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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 pyrazinamide 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 Testing 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].

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SUMMARY

A bioassay of the tuberculostatic drug pyrazinamide 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 pyrazinamide at one of two doses, either 5,000 or 10,000 ppm, for 78 weeks, and then observed for an additional 26 or 27 weeks. Matched controls consisted of groups of 15 untreated rats and 15 untreated mice of each sex. High-dose male mice died or were killed by week 92; all other surviving animals were killed at weeks 104 or 105.

Mean body weights of the dosed male rats were slightly lower than those of the matched controls, while mean body weights of the dosed females were more nearly comparable to those of the controls. A sufficient number of rats in each group was at risk to termination of the study at weeks 104-105 for the development of late-appearing tumors.

In mice, administration of pyrazinamide had no consistent effect on mean body weights. Survival to termination of the study was low, particularly among the control groups.

In rats, no lesions could clearly be related to administration of the chemical.

In mice, interstitial and suppurative myocarditis in the dosed animals and suppurative bronchopneumonias in both dosed and matched-control mice of each sex were associated with increased deaths. In the females, there was a significant positive dose-related trend ($P = 0.037$) in the incidence of lymphoma (matched controls 0/13, low-dose 2/25, high-dose 6/29); however, the incidences in each of the dosed groups were not significant when

compared with that in the matched controls. In addition, the poor survival and the small size of the control group precluded making a clear association of the incidence of these tumors with administration of the chemical.

It is concluded that under the conditions of this bioassay, the early deaths and small size of the control group precluded a conclusion regarding the carcinogenicity of pyrazinamide in female B6C3F1 mice. Pyrazinamide was not carcinogenic for Fischer 344 rats or for male mice.

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I. INTRODUCTION

Pyrazinamide (pyrazinecarboxamide; CAS 98-96-4; NCI C01785) is a tuberculostatic agent which differs from nicotinamide by the replacement of the pyridine ring with a pyrazine ring. The inhibition of bacterial growth that pyrazinamide causes is believed to be due to its promotion of an acidic environment (Smith, 1977). Pyrazinamide is used in the treatment of lymphadenitis, and pulmonary, miliary, renal, and meningeal tubercular infections caused by Mycobacterium tuberculosis and atypical mycobacteria. It is administered only where resistance has developed to primary drugs, or where there has been no response to these drugs (Pratt, 1973; Weinstein, 1975). It is one of the more toxic antitubercular agents; its most common and serious side effect is liver damage. Pyrazinamide is given orally at a daily dose not greater than 3 grams, and always in combination with another antitubercular drug (Weinstein, 1975; Smith, 1977). Pyrazinamide was selected for testing in the carcinogenesis program in an attempt to evaluate the carcinogenicity of certain drugs that may be used in humans for prolonged periods.

II. MATERIALS AND METHODS

A. Chemical

Pyrazinamide was obtained in several batches from the Aldrich Chemical Company, Inc., Milwaukee, Wisconsin. The purity and identity of the batch (Lot No. 083027) used in the chronic study was confirmed by analysis at Midwest Research Institute. The melting point was 190-192°C (literature: 189-191°C). Elemental analyses (C, H, N) were correct for $C_5H_5N_3O$, the molecular formula of pyrazinamide. Nuclear magnetic resonance, infrared, and ultraviolet spectra were in agreement with the structure.

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

B. Dietary Preparation

Test diets containing pyrazinamide were prepared every 2 weeks by mixing a known amount of the sifted chemical with a small amount of Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) in a portable mixer. The mixture was then added to the required amount of animal meal and mixed in a twin-shell blender for 10 minutes.

The prepared diets were stored at room temperature in sealed

plastic containers. No analyses for concentration or stability of pyrazinamide in feed were performed.

C. Animals

For the subchronic study, male Sprague-Dawley rats and male Swiss mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

For the chronic study, Fischer 344 rats and B6C3F1 mice of both sexes were obtained from Charles River Laboratories under a contract with the Division of Cancer Treatment, National Cancer Institute. The animals were 30 days of age on arrival at the laboratory and were quarantined for 12 days. Animals with no clinical 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%. The 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.). 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 pyrazinamide 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)
anthranilic acid (CAS 118-92-3)
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)

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)
anthranilic acid (CAS 118-92-3)
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)
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 feeding studies were conducted to estimate the maximum tolerated doses of pyrazinamide, 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. In the subchronic studies, pyrazinamide was added to the animal feed in concentrations of 1,000, 5,000, 10,000, 25,000, or 50,000 ppm for both male Sprague-Dawley rats and male Swiss mice. Treated groups each consisted of five animals. Control groups consisted of 19 untreated male rats and 20 untreated male mice. The treated groups were fed the test diets for 45 days, then observed for an additional 45 days.

In rats, one animal receiving 50,000 ppm died during week 10 of the study. After 45 days, mean body weight gains of the groups of rats at 1,000 to 10,000 ppm were 93-94% of the gains of the control group, gains of rats at 25,000 ppm were 70%, and gains of rats at 50,000 ppm were 43%. After 90 days, mean weight gains in

all treated groups of rats were at least 90% of those of the controls except in the 50,000 ppm group, where they were only 77%. No gross abnormalities were found at necropsy. The low and high doses for the chronic studies using rats were set at 5,000 and 10,000 ppm.

In mice, two animals receiving 50,000 ppm died during week 4, four animals at 25,000 ppm died during week 7, and one animal at 10,000 ppm died during week 13. Three control animals also died. After 45 days, mean body weight gains in the groups of mice treated at 1,000 and 5,000 ppm were comparable to those of the control groups, gains in mice at 10,000 and 25,000 ppm were 80% of those of the controls, and gains in mice at 50,000 ppm were 33%. After 90 days, mean weight gains in all treated groups of mice were comparable to those of the controls, except at 50,000 ppm, where gains were 83% of those of the controls. No gross abnormalities were found at necropsy. The low and high doses for the chronic studies using mice were set at 5,000 and 10,000 ppm.

F. Designs of Chronic Studies

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

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and

Table 1. Design of Pyrazinamide Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals[a]	Pyrazinamide in Diet[b] (ppm)	Time on Study	
			Treated (weeks)	Untreated (weeks)
Male				
Matched-Control	15	0		105
Low-Dose	35	5,000	78	26
High-Dose	36[c]	10,000	78	26
Female				
Matched-Control	15	0		105
Low-Dose	35	5,000	78	27
High-Dose	34[c]	10,000	78	26

[a]All animals were 42 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. All treated animals were fed control diets for 3 days during week 66.

[c]One high-dose animal was missexed at the start of the study.

Table 2. Design of Pyrazinamide Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals[a]	Pyrazinamide in Diet[b] (ppm)	Time on Study	
			Treated (weeks)	Untreated (weeks)
Male				
Matched-Control	15	0		104
Low-Dose	35	5,000	78	26
High-Dose	35	10,000	78	14[c]
Female				
Matched-Control	15	0		104
Low-Dose	35	5,000	78	26
High-Dose	35	10,000	78	26

[a]All animals were 42 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. All mice at 10,000 and 5,000 ppm were fed control diets for 3 days during week 66.

[c]All high-dose male mice died or were killed by week 92.

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 once every 2 weeks for 20 months and once per month 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 killed. 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 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 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 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 both low- and high-dose male rats were slightly lower than those of the matched controls throughout the study; those of the low- and high-dose female rats were similar to those of the matched controls during most of the study, except for several months in the second year when the weights of the high-dose group were lower (figure 1). 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. Yellowish discoloration of the hair and skin on the ears and feet appeared in a number of treated rats.

Some animals showed signs of respiratory disease, and all were treated with oxytetracycline in the drinking water at doses of 0.6 mg/ml during weeks 40-44 and 0.3 mg/ml during weeks 44-46.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats fed pyrazinamide 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

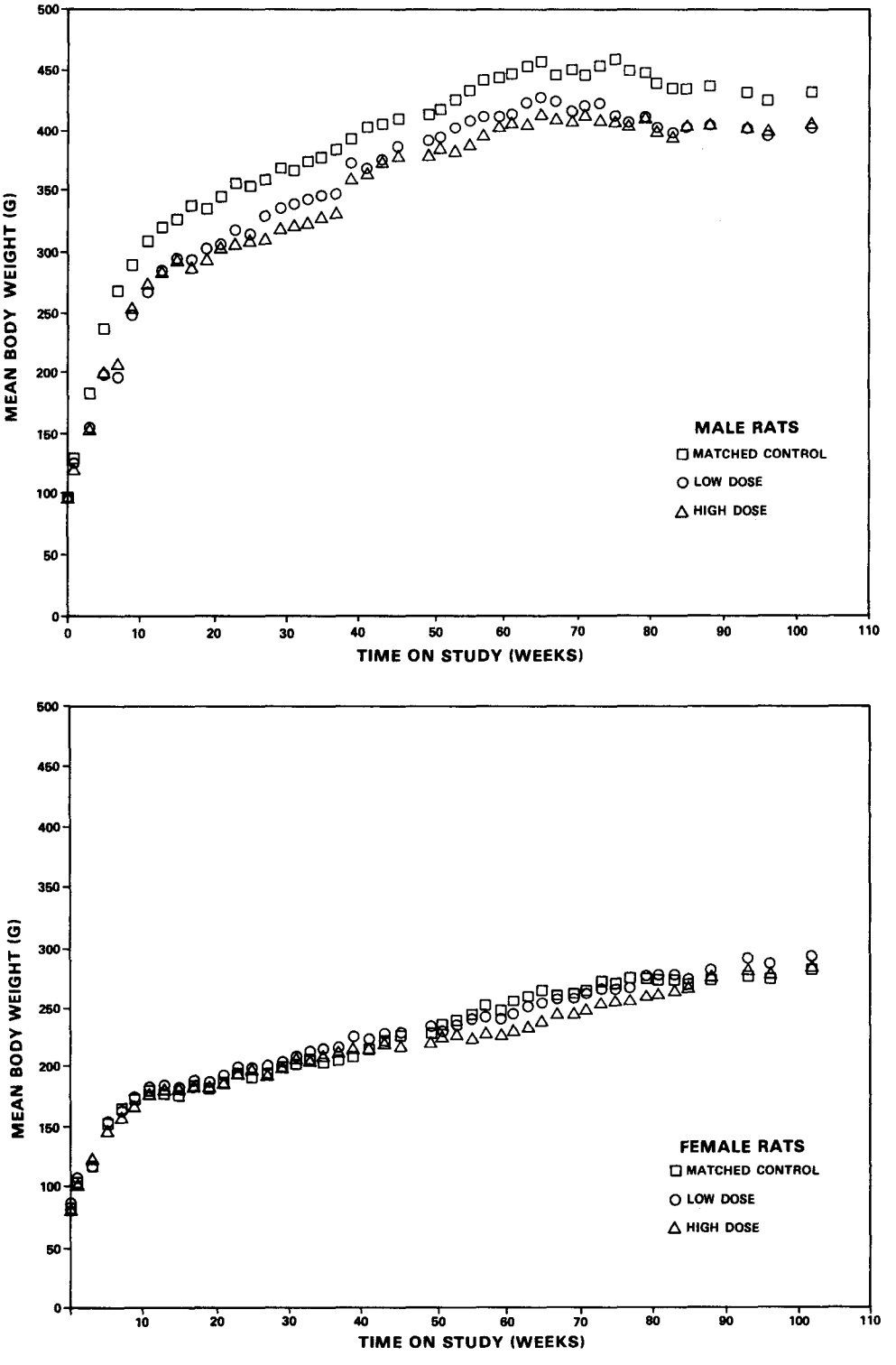


Figure 1. Growth Curves For Rats Fed Pyrazinamide In The Diet

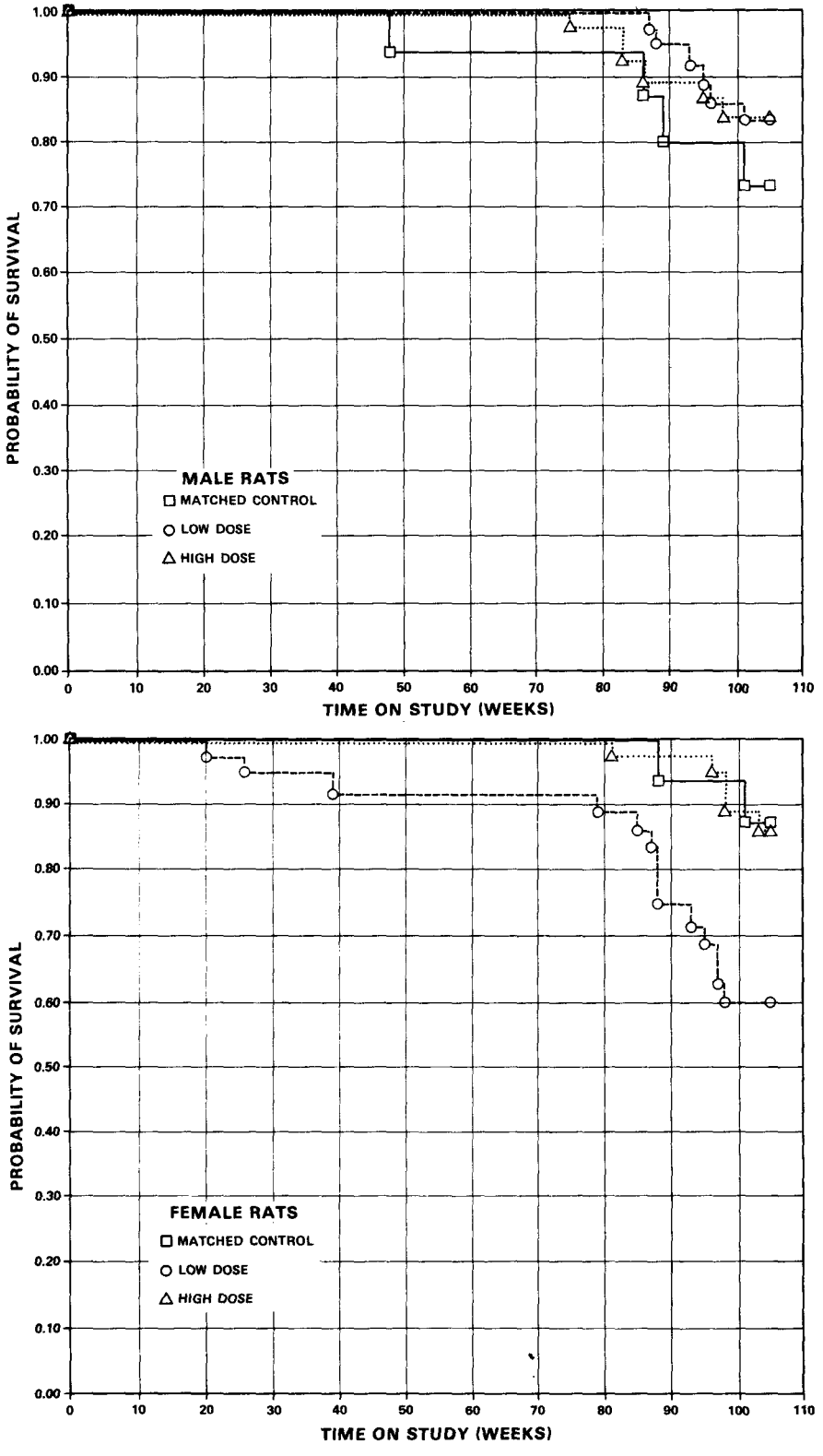


Figure 2. Survival Curves For Rats Fed Pyrazinamide In The Diet

trend in mortality over the period is not significant. In male rats, 30/36 (83%) of the high-dose, 29/35 (83%) of the low-dose, and 11/15 (73%) of the matched-control animals lived to the end of the study. In female rats, 29/34 (85%) of the high-dose, 21/35 (60%) of the low-dose, and 13/15 (87%) of the matched-control animals survived to termination of the study. A sufficient number of rats of each sex was 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 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 controls. These lesions, however, are not uncommon in this strain of rat independent of any chemical administration. Few malignant tumors were observed, and tumor metastases were present in only two treated rats.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were also encountered in

animals of the treated and control groups (Appendix C). These nonneoplastic lesions are commonly seen in aged rats.

In the judgment of the pathologists, pyrazinamide was not carcinogenic when fed to 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 occurred in at least two animals in one group and with an incidence of at least 5% in one or more than one group.

In male rats, the results of the Cochran-Armitage test for dose-related trend and of the Fisher exact test for direct comparison of the incidences of tumors in the treated groups with those in the matched-control group are not significant in a positive direction. The results of the Cochran-Armitage test for the incidence of leukemia are significant ($P = 0.037$), but in the negative direction, since the incidence in the control group exceeds those in the treated groups, but the Fisher exact test results were not significant.

In female rats, the result of the Cochran-Armitage test for the combined incidence of chromophobe adenoma and carcinoma of the

pituitary is not significant. The Fisher exact test shows that the incidence in the low-dose group is higher than that in the matched controls (P = 0.037); however, this significance level is above the 0.025 level required for significance by the Bonferroni inequality criterion.

In each of the 95% confidence intervals, shown in the tables, the value of one is included, indicating 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 pyrazinamide, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

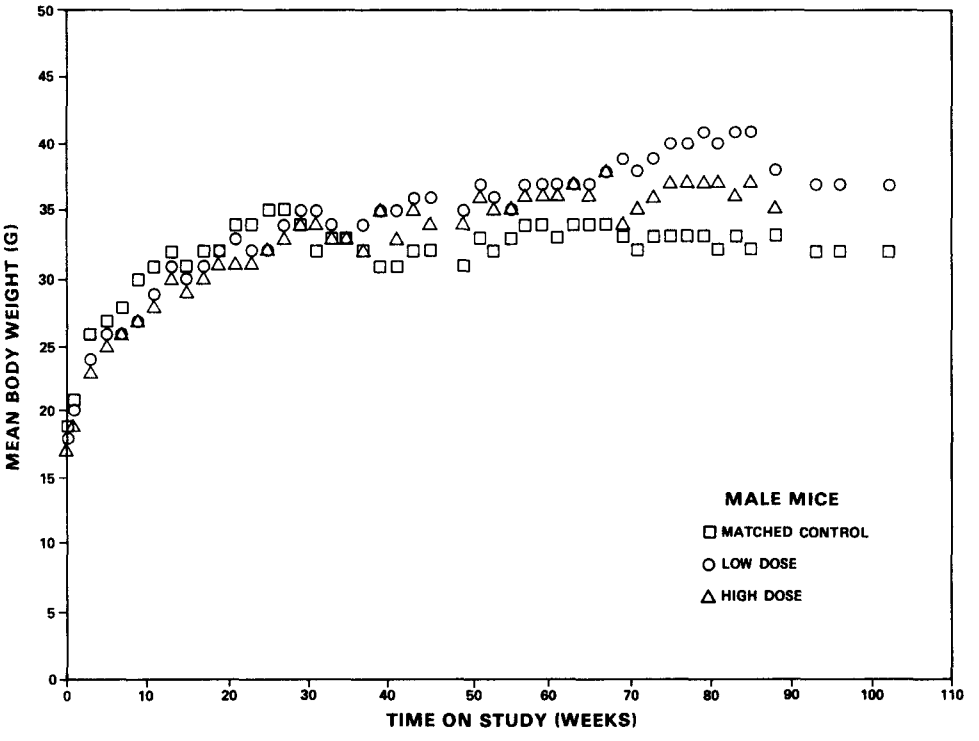
A. Body Weights and Clinical Signs (Mice)

Mean body weights of the treated male mice were higher than those of the matched controls from about week 25 to the end of the study (figure 3). Mean body weights of the high-dose females were generally equal to or higher than those of the matched controls, while the low-dose females had lower body weights after the feeding of pyrazinamide was completed. 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. Difficult breathing and progressive weight loss were noted in individual animals in all groups. No signs of chemical-related toxicity in the mice were recorded.

To control respiratory disease in the mouse colony, all animals were treated with oxytetracycline in the drinking water at doses of 0.6 mg/ml for 5 days during week 67 and 0.3 mg/ml for the following 5 days during week 68; also, rooms housing the mice were treated with propylene glycol vapor for about 2 months, beginning at week 67.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of



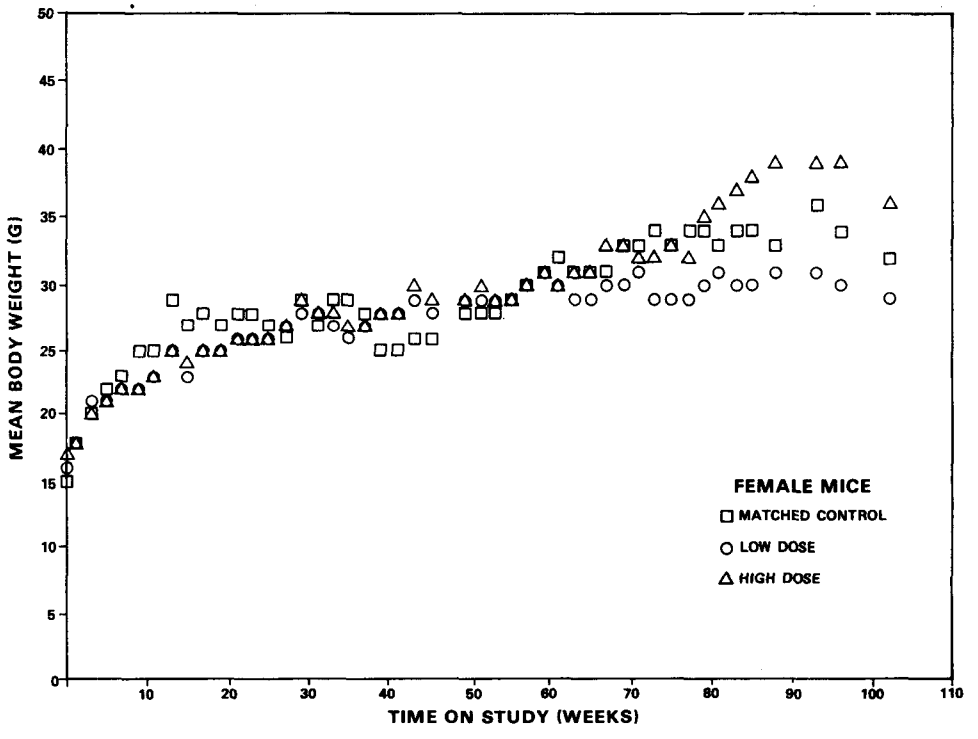


Figure 3. Growth Curves For Mice Fed Pyrazinamide In The Diet

survival for male and female mice fed pyrazinamide in the 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 positive dose-related trend in mortality over the period is not significant, with 29/35 (83%) of the high-dose group, 33/35 (94%) of the low-dose group, and 9/15 (60%) of the matched controls living beyond week 52 on study. In female mice, the Tarone test is significant (P = 0.016), but in the negative direction, with 31/35 (89%) of the high-dose group, 24/35 (69%) of the low-dose group, and 9/15 (60%) of the matched controls living beyond week 52 on study. In the female low-dose group, eight animals were reported missing: two at week 10, one at week 31, and five at week 39.

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 occurred in both the control and treated groups. Some types of neoplasms occurred only, or with a greater frequency, in mice of the treated groups when compared with the controls. These lesions, however, are not uncommon in this strain of mouse independent of any chemical administration.

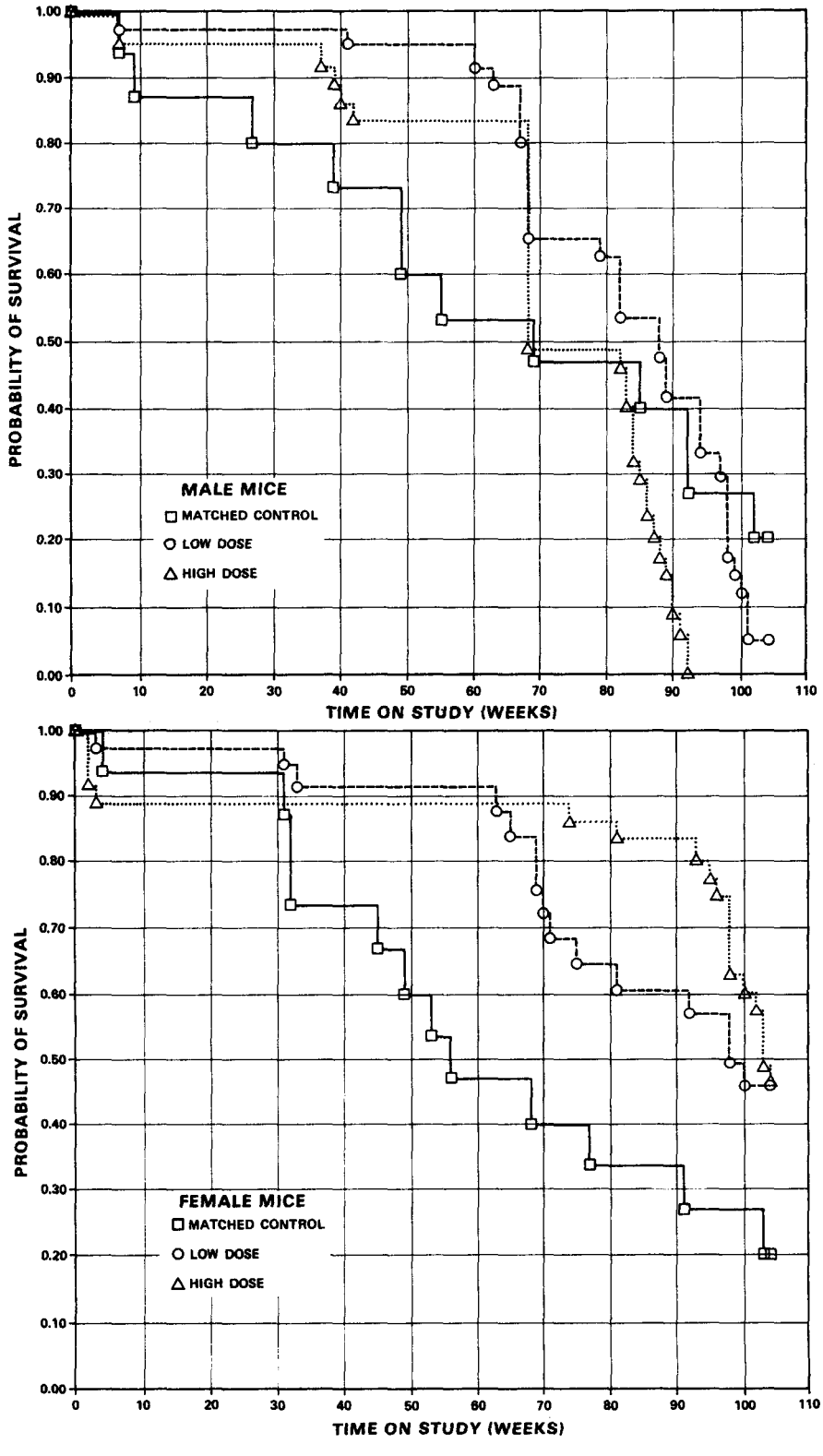


Figure 4. Survival Curves For Mice Fed Pyrazinamide In The Diet

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the treated and control groups (Appendix D). These nonneoplastic lesions are commonly seen in aged mice; however, the suppurative lesions involving the heart, trachea, and lungs were associated with increased deaths.

Suppurative bronchopneumonias accompanied by suppurative tracheitis occurred in 7/12 (58%) control males, 17/31 (55%) low-dose males, and 17/33 (52%) high-dose males. Similar lesions occurred in 5/13 (38%) control females, 12/24 (50%) low-dose females, and 12/29 (41%) high-dose females. Interstitial and suppurative myocarditis occurred in 10/31 (32%) low-dose males and 13/33 (39%) high-dose males in contrast to 1/24 (4%) low-dose females and 1/29 (3%) high-dose females. None of the control mice had myocarditis. The heart lesions resembled those associated with bacterial infections. In two high-dose male mice, a vegetative valvular endocarditis with large bacterial colonies was observed. The respiratory infections appeared to have resulted from intercurrent disease, whereas the myocarditis appeared to be sex and chemical related.

Although this bioassay was complicated by intercurrent disease, pyrazinamide appeared, in the judgment of the pathologists, to

have little or no carcinogenic activity when fed to 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 occurred in at least two animals in one group and with an incidence of at least 5% in one or more than one group.

In male mice, the results of the Cochran-Armitage test for dose-related trend and of the Fisher exact test for direct comparison of the incidences of tumors in the treated groups with those in the matched-control group are not significant.

In female mice, the result of the Cochran-Armitage test for the incidence of lymphoma is significant ($P = 0.037$), but the results of the Fisher exact test are not significant and the survival of the female controls was less than that of the treated groups.

In each of the 95% confidence intervals, shown in the tables, the value of one is included, indicating the absence of significant results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of induction of tumors by pyrazinamide, which could not be detected under the conditions of this test.

V. DISCUSSION

Administration of pyrazinamide did not appreciably affect the Fischer 344 rats in this bioassay. Mean body weights of the dosed males were only slightly lower than those of the matched controls; those of the dosed females were more nearly comparable to those of the controls; and survival of all groups was high. Mean body weights of the dosed male and female mice were equal to or higher than those of the matched controls throughout most of the study; however, mortality was high among the controls.

A sufficient number of each group of rats was at risk to termination of the study for the development of late-appearing tumors. However, survival of the dosed and control mice was too low for meaningful analyses of incidences of tumors appearing after week 52; interstitial and suppurative myocarditis in the dosed mice and suppurative bronchopneumonias in both dosed and matched-control groups of both sexes were associated with these early deaths.

In male rats, no tumor appeared in the dosed groups at an incidence significantly different from that in the control groups. In female rats, the incidence of chromophobe adenoma or carcinoma of the pituitary in the low-dose group 14/30 (47%) compared with the matched controls 2/14 (14%) had a probability

level of 0.037; however, this level is above that required for significance by the Bonferroni inequality criterion. The historical incidence of this tumor in female Fischer 344 rats in the bioassay program at this laboratory was 53/235 (23%), which is higher than the incidence in the low-dose group. In addition, the incidence in the high-dose group was not significant in any tests, and the tumor cannot be associated with administration of the chemical.

In mice, interstitial and suppurative myocarditis in the dosed animals and suppurative bronchopneumonias in both dosed and matched control mice of both sexes were associated with increased deaths. In female mice, a significant dose-related trend (P = 0.037) occurred in the incidence of lymphoma (matched controls 0/13, low-dose 2/25, high-dose 6/29); however, the incidences in the dosed groups were not statistically significant when compared with that in the matched controls. In addition, the survival of these controls was less than that of the dosed animals. The incidence in the high-dose group is higher than that of the historical incidence of lymphoma in female mice at this laboratory 42/538 (8%). Thus, the association of lymphoma with administration of the chemical in female mice is not clear and cannot be fully evaluted, due to the small size of the group and

poor survival among the female controls. In male mice, no tumors occurred in a significant incidence.

No long-term studies of the toxicity of pyrazinamide in laboratory animals have been reported in the literature. In a 7-month study in which pyrazinamide was fed at a concentration of 30,000 ppm to 10 female mice of strain dd, pulmonary adenomas occurred in six animals (Mori et al., 1960). Pulmonary tumors were not observed at significant incidences in dosed rats or mice in the present bioassay.

It is concluded that under the conditions of this bioassay, the early deaths and small size of the control group precluded a conclusion regarding the carcinogenicity of pyrazinamide in female B6C3F1 mice. Pyrazinamide was not carcinogenic for Fischer 344 rats or for male B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS FED PYRAZINAMIDE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED PYRAZINAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	36
ANIMALS NECROPSIED	14	35	36
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	35	36
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(14)	(35)	(36)
SARCOMA, NOS		1 (3%)	
FIBROMA		1 (3%)	
FIBROSARCOMA		1 (3%)	
RESPIRATORY SYSTEM			
#LUNG	(14)	(35)	(36)
FIBROSARCOMA, METASTATIC		1 (3%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(14)	(35)	(36)
UNDIFFERENTIATED LEUKEMIA	2 (14%)	2 (6%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(32)	(30)
CHROMOPHOBE ADENOMA	1 (10%)	4 (13%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CHROMOPHOBE CARCINOMA			1 (3%)
#ADRENAL	(14)	(35)	(36)
PHECCHROMOCYTOMA		1 (3%)	1 (3%)
#THYROID	(14)	(35)	(32)
FOLLICULAR-CELL CARCINOMA	1 (7%)	1 (3%)	
C-CELL ADENOMA	2 (14%)	1 (3%)	3 (9%)
C-CELL CARCINOMA		3 (9%)	
#PANCREATIC ISLETS	(14)	(35)	(36)
ISLET-CELL ADENOMA	1 (7%)	3 (9%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(14)	(35)	(36)
ADENOCARCINOMA, NOS		1 (3%)	
#TESTIS	(14)	(35)	(36)
INTERSTITIAL-CELL TUMOR	13 (93%)	30 (86%)	29 (81%)
NERVOUS SYSTEM			
#BRAIN	(13)	(34)	(36)
ASTROCYTOMA			1 (3%)
SPECIAL SENSE ORGANS			
*EAR CANAL	(14)	(35)	(36)
SQUAMOUS CELL PAPILLOMA		1 (3%)	
SQUAMOUS CELL CARCINOMA		1 (3%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(14)	(35)	(36)
MESOTHELIOMA, NOS			2 (6%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*PLEURA	(14)	(35)	(36)
FIBROSARCOMA, METASTATIC		1 (3%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	36
NATURAL DEATH@	1	1	
MORIBUND SACRIFICE	3	5	6
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	11	29	30
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	34	30
TOTAL PRIMARY TUMORS	20	51	37
TOTAL ANIMALS WITH BENIGN TUMORS	13	32	30
TOTAL BENIGN TUMORS	17	41	33
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	9	2
TOTAL MALIGNANT TUMORS	3	10	2
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		2	
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 PYRAZINAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	34
ANIMALS NECROPSIED	15	34	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	34	34
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(15)	(34)	(34)
BASAL-CELL CARCINOMA		1 (3%)	
RESPIRATORY SYSTEM			
#LUNG	(15)	(33)	(34)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (3%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(15)	(34)	(34)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (3%)	
UNDIFFERENTIATED LEUKEMIA	2 (13%)	2 (6%)	2 (6%)
#SPLEEN	(15)	(34)	(34)
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
#URINARY BLADDER	(15)	(34)	(33)
TRANSITIONAL-CELL PAPILLOMA		1 (3%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(14)	(30)	(24)
CHROMOPHOBE ADENOMA	2 (14%)	13 (43%)	7 (29%)
CHROMOPHOBE CARCINOMA		1 (3%)	
#THYROID	(15)	(33)	(32)
FOLLICULAR-CELL ADENOMA	1 (7%)		
C-CELL ADENOMA	1 (7%)	1 (3%)	
C-CELL CARCINOMA			1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(34)	(34)
PAPILLARY ADENOMA		1 (3%)	
FIBROADENOMA	4 (27%)	5 (15%)	4 (12%)
#UTERUS	(15)	(34)	(34)
ADENOCARCINOMA, NOS	1 (7%)	1 (3%)	1 (3%)
SARCOMA, NOS		2 (6%)	
LEIOMYOMA		1 (3%)	
ENDOMETRIAL STROMAL POLYP	3 (20%)	5 (15%)	5 (15%)
#OVARY	(15)	(34)	(34)
CYSTADENOMA, NOS			1 (3%)
NERVOUS SYSTEM			
#BRAIN	(15)	(33)	(34)
OLIGODENDROGLIOMA	1 (7%)		
SPECIAL SENSE ORGANS			
*EAR CANAL	(15)	(34)	(34)
SQUAMOUS CELL CARCINOMA			1 (3%)
KERATOACANTHOMA	1 (7%)		
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*PERITONEUM	(15)	(34)	(34)
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	34
NATURAL DEATH@	1	4	2
MORIBUND SACRIFICE	1	10	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	21	29
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	26	19
TOTAL PRIMARY TUMORS	16	35	23
TOTAL ANIMALS WITH BENIGN TUMORS	9	22	15
TOTAL BENIGN TUMORS	12	27	18
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	8	5
TOTAL MALIGNANT TUMORS	4	8	5
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE FED PYRAZINAMIDE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED PYRAZINAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING		1	
ANIMALS NECROPSIED	12	31	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	12	31	33
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(12)	(31)	(33)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (3%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (6%)	1 (3%)
#SPLEEN	(12)	(31)	(33)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (8%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(12)	(31)	(33)
HEPATOCELLULAR ADENOMA			2 (6%)
HEPATOCELLULAR CARCINOMA	1 (8%)		1 (3%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(12)	(31)	(33)
ADENOMA, NOS			1 (3%)
#TESTIS	(12)	(31)	(32)
INTERSTITIAL-CELL TUMOR			1 (3%)
NERVOUS SYSTEM			
*TRIGEMINAL GANGLION	(12)	(31)	(33)
NEURILEMOMA, MALIGNANT		1 (3%)	
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(12)	(31)	(33)
ADENOMA, NOS		1 (3%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	6	11	13
MORIBUND SACRIFICE	6	21	22
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	3	2	
ANIMAL MISSING		1	

@ 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*	2	5	6
TOTAL PRIMARY TUMORS	2	5	6
TOTAL ANIMALS WITH BENIGN TUMORS		1	4
TOTAL BENIGN TUMORS		1	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	4	2
TOTAL MALIGNANT TUMORS	2	4	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 PYRAZINAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING		8	
ANIMALS NECROPSIED	13	25	29
ANIMALS EXAMINED HISTOPATHOLOGICALLY	13	25	29
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(13)	(24)	(29)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (8%)		1 (3%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(13)	(25)	(29)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (4%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			4 (14%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (4%)	2 (7%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(13)	(25)	(28)
HEPATOCELLULAR CARCINOMA			1 (4%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(8)	(18)	(20)
CHROMOPHOBE ADENOMA			1 (5%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*PANCREATIC ISLETS	(13)	(25)	(29)
ISLET-CELL ADENOMA			1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(13)	(25)	(29)
ADENOMA, NOS		1 (4%)	
NERVOUS SYSTEM			
*TRIGEMINAL GANGLION	(13)	(25)	(29)
NEURILEMOMA, MALIGNANT		1 (4%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*BONE	(13)	(25)	(29)
OSTEOSARCOMA		1 (4%)	
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(13)	(25)	(29)
OSTEOSARCOMA, METASTATIC		1 (4%)	

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@	6	2	9
MORIBUND SACRIFICE	6	13	10
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	3	12	16
ANIMAL MISSING		8	
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	1	5	9
TOTAL PRIMARY TUMORS	1	5	10
TOTAL ANIMALS WITH BENIGN TUMORS		1	2
TOTAL BENIGN TUMORS		1	2
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	4	8
TOTAL MALIGNANT TUMORS	1	4	8
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 PYRAZINAMIDE IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED PYRAZINAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	36
ANIMALS NECROPSIED	14	35	36
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	35	36
INTEGUMENTARY SYSTEM			
*SKIN	(14)	(35)	(36)
INFLAMMATION, CHRONIC FOCAL			1 (3%)
*SUBCUT TISSUE	(14)	(35)	(36)
EPIDERMAL INCLUSION CYST		1 (3%)	
HEMORRHAGE			1 (3%)
RESPIRATORY SYSTEM			
#TRACHEA	(14)	(35)	(36)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (3%)	1 (3%)
INFLAMMATION, SUPPURATIVE	8 (57%)	16 (46%)	7 (19%)
PLASMA-CELL INFILTRATE		1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
#LUNG	(14)	(35)	(36)
EMPHYSEMA, NOS			1 (3%)
BRONCHOPNEUMONIA SUPPURATIVE	2 (14%)	2 (6%)	1 (3%)
ABSCESS, NOS			1 (3%)
BRONCHOPNEUMONIA CHRONIC SUPPURA		1 (3%)	1 (3%)
CALCIFICATION, METASTATIC			1 (3%)
HYPERPLASIA, ALVEOLAR EPITHELIUM			1 (3%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(14)	(35)	(35)
ATROPHY, NOS	5 (36%)	14 (40%)	11 (31%)
#SPLEEN	(14)	(35)	(36)
HEMATOPOIESIS		3 (9%)	1 (3%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#HEART	(14)	(35)	(36)
THROMBUS, MURAL			1 (3%)
#MYOCARDIUM	(14)	(35)	(36)
INFLAMMATION, INTERSTITIAL		1 (3%)	1 (3%)
PERIARTERITIS			1 (3%)
*AORTA	(14)	(35)	(36)
PERIARTERITIS		1 (3%)	
DIGESTIVE SYSTEM			
#LIVER	(14)	(35)	(36)
HEMORRHAGE			1 (3%)
HYPERPLASIA, NODULAR		2 (6%)	2 (6%)
HEMATOPOIESIS		1 (3%)	
#PANCREATIC ACINUS	(14)	(35)	(36)
ATROPHY, NOS	2 (14%)	3 (9%)	
ATROPHY, FOCAL			2 (6%)
#STOMACH	(14)	(35)	(36)
ULCER, FOCAL	1 (7%)		
#DUODENUM	(14)	(35)	(36)
ULCER, FOCAL	1 (7%)		
#COLON	(14)	(35)	(36)
ULCER, NOS		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(14)	(35)	(36)
INFLAMMATION, CHRONIC	13 (93%)	34 (97%)	34 (94%)
ENDOCRINE SYSTEM			
#ADRENAL	(14)	(35)	(36)
ANGIECTASIS		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
#THYROID	(14)	(35)	(32)
CYSTIC FOLLICLES			1 (3%)
HYPERPLASIA, C-CELL		1 (3%)	
HYPERPLASIA, FOLLICULAR-CELL	1 (7%)		
#PARATHYROID	(7)	(22)	(19)
HYPERPLASIA, NOS		1 (5%)	1 (5%)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(14)	(35)	(36)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
HYPERPLASIA, EPITHELIAL			1 (3%)
#PROSTATE	(14)	(35)	(36)
INFLAMMATION, SUPPURATIVE	2 (14%)	2 (6%)	
#TESTIS	(14)	(35)	(36)
INFLAMMATION, NECROTIZING			1 (3%)
ATROPHY, NOS			1 (3%)
NERVOUS SYSTEM			
#BRAIN	(13)	(34)	(36)
HEMORRHAGE	1 (8%)		
MALACIA	1 (8%)		
SPECIAL SENSE ORGANS			
*EYE	(14)	(35)	(36)
SYNECHIA, ANTERIOR		1 (3%)	
*EYE/CRYSTALLINE LENS	(14)	(35)	(36)
MINERALIZATION		1 (3%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(14)	(35)	(36)
HEMORRHAGE			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
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
AUTOLYSIS/NO NECROPSY	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 PYRAZINAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	34
ANIMALS NECROPSIED	15	34	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	34	34
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(34)	(34)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
*SUBCUT TISSUE	(15)	(34)	(34)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
RESPIRATORY SYSTEM			
#TRACHEA	(15)	(34)	(33)
INFLAMMATION, SUPPURATIVE	8 (53%)	13 (38%)	7 (21%)
PLASMA-CELL INFILTRATE			2 (6%)
#LUNG	(15)	(33)	(34)
BRONCHOPNEUMONIA SUPPURATIVE		1 (3%)	
PNEUMONIA INTERSTITIAL CHRONIC			1 (3%)
HYPERPLASIA, LYMPHOID			1 (3%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(15)	(34)	(33)
ATROPHY, NOS	9 (60%)	18 (53%)	16 (48%)
#SPLEEN	(15)	(34)	(34)
HYPERPLASIA, LYMPHOID			1 (3%)
HEMATOPOIESIS	2 (13%)	4 (12%)	3 (9%)
#RENAL LYMPH NODE	(9)	(21)	(17)
HYPERPLASIA, PLASMA CELL	1 (11%)		
CIRCULATORY SYSTEM			
NONE			

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(15)	(34)	(34)
NECROSIS, COAGULATIVE			1 (3%)
CYTOPLASMIC VACUOLIZATION			1 (3%)
CYTOLOGIC DEGENERATION		1 (3%)	
HEMATOPOIESIS	1 (7%)		2 (6%)
#PANCREATIC ACINUS	(15)	(34)	(34)
ATROPHY, NOS	1 (7%)		
#SMALL INTESTINE	(15)	(34)	(34)
INFLAMMATION, CHRONIC NECROTIZIN			1 (3%)
URINARY SYSTEM			
#KIDNEY	(15)	(34)	(34)
INFLAMMATION, NOS		1 (3%)	
INFLAMMATION, CHRONIC	10 (67%)	22 (65%)	13 (38%)
ENDOCRINE SYSTEM			
#ADRENAL	(15)	(34)	(34)
ANGIECTASIS	1 (7%)		
#THYROID	(15)	(33)	(32)
HYPERPLASIA, C-CELL			1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(34)	(34)
CYST, NOS	1 (7%)	1 (3%)	3 (9%)
*PREPUTIAL GLAND	(15)	(34)	(34)
INFLAMMATION, CHRONIC FOCAL	1 (7%)		
#UTERUS	(15)	(34)	(34)
METAPLASIA, SQUAMOUS			1 (3%)
DECIDUAL ALTERATION, NOS	1 (7%)	1 (3%)	
#UTERUS/ENDOMETRIUM	(15)	(34)	(34)
INFLAMMATION, SUPPURATIVE	4 (27%)	8 (24%)	7 (21%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC			1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	1 (3%)
INFLAMMATION, CHRONIC NECROTIZIN	1 (7%)		
HYPERPLASIA, CYSTIC			3 (9%)
#CVARY/OVIDUCT	(15)	(34)	(34)
INFLAMMATION, SUPPURATIVE	3 (20%)	3 (9%)	1 (3%)
#OVARY	(15)	(34)	(34)
CYST, NOS	4 (27%)	6 (18%)	11 (32%
INFLAMMATION, SUPPURATIVE	1 (7%)		1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(15)	(34)	(34)
SYNECHIA, ANTERIOR		1 (3%)	
*EYE/CORNEA	(15)	(34)	(34)
INFLAMMATION, CHRONIC		1 (3%)	
*HARDERIAN GLAND	(15)	(34)	(34)
INFLAMMATION, GRANULOMATOUS			1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(15)	(34)	(34)
INFLAMMATION, CHRONIC			1 (3%)
PERIARTERITIS	1 (7%)		
*MESENTERY	(15)	(34)	(34)
STEATITIS		1 (3%)	1 (3%)
ALL OTHER SYSTEMS			
NONE			

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			3
AUTOLYSIS/NO NECROPSY		1	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED PYRAZINAMIDE IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED PYRAZINAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING		1	
ANIMALS NECROPSIED	12	31	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	12	31	33
INTEGUMENTARY SYSTEM			
*SKIN	(12)	(31)	(33)
INFLAMMATION, NOS		1 (3%)	
*SUBCUT TISSUE	(12)	(31)	(33)
HEMORRHAGE		1 (3%)	2 (6%)
RESPIRATORY SYSTEM			
#TRACHEA	(12)	(31)	(33)
INFLAMMATION, SUPPURATIVE	2 (17%)	10 (32%)	9 (27%)
#LUNG/BRONCHUS	(12)	(31)	(33)
INFLAMMATION, SUPPURATIVE			3 (9%)
HYPERPLASIA, PLASMA CELL		1 (3%)	
#LUNG	(12)	(31)	(33)
INFLAMMATION, SUPPURATIVE			1 (3%)
BRONCHOPNEUMONIA SUPPURATIVE	7 (58%)	17 (55%)	17 (52%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (8%)	1 (3%)	
HYPERPLASIA, PLASMA CELL	3 (25%)	5 (16%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(11)	(30)	(31)
ATROPHY, NOS			1 (3%)
#SPLEEN	(12)	(31)	(33)
ATROPHY, NOS		1 (3%)	
HEMATOPOIESIS	2 (17%)	5 (16%)	4 (12%)
#MEDIASTINAL L.NODE	(3)	(22)	(12)
HYPERPLASIA, PLASMA CELL		1 (5%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE	(3)	(22)	(12)
CONGESTION, NOS	1 (33%)	1 (5%)	
INFLAMMATION, CHRONIC NECROTIZIN		1 (5%)	
ATROPHY, NOS		1 (5%)	
HYPERPLASIA, RETICULUM CELL		1 (5%)	
#THYMUS	(11)	(31)	(33)
HYPERPLASIA, PLASMA CELL			1 (3%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(12)	(31)	(33)
INFLAMMATION, INTERSTITIAL		6 (19%)	4 (12%
INFLAMMATION, SUPPURATIVE		4 (13%)	9 (27%
INFLAMMATION, NECROTIZING			1 (3%)
#ENDOCARDIUM	(12)	(31)	(33)
INFLAMMATION, SUPPURATIVE			1 (3%)
INFLAMMATION, ACUTE/CHRONIC			2 (6%)
*AORTA	(12)	(31)	(33)
INFLAMMATION, NECROTIZING			2 (6%)
DIGESTIVE SYSTEM			
#LIVER	(12)	(31)	(33)
HEMORRHAGE		1 (3%)	
INFLAMMATION, NECROTIZING			1 (3%)
INFLAMMATION, CHRONIC	1 (8%)		
NECROSIS, COAGULATIVE		2 (6%)	2 (6%)
HYPERPLASIA, NODULAR			2 (6%)
HYPERPLASIA, RETICULUM CELL		1 (3%)	
HEMATOPOIESIS	1 (8%)	1 (3%)	
#LIVER/CENTRILOBULAR	(12)	(31)	(33)
CYTOPLASMIC VACUOLIZATION		1 (3%)	1 (3%)
URINARY SYSTEM			
#KIDNEY	(12)	(31)	(33)
INFLAMMATION, CHRONIC	1 (8%)		1 (3%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#URINARY BLADDER	(10)	(30)	(31)
ULCER, FOCAL			1 (3%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(12)	(31)	(33)
METAPLASIA, SQUAMOUS			1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EAR CANAL	(12)	(31)	(33)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(12)	(31)	(33)
INFLAMMATION, CHRONIC FOCAL			1 (3%)
INFLAMMATION, CHRONIC NECROTIZIN		1 (3%)	
*PLEURA	(12)	(31)	(33)
INFLAMMATION, SUPPURATIVE			1 (3%)
*EPICARDIUM	(12)	(31)	(33)
INFLAMMATION, SUPPURATIVE			1 (3%)
*MESENTERY	(12)	(31)	(33)
HEMORRHAGE		1 (3%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

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

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED PYRAZINAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING		8	
ANIMALS NECROPSIED	13	25	29
ANIMALS EXAMINED HISTOPATHOLOGICALLY	13	25	29
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(13)	(25)	(29)
INFLAMMATION, SUPPURATIVE	1 (8%)	6 (24%)	6 (21%)
#LUNG	(13)	(24)	(29)
BRONCHOPNEUMONIA SUPPURATIVE	5 (38%)	12 (50%)	12 (41%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	1 (8%)		
INFLAMMATION, FOCAL GRANULOMATOU			1 (3%)
HYPERPLASIA, PLASMA CELL	3 (23%)	3 (13%)	2 (7%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(13)	(24)	(29)
ATROPHY, NOS			1 (3%)
#SPLEEN	(13)	(25)	(29)
FIBROSIS, FOCAL			1 (3%)
ATROPHY, NOS			1 (3%)
ANGIECTASIS			1 (3%)
HYPERPLASIA, RETICULUM CELL			1 (3%)
HEMATOPOIESIS			2 (7%)
#MANDIBULAR L. NODE	(7)	(12)	(23)
HYPERPLASIA, PLASMA CELL			1 (4%)
#BRONCHIAL LYMPH NODE	(7)	(12)	(23)
HYPERPLASIA, LYMPHOID	1 (14%)		
#MEDIASTINAL L.NODE	(7)	(12)	(23)
HYPERPLASIA, PLASMA CELL			2 (9%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC L.NODE	(7)	(12)	(23)
INFLAMMATION, CHRONIC			1 (4%)
#MESENTERIC L. NODE	(7)	(12)	(23)
CONGESTION, NOS		1 (8%)	
ATROPHY, NOS		1 (8%)	
#INGUINAL LYMPH NODE	(7)	(12)	(23)
HYPERPLASIA, LYMPHOID	1 (14%)		
#THYMUS	(13)	(25)	(29)
HYPERPLASIA, PLASMA CELL		1 (4%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(13)	(24)	(29)
INFLAMMATION, INTERSTITIAL		1 (4%)	
INFLAMMATION, SUPPURATIVE			1 (3%)
DIGESTIVE SYSTEM			
#LIVER	(13)	(25)	(28)
CYTOPLASMIC VACUOLIZATION			4 (14%)
HYPERPLASIA, NODULAR		1 (4%)	2 (7%)
ANGIECTASIS			1 (4%)
#PANCREATIC ACINUS	(13)	(25)	(29)
ATROPHY, NOS			1 (3%)
#STOMACH	(13)	(25)	(29)
ULCER, FOCAL		1 (4%)	
#PEYERS PATCH	(13)	(25)	(29)
HYPERPLASIA, LYMPHOID			1 (3%)
#DUODENUM	(13)	(25)	(29)
ULCER, FOCAL			1 (3%)
URINARY 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
ENDOCRINE SYSTEM			
#PITUITARY	(8)	(18)	(20)
HYPERPLASIA, CHROMOPHOBE-CELL			1 (5%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(13)	(25)	(29)
METAPLASIA, SQUAMOUS		1 (4%)	
#UTERUS	(13)	(24)	(29)
THROMBOSIS, NOS		1 (4%)	
#UTERUS/ENDOMETRIUM	(13)	(24)	(29)
INFLAMMATION, SUPPURATIVE		3 (13%)	3 (10%)
INFLAMMATION, CHRONIC SUPPURATIV		2 (8%)	
HYPERPLASIA, CYSTIC	5 (38%)	14 (58%)	23 (79%)
#OVARY	(13)	(23)	(28)
CYST, NOS		4 (17%)	2 (7%)
HEMORRHAGE			1 (4%)
INFLAMMATION, SUPPURATIVE		1 (4%)	
INFLAMMATION, CHRONIC SUPPURATIV		1 (4%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*MIDDLE EAR	(13)	(25)	(29)
INFLAMMATION, CHRONIC SUPPURATIV		1 (4%)	1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(13)	(25)	(29)
INFLAMMATION, CHRONIC FOCAL		1 (4%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, FAT			1 (3%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(13)	(25)	(29)
HYPERPLASIA, PLASMA CELL			2 (7%)
ADIPOSE TISSUE			
INFLAMMATION, GRANULOMATOUS			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	1	
ANIMAL MISSING/NO NECROPSY		8	
NO NECROPSY PERFORMED	1	1	4
AUTOLYSIS/NO NECROPSY	1	1	2

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS FED PYRAZINAMIDE IN THE DIET

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Subcutaneous Tissue: Sarcoma, NOS (not otherwise specified), or Fibrosarcoma[b]	0/14 (0)	2/35 (6)	0/36 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.125	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	95	--
Hematopoietic System: Leukemia[b]	2/14 (14)	2/35 (6)	0/36 (0)
P Values[c,d]	P = 0.037(N)	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.400	0.000
Lower Limit		0.033	0.000
Upper Limit		5.172	1.291
Weeks to First Observed Tumor	89	104	--

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Carcinoma[b]	0/10 (0)	0/32 (0)	1/30 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		--	Infinite
Lower Limit		--	0.019
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	104
Pituitary: Chromophobe Adenoma or Carcinoma[b]	1/10 (10)	4/32 (13)	1/30 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.250	0.333
Lower Limit		0.152	0.005
Upper Limit		59.496	25.331
Weeks to First Observed Tumor	105	87	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma[b]	0/14 (0)	3/35 (9)	0/32 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.258	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	101	--
Thyroid: C-cell Adenoma or Carcinoma[b]	2/14 (14)	4/35 (11)	3/32 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.800	0.656
Lower Limit		0.135	0.088
Upper Limit		8.252	7.314
Weeks to First Observed Tumor	105	101	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor[b]	13/14 (93)	30/35 (86)	29/36 (81)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.923	0.868
Lower Limit		0.831	0.780
Upper Limit		1.259	1.226
Weeks to First Observed Tumor	89	93	85
Ear Canal: Squamous-cell Papilloma or Carcinoma[b]	0/14 (0)	2/35 (6)	0/36 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.125	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	88	--

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

(continued)

[a]Treated groups received doses of 5,000 or 10,000 ppm in feed.

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

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

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

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma or Leukemia[b]	2/15 (13)	3/34 (9)	2/34 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.662	0.441
Lower Limit		0.087	0.036
Upper Limit		7.410	5.706
Weeks to First Observed Tumor	105	97	98
Pituitary: Chromophobe Carcinoma[b]	0/14 (0)	1/30 (3)	0/24 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.026	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	97	--

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma[b]	2/14 (14)	14/30 (47)	7/24 (29)
P Values[c,d]	N.S.	P = 0.037	N.S.
Departure from Linear Trend[e]	P = 0.033		
Relative Risk (Matched Control)[f]		3.267	2.042
Lower Limit		0.927	0.474
Upper Limit		26.414	18.084
Weeks to First Observed Tumor	105	79	96
Thyroid: C-cell Carcinoma[b]	0/15 (0)	0/33 (0)	1/32 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		--	Infinite
Lower Limit		--	0.026
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma or Carcinoma[b]	1/15 (7)	1/33 (3)	1/32 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.455	0.469
Lower Limit		0.006	0.006
Upper Limit		34.631	35.679
Weeks to First Observed Tumor	105	105	104
Mammary Gland: Fibroadenoma[b]	4/15 (27)	5/34 (15)	4/34 (12)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.551	0.441
Lower Limit		0.145	0.099
Upper Limit		2.468	2.121
Weeks to First Observed Tumor	105	105	98

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp[b]	3/15 (20)	5/34 (15)	5/34 (15)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.735	0.735
Lower Limit		0.172	0.172
Upper Limit		4.323	4.323
Weeks to First Observed Tumor	105	88	96
Uterus: Sarcoma, NOS[b]	0/15 (0)	2/34 (6)	0/34 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.138	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	88	--

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Adenocarcinoma, NOS[b]	1/15 (7)	1/34 (3)	1/34 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.441	0.441
Lower Limit		0.006	0.006
Upper Limit		33.649	33.649
Weeks to First Observed Tumor	101	105	96
Ear Canal: Keratoacanthoma or Squamous-cell Carcinoma[b]	1/15 (7)	0/34 (0)	1/34 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.000	0.441
Lower Limit		0.000	0.006
Upper Limit		8.176	33.649
Weeks to First Observed Tumor	105	--	--

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

(continued)

[a]Treated groups received doses of 5,000 or 10,000 ppm.

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

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

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

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE FED PYRAZINAMIDE IN THE DIET

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	1/12 (8)	3/31 (10)	1/33 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.161	0.364
Lower Limit		0.109	0.005
Upper Limit		58.909	27.718
Weeks to First Observed Tumor	104	67	86
Liver: Hepatocellular Carcinoma[b]	1/12 (8)	0/31 (0)	1/33 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.000	0.364
Lower Limit		0.000	0.005
Upper Limit		7.160	27.718
Weeks to First Observed Tumor	69	--	90

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	1/12 (8)	0/31 (0)	3/33 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.000	1.091
Lower Limit		0.000	0.103
Upper Limit		7.160	55.481
Weeks to First Observed Tumor	69	--	82

[a]Treated groups received doses of 5,000 or 10,000 ppm.

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

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

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

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	0/13 (0)	2/25 (8)	6/29 (21)
P Values[c,d]	P = 0.037	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.166	0.779
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	92	81

[a]Treated groups received doses of 5,000 or 10,000 ppm.

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

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

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

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

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

January 18, 1978

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 Pyrazinamide was reviewed.

The primary reviewer noted the relatively high incidence of myocarditis, particularly in the treated male mice, which resulted in a shorter survival time among the animals. She expressed reservations regarding the conclusion drawn in the report to the effect that an evaluation of the treated female mice cannot be made due to mortality and small size of the matched control groups. She said that the data suggest a possible relationship between treatment and an increased incidence of lymphomas in the female mice. She urged that the results be compared with historical control animals to determine their statistical significance and latent period. In regard to the male mice, the primary reviewer said that the animals did not survive long enough to be able to evaluate the carcinogenicity of Pyrazinamide. With respect to the rat portion of the study, she agreed with the conclusion given in the report that Pyrazinamide was not carcinogenic, under the conditions of test.

The secondary reviewer agreed with the conclusions in the report that Pyrazinamide was not carcinogenic in

either the rats or mice, under the conditions of test. However, he said that the small number of control animals made it difficult to exclude the possibility that the lymphomas in the female mice were not a result of treatment. He pointed out the treatment-related incidence of myocarditis in the male mice and the possible relationship of bronchial pneumonia to this condition. The secondary reviewer concluded that a possibility existed that the lymphomas in the female mice were induced by Pyrazinamide. In regard to human risk, he pointed out that the dose levels administered were 15 to 30 times the dosages used in man and were administered for the lifetime of the animals as opposed to a 1 or 2-year treatment period in humans. The secondary reviewer concluded that, if a retest of the mouse study is recommended, it should be given low priority.

A discussion ensued on the need of using historical control data. In some instances the decision is based on the adequacy of the bioassay study and whether a repeat bioassay should be undertaken.

It was moved that the staff's conclusion be accepted with respect to the rat portion of the study. In regard to the mouse portion, it was further moved that the data were inadequate for evaluating the carcinogenicity of Pyrazinamide, although the elevated incidence of lymphomas in the female mice requires special notice. It was recommended that the drug be considered by the Chemical Selection Working Group for possible retest in mice. The motion was seconded and approved by all the Subgroup members except Mr. Garfinkel, who opposed.

Members Present Were:

Arnold Brown (Acting Chairman), Mayo Clinic
Lawrence Garfinkel, American Cancer Society
Joseph Highland, Environmental Defense Fund
Charles Kensler, Arthur D. Little Company
Verald K. Rowe, Dow Chemical, U.S.A.
Sheldon Samuels, Industrial Union Department, AFL-CIO
Louise Strong, University of Texas Health Sciences Center
Sidney Wolfe, Health Research Group

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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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