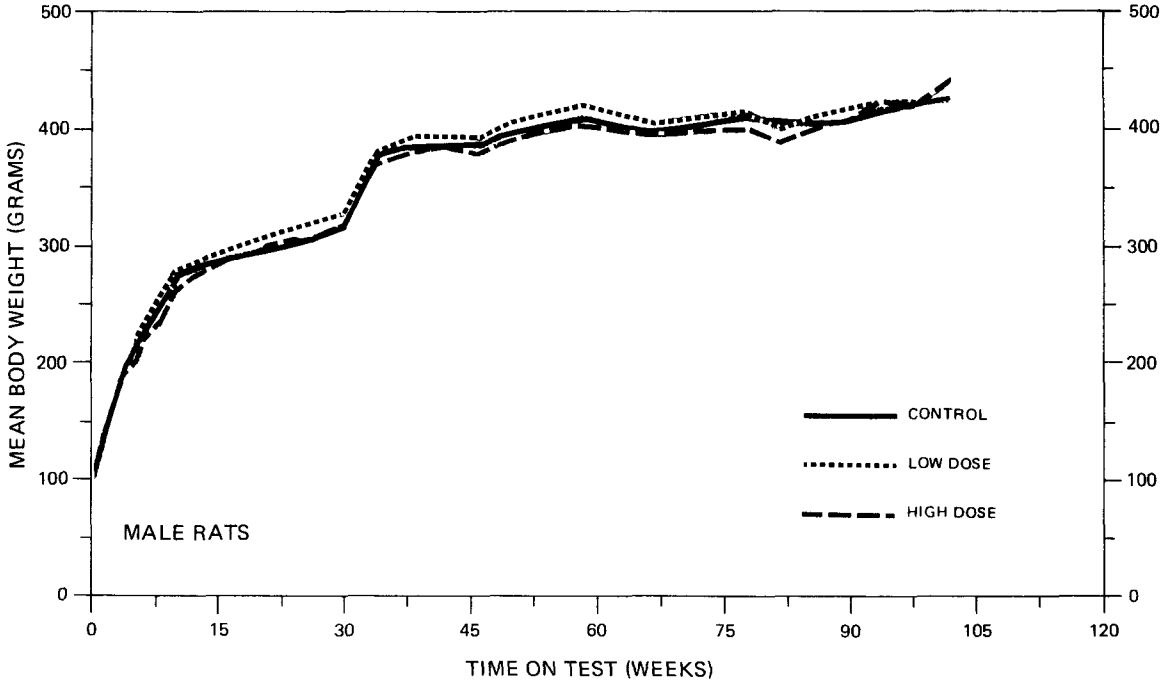
The estimated probabilities of survival for male and female rats in the control and p-chloroaniline-dosed groups are shown in Figure 3. The Tarone test indicated a significant ($P = 0.0294$) positive association between dosage and mortality in male rats, but not in female rats.

There were adequate numbers of male rats at risk from late-developing tumors as 76 percent (38/50) of the high dose, 92 percent (46/50) of the low dose, and 90 percent (18/20) of the controls survived on test for at least 102 weeks.

For females, with 90 percent (45/50) of the high dose, 98 percent (49/50) of the low dose, and 90 percent (18/20) of the controls surviving on test for at least 102 weeks, there were adequate numbers at risk from late-developing tumors.

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).



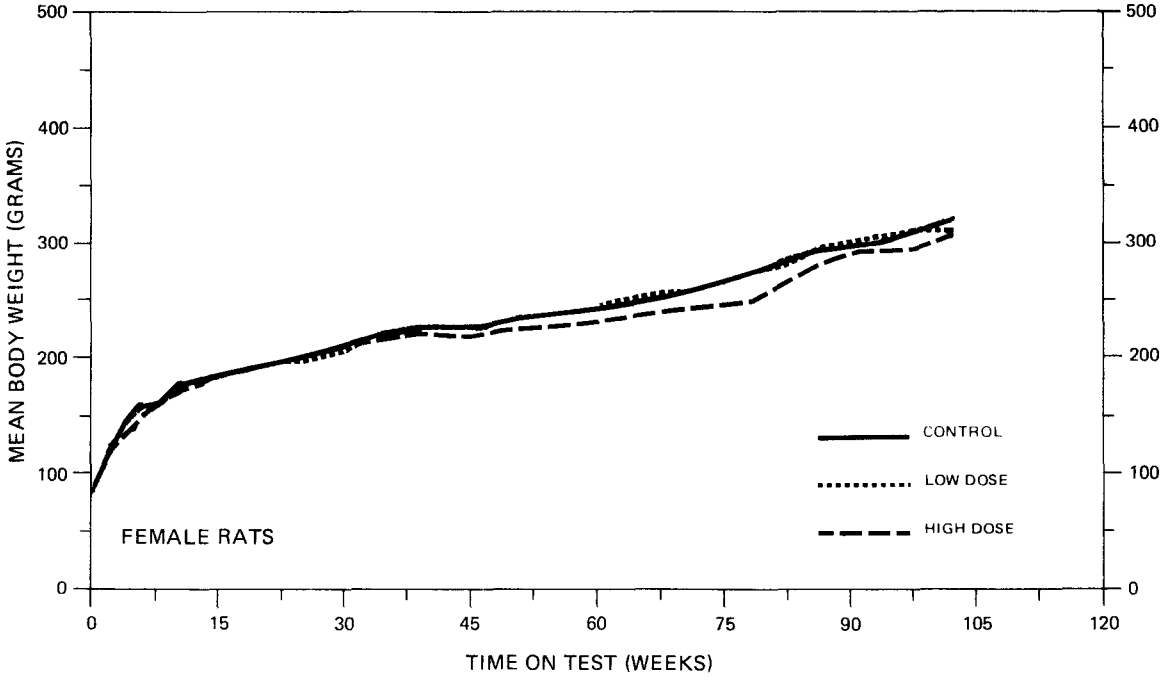
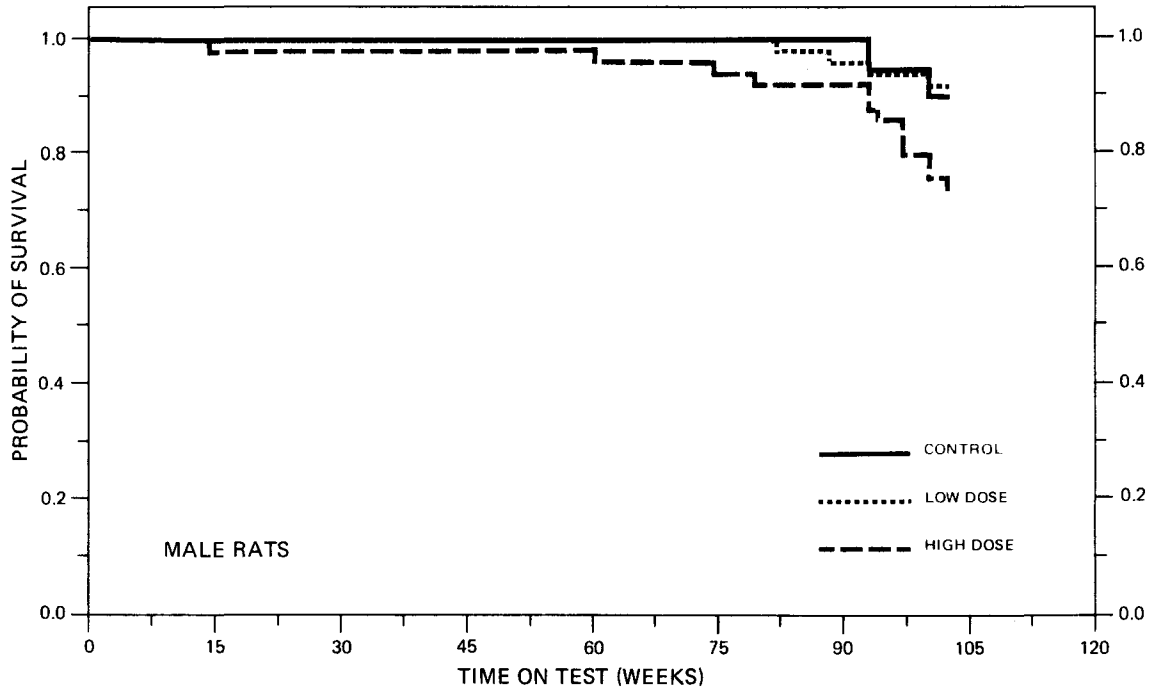


FIGURE 2
GROWTH CURVES FOR p-CHLOROANILINE CHRONIC STUDY RATS



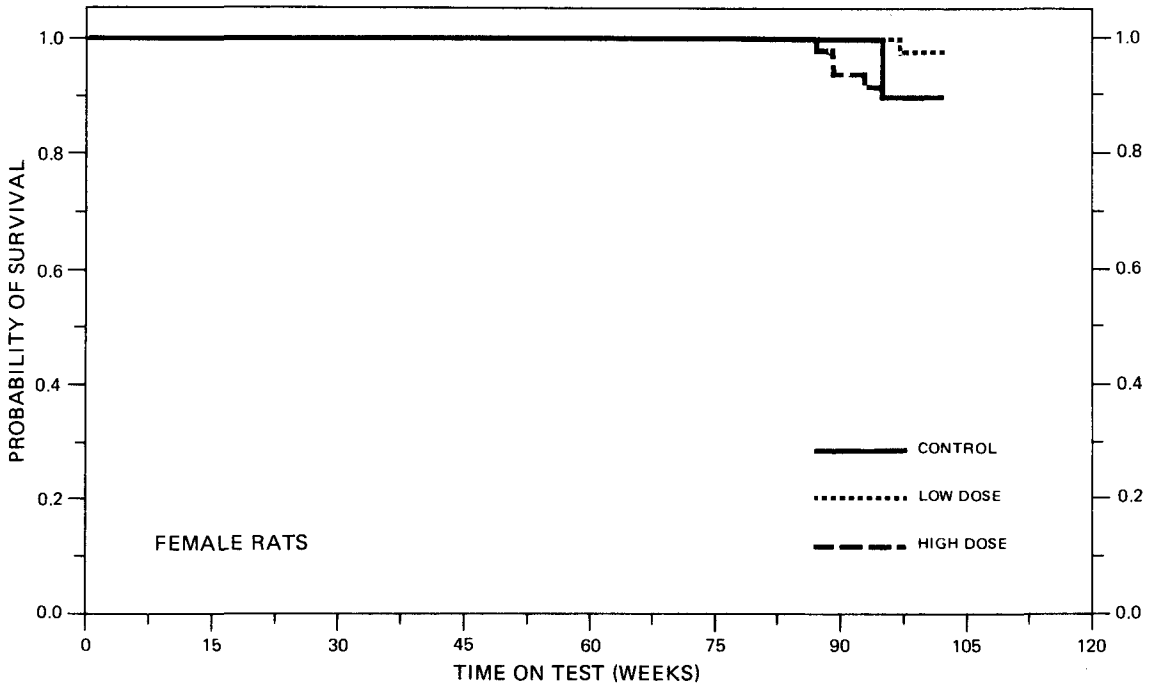


FIGURE 3
SURVIVAL COMPARISONS OF p-CHLOROANILINE CHRONIC STUDY RATS

A variety of neoplasms was present in both the dosed and control groups. The majority of these types has been encountered previously as a spontaneous lesion, except for those of the spleen.

In the high dose male rats, there was an increased incidence (i.e., 0/20 controls, 0/49 low dose, 10/49 high dose) of unusual splenic neoplasms (i.e., fibroma, fibrosarcoma, sarcoma NOS, hemangiosarcoma, and osteosarcoma). The rationale for combination of these lesions is twofold: (1) the fibromas are considered to be a benign form of sarcoma and (2) the neoplasms are all derived from cells of similar origin. The microscopic appearance of the fibromas varied from the cellular proliferation of fibroblasts to tumors containing abundant mature collagen and few cells. It should be noted that a metastatic sarcoma NOS was observed in the splenic capsule of one control male rat; however, no primary sarcoma NOS was observed in this animal.

Nonneoplastic, proliferative lesions were present to some degree in the spleen (splenic capsule) of most dosed rats. The most frequent abnormality involving the capsule was focal fibrosis. The capsule was irregularly thickened and often had eosinophilic collagenous tags, papillae or frond-like structures attached to the external surface of the capsule. Some of the latter occasionally contained a lumen filled with eosinophilic material resembling edema fluid. Frequently, subcapsular proliferation of undifferentiated mesenchymal cells with indistinct cytoplasmic borders and pleomorphic nuclei in varying

amounts was apparent. On occasion the capsular proliferation contained trapped iron-positive material. Also, fibrosis and fatty metamorphosis were noted in the splenic parenchyma of several high dose male and female rats. A few of the spleens contained more hemosiderin than is normally present in spleens of Fischer 344 rats.

The usual variety of other nonneoplastic lesions was present in both dosed and control rats. The majority of the lesions were interpreted as spontaneous and have been encountered previously in the Fischer 344 rat.

Based on the results of this pathology examination, p-chloroaniline was carcinogenic in male rats, inducing neoplasms of the spleen. In addition, the compound induced nonneoplastic proliferative splenic capsular and parenchymal lesions in male and female Fischer 344 rats under the conditions of this bioassay.

D. Statistical Analysis of Results

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

In male rats the Cochran-Armitage test indicated a significant ($P = 0.001$) positive association between dose and the combined incidence of fibromas, fibrosarcomas, hemangiosarcomas, osteosarcomas or

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH p-CHLOROANILINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Spleen: Fibroma[b]	0/20(0.00)	0/49(0.00)	6/49(0.12)
P Values[c]	P = 0.010	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.680
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	74
Spleen: Fibroma or Fibrosarcoma[b]	0/20(0.00)	0/49(0.00)	7/49(0.14)
P Values[c]	P = 0.005	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.826
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	74
Spleen or Splenic Capsule: Fibrosarcoma, Hemangiosarcoma, Osteosarcoma or Sarcoma NOS[b]	0/20(0.00)	0/49(0.00)	4/49(0.08)
P Values[c]	P = 0.039	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.394
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	93

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Spleen or Splenic Capsule: Fibroma, Fibrosarcoma, Hemangiosarcoma, Osteosarcoma or Sarcoma NOS[b]	0/20(0.00)	0/49(0.00)	10/49(0.20)
P Values[c]	P = 0.001	N.S.	P = 0.024
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	1.266
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	74
Pituitary: Chromophobe Adenoma[b]	1/17(0.06)	1/44(0.02)	3/43(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.386	1.186
Lower Limit	---	0.005	0.106
Upper Limit	---	29.672	60.801
Weeks to First Observed Tumor	100	102	93
Adrenal: Pheochromocytoma[b]	3/19(0.16)	4/46(0.09)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.551	0.388
Lower Limit	---	0.106	0.058
Upper Limit	---	3.503	2.710
Weeks to First Observed Tumor	102	102	102

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Carcinoma[b]	0/18(0.00)	2/38(0.05)	1/41(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.146	0.024
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	102	102
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	1/18(0.06)	4/38(0.11)	2/41(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.895	0.878
Lower Limit	---	0.210	0.050
Upper Limit	---	90.702	50.545
Weeks to First Observed Tumor	100	102	102
Pancreatic Islets: Islet-Cell Adenoma[b]	2/19(0.11)	2/49(0.04)	4/40(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.388	0.950
Lower Limit	---	0.031	0.153
Upper Limit	---	5.108	9.926
Weeks to First Observed Tumor	102	102	102

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Testis: Interstitial-Cell Tumor[b]	19/20(0.95)	49/50(0.98)	43/49(0.88)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.032	0.924
Lower Limit	---	0.962	0.859
Upper Limit	---	1.109	1.160
Weeks to First Observed Tumor	93	82	93

[a]Treated groups received doses of 250 or 500 ppm 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 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH p-CHLOROANILINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	4/18(0.22)	17/50(0.34)	13/45(0.29)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.530	1.300
Lower Limit	---	0.603	0.484
Upper Limit	---	5.615	4.914
Weeks to First Observed Tumor	102	97	89
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	0/20(0.00)	3/39(0.08)	2/37(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.321	0.166
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	102	102
Mammary Gland: Fibroadenoma[b]	1/20(0.05)	3/50(0.06)	1/50(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.200	0.400
Lower Limit	---	0.106	0.005
Upper Limit	---	61.724	30.802
Weeks to First Observed Tumor	102	97	102

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Uterus: Endometrial Stromal Polyp[b]	1/20(0.05)	4/50(0.08)	6/50(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.600	2.400
Lower Limit	---	0.175	0.325
Upper Limit	---	77.169	108.021
Weeks to First Observed Tumor	102	102	102

[a]Treated groups received doses of 250 or 500 ppm 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 $P < 0.05$.

sarcomas NOS of the spleen or splenic capsule. The Fisher exact test comparing the high dose group to the control group was also significant.

As indicated previously, a metastatic sarcoma NOS was observed in the splenic capsule of one control male rat; however, no primary sarcoma NOS was detected in this animal. If it is assumed that the missing primary tumor was in the spleen of this male rat the incidences for the combination of splenic fibromas, fibrosarcomas, hemangiosarcomas, osteosarcomas, and sarcomas NOS would be 1/20, 0/49, and 10/49 for the control, low dose, and high dose male rats, respectively. In this case the high dose to control Fisher exact comparison (P = 0.106) would not be significant under the Bonferroni criterion. For untreated control Fischer 344 male rats maintained by this laboratory for the NCI Carcinogenesis Testing Program the historical incidences of splenic fibromas, fibrosarcomas, osteosarcomas, and hemangiosarcomas are each 0/360 and the historical incidence of sarcomas NOS is 1/360 (0.27 percent). Therefore, even though the high dose to control Fisher exact comparison would not be significant if the missing sarcoma NOS was added to the control group, the data suggest a compound-related increase in the incidence of a combination of splenic fibromas, fibrosarcomas, hemangiosarcomas, osteosarcomas, and sarcomas NOS in male rats.

For male rats the Cochran-Armitage test indicated a significant (P = 0.010) positive association between dose and the incidence of

fibromas of the spleen. However, neither of the Fisher exact tests were significant. The Cochran-Armitage test also indicated a significant ($P = 0.039$) positive association between dose and the combined incidence of fibrosarcomas, hemangiosarcomas, osteosarcomas, or sarcomas NOS of the spleen or splenic capsule but neither of the Fisher exact tests were significant.

None of the statistical tests for any site in female rats indicated a significant positive association between chemical administration and tumor incidence.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

Distinct mean body weight depression relative to controls was observed in both male and female dosed mice throughout the bioassay (Figure 4).

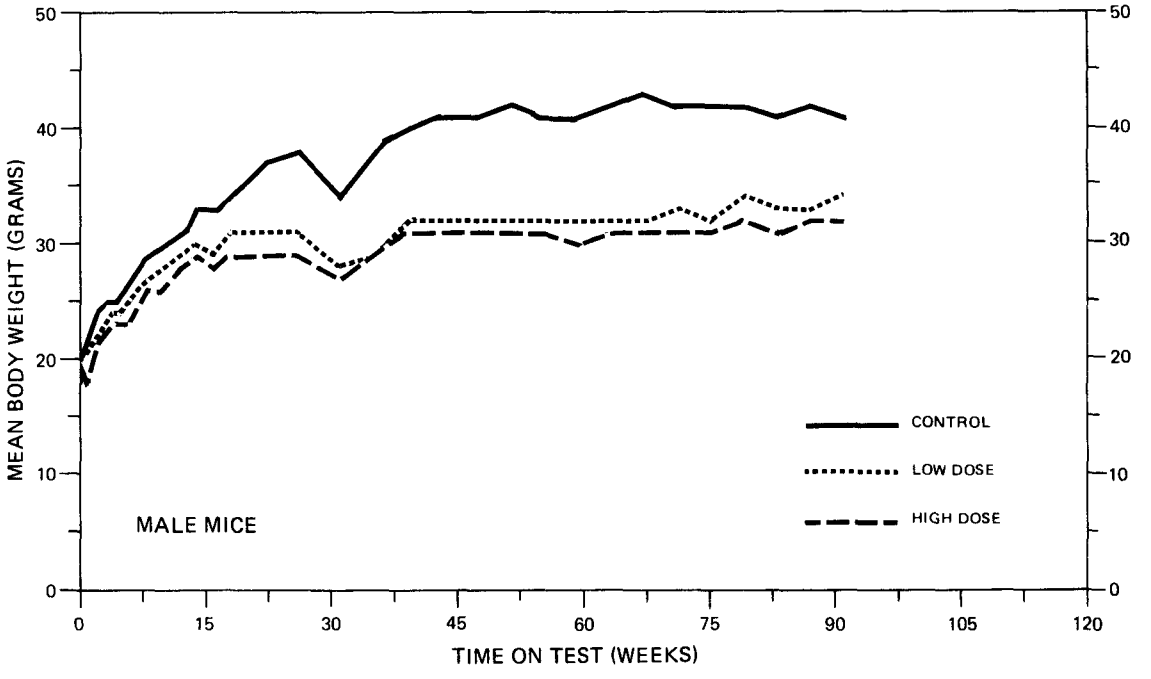
No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the control and p-chloroaniline-dosed groups are shown in Figure 5. Neither the Tarone test nor the Cox tests indicated a significant positive association between dosage and mortality in either male or female mice. In females, the test for departure from linear trend was significant.

There were adequate numbers of male mice at risk from late-developing tumors, as 88 percent (44/50) of the high dose, 88 percent (44/50) of the low dose and 90 percent (18/20) of the controls survived until the end of the study.

For females, with 78 percent (39/50) of the high dose, 82 percent (41/50) of the low dose and 100 percent (20/20) of the control group surviving until the end of the study, there were adequate numbers at risk from late-developing tumors. Eight of the high dose females were reported as missing; 2 in week 18 and 6 in week 50.



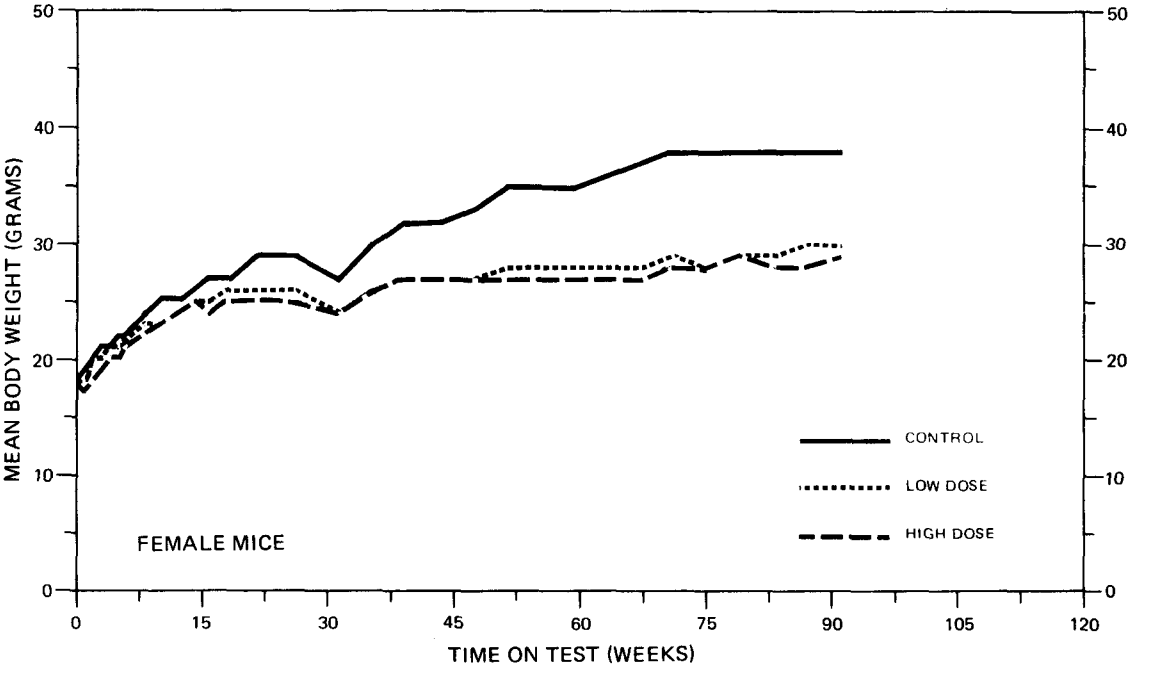
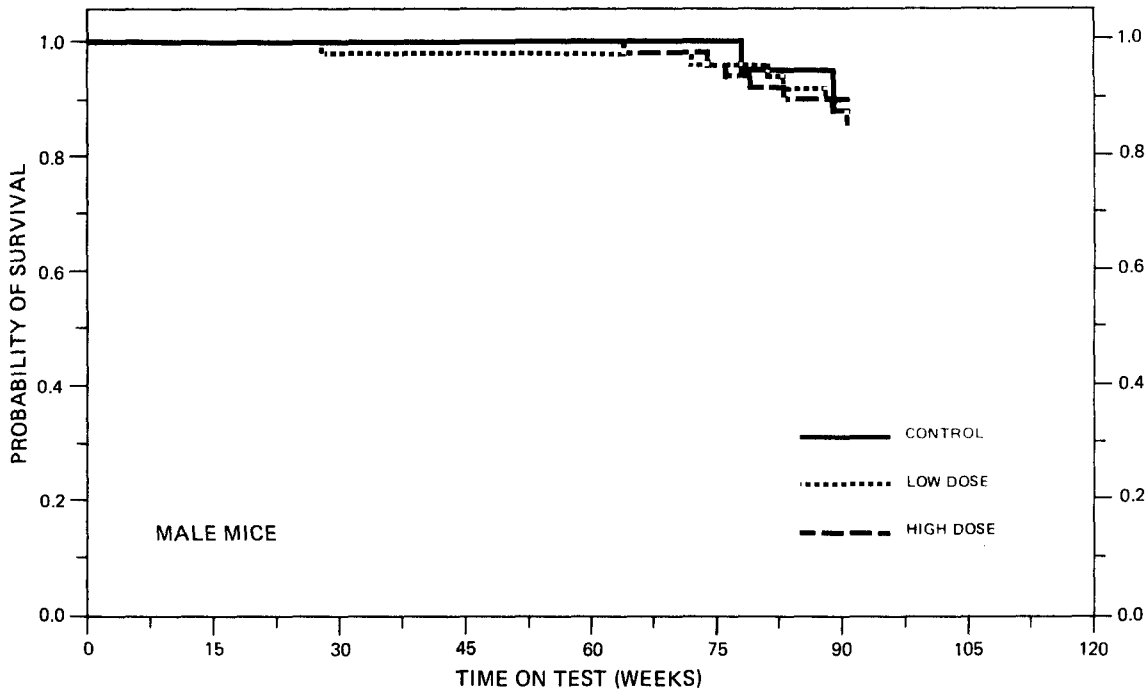


FIGURE 4
GROWTH CURVES FOR p-CHLOROANILINE CHRONIC STUDY MICE



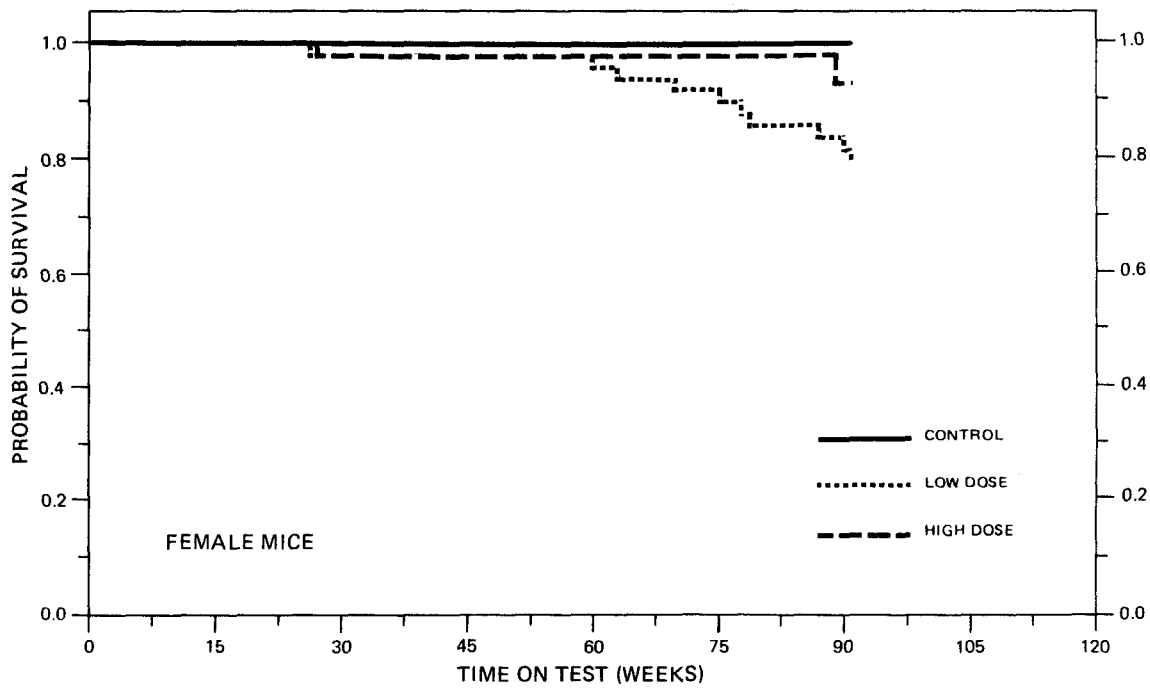


FIGURE 5
SURVIVAL COMPARISONS OF p-CHLOROANILINE CHRONIC STUDY MICE

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).

In the dosed males and females, there was an increased incidence of neoplasms of endothelial origin (i.e., hemangiomas and hemangiosarcomas) in the subcutaneous tissue, spleen, liver, kidney or multiple organs (i.e., 2/20 control males, 10/50 low dose males, 14/50 high dose males, 0/18 control females, 3/49 low dose females, and 8/42 high dose females). These tumors consisted of nonencapsulated, invading masses of vascular channels of various sizes lined with atypical endothelium. The histologic pattern frequently varied within the same tumor from large, cavernous spaces to dense proliferations of capillary structures. The neoplastic endothelial cells were hypertrophic with strongly basophilic nuclei and indistinct cytoplasm. In the more cellular areas, the vascular pattern was less pronounced and the neoplastic endothelium proliferated either in sheets or in a tangled haphazard fashion. These cells were spindle-shaped or in some cases pleomorphic with large basophilic vesiculated nuclei. Mitoses were present, but were not numerous. Foci of necrosis and hemorrhage, thrombosis and pigment laden macrophages were present in several of the neoplasms.

A variety of other neoplasms was present in both the dosed and control groups. Each of these types has been encountered previously

in the B6C3F1 mouse. However, in female mice hepatocellular adenomas and hepatocellular carcinomas were found only in dosed animals (i.e., 0/18, 1/49, and 6/41 in the control, low dose and high dose groups, respectively).

The variety of nonneoplastic lesions present in both the dosed and control animals, except for the pigmentation noted, has been encountered previously in aging B6C3F1 mice.

In both the dosed males and females there was moderate to heavy intracellular deposition of iron-positive pigment in most tissues; this was especially prominent in the spleen, liver and kidney. This pigment was interpreted as hemosiderin, and its presence was probably the result of excessive hemolysis induced by the compound.

Based on the results of this pathology examination, p-chloroaniline was carcinogenic in B6C3F1 mice under the conditions of this test, inducing hemangiosarcomas and hemangiomas in the liver, spleen, and other organs.

D. Statistical Analysis 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 malignant tumor in either sex where at least two such tumors were observed in at least one of the control or p-chloroaniline-dosed groups and where such tumors were observed in at least 5 percent of the group.

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH p-CHLOROANILINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	2/19(0.11)	1/48(0.02)	1/49(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.198	0.194
Lower Limit	---	0.004	0.003
Upper Limit	---	3.635	3.563
Weeks to First Observed Tumor	91	81	91
All Sites: Hemangiosarcoma[b]	2/20(0.10)	9/50(0.18)	14/50(0.28)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.800	2.800
Lower Limit	---	0.426	0.741
Upper Limit	---	16.255	23.979
Weeks to First Observed Tumor	89	72	79
All Sites: Hemangiosarcoma or Hemangioma[b]	2/20(0.10)	10/50(0.20)	14/50(0.28)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.000	2.800
Lower Limit	---	0.488	0.741
Upper Limit	---	17.808	23.979
Weeks to First Observed Tumor	89	72	79

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	1/19(0.05)	3/49(0.06)	1/49(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.163	0.388
Lower Limit	---	0.103	0.005
Upper Limit	---	59.809	29.845
Weeks to First Observed Tumor	91	81	91
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	3/19(0.16)	7/49(0.14)	2/49(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.905	0.259
Lower Limit	---	0.239	0.024
Upper Limit	---	5.042	2.118
Weeks to First Observed Tumor	91	81	91

[a]Treated groups received doses of 2500 or 5000 ppm 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 p-CHLOROANILINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	1/18(0.06)	2/49(0.04)	4/42(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.735	1.714
Lower Limit	---	0.042	0.190
Upper Limit	---	42.478	82.316
Weeks to First Observed Tumor	91	26	89
All Sites: Hemangiosarcoma[b]	0/18(0.00)	3/49(0.06)	7/42(0.17)
P Values[c]	P = 0.022	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.231	0.875
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	89
All Sites: Hemangiosarcoma or Hemangioma[b]	0/18(0.00)	3/49(0.06)	8/42(0.19)
P Values[c]	P = 0.012	N.S.	P = 0.046
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.231	1.030
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	89

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	0/18(0.00)	0/49(0.00)	3/41(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.277
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	91
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	0/18(0.00)	1/49(0.02)	6/41(0.15)
P Values[c]	P = 0.014	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.020	0.738
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	91

[a]Treated groups received doses of 2500 or 5000 ppm 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.

The Cochran-Armitage test indicated a significant (P = 0.014) positive association between dosage and the combined incidence of hepatocellular carcinomas or hepatocellular adenomas in female mice but the Fisher exact tests were not significant. The Cochran-Armitage test also indicated a significant (P = 0.022) positive association between dosage and the incidence of hemangiosarcomas. Neither the high dose to control nor the low dose to control Fisher exact comparisons were significant. The combined incidence of hemangiosarcomas or hemangiomas was also shown to have a significant (P = 0.012) positive association with dosage but again the Fisher exact tests were not significant.

For control female B6C3F1 mice maintained by this laboratory for the NCI Carcinogenesis Testing Program the combined incidence of hemangiosarcomas or hemangiomas is about 3 percent (7/260). Thus, even though the significant positive Cochran-Armitage tests at this site were not supported by the Fisher exact tests, when coupled with the difference between the incidences in the historical controls and the dosed female mice in this bioassay, the data suggest the possibility of an association between dose and tumor incidence in female mice.

In male mice, neither the Cochran-Armitage test nor the Fisher exact tests indicated a significant positive association between dose and tumor incidence for hemangiosarcomas or for the combination of hemangiosarcomas and hemangiomas. However, the historical control

incidence for the combined incidence of hemangiosarcomas or hemangiomas of the circulatory system in control male B6C3F1 mice maintained by this laboratory for the NCI Carcinogenesis Testing Program is about 3 percent (8/262). Thus, the observed incidences of 10 percent (2/20), 20 percent (10/50), and 28 percent (14/50) for the control, low, and high dose groups, respectively, suggest a compound-related increase.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by p-chloroaniline that could not be established under the conditions of this test.

V. DISCUSSION

There was a significant positive association between the dietary concentrations of p-chloroaniline administered and mortality in male rats; however, there were no significant positive associations between concentration and mortality in female rats or in mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Mean body weight depression, in relation to controls, was observed in high dose female rats and dosed mice of both sexes, indicating that the concentrations of p-chloroaniline administered to these animals may have approximated the maximum tolerated concentrations. Although splenic lesions were observed in dosed male rats, no mean body weight depression relative to controls was associated with administration of p-chloroaniline to these animals. Therefore, it is considered that these animals could have tolerated a higher dietary concentration of the compound.

Neoplastic and nonneoplastic splenic lesions were observed in dosed male rats. The splenic neoplasms appeared to arise in the areas of capsular or parenchymal fibrosis, possibly from reticular cells in the spleen (Saito, 1977). These proliferative and neoplastic lesions were histologically identical to those induced in Fischer 344 rats by aniline hydrochloride (U.S. Department of Health, Education, and Welfare, 1978a) and o-toluidine hydrochloride (U.S. Department of Health, Education, and Welfare, 1978b). There was a significant positive association between the concentrations of p-chloroaniline

administered and the incidence of fibromas of the spleen in male rats; however, the Fisher exact comparisons were not significant. The results of the statistical analysis were the same when the combination of fibromas and fibrosarcomas of the spleen and the combination of splenic osteosarcomas, fibrosarcomas, hemangiosarcomas, and sarcomas NOS were considered. However, when the incidences for the combination of fibromas, fibrosarcomas, hemangiosarcomas, osteosarcomas, and sarcomas NOS were analyzed statistically, there was a significant positive Cochran-Armitage test as well as a significant high dose to control Fisher exact comparison. It should be noted that a metastatic sarcomas NOS was observed in a control male rat; however, no primary sarcoma NOS was detected in this animal. When it was assumed that the missing tumor was in the spleen of this control male, the high dose to control Fisher exact comparison for the combination of splenic neoplasms was not significant. When the historical control incidences for these neoplasms are compared to the incidences observed in high dose male rats in this bioassay, the evidence is suggestive of a compound-related effect. None of the other statistical tests for tumors at any other site in male or female rats indicated a significant positive association between concentration and tumor incidence.

For female mice there was a significant positive association between the concentrations of p-chloroaniline administered and the incidences of hemangiosarcomas (i.e., 0/18, 3/49, and 7/42 in the

control, low dose and high dose, respectively) and the incidences of a combination of hemangiosarcomas and hemangiomas (i.e., 0/18, 3/49, and 8/42 in the control, low dose, and high dose, respectively). In neither situation were the Fisher exact comparisons significant. A similar situation existed for male mice. Although the incidences of a combination of hemangiosarcomas and hemangiomas in male mice in this bioassay were 2/20, 10/50, and 14/50 in the control, low dose, and high dose, respectively, neither the Cochran-Armitage test nor the Fisher exact comparisons were significant. The historical incidences for this combination of neoplasms among control male and control female B6C3F1 mice maintained by this laboratory for the NCI Carcinogenesis Testing Program are 8/262 and 7/260, respectively (approximately 3 percent). When the apparent dose-related increased incidences observed in this bioassay are considered in light of the divergence between the historical control incidences and the incidences observed in dosed male and female mice in this bioassay, the data suggest an association between the administration of p-chloroaniline and the incidences of these tumors in both sexes of mice.

The only other site in mice of either sex for which a significant positive association could be demonstrated between concentration administered and tumor incidence was the liver in females. However, the Fisher exact comparisons for the combination of hepatocellular carcinomas and hepatocellular adenomas in female mice were not significant, and the incidences observed were not unusual.

The pigmentation of the spleen and other tissues that was observed in both rats and mice dosed with p-chloroaniline may be a result of compound-induced methemeglobinemia.

The compound was not shown to be carcinogenic in female Fischer 344 rats. Under the conditions of this bioassay, there was suggestive evidence (connective tissue neoplasms) for the carcinogenic (sarcogenic) action of p-chloroaniline in male Fischer 344 rats. The increased incidences of a combination of hemangiosarcomas and hemangiomas in dosed B6C3F1 mice of both sexes was also suggestive of a carcinogenic effect of p-chloroaniline in these animals. However, it is concluded that, under the conditions of this bioassay, sufficient evidence was not found to establish the carcinogenicity of p-chloroaniline for Fischer 344 rats or B6C3F1 mice.

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Review of the Bioassay of *p*-Chloroaniline* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

October 25, 1978

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

A representative of ICI Americas presented a public statement regarding the bioassay of *p*-Chloroaniline. A major point concerned the discrepancy in pathologic diagnoses between those given in the report and the ones provided by consultant pathologists to ICI Americas. He noted that two pathologists not associated with ICI also reviewed the histologic materials and were in agreement with ICI pathologists. He recommended that an independent panel of pathologists be convened to review the diagnoses in question.

A second point made by the ICI representative regarded a sarcoma, observed in the spleen of a control rat, which was not considered in the statistical evaluation of the study. He said that its omission was inconsistent with the inclusion of an osteosarcoma found in the spleen of a treated rat. He added that if the tumor was included in the analysis, the incidence would not be statistically significant in the treated animals. The representative concluded that the bioassay did not provide evidence for the carcinogenicity of *p*-Chloroaniline.

A pathologist for ICI Americas discussed the pathology from the *p*-Chloroaniline bioassay. He said that the design of the bioassay was inadequate by current standards and that the quality of some of the histologic preparations made it difficult to assess the nature of a lesion. The pathologist objected to the practice of combining fibromas

with malignant forms of splenic neoplasms, particularly when they were of different cell types. He noted that a number of splenic fibromas were diagnosed in treated rats by himself and another ICI pathologist. In mice, however, they found no significant treatment-related effect in either sex. The ICI pathologist showed a number of slides in which he illustrated the discrepancies in diagnoses. In conclusion, he said that the only abnormal finding were fibromas in spleens of rats which, by themselves, were not sufficient evidence on which to base a judgment of carcinogenicity.

The primary reviewer for the report on the bioassay of *p*-Chloroaniline said that the experimental design of the bioassay was adequate, as was the animal survival and body weight gain. He noted the significant fibrosis in spleens of treated rats and pigmentation in spleens of treated mice. He also pointed out the increased incidence of endometrial polyps observed among treated high dose female rats. Because of the disagreement between pathologists, the primary reviewer agreed that the diagnoses should be reviewed by an independent panel of pathologists.

The secondary reviewer of the bioassay of *p*-Chloroaniline said that it was not possible to either agree or disagree with the conclusions in the report until the question regarding the diagnoses was resolved. Therefore, no conclusion could be made regarding the carcinogenicity of *p*-Chloroaniline. He said that it would be premature to consider the human risk posed by *p*-Chloroaniline before its carcinogenicity in animals was clarified.

A Program staff pathologist commented that essentially all the NCI and ICI pathologists were in agreement regarding the diagnoses of fibromas in treated rats -- both groups finding between five and seven fibromas and/or fibrosarcomas. He said that the Program staff considered the incidence of these tumors to be biologically significant, given their rare spontaneous occurrence in Fischer rats. Only one such lesion out of 360 animals had been found from the test laboratory. He noted that the Program staff concluded that the evidence was suggestive of a carcinogenic response and that it pointed the need for additional study.

In further discussion, it was pointed out that a variety of different lesions, including thrombosis, capsulitis, inflammation, hyperplasia, and atrophy were observed in spleens of treated rats. A Program staff member said that the major concern is whether the fibromas were induced by *p*-Chloroaniline, regardless of the mechanism of induction. He pointed out that similar lesions have been found in animals treated with *o*-Toluidine or Aniline hydrochloride.

A Subgroup member said that an additional pathology review would be unnecessary, since it probably would not resolve the need for further testing of *p*-Chloroaniline. He remarked that it would be inadvisable

to call for an independent pathology review since it could set a precedence for other studies when outside pathologists disagreed with the NCI diagnoses. He added that the report should be considered as a statement based on the NCI analysis of the study. Another Subgroup member interjected that the major concern should be with the proper pathology diagnoses.

The primary reviewer moved that the pathology from the bioassay of *p*-Chloroaniline be reviewed by pathologists not associated with NCI or industry and that their findings be submitted to the Program. The motion was carried by a vote of four to two.

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
(Michael B. Shimkin, University of California at San Diego, submitted a written review)
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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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH p-CHLOROANILINE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH p-CHLOROANILINE

	CONTROL(UNTR) 11-1115	LOW DOSE 11-1113	HIGH DOSE 11-1111
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA			1 (2%)
LIPOMA			1 (2%)
NEUROFIBROMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
CARCINOMA, NOS, METASTATIC		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	
SARCOMA, NOS, METASTATIC		1 (2%)	
OSTEOSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			2 (4%)
#SPLEEN	(20)	(49)	(49)
FIBROMA			6 (12%)
FIBROSARCOMA			1 (2%)
HEMANGIOSARCOMA			1 (2%)
OSTEOSARCOMA			1 (2%)
#SPLENIC CAPSULE	(20)	(49)	(49)
SARCOMA, NOS			1 (2%)
SARCOMA, NOS, METASTATIC	1 (5%)		
CIRCULATORY SYSTEM			
NONE			

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

TABLE A1 (CONTINUED)

	CONTROL(UNTR) 11-1115	LOW DOSE 11-1113	HIGH DOSE 11-1111
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(42)
HEPATOCELLULAR CARCINOMA			1 (2%)
SARCOMA, NOS, METASTATIC	1 (5%)		
#PANCREAS	(19)	(49)	(40)
SARCOMA, NOS, METASTATIC	1 (5%)		
URINARY SYSTEM			
#KIDNEY/CAPSULE	(20)	(48)	(50)
SARCOMA, NOS, METASTATIC	1 (5%)		
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(44)	(43)
CHROMOPHOBE ADENOMA	1 (6%)	1 (2%)	3 (7%)
#ADRENAL	(19)	(46)	(49)
CORTICAL ADENOMA		1 (2%)	
PHEOCHROMOCYTOMA	3 (16%)	4 (9%)	3 (6%)
#THYROID	(18)	(38)	(41)
FOLLICULAR-CELL ADENOMA			1 (2%)
C-CELL ADENOMA	1 (6%)	2 (5%)	1 (2%)
C-CELL CARCINOMA		2 (5%)	1 (2%)
#PANCREATIC ISLETS	(19)	(49)	(40)
ISLET-CELL ADENOMA	2 (11%)	2 (4%)	4 (10%)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(20)	(50)	(50)
ADENOMA, NOS			1 (2%)
#TESTIS	(20)	(50)	(49)
INTERSTITIAL-CELL TUMOR	19 (95%)	49 (98%)	43 (88%)
NERVOUS SYSTEM			
NONE			

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

TABLE A1 (CONTINUED)

	CONTROL(UNTR) 11-1115	LOW DOSE 11-1113	HIGH DOSE 11-1111
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*FRONTAL BONE	(20)	(50)	(50)
OSTEOSARCOMA			1 (2%)
BODY CAVITIES			
*ABDOMINAL VISCERA	(20)	(50)	(50)
SARCOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
SARCOMA, NOS		1 (2%)	
OSTEOSARCOMA			2 (4%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	2	6
MORIBUND SACRIFICE	1	2	7
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	46	37
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	CONTROL(UNTR) 11-1115	LOW DOSE 11-1113	HIGH DOSE 11-1111
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	49	49
TOTAL PRIMARY TUMORS	26	65	77
TOTAL ANIMALS WITH BENIGN TUMORS	20	49	46
TOTAL BENIGN TUMORS	26	61	65
TOTAL ANIMALS WITH MALIGNANT TUMORS		3	12
TOTAL MALIGNANT TUMORS		4	12
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	2	1
TOTAL SECONDARY TUMORS	4	2	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH p-CHLOROANILINE

	CONTROL(UNTR) 11-1116	LOW DOSE 11-1114	HIGH DOSE 11-1112
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%)
BASAL-CELL TUMOR			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	2 (4%)
C-CELL CARCINOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
LEUKEMIA,NOS	1 (5%)		
#SPLEEN	(20)	(48)	(50)
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(1)	(38)
ADENOMA, NOS	1 (5%)		
#LIVER	(20)	(10)	(43)
NEOPLASTIC NODULE			1 (2%)

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

TABLE A2 (CONTINUED)

	CONTROL(UNTR) 11-1116	LOW DOSE 11-1114	HIGH DOSE 11-1112
#SMALL INTESTINE	(20)	(50)	(49)
LEIOMYOMA	1 (5%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(50)	(45)
CHROMOPHOBE ADENOMA	4 (22%)	17 (34%)	13 (29%)
#THYROID	(20)	(39)	(37)
C-CELL ADENOMA		2 (5%)	2 (5%)
C-CELL CARCINOMA		1 (3%)	
#PANCREATIC ISLETS	(20)	(8)	(42)
ISLET-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
FIBROADENOMA	1 (5%)	3 (6%)	1 (2%)
*CLITORAL GLAND	(20)	(50)	(50)
CARCINOMA,NOS			1 (2%)
#UTERUS	(20)	(50)	(50)
ENDOMETRIAL STROMAL POLYP	1 (5%)	4 (8%)	6 (12%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(50)
GLIOMA, NOS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			

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

TABLE A2 (CONTINUED)

	CONTROL(UNTR) 11-1116	LOW DOSE 11-1114	HIGH DOSE 11-1112
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(50)	(50)
SARCOMA, NOS			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
SARCOMA, NOS			1 (2%)
SARCOMA, NOS, METASTATIC			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1		3
MORIBUND SACRIFICE	1	1	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	49	44
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL(UNTR) 11-1116	LOW DOSE 11-1114	HIGH DOSE 11-1112
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	24	24
TOTAL PRIMARY TUMORS	10	29	33
TOTAL ANIMALS WITH BENIGN TUMORS	6	24	22
TOTAL BENIGN TUMORS	8	28	26
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	1	6
TOTAL MALIGNANT TUMORS	2	1	6
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH p-CHLOROANILINE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH p-CHLOROANILINE

	CONTROL(UNTR) 22-2115	LOW DOSE 22-2113	HIGH DOSE 22-2111
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SEBACEOUS ADENOMA		1 (2%)	2 (4%)
*SUBCUT TISSUE	(20)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(48)	(49)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (11%)	1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(16)	(41)	(49)
HEMANGIOSARCOMA	1 (6%)	4 (10%)	10 (20%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(49)	(49)
HEPATOCELLULAR ADENOMA	2 (11%)	4 (8%)	1 (2%)
HEPATOCELLULAR CARCINOMA	1 (5%)	3 (6%)	1 (2%)
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA		4 (8%)	1 (2%)

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

TABLE B1 (CONTINUED)

	CONTROL(UNTR) 22-2115	LOW DOSE 22-2113	HIGH DOSE 22-2111
URINARY SYSTEM			
#KIDNEY	(19)	(44)	(50)
HEMANGIOSARCOMA			1 (2%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#PROSTATE	(18)	(35)	(34)
ADENOCARCINOMA, NOS	1 (6%)		
NERVOUS SYSTEM			
#BRAIN	(19)	(47)	(49)
MENINGIOMA	1 (5%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
SARCOMA, NOS	1 (5%)		
HEMANGIOSARCOMA	1 (5%)	1 (2%)	1 (2%)

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

TABLE B1 (CONCLUDED)

	CONTROL(UNTR) 22-2115	LOW DOSE 22-2113	HIGH DOSE 22-2111
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	5	6
MORIBUND SACRIFICE		1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	44	43
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	18	19
TOTAL PRIMARY TUMORS	10	19	20
TOTAL ANIMALS WITH BENIGN TUMORS	2	6	3
TOTAL BENIGN TUMORS	2	6	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	12	17
TOTAL MALIGNANT TUMORS	8	13	17
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH p-CHLOROANILINE

	CONTROL(UNTR) 22-2116	LOW DOSE 22-2114	HIGH DOSE 22-2112
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	2		8
ANIMALS NECROPSIED	18	49	42
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	18	49	42
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(18)	(49)	(42)
FIBROSARCOMA			1 (2%)
OSTEOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(18)	(48)	(42)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (6%)	2 (4%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA			2 (5%)
GRANULOSA-CELL CARCINOMA, METAST		1 (2%)	
OSTEOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(18)	(49)	(42)
MALIGNANT LYMPHOMA, NOS	1 (6%)	1 (2%)	3 (7%)
LEUKEMIA,NOS			1 (2%)
#SPLEEN	(18)	(44)	(39)
HEMANGIOMA			1 (3%)
HEMANGIOSARCOMA			5 (13%)
OSTEOSARCOMA, METASTATIC		1 (2%)	
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(18)	(49)	(41)
HEPATOCELLULAR ADENOMA		1 (2%)	3 (7%)

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

TABLE B2 (CONTINUED)

	CONTROL(UNTR) 22-2116	LOW DOSE 22-2114	HIGH DOSE 22-2112
HEPATOCELLULAR CARCINOMA			3 (7%)
SARCOMA, NOS, METASTATIC	1 (6%)		
HEMANGIOSARCOMA		2 (4%)	1 (2%)
URINARY SYSTEM			
#URINARY BLADDER	(13)	(38)	(34)
SARCOMA, NOS, METASTATIC	1 (8%)		
HEMANGIOSARCOMA			1 (3%)
ENDOCRINE SYSTEM			
#THYROID	(15)	(32)	(31)
FOLLICULAR-CELL ADENOMA		1 (3%)	1 (3%)
REPRODUCTIVE SYSTEM			
#UTERUS	(18)	(49)	(42)
SARCOMA, NOS	1 (6%)		
#UTERUS/ENDOMETRIUM	(18)	(49)	(42)
ADENOMA, NOS			1 (2%)
#OVARY	(17)	(48)	(37)
GRANULOSA-CELL CARCINOMA		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE B2 (CONCLUDED)

	CONTROL(UNTR) 22-2116	LOW DOSE 22-2114	HIGH DOSE 22-2112
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(18)	(49)	(42)
HEMANGIOSARCOMA		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@		10	3
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	40	39
ANIMAL MISSING	2		8
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	3	11	18
TOTAL PRIMARY TUMORS	3	11	23
TOTAL ANIMALS WITH BENIGN TUMORS	1	4	5
TOTAL BENIGN TUMORS	1	4	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	7	14
TOTAL MALIGNANT TUMORS	2	7	17
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	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 TREATED WITH p-CHLOROANILINE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH p-CHLOROANILINE

	CONTROL(UNTR) 11-1115	LOW DOSE 11-1113	HIGH DOSE 11-1111
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
PNEUMONIA, ASPIRATION			1 (2%)
PNEUMONIA, CHRONIC MURINE	2 (10%)	1 (2%)	6 (12%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(49)	(49)
HEMORRHAGIC CYST			1 (2%)
FIBROSIS			3 (6%)
FIBROSIS, FOCAL			2 (4%)
METAMORPHOSIS FATTY			2 (4%)
HYPERPLASIA, DIFFUSE			1 (2%)
HEMATOPOIESIS	2 (10%)		2 (4%)
#SPLENIC CAPSULE	(20)	(49)	(49)
FIBROSIS			9 (18%)
FIBROSIS, FOCAL		45 (92%)	38 (78%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(45)	(49)
FIBROSIS			3 (6%)
DEGENERATION, NOS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(42)
FIBROSIS			2 (5%)

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

TABLE C1 (CONTINUED)

	CONTROL(UNTR) 11-1115	LOW DOSE 11-1113	HIGH DOSE 11-1111
NECROSIS, FOCAL			2 (5%)
NECROSIS, DIFFUSE			1 (2%)
AMYLOIDOSIS			1 (2%)
METAMORPHOSIS FATTY	1 (5%)	1 (2%)	2 (5%)
BASOPHILIC CYTO CHANGE			1 (2%)
EOSINOPHILIC CYTO CHANGE	1 (5%)		
#LIVER/CENTRILOBULAR	(20)	(50)	(42)
NECROSIS, COAGULATIVE		1 (2%)	
#BILE DUCT	(20)	(50)	(42)
HYPERPLASIA, FOCAL			1 (2%)
#PANCREAS	(19)	(49)	(40)
FIBROSIS			1 (3%)
#PANCREATIC ACINUS	(19)	(49)	(40)
ATROPHY, NOS	2 (11%)	8 (16%)	4 (10%)
ATROPHY, FOCAL	1 (5%)		1 (3%)
#SMALL INTESTINE	(19)	(49)	(48)
HYPERPLASIA, LYMPHOID		2 (4%)	1 (2%)
#LARGE INTESTINE	(20)	(49)	(47)
NEMATODIASIS	1 (5%)	3 (6%)	3 (6%)
URINARY SYSTEM			
#KIDNEY	(20)	(48)	(50)
INFLAMMATION, CHRONIC	13 (65%)	33 (69%)	32 (64%)
#URINARY BLADDER	(17)	(42)	(45)
CALCULUS, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(44)	(43)
HYPERPLASIA, FOCAL	1 (6%)		1 (2%)
#ADRENAL	(19)	(46)	(49)
HEMORRHAGIC CYST		1 (2%)	2 (4%)
LIPOIDOSIS			1 (2%)
#ADRENAL CORTEX	(19)	(46)	(49)
HYPERPLASIA, NOS			1 (2%)

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

TABLE C1 (CONTINUED)

	CONTROL(UNTR) 11-1115	LOW DOSE 11-1113	HIGH DOSE 11-1111
#ADRENAL MEDULLA	(19)	(46)	(49)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL	1 (5%)		2 (4%)
#THYROID	(18)	(38)	(41)
HYPERPLASIA, CYSTIC			1 (2%)
HYPERPLASIA, C-CELL		3 (8%)	
HYPERPLASIA, FOLLICULAR-CELL			1 (2%)
#PANCREATIC ISLETS	(19)	(49)	(40)
HYPERPLASIA, NOS			1 (3%)
REPRODUCTIVE SYSTEM			
#PROSTATE	(15)	(31)	(34)
INFLAMMATION, ACUTE			1 (3%)
*SEMINAL VESICLE	(20)	(50)	(50)
ATROPHY, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(50)
HEMORRHAGE			1 (2%)
SPECIAL SENSE ORGANS			
*MIDDLE EAR	(20)	(50)	(50)
ABSCESS, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE C1 (CONCLUDED)

	CONTROL(UNTR) 11-1115	LOW DOSE 11-1113	HIGH DOSE 11-1111
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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 p-CHLOROANILINE

	CONTROL(UNTR) 11-1116	LOW DOSE 11-1114	HIGH DOSE 11-1112
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
PNEUMONIA, CHRONIC MURINE		15 (30%)	8 (16%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(48)	(50)
HEMATOMA, NOS			1 (2%)
FIBROSIS			1 (2%)
#SPLENIC CAPSULE	(20)	(48)	(50)
CYST, NOS		5 (10%)	
FIBROSIS		14 (29%)	3 (6%)
FIBROSIS, FOCAL		30 (63%)	43 (86%)
HYPERPLASIA, NOS			1 (2%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(47)	(49)
INFLAMMATION, FOCAL		1 (2%)	2 (4%)
FIBROSIS		2 (4%)	
DEGENERATION, NOS		3 (6%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(10)	(43)
INFLAMMATION, NOS		1 (10%)	

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

TABLE C2 (CONTINUED)

	CONTROL(UNTR) 11-1116	LOW DOSE 11-1114	HIGH DOSE 11-1112
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (10%)	
AMYLOIDOSIS		1 (10%)	
METAMORPHOSIS FATTY		2 (20%)	
LIPOIDOSIS		1 (10%)	
BASOPHILIC CYTO CHANGE		1 (10%)	
LYMPHOCYTOSIS		1 (10%)	
#LIVER/PERIPORTAL	(20)	(10)	(43)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (10%)	
AMYLOIDOSIS		1 (10%)	
#PANCREAS	(20)	(8)	(42)
FIBROSIS, FOCAL			1 (2%)
#PANCREATIC ACINUS	(20)	(8)	(42)
ATROPHY, NOS	1 (5%)	3 (38%)	2 (5%)
ATROPHY, FOCAL		1 (13%)	
#SMALL INTESTINE	(20)	(50)	(49)
HYPERPLASIA, LYMPHOID			1 (2%)
#LARGE INTESTINE	(19)	(50)	(49)
NEMATODIASIS	3 (16%)	4 (8%)	4 (8%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
HYDRONEPHROSIS			1 (2%)
HEMORRHAGIC CYST	1 (5%)		
INFLAMMATION, CHRONIC	3 (15%)	18 (36%)	12 (24%)
#KIDNEY/TUBULE	(20)	(50)	(50)
NECROSIS, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(50)	(45)
CYST, NOS	1 (6%)		
HEMORRHAGIC CYST		3 (6%)	1 (2%)
HEMOSIDEROSIS		1 (2%)	
HYPERPLASIA, NOS	1 (6%)		
ANGIECTASIS	2 (11%)		
#ADRENAL	(20)	(50)	(49)
HEMORRHAGIC CYST			1 (2%)

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

TABLE C2 (CONTINUED)

	CONTROL(UNTR) 11-1116	LOW DOSE 11-1114	HIGH DOSE 11-1112
LIPOIDOSIS		2 (4%)	
#ADRENAL/CAPSULE	(20)	(50)	(49)
FIBROSIS, FOCAL			1 (2%)
#ADRENAL CORTEX	(20)	(50)	(49)
HYPERPLASIA, NOS		1 (2%)	
#ADRENAL MEDULLA	(20)	(50)	(49)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	
#THYROID	(20)	(39)	(37)
HYPERPLASIA, C-CELL		1 (3%)	2 (5%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
CYST, NOS		1 (2%)	
#UTERUS	(20)	(50)	(50)
HYDROMETRA		1 (2%)	2 (4%)
#UTERUS/ENDOMETRIUM	(20)	(50)	(50)
CYST, NOS			1 (2%)
#OVARY	(20)	(49)	(50)
CYST, NOS		2 (4%)	
PAROVARIAN CYST	2 (10%)	2 (4%)	2 (4%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE C2 (CONCLUDED)

	CONTROL(UNTR) 11-1116	LOW DOSE 11-1114	HIGH DOSE 11-1112
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
NECROSIS, FAT			1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	1	
AUTO/NECROPSY/HISTO PERF	1		

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH p-CHLOROANILINE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH p-CHLOROANILINE

	CONTROL(UNTR) 22-2115	LOW DOSE 22-2113	HIGH DOSE 22-2111
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
CYST, NOS		2 (4%)	1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
GRANULOMA, NOS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(48)	(49)
PNEUMONIA, CHRONIC MURINE		2 (4%)	1 (2%)
PERIVASCULITIS			1 (2%)
#LUNG/ALVEOLI	(19)	(48)	(49)
HISTIOCYTOSIS	1 (5%)		1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(18)	(45)	(47)
HYPERPLASIA, NOS			1 (2%)
#SPLEEN	(16)	(41)	(49)
HEMORRHAGE		1 (2%)	
HEMORRHAGIC CYST			1 (2%)
PIGMENTATION, NOS		7 (17%)	5 (10%)
HYPERPLASIA, LYMPHOID			1 (2%)
#MESENTERIC L. NODE	(18)	(43)	(42)
PIGMENTATION, NOS			1 (2%)
HYPERPLASIA, RETICULUM CELL	1 (6%)		1 (2%)
CIRCULATORY SYSTEM			
#HEART	(19)	(45)	(44)
PERIARTERITIS		1 (2%)	

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

TABLE D1 (CONTINUED)

	CONTROL(UNTR) 22-2115	LOW DOSE 22-2113	HIGH DOSE 22-2111
#MYOCARDIUM	(19)	(45)	(44)
DEGENERATION, NOS		1 (2%)	3 (7%)
DIGESTIVE SYSTEM			
#LIVER	(19)	(49)	(49)
BILE STASIS			1 (2%)
HEMORRHAGIC CYST		1 (2%)	2 (4%)
NECROSIS, NOS		1 (2%)	1 (2%)
NECROSIS, FOCAL			1 (2%)
PIGMENTATION, NOS		3 (6%)	4 (8%)
HEPATOCYTOMEGALY		1 (2%)	
#PANCREAS	(18)	(40)	(46)
CYSTIC DUCTS		1 (3%)	
#STOMACH	(18)	(42)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#PEYERS PATCH	(19)	(45)	(49)
HYPERPLASIA, LYMPHOID	1 (5%)		
#COLON	(19)	(41)	(49)
NEMATODIASIS		1 (2%)	3 (6%)
URINARY SYSTEM			
#KIDNEY	(19)	(44)	(50)
HYDRONEPHROSIS	1 (5%)		
INFLAMMATION, CHRONIC	1 (5%)		
NEPHROPATHY		1 (2%)	1 (2%)
NEPHROSIS, CHOLEMIC		1 (2%)	2 (4%)
PIGMENTATION, NOS		3 (7%)	3 (6%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#TESTIS	(19)	(47)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		

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

TABLE D1 (CONCLUDED)

	CONTROL(UNTR) 22-2115	LOW DOSE 22-2113	HIGH DOSE 22-2111
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
PERIARTERITIS			1 (2%)
PIGMENTATION, NOS		35 (70%)	37 (74%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	7	2	1
AUTO/NECROPSY/HISTO PERF	1	1	

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

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
TREATED WITH p-CHLOROANILINE

	CONTROL(UNTR) 22-2116	LOW DOSE 22-2114	HIGH DOSE 22-2112
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	2		8
ANIMALS NECROPSIED	18	49	42
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	18	49	42
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(18)	(48)	(42)
PNEUMONIA, CHRONIC MURINE	2 (11%)	9 (19%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(44)	(39)
HEMORRHAGIC CYST			1 (3%)
PIGMENTATION, NOS		5 (11%)	3 (8%)
METAPLASIA, OSSEOUS		4 (9%)	4 (10%)
HYPERPLASIA, LYMPHOID	1 (6%)	2 (5%)	
#PANCREATIC L.NODE	(18)	(45)	(38)
HYPERPLASIA, LYMPHOID		1 (2%)	
#MESENTERIC L. NODE	(18)	(45)	(38)
HYPERPLASIA, LYMPHOID		1 (2%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(17)	(46)	(40)
DEGENERATION, NOS		3 (7%)	1 (3%)
DIGESTIVE SYSTEM			
#LIVER	(18)	(49)	(41)
NECROSIS, FOCAL	4 (22%)	2 (4%)	

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

TABLE D2 (CONTINUED)

	CONTROL(UNTR) 22-2116	LOW DOSE 22-2114	HIGH DOSE 22-2112
PIGMENTATION, NOS		2 (4%)	5 (12%)
FOCAL CELLULAR CHANGE		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	
#BILE DUCT	(18)	(49)	(41)
DILATATION, NOS			1 (2%)
INFLAMMATION, FOCAL		1 (2%)	
#PANCREAS	(18)	(46)	(39)
DILATATION/DUCTS			1 (3%)
#PANCREATIC ACINUS	(18)	(46)	(39)
ATROPHY, NOS			1 (3%)
#SMALL INTESTINE	(18)	(47)	(40)
AMYLOIDOSIS		1 (2%)	2 (5%)
#PEYERS PATCH	(18)	(47)	(40)
HYPERPLASIA, LYMPHOID			1 (3%)
URINARY SYSTEM			
#KIDNEY	(18)	(49)	(41)
HYDRONEPHROSIS			1 (2%)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
NEPHROPATHY		1 (2%)	
NEPHROSIS, CHOLEMIC		1 (2%)	
PIGMENTATION, NOS		1 (2%)	1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(15)	(32)	(31)
AMYLOIDOSIS			1 (3%)
HYPERPLASIA, CYSTIC	1 (7%)		
HYPERPLASIA, FOLLICULAR-CELL			1 (3%)
REPRODUCTIVE SYSTEM			
#UTERUS	(18)	(49)	(42)
DILATATION, NOS		1 (2%)	1 (2%)
PYOMETRA	1 (6%)	8 (16%)	4 (10%)

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

TABLE D2 (CONTINUED)

	CONTROL(UNTR) 22-2116	LOW DOSE 22-2114	HIGH DOSE 22-2112
ABSCESS, NOS		1 (2%)	
#CERVIX UTERI	(18)	(49)	(42)
INFLAMMATION, NOS			1 (2%)
#UTERUS/ENDOMETRIUM	(18)	(49)	(42)
INFLAMMATION, NOS		2 (4%)	1 (2%)
INFLAMMATION, NECROTIZING		1 (2%)	
HYPERPLASIA, CYSTIC	4 (22%)	13 (27%)	14 (33%)
#UTERUS/MYOMETRIUM	(18)	(49)	(42)
INFLAMMATION, NOS		1 (2%)	
#OVARY	(17)	(48)	(37)
CYST, NOS	1 (6%)	6 (13%)	2 (5%)
HEMORRHAGIC CYST			1 (3%)
ABSCESS, NOS		5 (10%)	
AMYLOIDOSIS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(18)	(49)	(42)
PERIARTERITIS			1 (2%)
PIGMENTATION, NOS		35 (71%)	33 (79%)

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

TABLE D2 (CONCLUDED)

	CONTROL(UNTR) 22-2116	LOW DOSE 22-2114	HIGH DOSE 22-2112
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5		
ANIMAL MISSING/NO NECROPSY	2		8
AUTO/NECROPSY/HISTO PERF		2	
AUTOLYSIS/NO NECROPSY		1	

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

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