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# BIOASSAY OF p-ANISIDINE HYDROCHLORIDE FOR POSSIBLE CARCINOGENICITY

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

# BIOASSAY OF

# p-ANISIDINE 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 p-ANISIDINE 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 p-anisidine 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 p-anisidine 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. A. Russfield (3), Dr. R. L. Schueler (6) (as a consultant), 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), using

methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (8).

This report was prepared at METREK, a Division of The MITRE Corporation (5) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (5), task leader Dr. M. R. Kornreich (5), senior biologist Ms. P. Walker (5), biochemist Mr. S. C. Drill (6), 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), Dr. R. A. Griesemer (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,9), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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## SUMMARY

A bioassay for possible carcinogenicity of p-anisidine hydrochloride was conducted using Fischer 344 rats and B6C3F1 mice. p-Anisidine hydrochloride was administered in the feed, at either of two concentrations, to groups of 55 male and 55 female animals of each species. Fifty-five animals of each sex and species were placed on test as controls. The high and low dietary concentrations of p-anisidine hydrochloride were, respectively, 0.6 and 0.3 percent for rats and 1.0 and 0.5 percent for mice. The compound was administered in the diet for 103 weeks, follwed by an observation period of 2 to 3 weeks for rats and 2 weeks for mice.

There were no significant positive associations for either species between the concentration of p-anisidine hydrochloride administered and mortality. In addition, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

In male rats there were significant associations between compound administration and the incidences of both squamous-cell carcinomas of the skin and alveolar/bronchiolar adenomas. None of the Fisher exact comparisons, however, supported these findings. When those males having adenomas NOS or carcinomas NOS of the preputial gland were combined and the resulting incidences statistically analyzed, the only test providing a significant result was the Fisher exact comparison of the low dose to the control. There were no significant positive associations between the administration of p-anisidine HCl and the incidence of any tumor in mice of either sex.

Although, under the conditions of this bioassay, there appeared to be an association between chemical administration and the increased incidence of preputial gland tumors in low dose male rats, the evidence was insufficient to establish the carcinogenicity of p-anisidine hydrochloride in Fischer 344 rats. The compound was not carcinogenic in B6C3F1 mice.

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# I. INTRODUCTION

p-Anisidine HCl (NCI No. C03758), the hydrochloride salt of an aromatic dye intermediate, was selected for bioassay by the National Cancer Institute because of the increased bladder cancer incidence noted among workers in the dye manufacturing industry (Wynder et al., 1963; Anthony and Thomas, 1970). Aromatic amines are one of several classes of chemicals thought to contribute to this high cancer risk (Clayson and Garner, 1976).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 4-methoxy-benzenamine HCl.* It is also known as p-aminoanisole HCl and 4-methoxyaniline HCl.

p-Anisidine is used as an intermediate for the production of C.I. (Colour Index) Azoic Coupling Components 11 and 13, C.I. Vat Red 29 (also called C.I. Pigment Red 190), C.I. Disperse Orange 15, Diazo Brilliant Scarlet ROD extra, Diazo Brilliant Scarlet BG extra, and Benzo Fast Scarlet 4FB (Society of Dyers and Colourists, 1956).

The hydrochloride salt of p-anisidine is not produced commercially (U.S. International Trade Commission [USITC], 1977); however, p-anisidine is produced in commercial quantities (in excess of 1000 pounds or $1000 in value annually) as are C.I. Azoic Coupling Components 11 and 13 and C.I. Vat Red 29 (USITC, 1977).

The potential for exposure to p-anisidine and p-anisidine HCl is greatest for workers in the dye and chemical industries.

*The CAS registry number is 20265-97-8.

p-Anisidine displays considerable acute and chronic systemic toxicity upon ingestion, inhalation, or skin absorption, and is a moderate local irritant (Sax, 1975).

## II. MATERIALS AND METHODS

### A. Chemicals

p-Anisidine hydrochloride (Figure 1) was purchased in two lots from Pfaltz and Bauer Chemical Company and chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri.

The first lot was used during the subchronic test and for the first 20 months of the chronic bioassay; the second lot was used in the final phase of the bioassay. The experimentally determined melting point range of 215° to 220°C was in general agreement with the range reported in the literature (216° to 218°C) (Dornow et al., 1957). Elemental analysis approximated that expected for $C_7H_{10}NOCl$, the molecular formula of p-anisidine hydrochloride. Thin-layer chromatography was performed utilizing two solvent systems (benzene: 1,4-dioxane; and ethyl acetate:ammonium hydroxide). Each plate indicated one nonmotile impurity. Vapor-phase chromatography revealed one homogeneous peak. Titration of the amine function with perchloric acid provided results close to those expected on a theoretical basis. This does not, however, preclude the possibility of other amine compounds being present. Infrared analysis was consistent with the structure of the compound.

A second batch of p-anisidine hydrochloride was purchased about two years later from the same supplier. The experimentally determined range in the melting point of 180° to 222°C suggested that this compound contained impurities; however, thin-layer chromatography

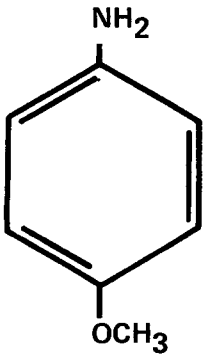



FIGURE 1
CHEMICAL STRUCTURE OF p-ANISIDINE (HYDROCHLORIDE)

utilizing the same solvent systems used previously only revealed one spot. In addition, elemental analysis agreed with that expected on a theoretical basis as did amine group titration. Vapor-phase chromatography also revealed only one homogeneous peak and infrared analysis was consistent with the structure of the compound.

The $\lambda_{max}$ and $\epsilon$ values reported by Sadtler Research Laboratories (Genero, 1977) for p-anisidine and p-anisidine hydrochloride and the values reported by Midwest Research Institute for the two batches of the compound purchased for this bioassay are indicated below. All analyses were performed using the same solvent systems:

| Sadtler p-anisidine | | Sadtler p-anisidine HCl | | Midwest Batch 1 | | Midwest Batch 2 | |
|---|---|---|---|---|---|---|---|
| $\lambda_{max}$ | $\epsilon$ | $\lambda_{max}$ | $\epsilon$ | $\lambda_{max}$ | $\epsilon$ | $\lambda_{max}$ | $\epsilon$ |
| --- | --- | 222.5 | 9570 | --- | --- | 223 | 8000 |
| 234 | 15573 | --- | --- | --- | --- | 236 | 30 |
| --- | --- | 274.5 | 1630 | 274 | 1570 | 275 | 1570 |
| --- | --- | 281 | 1400 | 281 | 1410 | 281 | 1390 |
| 299 | 4763 | --- | --- | 299.5 | 314 | 300 | 3100 |

The absence of the 222.5 nm peak from batch 1 is difficult to explain. The 236 and 300 nm peaks in batch 2 and the 299.5 nm peak in batch 1 suggest the presence of the free base in addition to the hydrochloride; however, the absence of a peak approximating 234 nm in batch 1 is anomalous with this suggestion. The noted discrepancies indicate that both batches may have contained impurities; however, no quantitative estimation of purity was made.

Throughout this report the term p-anisidine HCl is used to represent these materials.

B. Dietary Preparation

The basal laboratory diet for both treated and control animals was Wayne Lab-Blox® (Allied Mills, Inc., Chicago, Illinois). p-Anisidine HCl was administered to the treated animals as a component of the diet. The chemical was removed from its container and proper amounts were ground with a mortar and pestle and then mixed with an aliquot of the ground feed. Once visual homogeneity was attained, the mixture was placed in a 6 kg capacity Patterson-Kelley twin-shell stainless steel V-blender with the remainder of the meal. After 20 minutes of blending, the mixtures were placed in double plastic bags and stored in the dark at 4°C. Mixtures were prepared once weekly and the unused portions discarded 2 weeks after formulation.

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. Rats and mice were supplied by the Frederick Cancer Research Center, Frederick, Maryland. Treated 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. The remaining animals were quarantined by species for 2 weeks prior to initiation of test. The animals were assigned to groups and

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

D. Animal Maintenance

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

Rats were housed five per cage by sex. During quarantine and for the first 11 months of study rats were kept in galvanized-steel wire-mesh cages (Fenco Cage Products, Boston, Massachusetts) suspended over newspapers. Newspapers were replaced daily, and cages and racks washed weekly. For the remainder of the study, rats were housed in suspended polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) equipped with disposable nonwoven fiber filter sheets. Clean bedding and cages were provided twice weekly. Animals in polycarbonate cages were provided with Aspen hardwood chip bedding (American Excelsior Company, Baltimore, Maryland). Stainless steel cage racks were cleaned once every 2 weeks, and disposable filters were replaced at that time.

Mice were housed five per cage by sex in polycarbonate shoe box type cages. Cages were fitted with perforated stainless steel lids (Lab Products, Inc.). Nonwoven fiber filter bonnets were used over cage lids. Clean cages, lids, and bedding (Aspen bedding) were

provided twice per week. Reusable filter bonnets and pipe racks were sanitized every 2 weeks throughout the study.

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

Wayne Lab-Blox® meal was dispensed in Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc., Woburn, Massachusetts) equipped with stainless steel baffles to rats while in wire-mesh caging, and to mice for the first 2 months of study. For the remainder of the study, meal was supplied from stainless steel gangstyle hoppers (Scientific Cages, Inc., Bryan, Texas). During the 2-year period of compound administration, animals were fed meal containing the appropriate concentrations of p-anisidine HCl. Control animals had untreated meal available. Food hoppers were changed on the same schedule as were cages. Food was replenished daily in Alpine® feed cups.

p-Anisidine HCl-dosed rats were housed in a room with other rats receiving diets containing* 1,5-naphthalenediamine (2243-62-1); N-(1-naphthyl) ethylenediamine dihydrochloride (1465-25-4); 2-chloro-p-phenylenediamine sulfate (61702-44-1); and aniline hydrochloride (142-04-1). Control rats were in a room with other rats receiving

*CAS registry numbers are given in parentheses.

diets containing tris (2,3-dibromopropyl) phosphate (126-72-7) and o-anisidine hydrochloride (134-29-0).

All mice were in a room with other mice receiving diets containing o-anisidine hydrochloride (134-29-0); 4-chloro-o-phenylenediamine (95-83-0); cupferron (134-20-6); 2,5-dithiobiurea (142-46-1); and fenaminosulf (140-56-7).

E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of p-anisidine HCl for administration to treated animals in the chronic studies, subchronic toxicity studies were conducted with both rats and mice. Animals of each species were distributed among five groups, each consisting of five males and five females. p-Anisidine HCl was incorporated into the laboratory diet and supplied ad libitum to four of the five rat groups and four of the five mouse groups in concentrations of 0.1, 0.3, 1.0, and 3.0 percent. The sixth group of each species served as a control, receiving only the basal laboratory diet. The dosed dietary preparations were administered for 8 weeks.

The highest concentration causing no deaths, no compound-related gross abnormalities, and no mean group body weight depression in excess of 15 percent relative to controls during the 8-week subchronic test was selected as the high concentration utilized for the rat and mouse chronic bioassays.

One female rat receiving a concentration of 0.1 percent and all rats receiving concentrations of 3.0 percent died. Rats tested

at 1.0 percent were reported to have deep purple to black spleens in all cases. All rats receiving 0.3 percent appeared normal. A dietary concentration of 0.3 percent produced mean body weight depressions of 6.0 and 1.0 percent in male and female rats, respectively. A dietary concentration of 1.0 percent produced mean body weight depressions of 21.0 and 13.0 percent in male and female rats, respectively. The initial high concentration chosen for administration to rats in the chronic study was 0.6 percent.

One female mouse died at a concentration of 3.0 percent. Black spleens were noted in all mice receiving 3.0 percent. A dietary concentration of 1.0 percent produced mean body weight depressions of 13.0 and 5.0 percent in male and female mice, respectively. A dietary concentration of 3.0 percent produced mean body weight depressions of 38.0 and 29.0 percent in male and female mice, respectively. The initial high concentration utilized for administration to mice in the chronic study was 1.0 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.

All rats were approximately 6 weeks old at the time the test was initiated. The initial concentrations of p-anisidine HCl in diets were 0.6 and 0.3 percent, respectively. Throughout this report the

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
p-ANISIDINE HYDROCHLORIDE FEEDING EXPERIMENT

| | INITIAL GROUP SIZE | p-ANISIDINE HYDROCHLORIDE CONCENTRATION (PERCENT) | OBSERVATION PERIOD TREATED (WEEKS) | OBSERVATION PERIOD UNTREATED (WEEKS) |
|---|---|---|---|---|
| MALE | | | | |
| CONTROL | 55 | 0 | 0 | 106 |
| LOW DOSE | 55 | 0.3<br>0 | 103 | 2 |
| HIGH DOSE | 55 | 0.6<br>0 | 103 | 3 |
| FEMALE | | | | |
| CONTROL | 55 | 0 | 0 | 107 |
| LOW DOSE | 55 | 0.3<br>0 | 103 | 3 |
| HIGH DOSE | 55 | 0.6<br>0 | 103 | 3 |

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
p-ANISIDINE HYDROCHLORIDE FEEDING EXPERIMENT

| | INITIAL GROUP SIZE | p-ANISIDINE HYDROCHLORIDE CONCENTRATION (PERCENT) | OBSERVATION PERIOD | |
|---|---|---|---|---|
| | | | TREATED (WEEKS) | UNTREATED (WEEKS) |
| MALE | | | | |
| CONTROL | 55 | 0 | 0 | 105 |
| LOW DOSE | 55 | 0.5 | 103 | |
| | | 0 | | 2 |
| HIGH DOSE | 55 | 1.0 | 103 | |
| | | 0 | | 2 |
| FEMALE | | | | |
| CONTROL | 55 | 0 | 0 | 105 |
| LOW DOSE | 55 | 0.5 | 103 | |
| | | 0 | | 2 |
| HIGH DOSE | 55 | 1.0 | 103 | |
| | | 0 | | 2 |

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 treated rats were supplied with dosed feed for a total of 103 weeks, followed by a 2- to 3-week observation period.

All mice were approximately 6 weeks old at the time the test was initiated. The initial concentrations of p-anisidine HCl in diets were 1.0 and 0.5 percent, respectively. Throughout this report the 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 treated mice were supplied with dosed feed for a total of 103 weeks, followed by a 2-week observation period to detect any delayed toxicity.

G. Clinical and Histopathologic Examinations

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

A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was sacrificed at the end of the

bioassay. The animals were euthanized by carbon dioxide inhalation, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

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

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

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

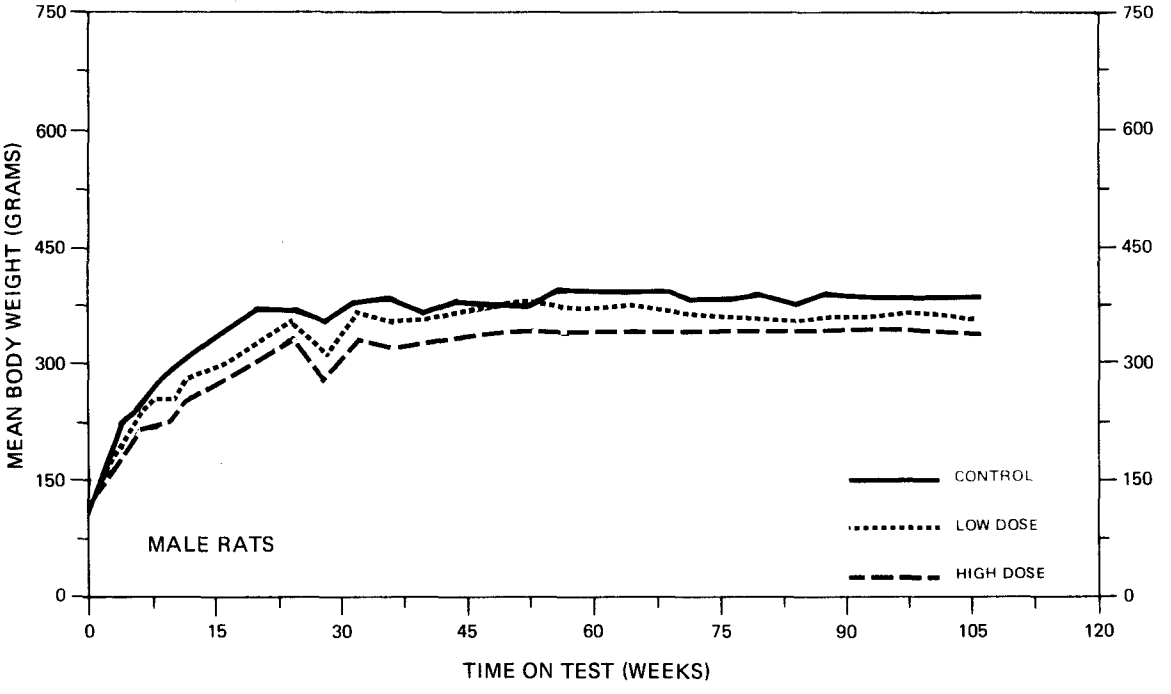
## III. CHRONIC TESTING RESULTS: RATS

### A. Body Weights and Clinical Observations

Mean body weight depression was apparent in all treated rat groups when compared to their control groups. Mean body weight depression in female high and low dose groups was more apparent after week 34 (Figure 2). White or yellow discoloration of the eye was recorded for 1 high dose male, 2 high dose females, 2 low dose males, 2 low dose females, 2 control males, and 1 control female. Red exudate around the eyes developed in 10 high dose females, 15 low dose males, and 17 low dose females. Swelling was observed in the eye region of 2 control females, the head of 2 control males, and the scrotum of 1 control male. Subcutaneous masses developed in 1 high dose male, 5 high dose females, 3 low dose males, 6 low dose females, 3 control males, and 18 control females. Cutaneous lesions and/or masses were recorded in 3 high dose males, 2 high dose females, 8 low dose males, 1 low dose female, 7 control males, and 3 control females. Two controls showed rectal prolapse. Jaundice was recorded for 1 control male. Emaciation was observed in 1 low dose female and 2 control females. Alopecia was reported in 30 high dose males, 14 low dose females, and 9 control females. No other clinical abnormalities were noted.

### B. Survival

The estimated probabilities of survival for male and female rats in the control and p-anisidine HCl-dosed groups are shown in Figure 3.



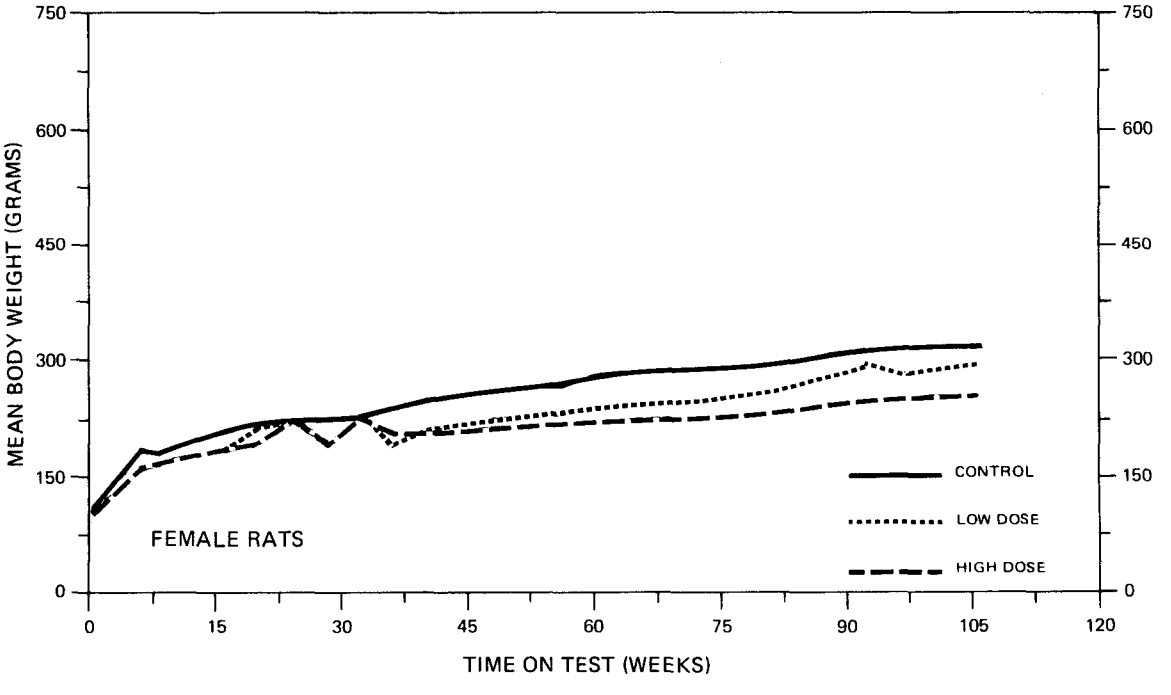
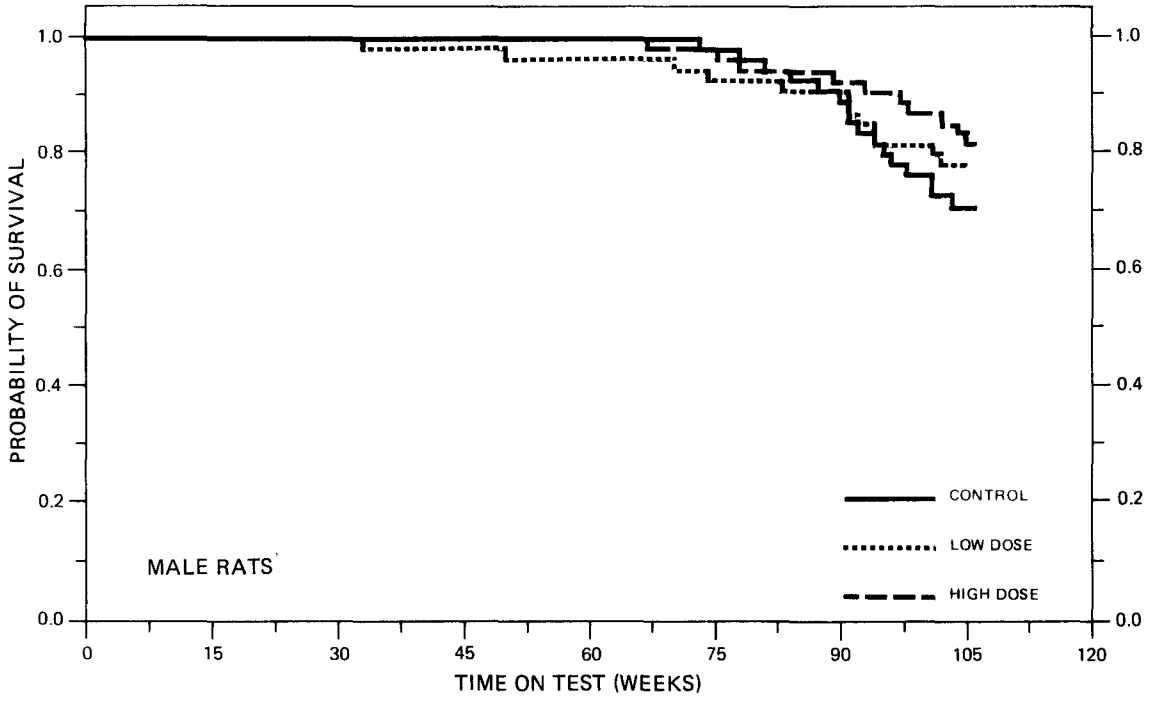




**FIGURE 2**
**GROWTH CURVES FOR p-ANISIDINE HYDROCHLORIDE CHRONIC STUDY RATS**



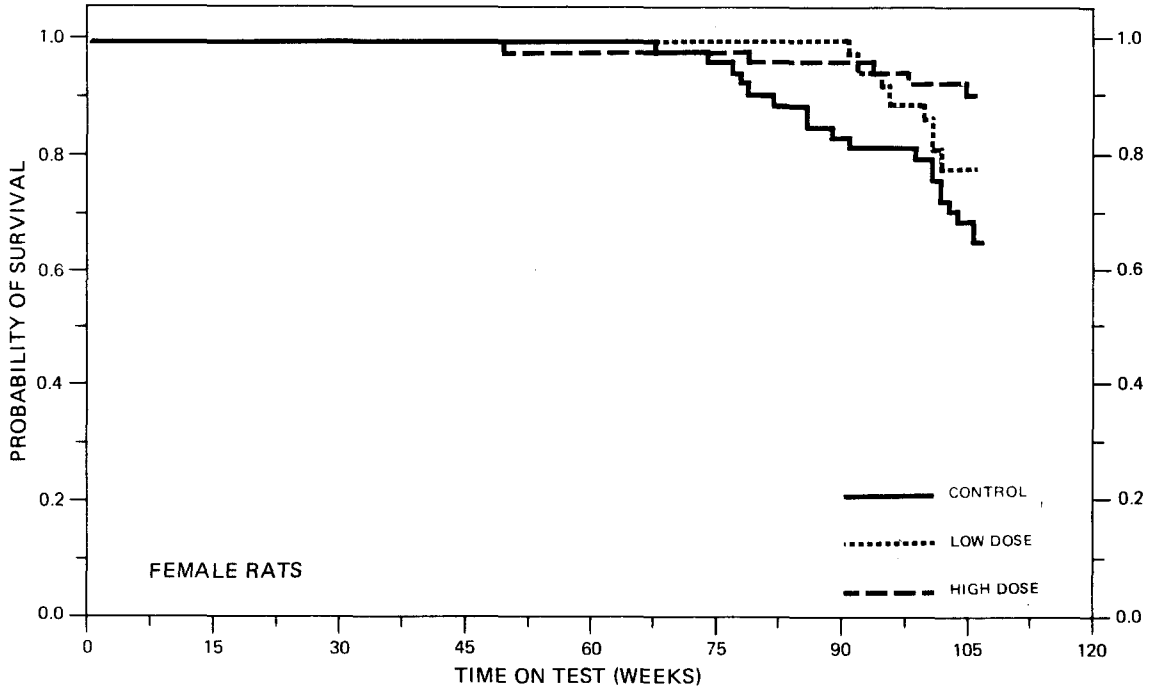




**FIGURE 3**
**SURVIVAL COMPARISONS OF p-ANISIDINE HYDROCHLORIDE CHRONIC STUDY RATS**

For both male and female rats, the Tarone test for association between dosage and mortality was not significant.

For males, adequate numbers of rats were at risk from late-developing tumors, as 82 percent (45/55) of the high dose, 78 percent (43/55) of the low dose, and 71 percent (39/55) of the control rats survived on test until the termination of the study.

For females, with 91 percent (50/55) of the high dose, 78 percent (43/55) of the low dose, and 65 percent (36/55) of the control rats alive on test until the termination of the study, survival was also adequate.

C. Pathology

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

A variety of neoplasms occurred both in the control and in the compound-treated groups. A few neoplasms occurred only in treated rats, but their numbers were too small to demonstrate convincing carcinogenicity. These included three transitional-cell neoplasms of the urinary bladder in both sexes, two neoplasms of intestinal smooth muscle in males, two gliomas of the brain in females, and preputial gland tumors (i.e., adenomas or carcinomas) in males (1/54, 8/54, and 3/55 of the control, low dose, and high dose groups, respectively).

In addition to the neoplastic lesions, a number of degenerative and inflammatory changes were found in both treated and control rats.

The only nonneoplastic lesions which appeared to be compound-related occurred in high dose females. These rats exhibited a high incidence of brown pigmentation in the reticuloendothelial cells of the spleen and in the tubular epithelium of the kidney; these changes were diagnosed as hemosiderosis and cholemic nephrosis, respectively.

Based upon this histopathologic examination, p-anisidine HCl was not carcinogenic in Fischer 344 rats under the conditions of this bioassay; however, the increase in preputial gland tumors may have been associated with the administration of the compound.

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

In male rats the incidences of squamous-cell carcinomas of the skin and of alveolar/bronchiolar adenomas were increased in the high dose treated group. In both cases the Cochran-Armitage test for association between compound administration and tumor incidence yielded a significant value ($P = 0.039$). These results, however, were not supported by significant Fisher exact tests.

For male rats the combined incidence of adenomas NOS or carcinomas NOS of the preputial gland was increased in both treated groups.

TABLE 3

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

| TOPOGRAPHY:MORPHOLOGY | CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| Skin and Subcutaneous Tissue: Fibroma[b] | 4/54(0.07) | 0/54(0.00) | 2/55(0.04) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.000 | 0.491 |
| Lower Limit | --- | 0.000 | 0.046 |
| Upper Limit | --- | 1.081 | 3.272 |
| Weeks to First Observed Tumor | 106 | --- | 106 |
| Skin: Squamous-Cell Carcinoma[b] | 0/54(0.00) | 0/54(0.00) | 3/55(0.05) |
| P Values[c] | P = 0.039 | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | --- | Infinite |
| Lower Limit | --- | --- | 0.589 |
| Upper Limit | --- | --- | Infinite |
| Weeks to First Observed Tumor | --- | --- | 97 |
| Lung: Alveolar/Bronchiolar Adenoma[b] | 0/54(0.00) | 0/54(0.00) | 3/55(0.05) |
| P Values[c] | P = 0.039 | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | --- | Infinite |
| Lower Limit | --- | --- | 0.589 |
| Upper Limit | --- | --- | Infinite |
| Weeks to First Observed Tumor | --- | --- | 106 |

TABLE 3 (CONTINUED)

| TOPOGRAPHY:MORPHOLOGY | CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| Hematopoietic System: Leukemia or Malignant Lymphoma[b] | 18/54(0.33) | 1/54(0.02) | 1/55(0.02) |
| P Values[c] | P < 0.001(N) | P < 0.001(N) | P < 0.001(N) |
| Departure from Linear Trend[e] | P = 0.004 | --- | --- |
| Relative Risk (Control)[d] | --- | 0.056 | 0.055 |
| Lower Limit | --- | 0.001 | 0.001 |
| Upper Limit | --- | 0.330 | 0.324 |
| Weeks to First Observed Tumor | 84 | 92 | 105 |
| Liver: Neoplastic Nodule or Hepatocellular Carcinoma[b] | 0/54(0.00) | 3/54(0.06) | 4/55(0.07) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | Infinite | Infinite |
| Lower Limit | --- | 0.600 | 0.908 |
| Upper Limit | --- | Infinite | Infinite |
| Weeks to First Observed Tumor | --- | 70 | 106 |
| Pituitary: Adenoma NOS, Chromophobe Adenoma, Acidophil Adenoma, or Basophil Adenoma[b] | 5/48(0.10) | 7/49(0.14) | 8/50(0.16) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 1.371 | 1.536 |
| Lower Limit | --- | 0.403 | 0.479 |
| Upper Limit | --- | 5.119 | 5.571 |
| Weeks to First Observed Tumor | 90 | 105 | 98 |

TABLE 3 (CONTINUED)

| TOPOGRAPHY:MORPHOLOGY | CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant[b] | 14/54(0.26) | 10/54(0.19) | 6/54(0.11) |
| P Values[c] | P = 0.032(N) | N.S. | P = 0.041(N) |
| Relative Risk (Control)[d] | --- | 0.714 | 0.429 |
| Lower Limit | --- | 0.312 | 0.146 |
| Upper Limit | --- | 1.571 | 1.090 |
| Weeks to First Observed Tumor | 73 | 70 | 106 |
| Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b] | 3/53(0.06) | 2/49(0.04) | 4/50(0.08) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.721 | 1.413 |
| Lower Limit | --- | 0.062 | 0.251 |
| Upper Limit | --- | 6.024 | 9.211 |
| Weeks to First Observed Tumor | 106 | 105 | 106 |
| Pancreatic Islets: Islet Cell-Adenoma or Islet-Cell Carcinoma[b] | 2/53(0.04) | 4/52(0.08) | 2/51(0.04) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 2.038 | 1.039 |
| Lower Limit | --- | 0.306 | 0.078 |
| Upper Limit | --- | 21.762 | 13.862 |
| Weeks to First Observed Tumor | 106 | 105 | 106 |

TABLE 3 (CONTINUED)

| TOPOGRAPHY:MORPHOLOGY | CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| Mammary Gland: Fibroadenoma[b] | 1/54(0.02) | 3/54(0.06) | 0/55(0.00) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 3.000 | 0.000 |
| Lower Limit | --- | 0.250 | 0.000 |
| Upper Limit | --- | 154.510 | 18.349 |
| Weeks to First Observed Tumor | 106 | 105 | --- |
| Preputial Gland: Adenoma NOS or Carcinoma NOS[b] | 1/54(0.02) | 8/54(0.15) | 3/55(0.05) |
| P Values[c] | N.S. | P = 0.016 | N.S. |
| Departure from Linear Trend[e] | P = 0.011 | --- | --- |
| Relative Risk (Control)[d] | --- | 8.000 | 2.945 |
| Lower Limit | --- | 1.131 | 0.246 |
| Upper Limit | --- | 347.530 | 151.741 |
| Weeks to First Observed Tumor | 106 | 92 | 93 |
| Testis: Interstitial-Cell Tumor[b] | 53/54(0.98) | 45/54(0.83) | 47/55(0.85) |
| P Values[c] | P = 0.026(N) | P = 0.008(N) | P = 0.017(N) |
| Relative Risk (Control)[d] | --- | 0.849 | 0.871 |
| Lower Limit | --- | 0.816 | 0.837 |
| Upper Limit | --- | 0.972 | 0.991 |
| Weeks to First Observed Tumor | 73 | 83 | 89 |

TABLE 3 (CONCLUDED)

| TOPOGRAPHY:MORPHOLOGY | CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| Body Cavities: Mesothelioma NOS or Mesothelioma, Malignant[b] | 2/54(0.04) | 0/54(0.00) | 3/55(0.05) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.000 | 1.473 |
| Lower Limit | --- | 0.000 | 0.176 |
| Upper Limit | --- | 3.387 | 17.071 |
| Weeks to First Observed Tumor | 87 | --- | 98 |

[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 regative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

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

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

TABLE 4

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

| TOPOGRAPHY:MORPHOLOGY | CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| Hematopoietic System: Leukemia or Malignant Lymphoma[b] | 9/54(0.17) | 3/55(0.05) | 2/55(0.04) |
| P Values[c] | P = 0.012(N) | N.S. | P = 0.024(N) |
| Relative Risk (Control)[d] | --- | 0.327 | 0.218 |
| Lower Limit | --- | 0.060 | 0.024 |
| Upper Limit | --- | 1.230 | 0.993 |
| Weeks to First Observed Tumor | 91 | 91 | 105 |
| Salivary Gland: Adenoma NOS[b] | 3/52(0.06) | 0/53(0.00) | 0/54(0.00) |
| P Values[c] | P = 0.035(N) | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.000 | 0.000 |
| Lower Limit | --- | 0.000 | 0.000 |
| Upper Limit | --- | 1.634 | 1.604 |
| Weeks to First Observed Tumor | 107 | --- | --- |
| Liver: Neoplastic Nodule or Hepatocellular Carcinoma[b] | 1/53(0.02) | 1/55(0.02) | 3/55(0.05) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.964 | 2.891 |
| Lower Limit | --- | 0.013 | 0.241 |
| Upper Limit | --- | 74.304 | 148.956 |
| Weeks to First Observed Tumor | 107 | 105 | 106 |

TABLE 4 (CONTINUED)

| TOPOGRAPHY:MORPHOLOGY | CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| Pituitary: Carcinoma NOS or Chromophobe Carcinoma[b] | 4/48(0.08) | 2/51(0.04) | 0/54(0.00) |
| P Values[c] | P = 0.028(N) | N.S. | P = 0.046(N) |
| Relative Risk (Control)[d] | --- | 0.471 | 0.000 |
| Lower Limit | --- | 0.044 | 0.000 |
| Upper Limit | --- | 3.123 | 0.960 |
| Weeks to First Observed Tumor | 89 | 92 | --- |
| Pituitary: Adenoma NOS, Chromophobe Adenoma, Acidophil Adenoma, Basophil Adenoma, Carcinoma NOS, or Chromophobe Carcinoma[b] | 21/48(0.44) | 19/51(0.37) | 19/54(0.35) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.852 | 0.804 |
| Lower Limit | --- | 0.503 | 0.442 |
| Upper Limit | --- | 1.442 | 1.312 |
| Weeks to First Observed Tumor | 74 | 92 | 94 |
| Adrenal: Pheochromocytoma[b] | 3/53(0.06) | 2/55(0.04) | 2/54(0.04) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.642 | 0.654 |
| Lower Limit | --- | 0.055 | 0.057 |
| Upper Limit | --- | 5.387 | 5.484 |
| Weeks to First Observed Tumor | 107 | 105 | 106 |

TABLE 4 (CONTINUED)

| TOPOGRAPHY:MORPHOLOGY | CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b] | 4/49(0.08) | 5/46(0.11) | 4/55(0.07) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 1.332 | 0.891 |
| Lower Limit | --- | 0.305 | 0.175 |
| Upper Limit | --- | 6.316 | 4.544 |
| Weeks to First Observed Tumor | 107 | 105 | 106 |
| Mammary Gland: Adenoma NOS or Adenocarcinoma NOS[b] | 3/54(0.06) | 1/55(0.02) | 2/55(0.04) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.327 | 0.655 |
| Lower Limit | --- | 0.006 | 0.056 |
| Upper Limit | --- | 3.925 | 5.490 |
| Weeks to First Observed Tumor | 101 | 105 | 50 |
| Mammary Gland: Fibroadenoma[b] | 16/54(0.30) | 4/55(0.07) | 4/55(0.07) |
| P Values[c] | P = 0.001(N) | P = 0.002(N) | P = 0.002(N) |
| Relative Risk (Control)[d] | --- | 0.245 | 0.245 |
| Lower Limit | --- | 0.064 | 0.064 |
| Upper Limit | --- | 0.702 | 0.702 |
| Weeks to First Observed Tumor | 99 | 96 | 94 |

TABLE 4 (CONCLUDED)

| TOPOGRAPHY:MORPHOLOGY | CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| Mammary Gland: Fibroadenoma, Adenoma NOS, or Adenocarcinoma[b] | 19/54(0.35) | 5/55(0.09) | 6/55(0.11) |
| P Values[c] | P = 0.001(N) | P = 0.001(N) | P = 0.002(N) |
| Departure from Linear Trend[e] | P = 0.028 | --- | --- |
| Relative Risk (Control)[d] | --- | 0.258 | 0.310 |
| Lower Limit | --- | 0.082 | 0.110 |
| Upper Limit | --- | 0.655 | 0.736 |
| Weeks to First Observed Tumor | 99 | 96 | 50 |
| Uterus: Endometrial Stromal Polyp[b] | 16/52(0.31) | 11/53(0.21) | 14/55(0.25) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.675 | 0.827 |
| Lower Limit | --- | 0.315 | 0.418 |
| Upper Limit | --- | 1.393 | 1.621 |
| Weeks to First Observed Tumor | 68 | 101 | 106 |

[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 $P < 0.05$.

The Fisher exact test for the low dose group showed a significant ($P = 0.016$) increase in these tumors compared to the control. In historical data collected by this laboratory for the NCI Carcinogenesis Testing Program, 3/250 (1 percent) of the untreated male rats had one of these tumors. Making the assumption of a binomial distribution with a 3/250 probability of spontaneous incidence, the probability of observing 8 or more rats with such tumors out of 54 males (as in the low dose group) was $P < 0.001$, a significant result. The high dose Fisher exact comparison and the Cochran-Armitage test, however, were not significant.

A number of possible negative associations between compound administration and tumor incidence were observed. For both sexes negative associations were observed from both the Cochran-Armitage and Fisher exact tests for the combined incidence of leukemia and malignant lymphoma. For females the incidence of mammary gland fibroadenomas also showed a possible negative association with dosage. In males the apparent negative association between dosage and the incidence of interstitial-cell tumors of the testis was noted. The significance of these results were doubtful, however, due to the variability of this tumor (Cockrell and Garner, 1976).

The Cochran-Armitage test indicated significant negative associations between dose and the incidences of pituitary neoplasms and of adenomas of the salivary gland in females and of adrenal

pheochromocytomas in males. For these cases, however, the Fisher exact tests were not significant under the Bonferroni criterion.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by p-anisidine HCl that could not be established under the conditions of this test. It should also be noted that for those sites with an upper limit less than one there is a statistically significant decrease in tumor incidence in the dosed group as compared to the control.

## IV. CHRONIC TESTING RESULTS: MICE

### A. Body Weights and Clinical Observations

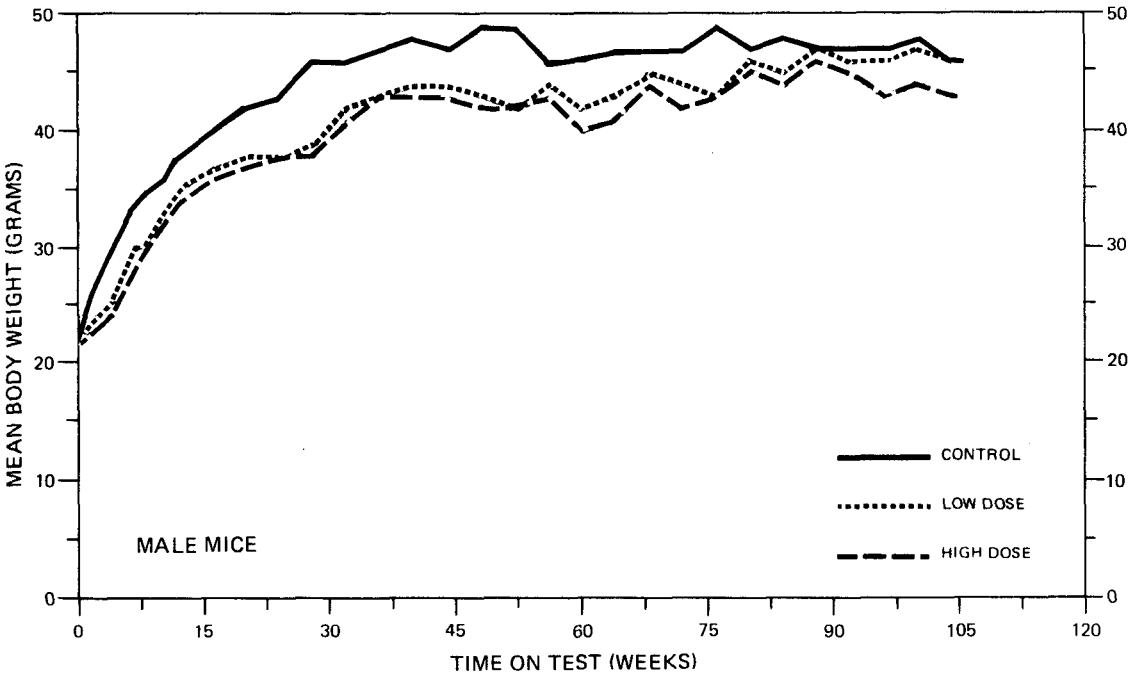
Mean body weight depression was apparent in all treated mouse groups when compared to their control groups (Figure 4). Alopecia was reported in 25 high dose males, 24 high dose females, 1 low dose male, 3 low dose females, 5 control males, and 3 control females. Cutaneous lesions were reported in 1 high dose female and 1 control male. Distention of the urogenital area was noted in 1 control male, and blood in the urogenital region was observed in 2 other control males. One control female displayed a distended abdomen. An open sore on the leg of 1 low dose female was detected. Edema of the eye region was observed in 1 high dose male and 1 high dose female. No other clinical abnormalities were observed.

### B. Survival

The estimated probabilities of survival for male and female mice in the control and p-anisidine HCl-dosed groups are shown in Figure 5. For both male and female mice, the Tarone test for association between dosage and mortality was not significant.

Adequate numbers of male mice were at risk from late-developing tumors, as 91 percent (50/55) of the high dose, 87 percent (48/55) of the low dose, and 80 percent (44/55) of the control mice survived on test until the termination of the study.

For female mice, with 78 percent (43/55) of the high dose, 76 percent (42/55) of the low dose, and 80 percent (44/55) of the control



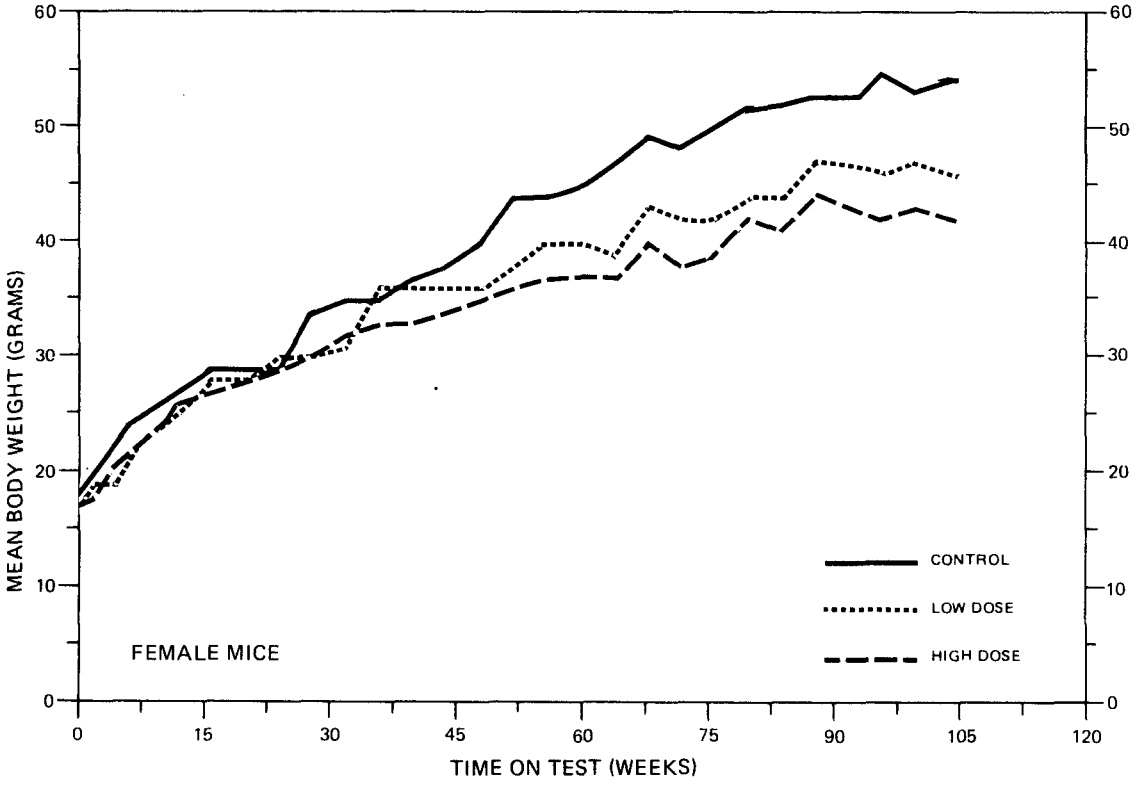
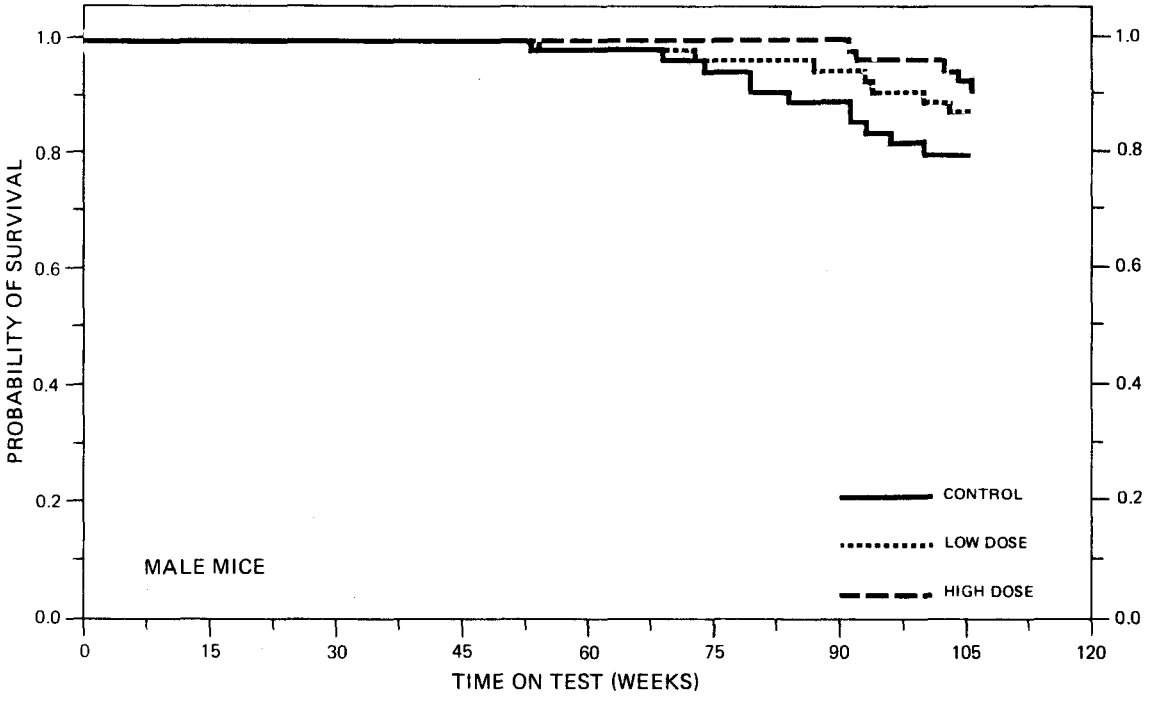




**FIGURE 4**
**GROWTH CURVES FOR p-ANISIDINE HYDROCHLORIDE CHRONIC STUDY MICE**



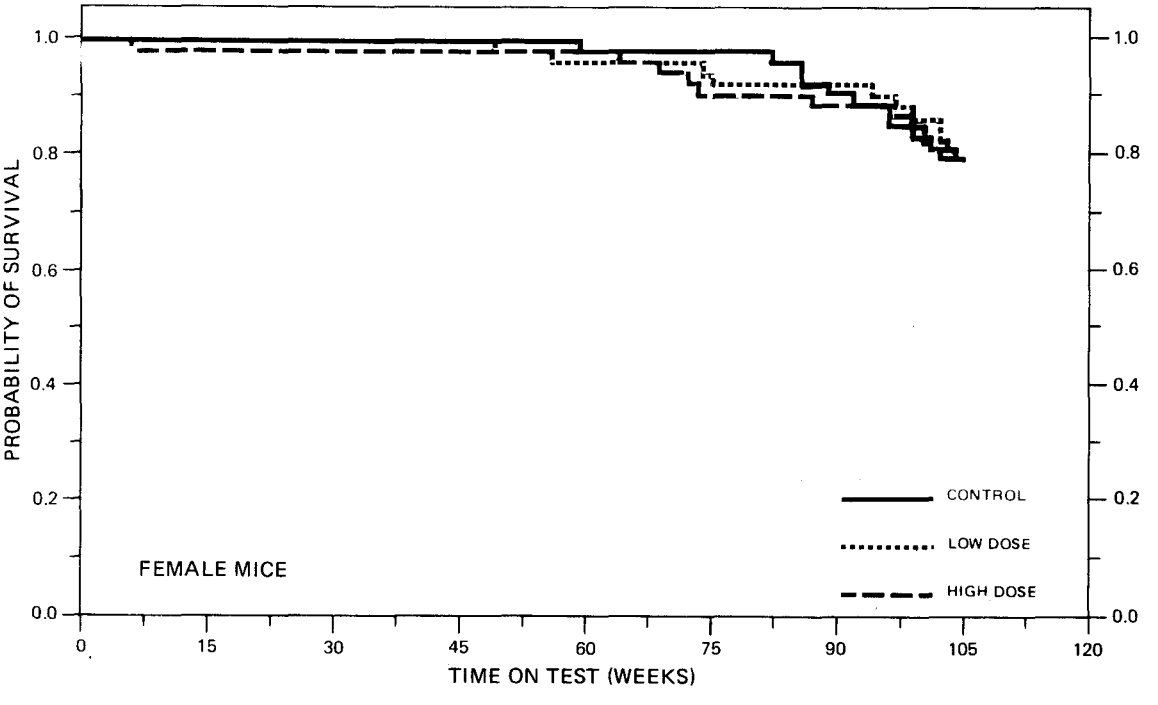




**FIGURE 5**
**SURVIVAL COMPARISONS OF p-ANISIDINE HYDROCHLORIDE CHRONIC STUDY MICE**

mice alive on test until the termination of the study, survival was also adequate.

C. Pathology

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

A variety of neoplasms occurred with approximately equal frequency in the compound-treated and control mice. Occasionally, as shown in the summary tables, neoplasms occurred only in the compound-treated mice or with an increased frequency in treated groups when compared with the controls. The nature and incidence of these neoplasms were similar to spontaneously occurring neoplasms in B6C3F1 mice.

There were no nonneoplastic lesions that could be attributed to compound administration. Degenerative, inflammatory and hyperplastic lesions, frequently observed in aging B6C3F1 mice, were noted among treated and control groups. Occasional lesions were found to be more frequent in treated mice; however, the incidences were within the limits of those observed in historical controls.

Based upon this histopathologic examination, p-anisidine HCl was not carcinogenic to B6C3F1 mice under the conditions of this bioassay.

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

TABLE 5

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

| TOPOGRAPHY:MORPHOLOGY | CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| Lung: Alveolar/Bronchiolar Carcinoma[b] | 6/54(0.11) | 3/54(0.06) | 7/54(0.13) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.500 | 1.167 |
| Lower Limit | --- | 0.085 | 0.359 |
| Upper Limit | --- | 2.211 | 3.934 |
| Weeks to First Observed Tumor | 105 | 105 | 105 |
| Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b] | 12/54(0.22) | 8/54(0.15) | 17/54(0.31) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.667 | 1.417 |
| Lower Limit | --- | 0.257 | 0.709 |
| Upper Limit | --- | 1.626 | 2.922 |
| Weeks to First Observed Tumor | 79 | 105 | 92 |
| Hematopoietic System: Leukemia or Malignant Lymphoma[b] | 4/55(0.07) | 3/54(0.06) | 4/55(0.07) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.764 | 1.000 |
| Lower Limit | --- | 0.117 | 0.196 |
| Upper Limit | --- | 4.302 | 5.110 |
| Weeks to First Observed Tumor | 105 | 94 | 105 |

TABLE 5 (CONTINUED)

| TOPOGRAPHY:MORPHOLOGY | CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| Liver: Hepatocellular Carcinoma[b] | 24/54(0.44) | 14/54(0.26) | 17/54(0.31) |
| P Values[c] | N.S. | P = 0.035(N) | N.S. |
| Relative Risk (Control)[d] | --- | 0.583 | 0.708 |
| Lower Limit | --- | 0.318 | 0.409 |
| Upper Limit | --- | 1.037 | 1.206 |
| Weeks to First Observed Tumor | 53 | 93 | 91 |
| Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma[b] | 28/54(0.52) | 22/54(0.41) | 23/54(0.43) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.786 | 0.821 |
| Lower Limit | --- | 0.501 | 0.529 |
| Upper Limit | --- | 1.226 | 1.271 |
| Weeks to First Observed Tumor | 53 | 93 | 91 |
| Adrenal: Cortical Adenoma or Adenoma NOS[b] | 6/50(0.12) | 0/52(0.00) | 0/53(0.00) |
| P Values[c] | P = 0.002(N) | P = 0.012(N) | P = 0.011(N) |
| Relative Risk (Control)[d] | --- | 0.000 | 0.000 |
| Lower Limit | --- | 0.000 | 0.000 |
| Upper Limit | --- | 0.002 | 0.591 |
| Weeks to First Observed Tumor | 105 | --- | --- |

TABLE 5 (CONCLUDED)

| TOPOGRAPHY:MORPHOLOGY | CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| Harderian Gland: Adenoma NOS, Papillary Adenoma, Cystadenoma NOS, or Papillary Cystadenoma NOS[b] | 1/55(0.02) | 2/54(0.04) | 3/55(0.05) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 2.037 | 3.000 |
| Lower Limit | --- | 0.109 | 0.250 |
| Upper Limit | --- | 117.954 | 154.535 |
| Weeks to First Observed Tumor | 105 | 105 | 105 |

[a]Treated groups received doses of 0.5 or 1.0 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 p-ANISIDINE HYDROCHLORIDE[a]

| TOPOGRAPHY:MORPHOLOGY | CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b] | 4/55(0.07) | 5/54(0.09) | 3/50(0.06) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 1.273 | 0.825 |
| Lower Limit | --- | 0.290 | 0.126 |
| Upper Limit | --- | 6.093 | 4.633 |
| Weeks to First Observed Tumor | 105 | 102 | 105 |
| Hematopoietic System: Leukemia or Malignant Lymphoma[b] | 18/55(0.33) | 10/54(0.19) | 12/50(0.24) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.566 | 0.733 |
| Lower Limit | --- | 0.258 | 0.360 |
| Upper Limit | --- | 1.167 | 1.437 |
| Weeks to First Observed Tumor | 86 | 75 | 69 |
| Liver: Hepatocellular Carcinoma[b] | 7/54(0.13) | 5/53(0.09) | 1/50(0.02) |
| P Values[c] | P = 0.033(N) | N.S. | P = 0.038(N) |
| Relative Risk (Control)[d] | --- | 0.728 | 0.154 |
| Lower Limit | --- | 0.194 | 0.003 |
| Upper Limit | --- | 2.492 | 1.138 |
| Weeks to First Observed Tumor | 101 | 105 | 105 |

TABLE 6 (CONCLUDED)

| TOPOGRAPHY:MORPHOLOGY | CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma[b] | 11/54(0.20) | 10/53(0.19) | 6/50(0.12) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.926 | 0.589 |
| Lower Limit | --- | 0.385 | 0.193 |
| Upper Limit | --- | 2.195 | 1.599 |
| Weeks to First Observed Tumor | 59 | 105 | 105 |
| Pituitary: Adenoma NOS, Chromophobe Adenoma, or Basophil Adenoma[b] | 3/42(0.07) | 3/48(0.06) | 2/38(0.05) |
| P Values[c] | N.S. | N.S. | N.S. |
| Relative Risk (Control)[d] | --- | 0.875 | 0.737 |
| Lower Limit | --- | 0.124 | 0.064 |
| Upper Limit | --- | 6.218 | 6.076 |
| Weeks to First Observed Tumor | 105 | 105 | 105 |

[a]Treated groups received doses of 0.5 or 1.0 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.

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

None of the statistical tests for any site in mice of either sex indicated a significant positive association between the administration of p-anisidine HCl and tumor incidence. Thus, at the dose levels used in this experiment there was no evidence that p-anisidine HCl was a carcinogen in B6C3F1 mice.

In male mice the possibility of a negative association between dose and the incidence of adrenal capsule adenomas NOS was observed.

For females the Cochran-Armitage test indicated a significant negative association between dose and the incidence of hepatocellular carcinomas. The Fisher exact tests, however, were not significant under the Bonferroni criterion.

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

# V. DISCUSSION

There were no significant positive associations for either species between the concentrations of p-anisidine HCl administered and mortality. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Mean body weight depression was apparent in treated animals of both species when compared to the corresponding controls, indicating that the concentrations administered may have approximated the maximum tolerated dosages.

In male rats there were significant associations between compound administration and the incidences of both squamous-cell carcinomas of the skin and alveolar/bronchiolar adenomas. None of the Fisher exact comparisons, however, supported these findings. When those males having adenomas NOS or carcinomas NOS of the preputial gland were combined and the resulting incidences statistically analyzed, the only test providing a significant result was the Fisher exact comparison of the low dose (8/54 [15 percent]) to the control (1/54 [2 percent]). Neither the Cochran-Armitage test nor the high dose to control Fisher exact test supported this finding. It was considered that insufficient evidence was provided by the study to establish a compound-related effect.

There were negative associations between compound administration and tumor incidence in rats (e.g., a combination of leukemia and

malignant lymphoma in rats of both sexes and mammary fibroadenoma in female rats).

There were no significant positive associations between the administration of p-anisidine HCl and the incidence of any tumor in mice of either sex.

Although, under the conditions of this bioassay, there appeared to be an association between chemical administration and the increased incidence of preputial gland tumors in low dose male rats, the evidence was insufficient to establish the carcinogenicity of p-anisidine HCl in Fischer 344 rats. The compound was not carcinogenic in B6C3F1 mice.

## VI. BIBLIOGRAPHY


Anthony, H.M. and G.M. Thomas, "Tumors of the Urinary Bladder: An Analysis of the Occupations of 1,030 Patients in Leeds, England." Journal of the National Cancer Institute 45:879-895, 1970.

Armitage, P., Statistical Methods in Medical Research, Chapter 14. J. Wiley & Sons, New York, 1971.

Berenblum, I., editor, Carcinogenicity Testing. International Union Against Cancer, Technical Report Series, Vol. 2. International Union Against Cancer, Geneva, 1969.

Chemical Abstracts Service. The Chemical Abstracts Service (CAS) Ninth Collective Index, Volumes 76-85, 1972-1976. American Chemical Society, Washington, D.C., 1977.

Clayson, D.B. and R.C. Garner, "Carcinogenic Aromatic Amines and Related Compounds." Chapter 8 in Carcinogenic Aromatic Amines, C.E. Searle, editor. American Chemical Society Monograph 173, Washington, D.C., 1976.

Cockrell, B.Y. and F.M. Garner, "Interstitial-Cell Tumors of the Testis in Rats." Comparative Pathology Bulletin 8:2-4, 1976.

Cox, D.R., Analysis of Binary Data, Chapters 4 and 5. Methuen and Co., Ltd., London, 1970.

Cox, D.R., "Regression Models and Life-Tables." Journal of the Royal Statistical Society, Series "B" 34:187-220, 1972.

Dornow, A., K.J. Fust, and H.D. Jordan. Berichte der Deutschen Chemischen Gesellschaft 90:2124-2137, 1957.

Gart, J.J., "The Comparison of Proportions: A Review of Significance Tests, Confidence Limits, and Adjustments for Stratification." International Statistical Institute Review 39:148-169, 1971.

Genero, A., UV consultant for Sadtler Research Laboratories, Philadelphia, Pennsylvania. Personal communication, July 1977.

Kaplan, E.L., and P. Meier, "Nonparametric Estimation from Incomplete Observations." Journal of the American Statistical Association 53:457-481, 1958.

Linhart, M.S., J.A. Cooper, R.L. Martin, N.P. Page, and J.A. Peters, "Carcinogenesis Bioassay Data System." Computers and Biomedical Research 7:230-248, 1974.

Miller, R.G., Simultaneous Statistical Inference. McGraw-Hill Book Co., New York, 1966.

Saffiotti, U., R. Montesano, A.R. Sellakumar, F. Cefis, and D.G. Kaufman, "Respiratory Tract Carcinogenesis in Hamsters Induced by Different Numbers of Administration of Benzo (a) Pyrene and Ferric Oxide." Cancer Research 32:1073-1079, 1972.

Sax, N.I., "General Chemicals." Dangerous Properties of Industrial Materials, N.I. Sax, editor. Van Nostrand Reinhold Company, New York, 1975.

Society of Dyers and Colourists, Colour Index, 2nd edition, Volume 3. Yorkshire, England, 1956.

Tarone, R.E., "Tests for Trend in Life-Table Analysis." Biometrika 62:679-682, 1975.

U.S. International Trade Commission, Synthetic Organic Chemicals, United States Production and Sales, 1975. USITC Publication 804, U.S. Government Printing Office, Washington, D.C., 1977.

Wynder, E.L., J. Onderdonk, and N. Mantel, "An Epidemiological Investigation of Cancer of the Bladder." Cancer 16:1388-1407, 1963.

# APPENDIX A

## SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH p-ANISIDINE HYDROCHLORIDE

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

| | CONTROL (UNTR) 01-0360 | LOW DOSE 01-0385 | HIGH DOSE 01-0390 |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 55 | 55 | 55 |
| ANIMALS NECROPSIED | 54 | 54 | 55 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY** | 54 | 54 | 55 |
| **INTEGUMENTARY SYSTEM** | | | |
| *SKIN | (54) | (54) | (55) |
| SQUAMOUS CELL PAPILLOMA | 2 (4%) | 1 (2%) | |
| SQUAMOUS CELL CARCINOMA | | | 3 (5%) |
| FIBROMA | 2 (4%) | | |
| *SUBCUT TISSUE | (54) | (54) | (55) |
| FIBROMA | 2 (4%) | | 2 (4%) |
| FIBROSARCOMA | | 2 (4%) | |
| **RESPIRATORY SYSTEM** | | | |
| #LUNG | (54) | (54) | (55) |
| HEPATOCELLULAR CARCINOMA, METAST | | | 1 (2%) |
| ALVEOLAR/BRONCHIOLAR ADENOMA | | | 3 (5%) |
| PHEOCHROMOCYTOMA, METASTATIC | | 1 (2%) | |
| SARCOMA, NOS, UNC PRIM OR META | | 1 (2%) | |
| FIBROSARCOMA, METASTATIC | | 1 (2%) | |
| **HEMATOPOIETIC SYSTEM** | | | |
| *MULTIPLE ORGANS | (54) | (54) | (55) |
| MALIGNANT LYMPHOMA, NOS | 1 (2%) | | |
| UNDIFFERENTIATED LEUKEMIA | 13 (24%) | | |
| MYELOMONOCYTIC LEUKEMIA | | | 1 (2%) |
| LYMPHOCYTIC LEUKEMIA | 4 (7%) | | |
| #SPLEEN | (54) | (54) | (55) |
| MALIG.LYMPHOMA, HISTIOCYTIC TYPE | | 1 (2%) | |
| #MANDIBULAR L. NODE | (53) | (51) | (49) |
| SQUAMOUS CELL CARCINOMA, METASTA | | | 1 (2%) |

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

TABLE A1 (CONTINUED)

| | CONTROL (UNTR) 01-0360 | LOW DOSE 01-0385 | HIGH DOSE 01-0390 |
|---|---|---|---|
| #THYMUS | (47) | (42) | (44) |
| THYMOMA | | 2 (5%) | |
| CIRCULATORY SYSTEM | | | |
| NONE | | | |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (54) | (54) | (55) |
| NEOPLASTIC NODULE | | 3 (6%) | 3 (5%) |
| HEPATOCELLULAR CARCINOMA | | | 1 (2%) |
| HEMANGIOMA | | 1 (2%) | |
| #STOMACH | (53) | (54) | (55) |
| SQUAMOUS CELL PAPILLOMA | | 2 (4%) | |
| SQUAMOUS CELL CARCINOMA | 2 (4%) | | |
| BASAL-CELL CARCINOMA | | 1 (2%) | 1 (2%) |
| #JEJUNUM | (52) | (53) | (55) |
| LEIOMYOMA | | 1 (2%) | |
| #ILEUM | (52) | (53) | (55) |
| LEIOMYOSARCOMA | | 1 (2%) | |
| URINARY SYSTEM | | | |
| #KIDNEY | (53) | (54) | (55) |
| HAMARTOMA + | | 1 (2%) | |
| #URINARY BLADDER | (51) | (52) | (55) |
| TRANSITIONAL-CELL PAPILLOMA | | | 1 (2%) |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (48) | (49) | (50) |
| ADENOMA, NOS | | 1 (2%) | |
| CHROMOPHOBE ADENOMA | 4 (8%) | 6 (12%) | 3 (6%) |
| ACIDOPHIL ADENOMA | 1 (2%) | | |
| BASOPHIL ADENOMA | | | 5 (10%) |

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

+ THIS IS CONSIDERED TO BE A BENIGN FORM OF THE MALIGNANT MIXED TUMOR OF THE KIDNEY AND CONSISTS OF PROLIFERATIVE LIPOCYTES, TUBULAR STRUCTURES, FIBROBLASTS, AND VASCULAR SPACES IN VARYING PROPORTIONS.

TABLE A1 (CONTINUED)

| | CONTROL (UNTR) 01-0360 | LOW DOSE 01-0385 | HIGH DOSE 01-0390 |
|---|---|---|---|
| BASOPHIL CARCINOMA | | | 1 (2%) |
| #ADRENAL | (54) | (54) | (54) |
| CORTICAL ADENOMA | 1 (2%) | 1 (2%) | 2 (4%) |
| PHEOCHROMOCYTOMA | 12 (22%) | 4 (7%) | 6 (11%) |
| PHEOCHROMOCYTOMA, MALIGNANT | 2 (4%) | 6 (11%) | |
| #THYROID | (53) | (49) | (50) |
| FOLLICULAR-CELL ADENOMA | | | 1 (2%) |
| FOLLICULAR-CELL CARCINOMA | | 1 (2%) | |
| C-CELL ADENOMA | 3 (6%) | 1 (2%) | 2 (4%) |
| C-CELL CARCINOMA | | 1 (2%) | 2 (4%) |
| PAPILLARY CYSTADENOMA, NOS | | 1 (2%) | |
| #PANCREATIC ISLETS | (53) | (52) | (51) |
| ISLET-CELL ADENOMA | 1 (2%) | 3 (6%) | 2 (4%) |
| ISLET-CELL CARCINOMA | 1 (2%) | 1 (2%) | |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (54) | (54) | (55) |
| INTRADUCTAL PAPILLOMA | 1 (2%) | | |
| FIBROADENOMA | 1 (2%) | 3 (6%) | |
| *PREPUTIAL GLAND | (54) | (54) | (55) |
| CARCINOMA,NOS | 1 (2%) | 6 (11%) | 1 (2%) |
| ADENOMA, NOS | | 2 (4%) | 2 (4%) |
| #PROSTATE | (52) | (50) | (53) |
| ADENOMA, NOS | | 1 (2%) | |
| #TESTIS | (54) | (54) | (55) |
| INTERSTITIAL-CELL TUMOR | 53 (98%) | 45 (83%) | 47 (85%) |
| HEMANGIOMA | | | 1 (2%) |
| NERVOUS SYSTEM | | | |
| #BRAIN | (54) | (53) | (55) |
| CERUMINOUS CARCINOMA, METASTATIC | 1 (2%) | | |
| SPECIAL SENSE ORGANS | | | |
| *EYE | (54) | (54) | (55) |
| SQUAMOUS CELL CARCINOMA | 1 (2%) | | |

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

TABLE A1 (CONTINUED)

| | CONTROL (UNTR) 01-0360 | LOW DOSE 01-0385 | HIGH DOSE 01-0390 |
|---|---|---|---|
| *EAR | (54) | (54) | (55) |
| CERUMINOUS CARCINOMA | 1 (2%) | | |
| *EAR CANAL | (54) | (54) | (55) |
| CERUMINOUS CARCINOMA | 1 (2%) | | |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| *BODY CAVITIES | (54) | (54) | (55) |
| MESOTHELIOMA, NOS | 2 (4%) | | 1 (2%) |
| MESOTHELIOMA, MALIGNANT | | | 2 (4%) |
| ALL OTHER SYSTEMS | | | |
| NONE | | | |
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 55 | 55 | 55 |
| NATURAL DEATH@ | 6 | 6 | 6 |
| MORIBUND SACRIFICE | 10 | 6 | 4 |
| SCHEDULED SACRIFICE | | | |
| ACCIDENTALLY KILLED | | | |
| TERMINAL SACRIFICE | 39 | 43 | 45 |
| ANIMAL MISSING | | | |

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

| | CONTROL (UNTR) 01-0360 | LOW DOSE 01-0385 | HIGH DOSE 01-0390 |
|---|---|---|---|
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 54 | 50 | 50 |
| TOTAL PRIMARY TUMORS | 112 | 100 | 93 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 53 | 49 | 49 |
| TOTAL BENIGN TUMORS | 83 | 76 | 77 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 26 | 18 | 11 |
| TOTAL MALIGNANT TUMORS | 27 | 20 | 12 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | 1 | 2 | 2 |
| TOTAL SECONDARY TUMORS | 1 | 2 | 2 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT | 2 | 3 | 4 |
| TOTAL UNCERTAIN TUMORS | 2 | 3 | 4 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC | | 1 | |
| TOTAL UNCERTAIN TUMORS | | 1 | |

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

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

| | CONTROL (UNTR) 02-0360 | LOW DOSE 02-0385 | HIGH DOSE 02-0390 |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 55 | 55 | 55 |
| ANIMALS NECROPSIED | 54 | 55 | 55 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY ** | 54 | 55 | 55 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (54) | (55) | (55) |
| SQUAMOUS CELL PAPILLOMA | 2 (4%) | | |
| SQUAMOUS CELL CARCINOMA | 2 (4%) | 2 (4%) | |
| *SUBCUT TISSUE | (54) | (55) | (55) |
| FIBROMA | 1 (2%) | | |
| LIPOMA | | | 1 (2%) |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (53) | (55) | (55) |
| ALVEOLAR/BRONCHIOLAR ADENOMA | 1 (2%) | 1 (2%) | 1 (2%) |
| OSTEOSARCOMA | | | 1 (2%) |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (54) | (55) | (55) |
| MALIG.LYMPHOMA, HISTIOCYTIC TYPE | | 1 (2%) | |
| UNDIFFERENTIATED LEUKEMIA | 8 (15%) | | |
| MYELOMONOCYTIC LEUKEMIA | | 1 (2%) | 2 (4%) |
| LYMPHOCYTIC LEUKEMIA | 1 (2%) | 1 (2%) | |
| #SPLEEN | (52) | (54) | (55) |
| NEUROFIBROSARCOMA, UNC PRIM OR M | 1 (2%) | | |
| #MEDIASTINAL L.NODE | (51) | (51) | (54) |
| UNDIFFERENTIATED CARCINOMA METAS | 1 (2%) | | |
| #MESENTERIC L. NODE | (51) | (51) | (54) |
| UNDIFFERENTIATED CARCINOMA METAS | 1 (2%) | | |

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

TABLE A2 (CONTINUED)

| | CONTROL (UNTR) 02-0360 | LOW DOSE 02-0385 | HIGH DOSE 02-0390 |
|---|---|---|---|
| **CIRCULATORY SYSTEM** | | | |
| #HEART | (53) | (54) | (55) |
| NEUROFIBROSARCOMA | | | 1 (2%) |
| NEUROFIBROSARCOMA, UNC PRIM OR M | 1 (2%) | | |
| **DIGESTIVE SYSTEM** | | | |
| #SALIVARY GLAND | (52) | (53) | (54) |
| ADENOMA, NOS | 3 (6%) | | |
| #LIVER | (53) | (55) | (55) |
| NEOPLASTIC NODULE | 1 (2%) | 1 (2%) | 2 (4%) |
| HEPATOCELLULAR CARCINOMA | | | 1 (2%) |
| NEUROFIBROSARCOMA, UNC PRIM OR M | 1 (2%) | | |
| #STOMACH | (51) | (55) | (55) |
| SQUAMOUS CELL PAPILLOMA | 1 (2%) | 1 (2%) | |
| SQUAMOUS CELL CARCINOMA | 1 (2%) | | |
| ADENOCARCINOMA, NOS | 1 (2%) | | |
| **URINARY SYSTEM** | | | |
| #URINARY BLADDER | (49) | (51) | (54) |
| TRANSITIONAL-CELL CARCINOMA | | 2 (4%) | |
| **ENDOCRINE SYSTEM** | | | |
| #PITUITARY | (48) | (51) | (54) |
| CARCINOMA,NOS | 3 (6%) | | |
| ADENOMA, NOS | 1 (2%) | | |
| CHROMOPHOBE ADENOMA | 15 (31%) | 17 (33%) | 18 (33%) |
| CHROMOPHOBE CARCINOMA | 1 (2%) | 2 (4%) | |
| ACIDOPHIL ADENOMA | 1 (2%) | | |
| BASOPHIL ADENOMA | | | 1 (2%) |
| #ADRENAL | (53) | (55) | (54) |
| CORTICAL ADENOMA | 1 (2%) | 2 (4%) | |
| PHEOCHROMOCYTOMA | 3 (6%) | 2 (4%) | 2 (4%) |
| ANGIOLIPOMA | 1 (2%) | | |
| #THYROID | (49) | (46) | (55) |
| UNDIFFERENTIATED CARCINOMA | 1 (2%) | | |

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

TABLE A2 (CONTINUED)

| | CONTROL (UNTR) 02-0360 | LOW DOSE 02-0385 | HIGH DOSE 02-0390 |
|---|---|---|---|
| C-CELL ADENOMA | 1 (2%) | 4 (9%) | 4 (7%) |
| C-CELL CARCINOMA | 3 (6%) | 1 (2%) | |
| #PANCREATIC ISLETS | (52) | (47) | (58) |
| ISLET-CELL ADENOMA | 1 (2%) | | |
| **REPRODUCTIVE SYSTEM** | | | |
| *MAMMARY GLAND | (54) | (55) | (55) |
| ADENOMA, NOS | 1 (2%) | 1 (2%) | 1 (2%) |
| ADENOCARCINOMA, NOS | 2 (4%) | | 1 (2%) |
| PAPILLARY ADENOCARCINOMA | | 1 (2%) | |
| INTRADUCTAL PAPILLOMA | | 1 (2%) | |
| FIBROSARCOMA | | 1 (2%) | |
| FIBROADENOMA | 16 (30%) | 4 (7%) | 4 (7%) |
| *CLITORAL GLAND | (54) | (55) | (55) |
| CARCINOMA,NOS | 2 (4%) | | |
| ADENOMA, NOS | | 1 (2%) | 1 (2%) |
| #UTERUS | (52) | (53) | (55) |
| ENDOMETRIAL STROMAL POLYP | 16 (31%) | 11 (21%) | 14 (25%) |
| ENDOMETRIAL STROMAL SARCOMA | | | 1 (2%) |
| #UTERUS/ENDOMETRIUM | (52) | (53) | (55) |
| ADENOCARCINOMA, NOS | | 2 (4%) | |
| #OVARY | (53) | (48) | (55) |
| GRANULOSA-CELL TUMOR | 1 (2%) | | |
| TUBULAR ADENOMA | 2 (4%) | 1 (2%) | |
| **NERVOUS SYSTEM** | | | |
| #BRAIN | (52) | (55) | (55) |
| CARCINOMA, NOS, METASTATIC | 2 (4%) | | |
| CHROMOPHOBE CARCINOMA, METASTATI | 1 (2%) | | |
| GLIOMA, NOS | | 2 (4%) | |
| **SPECIAL SENSE ORGANS** | | | |
| *EYE | (54) | (55) | (55) |
| SQUAMOUS CELL CARCINOMA | 2 (4%) | | |

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

TABLE A2 (CONTINUED)

| | CONTROL (UNTP) 02-0360 | LOW DOSE 02-0385 | HIGH DOSE 02-0390 |
|---|---|---|---|
| MIXED TUMOR, MALIGNANT | | 1 (2%) | |
| *EAR CANAL | (54) | (55) | (55) |
| SQUAMOUS CELL CARCINOMA | | | 1 (2%) |
| CERUMINOUS CARCINOMA | 1 (2%) | | |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| *ABDOMINAL WALL | (54) | (55) | (55) |
| HEMANGIOMA | | | 1 (2%) |
| ALL OTHER SYSTEMS | | | |
| *MULTIPLE ORGANS | (54) | (55) | (55) |
| ADENOCARCINOMA, NOS, METASTATIC | 1 (2%) | | |
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 55 | 55 | 55 |
| NATURAL DEATH@ | 6 | 5 | 1 |
| MORIBUND SACRIFICE | 13 | 7 | 4 |
| SCHEDULED SACRIFICE | | | |
| ACCIDENTALLY KILLED | | | |
| TERMINAL SACRIFICE | 36 | 43 | 50 |
| ANIMAL MISSING | | | |

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

| | CONTROL (UNTR) 02-0360 | LOW DOSE 02-0385 | HIGH DOSE 02-0390 |
|---|---|---|---|
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 52 | 38 | 42 |
| TOTAL PRIMARY TUMORS | 100 | 64 | 58 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 45 | 29 | 36 |
| TOTAL BENIGN TUMORS | 67 | 46 | 48 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 24 | 14 | 8 |
| TOTAL MALIGNANT TUMORS | 28 | 17 | 8 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | 5 | | |
| TOTAL SECONDARY TUMORS | 6 | | |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT | 2 | 1 | 2 |
| TOTAL UNCERTAIN TUMORS | 2 | 1 | 2 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC | 1 | | |
| TOTAL UNCERTAIN TUMORS | 3 | | |

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

# APPENDIX B

## SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH p-ANISIDINE HYDROCHLORIDE

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

| | CONTROL (UNTR) 05-0360 | LOW DOSE 05-0395 | HIGH DOSE 05-0400 |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 55 | 55 | 55 |
| ANIMALS MISSING | | 1 | |
| ANIMALS NECROPSIED | 55 | 54 | 55 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY** | 55 | 54 | 55 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (55) | (54) | (55) |
| SQUAMOUS CELL PAPILLOMA | 1 (2%) | | |
| *SUBCUT TISSUE | (55) | (54) | (55) |
| FIBROMA | 2 (4%) | | |
| FIBROSARCOMA | 1 (2%) | | |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (54) | (54) | (54) |
| HEPATOCELLULAR CARCINOMA, METAST | 4 (7%) | 2 (4%) | |
| ALVEOLAR/BRONCHIOLAR ADENOMA | 6 (11%) | 5 (9%) | 10 (19%) |
| ALVEOLAR/BRONCHIOLAR CARCINOMA | 6 (11%) | 3 (6%) | 7 (13%) |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (55) | (54) | (55) |
| MALIGNANT LYMPHOMA, NOS | | 1 (2%) | |
| MALIG.LYMPHOMA, UNDIFFER-TYPE | | 1 (2%) | 2 (4%) |
| MALIG.LYMPHOMA, LYMPHOCYTIC TYPE | | 1 (2%) | 2 (4%) |
| MALIG.LYMPHOMA, HISTIOCYTIC TYPE | 1 (2%) | | |
| MALIGNANT LYMPHOMA, MIXED TYPE | 2 (4%) | | |
| GRANULOCYTIC SARCOMA | | | 2 (4%) |
| #SPLEEN | (51) | (54) | (54) |
| HEMANGIOMA | 1 (2%) | | |
| #MESENTERIC L. NODE | (48) | (51) | (52) |
| HEPATOCELLULAR CARCINOMA, METAST | 1 (2%) | | |
| #JEJUNUM | (50) | (53) | (54) |
| MALIGNANT LYMPHOMA, MIXED TYPE | 1 (2%) | | |

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

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

| | CONTROL (UNTR) 05-0360 | LOW DOSE 05-0395 | HIGH DOSE 05-0400 |
|---|---|---|---|
| CIRCULATORY SYSTEM | | | |
| NONE | | | |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (54) | (54) | (54) |
| HEPATOCELLULAR ADENOMA | 4 (7%) | 8 (15%) | 7 (13%) |
| HEPATOCELLULAR CARCINOMA | 24 (44%) | 14 (26%) | 17 (31%) |
| ANGIOSARCOMA | | | 1 (2%) |
| #STOMACH | (51) | (53) | (51) |
| SQUAMOUS CELL PAPILLOMA | | 2 (4%) | |
| URINARY SYSTEM | | | |
| #KIDNEY/PELVIS | (54) | (54) | (54) |
| TRANSITIONAL-CELL PAPILLOMA | 1 (2%) | | |
| ENDOCRINE SYSTEM | | | |
| #ADRENAL | (50) | (52) | (53) |
| CORTICAL ADENOMA | 1 (2%) | | |
| #ADRENAL/CAPSULE | (50) | (52) | (53) |
| ADENOMA, NOS | 5 (10%) | | |
| #THYROID | (48) | (48) | (46) |
| FOLLICULAR-CELL ADENOMA | | 1 (2%) | |
| #PANCREATIC ISLETS | (49) | (52) | (52) |
| ISLET-CELL ADENOMA | 2 (4%) | | |
| REPRODUCTIVE SYSTEM | | | |
| NONE | | | |
| NERVOUS SYSTEM | | | |
| NONE | | | |

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

TABLE B1 (CONTINUED)

| | CONTROL (UNTR) 05-0360 | LOW DOSE 05-0395 | HIGH DOSE 05-0400 |
|---|---|---|---|
| SPECIAL SENSE ORGANS | | | |
| *HARDERIAN GLAND | (55) | (54) | (55) |
| ADENOMA, NOS | | 1 (2%) | |
| PAPILLARY ADENOMA | | | 1 (2%) |
| CYSTADENOMA, NOS | 1 (2%) | | |
| PAPILLARY CYSTADENOMA, NOS | | 1 (2%) | 2 (4%) |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| NONE | | | |
| ALL OTHER SYSTEMS | | | |
| NONE | | | |
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 55 | 55 | 55 |
| NATURAL DEATH@ | 9 | 6 | 4 |
| MORIBUND SACRIFICE | 2 | 1 | 1 |
| SCHEDULED SACRIFICE | | | |
| ACCIDENTALLY KILLED | | | |
| TERMINAL SACRIFICE | 44 | 47 | 50 |
| ANIMAL MISSING | | 1 | |

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1 (CONCLUDED)

| | CONTROL (UNTR) 05-0360 | LOW DOSE 05-0395 | HIGH DOSE 05-0400 |
|---|---|---|---|
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 43 | 29 | 36 |
| TOTAL PRIMARY TUMORS | 59 | 38 | 51 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 22 | 14 | 19 |
| TOTAL BENIGN TUMORS | 24 | 18 | 20 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 29 | 19 | 26 |
| TOTAL MALIGNANT TUMORS | 35 | 20 | 31 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | 4 | 2 | |
| TOTAL SECONDARY TUMORS | 5 | 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 p-ANISIDINE HYDROCHLORIDE

| | CONTROL (UNTR) 06-0360 | LOW DOSE 06-0395 | HIGH DOSE 06-0400 |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 55 | 55 | 55 |
| ANIMALS MISSING | | 1 | 1 |
| ANIMALS NECROPSIED | 55 | 54 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY** | 55 | 54 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SUBCUT TISSUE | (55) | (54) | (50) |
| BASAL-CELL CARCINOMA | | 2 (4%) | |
| HEMANGIOMA | 1 (2%) | | |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (55) | (54) | (50) |
| CARCINOMA, NOS, METASTATIC | | | 1 (2%) |
| HEPATOCELLULAR CARCINOMA, METAST | 2 (4%) | | |
| ALVEOLAR/BRONCHIOLAR ADENOMA | 3 (5%) | 3 (6%) | 2 (4%) |
| ALVEOLAR/BRONCHIOLAR CARCINOMA | 1 (2%) | 2 (4%) | 1 (2%) |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (55) | (54) | (50) |
| MALIGNANT LYMPHOMA, NOS | 1 (2%) | | 1 (2%) |
| MALIG.LYMPHOMA, UNDIFFER-TYPE | | 1 (2%) | |
| MALIG.LYMPHOMA, LYMPHOCYTIC TYPE | 2 (4%) | 1 (2%) | 2 (4%) |
| MALIG.LYMPHOMA, HISTIOCYTIC TYPE | 1 (2%) | 1 (2%) | 1 (2%) |
| MALIGNANT LYMPHOMA, MIXED TYPE | 6 (11%) | 2 (4%) | 4 (8%) |
| LYMPHOCYTIC LEUKEMIA | 4 (7%) | | |
| GRANULOCYTIC LEUKEMIA | | 1 (2%) | 1 (2%) |
| #SPLEEN | (53) | (53) | (49) |
| NEOPLASM, NOS | | 1 (2%) | |
| HEMANGIOSARCOMA | 2 (4%) | 1 (2%) | |
| MALIGNANT LYMPHOMA, MIXED TYPE | 1 (2%) | 1 (2%) | 1 (2%) |
| #MESENTERIC L. NODE | (47) | (49) | (40) |
| MALIG.LYMPHOMA, UNDIFFER-TYPE | | | 1 (3%) |
| MALIG.LYMPHOMA, LYMPHOCYTIC TYPE | | | 1 (3%) |

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

TABLE B2 (CONTINUED)

| | CONTROL (UNTR) 06-0360 | LOW DOSE 06-0395 | HIGH DOSE 06-0400 |
|---|---|---|---|
| MALIGNANT LYMPHOMA, MIXED TYPE | 2 (4%) | | |
| #LIVER | (54) | (53) | (50) |
| MALIGNANT LYMPHOMA, NOS | 1 (2%) | | |
| MALIG.LYMPHOMA, LYMPHOCYTIC TYPE | | 1 (2%) | |
| MALIG.LYMPHOMA, HISTIOCYTIC TYPE | | 1 (2%) | |
| #PEYERS PATCH | (52) | (52) | (49) |
| MALIG.LYMPHOMA, LYMPHOCYTIC TYPE | | 1 (2%) | |
| #THYMUS | (35) | (38) | (37) |
| THYMOMA | 1 (3%) | | |
| **CIRCULATORY SYSTEM** | | | |
| #HEART | (55) | (53) | (49) |
| HEMANGIOMA | 1 (2%) | | |
| **DIGESTIVE SYSTEM** | | | |
| #LIVER | (54) | (53) | (50) |
| HEPATOCELLULAR ADENOMA | 4 (7%) | 5 (9%) | 5 (10%) |
| HEPATOCELLULAR CARCINOMA | 7 (13%) | 5 (9%) | 1 (2%) |
| HEMANGIOMA | 1 (2%) | | |
| #STOMACH | (53) | (52) | (48) |
| SQUAMOUS CELL PAPILLOMA | 2 (4%) | | |
| #DUODENUM | (52) | (52) | (49) |
| CARCINOMA,NOS | | | 1 (2%) |
| **URINARY SYSTEM** | | | |
| NONE | | | |
| **ENDOCRINE SYSTEM** | | | |
| #PITUITARY | (42) | (48) | (38) |
| ADENOMA, NOS | | 3 (6%) | 2 (5%) |
| CHROMOPHOBE ADENOMA | 2 (5%) | | |
| BASOPHIL ADENOMA | 1 (2%) | | |

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

TABLE B2 (CONTINUED)

| | CONTROL (UNTR) 06-0360 | LOW DOSE 06-0395 | HIGH DOSE 06-0400 |
|---|---|---|---|
| #ADRENAL | (50) | (53) | (45) |
| PHEOCHROMOCYTOMA | 1 (2%) | | |
| #THYROID | (48) | (46) | (38) |
| FOLLICULAR-CELL ADENOMA | 1 (2%) | 2 (4%) | 1 (3%) |
| #PANCREATIC ISLETS | (49) | (52) | (47) |
| ISLET-CELL ADENOMA | | | 1 (2%) |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (55) | (54) | (50) |
| ACINAR-CELL CARCINOMA | 1 (2%) | | |
| FIBROADENOMA | 1 (2%) | | |
| #UTERUS | (54) | (50) | (49) |
| NEOPLASM, NOS, MALIGNANT | 1 (2%) | | |
| ENDOMETRIAL STROMAL POLYP | | 1 (2%) | 1 (2%) |
| #OVARY | (50) | (51) | (49) |
| PAPILLARY CYSTADENOMA, NOS | | | 1 (2%) |
| PAPILLARY CYSTADENOCARCINOMA,NOS | | 1 (2%) | |
| HEMANGIOSARCOMA | | 1 (2%) | |
| NERVOUS SYSTEM | | | |
| NONE | | | |
| SPECIAL SENSE ORGANS | | | |
| *HARDERIAN GLAND | (55) | (54) | (50) |
| PAPILLARY ADENOMA | | 1 (2%) | |
| PAPILLARY CYSTADENOMA, NOS | | 1 (2%) | 1 (2%) |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| *BODY CAVITIES | (55) | (54) | (50) |
| MESOTHELIOMA, NOS | 1 (2%) | | |

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

TABLE B2 (CONCLUDED)

| | CONTROL (UNTR) 06-0360 | LOW DOSE 06-0395 | HIGH DOSE 06-0400 |
|---|---|---|---|
| *ABDOMINAL CAVITY | (55) | (54) | (50) |
| GANGLIONEUROMA | | 1 (2%) | |
| ALL OTHER SYSTEMS | | | |
| *MULTIPLE ORGANS | (55) | (54) | (50) |
| NEOPLASM, NOS | | | 1 (2%) |
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 55 | 55 | 55 |
| NATURAL DEATH@ | 7 | 10 | 10 |
| MORIBUND SACRIFICE | 4 | 1 | 1 |
| SCHEDULED SACRIFICE | | | |
| ACCIDENTALLY KILLED | | 1 | |
| TERMINAL SACRIFICE | 44 | 42 | 43 |
| ANIMAL MISSING | | 1 | 1 |
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 34 | 33 | 24 |
| TOTAL PRIMARY TUMORS | 50 | 40 | 30 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 15 | 16 | 10 |
| TOTAL BENIGN TUMORS | 19 | 17 | 14 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 27 | 20 | 15 |
| TOTAL MALIGNANT TUMORS | 30 | 22 | 15 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | 2 | | 1 |
| TOTAL SECONDARY TUMORS | 2 | | 1 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT | 1 | 1 | 1 |
| TOTAL UNCERTAIN TUMORS | 1 | 1 | 1 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC | | | |
| TOTAL UNCERTAIN TUMORS | | | |

@ INCLUDES AUTOLYZED ANIMALS

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

# APPENDIX C

## SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH p-ANISIDINE HYDROCHLORIDE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
*TREATED WITH p-ANISIDINE HYDROCHLORIDE*

| | CONTROL (UNTR) 01-0360 | LOW DOSE 01-0385 | HIGH DOSE 01-0390 |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 55 | 55 | 55 |
| ANIMALS NECROPSIED | 54 | 54 | 55 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY ** | 54 | 54 | 55 |
| **INTEGUMENTARY SYSTEM** | | | |
| *SKIN | (54) | (54) | (55) |
| EPIDERMAL INCLUSION CYST | | | 2 (4%) |
| ULCER, NOS | | 1 (2%) | |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| POLYP, INFLAMMATORY | | | 1 (2%) |
| **RESPIRATORY SYSTEM** | | | |
| #LUNG/BRONCHUS | (54) | (54) | (55) |
| BRONCHIECTASIS | 1 (2%) | 4 (7%) | 11 (20%) |
| ABSCESS, NOS | | 2 (4%) | 2 (4%) |
| #LUNG | (54) | (54) | (55) |
| BRONCHOPNEUMONIA, NOS | 2 (4%) | 2 (4%) | 3 (5%) |
| ABSCESS, NOS | | 3 (6%) | 1 (2%) |
| PNEUMONIA, CHRONIC MURINE | 2 (4%) | 14 (26%) | 4 (7%) |
| GRANULOMA, FOREIGN BODY | | | 1 (2%) |
| #LUNG/ALVEOLI | (54) | (54) | (55) |
| CALCIFICATION, NOS | | 1 (2%) | |
| **HEMATOPOIETIC SYSTEM** | | | |
| #BONE MARROW | (52) | (54) | (55) |
| FIBROSIS, FOCAL | 1 (2%) | | |
| HYPERPLASIA, NOS | 7 (13%) | | |
| HYPERPLASIA, HEMATOPOIETIC | | | 1 (2%) |
| #SPLEEN | (54) | (54) | (55) |
| FIBROSIS, FOCAL | 1 (2%) | | |
| HEMOSIDEROSIS | 1 (2%) | | |

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

TABLE C1 (CONTINUED)

| | CONTROL (UNTR) 01-0360 | LOW DOSE 01-0385 | HIGH DOSE 01-0390 |
|---|---|---|---|
| ATROPHY, NOS | | | 1 (2%) |
| HEMATOPOIESIS | 1 (2%) | 1 (2%) | 2 (4%) |
| #MANDIBULAR L. NODE | (53) | (51) | (49) |
| INFLAMMATION, CHRONIC | | 1 (2%) | |
| INFLAMMATION, GRANULOMATOUS | | 1 (2%) | |
| HYPERPLASIA, NOS | | 1 (2%) | |
| #MEDIASTINAL L.NODE | (53) | (51) | (49) |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| HYPERPLASIA, NOS | | | 2 (4%) |
| #LUMBAR LYMPH NODE | (53) | (51) | (49) |
| INFLAMMATION, CHRONIC | | 1 (2%) | 1 (2%) |
| HYPERPLASIA, NOS | | 1 (2%) | 1 (2%) |
| #RENAL LYMPH NODE | (53) | (51) | (49) |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| HEMOSIDEROSIS | | 1 (2%) | 1 (2%) |
| HYPERPLASIA, NOS | | | 1 (2%) |
| #AXILLARY LYMPH NODE | (53) | (51) | (49) |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| CIRCULATORY SYSTEM | | | |
| #HEART | (54) | (53) | (55) |
| THROMBUS, MURAL | 1 (2%) | | |
| PERIARTERITIS | 1 (2%) | 2 (4%) | 1 (2%) |
| #MYOCARDIUM | (54) | (53) | (55) |
| INFLAMMATION, FOCAL | 1 (2%) | | |
| DEGENERATION, NOS | 16 (30%) | 14 (26%) | 12 (22%) |
| *CELIAC ARTERY | (54) | (54) | (55) |
| THROMBOSIS, NOS | 1 (2%) | | |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (54) | (54) | (55) |
| CONGESTION, CHRONIC PASSIVE | | | 3 (5%) |
| CHOLANGIOFIBROSIS | 9 (17%) | 4 (7%) | |
| NECROSIS, FOCAL | 1 (2%) | | 2 (4%) |

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

TABLE C1 (CONTINUED)

| | CONTROL (UNTR) 01-0360 | LOW DOSE 01-0385 | HIGH DOSE 01-0390 |
|---|---|---|---|
| NECROSIS, FAT | 1 (2%) | | |
| BASOPHILIC CYTO CHANGE | | 1 (2%) | 1 (2%) |
| CLEAR-CELL CHANGE | 1 (2%) | 2 (4%) | |
| *BILE DUCT | (54) | (54) | (55) |
| INFLAMMATION, NOS | 1 (2%) | | |
| #PANCREAS | (53) | (52) | (51) |
| DILATATION/DUCTS | 1 (2%) | | |
| PERIARTERITIS | | 1 (2%) | 1 (2%) |
| ATROPHY, FOCAL | | 1 (2%) | 2 (4%) |
| #STOMACH | (53) | (54) | (55) |
| ULCER, NOS | 2 (4%) | | |
| EROSION | 1 (2%) | 1 (2%) | |
| PERIARTERITIS | | 1 (2%) | 1 (2%) |
| HYPERPLASIA, BASAL CELL | 14 (26%) | 10 (19%) | 24 (44%) |
| #GASTRIC MUCOSA | (53) | (54) | (55) |
| CALCIFICATION, NOS | | 1 (2%) | |
| URINARY SYSTEM | | | |
| #KIDNEY | (53) | (54) | (55) |
| CYST, NOS | | 1 (2%) | 1 (2%) |
| NEPHROSIS, NOS | 26 (49%) | 33 (61%) | 20 (36%) |
| NEPHROSIS, CHOLEMIC | 2 (4%) | | |
| CALCIFICATION, FOCAL | | 1 (2%) | 2 (4%) |
| #KIDNEY/TUBULE | (53) | (54) | (55) |
| NECROSIS, NOS | 1 (2%) | | |
| REGENERATION, NOS | | 1 (2%) | |
| #KIDNEY/PELVIS | (53) | (54) | (55) |
| HYPERPLASIA, EPITHELIAL | | 1 (2%) | |
| #URINARY BLADDER | (51) | (52) | (55) |
| CALCULUS, NOS | | | 1 (2%) |
| HYPERPLASIA, EPITHELIAL | | | 1 (2%) |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (48) | (49) | (50) |
| HEMORRHAGE | 1 (2%) | | |

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

TABLE C1 (CONTINUED)

| | CONTROL (UNTR) 01-0360 | LOW DOSE 01-0385 | HIGH DOSE 01-0390 |
|---|---|---|---|
| HYPERPLASIA, FOCAL | 1 (2%) | | |
| HYPERPLASIA, BASOPHILIC | 2 (4%) | 1 (2%) | |
| #PITUITARY/BASOPHIL | (49) | (49) | (50) |
| NODULE | | 3 (6%) | 3 (6%) |
| #ADRENAL | (54) | (54) | (54) |
| CYST, NOS | 1 (2%) | | |
| HEMORRHAGE | | 1 (2%) | |
| HYPERPLASIA, NODULAR | | | 1 (2%) |
| #ADRENAL CORTEX | (54) | (54) | (54) |
| HYPERPLASIA, NOS | 1 (2%) | | |
| #ADRENAL MEDULLA | (54) | (54) | (54) |
| HYPERPLASIA, NOS | | 1 (2%) | 1 (2%) |
| #THYROID | (53) | (49) | (50) |
| HYPERPLASIA, C-CELL | | | 1 (2%) |
| #PANCREATIC ISLETS | (53) | (52) | (51) |
| HYPERPLASIA, NOS | 1 (2%) | | 2 (4%) |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (54) | (54) | (55) |
| GALACTOCELE | | | 1 (2%) |
| *PREPUTIAL GLAND | (54) | (54) | (55) |
| ABSCESS, NOS | 1 (2%) | | |
| INFLAMMATION, CHRONIC | 1 (2%) | | |
| #PROSTATE | (52) | (50) | (53) |
| INFLAMMATION, ACUTE | | | 1 (2%) |
| INFLAMMATION ACUTE AND CHRONIC | 1 (2%) | | |
| INFLAMMATION, CHRONIC | | 1 (2%) | |
| *TESTIS | (54) | (54) | (55) |
| PERIARTERITIS | | 1 (2%) | |
| ATROPHY, NOS | | 6 (11%) | 3 (5%) |
| HYPERPLASIA, INTERSTITIAL CELL | | | 1 (2%) |
| *SCROTUM | (54) | (54) | (55) |
| NECROSIS, FAT | | 1 (2%) | |

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

TABLE C1 (CONCLUDED)

| | CONTROL (UNTR) 01-0360 | LOW DOSE 01-0385 | HIGH DOSE 01-0390 |
|---|---|---|---|
| NERVOUS SYSTEM | | | |
| #CEREBRAL VENTRICLE | (54) | (53) | (55) |
| HEMORRHAGE | 1 (2%) | | |
| #BRAIN | (54) | (53) | (55) |
| HEMORRHAGE | 1 (2%) | | 2 (4%) |
| SPECIAL SENSE ORGANS | | | |
| *EYE | (54) | (54) | (55) |
| HEMORRHAGE | 1 (2%) | | |
| *EYE/LACRIMAL GLAND | (54) | (54) | (55) |
| INFLAMMATION, NOS | 1 (2%) | | |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| *ABDOMINAL CAVITY | (54) | (54) | (55) |
| NECROSIS, FAT | 9 (17%) | 6 (11%) | 6 (11%) |
| CALCIFICATION, NOS | | 1 (2%) | |
| ALL OTHER SYSTEMS | | | |
| NONE | | | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| AUTOLYSIS/NO NECROPSY | 1 | 1 | |

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

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
TREATED WITH p-ANISIDINE HYDROCHLORIDE

| | CONTROL (UNTR) 02-0360 | LOW DOSE 02-0385 | HIGH DOSE 02-0390 |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 55 | 55 | 55 |
| ANIMALS NECROPSIED | 54 | 55 | 55 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY ** | 54 | 55 | 55 |
| INTEGUMENTARY SYSTEM | | | |
| NONE | | | |
| RESPIRATORY SYSTEM | | | |
| #LUNG/BRONCHUS | (53) | (55) | (55) |
| BRONCHIECTASIS | | 2 (4%) | 1 (2%) |
| ABSCESS, NOS | | | 1 (2%) |
| #LUNG | (53) | (55) | (55) |
| BRONCHOPNEUMONIA, NOS | | 1 (2%) | |
| ABSCESS, NOS | | 1 (2%) | 1 (2%) |
| PNEUMONIA, CHRONIC MURINE | 3 (6%) | 9 (16%) | 8 (15%) |
| INFLAMMATION, FOCAL GRANULOMATOU | | | 1 (2%) |
| METAPLASIA, NOS | 1 (2%) | | 1 (2%) |
| HEMATOPOIETIC SYSTEM | | | |
| #BONE MARROW | (53) | (53) | (55) |
| HYPOPLASIA, NOS | | 1 (2%) | |
| HISTIOCYTOSIS | 1 (2%) | | 1 (2%) |
| HYPERPLASIA, HEMATOPOIETIC | | 1 (2%) | 1 (2%) |
| HYPERPLASIA, ERYTHROID | | | 1 (2%) |
| #SPLEEN | (52) | (54) | (55) |
| INFARCT, NOS | | 1 (2%) | |
| HEMOSIDEROSIS | | 1 (2%) | 41 (75%) |
| HEMATOPOIESIS | 1 (2%) | | 1 (2%) |
| #LUMBAR LYMPH NODE | (51) | (51) | (54) |
| INFLAMMATION, CHRONIC | 1 (2%) | | |
| #RENAL LYMPH NODE | (51) | (51) | (54) |
| INFLAMMATION, CHRONIC | 1 (2%) | | |

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

TABLE C2 (CONTINUED)

| | CONTROL (UNTR) 02-0360 | LOW DOSE 02-0385 | HIGH DOSE 02-0390 |
|---|---|---|---|
| #AXILLARY LYMPH NODE | (51) | (51) | (54) |
| HYPERPLASIA, NOS | | 1 (2%) | |
| **CIRCULATORY SYSTEM** | | | |
| #MYOCARDIUM | (53) | (54) | (55) |
| DEGENERATION, NOS | 6 (11%) | 3 (6%) | 4 (7%) |
| **DIGESTIVE SYSTEM** | | | |
| #LIVER | (53) | (55) | (55) |
| CONGESTION, PASSIVE | | 1 (2%) | |
| CHOLANGIOFIBROSIS | 2 (4%) | | |
| NECROSIS, FOCAL | | | 1 (2%) |
| METAMORPHOSIS FATTY | 6 (11%) | | |
| CALCIFICATION, FOCAL | | 1 (2%) | |
| BASOPHILIC CYTO CHANGE | 10 (19%) | 3 (5%) | 2 (4%) |
| FOCAL CELLULAR CHANGE | | | 1 (2%) |
| CLEAR-CELL CHANGE | | 1 (2%) | |
| HYPERPLASIA, FOCAL | | 1 (2%) | |
| *BILE DUCT | (54) | (55) | (55) |
| DILATATION, NOS | | 1 (2%) | |
| #PANCREAS | (52) | (47) | (54) |
| ATROPHY, NOS | | 1 (2%) | |
| ATROPHY, FOCAL | 1 (2%) | 4 (9%) | 3 (6%) |
| #STOMACH | (51) | (55) | (55) |
| ULCER, NOS | 1 (2%) | | |
| INFLAMMATION, ACUTE | | 1 (2%) | |
| HYPERPLASIA, BASAL CELL | 11 (22%) | 9 (16%) | 14 (25%) |
| **URINARY SYSTEM** | | | |
| #KIDNEY | (52) | (55) | (55) |
| NEPHROSIS, NOS | 2 (4%) | 2 (4%) | |
| NEPHROSIS, CHOLEMIC | 1 (2%) | | 31 (56%) |
| GLOMERULOSCLEROSIS, NOS | 1 (2%) | | |
| CALCIFICATION, FOCAL | 5 (10%) | 5 (9%) | 12 (22%) |
| #URINARY BLADDER | (49) | (51) | (54) |
| HYPERPLASIA, EPITHELIAL | | 3 (6%) | |

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

TABLE C2 (CONTINUED)

| | CONTROL (UNTR) 02-0360 | LOW DOSE 02-0385 | HIGH DOSE 02-0390 |
|---|---|---|---|
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (48) | (51) | (54) |
| CYST, NOS | | 1 (2%) | |
| HYPERPLASIA, NOS | 1 (2%) | | |
| #ADRENAL CORTEX | (53) | (55) | (54) |
| NODULE | | 1 (2%) | |
| HYPERPLASIA, NODULAR | | 4 (7%) | 2 (4%) |
| HYPERPLASIA, NOS | 1 (2%) | 1 (2%) | |
| #ADRENAL MEDULLA | (53) | (55) | (54) |
| NECROSIS, NOS | 1 (2%) | | |
| #THYROID | (49) | (46) | (55) |
| HYPERPLASIA, C-CELL | | 3 (7%) | 2 (4%) |
| #PARATHYROID | (15) | (23) | (27) |
| HYPERPLASIA, NOS | 1 (7%) | | |
| #PANCREATIC ISLETS | (52) | (47) | (54) |
| HYPERPLASIA, NOS | 1 (2%) | | |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (54) | (55) | (55) |
| GALACTOCELE | 6 (11%) | 1 (2%) | |
| #UTERUS | (52) | (53) | (55) |
| HYDROMETRA | 2 (4%) | 1 (2%) | |
| THROMBOSIS, NOS | 1 (2%) | | |
| PYOMETRA | | 2 (4%) | |
| ABSCESS, NOS | | 1 (2%) | |
| #CERVIX UTERI | (52) | (53) | (55) |
| POLYP, INFLAMMATORY | 1 (2%) | | |
| #UTERUS/ENDOMETRIUM | (52) | (53) | (55) |
| INFLAMMATION, ACUTE | | 1 (2%) | |
| HYPERPLASIA, NOS | | 1 (2%) | 1 (2%) |
| #OVARY/OVIDUCT | (52) | (53) | (55) |
| ABSCESS, NOS | 1 (2%) | 2 (4%) | |

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

TABLE C2 (CONCLUDED)

| | CONTROL (UNTR) 02-0360 | LOW DOSE 02-0385 | HIGH DOSE 02-0390 |
|---|---|---|---|
| #OVARY | (53) | (48) | (55) |
| CYST, NOS | | 2 (4%) | |
| INFLAMMATION, CHRONIC | 1 (2%) | | |
| NERVOUS SYSTEM | | | |
| #BRAIN | (52) | (55) | (55) |
| HYDROCEPHALUS, NOS | | 1 (2%) | |
| #CEREBELLUM | (52) | (55) | (55) |
| HEMORRHAGE | | | 1 (2%) |
| *SPINAL CORD | (54) | (55) | (55) |
| HEMORRHAGE | | 1 (2%) | |
| SPECIAL SENSE ORGANS | | | |
| *EYE | (54) | (55) | (55) |
| PHTHISIS BULBI | | 1 (2%) | 2 (4%) |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| *ABDOMINAL CAVITY | (54) | (55) | (55) |
| NECROSIS, FAT | 6 (11%) | 2 (4%) | 4 (7%) |
| ALL OTHER SYSTEMS | | | |
| NONE | | | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NO LESION REPORTED | | 3 | |
| AUTOLYSIS/NO NECROPSY | 1 | | |

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

# APPENDIX D

## SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH p-ANISIDINE HYDROCHLORIDE

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

| | CONTROL (UNTR) 05-0360 | LOW DOSE 05-0395 | HIGH DOSE 05-0400 |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 55 | 55 | 55 |
| ANIMALS MISSING | | 1 | |
| ANIMALS NECROPSIED | 55 | 54 | 55 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY ** | 55 | 54 | 55 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (55) | (54) | (55) |
| EPIDERMAL INCLUSION CYST | 1 (2%) | | |
| POLYP, INFLAMMATORY | 1 (2%) | | |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (54) | (54) | (54) |
| HYPEREMIA | | | 1 (2%) |
| HYPERPLASIA, ADENOMATOUS | | | 1 (2%) |
| LEUKEMOID REACTION | | | 1 (2%) |
| HEMATOPOIETIC SYSTEM | | | |
| #SPLEEN | (51) | (54) | (54) |
| HYPERPLASIA, LYMPHOID | | 2 (4%) | |
| HEMATOPOIESIS | 2 (4%) | 12 (22%) | 5 (9%) |
| #MANDIBULAR L. NODE | (48) | (51) | (52) |
| INFLAMMATION, GRANULOMATOUS | | 1 (2%) | |
| #MESENTERIC L. NODE | (48) | (51) | (52) |
| CONGESTION, NOS | 6 (13%) | | |
| INFLAMMATION, GRANULOMATOUS | | 1 (2%) | |
| HYPERPLASIA, NOS | 1 (2%) | | |
| HISTIOCYTOSIS | 1 (2%) | | 1 (2%) |
| ERYTHROCYTOSIS | | 1 (2%) | 1 (2%) |
| PLASMACYTOSIS | | 1 (2%) | |
| HYPERPLASIA, LYMPHOID | | 5 (10%) | 2 (4%) |
| HEMATOPOIESIS | | 6 (12%) | 8 (15%) |
| #RENAL LYMPH NODE | (48) | (51) | (52) |
| INFLAMMATION, NOS | | 1 (2%) | |

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

TABLE D1 (CONTINUED)

| | CONTROL (UNTR) 05-0360 | LOW DOSE 05-0395 | HIGH DOSE 05-0400 |
|---|---|---|---|
| HYPERPLASIA, LYMPHOID | | | 1 (2%) |
| #THYMUS | (39) | (34) | (27) |
| CYST, NOS | | | 1 (4%) |
| ATROPHY, NOS | | 1 (3%) | |
| CIRCULATORY SYSTEM | | | |
| NONE | | | |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (54) | (54) | (54) |
| INFLAMMATION, NECROTIZING | | | 1 (2%) |
| INFLAMMATION, ACUTE | | 1 (2%) | |
| INFLAMMATION, ACUTE/CHRONIC | | | 3 (6%) |
| INFLAMMATION, CHRONIC | | | 3 (6%) |
| INFLAMMATION, CHRONIC FOCAL | | 1 (2%) | |
| INFLAMMATION, PYOGRANULOMATOUS | | 1 (2%) | |
| NECROSIS, FOCAL | | | 1 (2%) |
| BASOPHILIC CYTO CHANGE | | | 1 (2%) |
| HYPERPLASIA, NOS | 1 (2%) | | |
| MYELOPOIESIS | | | 1 (2%) |
| #LIVER/HEPATOCYTES | (54) | (54) | (54) |
| POLYPOID HYPERPLASIA | | | 1 (2%) |
| *BILE DUCT | (55) | (54) | (55) |
| HYPERPLASIA, NOS | | 1 (2%) | 3 (5%) |
| #PANCREAS | (49) | (52) | (52) |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| PERIARTERITIS | | | 1 (2%) |
| ATROPHY, NOS | 1 (2%) | 1 (2%) | 6 (12%) |
| #STOMACH | (51) | (53) | (51) |
| INFLAMMATION, ACUTE | 1 (2%) | | |
| EROSION | | 3 (6%) | 1 (2%) |
| ATYPIA, NOS | 1 (2%) | | |
| HYPERKERATOSIS | | 3 (6%) | 3 (6%) |
| ACANTHOSIS | | 3 (6%) | 3 (6%) |
| #PEYERS PATCH | (50) | (53) | (54) |
| HYPERPLASIA, LYMPHOID | | 1 (2%) | |

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

TABLE D1 (CONTINUED)

| | CONTROL (UNTR) 05-0360 | LOW DOSE 05-0395 | HIGH DOSE 05-0400 |
|---|---|---|---|
| #COLON | (45) | (48) | (52) |
| PARASITISM | | | 1 (2%) |
| URINARY SYSTEM | | | |
| #KIDNEY | (54) | (54) | (54) |
| HYDRONEPHROSIS | 1 (2%) | | |
| INFLAMMATION, FOCAL | | 1 (2%) | |
| PYELONEPHRITIS, ACUTE | 1 (2%) | | |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| PYELONEPHRITIS, CHRONIC | 1 (2%) | | |
| INFLAMMATION, CHRONIC FOCAL | | 1 (2%) | |
| #KIDNEY/TUBULE | (54) | (54) | (54) |
| DEGENERATION, NOS | | 1 (2%) | |
| #URINARY BLADDER | (48) | (53) | (54) |
| CALCULUS, NOS | 1 (2%) | | |
| HYPERPLASIA, EPITHELIAL | 1 (2%) | | |
| ENDOCRINE SYSTEM | | | |
| #THYROID | (48) | (48) | (46) |
| ULTIMOBRANCHIAL CYST | | 2 (4%) | |
| #PANCREATIC ISLETS | (49) | (52) | (52) |
| HYPERPLASIA, NOS | 12 (24%) | 1 (2%) | 2 (4%) |
| REPRODUCTIVE SYSTEM | | | |
| *PREPUTIAL GLAND | (55) | (54) | (55) |
| CALCULUS, NOS | 1 (2%) | | |
| #PROSTATE | (52) | (52) | (50) |
| INFLAMMATION, ACUTE | 1 (2%) | | |
| #TESTIS | (54) | (54) | (54) |
| SPERMATOCELE | | | 1 (2%) |
| DEGENERATION, HYALINE | | | 1 (2%) |
| ATROPHY, NOS | | | 1 (2%) |
| NERVOUS SYSTEM | | | |
| NONE | | | |

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

TABLE D1 (CONCLUDED)

| | CONTROL (UNTR) 05-0360 | LOW DOSE 05-0395 | HIGH DOSE 05-0400 |
|---|---|---|---|
| SPECIAL SENSE ORGANS | | | |
| *EYE | (55) | (54) | (55) |
| INFLAMMATION, ACUTE | 1 (2%) | | |
| CATARACT | 1 (2%) | | |
| BAND KERATOPATHY | | | 1 (2%) |
| MUSCULOSKELETAL SYSTEM | | | |
| *SKELETAL MUSCLE | (55) | (54) | (55) |
| PARASITISM | | | 1 (2%) |
| BODY CAVITIES | | | |
| *ABDOMINAL CAVITY | (55) | (54) | (55) |
| NECROSIS, FAT | 5 (9%) | | |
| ALL OTHER SYSTEMS | | | |
| ADIPOSE TISSUE | | | |
| INFARCT, NOS | | 1 | |
| OMENTUM | | | |
| HEMATOMA, NOS | 1 | | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NO LESION REPORTED | 8 | 13 | 8 |
| ANIMAL MISSING/NO NECROPSY | | 1 | |
| AUTO/NECROPSY/HISTO PERF | 1 | 1 | |

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

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

| | CONTROL (UNTR) 06-0360 | LOW DOSE 06-0395 | HIGH DOSE 06-0400 |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 55 | 55 | 55 |
| ANIMALS MISSING | | 1 | 1 |
| ANIMALS NECROPSIED | 55 | 54 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY** | 55 | 54 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (55) | (54) | (50) |
| LYMPHOCYTIC INFLAMMATORY INFILTR | | | 1 (2%) |
| ULCER, ACUTE | | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (55) | (54) | (50) |
| ATELECTASIS | 1 (2%) | | |
| HYPERPLASIA, ADENOMATOUS | | | 1 (2%) |
| HISTIOCYTOSIS | | | 1 (2%) |
| HEMATOPOIETIC SYSTEM | | | |
| #BONE MARROW | (52) | (54) | (50) |
| INFLAMMATION, NOS | | 1 (2%) | |
| MYELOFIBROSIS | 31 (60%) | 35 (65%) | 30 (60%) |
| HYPERPLASIA, HEMATOPOIETIC | 1 (2%) | | 1 (2%) |
| #SPLEEN | (53) | (53) | (49) |
| MYELOFIBROSIS | | 1 (2%) | |
| HYPERPLASIA, LYMPHOID | | 6 (11%) | 2 (4%) |
| HEMATOPOIESIS | 1 (2%) | 13 (25%) | 22 (45%) |
| #MANDIBULAR L. NODE | (47) | (49) | (40) |
| HYPERPLASIA, LYMPHOID | | 1 (2%) | 1 (3%) |
| #MESENTERIC L. NODE | (47) | (49) | (40) |
| THROMBOSIS, NOS | | 1 (2%) | 1 (3%) |
| CONGESTION, NOS | 1 (2%) | | |
| HYPERPLASIA, NOS | 2 (4%) | | |
| PLASMACYTOSIS | | 1 (2%) | |

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

TABLE D2 (CONTINUED)

| | CONTROL (UNTR) 06-0360 | LOW DOSE 06-0395 | HIGH DOSE 06-0400 |
|---|---|---|---|
| HYPERPLASIA, LYMPHOID | | 5 (10%) | 2 (5%) |
| HEMATOPOIESIS | | | 3 (8%) |
| #THYMUS | (35) | (38) | (37) |
| ATROPHY, NOS | | 1 (3%) | |
| **CIRCULATORY SYSTEM** | | | |
| #HEART | (55) | (53) | (49) |
| PERIARTERITIS | 1 (2%) | | |
| **DIGESTIVE SYSTEM** | | | |
| #LIVER | (54) | (53) | (50) |
| INFLAMMATION, NECROTIZING | | 1 (2%) | |
| INFLAMMATION, CHRONIC FOCAL | | | 2 (4%) |
| NECROSIS, FOCAL | | 1 (2%) | |
| INFARCT, NOS | | | 1 (2%) |
| BASOPHILIC CYTO CHANGE | | 2 (4%) | 1 (2%) |
| HEMATOPOIESIS | | | 1 (2%) |
| #LIVER/HEPATOCYTES | (54) | (53) | (50) |
| CYTOPLASMIC VACUOLIZATION | | | 1 (2%) |
| *GALLBLADDER | (55) | (54) | (50) |
| CYTOPLASMIC VACUOLIZATION | | | 1 (2%) |
| #PANCREAS | (49) | (52) | (47) |
| DILATATION/DUCTS | 1 (2%) | 1 (2%) | 1 (2%) |
| INFLAMMATION, ACUTE NECROTIZING | | | 1 (2%) |
| INFLAMMATION, CHRONIC | 2 (4%) | | |
| DEGENERATION, HYALINE | | | 1 (2%) |
| ATROPHY, NOS | 1 (2%) | 2 (4%) | 6 (13%) |
| #STOMACH | (53) | (52) | (48) |
| MINERALIZATION | | 1 (2%) | |
| ULCER, NOS | 1 (2%) | | |
| INFLAMMATION, FOCAL | | | 1 (2%) |
| EROSION | 1 (2%) | | |
| HYPERPLASIA, EPITHELIAL | 2 (4%) | | |
| HYPERKERATOSIS | | 2 (4%) | 3 (6%) |
| ACANTHOSIS | | 2 (4%) | 3 (6%) |

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

TABLE D2 (CONTINUED)

| | CONTROL (UNTR) 06-0360 | LOW DOSE 06-0395 | HIGH DOSE 06-0400 |
|---|---|---|---|
| URINARY SYSTEM | | | |
| #KIDNEY | (55) | (53) | (50) |
| PYELONEPHRITIS, CHRONIC | 1 (2%) | | |
| NEPHROPATHY | | 1 (2%) | 1 (2%) |
| #KIDNEY/TUBULE | (55) | (53) | (50) |
| DEGENERATION, NOS | | | 2 (4%) |
| #URINARY BLADDER | (50) | (51) | (48) |
| INFLAMMATION, CHRONIC | 1 (2%) | | |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (42) | (48) | (38) |
| HYPERPLASIA, FOCAL | | 2 (4%) | 2 (5%) |
| #ADRENAL | (50) | (53) | (45) |
| CYTOPLASMIC VACUOLIZATION | | 1 (2%) | |
| #THYROID | (48) | (46) | (38) |
| FOLLICULAR CYST, NOS | | | 1 (3%) |
| #PANCREATIC ISLETS | (49) | (52) | (47) |
| HYPERPLASIA, NOS | 3 (6%) | | |
| REPRODUCTIVE SYSTEM | | | |
| #UTERUS | (54) | (50) | (49) |
| DILATATION, NOS | | 2 (4%) | 1 (2%) |
| HYDROMETRA | 3 (6%) | | |
| THROMBOSIS, NOS | | 1 (2%) | |
| INFLAMMATION, ACUTE NECROTIZING | | | 1 (2%) |
| DEGENERATION, HYALINE | | | 1 (2%) |
| HEMATOPOIESIS | | 1 (2%) | |
| #UTERUS/ENDOMETRIUM | (54) | (50) | (49) |
| HYPERPLASIA, CYSTIC | 15 (28%) | 24 (48%) | 26 (53%) |
| #OVARY | (50) | (51) | (49) |
| CYST, NOS | 7 (14%) | 4 (8%) | 7 (14%) |
| THROMBOSIS, NOS | | | 1 (2%) |

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

TABLE D2 (CONTINUED)

| | CONTROL (UNTR) 06-0360 | LOW DOSE 06-0395 | HIGH DOSE 06-0400 |
|---|---|---|---|
| HEMORRHAGIC CYST | 1 (2%) | 1 (2%) | 3 (6%) |
| ABSCESS, NOS | 1 (2%) | | |
| **NERVOUS SYSTEM** | | | |
| *BRAIN/MENINGES | (55) | (53) | (48) |
| INFLAMMATION, NOS | 1 (2%) | | |
| *BRAIN | (55) | (53) | (48) |
| HYDROCEPHALUS, NOS | 2 (4%) | | |
| PERIVASCULITIS | | 1 (2%) | |
| **SPECIAL SENSE ORGANS** | | | |
| NONE | | | |
| **MUSCULOSKELETAL SYSTEM** | | | |
| NONE | | | |
| **BODY CAVITIES** | | | |
| *ABDOMINAL CAVITY | (55) | (54) | (50) |
| NECROSIS, FAT | 7 (13%) | | |
| *MESENTERY | (55) | (54) | (50) |
| CYST, NOS | 1 (2%) | | |
| **ALL OTHER SYSTEMS** | | | |
| *MULTIPLE ORGANS | (55) | (54) | (50) |
| PERIARTERITIS | 1 (2%) | | |
| ADIPOSE TISSUE | | | |
| NECROSIS, FAT | | | 1 |
| **SPECIAL MORPHOLOGY SUMMARY** | | | |
| NO LESION REPORTED | 1 | 3 | |

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

TABLE D2 (CONCLUDED)

| | CONTROL (UNTR) 06-0360 | LOW DOSE 06-0395 | HIGH DOSE 06-0400 |
|---|---|---|---|
| ANIMAL MISSING/NO NECROPSY | | 1 | 1 |
| AUTO/NECROPSY/HISTO PERF | | | 1 |
| AUTOLYSIS/NC NECROPSY | | | 4 |

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

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

April 26, 1978

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

The primary reviewer noted the increase in preputial gland tumors in low (8/54) and high (3/55) dose treated male rats and in historic controls (3/250) from the test laboratories. Based on these findings, he questioned the statement in the report that "the evidence was insufficient to establish the carcinogenicity of *p*-Anisidine Hydrochloride" in rats. He suggested that the slides from the high dose treated aniamsl be reexamined to determine if the incidence of preputial gland tumors was higher than reported.

The secondary reviewer pointed out negative associations in which there were fewer tumors in treated animals than in controls. He questioned the need for a statement regarding the lower and upper confidence limits of the bioassay. A Program staff member explained that it was placed in reports to indicate that a compound cannot be proven to be unequivocally negative under the conditions of test.

A Program staff pathologist commented that tumors of the preputial gland are usually detected grossly, rather than by microscopic examination, and that slides of the preputial gland are not routinely prepared on every animal. A Subgroup member observed that there may be justification for combining the tumors from the low and high dose treated groups. He added that the biological significance of the tumors must be considered along with the statistical significance.

A motion was made that the report on the bioassay of *p*-Anisidine Hydrochloride be accepted with the provisos that: 1) the treated male rats would be reevaluated to determine if there were unreported preputial gland tumors and 2) the report would be reconsidered by the Subgroup if additional tumors are found. The motion was seconded and approved unanimously.

Members present were:

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

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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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