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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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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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CONTRIBUTORS: This report presents the results of the bioassay of anthranilic acid for possible carcinogenicity, conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), Bethesda, Maryland. The bioassay was conducted by Southern Research Institute, Birmingham, Alabama, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI carcinogenesis bioassay program.

The experimental design and doses were determined by Drs. D. P. Griswold[1], J. D. Prejean[1], E. K. Weisburger[2], and J. H. Weisburger[2,3]. Ms. J. Belzer[1] and Mr. I. Brown[1] were responsible for the care and feeding of the laboratory animals. Data management and retrieval were performed by Ms. C. A. Dominick[1]. Histopathologic examinations were performed by Drs. S. D. Kosanke[1] and J. C. Peckham[1], and the diagnoses included in this report represent their interpretation.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[4]. The statistical analyses were performed by Dr. J. R. Joiner[5], using methods selected for the bioassay program by Dr. J. J. Gart[6]. Chemicals used in this bioassay were analyzed under the direction of Dr. E. Murrill[7], and the analytical results were reviewed by Dr. C. W. Jameson[5].

This report was prepared at Tracor Jitco under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg[5], Director of the Bioassay Program; Drs. J. F. Robens[5] and C. H. Williams[5], toxicologists; Dr. R. L. Schueler[5], pathologist; Ms. L. A. Waitz[5] and Mr. W. D.

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SUMMARY

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

Groups of 35 rats and 35 mice of each sex were administered anthranilic acid at one of the following doses, either 15,000 or 30,000 ppm for the rats, and either 25,000 or 50,000 ppm for the mice, 5 days per week for 78 weeks, then observed for an additional 26-27 weeks. Matched controls consisted of groups of 15 rats and 15 mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 15 untreated male and 15 untreated female animals of each species from a similar bioassay of another test chemical. Except for the matched-control male mice, all surviving animals in the study were killed at 104-106 weeks. Half of the matched-control male mice, which were accidentally killed at week 12, were excluded from the report; the remaining matched-control males died by week 94.

Mean body weights of the low- and high-dose male and high-dose female rats were lower than those of the corresponding matched controls for the duration of the study. The weights of the low-dose females were similar to those of the matched controls for the first 45 weeks, after which they declined slightly. The weights of the low-dose male mice were similar to those of the matched controls, while those of the high-dose males and of the low- and high-dose females were slightly lower.

Survival of both treated and matched-control groups of rats of both sexes was high; survival of treated mice of both sexes and of female matched controls, although lower than that of the rats, was sufficient for meaningful statistical analyses of the incidences of tumors.

In rats, a variety of neoplasms were observed in both treated and control animals. Few malignant tumors were found, and no tumors occurred in treated animals in statistically significant incidences when compared with control animals.

In mice, a variety of neoplasms were observed in both treated and control animals. These neoplasms are not uncommon in this strain of mouse, and none occurred in treated animals in statistically significant incidences when compared with control animals.

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

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I. INTRODUCTION

Anthranilic acid (CAS 118-92-3; NCI C01730) is an aromatic amine which occurs physiologically as a metabolite of the amino acid tryptophan (Fruton and Simmonds, 1961). It is used commercially as an intermediate in dye synthesis (Noller, 1965). Anthranilic acid and other tryptophan metabolites have been studied for carcinogenicity as a result of the work of Dunning et al. (1950) and Boyland et al. (1954), who found that rats fed diets containing both 2-acetylaminofluorene and tryptophan developed bladder tumors, although 2-acetylaminofluorene itself induced mainly liver tumors. Since then, anthranilic acid and other tryptophan metabolites have been studied for carcinogenicity (Raushenbakh et al., 1961; Bryan, 1971), but no convincing evidence of the carcinogenicity of anthranilic acid was found. Certain tryptophan metabolites, including anthranilic acid, have been found in abnormally high concentrations in the urine of patients with bladder cancer (Boyland and Williams, 1956) and of persons who have increased risks of bladder cancer, such as cigarette smokers (Kerr et al., 1965) or patients with bilharzial bladder infestation (Abul-Fadl and Khalafallah, 1961). These observations suggested that an abnormality in tryptophan metabolism may be responsible for certain cases of endogenous bladder cancer (Bryan, 1969). Anthranilic acid was selected for testing to evaluate its carcinogenicity in a standard bioassay.

II. MATERIALS AND METHODS

A. Chemical

Anthranilic acid (2-aminobenzoic acid) was obtained in several batches from the Aldrich Chemical Company, Inc., Milwaukee, Wisconsin. The purity of the batch used for the chronic study (Lot No. 062927) was determined to be 98.5 $\pm$ 0.1% by analyses at Midwest Research Institute. The melting point of this batch was 145-147°C (literature: 144-146°C). Elemental analyses (C, H, N) were correct for $C_7H_7NO_2$, the molecular formula of anthranilic acid. The identity was confirmed by nuclear magnetic resonance, infrared, and ultraviolet spectra, which were in agreement with the structure and matched the spectra given in the literature.

The chemical used for the chronic study was stored in the original cardboard container at 5°C.

B. Dietary Preparation

Test diets were prepared at four concentrations of anthranilic acid: 1.5, 2.5, 3.0, and 5.0%. The chemical was sifted and a known amount was added to and mixed with a small quantity of Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.). This mixture was then added to a 2-week supply of animal meal and blended in a twin-shell blender for 10 minutes. No concentration

or stability analyses of the chemical in feed were performed. The prepared diets were stored at room temperature in sealed plastic containers.

C. Animals

Animals used for the subchronic studies were male Sprague-Dawley rats from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, and male Swiss mice from Purina Laboratories, St. Louis, Missouri. Animals used in the chronic studies were Fischer 344 rats of both sexes from Harlan Industries, Cumberland, Indiana, and B6C3F1 mice of both sexes from Charles River Laboratories. All animals were approximately 30 days of age when received at the laboratory. All animals were quarantined for an acclimation period (rats for 18 days, mice for 11 days). Animals with no visible signs of disease were then assigned to control and treated groups and earmarked for individual identification.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature range was 20-24°C, and the relative humidity was maintained at 40-60%. Room air was changed 15 times per hour and passed through both intake and exhaust fiberglass roughing filters. In addition to natural light, illumination was

provided by fluorescent light for 9 hours per day. Food and water were supplied daily and were available ad libitum.

Rats were housed five per cage and mice seven per cage in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). The bottoms of the rat cages were lined with Iso-Dri® hardwood chips (Carworth, Edison, N. J.), and cage tops were covered with disposable filter bonnets; mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N. Y.) and covered with disposable filter bonnets beginning at week 91. Bedding was replaced once per week; cages, water bottles, and feeders were sanitized at 82°C once per week; and racks were cleaned once per week.

The rats and mice were housed in separate rooms. Control animals were housed with respective treated animals. Animals treated with anthranilic acid were maintained in the same rooms as animals of the same species being treated with the following chemicals:

RATS

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide (acetohexamide) (CAS 968-81-0)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide (chlorpropamide) (CAS 94-20-2)

5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine
(pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride
(phenazopyridine hydrochloride) (CAS 136-40-3)
L-tryptophan (CAS 73-22-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea
(tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
4,4′-thiodianiline (CAS 139-65-1)
ethionamide (CAS 536-33-4)

MICE

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide
(acetohexamide) (CAS 968-81-0)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide
(chlorpropamide) (CAS 94-20-2)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine
(pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride
(phenazopyridine hydrochloride) (CAS 136-40-3)
L-tryptophan (CAS 73-22-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea
(tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
4,4′-thiodianiline (CAS 139-65-1)
ethionamide (CAS 536-33-4)

Gavage Studies

cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate
(phenesterin) (CAS 3546-10-9)
estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate)
(estradiol mustard) (CAS 22966-79-6)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride
(MAAM) (NSC 141549)

acronycine (CAS 7008-42-6)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGdR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride (phenoxybenzamine) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)

E. Subchronic Studies

Subchronic studies were conducted to estimate the maximum tolerated doses of anthranilic acid, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the chronic studies. Anthranilic acid was administered to male Sprague-Dawley rats and male Swiss mice in the feed in concentrations of 1,000, 5,000, 10,000, 25,000, or 50,000 ppm. Treated and untreated-control groups each consisted of five animals. Treated groups received test diets 7 days per week for 45 days and were observed for an additional 45 days.

No rats died at any dose administered. The mean body weight of the animals fed 50,000 ppm was 83% of that of the controls; the

weight of those fed 25,000 ppm was 89%; and the weights of those fed lower doses were close to those of the controls. The low and high doses for the chronic studies using rats were set at 15,000 and 30,000 ppm.

In mice, the doses administered had no effect, either on weight gain or mortality, although one animal died shortly before the end of the study. The low and high doses for the chronic studies using mice were set at 25,000 and 50,000 ppm.

F. Designs of Chronic Studies

The designs of the chronic studies are shown in tables 1 and 2.

Since the numbers of animals in the matched-control groups were small, pooled-control groups also were used for statistical comparisons. Matched controls from the current studies on anthranilic acid were combined with matched controls from studies performed on 4,4′-thiodianiline (CAS 139-65-1). The pooled controls for statistical tests using rats or mice consisted of 30 males and of 30 females. The studies on 4,4′-thiodianiline were also conducted at Southern Research Institute, administered in the feed, and overlapped the anthranilic acid study by at least 45 weeks using rats and 63 weeks using mice. The matched-control groups for the different test chemicals were of the same strain

Table 1. Design of Anthranilic Acid Chronic Feeding Studies in Rats

Sex and Treatment Group	Initial No. of Animals[a]	Anthranilic Acid in Diet[b] (ppm)	Time on Study	
			Treated[c] (weeks)	Untreated (weeks)
Male				
Matched-Control	15	0		106
Low-Dose	35	15,000	78	27
High-Dose	35	30,000	78	26
Female				
Matched-Control	15	0		106
Low-Dose	35	15,000	78	27
High-Dose	35	30,000	78	26-27

[a]All animals were 48 days of age when placed on study.

[b]Treated animals were fed test diets 5 days per week and control diets 2 days per week.

[c]All animals were placed on study on the same day.

Table 2. Design of Anthranilic Acid Chronic Feeding Studies in Mice

Sex and Treatment Group	Initial No. of Animals[a]	Anthranilic Acid in Diet[b] (ppm)	Time on Study: Treated[d] (weeks)	Time on Study: Untreated (weeks)
Male				
Matched-Control	15[c]	0		94
Low-Dose	35	25,000	78	27
High-Dose	35	50,000	78	27
Female				
Matched-Control	15	0		105
Low-Dose	35	25,000	78	27
High-Dose	35	50,000	78	27

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

[b]Treated animals were fed test diets 5 days per week and control diets 2 days per week.

[c]Seven matched-control mice were killed accidentally at week 12.

[d]All animals were placed on study on the same day.

and from the same supplier, and they were examined by the same pathologists.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and animals that were moribund were killed and necropsied, except for those dying prior to day 100, due, presumably, to toxicity of the test chemical. Rats and mice were weighed individually each week for 8 weeks and every 2 weeks thereafter. Palpation for masses was carried out at each weighing.

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, muscle, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder and bile duct (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, brain, and sensory organs. Peripheral blood smears were prepared from each animal. 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 were 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 treated animals at each dose level. When results for a number of treated groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) 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 relation-

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

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

cant 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 both low- and high-dose male and of high-dose female rats were lower than those of matched controls during administration of anthranilic acid (figure 1). The weights of the low-dose females were comparable to those of the controls for the first 50 weeks on test diets, and were lower thereafter. Fluctuation in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variation. No unusual clinical signs were reported in the treated groups of rats.

To control signs of respiratory disease, rats were treated with oxytetracycline in the drinking water at 0.6 mg/ml during weeks 31-35 and at 0.3 mg/ml during week 36.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats fed anthranilic acid in the diet at the doses of this experiment, together with those of the matched controls, are shown in figure 2.

In each sex, the Tarone test result for positive dose-related trends in mortality is not significant. In male rats, 89% of the

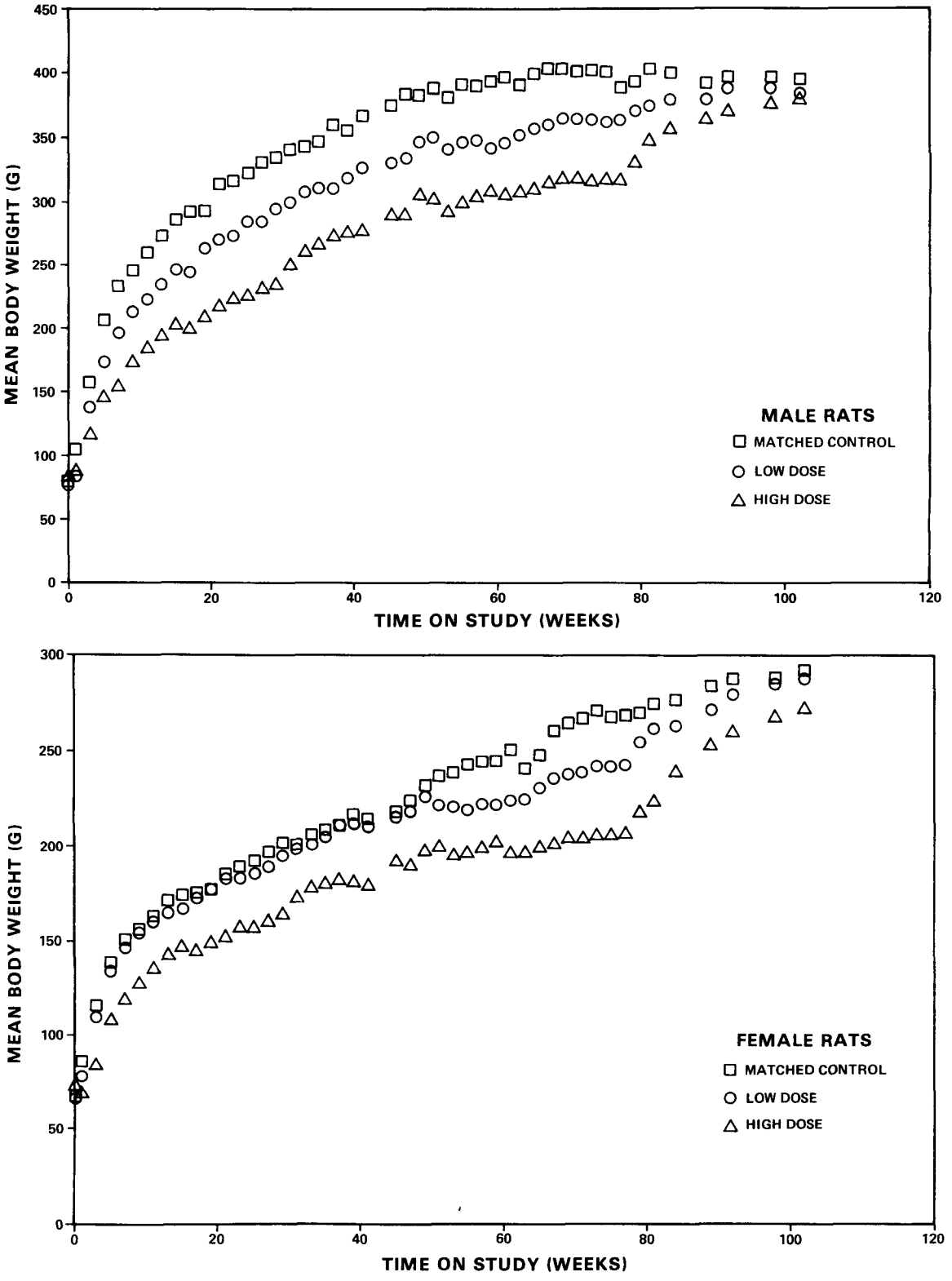

Figure 1. Growth Curves For Rats Fed Anthranilic Acid In The Diet

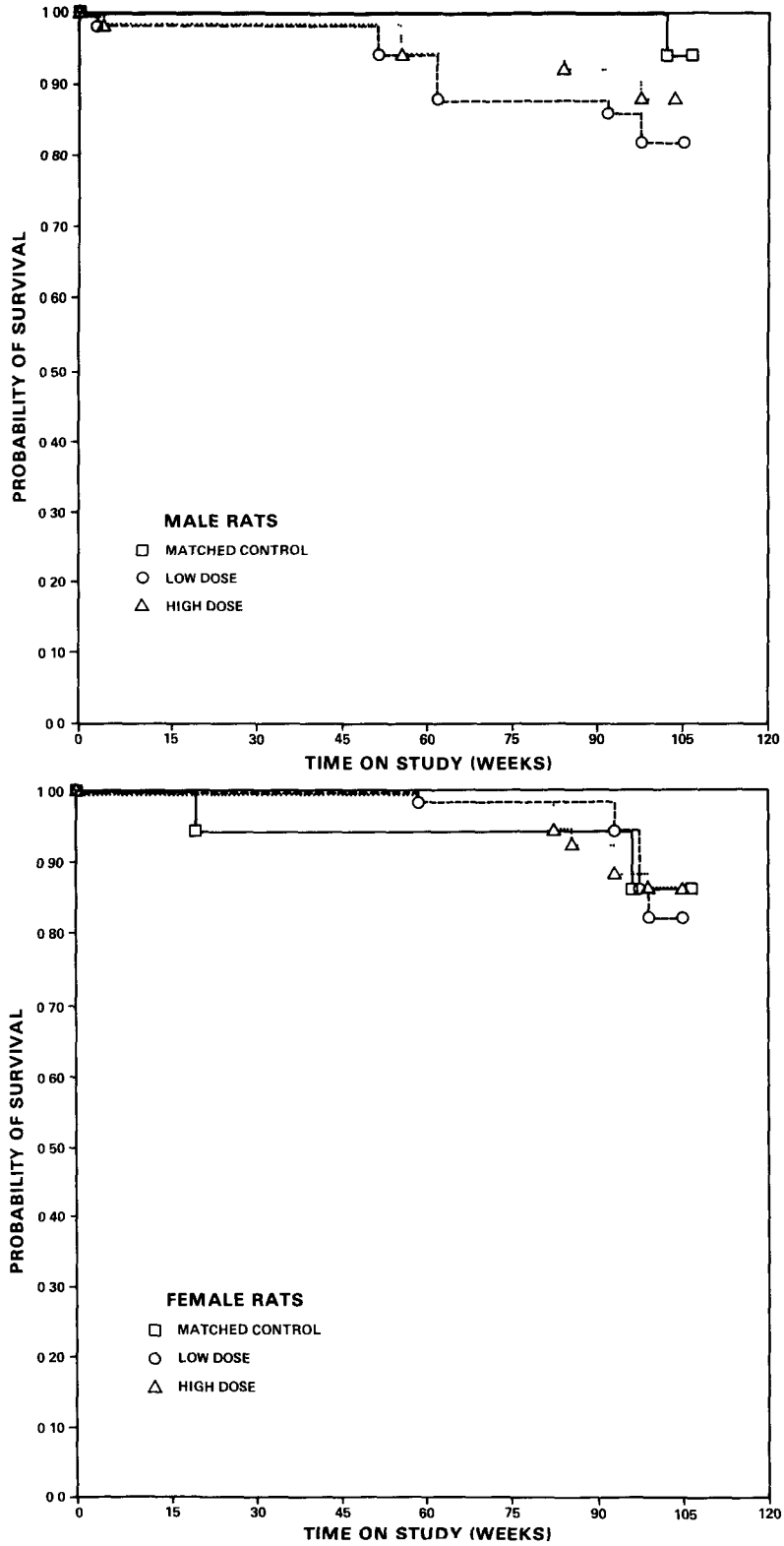

Figure 2. Survival Curves For Rats Fed Anthranilic Acid In The Diet

high-dose group, 83% of the low-dose group, and 93% of the matched controls lived to the end of the study. In females, the respective percentages of survival were 86%, 83%, and 87%. Sufficient numbers of animals were available in both male and female treated groups of rats for meaningful statistical analyses of the incidences 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 were observed in both the matched-control and treated groups. Some types of neoplasms occurred only, or with a greater frequency, in rats of the treated groups when compared with the matched controls. These lesions, however, are not uncommon in this strain of rat independent of any treatment. Few malignant tumors were observed, and tumor metastasis was observed in only one treated rat.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were encountered in animals of the treated and control groups (Appendix C). These nonneoplastic lesions are commonly seen in aged Fischer 344 rats.

In the judgment of the pathologists, anthranilic acid was not carcinogenic in Fischer 344 rats under the conditions of this study.

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 were observed in at least 5% of one or more of the groups.

In each sex, no tumors were observed in statistically significant incidences ($P < 0.05$) in the positive direction. Fibroadenoma of the mammary gland apppeared in statistically higher incidences in the control groups than in the treated groups. In each of the 95% confidence intervals for relative risk, shown in the tables, a 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 has an upper limit greater than one (except the incidence of fibroadenoma of the mammary gland in high-dose females), indicating the theoretical possibility of the induction of tumors by this chemical, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the low-dose male mice were similar to those of the matched controls, but those of the high-dose males and of the females at both the low and high doses were slightly lower than those of the matched controls (figure 3). Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variation.

The only clinical sign observed in the treated groups which was not observed in the control groups was bleeding from the perineal and anal regions.

To control respiratory disease, the animals were treated with oxytetracycline in the drinking water at 0.6 mg/ml for 5 days during week 71, followed by treatment for 5 days at 0.3 mg/ml. Beginning at week 70 and continuing for about 2 months, propylene glycol vapor was also used in the mouse room to help control the disease.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice fed anthranilic acid in the

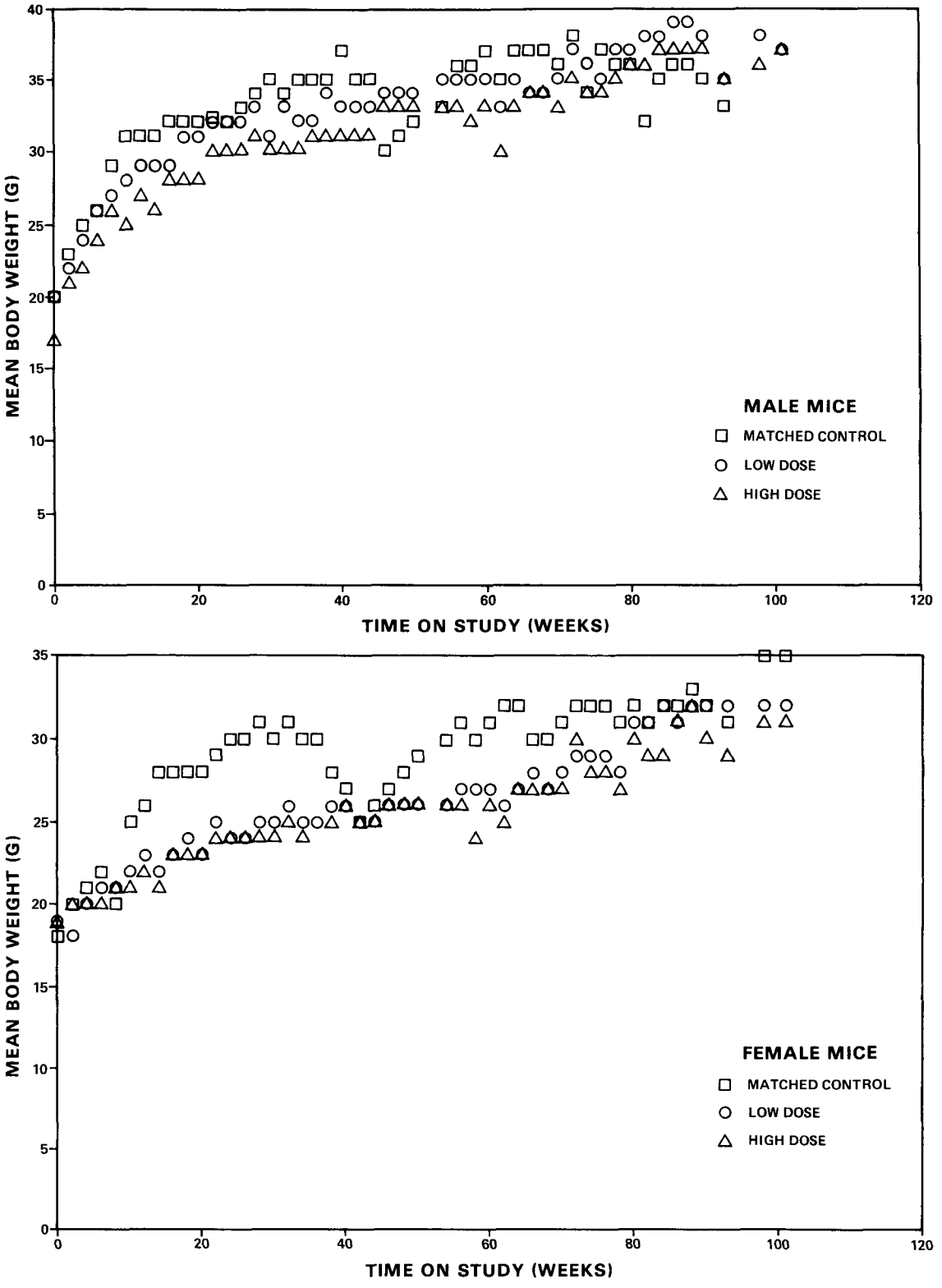

Figure 3. Growth Curves For Mice Fed Anthranilic Acid In The Diet

diet at the doses of this experiment, together with those of the matched controls, are shown in figure 4.

In male mice, the Tarone test result for negative dose-related trend in mortality is significant (P = 0.043), and an indicated departure from linearity is observed (P = 0.008). Of the males, 32/35 high-dose animals and 30/35 low-dose animals, but only 4/15 controls, lived at least as long as 52 weeks on study. In females, the Tarone test result is not significant. Fifty-six percent of the high-dose animals, 66% of the low-dose animals, and 41% of the controls survived to termination of the study. Sufficient numbers of treated animals of both sexes survived for meaningful statistical analyses of the incidences of late-appearing tumors, when pooled controls were used.

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 variety of neoplasms were observed in both matched-control and treated groups. Some types of neoplasms occurred only, or with a greater frequency, in mice of treated groups when compared with controls. These lesions, however, are not uncommon in this strain of mouse independent of any treatment.

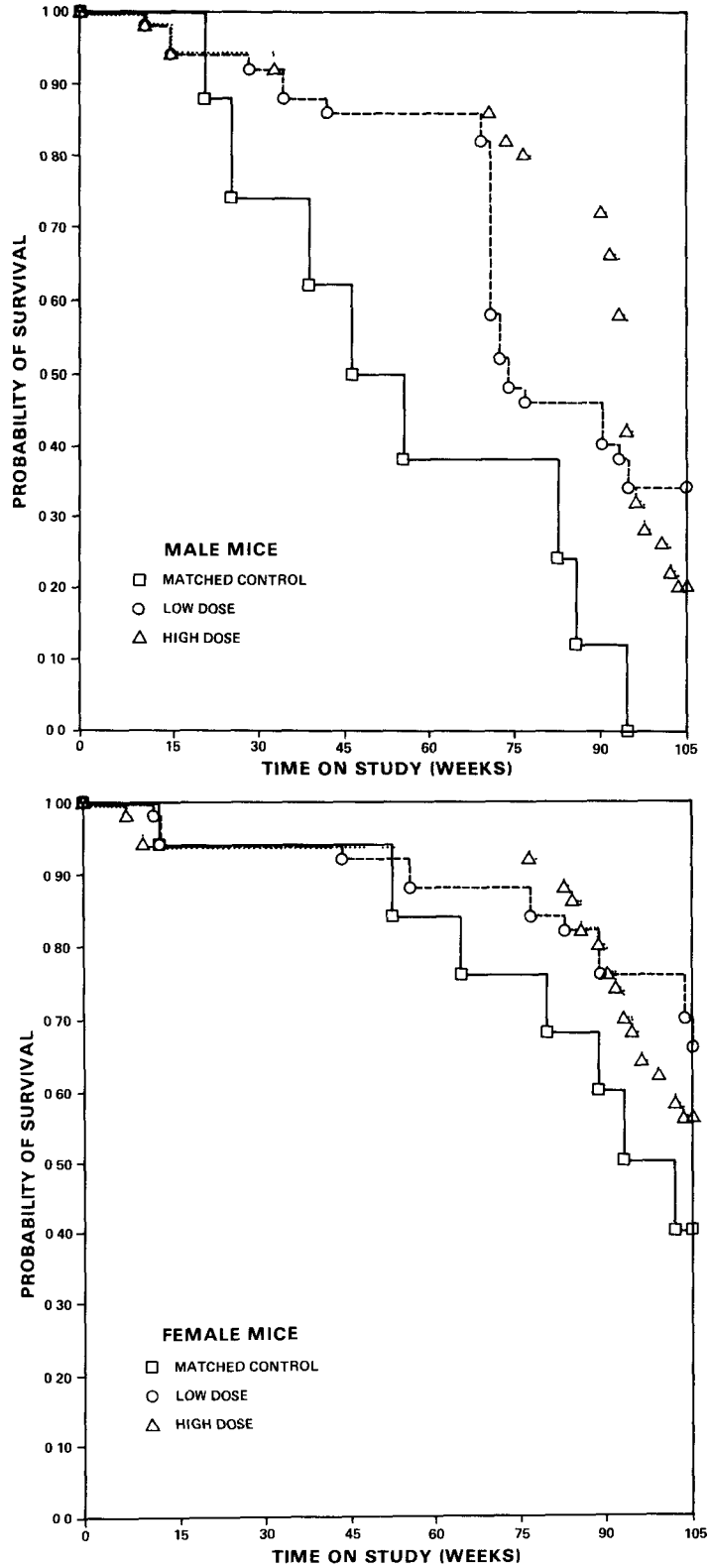

Figure 4. Survival Curves For Mice Fed Anthranilic Acid In The Diet

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were encountered in animals of the treated and control groups (Appendix D). These nonneoplastic lesions are commonly seen in aged B6C3F1 mice. The severe suppurative bronchopneumonias, however, were associated with increased deaths or decreased life spans. The incidence of bronchopneumonias was high in all the control and treated groups, except for the low-dose males.

Few matched-control mice lived to termination of the study, and many were not examined microscopically. Nearly half of the matched-control males were killed accidentally at week 12.

No specific types or incidences of tumors were considered to be associated with the chemical. All neoplasms have been encountered previously as spontaneous lesions in B6C3F1 mice. Few malignant tumors were observed, and tumor metastasis was observed in only one treated mouse.

In the judgment of the pathologists, anthranilic acid did not appear to be carcinogenic in B6C3F1 mice under the conditions of this study.

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 were observed in at least 5% of one or more of the groups.

No tumors occurred in either sex at a statistically significant incidence ($P < 0.05$) in the positive direction. In each of the 95% confidence intervals for 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 this chemical, which could not be detected under the conditions of this test.

V. DISCUSSION

Mean body weights of high-dose female rats and of both low- and high-dose males fed anthranilic acid were lower than those of the corresponding matched controls throughout the study. The weights of the low-dose females were the same as those of the controls for 45 weeks, then they decreased slightly. The weights of the low-dose male mice were similar to those of the matched controls; those of the high-dose male mice and of the low- and high-dose female mice were slightly lower. No unusual clinical signs were reported in the rats, although bleeding from the perineal and anal regions was observed in an occasional mouse in all the treated groups. The number of animals surviving in treated groups of rats of both sexes was sufficient for meaningful statistical analyses of the incidences of tumors. Survival of the treated mice was lower than that of the rats, but an adequate number of treated animals of both sexes was available for meaningful statistical analyses of the incidences of tumors. Since survival of the matched controls was low, pooled controls were used for comparisons.

A variety of neoplasms were observed in both treated and control rats of both sexes, but there was no incidence of tumors which could be attributed to the administration of anthranilic acid, and there was no dose-response pattern.

In mice, a variety of neoplasms were observed in both matched-control and treated groups. Some types of neoplasms occurred only, or with a greater frequency, in the treated groups when compared with the matched-control groups. They were of the types commonly found in this strain of mouse, however, and the incidences were low and not statistically significant.

Several metabolites of the essential amino acid L-tryptophan have been shown to produce bladder cancer when implanted in the bladder of experimental animals, but anthranilic acid, a known metabolite of tryptophan, was inactive using this method of administration (Bryan, 1969). When administered once per week by subcutaneous injection to mice over a period of 10-12 months, anthranilic acid induced a low incidence of leukemoid reaction, but did not induce tumors or leukemia (Raushenbakh, 1961). Anthranilic acid fed to male Fischer 344 rats for 16 weeks at 8,100 ppm did not induce liver tumors or other hepatic lesions (Yamamoto et al., 1970). No long-term feeding studies to measure the carcinogenic potential of anthranilic acid have previously been reported.

It is concluded that under the conditions of this bioassay, anthranilic acid 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 FED ANTHRANILIC ACID IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED ANTHRANILIC ACID IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	34	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	34	34
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(34)	(34)
PAPILLOMA, NOS		1 (3%)	1 (3%)
KERATOACANTHOMA		1 (3%)	
*SUBCUT TISSUE	(15)	(34)	(34)
SEBACEOUS ADENOMA		1 (3%)	
FIBROMA	1 (7%)	1 (3%)	
RESPIRATORY SYSTEM			
#TRACHEAL SUBMUCOSA	(15)	(33)	(34)
FIBROMA	1 (7%)		
#LUNG	(15)	(32)	(33)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (3%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(15)	(34)	(34)
UNDIFFERENTIATED LEUKEMIA		2 (6%)	1 (3%)
#MANDIBULAR L. NODE	(14)	(30)	(24)
C-CELL CARCINOMA, METASTATIC		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SMALL INTESTINE	(14)	(34)	(34)
MUCINOUS ADENOCARCINOMA			1 (3%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#ILEUM	(14)	(34)	(34)
ADENOMATOUS POLYP, NOS		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(15)	(34)	(34)
TUBULAR-CELL ADENOCARCINOMA	1 (7%)		
ENDOCRINE SYSTEM			
#PITUITARY	(14)	(31)	(30)
CHROMOPHOBE ADENOMA	1 (7%)	3 (10%)	
CHROMOPHOBE CARCINOMA	1 (7%)		
#ADRENAL	(15)	(34)	(32)
PHEOCHROMOCYTOMA		1 (3%)	
#THYROID	(15)	(32)	(34)
C-CELL ADENOMA	1 (7%)	2 (6%)	1 (3%)
C-CELL CARCINOMA		3 (9%)	1 (3%)
#PANCREATIC ISLETS	(14)	(34)	(34)
ISLET-CELL ADENOMA	1 (7%)	1 (3%)	1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(34)	(34)
FIBROADENOMA			1 (3%)
*PREPUTIAL GLAND	(15)	(34)	(34)
ADNEXAL ADENOMA		2 (6%)	1 (3%)
#TESTIS	(15)	(34)	(34)
INTERSTITIAL-CELL TUMOR	14 (93%)	27 (79%)	25 (74%)
NERVOUS SYSTEM			
#BRAIN	(15)	(33)	(34)
OLIGODENDROGLIOMA			1 (3%)
SPECIAL SENSE ORGANS			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*TUNICA VAGINALIS	(15)	(34)	(34)
MESOTHELIOMA, NOS			1 (3%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(15)	(34)	(34)
MESOTHELIOMA, NOS			1 (3%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@		3	1
MORIBUND SACRIFICE	1	3	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	14	29	31
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*	15	28	27
TOTAL PRIMARY TUMORS	21	46	37
TOTAL ANIMALS WITH BENIGN TUMORS	14	28	25
TOTAL BENIGN TUMORS	19	41	31
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	5	3
TOTAL MALIGNANT TUMORS	2	5	4
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED ANTHRANILIC ACID IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14	33	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	33	35
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(14)	(33)	(35)
FIBROMA	1 (7%)		
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(14)	(33)	(35)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (7%)		
UNDIFFERENTIATED LEUKEMIA		3 (9%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#COLON	(13)	(33)	(34)
ADENOMATOUS POLYP, NOS	1 (8%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(11)	(33)	(32)
CHROMOPHOBE ADENOMA		9 (27%)	6 (19%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#THYROID	(12)	(31)	(35)
C-CELL ADENOMA			2 (6%)
C-CELL CARCINOMA	1 (8%)		3 (9%)
#PANCREATIC ISLETS	(14)	(33)	(34)
ISLET-CELL ADENOMA		2 (6%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(14)	(33)	(35)
PAPILLARY ADENOMA	1 (7%)		
FIBROADENOMA	6 (43%)	4 (12%)	
*PREPUTIAL GLAND	(14)	(33)	(35)
ADNEXAL ADENOMA		1 (3%)	
#UTERUS	(14)	(33)	(35)
PAPILLARY ADENOCARCINOMA		1 (3%)	
SARCOMA, NOS		1 (3%)	
ENDOMETRIAL STROMAL POLYP	3 (21%)	4 (12%)	1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	1	3	2
MORIBUND SACRIFICE	1	3	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	29	30
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	10	19	11
TOTAL PRIMARY TUMORS	14	25	12
TOTAL ANIMALS WITH BENIGN TUMORS	9	15	8
TOTAL BENIGN TUMORS	12	20	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	5	3
TOTAL MALIGNANT TUMORS	2	5	3
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
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE FED ANTHRANILIC ACID IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED ANTHRANILIC ACID IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	6	31	29
ANIMALS EXAMINED HISTOPATHOLOGICALLY	6	31	29
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(6)	(31)	(29)
ALVEOLAR/BRONCHIOLAR ADENOMA		5 (16%)	
HEMATOPOIETIC SYSTEM			
#BRAIN	(3)	(30)	(29)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	
*MULTIPLE ORGANS	(6)	(31)	(29)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (3%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(5)	(31)	(29)
HEPATOCELLULAR ADENOMA		5 (16%)	4 (14%)
HEPATOCELLULAR CARCINOMA		1 (3%)	1 (3%)
URINARY SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#THYROID	(4)	(23)	(17)
FOLLICULAR-CELL ADENOMA		1 (4%)	
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	6	15	7
MORIBUND SACRIFICE	2	8	21
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED	7		
TERMINAL SACRIFICE		12	7
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*		13	6
TOTAL PRIMARY TUMORS		14	6
TOTAL ANIMALS WITH BENIGN TUMORS		10	4
TOTAL BENIGN TUMORS		11	4
TOTAL ANIMALS WITH MALIGNANT TUMORS		3	2
TOTAL MALIGNANT TUMORS		3	2
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
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED ANTHRANILIC ACID IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING	4	2	
ANIMALS NECROPSIED	11	30	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	11	30	33
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(11)	(30)	(33)
FIBROMA			1 (3%)
RESPIRATORY SYSTEM			
#LUNG	(11)	(30)	(33)
HEPATOCELLULAR CARCINOMA, METAST			1 (3%)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (3%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(11)	(30)	(33)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (3%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		3 (10%)	
LYMPHOCYTIC LEUKEMIA			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(11)	(30)	(33)
HEPATOCELLULAR ADENOMA		1 (3%)	1 (3%)
HEPATOCELLULAR CARCINOMA			1 (3%)
URINARY SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#UTERUS	(11)	(29)	(32)
LEIOMYOSARCOMA	1 (9%)		
#OVARY	(11)	(29)	(32)
PAPILLARY CYSTADENOMA, NOS			1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	4	9	6
MORIBUND SACRIFICE	3	2	9
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	1
TERMINAL SACRIFICE	4	21	19
ANIMAL MISSING	4	2	
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	1	5	5
TOTAL PRIMARY TUMORS	1	5	6
TOTAL ANIMALS WITH BENIGN TUMORS		1	4
TOTAL BENIGN TUMORS		1	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	4	2
TOTAL MALIGNANT TUMORS	1	4	2
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS FED ANTHRANILIC ACID IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED ANTHRANILIC ACID IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	34	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	34	34
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(34)	(34)
EPIDERMAL INCLUSION CYST		1 (3%)	1 (3%)
FIBROSIS, FOCAL			1 (3%)
RESPIRATORY SYSTEM			
#TRACHEA	(15)	(33)	(34)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (3%)	2 (6%)
INFLAMMATION, SUPPURATIVE	7 (47%)	4 (12%)	3 (9%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)		
#LUNG	(15)	(32)	(33)
PNEUMONIA, CHRONIC MURINE	1 (7%)	8 (25%)	1 (3%)
FIBROSIS			1 (3%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (3%)	1 (3%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(14)	(34)	(34)
HYPERPLASIA, NODULAR			1 (3%)
HYPERPLASIA, RETICULUM CELL		1 (3%)	
HEMATOPOIESIS		1 (3%)	6 (18%)
#MESENTERIC L. NODE	(14)	(30)	(24)
LYMPHANGIECTASIS			1 (4%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(15)	(33)	(34)
INFLAMMATION, INTERSTITIAL	2 (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, CHRONIC		1 (3%)	4 (12%)
INFLAMMATION, CHRONIC FOCAL		5 (15%)	7 (21%)
DIGESTIVE SYSTEM			
#LIVER	(15)	(34)	(34)
NECROSIS, FOCAL			1 (3%)
#LIVER/CENTRILOBULAR	(15)	(34)	(34)
METAMORPHOSIS FATTY	1 (7%)		
#PANCREAS	(14)	(34)	(34)
INFLAMMATION, INTERSTITIAL	1 (7%)		
INFLAMMATION, SUPPURATIVE			1 (3%)
INFLAMMATION, CHRONIC		1 (3%)	2 (6%)
INFLAMMATION, CHRONIC FOCAL	2 (14%)	1 (3%)	1 (3%)
FIBROSIS, DIFFUSE	1 (7%)	1 (3%)	
PERIARTERITIS		1 (3%)	
#PANCREATIC ACINUS	(14)	(34)	(34)
ATROPHY, FOCAL		1 (3%)	
#STOMACH	(15)	(34)	(33)
ULCER, NOS	1 (7%)		
URINARY SYSTEM			
#KIDNEY	(15)	(34)	(34)
INFLAMMATION, CHRONIC	11 (73%)	10 (29%)	3 (9%)
INFLAMMATION, CHRONIC DIFFUSE	3 (20%)	22 (65%)	21 (62%)
PERIARTERITIS		1 (3%)	
#KIDNEY/CORTEX	(15)	(34)	(34)
CYST, NOS		1 (3%)	
ENDOCRINE SYSTEM			
#THYROID	(15)	(32)	(34)
CYSTIC FOLLICLES	1 (7%)		
FOLLICULAR CYST, NOS			1 (3%)
HYPERPLASIA, C-CELL			1 (3%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(34)	(34)
CYST, NOS	1 (7%)		
*PREPUTIAL GLAND	(15)	(34)	(34)
HYPERPLASIA, NOS	1 (7%)		
#PROSTATE	(15)	(32)	(33)
INFLAMMATION, SUPPURATIVE	3 (20%)		1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV	5 (33%)		
#TESTIS	(15)	(34)	(34)
ATROPHY, NOS			1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	1
NO NECROPSY PERFORMED		1	1

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED ANTHRANILIC ACID IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14	33	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	33	35
INTEGUMENTARY SYSTEM			
*SKIN	(14)	(33)	(35)
EPIDERMAL INCLUSION CYST			1 (3%)
ULCER, FOCAL			1 (3%)
INFLAMMATION, CHRONIC			1 (3%)
RESPIRATORY SYSTEM			
#TRACHEA	(14)	(33)	(35)
LYMPHOCYTIC INFLAMMATORY INFILTR		2 (6%)	1 (3%)
INFLAMMATION, SUPPURATIVE	6 (43%)	2 (6%)	9 (26%)
#LUNG	(14)	(33)	(34)
CONGESTION, NOS			1 (3%)
PNEUMONIA, CHRONIC MURINE	1 (7%)	4 (12%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM			2 (6%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(14)	(32)	(35)
CONGESTION, NOS			1 (3%)
HYPERPLASIA, RETICULUM CELL		3 (9%)	
HEMATOPOIESIS		2 (6%)	10 (29%)
#MANDIBULAR L. NODE	(13)	(17)	(29)
CYST, NOS	1 (8%)		
CIRCULATORY SYSTEM			
#HEART	(14)	(33)	(35)
PERIARTERITIS	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
#MYOCARDIUM	(14)	(33)	(35)
INFLAMMATION, INTERSTITIAL	2 (14%)		1 (3%)
INFLAMMATION, CHRONIC FOCAL		1 (3%)	3 (9%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(14)	(32)	(34)
INFLAMMATION, ACUTE NECROTIZING	1 (7%)		
#LIVER	(14)	(33)	(35)
NECROSIS, COAGULATIVE		1 (3%)	
BASOPHILIC CYTO CHANGE	2 (14%)		
#PANCREAS	(14)	(33)	(34)
INFLAMMATION, INTERSTITIAL	4 (29%)		
#COLON	(13)	(33)	(34)
PERIARTERITIS	1 (8%)		
NEMATODIASIS	2 (15%)		
URINARY SYSTEM			
#KIDNEY	(14)	(33)	(35)
INFLAMMATION, CHRONIC	5 (36%)	7 (21%)	6 (17%)
INFLAMMATION, CHRONIC FOCAL	1 (7%)		
INFLAMMATION, CHRONIC DIFFUSE		9 (27%)	6 (17%)
#URINARY BLADDER	(13)	(32)	(33)
HYPERPLASIA, EPITHELIAL		1 (3%)	
ENDOCRINE SYSTEM			
#PITUITARY	(11)	(33)	(32)
HYPERPLASIA, CHROMOPHOBE-CELL	1 (9%)		
#ADRENAL CORTEX	(14)	(33)	(35)
NECROSIS, FOCAL	1 (7%)		
#THYROID	(12)	(31)	(35)
CYST, NOS	1 (8%)		
HYPERPLASIA, C-CELL			1 (3%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, FOLLICULAR-CELL	1 (8%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(14)	(33)	(35)
CYST, NOS	3 (21%)		
CYSTIC DUCTS		3 (9%)	8 (23%)
INFLAMMATION, FOCAL	1 (7%)		
INFLAMMATION, CHRONIC	1 (7%)		
*PREPUTIAL GLAND	(14)	(33)	(35)
INFLAMMATION, SUPPURATIVE		1 (3%)	
#UTERUS	(14)	(33)	(35)
HYDROMETRA			1 (3%)
CYST, NOS	1 (7%)		
PYOMETRA	8 (57%)		
INFLAMMATION, CHRONIC DIFFUSE		1 (3%)	
POLYP, INFLAMMATORY		1 (3%)	
DECIDUAL ALTERATION, NOS		1 (3%)	
#UTERUS/ENDOMETRIUM	(14)	(33)	(35)
INFLAMMATION, SUPPURATIVE		9 (27%)	2 (6%)
HYPERPLASIA, NOS		1 (3%)	1 (3%)
HYPERPLASIA, FOCAL		2 (6%)	
HYPERPLASIA, CYSTIC		2 (6%)	4 (11%)
#OVARY/OVIDUCT	(14)	(33)	(35)
INFLAMMATION, SUPPURATIVE	2 (14%)	3 (9%)	
#OVARY	(14)	(32)	(35)
CYST, NOS	1 (7%)		
FOLLICULAR CYST, NOS		3 (9%)	
ABSCESS, NOS	1 (7%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

\# 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			
*ABDOMINAL MUSCLE	(14)	(33)	(35)
FIBROSIS, FOCAL			1 (3%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		3	8
AUTOLYSIS/NO NECROPSY	1	2	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED ANTHRANILIC ACID IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED ANTHRANILIC ACID IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	6	31	29
ANIMALS EXAMINED HISTOPATHOLOGICALLY	6	31	29
INTEGUMENTARY SYSTEM			
*SKIN	(6)	(31)	(29)
INFLAMMATION, SUPPURATIVE			1 (3%)
*SUBCUT TISSUE	(6)	(31)	(29)
HEMORRHAGE		4 (13%)	
HEMATOMA, NOS			1 (3%) <-
RESPIRATORY SYSTEM			
#TRACHEA	(6)	(31)	(29)
INFLAMMATION, SUPPURATIVE			3 (10%)
#LUNG	(6)	(31)	(29)
THROMBOSIS, NOS		1 (3%)	
INFLAMMATION, INTERSTITIAL		1 (3%)	
BRONCHOPNEUMONIA SUPPURATIVE	1 (17%)		8 (28%)
PNEUMONIA INTERSTITIAL CHRONIC		1 (3%)	1 (3%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	2 (33%)		11 (38%)
PERIARTERITIS		1 (3%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(5)	(31)	(29)
ATROPHY, NOS			1 (3%)
#SPLEEN	(6)	(31)	(29)
HYPERPLASIA, RETICULUM CELL		1 (3%)	
HEMATOPOIESIS		3 (10%)	1 (3%)
#MESENTERIC L. NODE	(1)	(3)	(3)
INFLAMMATION, SUPPURATIVE			1 (33%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
<- MULTIPLE OCCURRENCE OF MORPHOLOGY IN THE SAME ORGAN TISSUES IS COUNTED ONCE ONLY

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#MYOCARDIUM	(6)	(31)	(29)
INFLAMMATION, SUPPURATIVE		2 (6%)	
INFLAMMATION, ACUTE NECROTIZING		2 (6%)	
ANGIECTASIS			1 (3%)
DIGESTIVE SYSTEM			
#LIVER	(5)	(31)	(29)
NECROSIS, FOCAL		2 (6%)	
NECROSIS, COAGULATIVE		3 (10%)	
HYPERPLASIA, RETICULUM CELL		1 (3%)	
#COLON	(5)	(31)	(29)
NEMATODIASIS	1 (20%)		
*RECTUM	(6)	(31)	(29)
INFLAMMATION, SUPPURATIVE	1 (17%)		
ULCER, CHRONIC		1 (3%)	
HYPERPLASIA, CYSTIC		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(6)	(31)	(29)
HYPERPLASIA, LYMPHOID		1 (3%)	
#URINARY BLADDER	(6)	(31)	(29)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
ENDOCRINE SYSTEM			
#THYROID	(4)	(23)	(17)
CYSTIC FOLLICLES			2 (12%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
*TRIGEMINAL GANGLION	(6)	(31)	(29)
INFLAMMATION, CHRONIC	1 (17%)		

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*MIDDLE EAR	(6)	(31)	(29)
INFLAMMATION, CHRONIC SUPPURATIV	1 (17%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		4	5
ACCIDENTAL DEATH	7		
NO NECROPSY PERFORMED		1	1
AUTO/NECROPSY/HISTO PERF	1		
AUTOLYSIS/NO NECROPSY	2	3	5

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED ANTHRANILIC ACID IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING	4	2	
ANIMALS NECROPSIED	11	30	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	11	30	33
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(11)	(30)	(33)
INFLAMMATION, SUPPURATIVE			3 (9%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (9%)		
#LUNG	(11)	(30)	(33)
BRONCHOPNEUMONIA SUPPURATIVE		2 (7%)	1 (3%)
PNEUMONIA INTERSTITIAL CHRONIC			1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	5 (45%)	5 (17%)	18 (55%)
HYPERPLASIA, LYMPHOID		3 (10%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(10)	(27)	(31)
ATROPHY, NOS		1 (4%)	
#SPLEEN	(11)	(30)	(33)
HYPERPLASIA, LYMPHOID		1 (3%)	
HEMATOPOIESIS		1 (3%)	5 (15%)
#MESENTERIC L. NODE	(1)	(8)	(7)
CONGESTION, NOS		1 (13%)	
INFLAMMATION, HEMORRHAGIC	1 (100%)		
INFLAMMATION, CHRONIC SUPPURATIV			1 (14%)
HYPERPLASIA, LYMPHOID		2 (25%)	
CIRCULATORY SYSTEM			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(11)	(30)	(33)
THROMBOSIS, NOS		1 (3%)	
INFLAMMATION, SUPPURATIVE		2 (7%)	
ANGIECTASIS	1 (9%)	1 (3%)	
HYPERPLASIA, LYMPHOID		1 (3%)	
*GALLBLADDER	(11)	(30)	(33)
HYPERPLASIA, EPITHELIAL			2 (6%)
HYPERPLASIA, CYSTIC			2 (6%)
#PEYERS PATCH	(10)	(30)	(33)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
HYPERPLASIA, LYMPHOID		2 (7%)	
#COLON	(11)	(30)	(32)
ULCER, NOS		1 (3%)	
ULCER, CHRONIC		1 (3%)	
NEMATODIASIS			1 (3%)
URINARY SYSTEM			
#KIDNEY	(11)	(30)	(33)
HYPERPLASIA, LYMPHOID		2 (7%)	
ENDOCRINE SYSTEM			
#THYROID	(5)	(16)	(25)
CYSTIC FOLLICLES		1 (6%)	1 (4%)
REPRODUCTIVE SYSTEM			
#UTERUS/ENDOMETRIUM	(11)	(29)	(32)
INFLAMMATION, SUPPURATIVE	1 (9%)	1 (3%)	2 (6%)
INFLAMMATION, CHRONIC SUPPURATIV			2 (6%)
HYPERPLASIA, CYSTIC	4 (36%)	14 (48%)	16 (50%)
#OVARY	(11)	(29)	(32)
CYST, NOS		1 (3%)	4 (13%)
INFLAMMATION, SUPPURATIVE			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
INFLAMMATICN, CHRONIC SUPPURATIV	1 (9%)		1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*MIDDLE EAR	(11)	(30)	(33)
INFLAMMATION, CHRONIC SUPPURATIV			4 (12%)
MUSCULOSKEIETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	4	2
ANIMAL MISSING/NO NECROPSY	4	2	
NC NECROPSY PERFORMED		2	2
AUTOLYSIS/NO NECROPSY		1	

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS FED ANTHRANILIC ACID IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Anthranilic Acid in the Diet[a]

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Hematopoietic System: Leukemia[b]	0/15 (0)	0/30 (0)	2/34 (6)	1/34 (3)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.138	0.025
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.264	0.048
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	98	98
Pituitary: Chromophobe Adenoma[b]	1/14 (7)	1/24 (4)	3/31 (10)	0/30 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			1.355	0.000
Lower Limit			0.125	0.000
Upper Limit			68.707	8.618
Relative Risk (Pooled Control)[f]			2.323	0.000
Lower Limit			0.204	0.000
Upper Limit			117.750	14.750
Weeks to First Observed Tumor	106	--	105	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Anthranilic Acid in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma[b]	0/15 (0)	1/30 (3)	3/32 (9)	1/34 (3)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.301	0.025
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			2.813	0.882
Lower Limit			0.243	0.011
Upper Limit			142.727	67.248
Weeks to First Observed Tumor	--	--	105	104
Thyroid: C-cell Adenoma or Carcinoma[b]	1/15 (7)	2/30 (7)	5/32 (16)	2/34 (6)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			2.344	0.882
Lower Limit			0.303	0.051
Upper Limit			106.950	50.523
Relative Risk (Pooled Control)[f]			2.344	0.882
Lower Limit			0.421	0.069
Upper Limit			23.115	11.541
Weeks to First Observed Tumor	106	--	105	104

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Anthranilic Acid in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Preputial Gland:				
Adnexal Adenoma[b]	0/15 (0)	0/30 (0)	2/34 (6)	1/34 (3)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.138	0.025
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.264	0.048
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	105	104
Testis: Interstitial-cell				
Tumor[b]	14/15 (93)	24/30 (80)	27/34 (79)	25/34 (74)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.851	0.788
Lower Limit			0.767	0.708
Upper Limit			1.189	1.130
Relative Risk (Pooled Control)[f]			0.993	0.919
Lower Limit			0.768	0.702
Upper Limit			1.305	1.247
Weeks to First Observed Tumor	106	--	98	84

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Anthranilic Acid in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Pancreatic Islets:				
Islet-cell Adenoma[b]	1/14 (7)	1/29 (3)	1/34 (3)	1/34 (3)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.412	0.412
Lower Limit			0.006	0.006
Upper Limit			31.405	31.405
Relative Risk (Pooled Control)[f]			0.853	0.853
Lower Limit			0.011	0.011
Upper Limit			65.021	65.021
Weeks to First Observed Tumor	106	--	105	104

[a]Treated groups received doses of 15,000 or 30,000 ppm.

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

[c]Beneath the incidence of tumors in a 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 matched-control group (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; otherwise, not significant (N.S.) is indicated.

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Anthranilic Acid in the Diet[a]

(continued)

[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 specified control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Anthranilic Acid in the Diet[a]

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Hematopoietic System: Leukemia[b]	0/14 (0)	0/29 (0)	3/33 (9)	0/35 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.039	P = 0.015		
Relative Risk (Matched Control)[f]			Infinite	--
Lower Limit			0.273	--
Upper Limit			Infinite	--
Relative Risk (Pooled Control)[f]			Infinite	--
Lower Limit			0.540	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor	--	--	99	--
Pituitary: Chromophobe Adenoma[b]	0/11 (0)	3/24 (13)	9/33 (27)	6/32 (19)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.972	0.608
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			2.182	1.500
Lower Limit			0.626	0.363
Upper Limit			11.387	8.481
Weeks to First Observed Tumor	--	--	97	82

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Anthranilic Acid in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma[b]	1/12 (8)	1/26 (4)	0/31 (0)	3/35 (9)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.000	1.029
Lower Limit			0.000	0.097
Upper Limit			7.160	52.416
Relative Risk (Pooled Control)[f]			0.000	2.229
Lower Limit			0.000	0.193
Upper Limit			15.478	113.497
Weeks to First Observed Tumor	106	--	--	104
Thyroid: C-cell Adenoma or Carcinoma[b]	1/12 (8)	1/26 (4)	0/31 (0)	5/35 (14)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.000	1.714
Lower Limit			0.000	0.231
Upper Limit			7.160	78.513
Relative Risk (Pooled Control)[f]			0.000	3.714
Lower Limit			0.000	0.456
Upper Limit			15.478	170.016
Weeks to First Observed Tumor	106	--	--	104

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Anthranilic Acid in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp[b]	3/14 (21)	3/29 (10)	4/33 (12)	1/35 (3)
P Values[c,d]	P = 0.036 (N)	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.566	0.133
Lower Limit			0.115	0.003
Upper Limit			3.496	1.528
Relative Risk (Pooled Control)[f]			1.172	0.276
Lower Limit			0.218	0.005
Upper Limit			7.410	3.231
Weeks to First Observed Tumor	106	--	105	105
Mammary Gland: Fibroadenoma[b]	6/14 (43)	8/29 (28)	4/33 (12)	0/35 (0)
P Values[c,d]	P < 0.001(N)	P = 0.001(N)	P = 0.028*(N)	P = 0.001**(N) P < 0.001*(N)
Relative Risk (Matched Control)[f]			0.283	0.000
Lower Limit			0.076	0.000
Upper Limit			1.025	0.240
Relative Risk (Pooled Control)[f]			0.439	0.000
Lower Limit			0.108	0.000
Upper Limit			1.460	0.356
Weeks to First Observed Tumor	104	--	98	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Anthranilic Acid in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Pancreatic Islets:				
Islet-cell Adenoma[b]	0/14 (0)	0/29 (0)	2/33 (6)	0/34 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]		P = 0.048		
Relative Risk (Matched Control)[f]			Infinite	--
Lower Limit			0.134	--
Upper Limit			Infinite	--
Relative Risk (Pooled Control)[f]			Infinite	--
Lower Limit			0.264	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor	--	--	97	--

[a]Treated groups received doses of 15,000 or 30,000 ppm.

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

[c]Beneath the incidence of tumors in a 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 matched-control group (*) or with the pooled-control group (**) when P < 0.05 for either control group; otherwise, not significant (N.S.) is indicated.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Anthranilic Acid in the Diet[a]

(continued)

[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 specified control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE FED ANTHRANILIC ACID IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Anthranilic Acid in the Diet[a]

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	0/6 (0)	0/20 (0)	5/31 (16)	0/29 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.033	P = 0.004		
Relative Risk (Matched Control)[f]			Infinite	--
Lower Limit			0.299	--
Upper Limit			Infinite	--
Relative Risk (Pooled Control)[f]			Infinite	--
Lower Limit			0.851	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor	--	--	71	--
Hematopoietic System: Lymphoma[b]	0/6 (0)	0/20 (0)	2/31 (6)	1/29 (3)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.069	0.013
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.198	0.038
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	71	101

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Anthranilic Acid in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	0/5 (0)	1/18 (6)	1/31 (3)	1/29 (3)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.010	0.011
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			0.581	0.621
Lower Limit			0.008	0.008
Upper Limit			44.138	47.076
Weeks to First Observed Tumor	--	--	105	93
Liver: Hepatocellular Adenoma or Carcinoma[b]	0/5 (0)	4/18 (22)	6/31 (19)	5/29 (17)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.322	0.279
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			0.871	0.776
Lower Limit			0.246	0.197
Upper Limit			3.729	3.468
Weeks to First Observed Tumor	--	--	70	76

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Anthranilic Acid in the Diet[a]

(continued)

[a]Treated groups received doses of 25,000 or 50,000 ppm.

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

[c]Beneath the incidence of tumors in a 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 matched-control group (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; 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 specified control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Anthranilic Acid in the Diet[a]

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	0/11 (0)	1/23 (4)	4/30 (13)	0/33 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.019	P = 0.036		
Relative Risk (Matched Control)[f]			Infinite	--
Lower Limit			0.375	--
Upper Limit			Infinite	--
Relative Risk (Pooled Control)[f]			3.067	0.000
Lower Limit			0.336	0.000
Upper Limit			145.421	12.890
Weeks to First Observed Tumor	--	--	103	--
Hematopoietic System: Lymphoma or Leukemia[b]	0/11 (0)	1/23 (4)	4/30 (13)	1/33 (3)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.375	0.019
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			3.067	0.697
Lower Limit			0.336	0.009
Upper Limit			142.540	53.545
Weeks to First Observed Tumor	--	--	103	99

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Anthranilic Acid in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	0/11 (0)	0/22 90)	0/30 (0)	1/33 93)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			--	Infinite
Lower Limit			--	0.019
Upper Limit			--	Infinite
Relative Risk (Pooled Control)[f]			--	Infinite
Lower Limit			--	0.037
Upper Limit			--	Infinite
Weeks to First Observed Tumor	--	--	--	105
Liver: Hepatocellular Adenoma or Carcinoma[b]	0/11 (0)	0/22 (0)	1/30 (3)	2/33 (6)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.021	0.108
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.040	0.203
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	77	105

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Anthranilic Acid in the Diet[a]

(continued)

[a]Treated groups received doses of 25,000 or 50,000 ppm.

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

[c]Beneath the incidence of tumors in a 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 matched-control group (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; 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 specified control group.

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

November 28, 1977

The Clearinghouse on Environmental Carcinogens was established in May, 1976 under the authority of the National Cancer Act of 1971 (P.L. 92-218). The purpose of the Clearinghouse is to advise on the National Cancer Institute's bioassay program to identify and 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 organic 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 NCI bioassay reports on chemicals studied for carcinogenicity. In this context, below is the edited excerpt from the minutes of the Subgroup's meeting at which Anthranilic Acid was reviewed.

Anthranilic Acid is a metabolite of tryptophan and was selected for test as part of a program to study the carcinogenicity of naturally occurring substances. After a brief description of the experiment and results, the primary reviewer concurred with the staff's conclusion that Anthranilic Acid was not carcinogenic under the conditions of test. The secondary reviewer agreed with this assessment of the study.

One Subgroup member pointed out the small animal groups, compared with today's standard, and the high early mortality. Another member commented that the performance of the study may be questionable in view of the number of animals that were lost.

A motion was made that the report on Anthranilic Acid be accepted. The motion was seconded and approved unanimously.

Members present were:

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology
Lawrence Garfinkel, American Cancer Society
Henry C. Pitot, University of Wisconsin Medical Center
George Roush, Jr., Monsanto Company
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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