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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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ANILINE HYDROCHLORIDE

FOR POSSIBLE CARCINOGENICITY

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 ANILINE HYDROCHLORIDE
FOR POSSIBLE CARCINOGENICITY

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

FOREWORD: This report presents the results of the bioassay of aniline hydrochloride conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. 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 aniline hydrochloride was conducted by Mason Research Institute, Worcester, Massachusetts, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

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

Histopathologic examinations were performed by Dr. D. W. Hayden (3) and Dr. D. S. Wyand (3) at the Mason Research Institute, and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (6).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (7); the statistical analysis was performed by Mr. W. W. Belew (5,8) and

Mr. R. M. Helfand (5), 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 (5) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (5), task leader Dr. M. R. Kornreich (5,10), senior biologist Ms. P. Walker (5), biochemist Mr. S. C. Drill (5), and technical editor Ms. P. A. Miller (5). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1,10), Dr. R. A. Griesemer (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,11), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay of aniline hydrochloride for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F1 mice. Aniline hydrochloride was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species, with the exception of 49 female mice in the high dose group. The high and low dietary concentrations of aniline hydrochloride were, respectively, 0.6 and 0.3 percent for rats and 1.2 and 0.6 percent for mice. After a 103-week period of compound administration, observation of the rats and mice continued for up to an additional 5 weeks.

For rats and mice, respectively, 25 and 50 animals of each sex were placed on test as controls and fed only the basal diet.

In male rats there were several types of mesenchymal tumors, primarily of the spleen, associated with administration of the compound. Hemangiosarcomas of the spleen and the combined incidence of fibrosarcomas and sarcomas NOS of the spleen were each statistically significant in male rats. The combined incidence of fibrosarcomas and sarcomas NOS of multiple body organs was also significant in male rats. The number of female rats having fibrosarcomas or sarcomas NOS of either the spleen alone or multiple organs of the body cavity was significantly associated with increased dietary concentration of aniline hydrochloride. This result was not supported by Fisher exact tests, but because of the rarity of these tumors, the observed incidences (0/24 in the control group, 1/50 [2 percent] in the low dose group, 7/50 [14 percent] in the high dose group) were considered indicative of a compound-related carcinogenic effect.

In mice of both sexes no tumors occurred in statistically significant increased incidences among dosed groups when compared to controls.

Under the conditions of this bioassay, dietary administration of aniline hydrochloride was carcinogenic to male and female Fischer 344 rats, inducing hemangiosarcomas and a combination of fibrosarcomas and sarcomas NOS of the spleen and a combination of fibrosarcomas and sarcomas NOS of multiple body organs. There was no evidence of compound-induced carcinogenicity in B6C3F1 mice of either sex.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF TABLES (Concluded)

I. INTRODUCTION

Aniline HCl (Figure 1) (NCI No. C03736), a dye intermediate and a commercially important salt of aniline, was selected for bioassay by the National Cancer Institute because of the increased incidence of bladder cancer among workers in the dye manufacturing industry and the historical association of aromatic amines with this increased cancer risk (Wynder et al., 1963; Hamblin, 1963).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is hydrochloride benzenamide.* It is also called aniline salt and aniline chloride.

The major industrial use of aniline HCl is as an intermediate in the manufacture of dyes. The oxidation of aniline HCl within textile fibers to produce the dye Aniline Black (C.I. [Colour Index] Pigment Black 1) (C.I. 50440) is one application (Kouris and Northcott, 1963).

Specific production statistics for aniline HCl are not available; however, the inclusion of aniline HCl in Synthetic Organic Chemicals, U.S. Production and Sales, 1975 (U.S. International Trade Commission, 1977) implies an annual commercial production in excess of 1000 pounds or $1000 in value. Approximately 4×10^8 pounds of aniline, the immediate precursor of aniline HCl, were produced in 1975 (U.S. International Trade Commission, 1977).

*The CAS registry number is 142-04-1

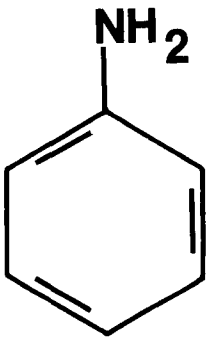


FIGURE 1
CHEMICAL STRUCTURE OF ANILINE (HYDROCHLORIDE)

The potential for exposure to aniline HCl is greatest for workers involved in the manufacture or use of the compound. The majority of these workers are employed by the dye and textile industries.

The most prominent effect of acute exposure to aniline hydrochloride is methemeglobinemia and the accompanying cyanosis; headache, vertigo and mental confusion may also occur (Hamblin, 1963; Windholz, 1976). In humans, chronic aniline HCl exposure can result in anemia, anorexia, weight loss, and cutaneous lesions (Windholz, 1976). Although aniline has been frequently associated with so-called "aniline tumors" of the urinary bladder, the majority of evidence indicates that other intermediates in the production of aniline dyes, especially 2-naphthylamine and benzidine, are responsible for these tumors (International Agency for Research on Cancer, 1974; Windholz, 1976).

II. MATERIALS AND METHODS

A. Chemicals

Aniline hydrochloride was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. Chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri. The narrow melting point range (197° to 199°C) and closeness of the observed melting point to the literature value (195°C) (Linstead and Braude, 1954) suggested a compound of high purity. Thin-layer chormatography was performed utilizing two solvent systems (acetone:benzene:ammonia and ethyl acetate:chloroform), and plates were visualized with ultraviolet light and furfural. Each plate showed one or more nonmotile impurities. Experimental elemental analysis approximated that expected on a theoretical basis. Results of nonaqueous titration of the amine functions were approximately 100 percent of the theoretical value. Vapor-phase chromatography showed one homogeneous peak. Infrared analysis was consistent with the structure of the compound. The data suggest a compound of high purity.

Throughout this report, the term aniline HCl is used to represent this compound.

B. Dietary Preparation

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

The chemical was removed from its container and proper amounts were ground to a powder in a Quaker City crystal mill, sifted and weighed out under an exhaust hood and ground with a mortar and pestle. The compound was hand-blended in an aluminum bowl with an aliquot of the ground feed. 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.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. Fischer 344 rats and B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. All rats and mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Dosed and control animals for both species were received in separate shipments.

Upon arrival, a sample of animals was examined for parasites and other signs of disease. All animals were treated for parasites with piperazine adipate at 3.0 gm/liter of water fed *ad libitum* for three days followed by three additional days of piperazine adipate dosing. Animals were then quarantined by species for 2 weeks prior to initiation of the test. Animals were assigned to groups and

distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenace

All animals were housed by species in rooms having a temperature range of 23° to 34°C. Incoming air was filtered through Tri-Dek® 15/40 denier Dacron® filters (Tri-Dim Filter Corp., Hawthorne, New Jersey) providing six changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

Rats were housed five per cage by sex. During quarantine and for the first 14 months of study, all rats were housed in wire-mesh cages (Fenco Cage Products, Boston, Massachusetts) suspended over newspapers. Newspapers under cages were replaced daily and cages and racks washed weekly. For the remainder of the study, all rats were held in suspended polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) equipped with nonwoven fiber filter sheets. Clean cages and bedding were provided twice weekly. SAN-I-CEL® corncob bedding (Paxton Processing Company, Paxton, Illinois) was used for the first 8 weeks in polycarbonate cages. Aspen hardwood chip bedding (American Excelsior Company, Baltimore, Maryland) was used for the remainder of the study. Stainless steel cage racks (Fenco Cage Products) were cleaned once every 2 weeks, and disposable filters were replaced at that time.

Mice were housed by sex in polycarbonate cages. Cages were fitted with perforated stainless steel lids (Lab Products, Inc.).

Nonwoven fiber filter bonnets were used over cage lids. Control mice were housed ten per cage for the first month of study, and five per cage thereafter. Dosed mice were housed five per cage throughout the study. Clean cages, lids, and bedding were provided three times per week when cage populations were ten and twice per week when cage populations were reduced to five. SAN-I-CEL® was supplied for the first 9 months of study, followed by Bed-o-Cobs® corncob bedding (The Andersons Cob Division, Maumee, Ohio) for 8 months. Aspen bedding was then used for the remainder of the study. Reusable filter bonnets and pipe racks were sanitized every 2 weeks throughout the study.

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

Wayne Lab-Blox® was supplied to all animals during the initial quarantine and final observation periods. During the period of compound administration, all dosed animals were fed Wayne Lab-Blox® meal containing the appropriate concentration of aniline HCl. Control animals had untreated meal available ad libitum. Meal was supplied to rats and mice for the first 12 and 11 months, respectively, in Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc., Woburn, Massachusetts). For the remainder of the study, meal was supplied from stainless steel gangstyle food hoppers (Scientific Cages, Inc., Bryan, Texas). Food hoppers were changed on the same

schedule as were cages. Food was replenished daily in Alpine® feed cups.

Dosed and control rats were housed in a room with other rats receiving diets containing* N-butylurea (592-31-4); N,N-dimethyl-p-nitrosoaniline (138-89-6); 2,5-toluenediamine sulfate (6369-59-1); 2,4-dinitrotoluene (121-14-2); 4-nitroanthranilic acid (619-17-0); 1,5-naphthalenediamine (2243-62-1); N-(1-naphthyl)ethylenediamine dihydrochloride (1465-25-4); 2-chloro-p-phenylenediamine sulfate (61702-44-1); and p-anisidine hydrochloride (20265-97-8).

All dosed and control mice were housed in a room with other mice receiving diets containing 2,3,5,6-tetrachloro-4-nitroanisole (2438-88-2); hydrazobenzene (530-50-7); tris(2,3-dibromopropyl) phosphate (126-72-7); N-(1-naphthyl)ethylenediamine dihydrochloride (1465-25-4); and 2-chloro-p-phenylenediamine sulfate (61702-44-1).

E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of aniline HCl for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among five groups, each consisting of five males and five females. Aniline HCl was incorporated into the basal diet and fed ad libitum to four of the five groups of each species in concentrations of 0.03, 0.01, 0.3, and 1.0 percent. The remaining group of each species served as a

*CAS registry numbers are given in parentheses.

control group, receiving only the basal laboratory diet. The dosed dietary preparations were administered for a period of 8 weeks. At the end of the observation period, all survivors were sacrificed and necropsied.

The highest concentration causing no deaths, no compound-related gross abnormalities, and no mean body weight depression in excess of 5 percent relative to controls during the 8 week subchronic test was selected as the high concentration utilized for the rat and mouse chronic bioassays.

At dietary concentrations of 0.3 percent or greater, black, granular and enlarged spleens were observed in both species. No deaths were observed at any dose tested. A dietary concentration of 0.3 percent produced no mean body weight depression, while a dietary concentration of 1.0 percent produced mean body weight depressions of 23 and 24 percent in male and female rats, respectively. The high concentration selected for administration to rats in the chronic bioassay was 0.6 percent.

A dietary concentration of 1.0 percent produced mean body weight depressions of 3 and 2 percent in male and female mice, respectively. The high concentration selected for administration to mice in the chronic bioassay was 1.2 percent.

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.

The dosed and control rats were all approximately 6 weeks old at the time the test was initiated. The initial concentrations of aniline HCl administered in the diet were 0.6 and 0.3 percent. 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. The dosed rats were supplied with feed containing aniline HCl for a total of 103 weeks followed by a 4- to 5-week observation period.

The dosed and control mice were all approximately 6 weeks old at the time the test was initiated. The initial concentrations of aniline HCl administered in the diet were 1.2 and 0.6 percent. 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. The dosed mice were supplied with feed containing aniline HCl for a total of 103 weeks followed by a 4-week observation period.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. Body weights were recorded twice weekly for the first 12 weeks of the study and at monthly intervals thereafter. From the first day, all animals were inspected twice daily for mortality. Food consumption, for two cages from each group, was monitored for

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
ANILINE HYDROCHLORIDE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	ANILINE HCl CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	25	0	0	110
LOW DOSE	50	0.3 0	103	4
HIGH DOSE	50	0.6 0	103	5
FEMALE				
CONTROL	25	0	0	110
LOW DOSE	50	0.3 0	103	4
HIGH DOSE	50	0.6 0	103	5

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
ANILINE HYDROCHLORIDE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	ANILINE HCl CONCENTRATION (PERCENT)	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	50	0	0	109
LOW DOSE	50	0.6 0	103	4
HIGH DOSE	50	1.2 0	103	4
FEMALE				
CONTROL	50	0	0	109
LOW DOSE	50	0.6 0	103	4
HIGH DOSE	49	1.2 0	103	4

seven consecutive days once a month for the first nine months of the bioassay and for three consecutive days each month thereafter. The presence of tissue masses and lesions was determined by monthly observation and palpation of each animal.

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

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

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, ear, 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 placed on experiment in each group.

H. Data Recording and Statistical Analyses

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

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

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

equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison

be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

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

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

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an

observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

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

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio

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

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

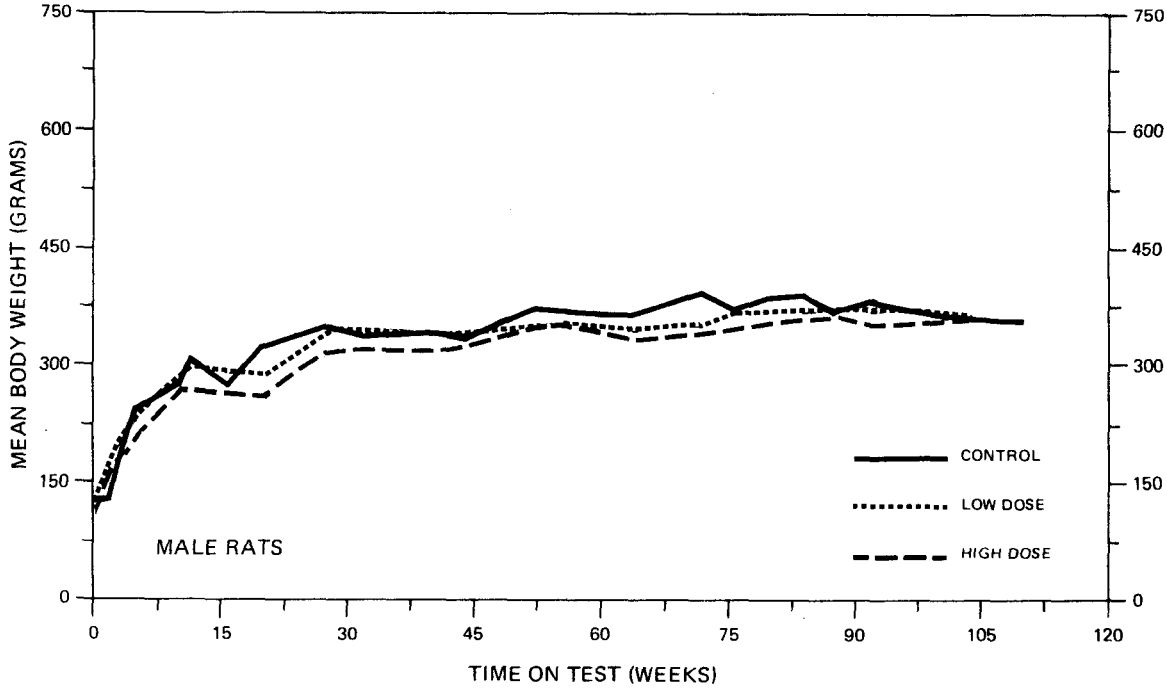
Slight mean body weight depression was apparent in dosed female rats and high dose male rats (Figure 2).

Subcutaneous masses developed in one high dose male, one low dose male, one control male, one high dose female, five low dose females, and three control females. Cutaneous lesions were observed in one low dose male, two control males, one high dose female, and one control female. Abdominal distention was noted in two low dose males and in one control female. Distention of the scrotal sac was seen in three low dose males. Posterior ataxia was displayed by one low dose male. Alopecia was seen in one high dose female. No other clinical abnormalities were noted.

B. Survival

The estimated probabilities of survival for male and female rats in the control and aniline HCl-dosed groups are shown in Figure 3. For both male and female rats the Tarone test did not indicate a significant positive association between dosage and mortality.

Adequate numbers of males were at risk from late-developing tumors, as 54 percent (27/50) of the high dose, 68 percent (34/50) of the low dose, and 68 percent (17/25) of the control rats survived on test until the end of the study. Adequate numbers of females were also at risk from late-developing tumors, as 82 percent (41/50) of



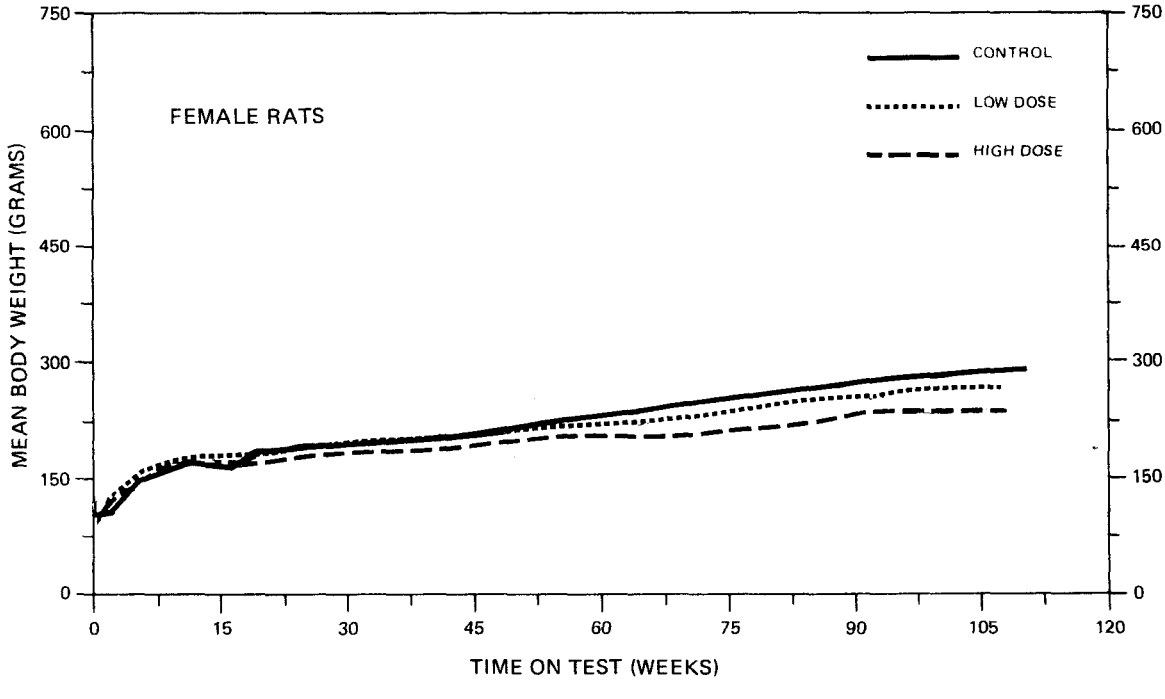
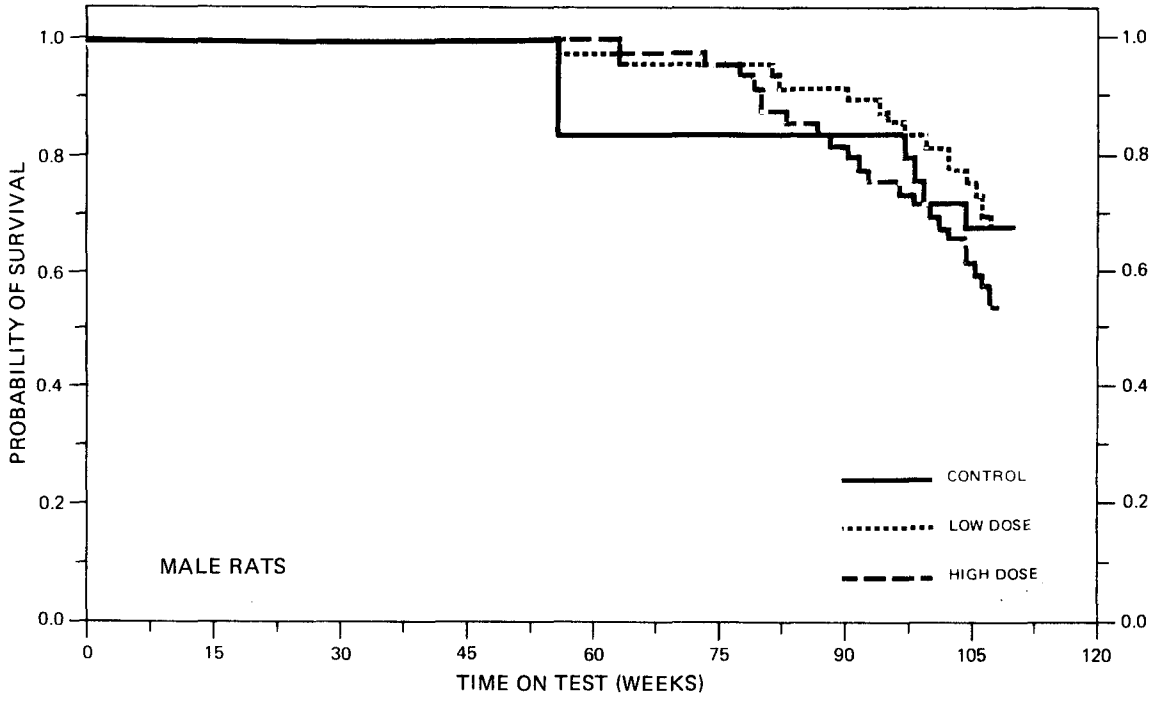


FIGURE 2
GROWTH CURVES FOR ANILINE HYDROCHLORIDE CHRONIC STUDY RATS



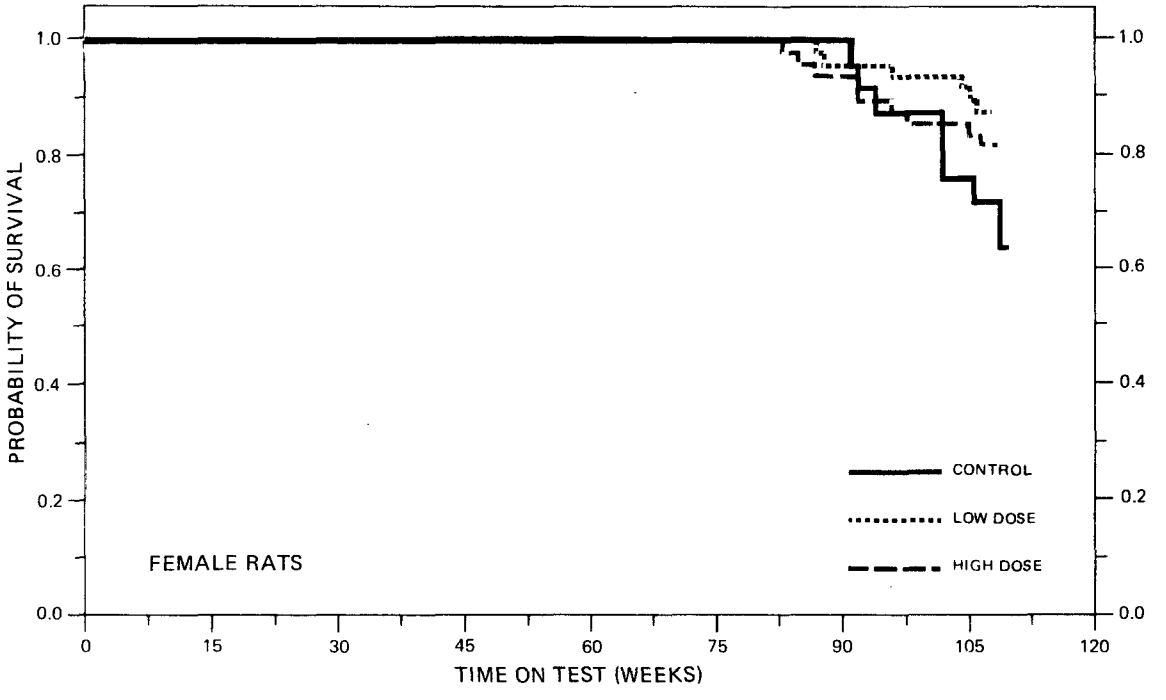


FIGURE 3
SURVIVAL COMPARISONS OF ANILINE HYDROCHLORIDE CHRONIC STUDY RATS

the high dose, 88 percent (44/50) of the low dose, and 64 percent (16/25) of the control rats survived on test until the end of the study.

C. Pathology

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

Several different mesenchymal tumors were associated with compound administration. These tumors occurred in large numbers in the spleens of dosed male rats; additionally, for dosed males and for high dose females, these tumors were often observed in multiple organs of the pleural and abdominal cavities in the same animal. These tumors are summarized in the following table:

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Spleens and Capsule						
Number of animals with tissues examined histopathologically	(25)	(50)	(46)	(23)	(50)	(50)
Sarcoma NOS	0	4	2	0	0	3
Fibroma	0	7	6	0	0	0
Fibrosarcoma	0	3	7	0	0	0
Hemangiosarcoma	0	19	20	0	1	2
Lipoma	0	0	0	0	0	1
Hemangioma	0	0	0	0	0	1

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Body Cavity, Multiple Organs						
Number of animals with tissues examined histopathologically	(25)	(50)	(48)	(24)	(50)	(50)
Fibrosarcoma	0	2	8	0	1	3
Leiomyosarcoma	0	0	2	1	0	0
Hemangiosarcoma	0	0	1	0	0	0
Sarcoma NOS	0	0	1	0	0	1

The incidence of hemangiosarcoma, the tumor most frequently observed, was elevated in dosed males (19/50 [38 percent] low dose and 21/48 [44 percent] high dose). Hemangiosarcomas were tumors of vascular endothelium with blood-filled spaces varying from capillary size to large hematomas enclosed by a rim of neoplastic cells which projected villous-like structures into the blood spaces. Capillary-like areas sometimes lacked blood in the spaces. Solid sarcomatous areas of spindle cells were common. Cells were often pleomorphic with numerous mitoses.

Fibrosarcomas consisted of pleomorphic fibroblasts and collagen. Mitotic figures were usually common. With loss of differentiation, there was increased hyperchromasia and pleomorphism, and the tumors became more cellular with decreased collagen. Fibrosarcomas were quite invasive with widespread extension seen in the abdominal cavity. In some fibrosarcomas there were prominent areas of osseous metaplasia. The diagnosis of sarcoma NOS may represent very poorly differentiated fibrosarcomas.

Fibromas were more circumscribed than fibrosarcomas, less cellular, and had mature fibroblasts and greater amounts of collagen. Mitotic figures were rare.

In a few instances, organs contained two distinct tumors, fibrosarcomas and hemangiosarcomas combined with fibromas. While the tumors were usually distinctly separate, in one spleen an area of fibrosarcoma blended imperceptibly with an area of endothelial proliferation which might have been early hemangiosarcoma.

Adrenal pheochromocytomas occurred in 6/50 (12 percent) low dose and 12/44 (27 percent) high dose males, and 5/48 (10 percent) high dose females. There were 2/24 (8 percent) in control males and 1/24 (4 percent) in control females.

Several proliferative nonneoplastic lesions occurred only in dosed rats. In the spleen these included fibrosis of the splenic capsule and trabeculae and fatty metamorphosis, the occurrence of scattered large fat cells in the splenic parenchyma. Many dosed rats (18/50 [36 percent] low dose and 7/46 [15 percent] high dose males; 23/50 [46 percent] low dose and 28/50 [56 percent] high dose females) exhibited fine papillary projections from the surface of the splenic capsule. These projections had dense fibrous cores and were covered by reactive mesothelial cells, as was the remainder of the splenic capsule. While these lesions had some resemblance to early mesotheliomas, they were considered to be hyperplastic rather than neoplastic lesions and were classifed as papillary hyperplasia.

Hemosiderosis of renal tubular epithelium was found in very high incidences in male (21/50 [42 percent] low dose, 34/48 [71 percent] high dose) and female (46/49 [94 percent] low dose, 45/50 [90 percent] high dose) rats. The tubular epithelial cells of the cortex contained many yellow-brown cytoplasmic pigment granules. Hemosiderosis of the liver characterized by Kupffer cells filled with the pigment occurred in both high dose males (26/47 [55 percent]) and high dose females (29/50 [58 percent]). Because of this marked degree of hemosiderosis compared to trace amounts in controls, the finding is considered to be compound-related. Hemosiderosis was occasionally found in other tissues.

The incidence of endometrial stromal polyp was increased in low dose females (15/48 [31 percent]) as compared with controls (2/24 [8 percent]). The increase was not dose-related as it occurred in only 7/50 (14 percent) high dose animals.

On the basis of this pathologic examination, it is concluded that aniline HCl was carcinogenic when fed to male and possibly to female rats, inducing mesenchymal tumors. An increased incidence of adrenal pheochromocytomas appeared related to compound administration. Renal and Kupffer-cell hemosiderosis in male and female rats were also related to compound administration.

D. Statistical Analyses 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

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH ANILINE HYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma NOS[b]	2/22(0.09)	7/45(0.16)	4/34(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.711	1.294
Lower Limit	---	0.367	0.206
Upper Limit	---	16.016	13.453
Weeks to First Observed Tumor	98	63	90
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant[b]	2/24(0.08)	6/50(0.12)	12/44(0.27)
P Values[c]	P = 0.022	N.S.	N.S.
Relative Risk (Control)[d]	---	1.440	3.273
Lower Limit	---	0.286	0.824
Upper Limit	---	13.931	28.393
Weeks to First Observed Tumor	110	102	105
Thyroid: C-Cell Carcinoma[b]	2/21(0.10)	2/39(0.05)	1/30(0.03)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.538	0.350
Lower Limit	---	0.042	0.006
Upper Limit	---	7.050	6.326
Weeks to First Observed Tumor	97	107	96

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	2/21(0.10)	3/39(0.08)	1/30(0.03)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.808	0.350
Lower Limit	---	0.102	0.006
Upper Limit	---	9.161	6.326
Weeks to First Observed Tumor	97	107	96
Thyroid: Follicular-Cell Adenoma or Follicular-Cell Carcinoma[b]	1/21(0.05)	2/39(0.05)	0/30(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.077	0.000
Lower Limit	---	0.060	0.000
Upper Limit	---	61.897	12.911
Weeks to First Observed Tumor	110	104	---
Testis: Interstitial-Cell Tumor[b]	21/25(0.84)	34/50(0.68)	29/46(0.63)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.810	0.751
Lower Limit	---	0.658	0.601
Upper Limit	---	1.122	1.061
Weeks to First Observed Tumor	97	90	93

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Skin: Squamous-Cell Papilloma[b]	2/25(0.08)	0/50(0.00)	0/48(0.00)
P Values[c]	P = 0.037(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	1.685	1.753
Weeks to First Observed Tumor	110	---	---
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	1/25(0.04)	3/50(0.06)	0/48(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.500	0.000
Lower Limit	---	0.130	0.000
Upper Limit	---	77.150	9.720
Weeks to First Observed Tumor	110	95	---
Spleen: Fibroma[b]	0/25(0.00)	7/50(0.14)	6/46(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.997	0.894
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	102	107

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Spleen: Fibrosarcoma or Sarcoma NOS[b]	0/25(0.00)	7/50(0.14)	9/46(0.20)
P Values[c]	P = 0.020	N.S.	P = 0.015
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.997	1.470
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	102	79
Spleen: Hemangiosarcoma[b]	0/25(0.00)	19/50(0.38)	20/46(0.43)
P Values[c]	P = 0.001	P < 0.001	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	3.140	3.616
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	94	93
Body Cavities, Multiple Organs: Fibrosarcoma or Sarcoma NOS[b]	0/25(0.00)	2/50(0.04)	9/48(0.19)
P Values[c]	P = 0.004	N.S.	P = 0.017
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.151	1.408
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	99	83

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Spleen or Body Cavities, Multiple Organs: Fibrosarcoma or Sarcoma NOS[b]	0/25(0.00)	5/50(0.10)	18/48(0.38)
P Values[c]	P < 0.001	N.S.	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.648	3.086
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	99	79
Spleen or Body Cavities, Multiple Organs: Hemangiosarcoma[b]	0/25(0.00)	19/50(0.38)	21/48(0.44)
P Values[c]	P = 0.001	P < 0.001	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	3.140	3.651
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	94	93

[a]Treated groups received doses of 0.3 or 0.6 percent in feed.

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

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

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

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH ANILINE HYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	3/24(0.13)	4/50(0.08)	0/50(0.00)
P Values[c]	P = 0.019(N)	N.S.	P = 0.031(N)
Relative Risk (Control)[d]	---	0.640	0.000
Lower Limit	---	0.120	0.000
Upper Limit	---	4.113	0.793
Weeks to First Observed Tumor	94	106	---
Spleen: Sarcoma NOS[b]	0/23(0.00)	0/50(0.00)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.285
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	108
Pituitary: Adenoma NOS[b]	6/21(0.29)	14/46(0.30)	8/40(0.20)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.065	0.700
Lower Limit	---	0.463	0.253
Upper Limit	---	2.980	2.167
Weeks to First Observed Tumor	91	87	85

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma NOS or Carcinoma NOS[b]	6/21(0.29)	15/46(0.33)	9/40(0.22)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.141	0.787
Lower Limit	---	0.505	0.299
Upper Limit	---	3.156	2.373
Weeks to First Observed Tumor	91	87	85
Adrenal: Cortical Adenoma or Cortical Carcinoma[b]	0/24(0.00)	2/50(0.04)	4/48(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.146	0.477
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	107	107
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant[b]	1/24(0.04)	0/50(0.00)	5/48(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	2.500
Lower Limit	---	0.000	0.306
Upper Limit	---	8.966	115.634
Weeks to First Observed Tumor	110	---	107

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	1/21(0.05)	2/38(0.05)	0/38(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.105	0.000
Lower Limit	---	0.062	0.000
Upper Limit	---	63.481	10.263
Weeks to First Observed Tumor	109	107	---
Thyroid: Follicular-Cell Adenoma or Follicular-Cell Carcinoma[b]	1/21(0.05)	0/38(0.00)	2/38(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	1.105
Lower Limit	---	0.000	0.062
Upper Limit	---	10.263	63.481
Weeks to First Observed Tumor	110	---	108
Mammary Gland: Fibroadenoma[b]	4/24(0.17)	5/50(0.10)	0/50(0.00)
P Values[c]	P = 0.006(N)	N.S.	P = 0.009(N)
Relative Risk (Control)[d]	---	0.600	0.000
Lower Limit	---	0.145	0.000
Upper Limit	---	2.807	0.514
Weeks to First Observed Tumor	109	107	---

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Uterus: Endometrial Stromal Polyp[b]	2/24(0.08)	15/48(0.31)	7/50(0.14)
P Values[c]	N.S.	P = 0.027	N.S.
Departure from Linear Trend[e]	P = 0.008	---	---
Relative Risk (Control)[d]	---	3.750	1.680
Lower Limit	---	0.988	0.356
Upper Limit	---	31.911	15.815
Weeks to First Observed Tumor	102	107	107
Body Cavities, Multiple Organs: Fibrosarcoma or Sarcoma NOS[b]	0/24(0.00)	1/50(0.02)	4/50(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.026	0.458
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	88	83
Spleen or Body Cavities, Multiple Organs: Fibrosarcoma or Sarcoma NOS[b]	0/24(0.00)	1/50(0.02)	7/50(0.14)
P Values[c]	P = 0.009	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.026	0.960
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	88	83

TABLE 4 (CONCLUDED)

[a]Treated groups received doses of 0.3 or 0.6 percent in feed.

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

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

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

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

every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or aniline HCI-dosed groups and where such tumors were observed in at least 5 percent of the group.

In dosed male rats numerous sarcomas were observed both in the spleen and in multiple organs of the body cavities.* The Cochran-Armitage test indicated a significant ($P = 0.001$) positive association between dosage and the incidence of hemangiosarcomas of the spleen. Both the Fisher exact tests showed a significantly ($P < 0.001$) greater incidence in comparing the respective dosed groups to the control. The Cochran-Armitage test also indicated a significant ($P = 0.002$) positive association between dosage and the combined incidence of fibrosarcomas or sarcomas NOS of the spleen. Again, the Fisher exact test comparing high dose to control was significant ($P = 0.015$). In historical control data compiled by this laboratory for the NCI Carcinogenesis Testing Program, none of the 250 male untreated Fischer 344 rats had one of these tumors--compared to the 20/46 (43 percent) hemangiosarcomas and 9/46 (20 percent) fibrosarcomas or sarcomas NOS observed in the male high dose group.

When the combined incidence of fibrosarcomas or sarcomas NOS of multiple organs of the body cavities was considered, for males the

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*In this report reference is made to neoplasms of "multiple organs of the body cavities." Such a neoplasm was observed in more than one of the organs located in the pleural or the abdominal cavity (or both).

Cochran-Armitage test indicated a significant ($P = 0.004$) positive association between dose and incidence. The high dose Fisher exact comparison was also significant ($P = 0.017$).

When the combined incidence of fibrosarcomas or sarcomas NOS either of the spleen alone or of multiple organs of the body cavities was considered, for males the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and incidence. For males the Fisher exact test comparing the high dose to the control was also significant ($P < 0.001$). For females the Cochran-Armitage test was significant ($P = 0.009$), but the Fisher exact tests were not. This tumor combination was also rare in untreated Fischer 344 historical control rats, as none of the 250 male or 249 females had one of these tumors. Making the assumption of a binomial distribution with a probability of 1/250 of spontaneous incidence (the most conservative estimate), the probability of observing 7 or more rats with one of these tumors out of 50 females (as in the high dose females) was $P < 0.00001$, a significant result.

Based upon these statistical results the administration of aniline HCl was associated with the increased incidence of hemangiosarcomas of the spleen and of fibrosarcomas or sarcomas NOS both of the spleen and of multiple organs of the body cavity in male rats. There was also the possibility of an association between administration and the increased combined incidence of fibrosarcomas or sarcomas NOS either

of the spleen or of multiple organs of the body cavities in female rats.

For males the Cochran-Armitage test indicated a significant ($P = 0.022$) positive association between dosage and the incidence of adrenal neoplasms. The Fisher exact tests, however, were not significant. No other tests for either males or females indicated a significant positive association under the Bonferroni criterion.

For males the Cochran-Armitage test indicated a significant negative association both between dosage and the incidence of squamous-cell papillomas of the skin and between dosage and the incidence of adrenal neoplasms. In both cases, however, no Fisher exact tests were significant. Similarly, for females the Cochran-Armitage test showed a significant negative association for the incidence of leukemia or malignant lymphomas, but no Fisher exact tests were significant under the Bonferroni criterion.

The possibility of a negative association between dosage and the incidence of mammary fibroadenomas was observed in female rats.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

Mean body weight depression occurred in dosed male mice, but not in dosed female mice (Figure 4).

Partial alopecia was observed in 35 high dose males, 33 low dose males, 39 high dose females, 21 low dose females, 28 control males, and 30 control females. Abdominal distention was reported in two low dose females, one control male, and one control female. Swollen eyes were observed in two high dose males. No other clinical abnormalities were noted.

B. Survival

The estimated probabilities of survival for male and female mice in the control and aniline HCl-dosed groups are shown in Figure 5. For both males and females the Tarone test did not indicate a significant association between dosage and mortality.

Adequate numbers of males were at risk from late-developing tumors, as 82 percent (41/50) of the high dose, 86 percent (43/50) of the low dose, and 66 percent (33/50) of the control mice survived on test until the end of the study. Seven control males were autolyzed in weeks 11 to 13. Survival was also adequate among females as 84 percent (41/49) of the high dose, 74 percent (37/50) of the low dose, and 60 percent (30/50) of the control mice survived on test until the end of the study.

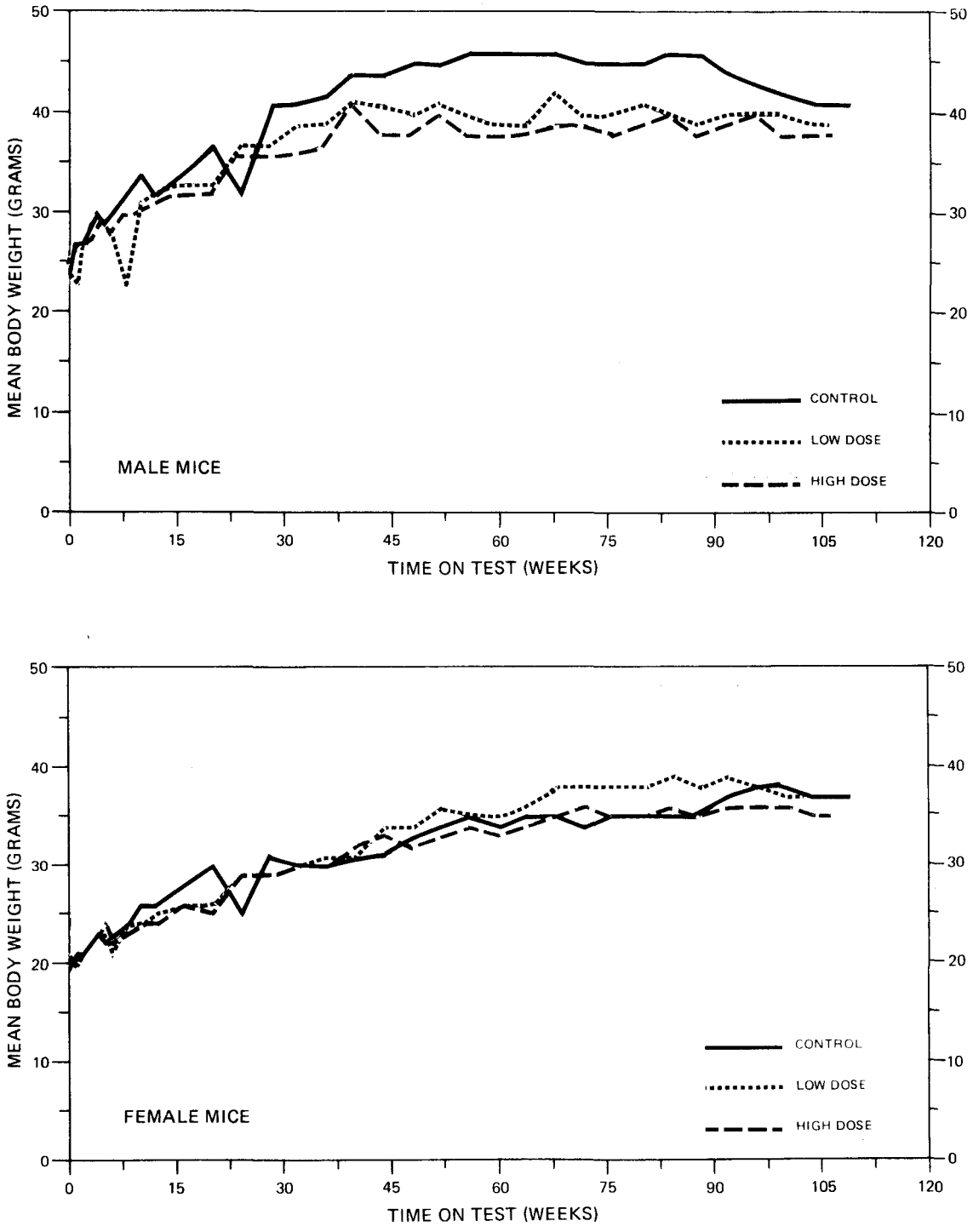

FIGURE 4
GROWTH CURVES FOR ANILINE HYDROCHLORIDE CHRONIC STUDY MICE

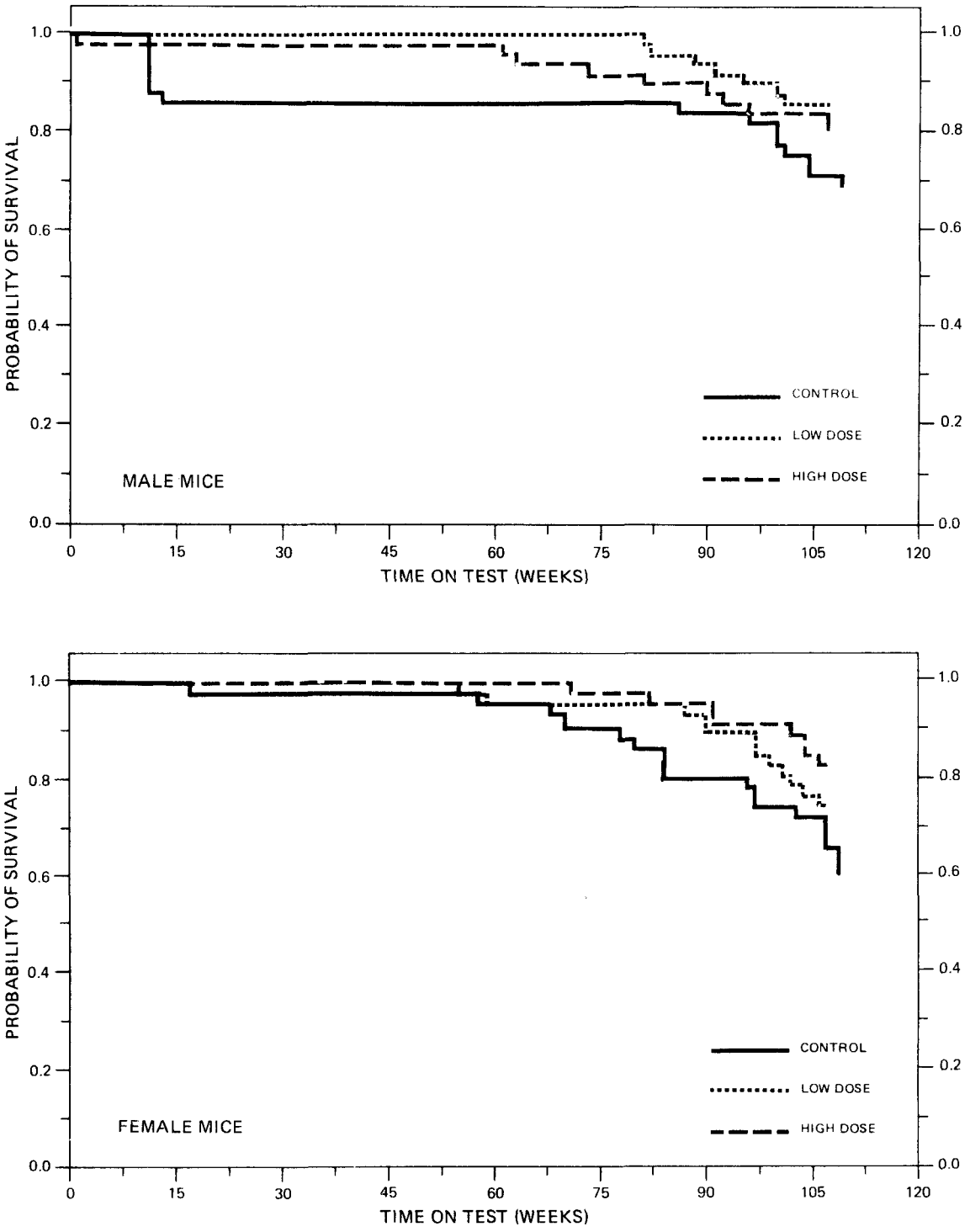

FIGURE 5
SURVIVAL COMPARISONS OF ANILINE HYDROCHLORIDE CHRONIC STUDY MICE

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).

None of the observed tumors were considered to be compound-induced, as they were found in approximately equal numbers in control and dosed groups.

There were also numerous degenerative and inflammatory lesions which commonly occur in aging mice of this strain. A chronic inflammation of bile ducts was only seen in dosed male mice. Other nonneoplastic lesions were not considered to be related to feeding of aniline HCl.

The pathologic examination provided no evidence for the carcinogenicity of aniline HCl in B6C3F1 mice under the conditions of this test.

D. Statistical Analyses of Results

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

For males the Cochran-Armitage test indicated a significant ($P = 0.042$) negative association between dosage and the incidence of

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH ANILINE HYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	2/39(0.05)	3/49(0.06)	1/49(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.194	0.398
Lower Limit	---	0.144	0.007
Upper Limit	---	13.758	7.377
Weeks to First Observed Tumor	109	107	107
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	4/39(0.10)	8/49(0.16)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.592	0.597
Lower Limit	---	0.465	0.093
Upper Limit	---	6.757	3.330
Weeks to First Observed Tumor	109	107	81
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	13/39(0.33)	7/49(0.14)	11/49(0.22)
P Values[c]	N.S.	P = 0.031(N)	N.S.
Relative Risk (Control)[d]	---	0.429	0.673
Lower Limit	---	0.162	0.311
Upper Limit	---	1.039	1.448
Weeks to First Observed Tumor	100	95	73

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	12/39(0.31)	9/49(0.18)	7/49(0.14)
P Values[c]	P = 0.042(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.597	0.464
Lower Limit	---	0.250	0.173
Upper Limit	---	1.384	1.154
Weeks to First Observed Tumor	86	81	107
Thyroid: Follicular-Cell Adenoma or Follicular-Cell Carcinoma[b]	0/38(0.00)	3/43(0.07)	1/43(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.536	0.048
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	82	107

[a]Treated groups received doses of 0.6 or 1.2 percent in feed.

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

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

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

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH ANILINE HYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	13/49(0.27)	17/49(0.35)	18/49(0.37)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.308	1.385
Lower Limit	---	0.675	0.726
Upper Limit	---	2.589	2.711
Weeks to First Observed Tumor	58	90	82
Circulatory System: Hemangioma or Hemangiosarcoma[b]	1/49(0.02)	0/49(0.00)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	3.000
Lower Limit	---	0.000	0.251
Upper Limit	---	18.651	154.197
Weeks to First Observed Tumor	109	---	71
Liver: Hepatocellular Carcinoma[b]	1/46(0.02)	5/48(0.10)	5/48(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	4.792	4.792
Lower Limit	---	0.566	0.566
Upper Limit	---	221.559	221.559
Weeks to First Observed Tumor	109	107	107

TABLE 6 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma NOS[b]	3/34(0.09)	2/32(0.06)	0/36(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.708	0.000
Lower Limit	---	0.062	0.000
Upper Limit	---	5.772	1.551
Weeks to First Observed Tumor	109	107	---
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant[b]	4/46(0.09)	1/45(0.02)	0/45(0.00)
P Values[c]	P = 0.027(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.256	0.000
Lower Limit	---	0.005	0.000
Upper Limit	---	2.452	1.099
Weeks to First Observed Tumor	68	107	---
Thyroid: Follicular-Cell Adenoma or Follicular-Cell Carcinoma[b]	4/44(0.09)	0/33(0.00)	0/36(0.00)
P Values[c]	P = 0.026(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	1.418	1.304
Weeks to First Observed Tumor	80	---	---

TABLE 6 (CONCLUDED)

[a]Treated groups received doses of 0.6 or 1.2 percent in feed.

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

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

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

hepatocellular carcinomas. The Fisher exact tests, however, were not significant. Similarly, for females Cochran-Armitage tests showed significant negative associations between dosage and the incidence of follicular-cell neoplasms of the thyroid and between dosage and the combined incidence of pheochromocytomas or malignant pheochromocytomas of the adrenal gland. In both cases, however, the Fisher exact tests were not significant.

There were no other statistically significant test results for either males or females under the Bonferroni criterion. Based upon these results there was no statistical evidence that indicated aniline HCl was a carcinogen in male or female mice.

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 all 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 all of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by aniline HCl that could not be established under the conditions of this test.

V. DISCUSSION

There were no significant positive associations between the administered dietary concentrations of aniline HCl and mortality in either sex of rats or mice. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Slight mean body weight depression, relative to controls, was observed in dosed female and high dose male rats and in dosed male mice.

In male rats there were several types of mesenchymal tumors, primarily of the spleen, associated with compound administration. There was a significant positive association between dietary concentration of the chemical and the incidence of hemangiosarcomas of the spleen. The high dose to control and low dose to control Fisher exact comparisons supported the finding. The combined incidence of fibrosarcomas and sarcomas NOS of the spleen was also statistically significant in male rats. Each of these types of sarcoma of the spleen is rare in untreated male Fischer 344 historical control rats. When the numbers of male rats having fibrosarcoma and/or sarcoma NOS of multiple body organs were combined, there was a significant positive association between aniline HCl concentration and incidence. This finding was supported by the high dose to control Fisher exact comparison. Therefore, under the conditions of this bioassay, aniline HCl was carcinogenic to male Fischer 344 rats.

The number of female rats having fibrosarcomas or sarcomas NOS of either the spleen alone or multiple organs of the body cavity was

significantly associated with increased dietary concentration of aniline HCl. Although this result was not supported by the Fisher exact tests, these tumors are rare in female Fischer 344 rats. Historical data indicate that none of the 249 female Fischer 344 control rats at this laboratory had any of these tumors. Assuming a binomial distribution, the probability of the incidence observed in the high dose group (7/50 [14 percent]) occurring by chance is less than 1 in 100,000. This result is, therefore, considered indicative of a carcinogenic effect of compound administration.

In mice no tumors occurred with significantly greater frequency in dosed groups than in control groups.

Experimental data reviewed by the International Agency for Research on Cancer was not considered adequate to indicate that aniline HCl is carcinogenic to rats or mice (International Agency for Research on Cancer, 1974). No tumors were observed in bladders, livers, spleens or kidneys of randomly bred rats (sex unspecified) surviving up to 750 days after initiation of oral intake of 22 mg/day of aniline HCl (Druckrey, 1950). Tumors were absent in 11 mice (strain unspecified) 12 months after they received 13 subcutaneous injections of aniline HCl (4 mg in aqueous solution) (Hartwell and Andervont, 1951; as cited in Hartwell, 1963).

Under the conditions of this bioassay, dietary administration of aniline HCl was carcinogenic to male and female Fischer 344 rats, inducing hemangiosarcomas and a combination of fibrosarcomas and

sarcomas NOS of the spleen and a combination of fibrosarcomas and sarcomas NOS of multiple body organs. There was no evidence of a carcinogenic effect due to the administration of aniline HCl to B6C3F1 mice of either sex.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH ANILINE HYDROCHLORIDE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS
TREATED WITH ANILINE HYDROCHLORIDE

	CONTROL (UNTR) 01-0330	LOW DOSE 01-0315	HIGH DOSE 01-0320
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	25	50	48
INTEGUMENTARY SYSTEM			
*SKIN	(25)	(50)	(48)
SQUAMOUS CELL PAPILLOMA	2 (8%)		
SQUAMOUS CELL CARCINOMA		1 (2%)	
LIPOSARCOMA		1 (2%)	
*SUBCUT TISSUE	(25)	(50)	(48)
FIBROMA	1 (4%)		
FIBROSARCOMA		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(25)	(50)	(46)
PHEOCHROMOCYTOMA, METASTATIC	1 (4%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(25)	(50)	(48)
MYELOMONOCYTIC LEUKEMIA	1 (4%)	2 (4%)	
*SUBCUT TISSUE/HEAD	(25)	(50)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
#BONE MARROW	(23)	(47)	(45)
HEMANGIOMA		1 (2%)	
#SPLEEN	(25)	(50)	(46)
SARCOMA, NOS		4 (8%)	2 (4%)
FIBROMA		7 (14%)	6 (13%)
FIBROSARCOMA		3 (6%)	7 (15%)
HEMANGIOSARCOMA		19 (38%)	20 (43%)
#MANDIBULAR L. NODE	(24)	(47)	(35)
C-CELL CARCINOMA, METASTATIC	1 (4%)		

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

TABLE A1 (CONTINUED)

	CONTROL(UNTR) 01-0330	LOW DOSE 01-0315	HIGH DOSE 01-0320
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(25)	(50)	(47)
NEOPLASTIC NODULE	1 (4%)		
#STOMACH	(24)	(48)	(45)
SQUAMOUS CELL PAPILLOMA		1 (2%)	
URINARY SYSTEM			
#U. BLADDER/MUCOSA	(25)	(49)	(44)
HEMANGIOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(22)	(45)	(34)
ADENOMA, NOS	2 (9%)	7 (16%)	4 (12%)
#ADRENAL	(24)	(50)	(44)
CORTICAL ADENOMA		1 (2%)	
PHEOCHROMOCYTOMA	1 (4%)	5 (10%)	11 (25%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (4%)	1 (2%)	1 (2%)
#THYROID	(21)	(39)	(30)
PAPILLARY ADENOCARCINOMA			1 (3%)
FOLLICULAR-CELL ADENOMA	1 (5%)	1 (3%)	
FOLLICULAR-CELL CARCINOMA		1 (3%)	
C-CELL ADENOMA		1 (3%)	
C-CELL CARCINOMA	2 (10%)	2 (5%)	1 (3%)
#PANCREATIC ISLETS	(25)	(45)	(37)
ISLET-CELL ADENOMA	1 (4%)	1 (2%)	
REPRODUCTIVE SYSTEM			
#TESTIS	(25)	(50)	(46)
INTERSTITIAL-CELL TUMOR	21 (84%)	34 (68%)	29 (63%)

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0330	LOW DOSE 01-0315	HIGH DOSE 01-0320
NERVOUS SYSTEM			
#BRAIN	(25)	(49)	(45)
ASTROCYTOMA		2 (4%)	
SPECIAL SENSE ORGANS			
*EAR CANAL	(25)	(50)	(48)
SQUAMOUS CELL CARCINOMA	1 (4%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*BODY CAVITIES	(25)	(50)	(48)
MESOTHELIOMA, NOS		2 (4%)	2 (4%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(25)	(50)	(48)
SARCOMA, NOS			1 (2%)
FIBROSARCOMA		2 (4%)	8 (17%)
LEIOMYOSARCOMA			2 (4%)
HEMANGIOSARCOMA			1 (2%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	25	50	50
NATURAL DEATH@	5	7	19
MORIBUND SACRIFICE	3	9	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	34	27
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	CONTROL(UNTR) 01-0330	LOW DOSE 01-0315	HIGH DOSE 01-0320
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	21	47	47
TOTAL PRIMARY TUMORS	35	102	97
TOTAL ANIMALS WITH BENIGN TUMORS	21	42	33
TOTAL BENIGN TUMORS	29	60	50
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	35	42
TOTAL MALIGNANT TUMORS	5	40	45
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		
TOTAL SECONDARY TUMORS	2		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	2	2
TOTAL UNCERTAIN TUMORS	1	2	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS
TREATED WITH ANILINE HYDROCHLORIDE

	CONTROL (UNTR) 02-0330	LOW DOSE 02-0315	HIGH DOSE 02-0320
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	24	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	24	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(24)	(50)	(50)
SARCOMA, NOS		1 (2%)	
FIBROSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(24)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (4%)		
LEUKEMIA,NOS		1 (2%)	
MYELOMONOCYTIC LEUKEMIA	2 (8%)	3 (6%)	
#SPLEEN	(23)	(50)	(50)
SARCOMA, NOS			3 (6%)
LIPOMA			1 (2%)
HEMANGIOMA			1 (2%)
HEMANGIOSARCOMA		1 (2%)	2 (4%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(24)	(50)	(50)
NEOPLASTIC NODULE			1 (2%)

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

TABLE A2 (CONTINUED)

	CONTROL(UNTR) 02-0330	LOW DOSE 02-0315	HIGH DOSE 02-0320
URINARY SYSTEM			
#URINARY BLADDER	(24)	(50)	(49)
PAPILLOMA, NOS			1 (2%)
TRANSITIONAL-CELL PAPILLOMA	1 (4%)		
ENDOCRINE SYSTEM			
#PITUITARY	(21)	(46)	(40)
CARCINOMA,NOS		1 (2%)	1 (3%)
ADENOMA, NOS	6 (29%)	14 (30%)	8 (20%)
#ADRENAL	(24)	(50)	(48)
CORTICAL ADENOMA		2 (4%)	3 (6%)
CORTICAL CARCINOMA			1 (2%)
PHEOCHROMOCYTOMA	1 (4%)		3 (6%)
PHEOCHROMOCYTOMA, MALIGNANT			2 (4%)
#THYROID	(21)	(38)	(38)
FOLLICULAR-CELL ADENOMA			1 (3%)
FOLLICULAR-CELL CARCINOMA	1 (5%)		1 (3%)
C-CELL ADENOMA		1 (3%)	
C-CELL CARCINOMA	1 (5%)	1 (3%)	
#PANCREATIC ISLETS	(22)	(49)	(49)
ISLET-CELL ADENOMA		1 (2%)	
ISLET-CELL CARCINOMA	1 (5%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(24)	(50)	(50)
FIBROADENOMA	4 (17%)	5 (10%)	
*CLITORAL GLAND	(24)	(50)	(50)
CARCINOMA,NOS	1 (4%)		
*VAGINA	(24)	(50)	(50)
SARCOMA, NOS			1 (2%)
*UTERUS	(24)	(48)	(50)
ADENOCARCINOMA, NOS	1 (4%)		1 (2%)
ENDOMETRIAL STROMAL POLYP	2 (8%)	15 (31%)	7 (14%)

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

TABLE A2 (CONTINUED)

	CONTROL(UNTR) 02-0330	LOW DOSE 02-0315	HIGH DOSE 02-0320
ENDOMETRIAL STROMAL SARCOMA	1 (4%)		
#UTERUS/ENDOMETRIUM	(24)	(48)	(50)
LEIOMYOSARCOMA			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(23)	(49)	(50)
ASTROCYTOMA		1 (2%)	
SPECIAL SENSE ORGANS			
*EAR CANAL	(24)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (4%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(24)	(50)	(50)
LEIOMYOSARCOMA	1 (4%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(24)	(50)	(50)
SARCOMA, NOS			1 (2%)
FIBROSARCOMA		1 (2%)	3 (6%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	25	50	50
NATURAL DEATH@	4	4	4
MORIBUND SACRIFICE	5	2	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	44	41
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 02-0330	LOW DOSE 02-0315	HIGH DOSE 02-0320
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	17	32	35
TOTAL PRIMARY TUMORS	25	48	44
TOTAL ANIMALS WITH BENIGN TUMORS	10	26	21
TOTAL BENIGN TUMORS	14	38	25
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	9	17
TOTAL MALIGNANT TUMORS	11	10	18
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
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
\# SECONDAPY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH ANILINE HYDROCHLORIDE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE
TREATED WITH ANILINE HYDROCHLORIDE

	CONTROL (UNTR) 05-0330	LOW DOSE 05-0320	HIGH DOSE 05-0325
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	2		
ANIMALS NECROPSIED	39	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	39	49	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(39)	(49)	(49)
HEPATOCELLULAR CARCINOMA, METAST	2 (5%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (5%)	5 (10%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (5%)	3 (6%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(39)	(49)	(49)
MALIGNANT LYMPHOMA, NOS	11 (28%)	4 (8%)	5 (10%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			2 (4%)
GRANULOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(38)	(49)	(49)
HEMANGIOMA			1 (2%)
HEMANGIOSARCOMA		1 (2%)	
MALIGNANT LYMPHOMA, NOS	1 (3%)		1 (2%)
#MESENTERIC L. NODE	(36)	(45)	(45)
MALIGNANT LYMPHOMA, NOS		3 (7%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (3%)		
#PEYERS PATCH	(37)	(48)	(48)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#THYMUS	(13)	(28)	(26)
MALIGNANT LYMPHOMA, NOS			1 (4%)

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 05-0330	LOW DOSE 05-0320	HIGH DOSE 05-0325
CIRCULATORY SYSTEM			
*CARDIOVASCULAR SYSTE	(39)	(49)	(49)
HEMANGIOSARCOMA			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(39)	(49)	(49)
HEPATOCELLULAR CARCINOMA	12 (31%)	9 (18%)	7 (14%)
#STOMACH	(37)	(49)	(47)
SQUAMOUS CELL PAPILLOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(38)	(43)	(43)
FOLLICULAR-CELL ADENOMA		2 (5%)	1 (2%)
FOLLICULAR-CELL CARCINOMA		1 (2%)	
#PANCREATIC ISLETS	(36)	(45)	(46)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
#TESTIS	(38)	(48)	(49)
INTERSTITIAL-CELL TUMOR		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(39)	(49)	(49)
ADENOMA, NOS		1 (2%)	

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 05-0330	LOW DOSE 05-0320	HIGH DOSE 05-0325
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	13	7	6
MORIBUND SACRIFICE	2		3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	33	43	41
ANIMAL MISSING	2		

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 05-0330	LOW DOSE 05-0320	HIGH DOSE 05-0325
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	24	27	22
TOTAL PRIMARY TUMORS	29	31	25
TOTAL ANIMALS WITH BENIGN TUMORS	2	9	5
TOTAL BENIGN TUMORS	2	10	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	22	20	19
TOTAL MALIGNANT TUMORS	27	21	20
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		
TOTAL SECONDARY TUMORS	2		
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 ANILINE HYDROCHLORIDE

	CONTROL (UNTR) 06-0330	LOW DOSE 06-0320	HIGH DOSE 06-0325
ANIMALS INITIALLY IN STUDY	50	50	@50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	49	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	49	48	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(49)	(49)
MALIGNANT LYMPHOMA, NOS	10 (20%)	7 (14%)	16 (33%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	4 (8%)	2 (4%)
GRANULOCYTIC LEUKEMIA		1 (2%)	
#SPLEEN	(45)	(48)	(49)
HEMANGIOSARCOMA	1 (2%)		
MALIGNANT LYMPHOMA, NOS		3 (6%)	
#MEDIASTINAL L.NODE	(44)	(37)	(46)
MALIGNANT LYMPHOMA, NOS		1 (3%)	
#PANCREATIC L.NODE	(44)	(37)	(46)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
#LIVER	(46)	(48)	(48)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
#KIDNEY	(46)	(48)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0330	LOW DOSE 06-0320	HIGH DOSE 06-0325
DIGESTIVE SYSTEM			
#LIVER	(46)	(48)	(48)
HEPATOCELLULAR CARCINOMA	1 (2%)	5 (10%)	5 (10%)
#STOMACH	(41)	(46)	(47)
SQUAMOUS CELL PAPILLOMA		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(34)	(32)	(36)
ADENOMA, NOS	3 (9%)	2 (6%)	
#ADRENAL	(46)	(45)	(45)
PHEOCHROMOCYTOMA	3 (7%)	1 (2%)	
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)		
#THYROID	(44)	(33)	(36)
FOLLICULAR-CELL ADENOMA	2 (5%)		
FOLLICULAR-CELL CARCINOMA	2 (5%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(44)	(46)	(43)
ENDOMETRIAL STROMAL POLYP	1 (2%)		
#OVARY	(44)	(45)	(47)
GRANULOSA-CELL TUMOR			1 (2%)
TUBULAR ADENOMA	1 (2%)		2 (4%)
HEMANGIOMA			2 (4%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0330	LOW DOSE 06-0320	HIGH DOSE 06-0325
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(49)	(49)	(49)
HEMANGIOSARCOMA			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	17	11	6
MORIBUND SACRIFICE	3	1	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	30	37	41
ANIMAL MISSING		1	
ANIMAL DELETED (WRONG SEX)			1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 06-0330	LOW DOSE 06-0320	HIGH DOSE 06-0325
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	21	22	28
TOTAL PRIMARY TUMORS	28	26	29
TOTAL ANIMALS WITH BENIGN TUMORS	9	4	4
TOTAL BENIGN TUMORS	10	4	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	16	20	24
TOTAL MALIGNANT TUMORS	18	22	24
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH ANILINE HYDROCHLORIDE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH ANILINE HYDROCHLORIDE

	CONTROL (UNTR) 01-0330	LOW DOSE 01-0315	HIGH DOSE 01-0320
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	25	50	48
INTEGUMENTARY SYSTEM			
*SKIN	(25)	(50)	(48)
ULCER, ACUTE		1 (2%)	
ABSCESS, NOS		1 (2%)	
SCAR	1 (4%)		
HYPERKERATOSIS		1 (2%)	
ACANTHOSIS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(25)	(50)	(46)
BRONCHIECTASIS		1 (2%)	
INFLAMMATION, SUPPURATIVE			1 (2%)
#LUNG/BRONCHIOLE	(25)	(50)	(46)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
#LUNG	(25)	(50)	(46)
BRONCHOPNEUMONIA NECROTIZING	1 (4%)		1 (2%)
PNEUMONIA, CHRONIC MURINE		2 (4%)	2 (4%)
FIBROSIS, DIFFUSE		1 (2%)	1 (2%)
HYPERPLASIA, ATYPICAL			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(25)	(50)	(46)
THROMBOSIS, NOS		1 (2%)	
THROMBUS, ORGANIZED			1 (2%)
CONGESTION, NOS	1 (4%)	2 (4%)	
CONGESTION, PASSIVE		1 (2%)	
HEMORRHAGE			1 (2%)

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

TABLE C1 (CONTINUED)

	CONTROL(UNTR) 01-0330	LOW DOSE 01-0315	HIGH DOSE 01-0320
FIBROSIS		2 (4%)	1 (2%)
NECROSIS, HEMORRHAGIC		1 (2%)	
METAMORPHOSIS FATTY		2 (4%)	1 (2%)
HEMOSIDEROSIS		3 (6%)	2 (4%)
HYPERPLASIA, PAPILLARY		18 (36%)	7 (15%)
METAPLASIA, OSSEOUS			1 (2%)
HEMATOPOIESIS		1 (2%)	
ERYTHROPOIESIS		6 (12%)	5 (11%)
#SPLENIC CAPSULE	(25)	(50)	(46)
INFLAMMATION, CHRONIC		1 (2%)	
#MANDIBULAR L. NODE	(24)	(47)	(35)
HYPERPLASIA, PLASMA CELL	2 (8%)		
#RENAL LYMPH NODE	(24)	(47)	(35)
HEMOSIDEROSIS			1 (3%)
HYPERPLASIA, NOS			1 (3%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(25)	(50)	(46)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, CHRONIC	1 (4%)		
DEGENERATION, NOS			1 (2%)
*CORONARY ARTERY	(25)	(50)	(48)
INFLAMMATION, NOS		1 (2%)	
*HEPATIC VEIN	(25)	(50)	(48)
THROMBUS, ORGANIZED			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(25)	(50)	(47)
THROMBOSIS, NOS			1 (2%)
HEMORRHAGE			2 (4%)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
DEGENERATION, NOS	1 (4%)		
NECROSIS, FOCAL			1 (2%)
INFARCT, NOS			1 (2%)
METAMORPHOSIS FATTY	1 (4%)		
BASOPHILIC CYTO CHANGE	1 (4%)	10 (20%)	

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

TABLE C1 (CONTINUED)

	CONTROL(UNTR) 01-0330	LOW DOSE 01-0315	HIGH DOSE 01-0320
HYPERPLASIA, FOCAL	1 (4%)		
ANGIECTASIS	1 (4%)		1 (2%)
#HEPATIC CAPSULE	(25)	(50)	(47)
NECROSIS, COAGULATIVE			1 (2%)
#LIVER/CENTRILOBULAR	(25)	(50)	(47)
NECROSIS, NOS			1 (2%)
NECROSIS, DIFFUSE			1 (2%)
METAMORPHOSIS FATTY			1 (2%)
#LIVER/KUPFFER CELL	(25)	(50)	(47)
HEMOSIDEROSIS		2 (4%)	26 (55%)
#PANCREATIC ACINUS	(25)	(45)	(37)
ATROPHY, NOS			2 (5%)
HYPERPLASIA, EPITHELIAL			1 (3%)
#STOMACH	(24)	(48)	(45)
ULCER, NOS			1 (2%)
#GASTRIC MUCOSA	(24)	(48)	(45)
NECROSIS, FOCAL			1 (2%)
#GASTRIC MUSCULARIS	(24)	(48)	(45)
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
URINARY SYSTEM			
#KIDNEY	(25)	(50)	(48)
CALCULUS, NOS			1 (2%)
HYDRONEPHROSIS		1 (2%)	
NEPHROSIS, NOS	21 (84%)	7 (14%)	8 (17%)
HEMOSIDEROSIS		21 (42%)	34 (71%)
#KIDNEY/CORTEX	(25)	(50)	(48)
MULTILOCULAR CYST	1 (4%)		
#URINARY BLADDER	(25)	(49)	(44)
INFLAMMATION, ACUTE		1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)	
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(24)	(50)	(44)
HYPERPLASIA, FOCAL	1 (4%)	1 (2%)	

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0330	LOW DOSE 01-0315	HIGH DOSE 01-0320
#ADRENAL MEDULLA	(24)	(50)	(44)
HYPERPLASIA, FOCAL	4 (17%)		2 (5%)
#THYROID	(21)	(39)	(30)
COLLOID CYST			1 (3%)
HYPERPLASIA, C-CELL		1 (3%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(25)	(50)	(48)
HYPERPLASIA, NOS			1 (2%)
*MAMMARY DUCT	(25)	(50)	(48)
HEMORRHAGE	1 (4%)		
#PROSTATE	(25)	(49)	(42)
INFLAMMATION, SUPPURATIVE	2 (8%)		
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, ACUTE FOCAL		1 (2%)	
*SEMINAL VESICLE	(25)	(50)	(48)
ATROPHY, NOS		1 (2%)	
#TESTIS	(25)	(50)	(46)
DEGENERATION, NOS		1 (2%)	
ATROPHY, NOS	5 (20%)	5 (10%)	5 (11%)
HYPERPLASIA, INTERSTITIAL CELL		5 (10%)	1 (2%)
#TESTIS/TUBULE	(25)	(50)	(46)
DEGENERATION, NOS		1 (2%)	1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 01-0330	LOW DOSE 01-0315	HIGH DOSE 01-0320
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC		3	
NECROSIS, NOS		3	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4		
AUTOLYSIS/NO NECROPSY			2

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

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
TREATED WITH ANILINE HYDROCHLORIDE

	CONTROL (UNTR) 02-0330	LOW DOSE 02-0315	HIGH DOSE 02-0320
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	24	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	24	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(24)	(50)	(50)
BRONCHOPNEUMONIA, ACUTE		1 (2%)	
INFLAMMATION, ACUTE DIFFUSE		1 (2%)	
PNEUMONIA, CHRONIC MURINE		1 (2%)	
HYPERPLASIA, ADENOMATOUS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(21)	(48)	(50)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
#SPLEEN	(23)	(50)	(50)
DILATATION, NOS		1 (2%)	1 (2%)
CYST, NOS			1 (2%)
CONGESTION, NOS		1 (2%)	11 (22%)
HEMORRHAGE			1 (2%)
FIBROSIS		1 (2%)	
FIBROSIS, FOCAL		1 (2%)	
METAMORPHOSIS FATTY		1 (2%)	1 (2%)
PIGMENTATION, NOS		1 (2%)	
HEMOSIDEROSIS		6 (12%)	5 (10%)
HYPERPLASIA, PAPILLARY		23 (46%)	28 (56%)
ERYTHROPOIESIS	1 (4%)	36 (72%)	30 (60%)
#SPLENIC CAPSULE	(23)	(50)	(50)
FIBROSIS		1 (2%)	1 (2%)
#SPLENIC SINUSOIDS	(23)	(50)	(50)
FIBROSIS			1 (2%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0330	LOW DOSE 02-0315	HIGH DOSE 02-0320
#MANDIBULAR L. NODE	(19)	(48)	(41)
HYPERPLASIA, PLASMA CELL	2 (11%)		
#MEDIASTINAL L.NODE	(19)	(48)	(41)
INFLAMMATION, ACUTE		1 (2%)	
#MESENTERIC L. NODE	(19)	(48)	(41)
LYMPHANGIECTASIS			1 (2%)
#RENAL LYMPH NODE	(19)	(48)	(41)
HYPERPLASIA, NOS			1 (2%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(24)	(50)	(50)
CALCIFICATION, FOCAL	1 (4%)		
#ENDOCARDIUM	(24)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
*AORTA	(24)	(50)	(50)
MEDIAL CALCIFICATION	1 (4%)		
*CORONARY ARTERY	(24)	(50)	(50)
MEDIAL CALCIFICATION	1 (4%)		
DIGESTIVE SYSTEM			
#LIVER	(24)	(50)	(50)
CYST, NOS			1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
METAMORPHOSIS FATTY		1 (2%)	1 (2%)
BASOPHILIC CYTO CHANGE	10 (42%)	38 (76%)	15 (30%)
HYPERPLASIA, FOCAL		1 (2%)	
ANGIECTASIS	1 (4%)	1 (2%)	
HYPERPLASIA, BASOPHILIC	1 (4%)	2 (4%)	
#HEPATIC CAPSULE	(24)	(50)	(50)
HEMORRHAGE		1 (2%)	
#LIVER/CENTRILOBULAR	(24)	(50)	(50)
CONGESTION, NOS			1 (2%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0330	LOW DOSE 02-0315	HIGH DOSE 02-0320
NECROSIS, DIFFUSE		1 (2%)	1 (2%)
NECROSIS, COAGULATIVE		1 (2%)	
#LIVER/KUPFFER CELL	(24)	(50)	(50)
HEMOSIDEROSIS			29 (58%)
#LIVER/HEPATOCYTES	(24)	(50)	(50)
NECROSIS, FOCAL			1 (2%)
*BILE DUCT	(24)	(50)	(50)
INFLAMMATION, CHRONIC DIFFUSE		1 (2%)	1 (2%)
#PANCREAS	(22)	(49)	(49)
METAMORPHOSIS FATTY			1 (2%)
#STOMACH	(24)	(49)	(48)
ULCER, ACUTE			1 (2%)
HYPERKERATOSIS			1 (2%)
ACANTHOSIS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(24)	(49)	(50)
HYDRONEPHROSIS			1 (2%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
NEPHROSIS, NOS	8 (33%)	1 (2%)	
METAMORPHOSIS FATTY			1 (2%)
HEMOSIDEROSIS		46 (94%)	45 (90%)
HYPERPLASIA, PAPILLARY		1 (2%)	
#KIDNEY/TUBULE	(24)	(49)	(50)
CALCIFICATION, NOS	1 (4%)		
ENDOCRINE SYSTEM			
#PITUITARY	(21)	(46)	(40)
HEMOSIDEROSIS	1 (5%)		
#ADRENAL MEDULLA	(24)	(50)	(48)
HYPERPLASIA, FOCAL		1 (2%)	2 (4%)
#THYROID	(21)	(38)	(38)
HYPERPLASIA, C-CELL	1 (5%)	2 (5%)	

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

TABLE C2 (CONCLUDED)

	CONTROL(UNTR) 02-0330	LOW DOSE 02-0315	HIGH DOSE 02-0320
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(24)	(50)	(50)
GALACTOCELE		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
#UTERUS/ENDOMETRIUM	(24)	(48)	(50)
INFLAMMATION, SUPPURATIVE	3 (13%)		1 (2%)
HYPERPLASIA, CYSTIC			1 (2%)
METAPLASIA, SQUAMOUS	1 (4%)		
#OVARY/OVIDUCT	(24)	(48)	(50)
INFLAMMATION, SUPPURATIVE	5 (21%)		
#OVARY	(24)	(49)	(50)
CYST, NOS	2 (8%)	1 (2%)	4 (8%)
INFLAMMATION, SUPPURATIVE	1 (4%)		1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CRYSTALLINE LENS	(24)	(50)	(50)
CALCIFICATION, NOS			1 (2%)
CALCIFICATION, FOCAL			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1		
AUTOLYSIS/NO NECROPSY	1		

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH ANILINE HYDROCHLORIDE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH ANILINE HYDROCHLORIDE

	CONTROL (UNTR) 05-0330	LOW DOSE 05-0320	HIGH DOSE 05-0325
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	2		
ANIMALS NECROPSIED	39	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	39	49	49
INTEGUMENTARY SYSTEM			
*SKIN	(39)	(49)	(49)
EPIDERMAL INCLUSION CYST	1 (3%)		
INFLAMMATION, CHRONIC	1 (3%)		
FIBROSIS	1 (3%)		
RESPIRATORY SYSTEM			
#LUNG/ALVEOLI	(39)	(49)	(49)
HISTIOCYTOSIS			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(38)	(49)	(49)
HYPERPLASIA, LYMPHOID	1 (3%)		1 (2%)
HEMATOPOIESIS	1 (3%)	1 (2%)	1 (2%)
ERYTHROPOIESIS	3 (8%)	2 (4%)	4 (8%)
#MANDIBULAR L. NODE	(36)	(45)	(45)
HYPERPLASIA, PLASMA CELL	1 (3%)		
#PANCREATIC L.NODE	(36)	(45)	(45)
HYPERPLASIA, NOS		1 (2%)	
#MESENTERIC L. NODE	(36)	(45)	(45)
LYMPHANGIECTASIS		2 (4%)	3 (7%)
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
HYPERPLASIA, NOS	4 (11%)	2 (4%)	2 (4%)
HYPERPLASIA, PLASMA CELL		2 (4%)	
HYPERPLASIA, LYMPHOID	4 (11%)		1 (2%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(39)	(49)	(49)
INFLAMMATION, CHRONIC		1 (2%)	

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0330	LOW DOSE 05-0320	HIGH DOSE 05-0325
DIGESTIVE SYSTEM			
#LIVER	(39)	(49)	(49)
DEGENERATION, NOS		1 (2%)	
HEMOSIDEROSIS			3 (6%)
HYPERPLASTIC NODULE		1 (2%)	1 (2%)
HYPERPLASIA, FOCAL	3 (8%)		
HEMATOPOIESIS			1 (2%)
*BILE DUCT	(39)	(49)	(49)
INFLAMMATION, CHRONIC		2 (4%)	
INFLAMMATION, CHRONIC DIFFUSE		14 (29%)	13 (27%)
HYPERPLASIA, DIFFUSE		1 (2%)	1 (2%)
#PANCREAS	(36)	(45)	(46)
PERIARTERITIS			1 (2%)
NECROSIS, DIFFUSE		1 (2%)	
#GASTRIC MUCOSA	(37)	(49)	(47)
HYPERPLASIA, FOCAL		1 (2%)	1 (2%)
HYPERPLASIA, ADENOMATOUS			1 (2%)
#GASTRIC SUBMUCOSA	(37)	(49)	(47)
INFLAMMATION, ACUTE FOCAL			1 (2%)
#PEYERS PATCH	(37)	(48)	(48)
HYPERPLASIA, LYMPHOID			1 (2%)
#JEJUNUM	(37)	(48)	(48)
AMYLOIDOSIS	1 (3%)		
#ILEUM	(37)	(48)	(48)
AMYLOIDOSIS	2 (5%)		
URINARY SYSTEM			
#KIDNEY	(39)	(49)	(49)
HYDRONEPHROSIS		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
PERIVASCULITIS		1 (2%)	
GLOMERULOSCLEROSIS, NOS	3 (8%)	8 (16%)	2 (4%)
#KIDNEY/CORTEX	(39)	(49)	(49)
SCAR	1 (3%)		

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0330	LOW DOSE 05-0320	HIGH DOSE 05-0325
#KIDNEY/GLOMERULUS	(39)	(49)	(49)
AMYLOIDOSIS	2 (5%)		
ENDOCRINE SYSTEM			
#ADRENAL	(36)	(45)	(46)
AMYLOIDOSIS	2 (6%)		
#THYROID	(38)	(43)	(43)
CYSTIC FOLLICLES	1 (3%)		1 (2%)
AMYLOIDOSIS	1 (3%)		
HYPERPLASIA, C-CELL			1 (2%)
HYPERPLASIA, FOLLICULAR-CELL	2 (5%)		
#PARATHYROID	(28)	(18)	(26)
HYPERPLASIA, NOS	1 (4%)		
#PANCREATIC ISLETS	(36)	(45)	(46)
HYPERPLASIA, NOS	1 (3%)		
REPRODUCTIVE SYSTEM			
#PROSTATE	(37)	(48)	(49)
PERIVASCULITIS		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 D1 (CONCLUDED)

	CONTROL (UNTR) 05-0330	LOW DOSE 05-0320	HIGH DOSE 05-0325
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(39)	(49)	(49)
AMYLOIDOSIS	1 (3%)		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	10	7
ANIMAL MISSING/NO NECROPSY	2		
AUTO/NECROPSY/HISTO PERF		1	1
AUTOLYSIS/NO NECROPSY	9	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 ANILINE HYDROCHLORIDE

	CONTROL(UNTR) 06-0330	LOW DOSE 06-0320	HIGH DOSE 06-0325
ANIMALS INITIALLY IN STUDY	50	50	@50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	49	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	49	48	49
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(49)	(49)
INFLAMMATION, ACUTE	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(49)	(48)	(49)
INFLAMMATION, CHRONIC			1 (2%)
#LUNG	(49)	(48)	(49)
PERIVASCULITIS			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(45)	(48)	(49)
HYPERPLASIA, LYMPHOID	1 (2%)	3 (6%)	
HEMATOPOIESIS			2 (4%)
ERYTHROPOIESIS	4 (9%)	6 (13%)	13 (27%)
#LYMPH NODE OF THORAX	(44)	(37)	(46)
HYPERPLASIA, NOS	1 (2%)		
#PANCREATIC L.NODE	(44)	(37)	(46)
HYPERPLASIA, NOS		1 (3%)	
HEMATOPOIESIS	1 (2%)		
#LUMBAR LYMPH NODE	(44)	(37)	(46)
HYPERPLASIA, NOS	1 (2%)		1 (2%)
#MESENTERIC L. NODE	(44)	(37)	(46)
HYPERPLASIA, NOS	1 (2%)	2 (5%)	2 (4%)
HYPERPLASIA, LYMPHOID	1 (2%)		1 (2%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0330	LOW DOSE 06-0320	HIGH DOSE 06-0325
HEMATOPOIESIS	1 (2%)		
*RENAL LYMPH NODE	(44)	(37)	(46)
HEMATOPOIESIS	1 (2%)		
CIRCULATORY SYSTEM			
*AORTA	(49)	(49)	(49)
MEDIAL CALCIFICATION		1 (2%)	
*PULMONARY ARTERY	(49)	(49)	(49)
MEDIAL CALCIFICATION		1 (2%)	
DIGESTIVE SYSTEM			
*LIVER	(46)	(48)	(48)
NECROSIS, FOCAL		1 (2%)	
HYPERPLASTIC NODULE	1 (2%)		
HEMATOPOIESIS	1 (2%)		1 (2%)
*GALLBLADDER	(49)	(49)	(49)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
*BILE DUCT	(49)	(49)	(49)
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION, CHRONIC DIFFUSE		1 (2%)	1 (2%)
#PANCREAS	(38)	(45)	(46)
CYSTIC DUCTS		1 (2%)	
#STOMACH	(41)	(46)	(47)
HYPERPLASIA, CYSTIC		1 (2%)	
#GASTRIC MUCOSA	(41)	(46)	(47)
CALCIFICATION, NOS		1 (2%)	
HYPERPLASIA, ADENOMATOUS		1 (2%)	
#GASTRIC FOVEOLAE	(41)	(46)	(47)
METAPLASIA, SQUAMOUS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(46)	(48)	(49)
GLOMERULOSCLEROSIS, NOS	2 (4%)	4 (8%)	

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0330	LOW DOSE 06-0320	HIGH DOSE 06-0325
HEMOSIDEROSIS			1 (2%)
#KIDNEY/TUBULE	(46)	(48)	(49)
CALCIFICATION, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#ADRENAL/CAPSULE	(46)	(45)	(45)
HYPERPLASIA, NOS		1 (2%)	
#THYROID	(44)	(33)	(36)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
INFLAMMATION, CHRONIC FOCAL			1 (3%)
HYPERPLASIA, FOLLICULAR-CELL	2 (5%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(44)	(46)	(43)
HYDROMETRA		1 (2%)	5 (12%)
#UTERUS/ENDOMETRIUM	(44)	(46)	(43)
HEMORRHAGE		1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, CYSTIC	30 (68%)	18 (39%)	13 (30%)
#OVARY	(44)	(45)	(47)
CYST, NOS	7 (16%)	6 (13%)	2 (4%)
HEMORRHAGIC CYST	4 (9%)		
ABSCESS, NOS	1 (2%)		
INFLAMMATION, CHRONIC	2 (5%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(49)	(49)	(49)
ABSCESS, NOS	1 (2%)		

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

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 06-0330	LOW DOSE 06-0320	HIGH DOSE 06-0325
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
STEATITIS	1		
INFLAMMATION, CHRONIC		1	
FIBROSIS		1	
NECROSIS, FAT	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	7	4
ANIMAL MISSING/NO NECROPSY		1	
NECROPSY PERF/NO HISTO PERFORMED		1	
AUTO/NECROPSY/HISTO PERF	3	1	
AUTOLYSIS/NO NECROPSY	1		

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

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

June 29, 1978

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

The reviewer agreed with the conclusion in the report that Aniline Hydrochloride was carcinogenic in treated rats, under the conditions of test. He pointed out that there was marked hemosiderosis in the renal tubular epithelium and in the liver of treated rats but none was reported in treated mice. Based on the experimental findings, he said that the compound may pose a possible carcinogenic risk to humans. The reviewer moved that the report on the bioassay of Aniline Hydrochloride be accepted as written. The motion was approved without objection.

Clearinghouse Members present:

Arnold L. Brown (Chairman), Mayo Clinic
Paul Nettesheim, National Institute of Environmental Health Sciences
Verne Ray, Pfizer Medical Research Laboratory
Verald K. Rowe, Dow Chemical U.S.A.
Michael B. Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center

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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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