National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 77
1978

BIOASSAY OF PYRIMETHAMINE FOR POSSIBLE CARCINOGENICITY

CAS No. 58-14-0

NCI-CG-TR-77

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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DHEW Publication No. (NIH) 78-1327

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FOREWORD: This report presents the results of the bioassay of pyrimethamine conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected environmental chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do no necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of pyrimethamine 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 of the laboratory animals and administration of the test chemical. Data management and retrieval were performed by Ms. C. A. Dominick[1]. Histopathologic examinations were performed by Drs. S. D. Kosanke[1], J. C. Peckham[1], and R. B. Thompson[1], and the diagnoses included in this report represent their interpretation.

Animal pathology tables and survival tables were compiled by 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 results of the analyses were reviewed by Dr. C. W. Jameson[5].

This report was prepared at Tracor Jitco[5] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg, Director of the Bioassay Program; Dr. L. A. Campbell, Deputy Director for Science; Drs. J. F. Robens and C. H. Williams, toxicologists; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Waitz, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of NCI[6]: Dr. John J. Gart, Mr. Jun-mo Nam, Dr. Hugh M. Pettigrew, and Dr. Robert E. Tarone.

The following other scientists at NCI were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings:

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SUMMARY

A bioassay of pyrimethamine, a prophylactic antimalarial, 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 pyrimethamine 5 days per week at one of two doses, either 200 or 400 ppm for the rats and either 500 or 1,000 ppm for the mice. The animals were administered the chemical for 78 weeks, then observed for 26 or 27 additional weeks. Matched controls consisted of 15 untreated rats and 15 untreated mice of each sex; pooled controls consisted of the matched controls combined with 30 untreated rats and 30 untreated mice from similar bioassays of two other test compounds. All surviving rats and mice were killed at 102-105 weeks.

Mean body weights of the rats and mice fed diets containing pyrimethamine were slightly lower than those of the matched controls. Survival of the rats was not affected adversely by the chemical. In mice, survival rates of both dosed and matched-control males were low, with nearly two-thirds of the dosed and one-half of the control mice dying by week 52. Some of the deaths were associated with respiratory infections and may not have been related to administration of the chemical. Numbers of animals at risk in the dosed and control groups of female mice were adequate, however, for the development of late-appearing tumors.

In rats of each sex, no neoplastic lesions were found at a statistically significant incidence in the groups fed the pyrimethamine as compared with control groups. An increased frequency of bone-marrow atrophy occurred in both male and female dosed groups.

In male mice, the markedly decreased life spans may have prevented the observation of late-appearing tumors, since only

two tumors were observed, one in a high-dose mouse and one in a low-dose mouse. In female mice, no neoplastic lesions were found at a statistically significant incidence in the groups fed the pyrimethamine as compared with control groups.

It is concluded that under the conditions of this bioassay, pyrimethamine was not carcinogenic for male or female Fischer 344 rats or for female B6C3F1 mice. The carcinogenic potential of pyrimethamine for male B6C3F1 mice cannot be assessed by this bioassay, because of the markedly reduced life span.

TABLE OF CONTENTS

APPENDIXES

I. INTRODUCTION

Pyrimethamine (CAS 58-14-0; NCI C01683) was developed in the early 1950's as an antimalarial drug with both prophylactic and suppressive properties. Currently, it is used to prevent falciparum malarial infection anu to suppress both vivax and falciparum infections in areas where these species of Plasmodium are not resistent to it. Repeated administration of the drug suppresses the erythrocytic stage of the infection and controls the periodic episodes of chills and fever, although it does not cure the infection. It has been used with either dapsone or a sulfonamide in areas where multiresistent strains are endemic. Pyrimethamine binds to and interferes with plasmodial dihydrofolate reductase, an enzyme converting folic acid to folinic acid; thus, it impairs the synthesis of the purine and pyrimidine bases needed for nucleic acid synthesis (Rollo, 1975; AMA Department of Drugs, 1973). Pyrimethamine has antitumor activity in cancer chemotherapeutic screens in vivo, and its mechanism is identical to that of the important antileukemic drug methotrexate (Johns and Bertino, 1973; Calabresi and Parks, 1975).

Pyrimethamine was selected for testing for carcinogenic activity because of its long-term administration to humans to prevent or control malaria.

II. MATERIALS AND METHODS

A. Chemical

Pyrimethamine (5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine) was obtained in two batches from a single lot (Lot No. 51416) from the Burroughs Wellcome Company, Research Triangle Park, North Carolina. The purity of this lot, according to the manufacturer, met United States Pharmacopeia specifications. The identity and purity of this batch was confirmed by analysis at Midwest Research Institute. The melting point was 241-244^{o}C, in agreement with the reported value of 238-242^{o}C (Stecker, 1968). Nonaqueous titration of the amine function with perchloric acid gave 100.3 $\pm$ 0.4% of the theoretical value. High-pressure liquid chromatography indicated a 0.2% impurity which was not identified. Elemental analyses (C, H, Cl, N) were correct for $C_{12}H_{13}ClN_4$, the molecular formula of pyrimethamine. Nuclear magnetic resonance and infrared spectra were consistent with the structure.

The chemical used for the chronic study was stored in the original container at 5^{o}C.

B. Dietary Preparation

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

No analysis of concentration or determination of stability of the chemical in feed were performed. The prepared diets were stored at room temperature in sealed plastic containers.

C. Animals

For the subchronic studies, female Sprague-Dawley rats and male and female Swiss mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Female Fischer 344 rats were obtained from Laboratory Supply Co., Indianapolis, Indiana. Rats were approximately 5 weeks of age, and mice 4 weeks of age when received from the supplier. All animals were quarantined for 1 week prior to testing.

For the chronic studies, male and female Fischer 344 rats and male and female B6C3F1 mice were obtained from Charles River Breeding Laboratories under a contract with the Division of Cancer Treatment, National Cancer Institute. Rats were received at 30 days of age and mice at 37 days of age. On arrival at the laboratory, all animals were quarantined (rats for 11 days, mice for 18 days). Animals with no visible signs of disease were assigned to control or dosed groups, and earmarked for individual identification.

D. Animal Maintenance

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 rat cages were provided with Iso-Dri® hardwood chip bedding (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 filter bonnets during the latter part of the study. 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 in the same room as the respective dosed animals. Animals fed pyrimethamine were maintained in the same rooms as animals of the same species being administered 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)
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)
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)
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 feeding studies were conducted to estimate the maximum tolerated doses of pyrimethamine, 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. Dosed animals were administered the chemical in the diet 7 days a week for 45 days and were then observed for an additional 45 days. Five animals were tested at each dose, and groups ranging in size from 5 to 20 animals were maintained as untreated controls.

Pyrimethamine was administered initially at doses of 1,200, 3,000, 6,000, 15,000 or 30,000 ppm to female Sprague-Dawley rats. Death occurred in all rats at doses of 3,000 ppm and above, and in 2/5 animals fed the test chemical at 1,200 ppm. The chemical was then retested at doses of 100, 300, 600, or 1,200 ppm in female Fischer 344 rats. At these doses, death occurred only in the group at 1,200 ppm, where there was one death at the end of the feeding period. Mean weight gain at the end of 45 days in animals fed pyrimethamine at 100 or 300 ppm was comparable to that of controls, and at 600 ppm was 74% of controls. The low and high doses for the chronic studies using rats were set at 200 and 400 ppm.

Pyrimethamine was initially tested in male Swiss mice at doses of 2,000, 5,000, 10,000, 25,000, or 50,000 ppm. Death occurred in all animals administered doses of 5,000 ppm and above, and in 3/5 animals administered a dose of 2,000 ppm. A second test was then performed using female Swiss mice and doses of 100, 250, 500, 1,000, or 2,000 ppm. There were no deaths in any of these dosed groups. Body weight gains in animals fed the chemical at doses of up to 500 ppm were comparable to those of controls, and weight gains in animals given doses of 1,000 or 2,000 ppm were only slightly lower than those of controls after 45 days of administration of the chemical. The low and high doses for the chronic studies using mice were set at 500 and 1,000 ppm.

F. Designs of the 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 of each species and sex from the current bioassay of pyrimethamine were combined with matched controls of each species and sex from bioassays of pyrazinamide and L-tryptophan. The pooled controls for statistical tests using either rats or mice consisted of 45 males and of 45 females. The bioassays of pyrimethamine, pyrazinamide, and L-tryptophan were all conducted at Southern Research Institute at the same time. The matched-control groups for the pyrazinamide and L-tryptophan bioassays were of the same strains (Fischer 344 rats and B6C3F1 mice) and from the same supplier as those for the pyrimethamine bioassay, and they were examined by the same pathologists.

Table 1. Design of Pyrimethamine Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals[a]	Pyrimethamine Dose (ppm)[b]	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male				
Matched-Control	15	0		105
Low-Dose	35	200	78	26
High-Dose	35	400	78	26
Female				
Matched-Control	15	0		105
Low-Dose	35	200	78	27
High-Dose	35	400	78	26

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

[b]Dosed animals were given test diets 5 days per week and control diets 2 days per week.

Table 2. Design of Pyrimethamine Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals[a]	Pyrimethamine Dose (ppm)[b]	Time on Study Dosed (weeks)	Time on Study Observed (weeks)
Male				
Matched-Control	15	0		102
Low-Dose	35	500	78	26
High-Dose	35	1,000	78	26
Female				
Matched-Control	15	0		104
Low-Dose	35	500	78	26
High-Dose	35	1,000	78	26

[a]Mice were 55 days of age when placed on study.

[b]Dosed animals were given test diets 5 days per week and control diets 2 days per week.

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 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 whenever possible. 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.

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 dosed animals at each dose level. When results for a number of dosed groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

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

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

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a dosed group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a dosed group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the dosed group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result ($P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

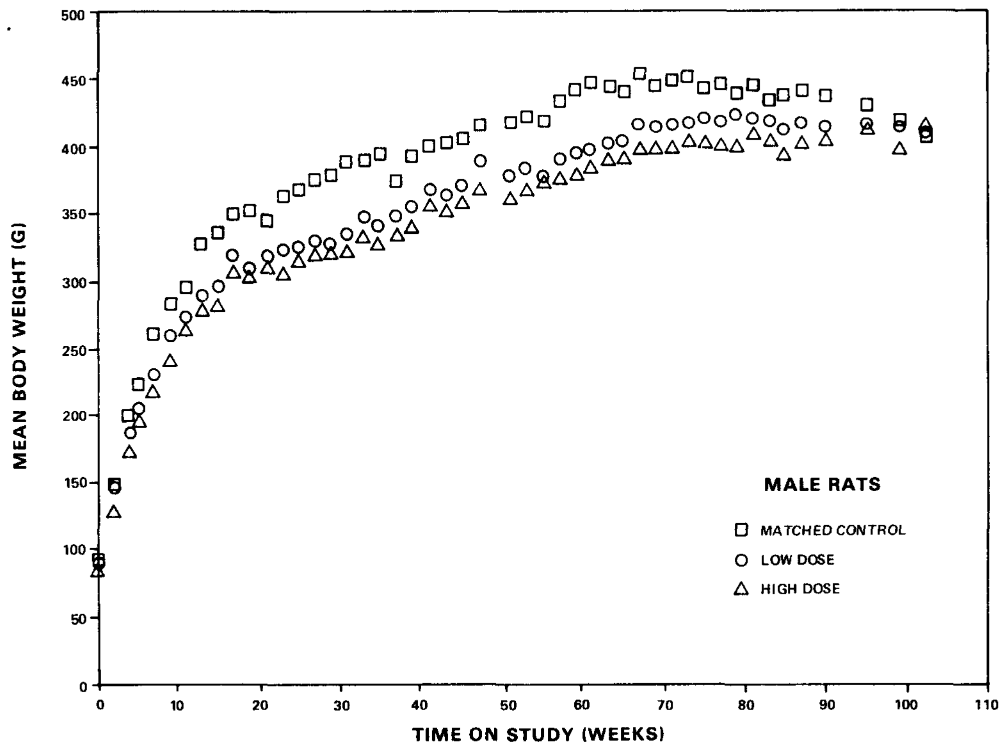
A. Body Weights and Clinical Signs (Rats)

During the period of administration of pyrimethamine in the diet, mean body weights of the low- and high-dose male rats were slightly lower than those of the matched controls. Mean body weights of the low- and high-dose female rats were comparable to those of controls for the first 30 weeks of administration, but were lower thereafter (figure 1). Fluctuations in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. No clinical signs related to administration of the test chemical were recorded.

B. Survival (Rats)

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

In male rats, the results of the Tarone test for positive dose-related trend in mortality are not significant, with 30/35 (86%) of the high-dose group, 28/35 (80%) of the low-dose group, and 10/15 (67%) of the matched controls living to the end of the



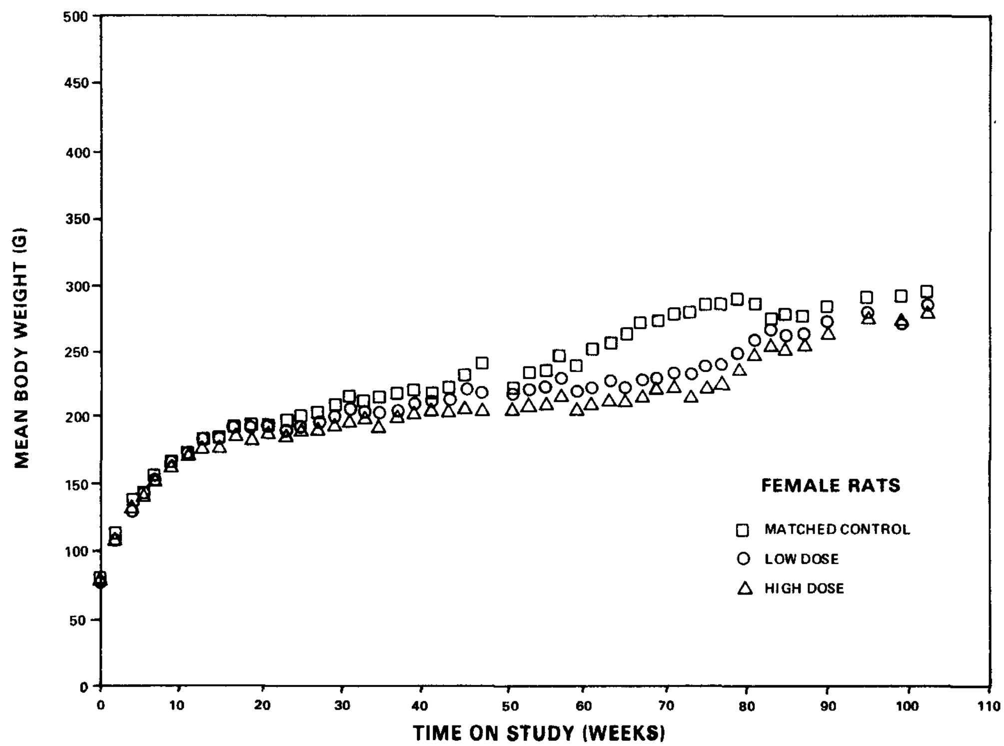


Figure 1. Growth Curves For Rats Fed Pyrimethamine In The Diet

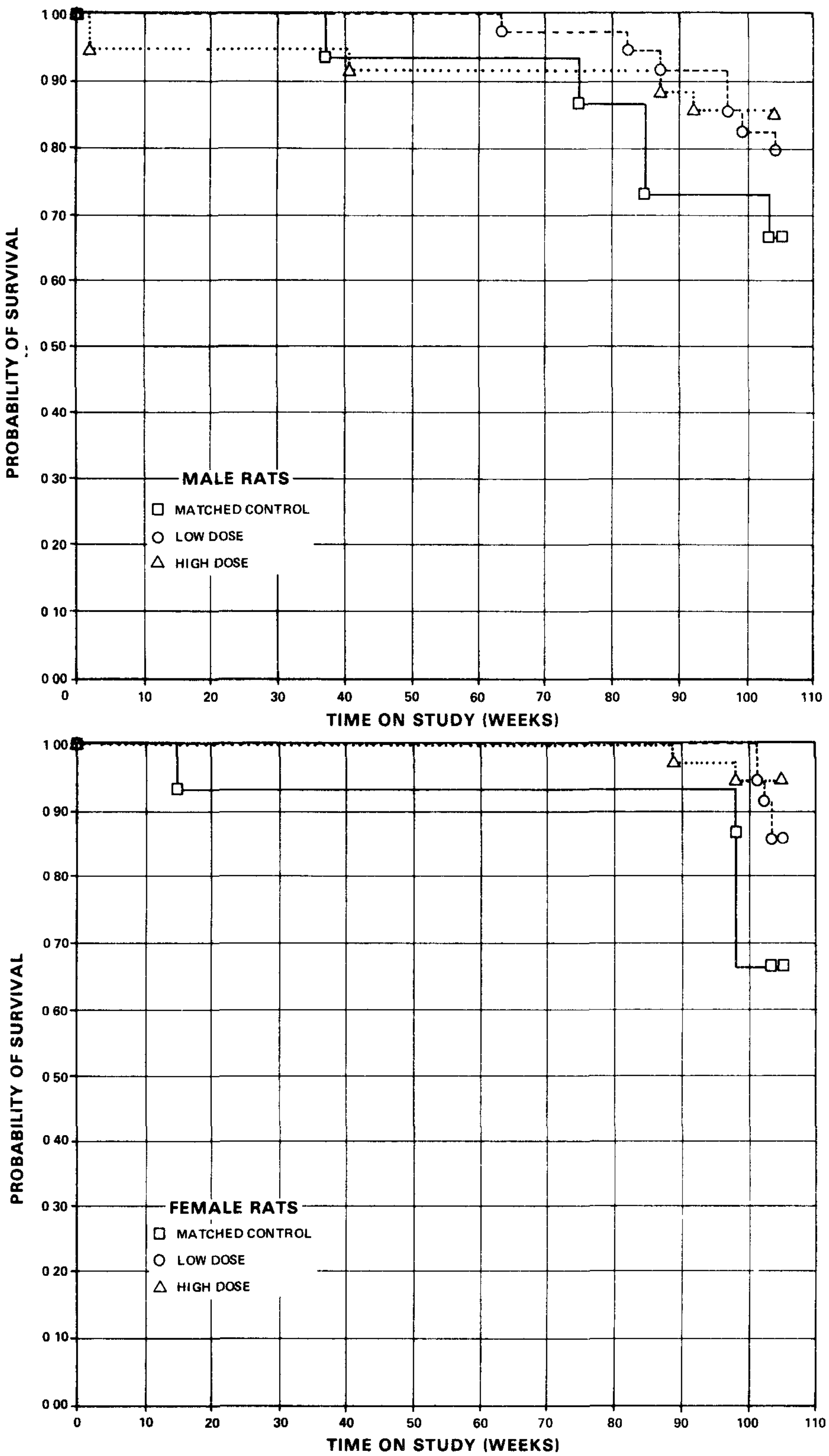


Figure 2. Survival Curves For Rats Fed Pyrimethamine In The Diet

study. In females, the results of the Tarone test are significant in the negative direction (P = 0.015), with 33/35 (94%) of the high-dose group, 30/35 (86%) of the low-dose group, and 10/15 (67%) of the matched controls living to end of the study. Sufficient numbers of dosed and control rats of each sex were at risk for the development of late-appearing tumors.

C. Pathology (Rats)

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

A variety of neoplasms occurred with approximately equal frequency in the matched-control and dosed groups. Some types of neoplasms occurred only in the dosed groups or with greater frequency in the dosed than in the control groups. These lesions, however, are not uncommon in this strain of rat independent of administration of the test chemical.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes also were encountered in animals of the dosed and control groups (Appendix C). These nonneoplastic lesions are commonly seen in aged Fischer 344 rats. An increased frequency of bone-marrow atrophy occurred in both male and female dosed groups (males: controls 1/15 [7%], low-dose 15/34 [44%], high-dose 15/32 [47%]; females: controls 2/14 [14%], low-dose 29/35 [83%], high-dose 23/35 [66%]).

In the judgment of the pathologists, pyrimethamine was not carcinogenic when fed to Fischer 344 rats at doses of 200 or 400 ppm for 78 weeks.

D. Statistical Analyses of Results (Rats)

Tables El 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 in incidences and those of the Fisher exact test for higher incidences in either dosed group than in either control group are not significant for any of the tumors that were observed. Significant results in the negative direction are observed in the combined incidence of lymphoma and leukemia and in the incidence of follicular-cell carcinoma of the thyroid, where the incidences in the control groups exceed those in the dosed groups; however, the dosed animals lived longer than the controls.

In females, the results of the Cochran-Armitage test for positive dose-related trend in the incidence of C-cell adenoma of the thyroid are significant ($P = 0.038$) using the matched controls, but the results of the Fisher exact tests for direct comparisons of incidences of this tumor in dosed and control groups are not significant.

In each of the 95% confidence intervals of 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 (except for the incidence of hematopoietic tumors in high-dose male rats compared with the corresponding pooled-control group) has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by pyrimethamine, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

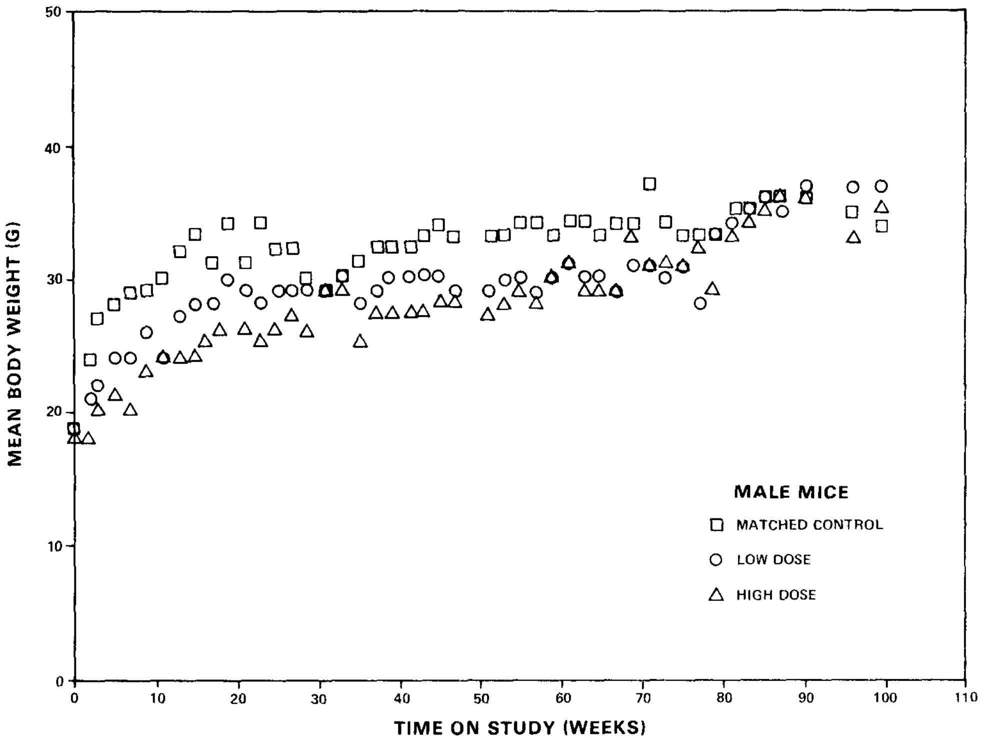
A. Body Weights and Clinical Signs (Mice)

Mean body weights of both male and female mice were slightly lower than those of the matched controls during the period of administration of pyrimethamine in the diet, but were roughly comparable during the observation period following administration of the chemical (figure 3). Fluctuations in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation.

No signs of toxicity related to administration of the test chemical were recorded in the mice. Some animals showed signs of respiratory disease, and all animals received oxytetracycline in the drinking water for 5 days at a dose of 0.6 mg/ml during week 69 and for 5 days at 0.3 mg/ml during week 70. To arrest the transmission of airborne microorganisms, propylene glycol was vaporized in the mouse room during weeks 68-79.

B. Survival (Mice)

Kaplan and Meier curves estimating the probabilities of survival for male and female mice fed pyrimethamine in the diet at the doses of this bioassay, together with those of the matched



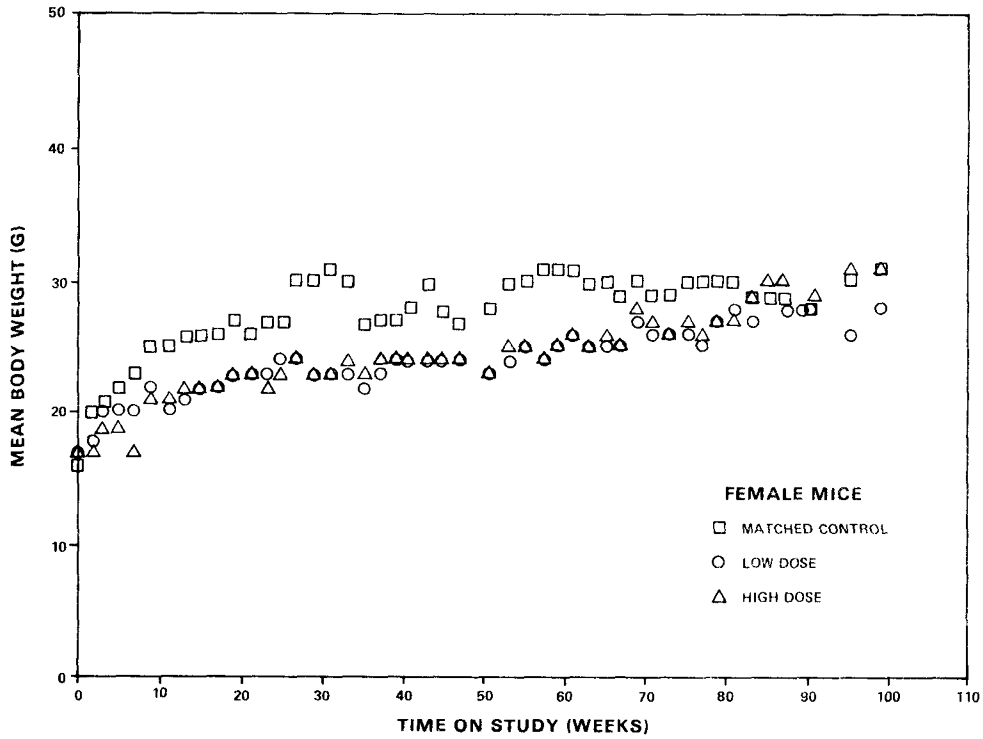


Figure 3. Growth Curves For Mice Fed Pyrimethamine In The Diet

controls, are shown in figure 4. This figure indicates that few animals of either sex survived to the end of the study.

The results of the Tarone test for dose-related trend in mortality are not significant in either sex. In male mice, the survival is low. Only 10/35 (29%) of the high-dose group, 12/35 (34%) of the low-dose group, and 8/15 (53%) of the matched controls lived beyond 1 year. Three of the matched-control animals were reported as missing at week 35. No tumor was observed in the control group and only two tumors in the dosed animals, one in the low-dose group at week 104 and one in the high-dose group at week 87. These early deaths may have prevented the observation of late-appearing tumors in the males. In females, however, over 50% of the animals in the three groups (22/35 [63%] of the high-dose group, 19/35 [54%] of the low-dose group, and 10/15 [67%] of the matched controls) were at risk for at least as long as 1 year; however, only 15/35 (45%) of the high-dose females survived to the end of the study.

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.

The small number of tumors observed in mice may have been

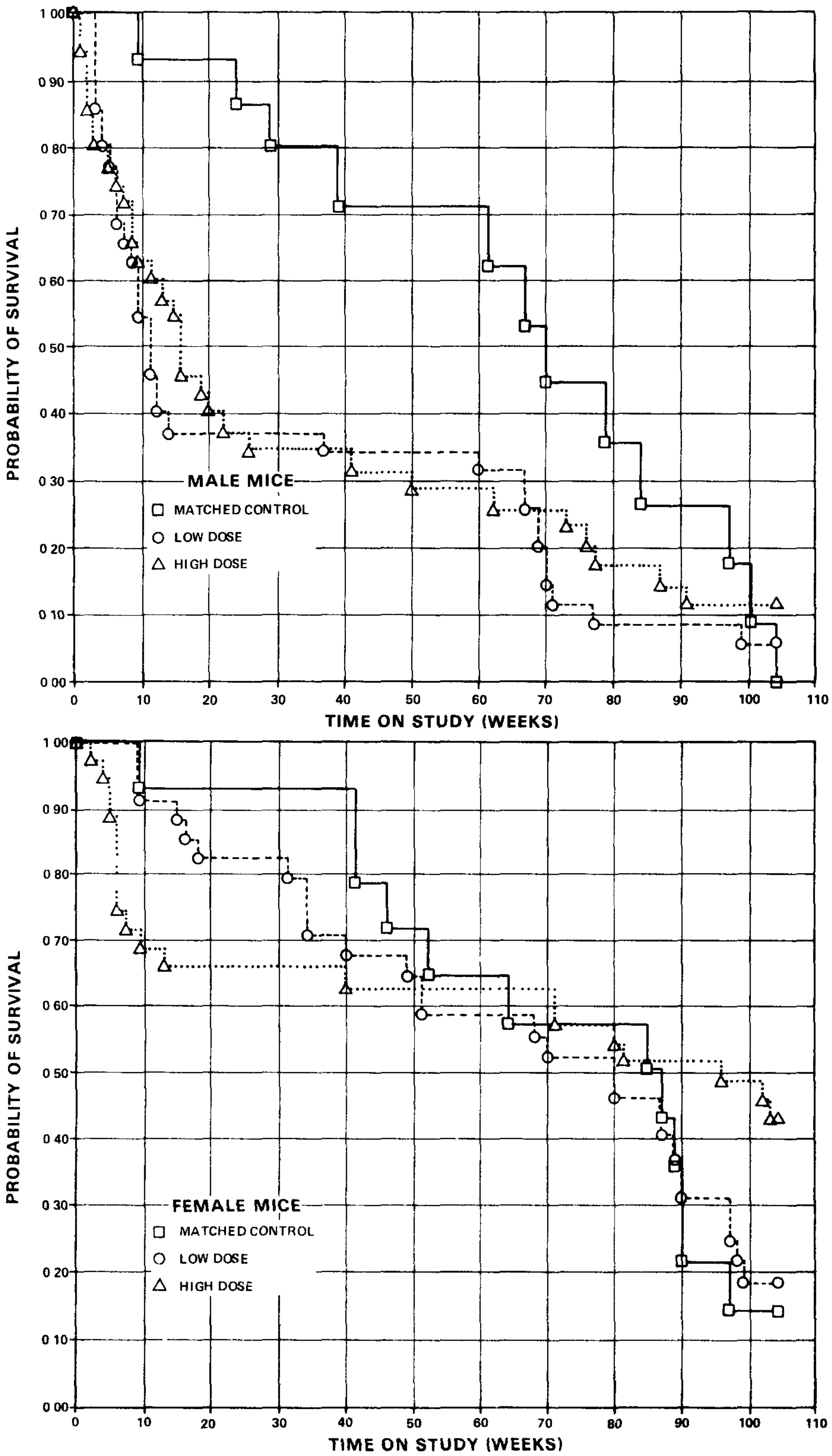


Figure 4. Survival Curves For Mice Fed Pyrimethamine In The Diet

influenced by the decreased life span in both the matched-control and dosed groups of male and female mice. Respiratory infections and inflammatory lesions in the lungs were a factor in the shortened life spans of all groups of mice. The incidences of animals with respiratory lesions were: 3/8 (38%) control males, 7/14 (50%) low-dose males, 2/13 (15%) high-dose males, 5/12 (42%) control females, 11/25 (44%) low-dose females, and 1/21 (5%) high-dose females.

Mortality was high, especially in the male mice. Some of these deaths were associated with respiratory infections, while in other animals the cause of death was undetermined. Many of the latter animals had advanced autolysis which precluded histologic evaluation. In addition, animals that died prior to 100 days on study were not evaluated grossly or histologically. The disposition of male and female mice was as follows:

	MICE					
	MALE			FEMALE		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Animals starting study	15	35	35	15	35	35
Autolysis/no necropsy	2	0	13	1	4	2
No necropsy performed	1	21	8	2	4	11
Animals missing	3	0	0	0	0	0
Number of animals at terminal sacrifice	0	2	4	2	6	15
Total animals necropsied (percent)	(60%)	(40%)	(40%)	(80%)	(77%)	(63%)

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes also were encountered in animals of the dosed and control groups (Appendix D). For the most part the nonneoplastic lesions are those commonly seen in aged mice.

Administration of the pyrimethamine to the mice resulted in few tumors. Most of the neoplastic lesions appeared unrelated to administration of the chemical. The effectiveness of the carcinogenesis bioassay was reduced by a decrease in life span.

In the judgment of the pathologists, the results of this bioassay failed to define the carcinogenic activity of pyrimethamine in B6C3F1 mice when fed at doses of 500 or 1,000 ppm for 78 weeks. This may have been due to the high early mortality, especially in the males, and to the small number of animals examined histologically.

D. Statistical Analyses of Results (Mice)

Table F1 in Appendix F contains 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 of the female mice. The incidences of tumors in the male mice are not included in the tables and analyses, because only two tumors were observed among the dosed and control groups of animals. A sebaceous adenoma of the skin was found in the low-dose group and a histiocytic type of malignant lymphoma of the hematopoietic system was found in the high-dose group. The early deaths of the male mice may have prevented the observation of late-appearing tumors.

In female mice, the results of the Cochran-Armitage test for positive dose-related trend and those of the Fisher exact test for direct comparisons of control and dosed groups are not significant. In each of the 95% confidence intervals of relative risk, shown in table F1, 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 pyrimethamine, which could not be detected under the conditions of this test.

V. DISCUSSION

In this bioassay of pyrimethamine administered in feed, mean body weights of rats were only slightly lower than those of controls, and mortality was not related to administration of the chemical. In the mice, mean body weights of the dosed animals were lower than those of the controls throughout the administration period, and mortality was high, particularly in the males. The early deaths may not have been related to administration of the pyrimethamine, since there was a high incidence of respiratory infections among all groups of mice.

In rats of each sex, no neoplastic lesions were found in dosed groups at incidences significantly different from those of either matched or pooled controls. An increased frequency of bone-marrow atrophy occurred in both male and female dosed groups.

In male mice, the markedly decreased life spans may have prevented the observation of late-appearing tumors, since only one tumor was found in a high-dose male and one in a low-dose male. No tumor occurred in the dosed female mice at an incidence significantly above those of the control animals.

No long-term studies on the carcinogenicity of pyrimethamine have been reported.

It is concluded that under the conditions of this bioassay, pyrimethamine was not carcinogenic for male or female Fischer 344 rats or for female B6C3F1 mice. The carcinogenic potential of pyrimethamine for male B6C3F1 mice cannot be assessed by this bioassay, because of the markedly reduced life span.

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APPENDIX A

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

TABLE A1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	35	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	35	33
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(15)	(35)	(33)
FIBROMA		1 (3%)	
FIBROSARCOMA		1 (3%)	1 (3%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(15)	(35)	(33)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (7%)		
LEUKEMIA,NOS		1 (3%)	
UNDIFFERENTIATED LEUKEMIA	2 (13%)	1 (3%)	1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#COLON	(14)	(35)	(32)
ADENOMATOUS POLYP, NOS			1 (3%)
URINARY SYSTEM			
#URINARY BLADDER	(15)	(30)	(32)
TRANSITIONAL-CELL CARCINOMA			1 (3%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(11)	(33)	(31)
CHROMOPHOBE ADENOMA	2 (18%)	8 (24%)	1 (3%)
#ADRENAL	(15)	(35)	(33)
PHEOCHROMOCYTOMA			1 (3%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (7%)	1 (3%)	
#THYROID	(14)	(34)	(32)
FOLLICULAR-CELL CARCINOMA	2 (14%)	1 (3%)	
C-CELL CARCINOMA		3 (9%)	3 (9%)
#PANCREATIC ISLETS	(14)	(35)	(33)
ISLET-CELL ADENOMA	1 (7%)	2 (6%)	1 (3%)
ISLET-CELL CARCINOMA			2 (6%)
REPRODUCTIVE SYSTEM			
#TESTIS	(14)	(35)	(33)
INTERSTITIAL-CELL TUMOR	13 (93%)	30 (86%)	27 (82%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EAR CANAL	(15)	(35)	(33)
SQUAMOUS CELL CARCINOMA		1 (3%)	1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(15)	(35)	(33)
MESOTHELIOMA, NOS		1 (3%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*PLEURA	(15)	(35)	(33)
MESOTHELIOMA BENIGN	1 (7%)		
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	4	3	2
MORIBUND SACRIFICE	1	4	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	10	28	30
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	33	30
TOTAL PRIMARY TUMORS	23	51	40
TOTAL ANIMALS WITH BENIGN TUMORS	14	32	29
TOTAL BENIGN TUMORS	17	41	31
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	8	9
TOTAL MALIGNANT TUMORS	6	9	9
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED PYRIMETHAMINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14	35	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	35	35
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(14)	(35)	(35)
SARCOMA, NOS		1 (3%)	
RESPIRATORY SYSTEM			
#LUNG	(13)	(34)	(35)
ADENOCARCINOMA, NOS, METASTATIC	1 (8%)		
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (3%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (3%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(14)	(35)	(35)
UNDIFFERENTIATED LEUKEMIA	1 (7%)	1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(11)	(34)	(32)
CHROMOPHOBE ADENOMA	4 (36%)	8 (24%)	14 (44%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#THYROID	(13)	(35)	(34)
FOLLICULAR-CELL CARCINOMA			1 (3%)
C-CELL ADENOMA			4 (12%)
#PANCREATIC ISLETS	(14)	(34)	(35)
ISLET-CELL CARCINOMA		1 (3%)	1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(14)	(35)	(35)
INFILTRATING DUCT CARCINOMA			1 (3%)
FIBROADENOMA	2 (14%)	4 (11%)	4 (11%)
#UTERUS	(14)	(35)	(35)
ADENOCARCINOMA, NOS	1 (7%)		
ENDOMETRIAL STROMAL POLYP		3 (9%)	3 (9%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(14)	(35)	(35)
ADENOCARCINOMA, NOS, METASTATIC	1 (7%)		
*PLEURA	(14)	(35)	(35)
ADENOCARCINOMA, NOS, METASTATIC	1 (7%)		
ALL OTHER SYSTEMS			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	3	3	1
MORIBUND SACRIFICE	2	2	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	10	30	33
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	16	26
TOTAL PRIMARY TUMORS	8	19	29
TOTAL ANIMALS WITH BENIGN TUMORS	6	14	24
TOTAL BENIGN TUMORS	6	16	25
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	3	4
TOTAL MALIGNANT TUMORS	2	3	4
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		
TOTAL SECONDARY TUMORS	3		
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN

MICE FED PYRIMETHAMINE IN THE DIET

TABLE B1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING	3		
ANIMALS NECROPSIED	9	14	14
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	14	14
INTEGUMENTARY SYSTEM			
*SKIN	(9)	(14)	(14)
SEBACEOUS ADENOMA		1 (7%)	
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(9)	(14)	(14)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (7%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
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@	8	31	29
MORIBUND SACRIFICE	4	2	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE		2	4
ANIMAL MISSING	3		

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*		1	1
TOTAL PRIMARY TUMORS		1	1
TOTAL ANIMALS WITH BENIGN TUMORS		1	
TOTAL BENIGN TUMORS		1	
TOTAL ANIMALS WITH MALIGNANT TUMORS			1
TOTAL MALIGNANT TUMORS			1
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 PYRIMETHAMINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	12	27	22
ANIMALS EXAMINED HISTOPATHOLOGICALLY	12	27	22
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(12)	(25)	(21)
ALVEOLAR/BRONCHIOLAR ADENOMA			2 (10%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(12)	(27)	(22)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (4%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (4%)	4 (18%)
#KIDNEY	(10)	(27)	(22)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (4%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#PANCREAS	(10)	(24)	(22)
ADENOCARCINOMA, NOS, METASTATIC			1 (5%)
URINARY SYSTEM			
#KIDNEY	(10)	(27)	(22)
ADENOCARCINOMA, NOS, METASTATIC			1 (5%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#URINARY BLADDER	(8)	(23)	(21)
ADENOCARCINOMA, NOS, METASTATIC			1 (5%)
ENDOCRINE SYSTEM			
#THYROID	(10)	(25)	(22)
FOLLICULAR-CELL ADENOMA			1 (5%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(12)	(27)	(22)
FIBROADENOMA		1 (4%)	
#UTERUS	(10)	(25)	(22)
ADENOMA, NOS		1 (4%)	
ADENOCARCINOMA, NOS			2 (9%)
ENDOMETRIAL STROMAL POLYP	1 (10%)		
#OVARY	(10)	(24)	(21)
ADENOCARCINOMA, NOS, METASTATIC			1 (5%)
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)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	8	15	14
MORIBUND SACRIFICE	4	12	6
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED	1	2	
TERMINAL SACRIFICE	2	6	15
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	1	5	9
TOTAL PRIMARY TUMORS	1	5	9
TOTAL ANIMALS WITH BENIGN TUMORS	1	2	3
TOTAL BENIGN TUMORS	1	2	3
TOTAL ANIMALS WITH MALIGNANT TUMORS		3	6
TOTAL MALIGNANT TUMORS		3	6
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			4
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 PYRIMETHAMINE IN THE DIET

TABLE C1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	35	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	35	33
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(35)	(33)
INFLAMMATION, CHRONIC			1 (3%)
*SUBCUT TISSUE	(15)	(35)	(33)
ULTIMOBRANCHIAL CYST			1 (3%)
EPIDERMAL INCLUSION CYST			1 (3%)
HEMORRHAGE			1 (3%)
RESPIRATORY SYSTEM			
#TRACHEA	(15)	(35)	(33)
INFLAMMATION, SUPPURATIVE		1 (3%)	7 (21%)
INFLAMMATION, CHRONIC SUPPURATIV			5 (15%)
#LUNG	(15)	(35)	(33)
PNEUMONIA, CHRONIC MURINE	3 (20%)	19 (54%)	4 (12%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(15)	(34)	(32)
ATROPHY, NOS	1 (7%)	15 (44%)	15 (47%)
#SPLEEN	(15)	(35)	(33)
HEMATOPOIESIS		2 (6%)	1 (3%)
#MANDIBULAR L. NODE	(11)	(33)	(27)
HYPERPLASIA, PLASMA CELL	1 (9%)	1 (3%)	
#CERVICAL LYMPH NODE	(11)	(33)	(27)
LYMPHANGIECTASIS			1 (4%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(15)	(35)	(32)
NECROSIS, COAGULATIVE	1 (7%)	1 (3%)	
CYTOPLASMIC VACUOLIZATION			1 (3%)
HYPERPLASIA, NODULAR			2 (6%)
#LIVER/CENTRILOBULAR	(15)	(35)	(32)
NECROSIS, COAGULATIVE		1 (3%)	
CYTOLOGIC DEGENERATION	1 (7%)		
#PANCREAS	(14)	(35)	(33)
FIBROSIS, DIFFUSE	1 (7%)		
#STOMACH	(15)	(35)	(33)
ULCER, FOCAL			1 (3%)
URINARY SYSTEM			
#KIDNEY	(15)	(35)	(33)
INFLAMMATION, CHRONIC	12 (80%)	30 (86%)	23 (70%)
INFARCT, NOS			1 (3%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(35)	(33)
CYST, NOS	1 (7%)		2 (6%)
#PROSTATE	(15)	(33)	(33)
INFLAMMATION, SUPPURATIVE		1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)		
NERVOUS SYSTEM			
#BRAIN	(13)	(34)	(33)
HEMORRHAGE			1 (3%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MALACIA			1 (3%)
SPECIAL SENSE ORGANS			
*MIDDLE EAR	(15)	(35)	(33)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(15)	(35)	(33)
INFLAMMATION, CHRONIC FOCAL			1 (3%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC			1
INFLAMMATION, CHRONIC FOCAL		1	
INFLAMMATION, CHRONIC DIFFUSE		1	
SPECIAL MORPHOLOGY SUMMARY			
NO NECROPSY PERFORMED			2

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED PYRIMETHAMINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14	35	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	35	35
INTEGUMENTARY SYSTEM			
*SKIN	(14)	(35)	(35)
ULCER, CHRONIC		1 (3%)	
INFLAMMATION, CHRONIC FOCAL		1 (3%)	
RESPIRATORY SYSTEM			
#TRACHEA	(14)	(35)	(35)
INFLAMMATION, SUPPURATIVE			1 (3%)
PLASMA-CELL INFILTRATE		1 (3%)	
HYPERPLASIA, PLASMA CELL			1 (3%)
#LUNG	(13)	(34)	(35)
PNEUMONIA, CHRONIC MURINE	4 (31%)	16 (47%)	3 (9%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (3%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(14)	(35)	(35)
ATROPHY, NOS	2 (14%)	29 (83%)	23 (66%)
#SPLEEN	(14)	(33)	(35)
HEMATOPOIESIS		1 (3%)	
#MEDIASTINAL L.NODE	(14)	(34)	(30)
HYPERPLASIA, PLASMA CELL			1 (3%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(14)	(34)	(35)
INFLAMMATION, CHRONIC FOCAL		1 (3%)	
#LIVER/CENTRILOBULAR	(14)	(34)	(35)
CYTOLOGIC DEGENERATION		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(14)	(35)	(35)
INFLAMMATION, CHRONIC	8 (57%)	7 (20%)	4 (11%)
ENDOCRINE SYSTEM			
#THYROID	(13)	(35)	(34)
CYSTIC FOLLICLES		1 (3%)	
HYPERPLASIA, C-CELL	1 (8%)	1 (3%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(14)	(35)	(35)
CYST, NOS			1 (3%)
#UTERUS/ENDOMETRIUM	(14)	(35)	(35)
INFLAMMATION, SUPPURATIVE	7 (50%)	8 (23%)	7 (20%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)	2 (6%)	4 (11%)
INFLAMMATION, FOCAL GRANULOMATOU			1 (3%)
HYPERPLASIA, CYSTIC			1 (3%)
#OVARY/OVIDUCT	(14)	(35)	(35)
INFLAMMATION, SUPPURATIVE		1 (3%)	3 (9%)
#OVARY	(14)	(35)	(35)
CYST, NOS		5 (14%)	12 (34%)
INFLAMMATION, SUPPURATIVE	1 (7%)	2 (6%)	4 (11%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)	1 (3%)	2 (6%)
NERVOUS SYSTEM			
NONE			

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC FOCAL	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1		
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 PYRIMETHAMINE IN THE DIET

TABLE D1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING	3		
ANIMALS NECROPSIED	9	14	14
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	14	14
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(7)	(14)	(13)
INFLAMMATION, SUPPURATIVE		1 (7%)	1 (8%)
#LUNG	(8)	(14)	(13)
CONGESTION, NOS		1 (7%)	1 (8%)
EDEMA, NOS		1 (7%)	
HEMORRHAGE		2 (14%)	
PNEUMONIA, ASPIRATION			1 (8%)
INFLAMMATION, SUPPURATIVE	1 (13%)		
BRONCHOPNEUMONIA SUPPURATIVE	2 (25%)	3 (21%)	1 (8%)
INFLAMMATION, CHRONIC FOCAL		1 (7%)	
INFLAMMATION, CHRONIC SUPPURATIV		2 (14%)	
BRONCHOPNEUMONIA CHRONIC SUPPURA		1 (7%)	
HYPERPLASIA, LYMPHOID			4 (31%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(8)	(14)	(13)
ATROPHY, NOS	2 (25%)		
DEPLETION	1 (13%)		
#SPLEEN	(8)	(13)	(14)
LYMPHOID DEPLETION	1 (13%)		
HYPERPLASIA, LYMPHOID		4 (31%)	
HEMATOPOIESIS	3 (38%)	6 (46%)	5 (36%)
#THYMUS	(6)	(9)	(12)
ATROPHY, NOS		1 (11%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#MYOCARDIUM	(8)	(14)	(13)
HEMORRHAGE		1 (7%)	
INFLAMMATION, CHRONIC		2 (14%)	3 (23%)
INFLAMMATION, CHRONIC FOCAL		2 (14%)	2 (15%)
INFLAMMATION, CHRONIC SUPPURATIV		2 (14%)	
DIGESTIVE SYSTEM			
#LIVER	(8)	(13)	(14)
INFLAMMATION, FOCAL		1 (8%)	
DEGENERATION, GRANULAR			1 (7%)
NECROSIS, COAGULATIVE		2 (15%)	
CYTOPLASMIC VACUOLIZATION	1 (13%)		1 (7%)
HYPERPLASIA, NODULAR	1 (13%)	1 (8%)	
#LIVER/HEPATOCYTES	(8)	(13)	(14)
DEGENERATION, GRANULAR			1 (7%)
*RECTUM	(9)	(14)	(14)
ULCER, NOS			1 (7%)
*PERIANAL TISSUE	(9)	(14)	(14)
INFLAMMATION, CHRONIC FOCAL			1 (7%)
URINARY SYSTEM			
#KIDNEY	(8)	(14)	(14)
LYMPHOCYTIC INFLAMMATORY INFILTR		2 (14%)	
INFLAMMATION, CHRONIC			2 (14%)
INFARCT, FOCAL		1 (7%)	
HYPERPLASIA, LYMPHOID		1 (7%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(9)	(14)	(14)
CYSTIC DUCTS			1 (7%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#TESTIS	(8)	(14)	(14)
HEMORRHAGE		1 (7%)	
INFLAMMATION, NOS		1 (7%)	
DEGENERATION, NOS		2 (14%)	4 (29%)
ATROPHY, NOS		1 (7%)	
NERVOUS SYSTEM			
#CEREBELLUM	(7)	(14)	(13)
HEMORRHAGE		1 (7%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3		2
ANIMAL MISSING/NO NECROPSY	3		
NO NECROPSY PERFORMED	1	21	8
AUTO/NECROPSY/HISTO PERF	1		2
AUTOLYSIS/NO NECROPSY	2		13

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

TABLE D2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	12	27	22
ANIMALS EXAMINED HISTOPATHOLOGICALLY	12	27	22
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(10)	(26)	(22)
INFLAMMATION, SUPPURATIVE		5 (19%)	
INFLAMMATION, CHRONIC SUPPURATIV		2 (8%)	
#LUNG	(12)	(25)	(21)
PNEUMONIA, ASPIRATION		1 (4%)	
INFLAMMATION, SUPPURATIVE		1 (4%)	1 (5%)
BRONCHOPNEUMONIA SUPPURATIVE	5 (42%)	11 (44%)	
HYPERPLASIA, LYMPHOID		2 (8%)	7 (33%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(10)	(25)	(22)
LYMPHOID DEPLETION			1 (5%)
HYPERPLASIA, RETICULUM CELL			1 (5%)
HYPERPLASIA, LYMPHOID			3 (14%)
HEMATOPOIESIS	1 (10%)	6 (24%)	4 (18%)
#THYMUS	(8)	(23)	(18)
ATROPHY, NOS		4 (17%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(12)	(26)	(22)
INFLAMMATION, NOS			1 (5%)
INFLAMMATION, INTERSTITIAL	1 (8%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, SUPPURATIVE		1 (4%)	
INFLAMMATION, CHRONIC			4 (18%)
INFLAMMATION, CHRONIC FOCAL	1 (8%)		
DIGESTIVE SYSTEM			
#LIVER	(9)	(25)	(22)
NECROSIS, FOCAL	2 (22%)		
NECROSIS, COAGULATIVE	1 (11%)		
CYTOPLASMIC VACUOLIZATION			1 (5%)
HYPERPLASIA, NODULAR			1 (5%)
#LIVER/HEPATOCYTES	(9)	(25)	(22)
DEGENERATION, GRANULAR			1 (5%)
#PANCREAS	(10)	(24)	(22)
INFLAMMATION, CHRONIC			1 (5%)
#PEYERS PATCH	(10)	(24)	(22)
HYPERPLASIA, LYMPHOID			1 (5%)
URINARY SYSTEM			
#KIDNEY	(10)	(27)	(22)
PERIARTERITIS		1 (4%)	
NECROSIS, ISCHEMIC		1 (4%)	
HYPERPLASIA, LYMPHOID			2 (9%)
#KIDNEY/PELVIS	(10)	(27)	(22)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (5%)
#URINARY BLADDER	(8)	(23)	(21)
HYPERPLASIA, LYMPHOID			1 (5%)
ENDOCRINE SYSTEM			
#THYROID	(10)	(25)	(22)
CYSTIC FOLLICLES		2 (8%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(12)	(27)	(22)
DILATATION/DUCTS		1 (4%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#UTERUS/ENDOMETRIUM	(10)	(25)	(22)
CYST, NOS			1 (5%)
INFLAMMATION, SUPPURATIVE	2 (20%)	1 (4%)	
HYPERPLASIA, FOCAL	1 (10%)		1 (5%)
HYPERPLASIA, CYSTIC	5 (50%)	7 (28%)	14 (64%)
#OVARY	(10)	(24)	(21)
FOLLICULAR CYST, NOS		3 (13%)	5 (24%)
INFLAMMATION, SUPPURATIVE	1 (10%)		
NERVOUS SYSTEM			
#BRAIN	(11)	(25)	(22)
LYMPHOCYTOSIS		1 (4%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(12)	(27)	(22)
INFLAMMATION, FIBRINOUS			1 (5%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		3	1
NO NECROPSY PERFORMED	2	4	11
AUTO/NECROPSY/HISTO PERF	1	2	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
AUTOLYSIS/NO NECROPSY	1	4	2

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

APPENDIX E

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

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

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia[b]	9/44 (20)	3/15 (20)	2/35 (6)	1/33 (3)
P Values[c,d]	P = 0.010(N)	N.S.	N.S.	P = 0.024**(N)
Relative Risk (Pooled Control)[f]			0.279	0.148
Lower Limit			0.031	0.003
Upper Limit			1.237	0.985
Relative Risk (Matched Control)[f]			0.286	0.152
Lower Limit			0.027	0.003
Upper Limit			2.289	1.737
Weeks to First Observed Tumor	--	103	63	92
Pituitary: Chromophobe Adenoma[b]	4/32 (13)	2/11 (18)	8/33 (24)	1/31 (3)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.026			
Relative Risk (Pooled Control)[f]			1.939	0.258
Lower Limit			0.582	0.005
Upper Limit			7.964	2.419
Relative Risk (Matched Control)[f]			1.333	0.177
Lower Limit			0.339	0.003
Upper Limit			11.794	3.167
Weeks to First Observed Tumor	--	85	97	104

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

(continued)

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma[b]	1/42 (2)	0/14 (0)	3/34 (9)	3/32 (9)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			3.706	3.938
Lower Limit			0.314	0.333
Upper Limit			188.485	199.811
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.265	0.282
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	104	104
Thyroid: Follicular-cell Carcinoma[b]	3/42 (7)	2/14 (14)	1/34 (3)	0/32 (0)
P Values[c,d]	N.S.	P = 0.039(N)	N.S.	N.S.
Relative Risk (Pooled Control)[f]			0.412	0.000
Lower Limit			0.008	0.000
Upper Limit			4.836	2.149
Relative Risk (Matched Control)[f]			0.206	0.000
Lower Limit			0.004	0.000
Upper Limit			3.714	1.447
Weeks to First Observed Tumor	--	105	104	--

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

(continued)

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Pancreatic Islets: Islet-cell Carcinoma[b]	0/43 (0)	0/14 (0)	0/35 (0)	2/33 (6)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			--	Infinite
Lower Limit			--	0.388
Upper Limit			--	Infinite
Relative Risk (Matched Control)[f]			--	Infinite
Lower Limit			--	0.134
Upper Limit			--	Infinite
Weeks to First Observed Tumor	--	--	--	104
Pancreatic Islets: Islet-cell Adenoma or Carcinoma[b]	2/43 (5)	1/14 (7)	2/35 (6)	3/33 (9)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			1.229	1.955
Lower Limit			0.093	0.237
Upper Limit			16.144	22.183
Relative Risk (Matched Control)[f]			0.800	1.273
Lower Limit			0.047	0.117
Upper Limit			45.853	64.698
Weeks to First Observed Tumor	--	75	104	92

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

(continued)

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor[b]	39/43 (91)	13/14 (93)	30/35 (86)	27/33 (82)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			0.945	0.902
Lower Limit			0.805	0.760
Upper Limit			1.125	1.100
Relative Risk (Matched Control)[f]			0.923	0.881
Lower Limit			0.831	0.790
Upper Limit			1.259	1.235
Weeks to First Observed Tumor	--	85	87	104

[a]Groups fed pyrimethamine received doses of 200 or 400 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 dosed group is the probability level for the Fisher exact test for the comparison of that dosed 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 dosed group than in a control group.

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

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

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

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	9/37 (24)	4/11 (36)	8/34 (24)	14/32 (44)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			0.967	1.799
Lower Limit			0.367	0.843
Upper Limit			2.487	3.978
Relative Risk (Matched Control)[f]			0.647	1.203
Lower Limit			0.236	0.520
Upper Limit			2.524	4.177
Weeks to First Observed Tumor	--	98	101	89
Thyroid: C-cell Adenoma[b]	2/42 (5)	0/13 (0)	0/35 (0)	4/34 (12)
P Values[c,d]	N.S.	P = 0.038	N.S.	N.S.
Relative Risk (Pooled Control)[f]			0.000	2.471
Lower Limit			0.000	0.378
Upper Limit			4.013	25.875
Relative Risk (Matched Control)[f]			--	Infinite
Lower Limit			--	0.383
Upper Limit			--	Infinite
Weeks to First Observed Tumor	--	--	--	104

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

(continued)

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	10/43 (23)	2/14 (14)	4/35 (11)	4/35 (11)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			0.491	0.491
Lower Limit			0.122	0.122
Upper Limit			1.536	1.536
Relative Risk (Matched Control)[f]			0.800	0.800
Lower Limit			0.135	0.135
Upper Limit			8.252	8.252
Weeks to First Observed Tumor	--	103	102	98
Uterus: Endometrial Stromal Polyp[b]	6/42 (14)	0/14 (0)	3/35 (9)	3/35 (9)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			0.600	0.600
Lower Limit			0.103	0.103
Upper Limit			2.581	2.581
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.258	0.258
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	105	104

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

(continued)

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[a]Groups fed pyrimethamine received doses of 200 or 400 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 dosed group is the probability level for the Fisher exact test for the comparison of that dosed 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 dosed group than in a control group.

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

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN FEMALE MICE FED PYRIMETHAMINE IN THE DIET

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

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	2/38 (5)	0/12 (0)	0/25 (0)	2/21 (10)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			0.000	1.810
Lower Limit			0.000	0.138
Upper Limit			5.012	23.025
Relative Risk (Matched Control)[f]			--	Infinite
Lower Limit			--	0.183
Upper Limit			--	Infinite
Weeks to First Observed Tumor	--	--	--	104
Hematopoietic System: Lymphoma[b]	2/38 (5)	0/12 (0)	3/27 (11)	4/22 (18)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			2.111	3.455
Lower Limit			0.258	0.537
Upper Limit			23.658	35.023
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.291	0.554
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	80	71

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

(continued)

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma, Histiocytic Type[b]	2/38 (5)	0/12 (0)	2/27 (7)	4/22 (18)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			1.407	3.455
Lower Limit			0.107	0.537
Upper Limit			18.229	35.023
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.142	0.554
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	80	71
Uterus: Adenocarcinoma, NOS[b]	0/36 (0)	0/10 (0)	0/25 (0)	2/22 (9)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			--	Infinite
Lower Limit			--	0.491
Upper Limit			--	Infinite
Relative Risk (Matched Control)[f]			--	Infinite
Lower Limit			--	0.149
Upper Limit			--	Infinite
Weeks to First Observed Tumor	--	--	--	96

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

(continued)

[a]Groups fed pyrimethamine received doses of 500 or 1000 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 dosed group is the probability level for the Fisher exact test for the comparison of that dosed 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 dosed group than in a control group.

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

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

Review of the Bioassay of Pyrimethamine* 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, biostatistics, biochemistry, 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 Pyrimethamine was reviewed.

The primary reviewer noted that the mode of action of Pyrimethamine is similar to cancer chemotherapeutic agents such as methotrexate. Dr. Kensler briefly described the conditions of test and pointed out that the subchronic study was conducted in different strains of rats and mice then used in the chronic phase. He agreed with the staff's conclusion that, under the conditions of test, Pyrimethamine was not carcinogenic in rats and that the mouse bioassay was inadequate for evaluation. Based on these results, he concluded that Pyrimethamine would not appear to pose a carcinogenic risk to humans.

The secondary reviewer opined that survival was sufficient in the female mice to conclude that a carcinogenic effect was not produced by Pyrimethamine. A subgroup member commented that there was an inadequate number of animals in the treated and control groups of both species to draw any conclusion regarding the carcinogenicity of Pyrimethamine.

It was moved that the report be accepted as written. The motion was seconded and approved by all the Subgroup members except Mr. Garfinkel, who opposed it.

<u>Members Present Were:</u>

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.

☆U.S. GOVERNMENT PRINTING OFFICE: 1978 260-899/3062 1-3

DHEW Publication No. (NIH) 78-1327