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BIOASSAY OF o-ANISIDINE HYDROCHLORIDE FOR POSSIBLE CARCINOGENICITY

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

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FOREWORD: This report presents the results of the bioassay of o-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 environmental chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that the test chemical is not a carcinogen, inasmuch as 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 that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: The bioassay of o-anisidine hydrochloride was conducted by EG&G Mason Research Institute, Worcester, Massachusetts, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The bioassay was conducted under the supervision of Drs. A. Handler[1] and E. Smith[2] and Mr. G. Wade[3]. NCI project officers were Drs. E. Weisburger[4], T. Cameron[4], and N. P. Page[4,5]. The program manager was Mr. J. Baker[3]. Ms. A. Good[3] supervised the technicians in charge of animal care, and Ms. E. Zepp[3] supervised the preparation of the feed mixtures and collected samples of the diets for analysis. Ms. D. Bouthot[3] kept all daily records of the test, and Ms. R. Monson[3] prepared a draft of the experimental design based on these records. Histopathologic examinations on rats and mice were performed by Drs. D. S. Wyand[3] and A.

Russfield[3], and the diagnoses included in this report represent their interpretations.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute, Rockville, Maryland[6]. The statistical analyses were performed by Dr. J. R. Joiner[7], using methods selected for the bioassay program by Dr. J. J. Gart[8].

Chemicals used in this bioassay were analyzed under the direction of Dr. E. Murrill[9], and dosed feed mixtures were analyzed by Dr. M. Hagopian[3]. The results of the analyses were reviewed by Dr. S. S. Olin[7]. The chemical structure was supplied by NCI.

This report was prepared at Tracor Jitco[7] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. L. A. Campbell, Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. J. F. Robens, toxicologist; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Waitz, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley and Ms. P. J. Graboske.

The following other scientists at NCI were responsible for evaluating the bioassay, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Dawn G. Goodman, Dr. Richard A. Griesemer, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Robert A. Squire[10], Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

A bioassay of o-anisidine hydrochloride for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F1 mice.

Groups of 55 rats of each sex and 55 mice of each sex were administered o-anisidine hydrochloride at one of the following doses, either 5,000 or 10,000 ppm for rats and either 2,500 or 5,000 ppm for mice, for 103 weeks, then observed for 1 or 2 additional weeks. Controls consisted of groups of 55 untreated rats of each sex and 55 untreated mice of each sex. All surviving rats were killed at 103-107 weeks, and all surviving mice at 104 or 105 weeks.

Mean body weights of the dosed male and female rats and mice were lower than those of the corresponding controls throughout the bioassay. Bloody exudates and stained fur in the urogenital area were noted in many dosed animals. Sufficient numbers of animals were at risk in the mice, but not in the rats, for development of late-appearing tumors; however, survival in the rats was 80% or more at week 52.

Transitional-cell carcinomas or papillomas of the urinary bladder occurred at statistically significant incidences ($P < 0.001$) in the low- and high-dose groups of rats (males: controls 0/51, low-dose 52/54, high-dose 52/52; females: controls 0/49, low-dose 46/49, high-dose 50/51) and in high-dose groups of mice (males: controls 0/48, low-dose 2/55, high-dose 22/53; females: controls 0/50, low-dose 1/51, high-dose 22/50); the incidences also had significant dose-related trends ($P < 0.001$) in both species. These lesions were observed as early as week 36 in female rats, week 40 in male rats, and week 45 in male mice. Transitional-cell carcinomas of the pelvis of the kidney occurred with a significant dose-related trend ($P = 0.005$) in the male rats, and the incidence in the high-dose group was significantly higher ($P = 0.006$) than that in the control group (controls 0/53, low-dose 3/55, high-dose 7/53); all rats having this tumor also had a transitional-cell carcinoma of the urinary bladder. Only one animal in the control groups of rats or mice had any tumor of the

urinary system (a transitional-cell papilloma of the pelvis of the kidney in a male mouse).

Follicular-cell tumors of the thyroid (carcinomas, cystadenocarcinomas, adenomas, cystadenomas, and papillary cystadenomas) occurred at statistically significant incidences ($P \leq 0.005$) in low- and high-dose groups of male rats (controls 0/53, low-dose 7/40, high-dose 6/40); the incidences also had a dose-related trend ($P = 0.009$). These tumors did not occur at significant incidences in dosed groups of female rats.

It is concluded that under the conditions of this bioassay, o-anisidine hydrochloride was carcinogenic for Fischer 344 rats and B6C3F1 mice, inducing transitional-cell carcinomas or papillomas of the bladder in both rats and mice and in both sexes of each species, transitional-cell carcinomas of the pelvis of the kidney in male rats, and follicular-cell tumors of the thyroid in male rats.

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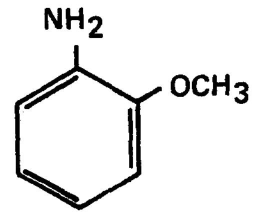
I. INTRODUCTION

Ortho-anisidine (CAS 134-29-0; NCI C03747) is the common name for 2-methoxyaniline and has been marketed in Germany under the trade name Fast Red BB Base (Society of Dyers and Colourists, 1971).



o-ANISIDINE (HYDROCHLORIDE)

The chemical is used chiefly in the manufacture of dyes, one method being the diazotization of o-anisidine and coupling with other aromatic amines or phenols to yield a large number of the azo dyes (Noller, 1965). Chloro, nitro, alkyl, and aryl derivatives of o-anisidine exist and are used similarly in the synthesis of other azo dyes. o-Anisidine is listed as a possible ingredient in permanent oxidation hair dyes (Wall, 1972) although it is not currently used in any hair dyes in the United States (FDA, 1977). Another use of o-anisidine is as a starting material in the synthesis of guaiacol (o-methoxyphenol) (Stecher, 1968).

o-Anisidine was selected for study in the Carcinogenesis Testing Program because of its industrial importance, which indicated that there was long-term exposure to the chemical among many persons employed in the dye manufacturing industry. The earlier finding that o-toluidine, an aromatic amine analog of o-anisidine, was carcinogenic in long-term feeding studies (Homburger et al., 1972; Russfield et al., 1973) was a further reason for testing o-anisidine.

II. MATERIALS AND METHODS

A. Chemical

o-Anisidine hydrochloride was obtained from Pfaltz and Bauer, Flushing, New York, in two batches. When the identity and purity of Lot No. M7024-4, which was used during the subchronic studies, and of Lot No. M7270, used during the chronic studies, were analytically determined, both lots showed a single homogeneous peak by vapor-phase chromatography and a trace impurity at the origin in two different solvent systems by thin-layer chromatography. Nonaqueous titration of the amine group with perchloric acid was 99.5% $\pm$ 0.2% of the theoretical value for Lot No. M7024-4 and 100.1 $\pm$ 0.3% for Lot No. M7270. By Karl Fischer analysis, 0.23 $\pm$ 0.01% water was detected in Lot No. M7024-4 and less than 0.5% water in Lot No. M7270. Elemental analyses for both lots were consistent with $C_7H_{10}NOCl$, the molecular formula of o-anisidine hydrochloride. Infrared, ultraviolet, and nuclear magnetic resonance spectra were consistent with expectations based on the structure and spectra in the literature for o-anisidine (Sadtler, 1966, 1970; Ungnade, 1954; Pawlawski, 1967).

The bulk chemical was stored at $4^{\circ}C$ in a plastic-lined drum. A small quantity was stored in an amber glass bottle for daily use.

B. Dietary Preparation

Diets were prepared once per week by first mixing a weighed amount of chemical with an aliquot of ground Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) in a mortar. When this premix appeared homogeneous, it was placed in a Patterson-Kelly twin-shell blender with the remaining feed and mixed for 20 minutes. Formulated diets were stored in double plastic bags at 4°C and used within 1 week of preparation.

As a quality control test on the accuracy of preparation of the diets, the concentration of o-anisidine hydrochloride was determined at Midwest Research Institute in selected batches of formulated diets during the chronic studies. The results are summarized in Appendix G. At each dietary concentration, the mean of the analytical concentrations for the checked samples was within 12% of the theoretical concentration, and the coefficient of variation ranged from 9.5% to 24.4%. In temperature-dependent stability studies performed at Midwest, some loss of the test chemical in feed was observed after 2 weeks at 25°C, which may account in part for the unusually large variations.

C. Animals

For the subchronic studies, Fischer 344 rats and B6C3F1 mice of each sex were obtained from Charles River Laboratories, Inc., Wilmington, Massachusetts. For the chronic studies, Fischer 344 rats and B6C3F1 mice of each sex were obtained from Frederick Cancer Research Center, Frederick, Maryland. Control rats and mice were received earlier than, and placed on test prior to, animals in the dosed groups. All animals were approximately 28 days of age when received at the laboratory and were quarantined for 2 weeks prior to the start of the bioassay. Animals in a random sample were found to be free of parasites and signs of disease. At the end of the quarantine period, animals were assigned to control or dosed groups in such a way that the mean weights of animals in each cage were approximately the same within a given group.

D. Animal Maintenance

Animal rooms were maintained at temperatures ranging from 23-34°C. Air was filtered through Tri-Dek® 15/40 denier Dacron filters and air flow was maintained at a velocity permitting six changes of room air per hour. Rooms were illuminated by fluorescent lighting for 12 hours per day.

Rats were housed five per cage in galvanized steel wire mesh cages (Fenco Cage Products, Boston, Mass.), suspended over drop trays lined with newspaper. Cages and racks were sanitized every week and paper in the drop trays was replaced daily. After 48 weeks on study, rats were transferred to suspended solid polycarbonate cages (Lab Products, Inc., Garfield, N. J.), each of which housed five animals per cage. These cages were equipped with disposable nonwoven fiber filter sheets. The polycarbonate cages were sanitized and supplied with fresh bedding two times per week. A hardwood chip bedding (Aspen-bed®, American Excelsior, Sommerville, Mass.) was used in the polycarbonate cages.

Mice were housed five per cage in solid polycarbonate cages fitted with perforated stainless steel lids and disposable filter bonnets. A hardwood chip bedding (Aspen-Bed®, American Excelsior) or a corn cob bedding (Bed-o-Cobs®, Anderson Cob Mills, Inc., Maumee, Ohio) was used in the mouse cages. Cages were sanitized and furnished with fresh bedding two times per week. Cage racks were sanitized every 2 weeks. All equipment that was sanitized was washed with detergents and rinsed at 82°C.

Tap water (0.75-1.0 ppm chlorine) was provided in 250-ml polycarbonate bottles which were sanitized two times per week and refilled as necessary. Sipper tubes and stoppers were soaked in a disinfectant (Environ, Vestal Laboratories, St. Louis, Mo.) and rinsed before use, once per week. Control animals were fed Wayne® Lab Blox animal meal, and dosed animals were fed the same product which had been mixed with the test chemical. All diets were available ad libitum 7 days per week in Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc., Woburn, Mass.) for 8 weeks in the mice and for 48 weeks in the rats. Thereafter, feed was placed in stainless steel hoppers (Scientific Cages, Inc., Bryan, Texas). The Alpine® cups were emptied and filled with fresh feed every day. The stainless steel hoppers were changed two times per week and were filled with fresh feed and any that had not been consumed in the earlier part of the week. However, no feed was more than 1 week old when presented to the animals.

Rats and mice were housed in separate rooms. Control animals matched with o-anisidine hydrochloride animals were in the same room as the respective dosed animals. Rats on study with o-anisidine hydrochloride were housed in the same room with other rats being fed the following chemicals:

Rats

(CAS 615-66-7) 2-chloro-p-phenylenediamine sulfate
(CAS 20265-97-8) p-anisidine hydrochloride
(CAS 126-72-7) tris (2,3-dibromopropyl)phosphate

Mice were housed in a room with other mice being fed the following chemicals:

Mice

(CAS 615-66-7) 2-chloro-p-phenylenediamine sulfate
(CAS 126-72-7) tris (2,3-dibromopropyl)phosphate
(CAS 20265-97-8) p-anisidine hydrochloride
(CAS 2438-88-2) 2,3,5,6-tetrachloro-4-nitroanisole

(CAS 1465-25-4) N-1-naphthylethylenediamine dihydrochloride
(CAS 142-04-1) aniline hydrochloride

E. Subchronic Studies

Subchronic feeding studies were conducted with Fischer 344 rats and B6C3F1 mice to estimate the maximum tolerated doses of o-anisidine hydrochloride, on the basis of which two concentrations (hereinafter referred to as "low doses" and "high doses") were determined for use in the chronic studies. In the subchronic studies, the chemical was administered in feed for 7 weeks at doses of 1,000, 3,000, 10,000, or 30,000 ppm. Five males and five females of each species were administered each dose, and five males and five females of each species were given basal diets. All animals were killed by inhalation of carbon dioxide and necropsied 1 week after the end of the administration of the test chemical.

The rats at doses of 1,000 and 3,000 ppm showed weight depressions of less than 10%. At 10,000 ppm, weight depressions were 21% for males and 11% for females; at 30,000 ppm, weight depressions were 52% for males and 27% for females. No deaths occurred among the rats. However, on gross pathology examination, all animals administered 10,000 or 30,000 ppm had moderately enlarged spleens which were black and granular in appearance. Spleens of males administered 1,000 or 3,000 ppm were granular, whereas in the females administered the chemical at these doses, no effects were noted. Based on these data, the low and high doses for the chronic studies using rats were set at 5,000 and 10,000 ppm.

In the male mice, mean body weights were depressed 6% at 1,000 ppm, 14% at 3,000 ppm, 28% at 10,000, and 40% at 30,000 ppm. In the female mice, mean body weights were depressed 9% at 1,000 ppm, 19% at 3,000 ppm, 23% at 10,000 ppm, and 37% at 30,000 ppm. One female died during the study at 30,000 ppm. Spleens were black and enlarged in both the males and the females administered 10,000 and 30,000 ppm. Based on these data, the low and high doses for the chronic studies using mice were set at 2,500 and 5,000 ppm.

F. Chronic Studies

The test groups, doses administered, and times on study of the chronic feeding studies are shown in tables 1 and 2.

G. Clinical and Pathologic Examinations

Inspections for mortality and morbidity were carried out twice daily. Body weights were recorded every 2 weeks for the first 12 weeks and monthly thereafter. Clinical observations were recorded every month.

Table 1. o-Anisidine Hydrochloride Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals[a]	o-Anisidine Hydrochloride in Diet[b] (ppm)	Time on Study Dosed (weeks)	Time on Study Observed (weeks)
Male				
Control[c]	55	0		106
Low-Dose	55	5,000	103	1
High-Dose	55	10,000	88[d]	
Female				
Control[c]	55	0		107
Low-Dose	55	5,000	103	
High-Dose	55	10,000	83[d]	

[a]Rats were 41 days of age when placed on study.

[b]Diets were available *ad libitum* 7 days per week.

[c]Controls were placed on study 3 weeks earlier than the dosed groups.

[d]Period of administration terminated at time indicated, due to death of all animals.

Table 2. o-Anisidine Hydrochloride Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals[a]	o-Anisidine Hydrochloride in Diet[b] (ppm)	Time on Study Dosed (weeks)	Time on Study Observed (weeks)
Male				
Control[c]	55	0		105
Low-Dose	55	2,500	103	1-2
High-Dose	55	5,000	103	2
Female				
Control[c]	55	0		105
Low-Dose	55	2,500	103	2
High-Dose	55	5,000	103	2

[a]Mice were 41 days of age when placed on study.

[b]Diets were available ad libitum 7 days per week.

[c]Controls were placed on study 3 weeks earlier than the dosed groups.

Moribund animals and animals that survived to the end of the bioassay were killed using CO_2 anesthesia and necropsied. Necropsies were also performed on all animals found dead, unless precluded by autolysis or severe cannibalization. The following tissues were examined where possible: tissue masses, abnormal regional lymph nodes, skin, mandibular lymph nodes, mammary gland, salivary gland, thigh muscle, sciatic nerve, bone marrow, costochondral junction (rib), thymus, larynx, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, duodenum, jejunum, ileum, colon, mesenteric lymph nodes, liver, gall bladder (mice), pancreas, spleen, kidney, adrenal, bladder, seminal vesicles/prostate/testis (males), ovary/uterus (females), nasal cavity, brain, pituitary, eyes, external and middle ear, and spinal cord. Peripheral blood smears were prepared from each animal whenever possible. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definite diagnosis.

A few tissues from some animals were not examined, particularly from those animals that died early. Also, some animals may have been missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Thus, the number of animals from which particular organs or tissues were examined microscopically varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

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

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

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

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

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

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

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, 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 dosed group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a dosed group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the dosed group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result ($P < 0.025$ 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. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of dosed male and female rats were lower than those of corresponding controls throughout the bioassay, and the depressions in weight were dose related (figure 1). Fluctuations in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation.

Palpable subcutaneous masses were seen in 4 control males, 17 control females, 8 low-dose males, and 7 low-dose females. Cutaneous lesions and/or growths developed in five control males, four control females, five low-dose males, seven low-dose females, and three high-dose females. Discoloration, reddening, and crusting of the eyes was noted in 2 control males, 19 low-dose males, 17 low-dose females, and 1 high-dose male. Rectal prolapses occurred in three control males and one high-dose female. Two control males displayed jaundice and one had a distended scrotal sac. Nine control females had facial alopecia. Emaciation was noted in two control females, two low-dose males, five low-dose females, two high-dose males, and one high-dose female. Abdominal distention occurred in one low-dose male, two low-dose females, and two high-dose males.

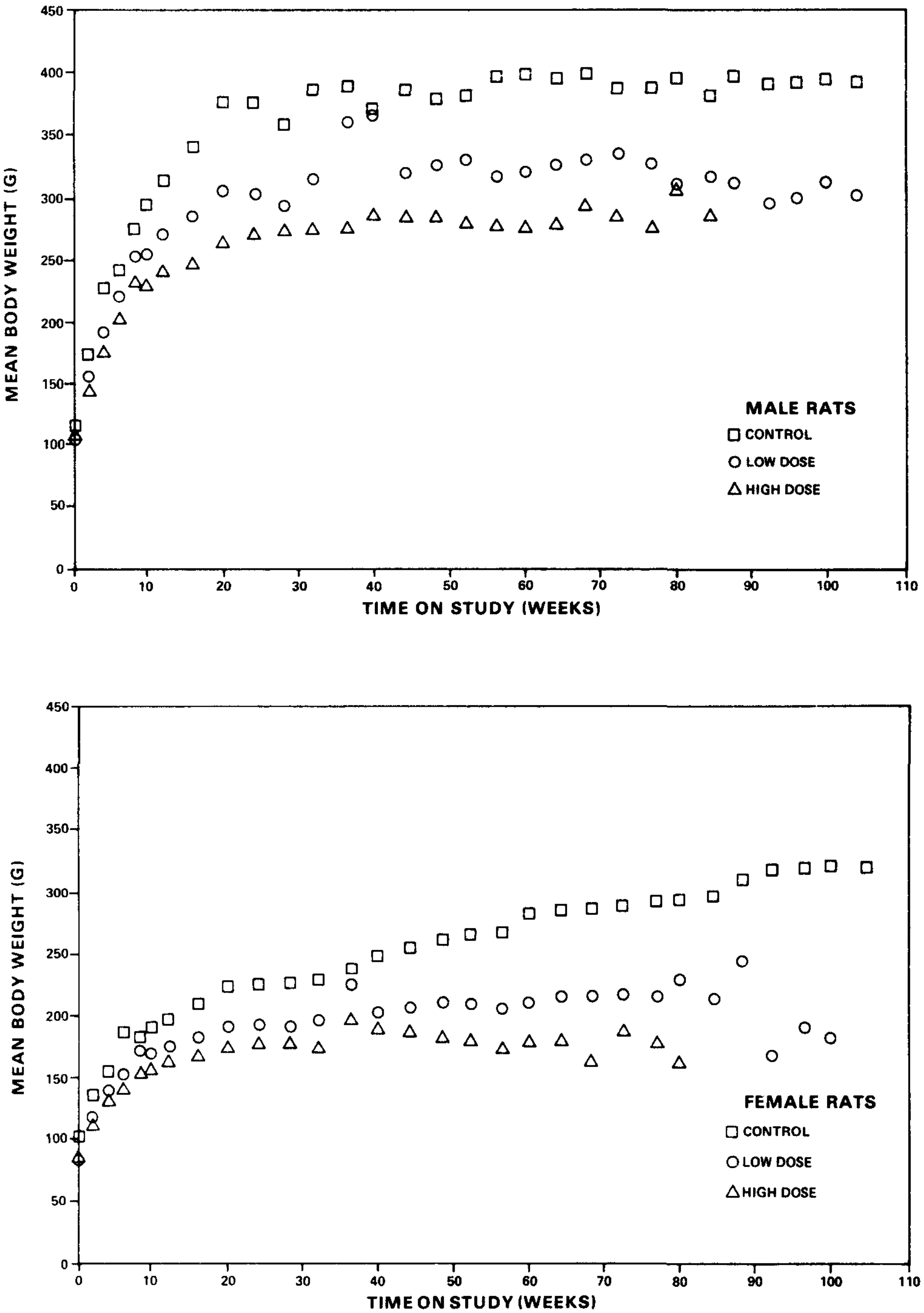


Figure 1. Growth Curves For Rats Fed o-Anisidine Hydrochloride In The Diet

Two low-dose males displayed posterior ataxia, and three high-dose males were badly hunched over prior to death.

Blood/exudates in the urogenital area and/or black/brown stained fur in the urogenital region were seen in 45 low-dose males, 18 low-dose females, 5 high-dose males, and 4 high-dose females.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats fed o-anisidine hydrochloride in the diet at the doses of this bioassay, together with those of the controls, are shown in figure 2.

The result of the Tarone test for positive dose-related trend in mortality is significant ($P < 0.001$) in each sex. A departure from linear trend is observed ($P < 0.001$), because of the steep decrease in survival in the dosed groups. In the high-dose group of each sex, no animal survived to termination of the study; however, 49/55 (89%) of the high-dose males and 44/55 (80%) of the high-dose females were still alive at week 52. All 55 of the low-dose animals and all 55 of the control animals of each sex lived beyond week 52 on study.

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in

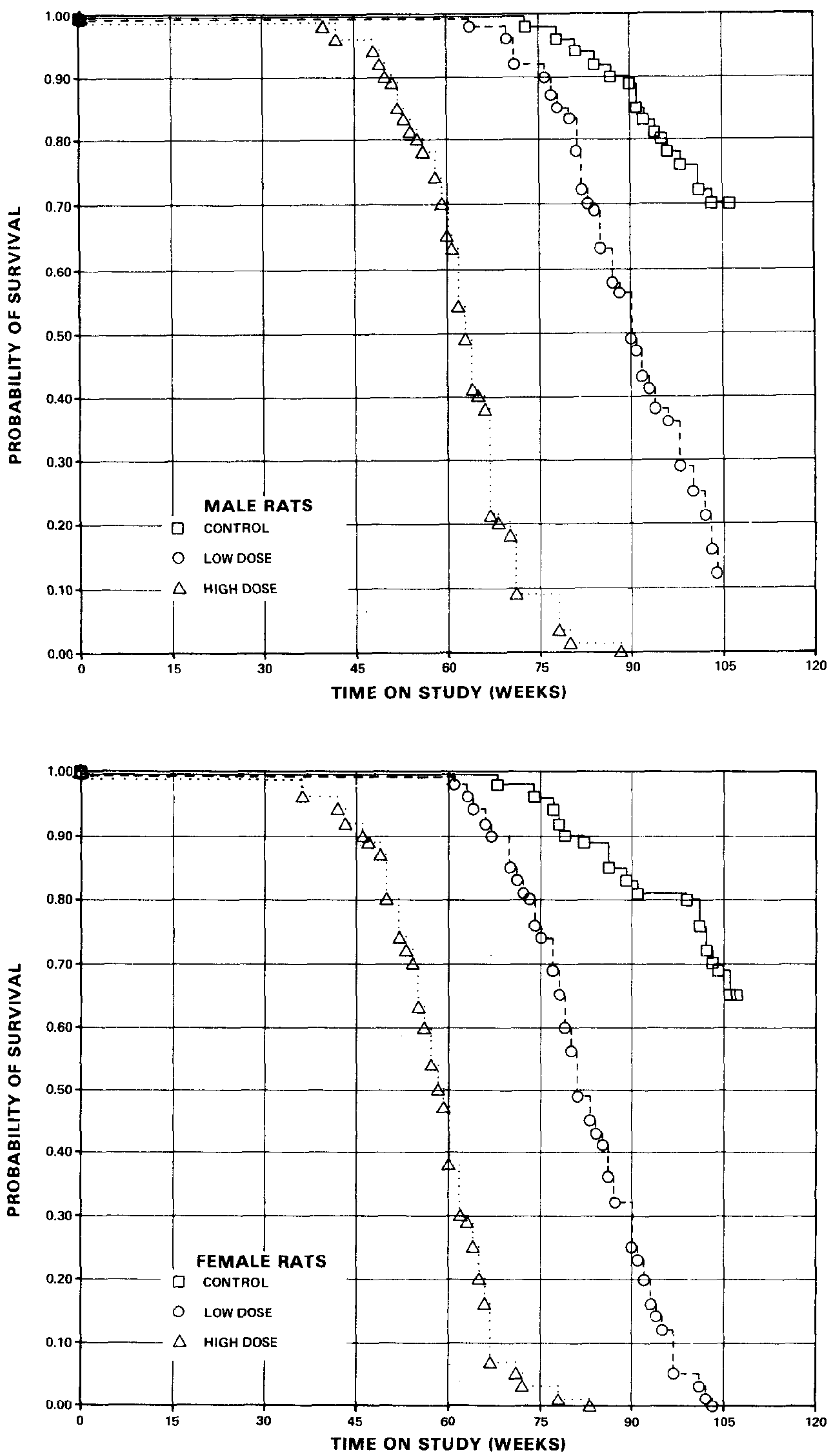


Figure 2. Survival Curves For Rats Fed o-Anisidine Hydrochloride In The Diet

Appendix A, tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix C, tables C1 and C2.

Transitional-cell carcinomas of the urinary bladder were found at a high incidence in both males (controls 0/51, low-dose 50/54, high-dose 51/52) and females (controls 0/49, low-dose 41/49, high-dose 50/51). Transitional-cell carcinomas of the bladder had varying morphological patterns. The more well-differentiated tumors consisted of transitional cells growing in solid sheets and serpentine strands two or three cells thick in a fibrous stroma. There were scattered foci of necrosis and mitotic figures were uncommon. The bladder wall was usually extensively invaded and the tumor often nearly filled the lumen. In some tumors, there was a marked desmoplasia and a mucinous degeneration of the stroma. Many tumors were very pleomorphic with spindle cells, tumor giant cells, bizarre hyperchromatic nuclei, and numerous abnormal mitotic figures. Areas of squamous metaplasia were common. In a few tumors, extensive formation of cystic spaces occurred. The tumor appeared to have a multicentric origin in a few instances with transitional-cell carcinomas appearing in the bladder and one or more additional sites such as renal pelvis, prostatic urethra, and kidney. One low-dose and one high-dose rat had two types of malignant primary tumors involving the bladder. A transitional-cell carcinoma filled the bladder lumen and a leiomyosarcoma originated from the muscularis. Although distant metastases were not seen in rats with bladder carcinomas, the bladder was heavily invaded, and in one case, the tumor extended to the serosa of the spleen and prostate. Diffuse and focal transitional-cell hyperplasias were seen in many bladders with carcinomas.

A variety of other neoplasms were observed with approximately equal frequency in the control and dosed animals. There were instances in this bioassay, as noted in the summary tables, where these neoplastic lesions occurred only in dosed animals or with increased frequency when compared with the control group. The nature and incidence of these neoplasms are similar to those known to occur spontaneously in aged Fischer 344 rats. However, follicular-cell tumors of the thyroid in males may be related to administration of the test chemical.

Nonneoplastic lesions which commonly occur in rats of this strain were seen. These were not considered to be compound induced.

Based on the histopathologic examination, there was evidence that under the conditions of this bioassay o-anisidine hydrochloride was carcinogenic when fed to Fischer 344 rats, inducing transitional-cell carcinomas of the urinary system.

D. Statistical Analyses of Results (Rats)

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

In each sex, the incidence of transitional-cell carcinomas or papillomas of the urinary bladder is high in either dosed group (males: low-dose 52/54 [96%], high-dose 52/52 [100%]; females: low-dose 46/49 [94%], high-dose 50/51 [98%]). These lesions were observed as early as week 36 in female and week 40 in male rats; none were observed in the control groups. The results of the Cochran-Armitage test for positive dose-related trend are significant ($P < 0.001$) in each sex. A departure from linear trend is observed ($P < 0.001$), because of the steep increase in incidence in the dosed groups. The results of the Fisher exact test comparing the incidence of these tumors in each dosed group with the incidence in the control group are significant ($P < 0.001$) in each sex. The statistical conclusion is that the incidence of these transitional-cell tumors of the urinary bladder in rats of each sex is dose associated.

In male rats, the results of the Cochran-Armitage test are significant ($P = 0.005$) for the incidence of transitional-cell carcinomas of the kidney or kidney pelvis, and the results of the Fisher exact test show that the incidence of this tumor is significantly higher (P = 0.006) in the high-dose group than in the control group. The statistical conclusion is that the occurrence of transitional-cell carcinomas of the kidney or kidney pelvis in the male rats was associated with the administration of o-anisidine hydrochloride. In female rats the incidence of this tumor was 1/54 in the high-dose group and 0/52 in both the low-dose and the control groups.

The results of the Cochran-Armitage test are also significant in the male rats for the incidence of follicular-cell adenomas, cystadenomas, or papillary cystadenomas of the thyroid or thyroid follicle (P = 0.030). The corresponding results of the Fisher exact test show P values of 0.031 for each dosed group; however, these values are not significant when the Bonferroni inequality criterion for multiple comparisons is applied. The results of the Cochran-Armitage test on the combined incidence of all follicular-cell tumors of the thyroid (carcinomas, adenomas, cystadenomas, papillary cystadenomas, or papillary cystadenocarcinomas) show an increased significant trend (P = 0.009), and the Fisher exact comparisons of the combined incidence in each dosed group with that in the control group also show an increased significance ($P \leq 0.005$). The incidence of follicular-cell tumors in female rats is not significant. The historical controls at this laboratory indicate an incidence of follicular-cell thyroid tumors in male rats of 3/250 (1.2%) and in female rats of 2/249 (0.8%).

In each sex, significant results in the negative direction are observed in incidences of several of the tumors, due to higher incidences in the control groups than in the dosed groups. This negative significance may be caused by the fact that the dosed animals did not live as long as the controls.

In summary, the results of the statistical tests show that the administration of o-anisidine hydrochloride is associated with the occurrence of transitional-cell carcinomas or papillomas of the urinary bladder in both sexes of Fischer 344 rats, and with the occurrence of transitional-cell carcinomas of the kidney or kidney pelvis and follicular-cell tumors of the thyroid in male rats.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of dosed male and female mice were lower than those of corresponding controls throughout the bioassay, and the depressions in weight were dose related (figure 3). Fluctuations in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation.

Subcutaneous palpable masses were recorded in four control males, three control females, and one low-dose female. Urogenital distention, nodules and/or bleeding was seen in three control males and one control female. Cutaneous growths developed in two control males, one low-dose male, and two high-dose females. Swelling of the eyes was seen in two low-dose females, two high-dose males, and two high-dose females. Alopecia, at one time or another during the bioassay, was noted in 42 control males, 54 control females, 49 low-dose males, 34 low-dose females, 25 high-dose males, and 26 high-dose females. One high-dose female had labored breathing. These signs were not considered to be related to administration of the test chemical.

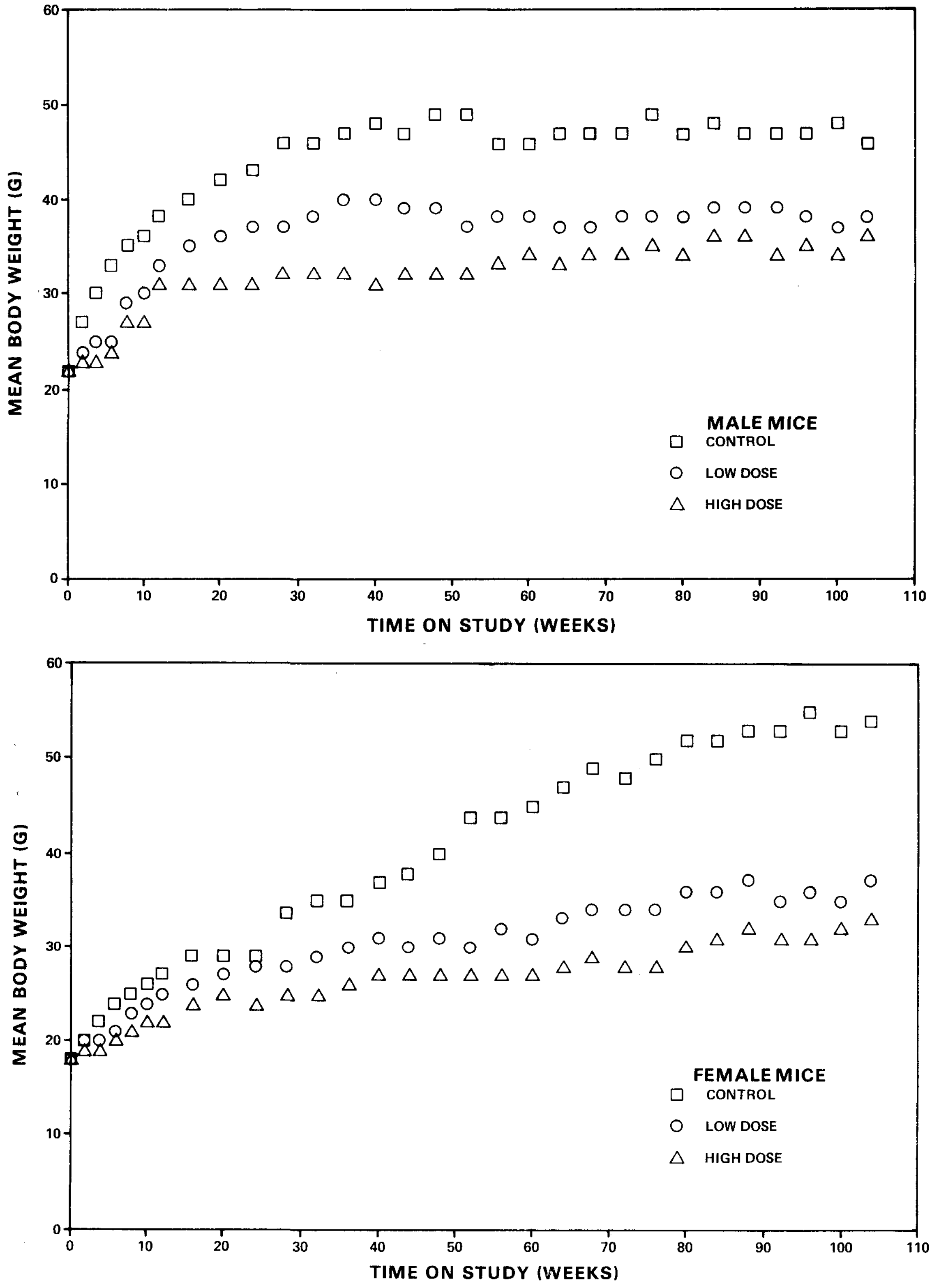


Figure 3. Growth Curves For Mice Fed o-Anisidine Hydrochloride In The Diet

B. Survival (Mice)

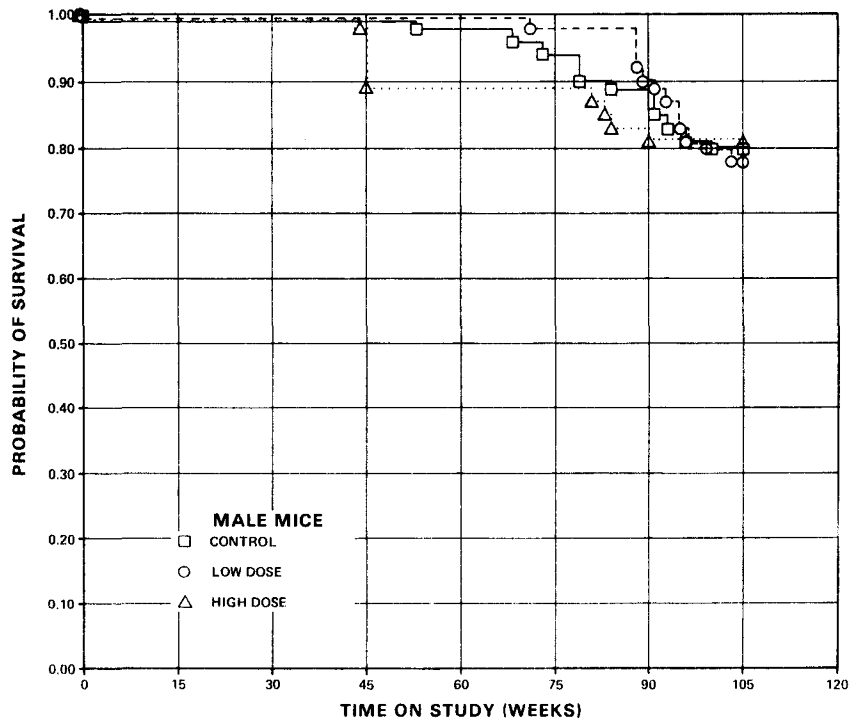
The Kaplan and Meier curves estimating the probabilities of survival for male and female mice fed o-anisidine hydrochloride in the diet at the doses of this bioassay, together with those of the controls, are shown in figure 4.

The result of the Tarone test for dose-related trend in mortality is not significant in either sex. In male mice, 43/55 (78%) of the high-dose group, 43/55 (78%) of the low-dose group, and 44/55 (80%) of the controls lived to the end of the bioassay. In females, the proportions which survived to termination of the study were 42/55 (76%) of the high-dose group, 38/55 (69%) of the low-dose group, and 44/55 (80%) of the controls. Sufficient numbers of mice of each sex were at risk for the development of tumors.

C. Pathology (Mice)

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

Transitional-cell papillomas of the urinary bladder were significantly elevated in dosed male mice (controls 0/48, low-dose 2/55, high-dose 7/53). Transitional-cell carcinomas of the bladder



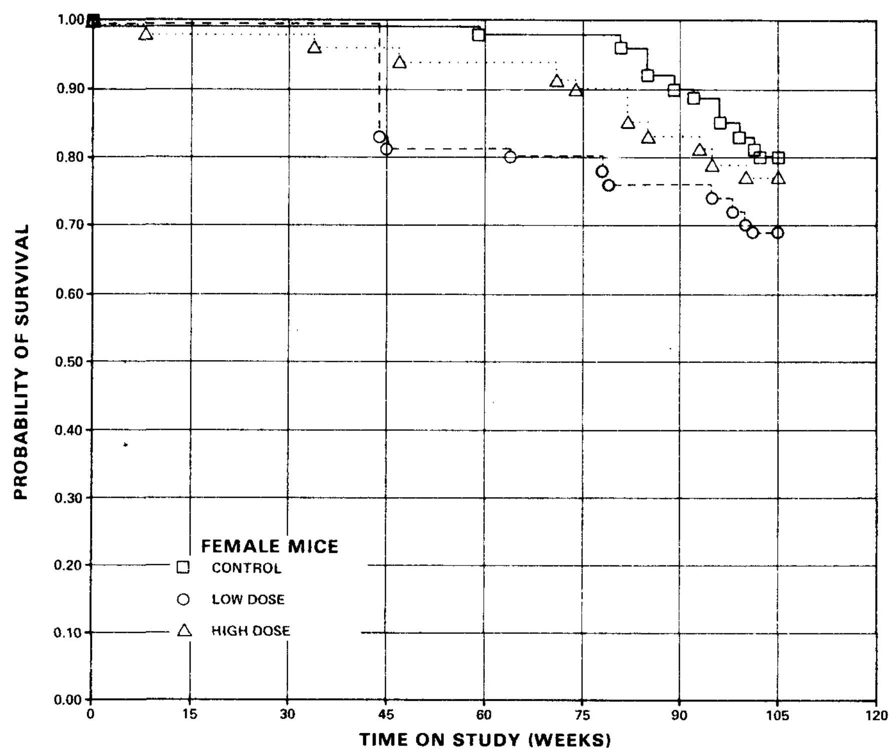


Figure 4. Survival Curves For Mice Fed o-Anisidine Hydrochloride In The Diet

were found to be significantly elevated in both males (high-dose 15/53) and females (high-dose 18/50). No transitional-cell carcinomas were seen in male and female low-dose or control groups. The earliest bladder changes recognized were focal hyperplasias (males: controls 1/48, low-dose 2/55, high-dose 21/53; females: controls 0/50, low-dose 1/51, high-dose 12/50) consisting of a few transitional cells with slight atypia piling up and projecting into the lumen and diffuse mild thickening of the epithelium with occasional piling up of cells and short papillary formations. There was normal cellular polarity and an intact basement membrane. The transitional-cell carcinomas invaded the bladder wall with strands and finger-like sheets of transitional cells. In some tumors, many small cystic spaces were formed, giving a pseudoacinar appearance. Extensive invasion of the bladder wall was often seen. Occasionally, a relatively small transitional-cell carcinoma invaded to the serosa. Some of the invasive tumors had extensive lymphocytic and plasma-cell infiltrates both surrounding and within the tumor. There were no distant metastases.

A variety of other neoplasms were observed with approximately equal frequency in the control and dosed mice. There were instances in this study, as noted in the summary tables, where these neoplastic lesions occurred only in dosed animals or with increased frequency when compared with the control group. The nature and incidence of these neoplasms are similar to those known to occur spontaneously in aged mice of this strain.

A variety of inflammatory and degenerative lesions which commonly occur in aging B6C3F1 mice were seen. None of these lesions were considered to be compound related.

Based on the histopathologic examination, there was evidence that o-anisidine hydrochloride was carcinogenic when fed to both male and female B6C3F1 mice, inducing transitional-cell carcinomas, under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

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

In each sex, transitional-cell carcinomas or papillomas of the urinary bladder occur in significant incidences ($P < 0.001$) exclusively in the high-dose groups (high-dose males 22/53 [42%] and high-dose females 22/50 [44%]), compared with the controls. These lesions were observed as early as week 45 in male mice. The results of the Cochran-Armitage test are also significant

($P < 0.001$) in each sex. A departure from linear trend is observed ($P = 0.005$ in male mice and $P = 0.001$ in female mice), because of the steep increase in the incidence of tumors in the high-dose group. The statistical conclusion is that the incidence of transitional-cell carcinomas or papillomas of the urinary bladder in mice is dose associated.

Significant results in the negative direction are observed in several of the incidences of tumors in each sex, where the incidences in the control group exceed those in the dosed groups.

In summary, the statistical conclusion indicates that the incidence of transitional-cell tumors of the urinary bladder in B6C3F1 mice is associated with the administration of o-anisidine hydrochloride.

V. DISCUSSION

The toxicity of o-anisidine hydrochloride for Fischer 344 rats and B6C3F1 mice was shown by consistently lowered mean body weights of all dosed groups when compared with corresponding controls throughout the bioassay; further, the data indicated dose-related effects of the test chemical on the mean body weights. Bloody exudates and stained fur in the urogenital area were noted in many dosed animals.

Survival of the rats at the end of the bioassay was low (males: controls 71%, low-dose 13%, high-dose 0%; females: controls 65%, low-dose 0%, high-dose 0%); however, survival was 80% or greater at week 52, and early development of tumors established the carcinogenicity of the test chemical, as given below. Survival of the mice at the end of the bioassay was high (males 78-80%; females 76-80%), and sufficient numbers of animals were at risk for the development of late-appearing tumors.

Transitional-cell carcinomas or papillomas of the urinary bladder occurred at statistically significant incidences ($P < 0.001$) in the low- and high-dose groups of rats (males: controls 0/51, low-dose 52/54, high-dose 52/52; females: controls 0/49, low-dose 46/49, high-dose 50/51) and in high-dose groups of mice (males: controls 0/48, low-dose 2/55, high-dose 22/53; females:

controls 0/50, low-dose 1/51, high-dose 22/50); the incidences also had significant dose-related trends ($P < 0.001$) in both species. These lesions were observed as early as week 36 in female rats, week 40 in male rats, and week 45 in male mice. Transitional-cell carcinomas of the pelvis of the kidney occurred with a significant dose-related trend ($P = 0.005$) in the male rats, and the incidence in the high-dose group was significantly higher ($P = 0.006$) than in the control group (controls 0/53, low-dose 3/55, high-dose 7/53); all rats having this tumor also had a transitional-cell carcinoma of the urinary bladder. A transitional-cell carcinoma also occurred in the pelvis of the kidney in one high-dose female rat. Only one animal in the control groups of rats or mice had any tumor of the urinary system (a transitional-cell papilloma of the pelvis of the kidney in a male mouse).

Follicular-cell tumors of the thyroid (carcinomas, cystadenocarcinomas, adenomas, cystadenomas, and papillary cystadenomas) occurred at statistically significant incidences ($P \leq 0.005$) in low- and high-dose groups of male rats (controls 0/53, low-dose 7/40, high-dose 6/40); the incidences also had a dose-related trend ($P = 0.009$). These tumors did not occur at significant incidences in dosed groups of female rats.

In humans, o-anisidine can be absorbed through the skin and is an irritant and a sensitizer (Stecher, 1968). No previous work has been reported on long-term studies of toxicity or carcinogenicty of o-anisidine.

It is concluded that under the conditions of the bioassay, o-anisidine hydrochloride was carcinogenic for Fischer 344 rats and B6C3F1 mice, inducing transitional-cell carcinomas or papillomas of the bladder in both rats and mice and in both sexes of each species, transitional-cell carcinomas of the pelvis of the kidney in male rats, and follicular-cell tumors of the thyroid in male rats.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN

RATS FED o-ANISIDINE HYDROCHLORIDE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED o-ANISIDINE HYDROCHLORIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	55	55	55
ANIMALS NECROPSIED	54	55	53
ANIMALS EXAMINED HISTOPATHOLOGICALLY	54	55	53
INTEGUMENTARY SYSTEM			
*SKIN	(54)	(55)	(53)
SQUAMOUS CELL PAPILLOMA	2 (4%)		
BASAL-CELL TUMOR		1 (2%)	
FIBROMA	2 (4%)		
*SUBCUT TISSUE	(54)	(55)	(53)
FIBROMA	2 (4%)		
RESPIRATORY SYSTEM			
*NASAL CAVITY	(54)	(55)	(53)
UNDIFFERENTIATED CARCINOMA		2 (4%)	
#LUNG	(54)	(55)	(51)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BRAIN/MENINGES	(54)	(53)	(51)
MALIGNANT LYMPHOMA, NOS			1 (2%)
*MULTIPLE ORGANS	(54)	(55)	(53)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
UNDIFFERENTIATED LEUKEMIA	13 (24%)		
LYMPHOCYTIC LEUKEMIA	4 (7%)		
#BONE MARROW	(52)	(55)	(47)
OSTEOSARCOMA, INVASIVE		1 (2%)	
#SPLENIC CAPSULE	(54)	(55)	(52)
TRANSITIONAL-CELL CARCINOMA			1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*MANDIBULAR L. NODE	(53)	(47)	(34)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*LIVER	(54)	(55)	(52)
NEOPLASTIC NODULE		2 (4%)	
HEPATOCELLULAR CARCINOMA		2 (4%)	
LEIOMYOSARCOMA, METASTATIC			1 (2%)
*PANCREAS	(53)	(54)	(51)
LEIOMYOSARCOMA, METASTATIC			1 (2%)
*STOMACH	(53)	(55)	(52)
SQUAMOUS CELL CARCINOMA	2 (4%)		
LEIOMYOSARCOMA			1 (2%)
*DUODENAL SEROSA	(52)	(53)	(51)
LEIOMYOSARCOMA, METASTATIC			1 (2%)
URINARY SYSTEM			
*KIDNEY	(53)	(55)	(53)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	3 (6%)
*KIDNEY/PELVIS	(53)	(55)	(53)
TRANSITIONAL-CELL CARCINOMA		2 (4%)	4 (8%)
*URINARY BLADDER	(51)	(54)	(52)
TRANSITIONAL-CELL PAPILLOMA		2 (4%)	1 (2%)
TRANSITIONAL-CELL CARCINOMA		50 (93%)	51 (98%)
LEIOMYOSARCOMA		1 (2%)	1 (2%)
HEMANGIOMA			1 (2%)
*PROSTATIC URETHRA	(54)	(55)	(53)
TRANSITIONAL-CELL CARCINOMA			2 (4%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(49)	(47)
ADENOMA, NOS		2 (4%)	
CHROMOPHOBE ADENOMA	4 (8%)		
ACIDOPHIL ADENOMA	1 (2%)		
#ADRENAL	(54)	(55)	(53)
CORTICAL ADENOMA	1 (2%)		
PHEOCHROMOCYTOMA	12 (22%)		
PHEOCHROMOCYTOMA, MALIGNANT	2 (4%)		
LEIOMYOSARCOMA, METASTATIC			1 (2%)
#THYROID	(53)	(40)	(40)
FOLLICULAR-CELL ADENOMA		3 (8%)	1 (3%)
FOLLICULAR-CELL CARCINOMA		2 (5%)	2 (5%)
C-CELL ADENOMA	3 (6%)	1 (3%)	
C-CELL CARCINOMA		2 (5%)	
CYSTADENOMA, NOS		1 (3%)	
#THYROID FOLLICLE	(53)	(40)	(40)
PAPILLARY CYSTADENOMA, NOS			3 (8%)
PAPILLARY CYSTADENOCARCINOMA,NOS		1 (3%)	
#PANCREATIC ISLETS	(53)	(54)	(51)
ISLET-CELL ADENOMA	1 (2%)	1 (2%)	
ISLET-CELL CARCINOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(54)	(55)	(53)
INTRADUCTAL PAPILLOMA	1 (2%)		
FIBROADENOMA	1 (2%)		
*PREPUTIAL GLAND	(54)	(55)	(53)
CARCINOMA,NOS	1 (2%)	2 (4%)	2 (4%)
#PROSTATE	(52)	(52)	(52)
TRANSITIONAL-CELL CARCINOMA			1 (2%)
#TESTIS	(54)	(52)	(51)
INTERSTITIAL-CELL TUMOR	53 (98%)	43 (83%)	4 (8%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(54)	(53)	(51)
CERUMINOUS CARCINOMA, METASTATIC	1 (2%)		
ASTROCYTOMA		2 (4%)	
SPECIAL SENSE ORGANS			
*EYE	(54)	(55)	(53)
SQUAMOUS CELL CARCINOMA	1 (2%)		
*EAR	(54)	(55)	(53)
CERUMINOUS CARCINOMA	1 (2%)		
*EAR CANAL	(54)	(55)	(53)
CERUMINOUS CARCINOMA	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*VERTEBRA	(54)	(55)	(53)
OSTEOSARCOMA		1 (2%)	
BODY CAVITIES			
*BODY CAVITIES	(54)	(55)	(53)
MESOTHELIOMA, NOS	2 (4%)	4 (7%)	1 (2%)
MESOTHELIOMA, MALIGNANT		2 (4%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	55	55	55
NATURAL DEATH@	6	21	37
MORIBUND SACRIFICE	10	27	18
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	39	7	
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	54	53	53
TOTAL PRIMARY TUMORS	112	132	80
TOTAL ANIMALS WITH BENIGN TUMORS	53	43	8
TOTAL BENIGN TUMORS	83	55	10
TOTAL ANIMALS WITH MALIGNANT TUMORS	26	51	53
TOTAL MALIGNANT TUMORS	27	71	69
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	1
TOTAL SECONDARY TUMORS	1	1	4
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	2	6	1
TOTAL UNCERTAIN TUMORS	2	6	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED o-ANISIDINE HYDROCHLORIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	55	55	55
ANIMALS NECROPSIED	54	53	54
ANIMALS EXAMINED HISTOPATHOLOGICALLY	54	53	54
INTEGUMENTARY SYSTEM			
*SKIN	(54)	(53)	(54)
SQUAMOUS CELL PAPILLOMA	2 (4%)		
SQUAMOUS CELL CARCINOMA	2 (4%)		
*SUBCUT TISSUE	(54)	(53)	(54)
SQUAMOUS CELL CARCINOMA			1 (2%)
ADENOCARCINOMA, NOS		1 (2%)	
FIBROMA	1 (2%)	2 (4%)	
FIBROADENOMA		1 (2%)	
RESPIRATORY SYSTEM			
*NASAL CAVITY	(54)	(53)	(54)
CARCINOMA, NOS		1 (2%)	
#LUNG	(53)	(53)	(54)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(54)	(53)	(54)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
UNDIFFERENTIATED LEUKEMIA	8 (15%)		
LYMPHOCYTIC LEUKEMIA	1 (2%)		
#SPLEEN	(52)	(52)	(51)
NEUROFIBROSARCOMA, UNC PRIM OR M	1 (2%)		
#MEDIASTINAL L.NODE	(51)	(46)	(36)
UNDIFFERENTIATED CARCINOMA METAS	1 (2%)		
#MESENTERIC L. NODE	(51)	(46)	(36)
UNDIFFERENTIATED CARCINOMA METAS	1 (2%)		

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#HEART	(53)	(53)	(53)
NEUROFIBROSARCOMA, UNC PRIM OR M	1 (2%)		
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(52)	(51)	(47)
ADENOMA, NOS	3 (6%)		
#LIVER	(53)	(53)	(53)
NEOPLASTIC NODULE	1 (2%)		
NEUROFIBROSARCOMA, UNC PRIM OR M	1 (2%)		
#STOMACH	(51)	(51)	(49)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
SQUAMOUS CELL CARCINOMA	1 (2%)		
ADENOCARCINOMA, NOS	1 (2%)		
URINARY SYSTEM			
#KIDNEY/PELVIS	(52)	(52)	(54)
TRANSITIONAL-CELL CARCINOMA			1 (2%)
#URINARY BLADDER	(49)	(49)	(51)
TRANSITIONAL-CELL PAPILLOMA		5 (10%)	
TRANSITIONAL-CELL CARCINOMA		41 (84%)	50 (98%)
FIBROMA		1 (2%)	
LEIOMYOSARCOMA		1 (2%)	1 (2%)
HEMANGIOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(51)	(45)
CARCINOMA,NOS	3 (6%)		
ADENOMA, NOS	1 (2%)	9 (18%)	1 (2%)
CHROMOPHOBE ADENOMA	15 (31%)		
CHROMOPHOBE CARCINOMA	1 (2%)		
ACIDOPHIL ADENOMA	1 (2%)		
#ADRENAL	(53)	(53)	(54)
CORTICAL ADENOMA	1 (2%)		

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
PHEOCHROMOCYTOMA	3 (6%)	1 (2%)	1 (2%)
ANGIOLIPOMA	1 (2%)		
#THYROID	(49)	(45)	(46)
UNDIFFERENTIATED CARCINOMA	1 (2%)		
FOLLICULAR-CELL ADENOMA			2 (4%)
FOLLICULAR-CELL CARCINOMA		3 (7%)	
C-CELL ADENOMA	1 (2%)		
C-CELL CARCINOMA	3 (6%)	1 (2%)	
#THYROID FOLLICLE	(49)	(45)	(46)
PAPILLARY CYSTADENOMA, NOS		1 (2%)	1 (2%)
#PANCREATIC ISLETS	(52)	(50)	(43)
ISLET-CELL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(54)	(53)	(54)
ADENOMA, NOS	1 (2%)		
ADENOCARCINOMA, NOS	2 (4%)		
FIBROADENOMA	16 (30%)	1 (2%)	
*CLITORAL GLAND	(54)	(53)	(54)
CARCINOMA,NOS	2 (4%)	2 (4%)	1 (2%)
#UTERUS	(52)	(50)	(50)
ENDOMETRIAL STROMAL POLYP	16 (31%)	6 (12%)	
HEMANGIOMA		1 (2%)	
#OVARY	(53)	(49)	(45)
GRANULOSA-CELL TUMOR	1 (2%)		
TUBULAR ADENOMA	2 (4%)		
NERVOUS SYSTEM			
*OLFACTORY SYSTEM	(54)	(53)	(54)
MENINGIOMA		1 (2%)	
#BRAIN/MENINGES	(52)	(53)	(52)
CARCINOMA, NOS, INVASIVE		1 (2%)	
#BRAIN	(52)	(53)	(52)
CARCINOMA, NOS, METASTATIC	2 (4%)		

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CHROMOPHOBE CARCINOMA, METASTATI	1 (2%)		
ASTROCYTOMA			1 (2%)
SPECIAL SENSE ORGANS			
*EYE	(54)	(53)	(54)
SQUAMOUS CELL CARCINOMA	2 (4%)		
*EAR CANAL	(54)	(53)	(54)
CERUMINOUS CARCINOMA	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(54)	(53)	(54)
ADENOCARCINOMA, NOS, METASTATIC	1 (2%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	55	55	55
NATURAL DEATH@	6	24	41
MORIBUND SACRIFICE	13	31	14
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36		
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	52	50	51
TOTAL PRIMARY TUMORS	100	81	60
TOTAL ANIMALS WITH BENIGN TUMORS	45	24	5
TOTAL BENIGN TUMORS	67	29	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	24	44	51
TOTAL MALIGNANT TUMORS	28	52	55
TOTAL ANIMALS WITH SECONDARY TUMORS#	5	1	
TOTAL SECONDARY TUMORS	6	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	2		
TOTAL UNCERTAIN TUMORS	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 FED o-ANISIDINE HYDROCHLORIDE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED o-ANISIDINE HYDROCHLORIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	55	55	55
ANIMALS MISSING			1
ANIMALS NECROPSIED	55	55	53
ANIMALS EXAMINED HISTOPATHOLOGICALLY	55	55	53
INTEGUMENTARY SYSTEM			
*SKIN	(55)	(55)	(53)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
*SUBCUT TISSUE	(55)	(55)	(53)
SQUAMOUS CELL PAPILLOMA			1 (2%)
FIBROMA	2 (4%)		
FIBROSARCOMA	1 (2%)	1 (2%)	
RESPIRATORY SYSTEM			
*NASAL CAVITY	(55)	(55)	(53)
SQUAMOUS CELL CARCINOMA			1 (2%)
#LUNG	(54)	(54)	(52)
HEPATOCELLULAR CARCINOMA, METAST	4 (7%)	1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	6 (11%)	3 (6%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	6 (11%)	6 (11%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(55)	(55)	(53)
MALIGNANT LYMPHOMA, NOS		3 (5%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
MALIGNANT LYMPHOMA, MIXED TYPE	2 (4%)		
UNDIFFERENTIATED LEUKEMIA		1 (2%)	
#SPLEEN	(51)	(55)	(52)
HEMANGIOMA	1 (2%)		
#MESENTERIC L. NODE	(48)	(44)	(47)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#RENAL LYMPH NODE	(48)	(44)	(47)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#JEJUNUM	(50)	(54)	(53)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (2%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(54)	(54)	(52)
HEPATOCELLULAR ADENOMA	4 (7%)		
HEPATOCELLULAR CARCINOMA	24 (44%)	13 (24%)	7 (13%)
FIBROSARCOMA		1 (2%)	
#PANCREAS	(49)	(53)	(53)
FIBROSARCOMA		1 (2%)	
#STOMACH	(51)	(53)	(51)
FIBROSARCOMA		1 (2%)	
URINARY SYSTEM			
#KIDNEY/PELVIS	(54)	(55)	(51)
TRANSITIONAL-CELL PAPILLOMA	1 (2%)		
#URINARY BLADDER	(48)	(55)	(53)
NEOPLASM, NOS		1 (2%)	
TRANSITIONAL-CELL PAPILLOMA		2 (4%)	7 (13%)
TRANSITIONAL-CELL CARCINOMA			15 (28%)
ENDOCRINE SYSTEM			
#ADRENAL	(50)	(53)	(52)
CORTICAL ADENOMA	1 (2%)		
#ADRENAL/CAPSULE	(50)	(53)	(52)
ADENOMA, NOS	5 (10%)		

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#THYROID	(48)	(49)	(39)
FOLLICULAR-CELL ADENOMA		1 (2%)	
FOLLICULAR-CELL CARCINOMA		1 (2%)	
#PANCREATIC ISLETS	(49)	(53)	(53)
ISLET-CELL ADENOMA	2 (4%)		
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(55)	(55)	(53)
CYSTADENOMA, NOS	1 (2%)		1 (2%)
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(55)	(55)	(53)
FIBROSARCOMA		1 (2%)	
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM			
FIBROSARCOMA		1	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	55	55	55
NATURAL DEATH@	9	4	6
MORIBUND SACRIFICE	2	8	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	44	43	43
ANIMAL MISSING			1

@ INCLUDES AUTOLYZED ANIMALS

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	43	27	30
TOTAL PRIMARY TUMORS	59	37	35
TOTAL ANIMALS WITH BENIGN TUMORS	22	5	11
TOTAL BENIGN TUMORS	24	6	11
TOTAL ANIMALS WITH MALIGNANT TUMORS	29	25	23
TOTAL MALIGNANT TUMORS	35	30	24
TOTAL ANIMALS WITH SECONDARY TUMORS#	4	1	
TOTAL SECONDARY TUMORS	5	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED *o*-ANISIDINE HYDROCHLORIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	55	55	55
ANIMALS NECROPSIED	55	52	54
ANIMALS EXAMINED HISTOPATHOLOGICALLY	55	52	54
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(55)	(52)	(54)
CARCINOMA, NOS			1 (2%)
SQUAMOUS CELL CARCINOMA			1 (2%)
ADENOCARCINOMA, NOS			1 (2%)
LEIOMYOSARCOMA			1 (2%)
HEMANGIOMA	1 (2%)		
HEMANGIOSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(55)	(51)	(52)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
HEPATOCELLULAR CARCINOMA, METAST	2 (4%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (5%)	2 (4%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(55)	(52)	(54)
MALIGNANT LYMPHOMA, NOS	1 (2%)	9 (17%)	5 (9%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (4%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
MALIGNANT LYMPHOMA, MIXED TYPE	6 (11%)		
LYMPHOCYTIC LEUKEMIA	4 (7%)		
#SPLEEN	(53)	(52)	(51)
NEOPLASM, NOS		1 (2%)	
HEMANGIOSARCOMA	2 (4%)	1 (2%)	
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIGNANT LYMPHOMA, MIXED TYPE	1 (2%)		
#MANDIBULAR L. NODE	(47)	(45)	(47)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE	(47)	(45)	(47)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
MALIGNANT LYMPHOMA, MIXED TYPE	2 (4%)		
#LIVER	(54)	(52)	(53)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
#THYMUS	(35)	(31)	(41)
THYMOMA	1 (3%)		
MALIGNANT LYMPHOMA, NOS		1 (3%)	1 (2%)
CIRCULATORY SYSTEM			
#HEART	(55)	(51)	(51)
HEMANGIOMA	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(54)	(52)	(53)
HEPATOCELLULAR ADENOMA	4 (7%)		
NEOPLASTIC NODULE		1 (2%)	
HEPATOCELLULAR CARCINOMA	7 (13%)		4 (8%)
HEMANGIOMA	1 (2%)		
#PANCREAS	(49)	(52)	(52)
NEOPLASM, NOS		1 (2%)	
#STOMACH	(53)	(52)	(53)
NEOPLASM, NOS		1 (2%)	
SQUAMOUS CELL PAPILLOMA	2 (4%)		
#DUODENUM	(52)	(52)	(52)
NEOPLASM, NOS		1 (2%)	
*ANUS	(55)	(52)	(54)
SQUAMOUS CELL PAPILLOMA			1 (2%)
URINARY SYSTEM			
#URINARY BLADDER	(50)	(51)	(50)
NEOPLASM, NOS		1 (2%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TRANSITIONAL-CELL PAPILLOMA		1 (2%)	4 (8%)
TRANSITIONAL-CELL CARCINOMA			18 (36%)
ENDOCRINE SYSTEM			
#PITUITARY	(42)	(40)	(43)
CHROMOPHOBE ADENOMA	2 (5%)		
BASOPHIL ADENOMA	1 (2%)		
#ADRENAL	(50)	(52)	(52)
PHEOCHROMOCYTOMA	1 (2%)		
#THYROID	(48)	(39)	(38)
FOLLICULAR-CELL ADENOMA	1 (2%)	1 (3%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(55)	(52)	(54)
ADENOCARCINOMA, NOS		1 (2%)	
ACINAR-CELL CARCINOMA	1 (2%)		
FIBROADENOMA	1 (2%)		
#UTERUS	(54)	(51)	(50)
NEOPLASM, NOS, MALIGNANT	1 (2%)		
ENDOMETRIAL STROMAL POLYP		1 (2%)	
#OVARY	(50)	(46)	(52)
TERATOMA, NOS		1 (2%)	
HEMANGIOMA		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(55)	(52)	(54)
ADENOMA, NOS			2 (4%)
*EXTERNAL EAR	(55)	(52)	(54)
FIBROSARCOMA		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*BODY CAVITIES	(55)	(52)	(54)
MESOTHELIOMA, NOS	1 (2%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(55)	(52)	(54)
NEOPLASM, NOS		1 (2%)	
OMENTUM			
NEOPLASM, NOS		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	55	55	55
NATURAL DEATH@	7	12	5
MORIBUND SACRIFICE	4	5	7
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	44	38	42
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	34	20	33
TOTAL PRIMARY TUMORS	50	30	41
TOTAL ANIMALS WITH BENIGN TUMORS	15	6	7
TOTAL BENIGN TUMORS	19	6	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	27	13	29
TOTAL MALIGNANT TUMORS	30	15	33
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		1
TOTAL SECONDARY TUMORS	2		2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	4	
TOTAL UNCERTAIN TUMORS	1	9	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN RATS FED o-ANISIDINE HYDROCHLORIDE IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED o-ANISIDINE HYDROCHLORIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	55	55	55
ANIMALS NECROPSIED	54	55	53
ANIMALS EXAMINED HISTOPATHOLOGICALLY	54	55	53
INTEGUMENTARY SYSTEM			
*SKIN	(54)	(55)	(53)
INFLAMMATION, NECROTIZING			1 (2%)
*SUBCUT TISSUE	(54)	(55)	(53)
ABSCESS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#TRACHEA	(51)	(53)	(47)
INFLAMMATION, CHRONIC		1 (2%)	
METAPLASIA, SQUAMOUS		1 (2%)	
#LUNG/BRONCHUS	(54)	(55)	(51)
BRONCHIECTASIS	1 (2%)		
#LUNG	(54)	(55)	(51)
BRONCHOPNEUMONIA, NOS	2 (4%)		
BRONCHOPNEUMONIA, ACUTE			1 (2%)
PNEUMONIA, CHRONIC MURINE	2 (4%)	2 (4%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(52)	(55)	(47)
FIBROSIS, FOCAL	1 (2%)		
HYPERPLASIA, NOS	7 (13%)		
#SPLEEN	(54)	(55)	(52)
FIBROSIS, FOCAL	1 (2%)		
METAMORPHOSIS FATTY			1 (2%)
HEMOSIDEROSIS	1 (2%)		
HEMATOPOIESIS	1 (2%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ERYTHROPOIESIS		1 (2%)	
#LYMPH NODE	(53)	(47)	(34)
HYPERPLASIA, NOS		1 (2%)	
#MANDIBULAR L. NODE	(53)	(47)	(34)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, PLASMA CELL		1 (2%)	
#LUMBAR LYMPH NODE	(53)	(47)	(34)
HYPERPLASIA, PLASMA CELL		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(54)	(55)	(51)
THROMBUS, MURAL	1 (2%)		
PERIARTERITIS	1 (2%)		
NECROSIS, FOCAL			2 (4%)
CALCIFICATION, NOS			3 (6%)
#MYOCARDIUM	(54)	(55)	(51)
INFLAMMATION, FOCAL	1 (2%)		
DEGENERATION, NOS	16 (30%)		
#CARDIAC VALVE	(54)	(55)	(51)
INFLAMMATION, CHRONIC		1 (2%)	
*AORTA	(54)	(55)	(53)
MEDIAL CALCIFICATION		1 (2%)	3 (6%)
*CORONARY ARTERY	(54)	(55)	(53)
MEDIAL CALCIFICATION			2 (4%)
*CELIAC ARTERY	(54)	(55)	(53)
THROMBOSIS, NOS	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(54)	(55)	(52)
CHOLANGIOFIBROSIS	9 (17%)		
NECROSIS, FOCAL	1 (2%)		
NECROSIS, FAT	1 (2%)		
METAMORPHOSIS FATTY		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BASOPHILIC CYTO CHANGE		2 (4%)	1 (2%)
CLEAR-CELL CHANGE	1 (2%)		
*BILE DUCT	(54)	(55)	(53)
INFLAMMATION, NOS	1 (2%)		
#PANCREAS	(53)	(54)	(51)
DILATATION/DUCTS	1 (2%)		
INFLAMMATION, CHRONIC		1 (2%)	
FIBROSIS		1 (2%)	
#STOMACH	(53)	(55)	(52)
ULCER, NOS	2 (4%)		
EROSION	1 (2%)		
HYPERPLASIA, BASAL CELL	14 (26%)		
#GASTRIC MUCOSA	(53)	(55)	(52)
ULCER, NOS		1 (2%)	
CALCIFICATION, NOS		1 (2%)	2 (4%)
CALCIFICATION, FOCAL			1 (2%)
URINARY SYSTEM			
#KIDNEY	(53)	(55)	(53)
HYDRONEPHROSIS		2 (4%)	3 (6%)
PYELONEPHRITIS, NOS		1 (2%)	
PYELONEPHRITIS, ACUTE			1 (2%)
NEPHROSIS, NOS	26 (49%)	43 (78%)	5 (9%)
NEPHROSIS, CHOLEMIC	2 (4%)		
GLOMERULOSCLEROSIS, NOS			2 (4%)
#KIDNEY/MEDULLA	(53)	(55)	(53)
CALCIFICATION, NOS			1 (2%)
#RENAL PAPILLA	(53)	(55)	(53)
INFLAMMATION, SUPPURATIVE			1 (2%)
NECROSIS, NOS		5 (9%)	3 (6%)
CALCIFICATION, NOS		1 (2%)	3 (6%)
#KIDNEY/TUBULE	(53)	(55)	(53)
NEPHROSIS, NOS			1 (2%)
NECROSIS, NOS	1 (2%)		1 (2%)
CALCIFICATION, NOS		2 (4%)	2 (4%)
#KIDNEY/PELVIS	(53)	(55)	(53)
INFLAMMATION, SUPPURATIVE			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, ACUTE FOCAL			1 (2%)
HYPERPLASIA, EPITHELIAL			2 (4%)
#URINARY BLADDER	(51)	(54)	(52)
HYPERPLASIA, EPITHELIAL		1 (2%)	
#U.BLADDER/SUBMUCOSA	(51)	(54)	(52)
HYPERPLASIA, NOS		1 (2%)	
*PROSTATIC URETHRA	(54)	(55)	(53)
INFLAMMATION, SUPPURATIVE			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(49)	(47)
HEMORRHAGE	1 (2%)		
HYPERPLASIA, FOCAL	1 (2%)		
HYPERPLASIA, BASOPHILIC	2 (4%)		
#ADRENAL	(54)	(55)	(53)
CYST, NOS	1 (2%)		
#ADRENAL CORTEX	(54)	(55)	(53)
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, FOCAL		1 (2%)	
#THYROID	(53)	(40)	(40)
CYSTIC FOLLICLES		10 (25%)	3 (8%)
FOLLICULAR CYST, NOS			3 (8%)
CALCIFICATION, FOCAL			1 (3%)
HYPERPLASIA, FOLLICULAR-CELL			3 (8%)
#PANCREATIC ISLETS	(53)	(54)	(51)
HYPERPLASIA, NOS	1 (2%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(54)	(55)	(53)
INFLAMMATION, ACUTE		1 (2%)	
ABSCESS, NOS	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
#PROSTATE	(52)	(52)	(52)
INFLAMMATION, SUPPURATIVE		6 (12%)	7 (13%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION ACUTE AND CHRONIC	1 (2%)		
#TESTIS	(54)	(52)	(51)
ATROPHY, NOS			3 (6%)
*SCROTUM	(54)	(55)	(53)
ULCER, ACUTE		1 (2%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(54)	(53)	(51)
INFLAMMATION, NOS		1 (2%)	
#CEREBRAL VENTRICLE	(54)	(53)	(51)
HEMORRHAGE	1 (2%)		
#BRAIN	(54)	(53)	(51)
HEMORRHAGE	1 (2%)		
SPECIAL SENSE ORGANS			
*EYE	(54)	(55)	(53)
HEMORRHAGE	1 (2%)		
*EYE/IRIS	(54)	(55)	(53)
INFLAMMATION, CHRONIC		1 (2%)	
*EYE/CRYSTALLINE LENS	(54)	(55)	(53)
CALCIFICATION, NOS		1 (2%)	
*EYE/LACRIMAL GLAND	(54)	(55)	(53)
INFLAMMATION, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(54)	(55)	(53)
NECROSIS, FAT	9 (17%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	
AUTOLYSIS/NO NECROPSY	1		2

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED o-ANISIDINE HYDROCHLORIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	55	55	55
ANIMALS NECROPSIED	54	53	54
ANIMALS EXAMINED HISTOPATHOLOGICALLY	54	53	54
INTEGUMENTARY SYSTEM			
*SKIN	(54)	(53)	(54)
ULCER, FOCAL		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(53)	(53)	(54)
INFLAMMATION, SUPPURATIVE		2 (4%)	
#LUNG	(53)	(53)	(54)
BRONCHOPNEUMONIA, NOS		1 (2%)	
PNEUMONIA, CHRONIC MURINE	3 (6%)		
METAPLASIA, NOS	1 (2%)		
#ALVEOLAR WALL	(53)	(53)	(54)
CALCIFICATION, NOS		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(53)	(49)	(51)
HISTIOCYTOSIS	1 (2%)		
#SPLEEN	(52)	(52)	(51)
HEMATOPOIESIS	1 (2%)		
ERYTHROPOIESIS		4 (8%)	
#CERVICAL LYMPH NODE	(51)	(46)	(36)
HYPERPLASIA, PLASMA CELL		1 (2%)	
#LUMBAR LYMPH NODE	(51)	(46)	(36)
INFLAMMATION, CHRONIC	1 (2%)		
HYPERPLASIA, NOS		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#RENAL LYMPH NODE	(51)	(46)	(36)
INFLAMMATION, CHRONIC	1 (2%)		
HYPERPLASIA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(53)	(53)	(53)
CALCIFICATION, NOS		1 (2%)	
#MYOCARDIUM	(53)	(53)	(53)
DEGENERATION, NOS	6 (11%)	1 (2%)	
*AORTA	(54)	(53)	(54)
MEDIAL CALCIFICATION		2 (4%)	1 (2%)
*CORONARY ARTERY	(54)	(53)	(54)
MEDIAL CALCIFICATION		2 (4%)	
DIGESTIVE SYSTEM			
#LIVER	(53)	(53)	(53)
CHOLANGIOFIBROSIS	2 (4%)		
NECROSIS, NOS		1 (2%)	
METAMORPHOSIS FATTY	6 (11%)		
BASOPHILIC CYTO CHANGE	10 (19%)	10 (19%)	
ANGIECTASIS		1 (2%)	
#PANCREAS	(52)	(50)	(43)
ATROPHY, FOCAL	1 (2%)		
#STOMACH	(51)	(51)	(49)
INFLAMMATION, NOS		1 (2%)	
ULCER, NOS	1 (2%)		
ULCER, ACUTE		1 (2%)	
CALCIFICATION, NOS		1 (2%)	
HYPERPLASIA, BASAL CELL	11 (22%)		
#GASTRIC MUCOSA	(51)	(51)	(49)
ULCER, FOCAL		1 (2%)	
CALCIFICATION, NOS		1 (2%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(52)	(52)	(54)
EMBRYONAL DUCT CYST		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYDRONEPHROSIS		7 (13%)	14 (26%)
PYELONEPHRITIS, NOS			3 (6%)
NEPHROSIS, NOS	2 (4%)	3 (6%)	
NEPHROSIS, CHOLEMIC	1 (2%)		
GLOMERULOSCLEROSIS, NOS	1 (2%)		
CALCIFICATION, NOS		1 (2%)	
CALCIFICATION, FOCAL	5 (10%)		
HYPERPLASIA, TUBULAR CELL			1 (2%)
#RENAL PAPILLA	(52)	(52)	(54)
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, ACUTE			1 (2%)
NECROSIS, NOS		6 (12%)	20 (37%)
CALCIFICATION, NOS		5 (10%)	5 (9%)
#KIDNEY/TUBULE	(52)	(52)	(54)
CALCIFICATION, NOS		3 (6%)	2 (4%)
#KIDNEY/PELVIS	(52)	(52)	(54)
INFLAMMATION, ACUTE		1 (2%)	
HYPERPLASIA, EPITHELIAL		2 (4%)	
#URINARY BLADDER	(49)	(49)	(51)
CALCIFICATION, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(51)	(45)
HYPERPLASIA, NOS	1 (2%)		
ANGIECTASIS		1 (2%)	
#ADRENAL CORTEX	(53)	(53)	(54)
HYPERPLASIA, NOS	1 (2%)		
#ADRENAL MEDULLA	(53)	(53)	(54)
NECROSIS, NOS	1 (2%)		
#THYROID	(49)	(45)	(46)
CYSTIC FOLLICLES		3 (7%)	3 (7%)
HYPERPLASIA, C-CELL			2 (4%)
HYPERPLASIA, FOLLICULAR-CELL			2 (4%)
#PARATHYROID	(15)	(12)	(17)
HYPERPLASIA, NOS	1 (7%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC ISLETS	(52)	(50)	(43)
HYPERPLASIA, NOS	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(54)	(53)	(54)
GALACTOCELE	6 (11%)		
*CLITORAL GLAND	(54)	(53)	(54)
INFLAMMATION, ACUTE		4 (8%)	1 (2%)
#UTERUS	(52)	(50)	(50)
HYDROMETRA	2 (4%)		
THROMBOSIS, NOS	1 (2%)		
ABSCESS, NOS		1 (2%)	
#CERVIX UTERI	(52)	(50)	(50)
POLYP, INFLAMMATORY	1 (2%)		
#UTERUS/ENDOMETRIUM	(52)	(50)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, ACUTE		1 (2%)	
ABSCESS, NOS		1 (2%)	
HYPERPLASIA, CYSTIC		3 (6%)	1 (2%)
#OVARY/OVIDUCT	(52)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
ABSCESS, NOS	1 (2%)		
#OVARY	(53)	(49)	(45)
CYST, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(54)	(53)	(54)
INFLAMMATION, ACUTE		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(54)	(53)	(54)
METAPLASIA, OSSEOUS			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(54)	(53)	(54)
NECROSIS, FAT	6 (11%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			1
AUTOLYSIS/NO NECROPSY	1	2	1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN MICE FED o-ANISIDINE HYDROCHLORIDE IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED o-ANISIDINE HYDROCHLORIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	55	55	55
ANIMALS MISSING			1
ANIMALS NECROPSIED	55	55	53
ANIMALS EXAMINED HISTOPATHOLOGICALLY	55	55	53
INTEGUMENTARY SYSTEM			
*SKIN	(55)	(55)	(53)
EPIDERMAL INCLUSION CYST	1 (2%)		
POLYP, INFLAMMATORY	1 (2%)		
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(51)	(55)	(52)
HEMATOPOIESIS	2 (4%)		1 (2%)
#MESENTERIC L. NODE	(48)	(44)	(47)
CONGESTION, NOS	6 (13%)		
HYPERPLASIA, NOS	1 (2%)	2 (5%)	
HISTIOCYTOSIS	1 (2%)		
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(54)	(54)	(52)
NECROSIS, NOS		1 (2%)	
METAMORPHOSIS FATTY			3 (6%)
MEGALOCYTOSIS			3 (6%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASTIC NODULE		1 (2%)	1 (2%)
HYPERPLASIA, NOS	1 (2%)		
ANGIECTASIS			1 (2%)
HEMATOPOIESIS			2 (4%)
#PANCREAS	(49)	(53)	(53)
CYSTIC DUCTS		1 (2%)	
ATROPHY, NOS	1 (2%)		
#STOMACH	(51)	(53)	(51)
INFLAMMATION, ACUTE	1 (2%)		
ATYPIA, NOS	1 (2%)		
HYPERPLASTIC NODULE		1 (2%)	
#PEYERS PATCH	(50)	(54)	(53)
HYPERPLASIA, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(54)	(55)	(51)
HYDRONEPHROSIS	1 (2%)		
CYST, NOS		1 (2%)	
PYELONEPHRITIS, ACUTE	1 (2%)		
PYELONEPHRITIS, CHRONIC	1 (2%)		
#KIDNEY/GLOMERULUS	(54)	(55)	(51)
AMYLOIDOSIS			1 (2%)
#URINARY BLADDER	(48)	(55)	(53)
CALCULUS, NOS	1 (2%)		
HYPERPLASIA, EPITHELIAL	1 (2%)	2 (4%)	21 (40%)
ENDOCRINE SYSTEM			
#PANCREATIC ISLETS	(49)	(53)	(53)
HYPERPLASIA, NOS	12 (24%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(55)	(55)	(53)
CALCULUS, NOS	1 (2%)		
#PROSTATE	(52)	(53)	(51)
INFLAMMATION, ACUTE	1 (2%)		

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(55)	(55)	(53)
INFLAMMATION, ACUTE	1 (2%)		
CATARACT	1 (2%)		
*HARDERIAN GLAND	(55)	(55)	(53)
INFLAMMATION, ACUTE			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(55)	(55)	(53)
NECROSIS, FAT	5 (9%)		
*MESENTERY	(55)	(55)	(53)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
ALL OTHER SYSTEMS			
OMENTUM			
HEMATOMA, NOS	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	8	23	6
ANIMAL MISSING/NO NECROPSY			1
AUTO/NECROPSY/HISTO PERF	1	1	
AUTOLYSIS/NO NECROPSY			1

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED o-ANISIDINE HYDROCHLORIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	55	55	55
ANIMALS NECROPSIED	55	52	54
ANIMALS EXAMINED HISTOPATHOLOGICALLY	55	52	54
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(55)	(51)	(52)
ATELECTASIS	1 (2%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(52)	(51)	(51)
MYELOFIBROSIS	31 (60%)		
HYPERPLASIA, HEMATOPOIETIC	1 (2%)		
#SPLEEN	(53)	(52)	(51)
HYPERPLASIA, LYMPHOID			1 (2%)
HEMATOPOIESIS	1 (2%)	1 (2%)	
#MESENTERIC L. NODE	(47)	(45)	(47)
CONGESTION, NOS	1 (2%)		
HYPERPLASIA, NOS	2 (4%)		
HYPERPLASIA, LYMPHOID			2 (4%)
CIRCULATORY SYSTEM			
#HEART	(55)	(51)	(51)
PERIARTERITIS	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(54)	(52)	(53)
NODULE		1 (2%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
METAMORPHOSIS FATTY		2 (4%)	
MEGALOCYTOSIS		1 (2%)	
HEMATOPOIESIS		1 (2%)	
#PANCREAS	(49)	(52)	(52)
DILATATION/DUCTS	1 (2%)		
CYSTIC DUCTS			1 (2%)
INFLAMMATION, CHRONIC	2 (4%)		
METAMORPHOSIS FATTY			1 (2%)
ATROPHY, NOS	1 (2%)		
#STOMACH	(53)	(52)	(53)
ULCER, NOS	1 (2%)		
EROSION	1 (2%)		
HYPERPLASIA, EPITHELIAL	2 (4%)		
#GASTRIC SEROSA	(53)	(52)	(53)
STEATITIS		1 (2%)	
NECROSIS, FAT		1 (2%)	
#PEYERS PATCH	(52)	(52)	(52)
HYPERPLASIA, LYMPHOID			1 (2%)
URINARY SYSTEM			
#KIDNEY	(55)	(52)	(54)
HEMATOMA, ORGANIZED			1 (2%)
STEATITIS		1 (2%)	
PYELONEPHRITIS, CHRONIC	1 (2%)		
NECROSIS, FAT		1 (2%)	
#URINARY BLADDER	(50)	(51)	(50)
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		3 (6%)
INFLAMMATION, CHRONIC DIFFUSE		1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)	12 (24%)
ENDOCRINE SYSTEM			
#ADRENAL	(50)	(52)	(52)
INFLAMMATION, ACUTE		1 (2%)	
#THYROID	(48)	(39)	(38)
CYSTIC FOLLICLES			1 (3%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC ISLETS	(49)	(52)	(52)
HYPERPLASIA, NOS	3 (6%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(54)	(51)	(50)
HYDROMETRA	3 (6%)		1 (2%)
#UTERUS/ENDOMETRIUM	(54)	(51)	(50)
HYPERPLASIA, CYSTIC	15 (28%)	36 (71%)	39 (78%)
#OVARY	(50)	(46)	(52)
CYST, NOS	7 (14%)	6 (13%)	5 (10%)
HEMORRHAGIC CYST	1 (2%)		
INFLAMMATION, ACUTE		1 (2%)	
ABSCESS, NOS	1 (2%)		
NERVOUS SYSTEM			
#BRAIN/MENINGES	(55)	(48)	(52)
INFLAMMATION, NOS	1 (2%)		
FIBROSIS, FOCAL			1 (2%)
#BRAIN	(55)	(48)	(52)
HYDROCEPHALUS, NOS	2 (4%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(55)	(52)	(54)
NECROSIS, FAT	7 (13%)		
*MESENTERY	(55)	(52)	(54)
CYST, NOS	1 (2%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(55)	(52)	(54)
PERIARTERITIS	1 (2%)		
OMENTUM			
STEATITIS		1	
NECROSIS, FAT		1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	4	1
AUTO/NECROPSY/HISTO PERF			1
AUTOLYSIS/NO NECROPSY		3	1

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS

IN RATS FED o-ANISIDINE HYDROCHLORIDE IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed o-Anisidine Hydrochloride in the Diet[a]

Topography: Morphology	Control	Low Dose	High Dose
Integumentary System: Fibroma[b]	4/54 (7)	0/55 (0)	0/53 (0)
P Values[c,d]	P = 0.015(N)	N.S.	N.S.
Relative Risk[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.062	1.101
Weeks to First Observed Tumor	106	--	--
Hematopoietic System: Lymphoma or Leukemia[b]	18/54 (33)	1/55 (2)	1/53 (2)
P Values[c,d]	P < 0.001(N)	P < 0.001(N)	P < 0.001(N)
Departure from Linear Trend[e]	P = 0.004		
Relative Risk[f]		0.055	0.057
Lower Limit		0.001	0.001
Upper Limit		0.324	0.336
Weeks to First Observed Tumor	84	98	40

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Neoplastic Nodule or Hepatocellular Carcinoma[b]	0/54 (0)	4/55 (7)	0/52 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.005		
Relative Risk[f]		Infinite	--
Lower Limit		0.908	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	85	--
Kidney or Kidney Pelvis: Transitional-cell Carcinoma[b]	0/53 (0)	3/55 (5)	7/53 (13)
P Values[c,d]	P = 0.005	N.S.	P = 0.006
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.578	1.937
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	64	51

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Urinary Bladder: Transitional-cell Carcinoma[b]	0/51 (0)	50/54 (93)	51/52 (98)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk[f]		Infinite	Infinite
Lower Limit		17.045	19.525
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	64	48
Urinary Bladder: Transitional-cell Papilloma or Carcinoma[b]	0/51 (0)	52/54 (96)	52/52 (100)
P Values[c,d]	P < 0.001	P <0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk[f]		Infinite	Infinite
Lower Limit		18.455	21.590
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	64	40

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	4/48 (8)	0/49 (0)	0/47 (0)
P Values[c,d]	P = 0.015(N)	N.S.	N.S.
Relative Risk[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.056	1.100
Weeks to First Observed Tumor	90	--	--
Pituitary: Adenoma, NOS, or Chromophobe Adenoma[b]	4/48 (8)	2/49 (4)	0/47 (0)
P Values[c,d]	P = 0.038(N)	N.S.	N.S.
Relative Risk[f]		0.490	0.000
Lower Limit		0.046	0.000
Upper Limit		3.246	1.100
Weeks to First Observed Tumor	90	103	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant[b]	14/54 (26)	0/55 (0)	0/53 (0)
P Values[c,d]	P < 0.001(N)	P < 0.001(N)	P < 0.001(N)
Departure from Linear Trend[e]	P = 0.005		
Relative Risk[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		0.226	0.234
Weeks to First Observed Tumor	73	--	--
Thyroid: C-cell Carcinoma[b]	0/53 (0)	2/40 (5)	0/40 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.030		
Relative Risk[f]		Infinite	--
Lower Limit		0.392	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	87	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-cell Adenoma or Carcinoma[b]	3/53 (6)	3/40 (8)	0/40 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.325	0.000
Lower Limit		0.186	0.000
Upper Limit		9.370	2.190
Weeks to First Observed Tumor	106	87	--
Thyroid: Follicular-cell Carcinoma[b]	0/53(0)	2/40 (5)	2/40 (5)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.392	0.392
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	104	78

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid or Thyroid Follicle: Follicular-cell Carcinoma or Papillary Cystadenocarcinoma, NOS[b]	0/53 (0)	3/40 (8)	2/40 (5)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.797	0.392
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	91	78
Thyroid or Thyroid Follicle: Follicular-cell Adenoma, Cystadenoma, or Papillary Cystadenoma, NOS[b]	0/53 (0)	4/40 (10)	4/40 (10)
P Values[c,d]	P = 0.030	P = 0.031	P = 0.031
Relative Risk[f]		Infinite	Infinite
Lower Limit		1.229	1.229
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	92	55

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: All Follicular-cell Tumors[g]	0/53 (0)	7/40 (17)	6/40 (15)
P Values[c,d]	P = 0.009	P = 0.002	P = 0.005
Relative Risk[f]		Infinite	Infinite
Lower Limit		2.574	2.121
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	91	55
Testis: Interstitial-cell Tumor[b]	53/54 (98)	43/52 (83)	4/51 (8)
P Values[c,d]	P < 0.001(N)	P = 0.007(N)	P < 0.001(N)
Departure from Linear Trend[e]	P < 0.001		
Relative Risk[f]		0.843	0.080
Lower Limit		0.809	0.060
Upper Limit		0.968	0.161
Weeks to First Observed Tumor	73	71	62

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Body Cavities: Mesothelioma or Mesothelioma, Malignant[b]	2/54 (4)	6/55 (11)	1/53 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.033		
Relative Risk[f]		2.945	0.509
Lower Limit		0.556	0.009
Upper Limit		28.827	9.485
Weeks to First Observed Tumor	87	77	61

[a]Dosed groups received 5,000 or 10,000 ppm in feed.

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

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

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

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

[g]These tumors include follicular-cell adenoma, follicular-cell carcinoma, cystadenoma, NOS, papillary cystadenoma, NOS and papillary cystadenocarcinoma, NOS.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed o-Anisidine Hydrochloride in the Diet[a]

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia[b]	9/54 (17)	1/53 (2)	0/54 (0)
P Values[c,d]	$P < 0.001$(N)	$P = 0.009$(N)	$P = 0.001$(N)
Relative Risk[f]		0.113	0.000
Lower Limit		0.003	0.000
Upper Limit		0.773	0.382
Weeks to First Observed Tumor	91	78	--
Salivary Gland: Adenoma, NOS[b]	3/52 (6)	0/51 (0)	0/47 (0)
P Values[c,d]	$P = 0.043$(N)	N.S.	N.S.
Relative Risk[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.697	1.837
Weeks to First Observed Tumor	107	--	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Urinary Bladder: Transitional-cell Papilloma[b]	0/49 (0)	5/49 (10)	0/51 (0)
P Values[c,d]	N.S.	P = 0.028	N.S.
Departure from Linear Trend[e]	P = 0.002		
Relative Risk[f]		Infinite	--
Lower Limit		1.262	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	74	--
Urinary Bladder: Transitional-cell Carcinoma[b]	0/49 (0)	41/49 (84)	50/51 (98)
P Values[c,d]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk[f]		Infinite	Infinite
Lower Limit		14.250	18.754
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	61	36

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Urinary Bladder: Transitional-cell Papilloma or Carcinoma[b]	0/49 (0)	46/49 (94)	50/51 (98)
P Values[c,d]	$P < 0.001$	$P < 0.001$	$P < 0.001$
Departure from Linear Trend[e]	$P < 0.001$		
Relative Risk[f]		Infinite	Infinite
Lower Limit		16.833	18.754
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	61	36
Pituitary: Carcinoma, NOS[b]	3/48 (6)	0/51 (0)	0/45 (0)
P Values[c,d]	$P = 0.039$(N)	N.S.	N.S.
Relative Risk[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.565	1.768
Weeks to First Observed Tumor	89	--	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	15/48 (31)	0/51 (0)	0/45 (0)
P Values[c,d]	P < 0.001(N)	P < 0.001(N)	P < 0.001(N)
Departure from Linear Trend[e]	P = 0.003		
Relative Risk[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		0.200	0.226
Weeks to First Observed Tumor	74	--	--
Pituitary : Chromophobe Carcinoma or Carcinoma, NOS[b]	4/48 (8)	0/51 (0)	0/45 (0)
P Values[c,d]	P = 0.016(N)	N.S.	N.S.
Relative Risk[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.016	1.147
Weeks to First Observed Tumor	89	--	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Adenoma, NOS, Chromophobe Adenoma, or Acidophil Adenoma[b]	17/48 (35)	9/51 (18)	1/45 (2)
P Values[c,d]	$P < 0.001$(N)	$P = 0.037$(N)	$P < 0.001$(N)
Relative Risk[f]		0.498	0.063
Lower Limit		0.219	0.002
Upper Limit		1.060	0.372
Weeks to First Observed Tumor	74	72	66
Adrenal: Pheochromocytoma[b]	3/53 (6)	1/53 (2)	1/54 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.333	0.327
Lower Limit		0.006	0.006
Upper Limit		3.993	3.921
Weeks to First Observed Tumor	107	97	55

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma[b]	3/49 (6)	1/45 (2)	0/46 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.363	0.000
Lower Limit		0.007	0.000
Upper Limit		4.321	1.767
Weeks to First Observed Tumor	107	95	--
Thyroid C-cell Adenoma or Carcinoma[b]	4/49 (8)	1/45 (2)	0/46 (0)
P Values[c,d]	P = 0.031(N)	N.S.	N.S.
Relative Risk[f]		0.272	0.000
Lower Limit		0.006	0.000
Upper Limit		2.615	1.146
Weeks to First Observed Tumor	107	95	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: Follicular-cell Carcinoma[b]	0/49 (0)	3/45 (7)	0/46 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.011		
Relative Risk[f]		Infinite	--
Lower Limit		0.656	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	79	--
Thyroid: All Follicular-cell Tumors[g]	1/49 (2)	4/45 (9)	3/46 (7)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		4.356	3.196
Lower Limit		0.453	0.268
Upper Limit		209.417	164.012
Weeks to First Observed Tumor	77	79	57

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Subcutaneous Tissue or Mammary Gland: Fibroadenoma[b]	16/54 (30)	2/53 (4)	0/54 (0)
P Values[c,d]	P < 0.001(N)	P < 0.001(N)	P < 0.001(N)
Departure from Linear Trend[e]	P = 0.033		
Relative Risk[f]		0.127	0.000
Lower Limit		0.015	0.000
Upper Limit		0.505	0.198
Weeks to First Observed Tumor	99	90	--
Uterus: Endometrial Stromal Polyp[b]	16/52 (31)	6/50 (12)	0/50 (0)
P Values[c,d]	P < 0.001(N)	P = 0.019(N)	P < 0.001(N)
Relative Risk[f]		0.390	0.000
Lower Limit		0.136	0.000
Upper Limit		0.955	0.206
Weeks to First Observed Tumor	68	66	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

---

[a]Dosed groups received 5,000 or 10,000 ppm in feed.

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

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

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

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

[g]These tumors include undifferentiated carcinoma, follicular-cell adenoma, follicular-cell carcinoma or papillary cystadenoma, NOS.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE FED o-ANISIDINE HYDROCHLORIDE IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed o-Anisidine Hydrochloride in the Diet[a]

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	6/54 (11)	6/54 (11)	0/52 (0)
P Values[c,d]	P = 0.024(N)	N.S.	P = 0.015(N)
Relative Risk[f]		1.000	0.000
Lower Limit		0.285	0.000
Upper Limit		3.508	0.650
Weeks to First Observed Tumor	105	104	--
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	12/54 (22)	9/54 (17)	2/52 (4)
P Values[c,d]	P = 0.006(N)	N.S.	P = 0.005(N)
Relative Risk[f]		0.750	0.173
Lower Limit		0.304	0.020
Upper Limit		1.774	0.727
Weeks to First Observed Tumor	79	104	105

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia[b]	4/55 (7)	4/55 (7)	1/53 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.000	0.259
Lower Limit		0.196	0.005
Upper Limit		5.110	2.510
Weeks to First Observed Tumor	105	71	88
Liver: Hepatocellular Carcinoma[b]	24/54 (44)	13/54 (24)	7/52 (13)
P Values[c,d]	P < 0.001(N)	P = 0.021(N)	P < 0.001(N)
Relative Risk[f]		0.542	0.303
Lower Limit		0.288	0.122
Upper Limit		0.980	0.651
Weeks to First Observed Tumor	53	88	83

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	28/54 (52)	13/54 (24)	7/52 (13)
P Values[c,d]	P < 0.001(N)	P = 0.003(N)	P < 0.001(N)
Relative Risk[f]		0.464	0.260
Lower Limit		0.254	0.108
Upper Limit		0.815	0.543
Weeks to First Observed Tumor	53	88	83
Urinary Bladder: Transitional-cell Carcinoma[b]	0/48 (0)	0/55 (0)	15/53 (28)
P Values[c,d]	P < 0.001	N.S.	P < 0.001
Departure from Linear Trend[e]	P = 0.004		
Relative Risk[f]		--	Infinite
Lower Limit		--	4.267
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	45

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Urinary Bladder: Transitional-cell Papilloma or Carcinoma[b]	0/48 (0)	2/55 (4)	22/53 (42)
P Values[c,d]	P < 0.001	N.S.	P < 0.001
Departure from Linear Trend[e]	P = 0.005		
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.258	6.486
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	104	45
Adrenal/Capsule: Adenoma, NOS[b]	5/50 (10)	0/53 (0)	0/52 (0)
P Values[c,d]	P = 0.006(N)	P = 0.024(N)	P = 0.025(N)
Relative Risk[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		0.749	0.763
Weeks to First Observed Tumor	105	--	--

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

---

[a]Dosed groups received 2,500 or 5,000 ppm in feed.

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

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

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

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed o-Anisidine Hydrochloride in the Diet[a]

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	4/55 (7)	2/51 (4)	1/52 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.539	0.264
Lower Limit		0.050	0.005
Upper Limit		3.586	2.557
Weeks to First Observed Tumor	105	105	105
Hematopoietic System: Lymphoma or Leukemia[b]	18/55 (33)	12/52 (23)	6/54 (11)
P Values[c,d]	P = 0.005(N)	N.S.	P = 0.006(N)
Relative Risk[f]		0.705	0.340
Lower Limit		0.345	0.120
Upper Limit		1.385	0.815
Weeks to First Observed Tumor	86	78	34

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	7/54 (13)	0/52 (0)	4/53 (8)
P Values[c,d]	N.S.	P = 0.007(N)	N.S.
Departure from Linear Trend[e]	P = 0.017		
Relative Risk[f]		0.000	0.582
Lower Limit		0.000	0.132
Upper Limit		0.536	2.148
Weeks to First Observed Tumor	101	--	81
Liver: Hepatocellular Adenoma, Carcinoma, or Neoplastic Nodule[b]	11/54 (20)	1/52 (2)	4/53 (8)
P Values[c,d]	P = 0.020(N)	P = 0.002(N)	N.S.
Departure from Linear Trend[e]	P = 0.018		
Relative Risk[f]		0.094	0.370
Lower Limit		0.002	0.091
Upper Limit		0.613	1.161
Weeks to First Observed Tumor	59	105	81

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Urinary Bladder: Transitional-cell Carcinoma[b]	0/50 (0)	0/51 (0)	18/50 (36)
P Values[c,d]	P < 0.001	N.S.	P < 0.001
Departure from Linear Trend[e]	P = 0.001		
Relative Risk[f]		--	Infinite
Lower Limit		--	5.758
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	81
Urinary Bladder: Transitional-cell Papilloma or Carcinoma[b]	0/50 (0)	1/51 (2)	22/50 (44)
P Values[c,d]	P < 0.001	N.S.	P < 0.001
Departure from Linear Trend[e]	P = 0.001		
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.053	7.163
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	81

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed o-Anisidine Hydrochloride in the Diet[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	2/42 (5)	0/40 (0)	0/43 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		3.526	3.287
Weeks to First Observed Tumor	105	--	--

[a]Treated groups received doses of 2,500 or 5,000 ppm in feed.

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

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

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

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the control group.

APPENDIX G

ANALYSIS OF FORMULATED DIETS FOR

o-ANISIDINE HYDROCHLORIDE CONCENTRATION

APPENDIX G

Analysis of Formulated Diets for o-Anisidine Hydrochloride Concentration

Duplicate 2-g samples of the diet mixtures were each shaken with 50 ml of 95% ethanol for 15 minutes. The mixture was allowed to settle overnight, and the absorbance of the supernatant, after appropriate dilution, was measured at 270 nm against a "blank" extracted from 2 g of the same feed used to prepare the diet mixtures. Concentrations were determined by comparison with standard solutions. Recoveries were determined from duplicate spiked feed samples worked up simultaneously with each set of diet samples. The average recovery from the 0.5% spiked feed samples was 80%.

Theoretical Concentrations In Diet (% in Feed)	No. of Samples	Sample Analytical Mean (% in Feed)	Coefficient of Variation (%)	Range (% in feed)
0.25	6	0.26	24.4	0.16-0.33
0.5	6	0.44	11.8	0.36-0.51
1.0	5	0.97	9.5	0.86-1.11

Review of the Bioassay of *o*-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 *o*-Anisidine Hydrochloride for carcinogenicity.

The primary reviewer said that the compound was carcinogenic in both the treated rats and mice, under the conditions of test. Although a different batch of the test chemical was used for the subchronic and chronic phases, each was reported to be more than 99% pure. Transitional-cell carcinomas and papillomas of the urinary bladder were induced, in a dose-related fashion, in both species. Transitional-cell carcinomas of the renal pelvis and follicular-cell tumors of the thyroid also were induced in treated male rats. Although there were a number of experimental shortcomings, including poor survival, the primary reviewer said that the conclusion on the carcinogenicity of *o*-Anisidine Hydrochloride was still valid.

The secondary reviewer also agreed that *o*-Anisidine Hydrochloride was carcinogenic under the conditions of test. He added that the chemical may pose a carcinogenic risk to humans, especially in occupational situations.

It was moved that the report on the bioassay of *o*-Anisidine Hydrochloride be accepted as written. 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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