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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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FOREWORD: This report presents the results of the bioassay of anilazine 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: This bioassay of anilazine was conducted by Gulf South Research Institute, New Iberia, Louisiana, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design for this bioassay is based on guidelines for carcinogen bioassays in small animals that have been established by NCI[1]. The doses for the chronic studies were selected by Drs. E. E. Storrs[2] and O. G. Fitzhugh[3,4], and the principal investigator was Mr. R. J. Wheeler[2]. Chemicals were analyzed by Mr. Wheeler and dosed feed mixtures by Mr. S. M. Billedeau[2]. The results of these analyses were reviewed by Dr. C. W. Jameson[3]. Histologic examination of animal tissues was performed by Drs. R. A. Ball[2] and E. Bernal[2], and the diagnoses

included in this report represent the interpretation of these pathologists.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[5]. Statistical analyses were performed by Dr. J. R. Joiner[3] and Ms. P. L. Yong[3], using methods selected for the bioassay program by Dr. J. J. Gart[6].

This report was prepared at Tracor Jitco[3] 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, Mr. W. D. Reichardt, and Ms. L. A. Waitz, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley and Ms. P. J. Graboske.

The following scientists at NCI were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Dawn G. Goodman[7], Dr. Richard A. Griesemer, Dr. Morton H. Levitt, Dr. Harry A. Milman, Dr. Thomas Orme, Dr. Robert A. Squire[8], Dr. Sherman Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

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

Groups of 50 rats and 50 mice of each sex were administered anilazine at one of two doses, either 500 or 1,000 ppm, for 103 weeks and then observed for 1-6 additional weeks. Matched controls consisted of 25 untreated rats and 25 untreated mice of each sex. All surviving rats were killed at 103-104 weeks; all surviving mice were killed at 107-109 weeks.

Administration of the anilazine had no appreciable effect on body weight in the rats and female mice; however, there was a decreased gain in mean body weight in the dosed male mice. Survival also was essentially unaffected. Survival in all groups of dosed and control rats and mice was at least 80% at the end of 90 weeks on study, except for the male control mice; thus, sufficient numbers of animals were at risk in most groups for development of late-appearing tumors.

No tumors occurred in dosed rats or mice of either sex at incidences that were significantly higher than those in corresponding controls. Male and female rats and female mice may have been able to tolerate higher doses.

It is concluded that under the conditions of this bioassay, anilazine was not carcinogenic for either Fischer 344 rats or B6C3F1 mice.

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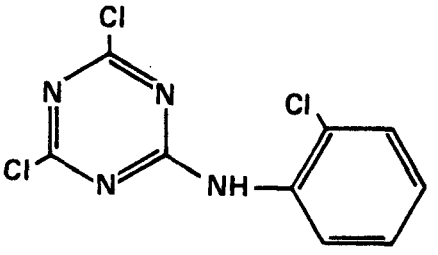
I. INTRODUCTION



Anilazine

Anilazine (CAS 101-05-03; NCI C08684) is the internationally accepted (ISO) generic name for the triazine fungicide 2,4-dichloro-6-(o-chloroanilino)-s-triazine. Anilazine was originally synthesized and screened for herbicidal activity. Although anilazine is virtually nonphytotoxic, it was found to have broad-spectrum fungicidal effects, and was marketed in 1955 as an agricultural fungicide (Spencer, 1973; Gysin, 1972).

Since that time, anilazine has been used to control fungal infections of plant foliage and in some cases, of seeds. In 1975, an estimated 200,000 pounds of anilazine were applied to vegetable

crops in the United States. Smaller amounts of anilazine were used on lawns and turf (Ayers and Johnson, 1976).

In general, many heterocyclic nitrogen compounds are toxic to fungi (Lukens, 1969). Anilazine is a nonspecific fungicide, functioning as an alkylating agent. The triazine ring loses a chlorine atom, and then reacts rapidly with amino and thiol groups by nucleophilic substitution. The second chlorine atom on the triazine ring is equally reactive initially, but loses reactivity following the removal of the first chlorine (Lukens, 1969; Burchfield, 1967). In this way it is conserved for a future reaction at a more specific site. High concentrations are required for fungicidal activity, presumably because the chemical must weaken the cell membrane of the fungus before it can have any critical effects on cell organelles (Lukens, 1969).

This compound was selected for testing because its use results in its distribution in the environment and in food products. It is structurally related to cyanuric acid, which was thought to be carcinogenic at the time anilazine was considered for testing (Pliss and Zabezhinsky, 1970). As an anilino compound, anilazine is related to the monocyclic aromatic amines, such as o-toluidine, which are also carcinogens (Homburger et al., 1972; Russfield et al., 1973).

II. MATERIALS AND METHODS

A. Chemical

Anilazine was obtained in several batches as technical-grade Dyrene® from the Chemagro Agricultural Division of the Mobay Chemical Corp., Kansas City, Missouri. The identity and purity of Lot No. 4050279, which was used in the subchronic studies and part of the chronic studies, was confirmed in analysis at Gulf South Research Institute. The melting range was 151-154°C (literature:159-160°C) (Farm Chemicals Handbook, 1977). Vapor phase chromatography performed using two different stationery phases of differing polarities gave a single peak in each case. Elemental analyses (C, H, Cl, N) were correct for $C_9H_5N_4Cl_3$, the molecular formula of anilazine. Nuclear magnetic resonance, infrared, and ultraviolet spectra were consistent with the structure. No analysis was performed on Lot No. 4050432 of anilazine, which was used for the remainder of the chronic studies.

B. Dietary Preparation

All diets were formulated every week using ground Wayne® Lab Blox animal feed (Allied Mills, Inc., Chicago, Ill.) to which was added the required amount of anilazine. The test compound was first dissolved in a small amount of acetone (Mallinckrodt Inc.,

St. Louis, Mo.) and this solution was then added to the feed. Corn oil (LouAna®, Opelousas Refinery, Opelousas, La.) was also added to the feed, primarily as a dust suppressant. The diets were mixed mechanically in a Hobart blender for not less than 25 minutes to assure homogeneity and to allow for evaporation of the acetone. Final diets, including those for the control groups of animals, contained corn oil equal to 2% of the final weight of feed.

The stability of anilazine in feed was tested by determining the concentration of the compound in formulated diets at intervals over a 7-day period. Diets containing 200 or 2,000 ppm anilazine showed a significant decrease in concentration (57 and 70% compound remaining, respectively) on standing at ambient temperature in an open feeder for this period. Therefore, diet mixtures used in the actual tests were stored at -20°C and were kept for no longer than 1 week.

As a quality control test on the accuracy of preparation of the diets, the concentration of anilazine was determined in randomly selected batches of formulated diets at 8-week intervals during the chronic studies. The results are summarized in Appendix G. At each dietary concentration used in the chronic studies (500 or 1,000 ppm), the mean of the analytical concentrations for the checked samples was within 1.6% of the theoretical concentration,

and the coefficient of variation was never more than 0.04. Thus, the evidence indicates that the formulated diets were prepared accurately.

C. Animals

Fischer 344 rats and B6C3F1 hybrid mice of each sex, obtained through contracts of the Division of Cancer Treatment, NCI, were used in these bioassays. The rats and mice were bred at and supplied from the Frederick Cancer Research Center, Frederick, Maryland. On arrival at the laboratory, the rats were quarantined for 14 days and the mice for 30 days. Following quarantine, animals were assigned to dosed or control groups. The rats were 45 days of age and the mice were 73 days of age when placed on study.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature range was 22-24°C, and the relative humidity was maintained at 40-70%. The air in each room was filtered through permanent air maze filters (Air Maze Incom International, Cleveland, Ohio), and room air was changed 10-12 times per hour. Fluorescent lighting provided illumination 10 hours per day. Food and tap water were provided ad libitum.

Fresh feed was provided daily, and any feed remaining from the previous day was discarded.

The rats were housed individually in hanging galvanized steel mesh cages (Hoeltge, Cincinnati, Ohio), and the mice were housed in polypropylene cages (Lab Products, Inc., Garfield, N.J.) containing five animals per cage. Mouse cages were covered with polyester filter bonnets (Lab Products, Inc.). The rat cages were sanitized every 2 weeks. The mouse cages were sanitized two times per week. Cages and racks were washed in an industrial washer (Industrial Washing Machine Corp., Matawan, N.J.) at 82°C with Acclaim® detergent (Economics Laboratory, Inc., St. Paul, Minn.) and then rinsed. Absorbent Kimpak® cage liners (Kimberly Clark Corp., Neenah, Wis.) under the rat cages were changed two times per week. Absorb-dri® hardwood chip bedding (Lab Products, Inc.) used in the mouse cages was provided two times per week. Filter bonnets were sanitized each week. Feed jars and water bottles were changed and sanitized two times per week. Sipper tubes and stoppers were sanitized two times per week.

Filter bonnets, feed jars, water bottles, sipper tubes, and stoppers were washed in a Vulcan Autosan washer (Louisville, Ky.) at 82°C, using Acclaim® detergent, and then rinsed.

Cage racks for each species were rotated to a new position in the

room once per week; at the same time, each cage was moved to a different row within the same column of a rack. Rats and mice were housed in separate rooms. Control and dosed rats were housed on the same rack, whereas cages for control and dosed mice were placed in separate racks in the same room. Anilazine was the only compound on test in each room.

E. Subchronic Studies

Subchronic feeding studies were conducted with rats and mice to estimate the maximum tolerated doses of anilazine, on the basis of which two concentrations (hereinafter referred to as the "high dose" and the "low dose"), were chosen for administration in the chronic studies. In the subchronic studies, anilazine was added to the animal feed at concentrations of 250, 500, 1,000, 2,000, 4,000, 8,000, and 16,000 ppm for 13 weeks for both rats and mice. Each dosed group, as well as the untreated controls, consisted of 10 male and 10 female animals. Diets were stored at -20°C and fresh feed was provided each day. Animal weights were measured each week. All animals were killed at the end of the 13-week test period.

After 13 weeks on study, the mean weight gain in male rats was unaffected at doses of up to 4,000 ppm, 87% of controls at 8,000 ppm, and 59% of controls at 16,000 ppm. In females, the mean

weight gains were unaffected at doses up to 8,000 ppm, and were 79% of controls at 16,000 ppm.

In both male and female mice, the mean body weight gains were above controls at all doses up to 8,000 ppm. At 16,000 ppm, weight gain in males was 76% of controls, and weight gain in females was 94% of controls.

Gross and microscopic examination of tissues of the rats and mice gave no evidence of any effect of anilazine. Analysis of rat and mouse feces showed unreacted anilazine present in the feces, especially at the higher doses. No unreacted anilazine was detected in any of the rat and mouse tissues tested from the group receiving 16,000 ppm, including the heart, lung, brain, fat, muscle, and kidney, at a minimal detectable concentration of 0.1 micrograms per gram of tissue.

The low and high doses for the chronic studies in both rats and mice were set at 500 and 1,000 ppm, respectively.

F. Chronic Studies

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

Table 1. Anilazine Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals[a]	Anilazine in Diet[b] (ppm)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Matched-Control	25	0		103-104
Low-Dose	50	500	103	0-1
High-Dose	50	1,000	103	0-1
Female				
Matched-Control	25	0		103-104
Low-Dose	50	500	103	0-1
High-Dose	50	1,000	103	0-1

[a]All rats were at 45 days of age when placed on study.

[b]Diets were provided ad libitum.

Table 2. Anilazine Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals[a]	Anilazine in Diet[b] (ppm)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Matched-Control	25	0		107-109
Low-Dose	50	500	103	4-5
High-Dose	50	1,000	103	4-6
Female				
Matched-Control	25	0		107-109
Low-Dose	50	500	103	4-6
High-Dose	50	1,000	103	4-6

[a]All mice were at 73 days of age when placed on study.

[b]Diets were provided ad libitum.

G. Clinical and Pathologic Examinations

All animals were observed twice per day for signs of toxicity, weighed at regular intervals, and palpated for masses at each weighing. Animals that were moribund at the time of clinical examination and those that survived to the end of the bioassay were sacrificed under pentobarbital anesthesia and necropsied.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, gallbladder (mice), pancreas, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, and brain. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive 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 appropriate 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 the dosed male and female rats were slightly lower than those of the corresponding controls throughout the bioassay (figure 1).

During the first 6 months of the bioassay, the appearance and behavior of the dosed rats was generally comparable to that of the control rats. During the second 6 months, adverse clinical signs were noted at a low incidence among dosed rats. These signs included loss of weight, rough hair coats, discolored (dark) urine, loose stools, and pale mucous membranes. Adverse clinical signs were noted with an increased frequency during the second year of the bioassay, among all groups, dosed and control, but predominantly in dosed groups. These signs included loss of weight, rough hair coats, poor food consumption, loose stools, hematuria, vaginal bleeding, tachypnea, dyspnea, discolored (dark) urine, impaired equilibrium, pale mucous membranes, mucous-like vaginal discharge, hyporeactivity, and tissue masses. At week 84, a majority of low-dose females appeared hyperactive, but stabilized. At week 103, when placed on control diet, the high-dose males, low-dose females, and high-dose females appeared hyperactive.

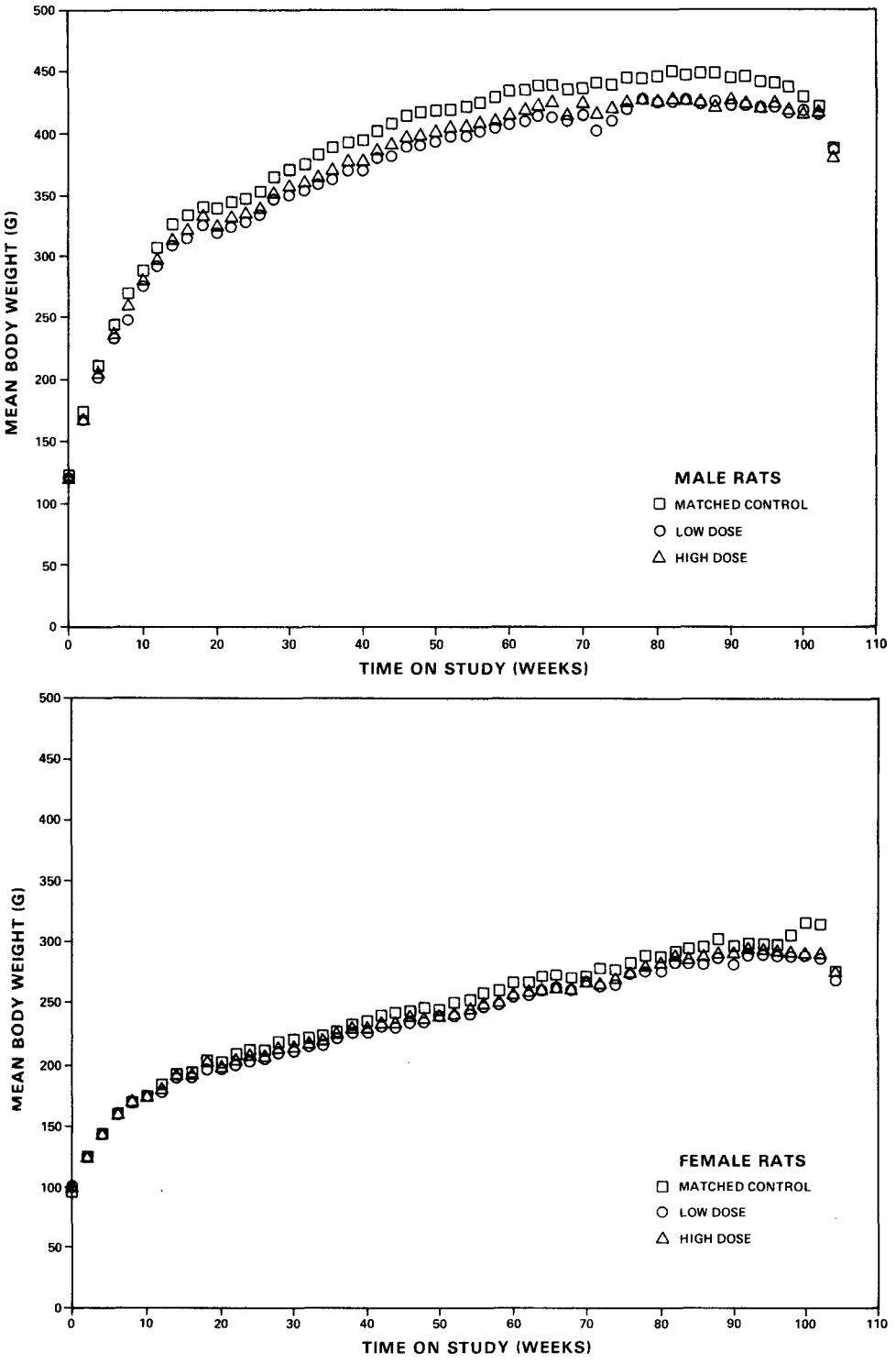


Figure 1. Growth Curves for Rats Administered Anilazine in the Diet

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered anilazine 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 dose-related trend in mortality is not significant in either sex.

In male rats, 37/50 (74%) of each dose group and 20/25 (80%) of the control group were still alive at 2 years on study. In females, 34/50 (68%) of the high-dose group, 37/50 (74%) of the low-dose group, and 16/25 (64%) of the control group were alive at 2 years on study.

Sufficient numbers of rats of each sex were at risk for the development of late-appearing tumors.

C. Pathology (Rats)

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

A variety of neoplasms occurred in both the dosed and control animals. An increased incidence of certain types of endocrine neoplasms occurred in the dosed animals.

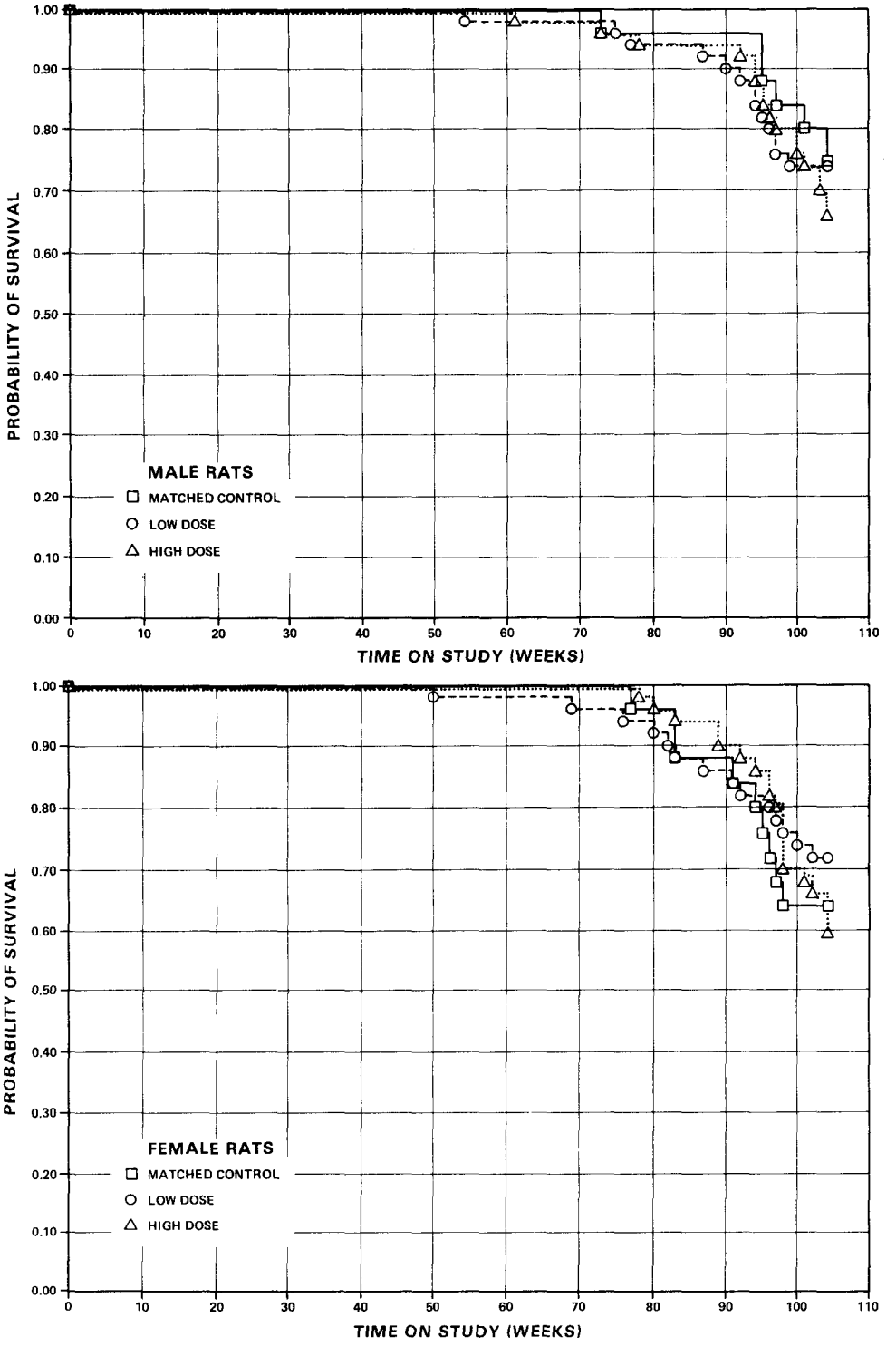


Figure 2. Survival Curves for Rats Administered Anilazine in the Diet

The finding of adenocarcinoma in the jejunum of 2/47 (4%) of the high-dose male rats is of some interest, because of the relative rarity of this neoplasm as a spontaneous entity. The primary neoplasm in one animal was characterized by pleomorphic epithelial cells which were invading the subjacent lamina propria, submucosa, and muscularis externa. The less differentiated neoplastic cells were compact cells whose nuclei and cytoplasm stained deeply with hematoxylin. Differentiation to goblet cells was frequently noted. Metastatic foci having morphologic features closely resembling those of the primary tumor were found in the lungs. Implants were also noted on the serosa of the gastrointestinal tract.

The primary neoplasm in the jejunum of the second animal was a broad-based tumor with increased mitoses in the epithelial cells, moderate cellular pleomorphism, and increased cytoplasmic basophilia. In some areas the basement membrane was indistinct. In one area there was morphologic evidence of invasion of tumor cells in the submucosa, with encroachment upon the inner layer of the muscularis externa.

Other neoplasms occurred at approximately the same incidences in dosed and control animals in this bioassay, or were neoplasms whose rates of occurrence were not appreciably above those in control animals in this and other laboratories.

A variety of nonneoplastic lesions occurred in both dosed and control animals. There were instances in which lesions occurred only or with increased incidence in dosed animals; however, the incidence, distribution, and severity of these lesions are similar to those which are known to occur spontaneously in aged Fischer 344 rats.

Based on the histopathologic examination, there was no clear evidence for the carcinogenicity of anilazine in Fischer 344 rats under the conditions of this bioassay.

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.

The results of the Cochran-Armitage test for dose-related trend in incidences of tumors and those of the Fisher exact test comparing the incidence of tumors in the dosed groups with those in the corresponding control groups are not significant in either sex.

In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one is included; this indicates the

absence of significant positive results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by anilazine, which could not be detected under the conditions of this bioassay.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the dosed male mice were markedly lower than those of the corresponding controls, beginning at about week 30; however, there was no relationship to dose. The mean body weights of the dosed females were essentially unaffected (figure 3).

During the first 6 months of the bioassay, the appearance and behavior of the dosed mice was generally comparable to that of the control mice. During the next 6 months, adverse clinical signs were noted at a fairly low incidence. These signs included alopecia, hyperactivity, hyporeactivity, mucous in feces, obesity, bloating (or abdominal distention), and discolored (yellow) hair coats. Fighting was observed among all male mice, but predominantly among low-dose males. This increased aggression persisted until termination of the bioassay. A few high-dose females and low- and high-dose males had single areas of depigmentation of both skin and hair coats. Hyperemia of ears and feet was also noted on a few animals in these same groups. During the second year of the bioassay, adverse clinical signs were noted at an increased frequency in all groups, but predominantly in the dosed groups of both sexes. These signs

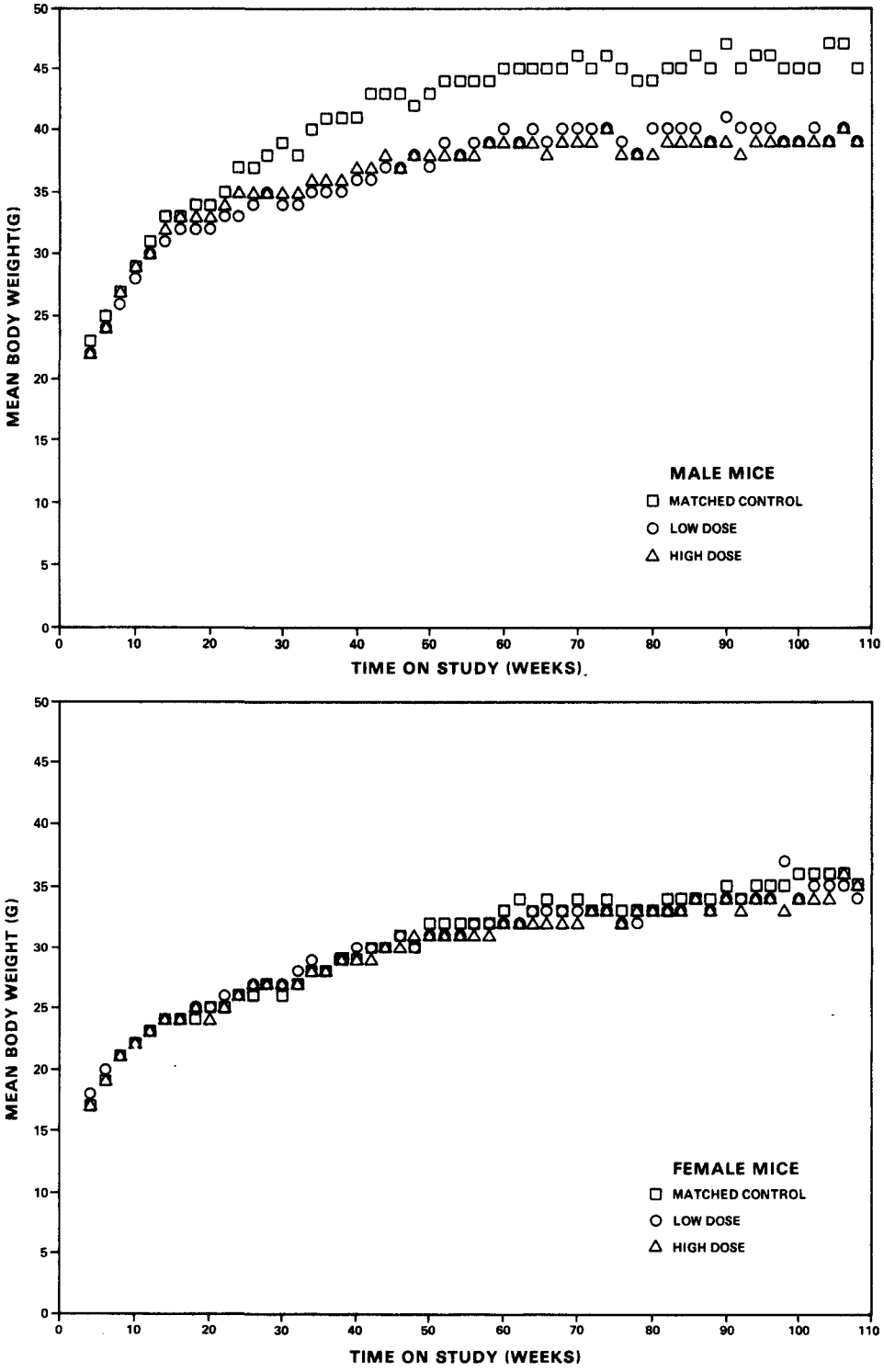


Figure 3. Growth Curves for Mice Administered Anilazine in the Diet

included tachypnea, obesity, mucous in feces, rough hair coats, and abdominal distention.

B. Survival (Mice)

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

In male mice, the result of the Tarone test for dose-related trend in mortality is significant (P = 0.006), but in the negative direction. There were 44/50 (88%) of the high-dose group, 40/50 (80%) of the low-dose group, and 16/25 (64%) of the control group alive at 2 years on study. In females, the result of the Tarone test is not significant. An indicated departure from linear trend is observed (P = 0.017), because the high-dose female mice survived longer than the low-dose group. All 50 of the high-dose female mice, 49/50 (98%) of the low-dose group, and all 25 of the control group survived beyond week 52 on study.

Sufficient numbers of mice of each sex were at risk for the development of late-appearing tumors.

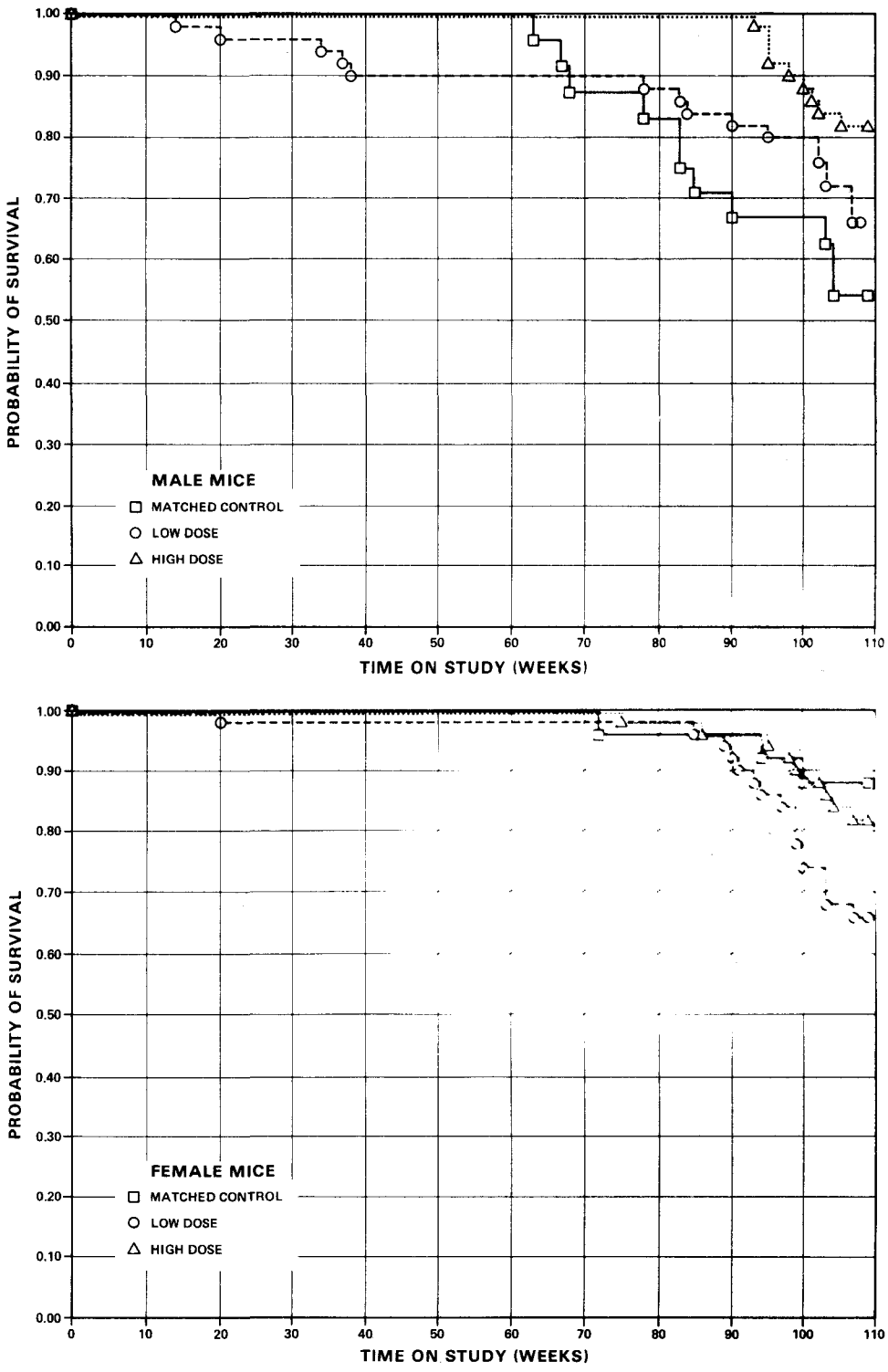


Figure 4. Survival Curves for Mice Administered Anilazine in the Diet

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.

A number of neoplastic and nonneoplastic lesions were observed in mice of the control and dosed groups with approximately equal frequency. One exception was the occurrence of diverse types of sarcomas in the subcutaneous tissue in dosed mice, but not in control mice. The incidence of these neoplasms was 5/49 (10%) in the low-dose males, 0/50 in the high-dose males, and 1/50 (2%) in the high-dose females. The absence of sarcomas in the high-dose males and the low number in the high-dose females suggests that they occurred spontaneously.

Based on the histopathologic examination, there was no evidence for the carcinogenicity of anilazine in B6C3F1 mice 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.

The results of the Cochran-Armitage test for dose-related trend in incidences of tumors and those of the Fisher exact test comparing the incidences of tumors in the dosed groups with those in the corresponding control groups are not significant in the positive direction in either sex.

In male mice, the incidence of liver tumors in the control group is significantly higher than that in the low-dose group.

In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one or less than one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals (except that for the incidence of liver tumors in the low-dose male mice) has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by anilazine, which could not be detected under the conditions of this bioassay.

V. DISCUSSION

The anilazine was generally nontoxic for the rats and mice under the conditions of this bioassay. The chemical in the feed was unstable at room temperature; however, the feed was changed each day to compensate for the loss at ambient temperatures. Administration of the chemical had only a slight effect on mean body weights in male or female rats and female mice; however, there was a more obvious decrease in mean body weight gain in the dosed male mice. Survival also was essentially unaffected. Except for the control male mice, survival in all groups of rats and mice was at least 80% at the end of 90 weeks on study; thus, sufficient numbers of animals were at risk in most groups for the development of late-appearing tumors. Male and female rats and female mice may have been able to tolerate higher doses.

No tumors occurred in dosed male or female rats or mice at incidences that were statistically significantly higher than those in corresponding controls. However, adenocarcinomas occurred in the intestines of two high-dose male rats. Although these lesions are generally rare in rats, their low incidence in this study does not permit implication of a compound-related effect.

The acute oral LD_{50} for anilazine in rats (strain not specified)

has been reported as 2,700 mg/kg body weight; also, feeding of the test chemical to rats (strain not specified) at doses of 50 to 5,000 ppm in the diet over a period of 2 years had no effect on weight gain, survival, histological appearance of tissues, or incidence of tumors (Lehman, 1965). A number of triazine analogues related to anilazine have been reported to induce tumors in rats or mice or both. These include 2,4,6-tris(1-aziridinyl)-s-triazine (Hendry et al., 1951; Shimkin, 1954; Roe and Salaman, 1955; Walpole, 1958; Conklin et al., 1965), 2,4,6-trichloro-s-triazine (Pliss, 1966), 2-chloro-4,6-bis(ethyl-amino)-s-triazine (Pliss and Zabezhinsky, 1970), 4,6-diamino-2-(5-nitro-2-furyl)-s-triazine and its bisacetamide (Cohen et al., 1973), hexamethylmelamine (Cohen et al., 1973), azacytidine (Stoner et al., 1973), and hexahydro-1,3,5-trinitroso-s-triazine (Urban and Danz, 1976). Prolonged contact with anilazine can cause skin irritation in humans (Kambe et al., 1970; Gosselin et al., 1976).

It is concluded that under the conditions of this bioassay, anilazine was not carcinogenic for either Fischer 344 rats or B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED ANILAZINE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED ANILAZINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	25	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(25)	(50)	(50)
FIBROSARCOMA			1 (2%)
*SUBCUT TISSUE	(25)	(50)	(50)
FIBROMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(25)	(49)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (8%)		2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(25)	(50)	(50)
UNDIFFERENTIATED LEUKEMIA	2 (8%)	1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#JEJUNUM	(22)	(47)	(47)
ADENOCARCINOMA, NOS			2 (4%)
URINARY SYSTEM			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(25)	(44)	(46)
CARCINOMA,NOS			2 (4%)
ADENOMA, NOS	6 (24%)	16 (36%)	15 (33%)
#ADRENAL	(23)	(49)	(49)
PHEOCHROMOCYTOMA		1 (2%)	1 (2%)
#ADRENAL MEDULLA	(23)	(49)	(49)
NEUROBLASTOMA		1 (2%)	
#THYROID	(23)	(38)	(47)
PAPILLARY ADENOMA		2 (5%)	
C-CELL ADENOMA	1 (4%)	4 (11%)	1 (2%)
#PARATHYROID	(16)	(29)	(33)
ADENOMA, NOS		1 (3%)	
#PANCREATIC ISLETS	(24)	(47)	(50)
ISLET-CELL ADENOMA	1 (4%)	4 (9%)	5 (10%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(25)	(50)	(50)
FIBROMA		1 (2%)	1 (2%)
#TESTIS	(24)	(49)	(49)
INTERSTITIAL-CELL TUMOR	20 (83%)	43 (88%)	44 (90%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKULL	(25)	(50)	(50)
OSTEOSARCOMA		1 (2%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*PERITONEUM	(25)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(25)	(50)	(50)
CARCINOMA,NOS	1 (4%)		
ADENOCARCINOMA, NOS		1 (2%)	
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
SARCOMA, NOS	1 (4%)		
MESOTHELIOMA, NOS			1 (2%)
SITE UNKNOWN			
PARAGANGLIOMA, NOS			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	25	50	50
NATURAL DEATH@	1	2	3
MORIBUND SACRIFICE	5	11	14
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED	1		
TERMINAL SACRIFICE	18	37	33
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	24	50	50
TOTAL PRIMARY TUMORS	34	77	79
TOTAL ANIMALS WITH BENIGN TUMORS	23	49	50
TOTAL BENIGN TUMORS	30	72	70
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	4	7
TOTAL MALIGNANT TUMORS	4	4	7
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	2
TOTAL UNCERTAIN TUMORS		1	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED ANILAZINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	25	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(25)	(50)	(50)
BASAL-CELL CARCINOMA			1 (2%)
*SUBCUT TISSUE	(25)	(50)	(50)
SARCOMA, NOS	1 (4%)		
RESPIRATORY SYSTEM			
#LUNG	(24)	(49)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(25)	(50)	(50)
UNDIFFERENTIATED LEUKEMIA	3 (12%)	3 (6%)	1 (2%)
*MUSCLE OF TRUNK	(25)	(50)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(25)	(48)	(49)
NEOPLASTIC NODULE		1 (2%)	
HEPATOCELLULAR CARCINOMA			1 (2%)
#JEJUNUM	(24)	(50)	(46)
SARCOMA, NOS			1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(22)	(43)	(46)
CARCINOMA,NOS	1 (5%)	3 (7%)	1 (2%)
ADENOMA, NOS	11 (50%)	22 (51%)	32 (70%)
#THYROID	(24)	(35)	(46)
PAPILLARY ADENOMA			1 (2%)
C-CELL ADENOMA		1 (3%)	3 (7%)
#PANCREATIC ISLETS	(24)	(49)	(50)
ISLET-CELL ADENOMA	1 (4%)	2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(25)	(50)	(50)
ADENOCARCINOMA, NOS			1 (2%)
FIBROSARCOMA			1 (2%)
FIBROADENOMA	1 (4%)	3 (6%)	7 (14%)
#UTERUS	(22)	(46)	(43)
ADENOMA, NOS		1 (2%)	
ENDOMETRIAL STROMAL POLYP	6 (27%)	13 (28%)	11 (26%)
ENDOMETRIAL STROMAL SARCOMA		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(25)	(50)	(50)
SARCOMA, NOS			1 (2%)
CARCINOSARCOMA			1 (2%)
THORACIC CAVITY			
PARAGANGLIOMA, NOS		1	
ADIPOSE TISSUE			
LIPOMA	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	25	50	50
NATURAL DEATH@	1	2	5
MORIBUND SACRIFICE	8	12	15
**SCHEDULED SACRIFICE		1	
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	35	30
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** Animal is in fact an early terminal sacrifice, but will appear as a scheduled sacrifice due to system interpretation.

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	22	42	43
TOTAL PRIMARY TUMORS	25	53	66
TOTAL ANIMALS WITH BENIGN TUMORS	17	36	40
TOTAL BENIGN TUMORS	20	44	56
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	7	9
TOTAL MALIGNANT TUMORS	5	7	10
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		2	
TOTAL UNCERTAIN TUMORS		2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED ANILAZINE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED ANILAZINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	23	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	23	49	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(23)	(49)	(50)
SARCOMA, NOS		3 (6%)	
FIBROSARCOMA		1 (2%)	
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
HEMANGIOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(23)	(48)	(50)
CARCINOMA, NOS, METASTATIC		1 (2%)	
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (9%)	5 (10%)	4 (8%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (9%)	2 (4%)	2 (4%)
FIBROUS HISTIOCYTOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(23)	(49)	(50)
MALIGNANT LYMPHOMA, NOS			2 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (9%)		1 (2%)
#SPLEEN	(23)	(49)	(50)
HEMANGIOMA			1 (2%)
HEMANGIOSARCOMA	1 (4%)	1 (2%)	1 (2%)
#LYMPH NODE	(18)	(45)	(40)
ALVEOLAR/BRONCHIOLAR CA, METASTA	1 (6%)		
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(23)	(49)	(50)
HEPATOCELLULAR ADENOMA		1 (2%)	
HEPATOCELLULAR CARCINOMA	9 (39%)	5 (10%)	12 (24%)
HEMANGIOSARCOMA		1 (2%)	
*BILE DUCT	(23)	(49)	(50)
CARCINOMA,NOS		1 (2%)	
#COLON	(20)	(43)	(47)
LEIOMYOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY	(23)	(49)	(50)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (2%)	1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(20)	(43)	(39)
FOLLICULAR-CELL ADENOMA			1 (3%)
REPRODUCTIVE SYSTEM			
#TESTIS	(23)	(49)	(50)
SEMINOMA/DYSGERMINOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(23)	(49)	(50)
ADENOMA, NOS		2 (4%)	
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*PERITONEUM	(23)	(49)	(50)
ALVEOLAR/BRONCHIOLAR CA, METASTA	1 (4%)		
*PLEURA	(23)	(49)	(50)
ALVEOLAR/BRONCHIOLAR CA, INVASIV			1 (2%)
ALVEOLAR/BRONCHIOLAR CA, METASTA	1 (4%)	1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	25	50	50
NATURAL DEATH@	6	8	2
MORIBUND SACRIFICE	5	9	7
**SCHEDULED SACRIFICE	5		7
ACCIDENTALLY KILLED	1		
TERMINAL SACRIFICE	8	33	34
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** Animals are in fact early terminal sacrifices, but will appear as scheduled sacrifices due to system interpretation.

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	15	20	25
TOTAL PRIMARY TUMORS	16	25	27
TOTAL ANIMALS WITH BENIGN TUMORS	2	8	6
TOTAL BENIGN TUMORS	2	8	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	14	16	20
TOTAL MALIGNANT TUMORS	14	17	20
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	3	2
TOTAL SECONDARY TUMORS	3	4	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED ANILAZINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	47	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	25	47	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(25)	(47)	(50)
SARCOMA, NOS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(23)	(47)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(25)	(47)	(50)
MALIGNANT LYMPHOMA, NOS	1 (4%)	4 (9%)	8 (16%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (4%)	2 (4%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (4%)	1 (2%)	
MYELOMONOCYTIC LEUKEMIA			1 (2%)
MONOCYTIC LEUKEMIA		1 (2%)	
#SPLEEN	(25)	(47)	(49)
HEMANGIOMA		1 (2%)	
#LIVER	(24)	(45)	(48)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(24)	(45)	(48)
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(23)	(43)	(44)
CHROMOPHOBE ADENOMA	1 (4%)	3 (7%)	1 (2%)
#THYROID	(25)	(44)	(48)
FOLLICULAR-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(25)	(47)	(50)
CARCINOMA,NOS		1 (2%)	
ADENOMA, NOS		1 (2%)	
ADENOCARCINOMA, NOS			1 (2%)
#UTERUS	(25)	(43)	(48)
FIBROMA		1 (2%)	
ENDOMETRIAL STROMAL POLYP		1 (2%)	1 (2%)
#OVARY	(25)	(46)	(49)
CYSTADENOMA, NOS		1 (2%)	1 (2%)
TERATOMA, BENIGN		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(25)	(47)	(50)
ADENOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(25)	(47)	(50)
LIPOMA		2 (4%)	
*PERITONEUM	(25)	(47)	(50)
LIPOMA		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	25	50	50
NATURAL DEATH@		6	3
MORIBUND SACRIFICE	3	11	6
**SCHEDULED SACRIFICE	5	5	8
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	17	27	33
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	22	18
TOTAL PRIMARY TUMORS	4	25	19
TOTAL ANIMALS WITH BENIGN TUMORS	1	14	5
TOTAL BENIGN TUMORS	1	14	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	11	14
TOTAL MALIGNANT TUMORS	3	11	14
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

** Animals are in fact early terminal sacrifices, but will appear as scheduled sacrifices due to system interpretation.

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN RATS ADMINISTERED ANILAZINE IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED ANILAZINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	25	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(25)	(49)	(49)
HEMORRHAGE		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(24)	(50)	(48)
ATROPHY, NOS		1 (2%)	
HYPOPLASIA, HEMATOPOIETIC			1 (2%)
#SPLEEN	(25)	(46)	(50)
CONGESTION, NOS		2 (4%)	
INFLAMMATION, ACUTE	1 (4%)		
ATROPHY, NOS			1 (2%)
HEMATOPOIESIS		1 (2%)	3 (6%)
#MANDIBULAR L. NODE	(23)	(47)	(46)
INFLAMMATION, ACUTE	1 (4%)		
HYPERPLASIA, NOS		2 (4%)	
#MESENTERIC L. NODE	(23)	(47)	(46)
PERIARTERITIS			1 (2%)
#RENAL LYMPH NODE	(23)	(47)	(46)
CYST, NOS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(25)	(50)	(50)
THROMBOSIS, NOS			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#MYOCARDIUM	(25)	(50)	(50)
INFLAMMATION, ACUTE		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(25)	(47)	(49)
INFLAMMATION, NOS	11 (44%)	21 (45%)	12 (24%)
HYPERPLASIA, NOS	11 (44%)	21 (45%)	12 (24%)
#LIVER	(24)	(48)	(49)
INFLAMMATION, NOS	1 (4%)		
NECROSIS, CENTRAL			1 (2%)
METAMORPHOSIS FATTY	3 (13%)	1 (2%)	4 (8%)
FOCAL CELLULAR CHANGE		1 (2%)	2 (4%)
ANGIECTASIS		3 (6%)	1 (2%)
HEMATOPOIESIS	1 (4%)	3 (6%)	4 (8%)
#LIVER/CENTRILOBULAR	(24)	(48)	(49)
DEGENERATION, NOS			1 (2%)
*BILE DUCT	(25)	(50)	(50)
CYST, NOS			1 (2%)
HYPERPLASIA, NOS	5 (20%)	18 (36%)	15 (30%)
#PANCREAS	(24)	(47)	(50)
PERIARTERITIS			1 (2%)
ATROPHY, NOS	2 (8%)	1 (2%)	
ATROPHY, FOCAL	1 (4%)	1 (2%)	
#STOMACH	(24)	(50)	(48)
INFLAMMATION, NOS	1 (4%)		
INFLAMMATION, ACUTE	1 (4%)	2 (4%)	1 (2%)
ULCER, ACUTE	1 (4%)		1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	1 (2%)
ULCER, CHRONIC	1 (4%)		1 (2%)
URINARY SYSTEM			
#KIDNEY	(25)	(49)	(49)
INFLAMMATION, CHRONIC	14 (56%)	36 (73%)	36 (73%)
ENDOCRINE SYSTEM			
#PITUITARY	(25)	(44)	(46)
CYST, NOS		1 (2%)	4 (9%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, FOCAL	2 (8%)		
#ADRENAL	(23)	(49)	(49)
HEMORRHAGE		1 (2%)	
HEMORRHAGIC CYST		1 (2%)	
#ADRENAL CORTEX	(23)	(49)	(49)
ANGIECTASIS			2 (4%)
#ADRENAL MEDULLA	(23)	(49)	(49)
HYPERPLASIA, NOS			1 (2%)
#THYROID	(23)	(38)	(47)
HYPERPLASIA, C-CELL	3 (13%)	1 (3%)	3 (6%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(25)	(50)	(50)
INFLAMMATION, CHRONIC			1 (2%)
*PREPUTIAL GLAND	(25)	(50)	(50)
HYPERPLASIA, NOS		1 (2%)	
#PROSTATE	(18)	(45)	(45)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE		1 (2%)	
ABSCESS, NOS	1 (6%)		
#TESTIS	(24)	(49)	(49)
ATROPHY, NOS		2 (4%)	
NERVOUS SYSTEM			
#BRAIN	(25)	(47)	(49)
HYDROCEPHALUS, NOS			2 (4%)
INFLAMMATION, NOS			1 (2%)
#CEREBELLUM	(25)	(47)	(49)
GLIOSIS		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(25)	(50)	(50)
ABSCESS, NOS	1 (4%)		
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC	1	3	1
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED ANILAZINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	25	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(24)	(49)	(50)
HYPERPLASIA, NOS			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(25)	(47)	(50)
THROMBOSIS, NOS			1 (2%)
HYPERPLASIA, NOS			1 (2%)
HEMATOPOIESIS		1 (2%)	
#MANDIBULAR L. NODE	(23)	(43)	(44)
INFLAMMATION, ACUTE		4 (9%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(22)	(48)	(49)
INFLAMMATION, NOS	7 (32%)	23 (48%)	20 (41%)
DEGENERATION, NOS	1 (5%)		
HYPERPLASIA, NOS	8 (36%)	23 (48%)	20 (41%)
#LIVER	(25)	(48)	(49)
INFLAMMATION, ACUTE		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
METAMORPHOSIS FATTY		5 (10%)	1 (2%)
BASOPHILIC CYTO CHANGE	2 (8%)	3 (6%)	6 (12%)
FOCAL CELLULAR CHANGE	1 (4%)	2 (4%)	3 (6%)
HEMATOPOIESIS		2 (4%)	2 (4%)
REGENERATION, NOS		1 (2%)	
*BILE DUCT	(25)	(50)	(50)
HYPERPLASIA, NOS	1 (4%)	4 (8%)	6 (12%)
#PANCREAS	(24)	(49)	(50)
ATROPHY, NOS		1 (2%)	2 (4%)
#STOMACH	(24)	(48)	(48)
ULCER, CHRONIC	1 (4%)		
URINARY SYSTEM			
#KIDNEY	(24)	(48)	(49)
INFLAMMATION, CHRONIC	8 (33%)	12 (25%)	6 (12%)
DEGENERATION, NOS			1 (2%)
DYSPLASIA, NOS			1 (2%)
#URINARY BLADDER	(22)	(46)	(44)
HYPERPLASIA, EPITHELIAL			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(22)	(43)	(46)
CYST, NOS	2 (9%)	1 (2%)	4 (9%)
HEMORRHAGIC CYST		1 (2%)	1 (2%)
HYPERPLASIA, FOCAL	2 (9%)	1 (2%)	
#ADRENAL	(24)	(47)	(48)
CYST, NOS			1 (2%)
DEGENERATION, NOS		1 (2%)	
ANGIECTASIS			1 (2%)
#ADRENAL CORTEX	(24)	(47)	(48)
LIPOIDOSIS	3 (13%)		1 (2%)
ANGIECTASIS			1 (2%)
#THYROID	(24)	(35)	(46)
HYPERPLASIA, C-CELL	2 (8%)	2 (6%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY DUCT	(25)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
#UTERUS	(22)	(46)	(43)
HEMATOMA, NOS	1 (5%)		
METAPLASIA, SQUAMOUS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(24)	(49)	(50)
HYDROCEPHALUS, NOS	1 (4%)	2 (4%)	
HEMORRHAGE		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
STEATITIS	1		
INFLAMMATION, ACUTE		1	
INFLAMMATION, CHRONIC		2	1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2		2

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN MICE ADMINISTERED ANILAZINE IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED ANILAZINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	23	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	23	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(23)	(49)	(50)
ABSCESS, NOS			3 (6%)
RESPIRATORY SYSTEM			
#LUNG	(23)	(48)	(50)
HYPERPLASIA, ALVEOLAR EPITHELIUM		3 (6%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(23)	(49)	(50)
ANGIECTASIS			1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	
HEMATOPOIESIS		1 (2%)	
#LYMPH NODE	(18)	(45)	(40)
CONGESTION, NOS			1 (3%)
INFLAMMATION, NOS			1 (3%)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (3%)
#MESENTERIC L. NODE	(18)	(45)	(40)
CONGESTION, NOS			1 (3%)
#THYMUS	(1)	(2)	(3)
HYPERPLASIA, LYMPHOID			1 (33%)
CIRCULATORY SYSTEM			
#HEART	(23)	(48)	(50)
PERIARTERITIS	1 (4%)		

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(23)	(49)	(50)
NECROSIS, NOS		1 (2%)	
NECROSIS, COAGULATIVE	2 (9%)		
NECROSIS, HEMORRHAGIC	1 (4%)		
HYPERPLASIA, NODULAR		1 (2%)	2 (4%)
*BILE DUCT	(23)	(49)	(50)
HYPERPLASIA, CYSTIC			1 (2%)
#PANCREAS	(23)	(49)	(49)
INFLAMMATION, NOS			1 (2%)
#SMALL INTESTINE	(20)	(44)	(43)
ULCER, NOS	1 (5%)		
HYPERPLASIA, LYMPHOID			1 (2%)
URINARY SYSTEM			
#KIDNEY	(23)	(49)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
#URINARY BLADDER	(18)	(47)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#PROSTATE	(18)	(44)	(48)
HEMORRHAGE		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(23)	(49)	(50)
INFLAMMATION, NOS		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(23)	(49)	(50)
INFLAMMATION, GRANULOMATOUS		2 (4%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(23)	(49)	(50)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	6	19	19
ACCIDENTAL DEATH	1		
AUTOLYSIS/NO NECROPSY	1	1	

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

NOTE: ANIMAL #049 IS IN FACT AN EARLY TSAC BUT WILL APPEAR AS SSAC DUE TO SYSTEM INTERPRETATION.

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED ANILAZINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	47	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	25	47	50
INTEGUMENTARY SYSTEM			
*SKIN	(25)	(47)	(50)
INFLAMMATION, FOCAL			1 (2%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(25)	(47)	(49)
HYPERPLASIA, LYMPHOID			1 (2%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(25)	(47)	(50)
INFLAMMATION, FOCAL	1 (4%)		
*PULMONARY ARTERY	(25)	(47)	(50)
SCLEROSIS	4 (16%)	3 (6%)	
DIGESTIVE SYSTEM			
#LIVER	(24)	(45)	(48)
INFLAMMATION, NOS		1 (2%)	1 (2%)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, GRANULOMATOUS	2 (8%)		
NECROSIS, NOS		1 (2%)	
FOCAL CELLULAR CHANGE			1 (2%)
#PEYERS PATCH	(24)	(41)	(44)
HYPERPLASIA, LYMPHOID	1 (4%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#JEJUNUM	(24)	(41)	(44)
HYPERPLASIA, LYMPHOID		1 (2%)	
#ILEUM	(24)	(41)	(44)
HYPERPLASIA, LYMPHOID			2 (5%)
URINARY SYSTEM			
#KIDNEY	(25)	(47)	(50)
NECROSIS, DIFFUSE			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(23)	(43)	(44)
HEMORRHAGIC CYST		1 (2%)	
HYPERPLASIA, NOS			1 (2%)
#THYROID	(25)	(44)	(48)
CYSTIC FOLLICLES			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(25)	(47)	(50)
GALACTOCELE		1 (2%)	
#UTERUS/ENDOMETRIUM	(25)	(43)	(48)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
HYPERPLASIA, CYSTIC	1 (4%)		2 (4%)
#OVARY	(25)	(46)	(49)
CYST, NOS	1 (4%)	2 (4%)	1 (2%)
FOLLICULAR CYST, NOS		1 (2%)	2 (4%)
HEMORRHAGIC CYST			1 (2%)
INFLAMMATION, NOS		1 (2%)	
ENDOMETRIOSIS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(25)	(47)	(50)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	14	15	22
AUTOLYSIS/NO NECROPSY		3	

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED ANILAZINE IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Anilazine in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	2/25 (8)	0/49 (0)	3/49 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.000	0.765
Lower Limit		0.000	0.095
Upper Limit		1.718	8.775
Weeks to First Observed Tumor	95	--	104
Hematopoietic System: Undifferentiated Leukemia[b]	2/25 (8)	1/50 (2)	1/50 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.250	0.250
Lower Limit		0.004	0.004
Upper Limit		4.616	4.616
Weeks to First Observed Tumor	95	92	94

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Anilazine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Adenoma or Carcinoma, NOS[b]	6/25 (24)	16/44 (36)	17/46 (37)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.515	1.540
Lower Limit		0.663	0.683
Upper Limit		4.167	4.213
Weeks to First Observed Tumor	95	75	61
Thyroid: C-cell Adenoma[b]	1/23 (4)	4/38 (11)	1/47 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		2.421	0.489
Lower Limit		0.263	0.007
Upper Limit		115.840	37.631
Weeks to First Observed Tumor	104	103	104

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Anilazine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Papillary Adenoma	0/23 (0)	2/38 (5)	0/47 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	--
Lower Limit		0.184	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	104	--
Pancreatic Islets: Islet-cell Adenoma[b]	1/24 (4)	4/47 (9)	5/50 (10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		2.043	2.400
Lower Limit		0.220	0.294
Upper Limit		98.366	111.118
Weeks to First Observed Tumor	104	94	92

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Anilazine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor[b]	20/24 (83)	43/49 (88)	44/49 (90)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.053	1.078
Lower Limit		0.870	0.892
Upper Limit		1.340	1.345
Weeks to First Observed Tumor	95	77	92

[a]Dosed groups received 500 or 1,000 ppm in feed.

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

[c]Beneath the incidence of tumors in the matched-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 matched-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 E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Anilazine in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System:			
Undifferentiated Leukemia[b]	3/25 (12)	3/50 (6)	1/50 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.500	0.167
Lower Limit		0.073	0.003
Upper Limit		3.524	1.971
Weeks to First Observed Tumor	77	69	102
Hematopoietic System:			
Lymphoma or Leukemia[b]	3/25 (12)	3/50 (6)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.500	0.333
Lower Limit		0.073	0.030
Upper Limit		3.524	2.753
Weeks to First Observed Tumor	77	69	89

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Anilazine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Carcinoma, NOS[b]	1/22 (5)	3/43 (7)	1/46 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.535	0.478
Lower Limit		0.134	0.006
Upper Limit		78.651	36.761
Weeks to First Observed Tumor	83	83	104
Pituitary: Adenoma or Carcinoma, NOS[b]	12/22 (55)	25/43 (58)	33/46 (72)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.066	1.315
Lower Limit		0.673	0.868
Upper Limit		1.867	2.168
Weeks to First Observed Tumor	83	50	89

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Anilazine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma[b]	0/24 (0)	1/35 (3)	3/46 (7)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.038	0.323
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	82	96
Mammary Gland: Fibroadenoma[b]	1/25 (4)	3/50 (6)	7/50 (14)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.500	3.500
Lower Limit		0.130	0.494
Upper Limit		77.150	154.214
Weeks to First Observed Tumor	104	76	98

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Anilazine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp[b]	6/22 (27)	13/46 (28)	11/43 (26)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.036	0.930
Lower Limit		0.438	0.378
Upper Limit		2.945	2.734
Weeks to First Observed Tumor	95	76	89
Uterus: Endometrial Stromal Polyp or Sarcoma[b]	6/22 (27)	14/46 (30)	11/43 (26)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.116	0.938
Lower Limit		0.481	0.378
Upper Limit		3.131	2.734
Weeks to First Observed Tumor	95	76	89

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Anilazine in the Diet[a]

(continued)

[a]Dosed groups received 500 or 1,000 ppm in feed.

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

[c]Beneath the incidence of tumors in the matched-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 matched-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.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED ANILAZINE IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Anilazine in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Sarcoma, NOS, of the Subcutaneous Tissue[b]	0/23 (0)	3/49 (6)	0/50 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.038		
Relative Risk[f]		Infinite	--
Lower Limit		0.291	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	78	--
Lung: Alveolar/Bronchiolar Carcinoma[b]	2/23 (9)	2/48 (4)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.479	0.460
Lower Limit		0.037	0.036
Upper Limit		6.328	6.082
Weeks to First Observed Tumor	107	83	102

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Anilazine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	4/23 (17)	7/48 (15)	6/50 (12)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.839	0.690
Lower Limit		0.243	0.186
Upper Limit		3.600	3.075
Weeks to First Observed Tumor	83	83	102
Hematopoietic System: Lymphoma[b]	2/23 (9)	1/49 (2)	4/50 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.235	0.920
Lower Limit		0.004	0.145
Upper Limit		4.326	9.724
Weeks to First Observed Tumor	78	103	95

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Anilazine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	9/23 (39)	5/49 (10)	12/50 (24)
P Values[c,d]	N.S.	P = 0.006(N)	N.S.
Departure from Linear Trend[e]	P = 0.007		
Relative Risk[f]		0.261	0.613
Lower Limit		0.081	0.289
Upper Limit		0.771	1.439
Weeks to First Observed Tumor	68	95	93
Liver: Hepatocellular Adenoma or Carcinoma[b]	9/23 (39)	6/49 (12)	12/50 (24)
P Values[c,d]	N.S.	P = 0.012(N)	N.S.
Departure from Linear Trend[e]	P = 0.015		
Relative Risk[f]		0.313	0.613
Lower Limit		0.109	0.289
Upper Limit		0.872	1.439
Weeks to First Observed Tumor	68	95	93

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Anilazine in the Diet[a]

(continued)

[a]Dosed groups received 500 or 1,000 ppm in feed.

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

[c]Beneath the incidence of tumors in the matched-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 matched-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 Administered Anilazine in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma or Leukemia[b]	3/25 (12)	9/47 (19)	10/50 (20)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.596	1.667
Lower Limit		0.449	0.485
Upper Limit		8.552	8.811
Weeks to First Observed Tumor	72	85	86
Pituitary: Chromophobe Adenoma[b]	1/23 (4)	3/43 (7)	1/44 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.605	0.523
Lower Limit		0.140	0.007
Upper Limit		82.217	40.132
Weeks to First Observed Tumor	109	107	109

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Anilazine in the Diet[a]

(continued)

[a]Dosed groups received 500 or 1,000 ppm in feed.

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

[c]Beneath the incidence of tumors in the matched-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 matched-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.

APPENDIX G

ANALYSIS OF FORMULATED DIETS FOR CONCENTRATIONS OF ANILAZINE

APPENDIX G

Analysis of Formulated Diets for

Concentrations of Anilazine

Duplicate 10-g samples of formulated diets were extracted in 250 ml of a 50:50 acetone:benzene solution and agitated mechanically for 3-4 hours. Suitable diluted aliquots of the extract were analyzed by gas-liquid chromatography using an electron capture detector. Spiked samples were worked up simultaneously with the dosed feed samples and data from these analyses were used to correct the recoveries from the dosed feed samples.

Theoretical Concentration (ppm)	No. of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
500	12	492	4.05	464-530
1,000	14	988	4.46	894-1056

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

June 29, 1978

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

The reviewer agreed with the conclusion in the report that Anilazine was not carcinogenic in treated rats or mice, under the conditions of test. After a brief description of the experimental design, the reviewer commented on the small number of control animals and low dose levels administered. He said that the results from the subchronic study indicated that higher dosages should have been used in the chronic phase. The experimental flaw detracted from the value of the bioassay. The reviewer moved that the report on the bioassay of Anilazine be accepted as written. The motion was approved without objection.

Clearinghouse Members present:

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

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

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