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

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FOREWORD: This report presents the results of the bioassay of phenazopyridine hydrochloride conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of phenazopyridine hydrochloride 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 the administration of the test chemical. Data management and retrieval were performed by Ms. C. A. Dominick[1]. Histopathologic examinations were performed by Drs. R. B. Thompson[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 results of the analyses were reviewed by Dr. C. W. Jameson[5]. The chemical structure was supplied by NCI.

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

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

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SUMMARY

A bioassay of phenazopyridine hydrochloride 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 phenazopyridine hydrochloride at one of the following doses, either 3,700 or 7,500 ppm for rats and either 600 ppm or 1,200 ppm for mice. The rats were administered the chemical for 78 weeks, then observed for 26 or 27 additional weeks; the mice were administered the chemical for 80 weeks, then observed for 25-27 additional weeks. Matched controls consisted of 15 untreated rats and 15 untreated mice of each sex. All surviving rats were killed at 104 or 105 weeks, all surviving mice at 105-107 weeks.

Mean body weights of the dosed rats and mice of each sex were consistently lower than those of corresponding control animals, and the depressions in mean body weight were dose related. Mortality in the groups of rats and mice did not, however, show dose-related trends, and sufficient numbers of animals of both dosed and control groups were at risk for the development of late-appearing tumors.

In male rats, adenomas or adenocarcinomas of the large intestine (colon or rectum) occurred at incidences having a significant dose-related trend ($P = 0.015$). The direct comparison of the incidences in each of the dosed groups with that in the control group was not significant (controls 0/14, low-dose 4/34, high-dose 8/35). In the females, 3/33 low-dose and 5/32 high-dose animals, but no control animals, had this tumor. In addition, sarcomas were observed in the colon of one low-dose male and one high-dose female. The laboratory historical records showed no incidence of adenomas or adenocarcinomas of the large intestine in 260 females and only one adenomatous polyp in 260 males. Assuming a spontaneous incidence of 1/261 (0.038%) and a binomial distribution of such tumors, the occurrence seen in the male and female high-dose groups are both significantly ($P < 0.001$) different from the expected value. Thus, these tumors are considered to be related to administration of the test chemical.

In female mice, the combined incidence of hepatocellular adenomas and carcinomas showed a significant dose-related trend (P = 0.002), and the incidence in the high-dose group was significant (P = 0.003) when compared with that in the control group (controls 2/15, low-dose 11/34, high-dose 19/32). The incidence of hepatocellular carcinomas, considered alone, also was significant in the female mice, showing a dose-related trend (P = 0.010) and a P value of 0.039 for the comparison of the high-dose group with the control group. In the males, the combined incidence of hepatocellular adenomas and carcinomas was not significant.

It is concluded that under the conditions of this bioassay, phenazopyridine hydrochloride was carcinogenic in Fischer 344 rats, inducing adenocarcinomas of the colon in both males and females. Although phenazopyridine hydrochloride was not carcinogenic in male B6C3F1 mice, the chemical was carcinogenic in females, inducing hepatocellular adenomas and carcinomas.

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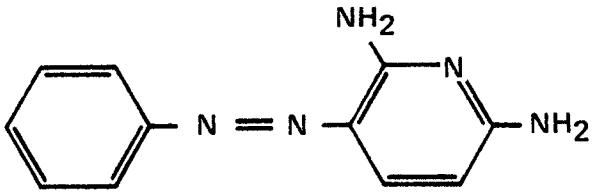
I. INTRODUCTION



PHENAZOPYRIDINE (HYDROCHLORIDE)

Phenazopyridine hydrochloride (CAS 136-40-3; NCI C01672) is the generic name for an azo dye which has been used for 40 years as an analgesic drug to reduce pain associated with urinary tract infections (American Medical Association, 1973; Billups, 1977; Harvey, 1975). It is marketed both alone and in combination with the sulfonamide urinary tract antiseptics.

This chemical was selected for testing because it is in common use and could be used on a prolonged basis in the treatment of persistent urinary tract infections.

II. MATERIALS AND METHODS

A. Chemical

Phenazopyridine hydrochloride (2,6-diamino-3-(phenylazo)-pyridine monohydrochloride, hereinafter referred to as phenazopyridine) was obtained for the chronic studies in a single batch (Lot No. 26A-2023-DR4) from the Warner-Lambert Pharmaceutical Company, Morris Plains, New Jersey. The identity and purity of this batch was confirmed in analyses at Midwest Research Institute. The melting point was 244-245°C with decomposition, (literature: 235°C with decomposition, National Formulary, 1970). Nonaqueous titration of the amine function indicated 96.5 $\pm$ 1.4% purity. High-pressure liquid chromatography showed two impurities which totaled ~1%; the impurities were not identified. Elemental analyses (C, H, Cl, N) for $C_{11}H_{12}ClN_5$ were slightly low for carbon, chlorine, and nitrogen. The infrared and nuclear magnetic resonance spectra were consistent with the structure. The chemical used for the chronic study was stored at 5°C.

B. Dietary Preparation

Test diets were prepared every 2 weeks by mixing a known amount of the sifted phenazopyridine 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 not less than 10 minutes, to assure homogeneity of the mixture.

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

C. Animals

For the subchronic studies, male and female Sprague-Dawley rats and male Swiss mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. All animals were quarantined for approximately 1 week prior to the start of the studies.

For the chronic studies, male and female Fischer 344 rats were obtained from Laboratory Supply Company, Indianapolis, Indiana, and male and female B6C3F1 mice from Charles River Breeding Laboratories. These animals were 30 days of age when received. On arrival at the laboratory, all animals were quarantined (rats for 12 days, mice for 19 days). Animals with no visible signs of disease were then assigned to control or dosed 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%. There were 15 changes of room air per hour. Air was 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 consumed 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.). 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 phenazopyridine were maintained in the same rooms as

animals of the same species being administered the following chemicals:

RATS

Feed Studies

(CAS 968-81-0) 4-acetyl-N-((cyclohexylamino)carbonyl)benzene-sulfonamide (acetohexamide)
(CAS 118-92-3) anthranilic acid
(CAS 64-77-7) 1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide)
(CAS 94-20-2) 4-chloro-N-((propylamino)carbonyl)benzene-sulfonamide (chlorpropamide)
(CAS 58-14-0) 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (pyrimethamine)
(CAS 73-22-3) L-tryptophan
(CAS 53-96-3) N-9H-fluoren-2-ylacetamide
(CAS 1156-19-0) N-(p-toluenesulfonyl)-N′-hexamethyleniminourea (tolazamide)
(CAS 114-86-3) 1-phenethylbiguanide hydrochloride (phenformin)
(CAS 98-96-4) pyrazinecarboxamide (pyrazinamide)
(CAS 80-08-0) 4,4′-sulfonyldianiline (dapsone)
(CAS 139-65-1) 4,4′-thiodianiline
(CAS 536-33-4) ethionamide

MICE

Feed Studies

(CAS 968-81-0) 4-acetyl-N-((cyclohexylamino)carbonyl)benzene-sulfonamide (acetohexamide)
(CAS 118-92-3) anthranilic acid
(CAS 64-77-7) 1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide)
(CAS 94-20-2) 4-chloro-N-((propylamino)carbonyl)benzene-sulfonamide (chlorpropamide)
(CAS 58-14-0) 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (pyrimethamine)
(CAS 73-22-3) L-tryptophan
(CAS 53-96-3) N-9H-fluoren-2-ylacetamide
(CAS 1156-19-0) N-(p-toluenesulfonyl)-N′-hexamethyleniminourea (tolazamide)
(CAS 114-86-3) 1-phenethylbiguanide hydrochloride (phenformin)
(CAS 98-96-4) pyrazinecarboxamide (pyrazinamide)
(CAS 80-08-0) 4,4′-sulfonyldianiline (dapsone)

(CAS 139-65-1) 4,4'-thiodianiline
(CAS 536-33-4) ethionamide

Gavage Studies

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

Intraperitoneal Injection Studies

(NSC 141549) 4'-(9-acridinylamino)methansulfon-m-aniside monohydrochloride (MAAM)
(CAS 7008-42-6) acronycine
(CAS 320-67-2) 5-azacytidine
(CAS 789-61-7) beta-2'-deoxy-6-thioguanosine monohydrate (beta-TGdR)
(CAS 55-98-1) 1,4-butanediol dimethanesulfonate (busulfan)
(CAS 316-42-7) emetine dihydrochloride tetrahydrate
(CAS 3458-22-8) 3,3'-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD]
(CAS 21416-87-5) (+)-4,4'-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159)
(CAS 3778-73-2) N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide)
(CAS 63-92-3) N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride (phenoxybenzamine hydrochloride)
CAS 366-70-1) N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride (procarbazine)
(CAS 52-24-4) tris(1-aziridinyl)phosphine sulfide (thio-TEPA)

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses of phenazopyridine, on the basis of which two concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the chronic studies. In these subchronic studies, phenazopyridine was added to the animal feed at concentrations of 300, 800, 1,500, 3,000, or 6,000 ppm for the male Sprague-Dawley rats; concentrations of

7,500 or 15,000 ppm for the female Sprague-Dawley rats; and concentrations of 500, 1,200, 2,500, 5,000, or 10,000 ppm for the male Swiss mice. Dosed animals received the diets containing the test chemical 7 days per week for 45 days and were then observed for an additional 45 days. Five animals of each species were tested at each dose, and 10 animals of each species were maintained as untreated controls.

In rats, the only deaths occurred during weeks 4-6, when 3/5 females administered 15,000 ppm died. After 45 days of administration of the chemical, mean body weight gain of male rats was depressed 11% at 300 ppm, 18% at 800 ppm, 33% at 1,500 ppm, 41% at 3,000 ppm, and 57% at 6,000 ppm. In the female rats, mean body weight gain was depressed 54% at 7,500 ppm, and the mean body weight of females administered 15,000 ppm at day 45 fell below the initial value. All groups showed signs of recovery during the observation period and had final weight gains comparable to those of the controls. No gross abnormalities were noted at necropsy. The low and high doses for the chronic studies using rats were set at 3,700 and 7,500 ppm.

In mice, deaths occurred in 0/5 animals at doses of 500 ppm, 1/5 at 1,200 ppm, 2/5 at 2,500 ppm, 1/5 at 5,000 ppm, and 5/5 at 10,000 ppm. Most deaths occurred after week 4. After 45 days of administration of the chemical, mean body weight gains in animals

administered 500 and 1,200 ppm were comparable with those of the controls. Weight gain was depressed 10% at 2,500 ppm and 70% at 5,000 ppm. No gross abnormalities were seen at necropsy. The low and high doses for the chronic studies using mice were set at 600 and 1,200 ppm.

F. Chronic Studies

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

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity. Rats and mice were individually weighed every 2 weeks for 21-23 months, then once per month thereafter. Palpation for masses was carried out at each weighing. Moribund animals and those animals that survived to the end of the bioassay were killed using CO_2 anesthesia and necropsied.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, muscle, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice),

Table 1. Phenazopyridine Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals[a]	Phenazopyridine in Diet[b] (ppm)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Matched-Control	15	0		105
Low-Dose	35	3,700	78	27
High-Dose	35	7,500	78	26
Female				
Matched-Control	15	0		105
Low-Dose	35	3,700	78	27
High-Dose	35	7,500	78	27

[a]Rats were 42 days of age when placed on study.

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

Table 2. Phenazopyridine Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals[a]	Phenazopyridine in Diet[b] (ppm)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male				
Matched-Control	15	0		106
Low-Dose	35	600	80	25
High-Dose	35	1,200	80	25
Female				
Matched-Control	15	0		106
Low-Dose	35	600	80	27
High-Dose	35	1,200	80	26

[a]Mice were 49 days of age when placed on study.

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

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.

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

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descrip-

tive 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)

Mean body weights of the dosed rats were consistently lower than those of corresponding control rats, and the depressions in mean body weight were dose related (figure 1). Fluctuation 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. Clinical signs, such as noisy breathing and presence of palpable masses, were noted in both dosed and control animals.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats fed phenazopyridine in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2. The result of the Tarone test for positive dose-related trend in mortality is not significant in either sex.

In the males, over 70% of the animals in each of the groups (28/35 [80%] in the high-dose group, 25/35 [71%] in the low-dose group, and 12/15 [80%] in the control group) were still alive at week 104. In the females, 28/35 (80%) of the high-dose group, 30/34 (88%) of the low-dose group, and 8/15 (53%) of the controls

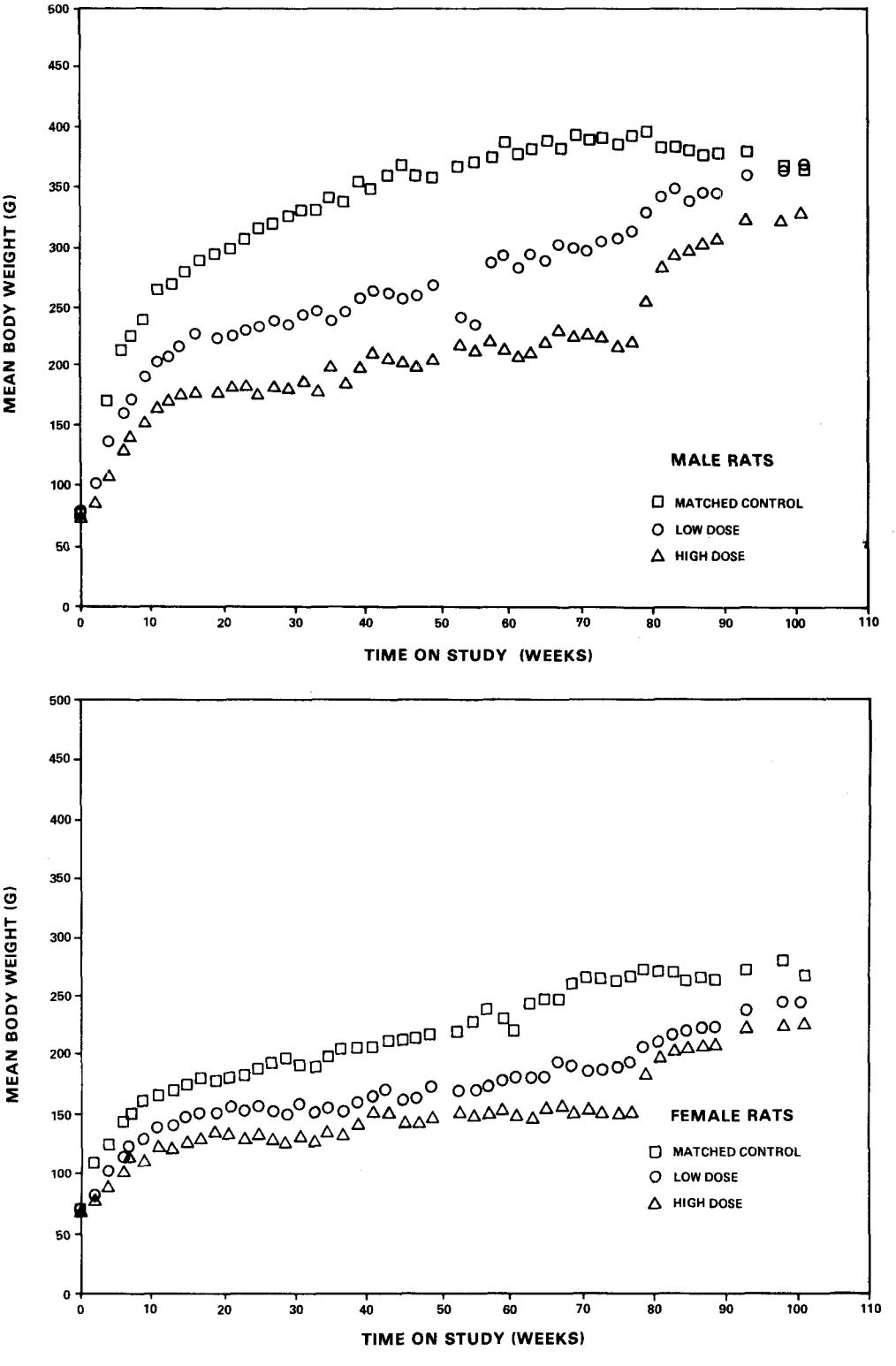


Figure 1. Growth Curves for Rats Fed Phenazopyridine in the Diet

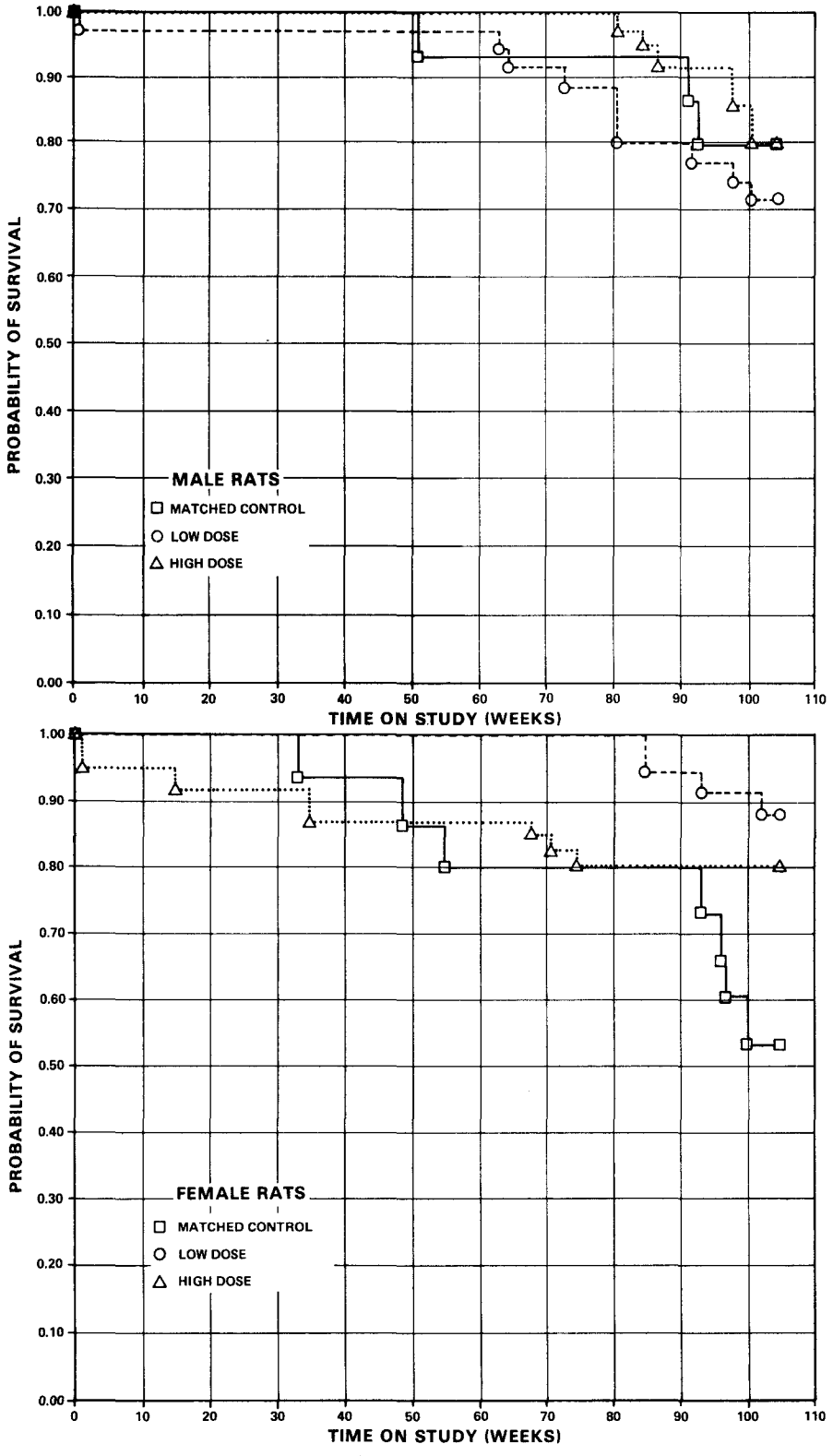


Figure 2. Survival Curves for Rats Fed Phenazopyridine in the Diet

were alive at week 104. Sufficient numbers of animals 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.

The incidence and distribution of tumors in the large intestine (colon and rectum) were as follows:

	MALES			FEMALES		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Number of Animals with Tissues Examined Microscopically	(14)	(34)	(33)	(14)	(33)	(32)
Adenoma, NOS*	0	0	1	0	0	0
Adenocarcinoma, NOS	0	4	9	0	3	5
Sarcoma, NOS	0	1	0	0	0	1

*Not otherwise specified

The most common tumors observed in the colon were adenocarcinomas. These tumors were polypoid masses that projected into the lumen of the colon and were supported by a stalk composed of fibrous connnective tissue. In some cases the muscularis mucosa was pulled upward into the stalk. The epithelial cells of these tumors usually formed glands and crypts. Neoplastic epithelial

cells were usually tall columnar cells with elongated or oval basilar nuclei and acidophilic cytoplasm. In many instances, the epithelial cells stained darkly basophilic. The deep portions of the crypts had extensive hyperplasia and piling up of cells. Dark-staining carcinomatous cells were usually present on the surface of the tumors, and mitotic figures were commonly seen. Many of the neoplastic cells appeared to have lost their mucous-secreting ability and were distinguishable from normal epithelial cells adjacent to the polypoid structures. Some ducts and glands in the tumors were cystic and filled with necrotic cellular debris.

The stalks of the polypoid colonic adenocarcinomas were composed of loose or dense connective tissue and contained numerous vessels in addition to an inflammatory cell infiltrate composed of lymphocytes, plasma cells, histiocytes, and neutrophils. There was carcinomatous infiltration into the stalk of some tumors; however, the incidence of this type of change was difficult to evaluate because the stalk was not always sectioned with the tumors.

Metastatic lesions were not observed in other organs, which may indicate that the tumors were low-grade, polypoid adenocarcinomas and were less agressive than highly invasive flat carcinomas.

A colonic adenoma was observed in one high-dose male rat. The adenoma was an elevated spherical mass on the mucosal surface, consisting of a fibrous connective tissue stroma and a moderate number of intestinal crypts. The cells covering the adenoma and lining the crypts were well-differentiated colonic epithelial cells with mucous-secreting ability. The adenoma lacked areas of epithelial proliferation, cellular basophilia, and numerous mitotic figures seen in the polypoid colonic adenocarcinomas.

A poorly differentiated sarcoma was detected in the colon of 1/34 (3%) of the low-dose males and 1/32 (3%) of the high-dose females. An additional polypoid adenocarcinoma occurred in the rectum of 1/35 (3%) of the high-dose male rats. No lesions of the colon or rectum occurred in the control groups. Furthermore, tumors of the intestine are rare in this strain of rat.

There was a decreased incidence of interstitial-cell adenomas of the testis in the high-dose male rats. The incidence of interstitial-cell tumors were: control males 14/14 (100%), low-dose males 22/34 (65%), high-dose males 7/33 (21%). The decreased incidence of these tumors in the high-dose male rats was not associated with increased mortality or decreased life spans.

A variety of other neoplasms occurred in both the matched-control and dosed groups. Some types of these neoplasms occurred only in

rats of dosed groups, or with greater frequency in dosed groups when compared with controls. These lesions, however, are not uncommon in this strain of rat independent of any treatment.

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

Based on the histopathologic examination, it was concluded that phenazopyridine fed in the diet increased the incidence of polypoid adenocarcinomas in the colon and rectum of Fischer 344 rats under the conditions of this bioassay. A reduction of testicular adenomas in high-dose male rats appeared to be compound and dose related. However, verification of this relationship would require further evaluation.

D. Statistical Analyses of Results (Rats)

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

Adenomas or adenocarcinomas of the large intestine (colon or rectum) were found in both male and female dosed groups of rats.

The result of the Cochran-Armitage test for positive dose-related trend in the incidence of the tumors is significant (P = 0.015) in the male rats, but not in the females. The result of the Fisher exact comparison of the incidence of the tumor in the high-dose group with that in the control group in the males shows a P value of 0.034, which is above the 0.025 level required for significance when the Bonferroni inequality criterion is used for multiple comparison. The historical records of the bioassays conducted for the program at this laboratory show no incidence of adenoma or adenocarcinoma of the large intestine in 260 females and only one adenomatous polyp in 260 males. Assuming a spontaneous incidence of 1/261 (0.38%) and a binomial distribution of such tumors, the occurrences of 8/35 (23%) and 5/32 (16%) seen in the male and female high-dose groups, respectively, are both significantly (P < 0.001) different from the expected value (Fears et al., 1977).

Significant results in the negative direction are observed in the incidence of interstitial-cell tumors of the testis in the male rats and in the incidence of fibroadenomas of the mammary gland in the females, due to higher incidences in the control groups than in the dosed groups.

In summary, the tumors of the large intestine appear to be related to the administration of the chemical.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

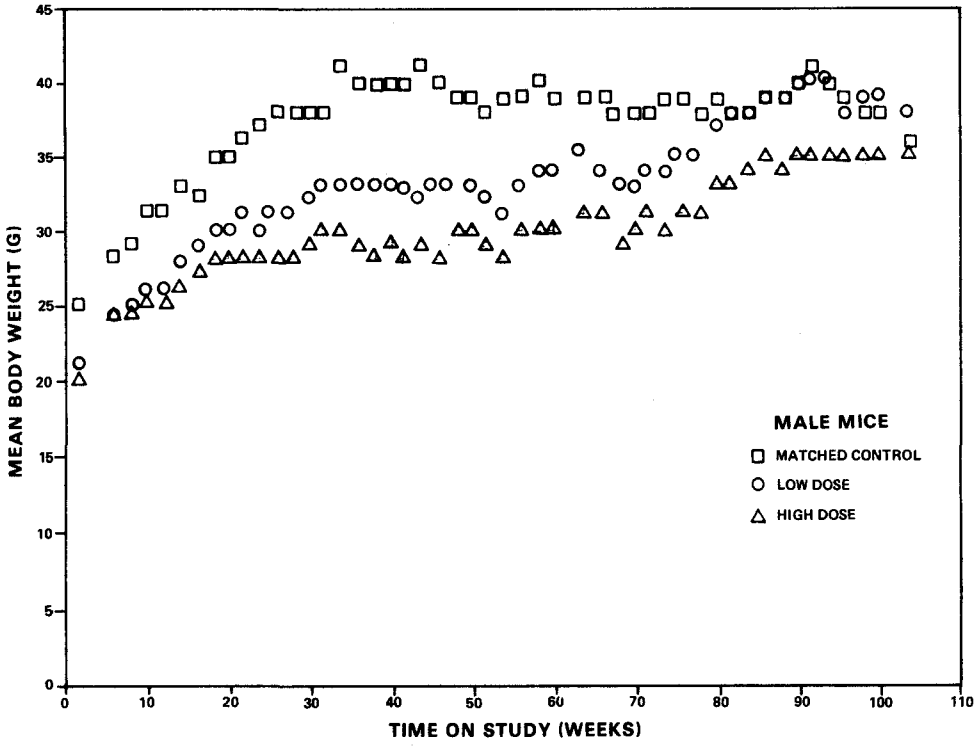
Mean body weights of the dosed mice were consistently lower than those of corresponding control mice, and the depressions in mean body weight were dosed related (figure 3). Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. Other clinical signs were common to both control and dosed animals.

To control respiratory disease, oxytetracycline was added to the drinking water at 0.6 mg/ml during weeks 81-82 and at 0.3 mg/ml during week 82; also, propylene glycol was vaporized in the mouse room for approximately 2 months, beginning at week 81.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice fed phenazopyridine in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 4. The result of the Tarone test for positive dose-related trend in mortality is not significant in either sex.

At least 60% of the animals in each group lived to the end of the



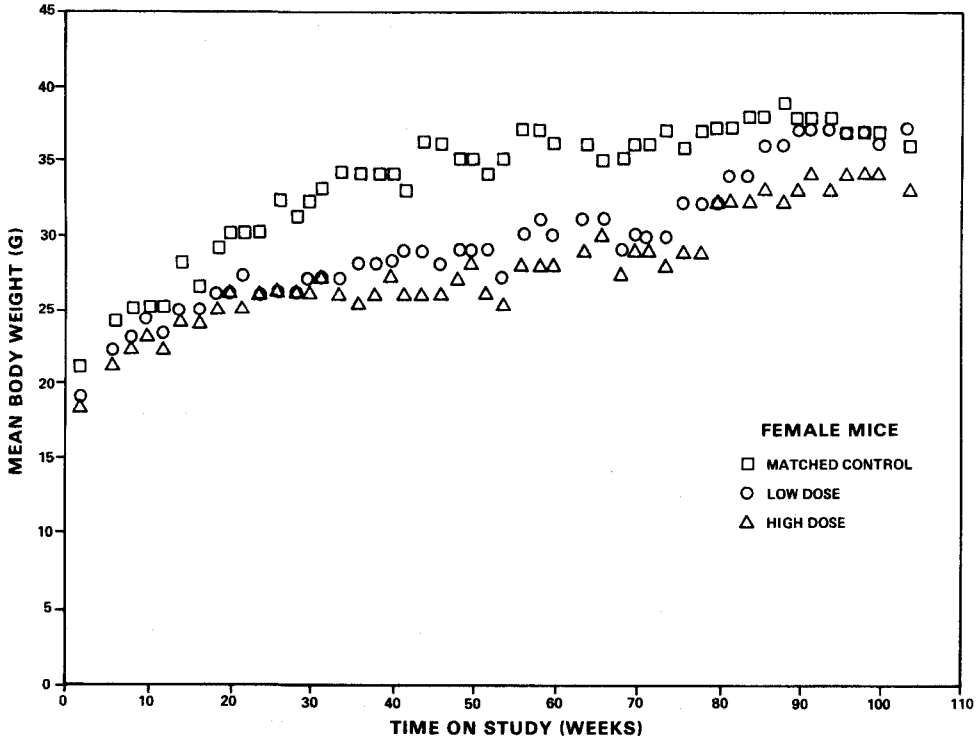


Figure 3. Growth Curves for Mice Fed Phenazopyridine in the Diet

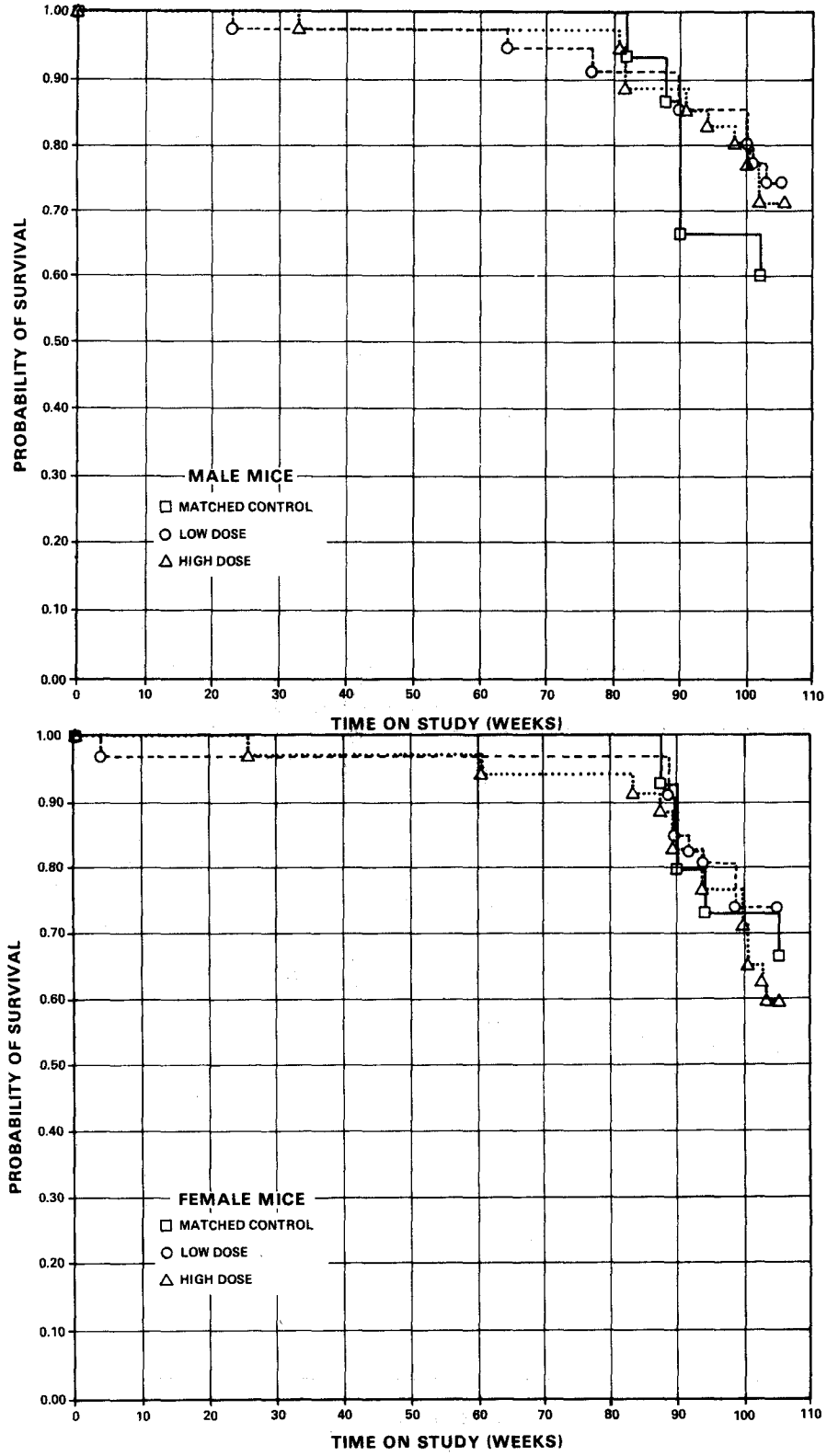


Figure 4. Survival Curves for Mice Fed Phenazopyridine in the Diet

bioassay (25/35 [71%] in the high-dose males, 26/35 [74%] in the low-dose males, and 9/15 [60%] in the male controls; 21/35 [60%] in the high-dose females, 26/35 [74%] in the low-dose females, and 10/15 [67%] in the female controls). Sufficient numbers of mice were at risk for the development of late-appearing tumors.

C. Pathology (Mice)

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

With the exception of hepatocellular neoplasms in male and female mice, the neoplasms listed in Appendix B appeared with approximately equal frequency in dosed and control mice or appeared in insignificant numbers.

There was an increased incidence of hepatocellular adenomas and carcinomas in dosed mice when compared with corresponding controls, as follows:

	MALES			FEMALES		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Number of Animals with Tissues Examined Microscopically	(15)	(35)	(33)	(15)	(34)	(32)
Liver						
Hepatocellular Carcinoma	2	5	9	2	6	14
Hepatocellular Adenoma or Carcinoma	5	15	15	2	11	19

Hepatocellular adenomas were variable in size, ranging from small spherical lesions to large irregular lesions equivalent in size to several liver lobules. These lesions were usually well demarcated from the surrounding parenchyma. Compression of the surrounding parenchyma was occasionally seen but was not severe. Cells were arranged in solid sheets or irregular plates that were one or more cells thick. Sinusoids were dilated or compressed. The adenomas frequently contained acidophilic, basophilic, or clear cell foci. Cytologic alterations included nuclear atypia which was characterized by enlargement, hyperchromasia, and sometimes enlarged nucleoli. Marked cytomegaly was present in some adenomas. Mitotic figures were occasionally seen but were not abundant.

In contrast to adenomas, hepatocellular carcinomas were extensive, irregularly shaped lesions involving numerous lobules

or entire lobes of the liver. Extensive peripheral compression was frequently seen. The carcinomas were characterized by the formation of trabeculae, sheets, or plates of irregularly arranged cells in linar, papillar, or pseudoacinar patterns. Plates and trabeculae of tumor cells were often 2 to 4 cells thick. Cytologic alterations included nuclear atypia, cytomegaly, and variable numbers of mitotic figures. Hepatic sinusoids were extensively dilated in some tumors and compressed in others. Metastasis of a hepatocellular carcinoma to an other organ was observed in the female mice. Pulmonary metastases were observed in 1/15 (7%) control females and 2/32 (6%) high-dose females. Renal metastasis was detected in 1/5 (7%) control females. Metastatic hepatocellular carcinomas were not observed in the male mice.

In addition to the neoplastic lesions, a large number of degenerative, proliferative, and inflammatory changes were encountered in animals of the dosed and control groups (Appendix D). For the most part, these nonneoplastic lesions are commonly seen in aged mice and were not associated with increased mortality.

Based on the histopathologic examination, it was concluded that phenazopyridine fed in the diet increased the incidence of hepato-

cellular adenomas and carcinomas in B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

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

In female mice, the result of the Cochran-Armitage test for positive dose-related trend in the incidence of hepatocellular carcinomas is significant (P = 0.010); however, the result of the Fisher exact comparison of the incidence of the tumors in the high-dose group with that in the control group shows a P value of 0.039, which is above the 0.025 level for significance when the Bonferroni inequality criterion is used for multiple comparison. When hepatocellular adenomas and carcinomas are combined for analyses in female mice, the significance of the Cochran-Armitage test increases (P = 0.002), and the Fisher exact test shows that the incidence in the high-dose group is significantly higher (P = 0.003) than that in the controls. The incidence seen in the control group of female mice, 2/15 (13%), is the highest percentage of any control group reported from this laboratory and may reflect the small sample size. Despite this high incidence

in the control group, a significant difference is indicated by the Fisher exact test when the incidence in the high-dose group, 19/32 (59%), is compared with this control group. The statistical conclusion is that the combined incidence of hepatocellular adenomas and carcinomas in female mice is associated with administration of the phenazopyridine. Results of the statistical tests on the incidence of the combination of hepatocellular carcinomas and adenomas in male mice are not significant.

A significant linear trend in the negative direction is observed in the incidence of lymphoma of the hematopoietic system in female mice, due to a higher incidence in the control group than in the dosed group.

V. DISCUSSION

Both rats and mice administered phenazopyridine had a dose-related depression in mean body weights. Mortality in the groups of rats and mice did not, however, show dose-related trends, and sufficient numbers of animals in both dosed and control groups were at risk for the development of late-appearing tumors.

In male rats, adenomas or adenocarcinomas of the large intestine (colon or rectum) occurred at incidences having a significant dose-related trend ($P = 0.015$). Direct comparisons of the incidence in each dosed group with that in the control group were not significant (controls 0/14, low-dose 4/34, high-dose 8/35). In the females, 3/33 low-dose and 5/32 high-dose animals, but no control animals, had this tumor. In addition, sarcomas were observed in the colon of one low-dose male and one high-dose female. The laboratory historical records showed no incidence of adenomas or adenocarcinomas of the large intestine in 260 females and only one adenomatous polyp in 260 males. Assuming a spontaneous incidence of 1/261 (0.038%) and a binomial distribution of such tumors, the occurrence seen in the male and female high-dose groups are both significantly ($P < 0.001$) different from the expected value. Thus, these tumors are considered to be related to administration of the test chemical.

In female mice, the combined incidence of hepatocellular adenomas and carcinomas showed a significant dose-related trend (P = 0.002), and the incidence in the high-dose group was significant (P = 0.003) when compared with that in the control group (controls 2/15, low-dose 11/34, high-dose 19/32). The incidence of hepatocellular carcinomas, considered alone, also was significant in the female mice, showing a dose-related trend (P = 0.010) and a P value of 0.039 for the comparison of the high-dose group with the control group. In the males, the combined incidence of hepatocellular adenomas and carcinomas was not significant.

When previously tested by intraperitoneal injection of total doses of 0.31, 0.78, and 1.55 mg/kg body weight over a 24-week period in an "A" mouse pulmonary tumor system (Stoner et al., 1973), phenazopyridine induced no significant increases in incidences of pulmonary tumors. When pellets of phenazopyridine in cholesterol were implanted into the urinary bladders of 20 "stock" mice in a 52-week study (Allen et al., 1957), two dosed mice out of 14 that survived longer than 30 weeks developed carcinoma of the bladder and two others developed papilloma or adenoma of the bladder; of 28 controls implanted with cholesterol alone, 24 survived 30 weeks, and one of these developed carcinoma of the bladder.

It is concluded that under the conditions of this bioassay,

phenazopyridine hydrochloride was carcinogenic in Fischer 344 rats, inducing adenocarcinomas of the colon in both males and females. Although administration of phenazopyridine hydrochloride was not carcinogenic in male B6C3F1 mice, the chemical was carcinogenic in females, inducing hepatocellular adenomas and carcinomas.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS FED PHENAZOPYRIDINE HYDROCHLORIDE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED PHENAZOPYRIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14	34	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	34	35
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(14)	(34)	(35)
SQUAMOUS CELL PAPILLOMA	1 (7%)		
FIBROMA	1 (7%)	1 (3%)	
RESPIRATORY SYSTEM			
#LUNG	(12)	(33)	(35)
SQUAMOUS CELL CARCINOMA, METASTA			1 (3%)
ALVEOLAR/BRONCHIOLAR ADENOMA		3 (9%)	
HEMATOPOIETIC SYSTEM			
#BRAIN	(13)	(33)	(35)
MALIGNANT RETICULOSIS		2 (6%)	
*MULTIPLE ORGANS	(14)	(34)	(35)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (7%)		1 (3%)
#SPLEEN	(14)	(34)	(35)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(14)	(34)	(34)
HEPATOCELLULAR ADENOMA		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
HEPATOCELLULAR CARCINOMA			1 (3%)
#ESOPHAGUS	(14)	(33)	(35)
PAPILLOMA, NOS			1 (3%)
#COLON	(14)	(34)	(33)
ADENOMA, NOS			1 (3%)
ADENOCARCINOMA, NOS		4 (12%)	8 (24%)
SARCOMA, NOS		1 (3%)	
*RECTUM	(14)	(34)	(35)
ADENOCARCINOMA, NOS			1 (3%)
URINARY SYSTEM			
#KIDNEY	(14)	(34)	(35)
TUBULAR-CELL ADENOCARCINOMA		1 (3%)	
#URINARY BLADDER	(11)	(30)	(32)
UNDIFFERENTIATED CARCINOMA	1 (9%)		
ENDOCRINE SYSTEM			
#PITUITARY	(5)	(25)	(23)
CHROMOPHOBE ADENOMA	1 (20%)		
#ADRENAL	(13)	(34)	(34)
CORTICAL ADENOMA			1 (3%)
PHEOCHROMOCYTOMA	2 (15%)	1 (3%)	1 (3%)
#THYROID	(14)	(32)	(32)
FOLLICULAR-CELL ADENOMA		1 (3%)	
C-CELL ADENOMA	2 (14%)		1 (3%)
#PANCREATIC ISLETS	(14)	(34)	(35)
ISLET-CELL ADENOMA	1 (7%)	1 (3%)	1 (3%)
REPRODUCTIVE SYSTEM			
#TESTIS	(14)	(34)	(33)
INTERSTITIAL-CELL TUMOR	14 (100%)	22 (65%)	7 (21%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(13)	(33)	(35)
GLIOMA, NOS		1 (3%)	
SPECIAL SENSE ORGANS			
*EYE	(14)	(34)	(35)
SARCOMA, NOS		1 (3%)	1 (3%)
*EAR CANAL	(14)	(34)	(35)
SQUAMOUS CELL CARCINOMA		1 (3%)	1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(14)	(34)	(35)
MESOTHELIOMA BENIGN		1 (3%)	
*PELVIS	(14)	(34)	(35)
LIPOMA		1 (3%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	2	3	2
MORIBUND SACRIFICE	1	7	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	12	25	28
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	29	17
TOTAL PRIMARY TUMORS	24	44	26
TOTAL ANIMALS WITH BENIGN TUMORS	14	24	10
TOTAL BENIGN TUMORS	22	32	13
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	12	11
TOTAL MALIGNANT TUMORS	2	12	13
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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED PHENAZOPYRIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	33	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	33	32
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(14)	(33)	(32)
ADENOCARCINOMA, NOS, METASTATIC		1 (3%)	
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (3%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(15)	(33)	(32)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (7%)		
UNDIFFERENTIATED LEUKEMIA		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#COLON	(14)	(33)	(32)
ADENOCARCINOMA, NOS		3 (9%)	5 (16%)
SARCOMA, NOS			1 (3%)
URINARY SYSTEM			
#KIDNEY	(15)	(33)	(32)
ADENOCARCINOMA, NOS, METASTATIC		1 (3%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#URINARY BLADDER	(13)	(28)	(27)
PAPILLOMA, NOS	1 (8%)		
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(30)	(25)
CHROMOPHOBE ADENOMA	2 (22%)	1 (3%)	1 (4%)
#ADRENAL	(14)	(33)	(31)
PHEOCHROMOCYTOMA			1 (3%)
#THYROID	(11)	(32)	(31)
FOLLICULAR-CELL ADENOMA		1 (3%)	1 (3%)
C-CELL ADENOMA		1 (3%)	1 (3%)
#PANCREATIC ISLETS	(14)	(33)	(32)
ISLET-CELL ADENOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(33)	(32)
FIBROADENOMA	2 (13%)		
#UTERUS	(15)	(33)	(32)
ADENOCARCINOMA, NOS		1 (3%)	
ENDOMETRIAL STROMAL POLYP	1 (7%)	1 (3%)	1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EAR CANAL	(15)	(33)	(32)
SQUAMOUS CELL CARCINOMA			1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*PLEURA	(15)	(33)	(32)
MESOTHELIOMA, MALIGNANT			1 (3%)
ALL OTHER SYSTEMS			
DIAPHRAGM			
ADENOCARCINOMA, NOS, METASTATIC		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	3	2	5
MORIBUND SACRIFICE	4	2	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	8	30	28
ANIMAL MISSING			
ANIMAL DELETED (WRONG SEX)		1	
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	5	10	13
TOTAL PRIMARY TUMORS	7	10	14
TOTAL ANIMALS WITH BENIGN TUMORS	4	5	5
TOTAL BENIGN TUMORS	6	5	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	5	8
TOTAL MALIGNANT TUMORS	1	5	8
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 PHENAZOPYRIDINE HYDROCHLORIDE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED PHENAZOPYRIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	35	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	35	34
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(15)	(35)	(34)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (7%)	3 (9%)	4 (12%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (3%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(15)	(35)	(34)
UNDIFFERENTIATED LEUKEMIA			1 (3%)
#MESENTERIC L. NODE	(13)	(31)	(31)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (3%)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (3%)
#PEYERS PATCH	(15)	(35)	(34)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (7%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(15)	(35)	(33)
HEPATOCELLULAR ADENOMA	3 (20%)	10 (29%)	6 (18%)
HEPATOCELLULAR CARCINOMA	2 (13%)	5 (14%)	9 (27%)
URINARY 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
ENDOCRINE SYSTEM			
#THYROID	(14)	(30)	(31)
FOLLICULAR-CELL ADENOMA	1 (7%)		
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(15)	(35)	(34)
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@	2	7	4
MORIBUND SACRIFICE	4	2	6
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	9	26	25
ANIMAL MISSING			

@ 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*	7	17	20
TOTAL PRIMARY TUMORS	8	19	23
TOTAL ANIMALS WITH BENIGN TUMORS	5	13	10
TOTAL BENIGN TUMORS	5	13	11
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	5	12
TOTAL MALIGNANT TUMORS	3	6	12
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 ADMINISTERED PHENAZOPYRIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	34	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	34	32
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(15)	(34)	(32)
MALIGNANT MELANOMA	1 (7%)		
SARCOMA, NOS			2 (6%)
HEMANGIOMA		1 (3%)	
RESPIRATORY SYSTEM			
#LUNG	(15)	(34)	(32)
HEPATOCELLULAR CARCINOMA, METAST	1 (7%)		2 (6%)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (7%)	1 (3%)	2 (6%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(15)	(34)	(32)
MALIGNANT LYMPHOMA, NOS		1 (3%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (7%)		1 (3%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	1 (3%)
MALIGNANT LYMPHOMA, MIXED TYPE	3 (20%)	2 (6%)	
#PEYERS PATCH	(15)	(34)	(32)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (3%)	
#UTERUS	(15)	(34)	(32)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(15)	(34)	(32)
HEPATOCELLULAR ADENOMA	1 (7%)	5 (15%)	7 (22%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA	2 (13%)	6 (18%)	14 (44%)
URINARY SYSTEM			
#KIDNEY	(15)	(34)	(32)
HEPATOCELLULAR CARCINOMA, METAST	1 (7%)		
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(30)	(23)
CHROMOPHOBE ADENOMA	1 (10%)	2 (7%)	
#THYROID	(14)	(30)	(29)
FOLLICULAR-CELL ADENOMA			1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(34)	(32)
FIBROADENOMA			1 (3%)
#UTERUS	(15)	(34)	(32)
ENDOMETRIAL STROMAL POLYP		1 (3%)	
#OVARY	(15)	(34)	(32)
PAPILLARY CYSTADENOMA, NOS		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(15)	(34)	(32)
ADENOMA, NOS		2 (6%)	3 (9%)
*EAR CANAL	(15)	(34)	(32)
SQUAMOUS CELL CARCINOMA			1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(15)	(34)	(32)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (3%)	
*PLEURA	(15)	(34)	(32)
SARCOMA, NOS			1 (3%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	2	5	7
MORIBUND SACRIFICE	3	4	7
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	10	26	21
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	20	27
TOTAL PRIMARY TUMORS	10	26	34
TOTAL ANIMALS WITH BENIGN TUMORS	2	11	14
TOTAL BENIGN TUMORS	3	13	14
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	12	18
TOTAL MALIGNANT TUMORS	7	13	20
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		2
TOTAL SECONDARY TUMORS	2		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 C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN RATS FED PHENAZOPYRIDINE HYDROCHLORIDE IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED PHENAZOPYRIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14	34	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	34	35
INTEGUMENTARY SYSTEM			
*SKIN	(14)	(34)	(35)
EPIDERMAL INCLUSION CYST	1 (7%)		
*SUBCUT TISSUE	(14)	(34)	(35)
HYPERPLASIA, BASAL CELL	1 (7%)		
RESPIRATORY SYSTEM			
#TRACHEA	(14)	(33)	(35)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (7%)	5 (15%)	10 (29%)
INFLAMMATION, SUPPURATIVE	12 (86%)	20 (61%)	17 (49%)
INFLAMMATION, ACUTE/CHRONIC			2 (6%)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
#LUNG/BRONCHUS	(12)	(33)	(35)
BRONCHIECTASIS	2 (17%)	1 (3%)	
INFLAMMATION, SUPPURATIVE	1 (8%)		
POLYP, INFLAMMATORY		1 (3%)	
HYPERPLASIA, LYMPHOID	1 (8%)	1 (3%)	
#LUNG	(12)	(33)	(35)
HEMORRHAGE		1 (3%)	
INFLAMMATION, INTERSTITIAL	5 (42%)	1 (3%)	9 (26%)
BRONCHOPNEUMONIA SUPPURATIVE		3 (9%)	
ABSCESS, NOS		1 (3%)	
PNEUMONIA INTERSTITIAL CHRONIC	1 (8%)		
BRONCHOPNEUMONIA CHRONIC SUPPURA			1 (3%)
INFLAMMATION, FOCAL GRANULOMATOU			1 (3%)
FIBROSIS			1 (3%)
#LUNG/ALVEOLI	(12)	(33)	(35)
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
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(14)	(33)	(35)
ATROPHY, NOS			3 (9%)
#SPLEEN	(14)	(34)	(35)
FIBROSIS			1 (3%)
ATROPHY, NOS			1 (3%)
HEMATOPOIESIS		19 (56%)	5 (14%)
#LYMPH NODE	(14)	(10)	(14)
CYST, NOS			1 (7%)
#MESENTERIC L. NODE	(14)	(10)	(14)
LYMPHANGIECTASIS			2 (14%)
#THYMUS	(13)	(26)	(26)
ABSCESS, NOS		1 (4%)	
ATROPHY, NOS			4 (15%)
CIRCULATORY SYSTEM			
#HEART	(14)	(33)	(35)
INFLAMMATION, INTERSTITIAL			1 (3%)
#MYOCARDIUM	(14)	(33)	(35)
INFLAMMATION, INTERSTITIAL	6 (43%)	30 (91%)	32 (91%)
FIBROSIS	2 (14%)		3 (9%)
FIBROSIS, DIFFUSE	1 (7%)		
ATROPHY, NOS	2 (14%)		
#ENDOCARDIUM	(14)	(33)	(35)
INFLAMMATION, FOCAL			1 (3%)
*AORTA	(14)	(34)	(35)
MEDIAL CALCIFICATION	1 (7%)		
*PULMONARY ARTERY	(14)	(34)	(35)
MEDIAL CALCIFICATION		2 (6%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(14)	(30)	(34)
INFLAMMATION, CHRONIC DIFFUSE			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
#LIVER	(14)	(34)	(34)
NECROSIS, FOCAL		2 (6%)	1 (3%)
METAMORPHOSIS FATTY		1 (3%)	
CYTOPLASMIC VACUOLIZATION		2 (6%)	1 (3%)
CYTOLOGIC DEGENERATION			1 (3%)
HYPERPLASIA, NODULAR		1 (3%)	1 (3%)
ANGIECTASIS		1 (3%)	1 (3%)
#LIVER/CENTRILOBULAR	(14)	(34)	(34)
CYTOPLASMIC VACUOLIZATION		2 (6%)	1 (3%)
*BILE DUCT	(14)	(34)	(35)
HYPERPLASIA, NOS	1 (7%)	3 (9%)	1 (3%)
#PANCREAS	(14)	(34)	(35)
FIBROSIS, DIFFUSE	1 (7%)		
PERIARTERITIS	1 (7%)		
#PANCREATIC ACINUS	(14)	(34)	(35)
ATROPHY, NOS	1 (7%)	2 (6%)	
ATROPHY, FOCAL		8 (24%)	
#ESOPHAGUS	(14)	(33)	(35)
DIVERTICULUM			1 (3%)
#STOMACH	(14)	(34)	(35)
ULCER, FOCAL			1 (3%)
INFLAMMATION, SUPPURATIVE		1 (3%)	
HYPERKERATOSIS		1 (3%)	
#ILEUM	(14)	(33)	(34)
ULCER, FOCAL			1 (3%)
#CECUM	(14)	(34)	(33)
ULCER, NOS		1 (3%)	
METAPLASIA, OSSEOUS		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(14)	(34)	(35)
INFLAMMATION, CHRONIC	13 (93%)	22 (65%)	5 (14%)
NECROSIS, MEDULLARY	1 (7%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(13)	(34)	(34)
HYPERPLASIA, FOCAL	1 (8%)		
#THYROID	(14)	(32)	(32)
HYPERPLASIA, C-CELL		2 (6%)	5 (16%)
#PARATHYROID	(12)	(22)	(24)
HYPERPLASIA, NOS		1 (5%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(14)	(34)	(35)
DILATATION/DUCTS			1 (3%)
CYST, NOS	1 (7%)	1 (3%)	
#PROSTATE	(11)	(34)	(34)
INFLAMMATION, SUPPURATIVE		6 (18%)	1 (3%)
*SEMINAL VESICLE	(14)	(34)	(35)
CYST, NOS			1 (3%)
#TESTIS	(14)	(34)	(33)
ATROPHY, NOS			5 (15%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(13)	(33)	(35)
INFLAMMATION, ACUTE/CHRONIC			1 (3%)
SPECIAL SENSE ORGANS			
*EYE	(14)	(34)	(35)
SYNECHIA, POSTERIOR			1 (3%)
*EYE/RETINA	(14)	(34)	(35)
ATROPHY, NOS			1 (3%)
*EAR	(14)	(34)	(35)
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*EAR CANAL	(14)	(34)	(35)
INFLAMMATION, SUPPURATIVE			1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(14)	(34)	(35)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
FIBROSIS			1 (3%)
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC FOCAL	1		
SPECIAL MORPHOLOGY SUMMARY			
NO NECROPSY PERFORMED		1	
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 ADMINISTERED PHENAZOPYRIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	33	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	33	32
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(15)	(33)	(32)
EDEMA, NOS			1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)		
RESPIRATORY SYSTEM			
#TRACHEA	(15)	(33)	(32)
LYMPHOCYTIC INFLAMMATORY INFILTR	3 (20%)	6 (18%)	6 (19%)
INFLAMMATION, SUPPURATIVE	7 (47%)	18 (55%)	18 (56%)
METAPLASIA, SQUAMOUS	1 (7%)		
#LUNG/BRONCHUS	(14)	(33)	(32)
BRONCHIECTASIS			1 (3%)
HYPERPLASIA, LYMPHOID		3 (9%)	
#LUNG/BRONCHIOLE	(14)	(33)	(32)
ABSCESS, NOS			1 (3%)
#LUNG	(14)	(33)	(32)
INFLAMMATION, INTERSTITIAL	5 (36%)		
BRONCHOPNEUMONIA SUPPURATIVE		1 (3%)	2 (6%)
PNEUMONIA INTERSTITIAL CHRONIC	1 (7%)		
BRONCHOPNEUMONIA CHRONIC SUPPURA			1 (3%)
FIBROSIS, FOCAL			1 (3%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(14)	(33)	(30)
ATROPHY, NOS			17 (57%)
HYPERPLASIA, RETICULUM CELL		1 (3%)	
#SPLEEN	(15)	(33)	(32)
HEMOSIDEROSIS			1 (3%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIESIS	1 (7%)	27 (82%)	21 (66%)
#MESENTERIC L. NODE	(13)	(16)	(12)
LYMPHANGIECTASIS		1 (6%)	5 (42%)
#THYMUS	(11)	(27)	(26)
HEMORRHAGE			1 (4%)
ATROPHY, NOS			1 (4%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(15)	(32)	(32)
INFLAMMATION, INTERSTITIAL	2 (13%)	30 (94%)	25 (78%)
INFLAMMATION, SUPPURATIVE			1 (3%)
FIBROSIS, DIFFUSE	1 (7%)		
DIGESTIVE SYSTEM			
#LIVER	(15)	(33)	(31)
NECROSIS, FOCAL			2 (6%)
FOCAL CELLULAR CHANGE			1 (3%)
HEMATOPOIESIS	1 (7%)		
#LIVER/CENTRILOBULAR	(15)	(33)	(31)
CONGESTION, NOS			1 (3%)
#PANCREAS	(14)	(33)	(32)
FIBROSIS, DIFFUSE	3 (21%)		
#PANCREATIC ACINUS	(14)	(33)	(32)
ATROPHY, NOS	2 (14%)		3 (9%)
ATROPHY, FOCAL		6 (18%)	
URINARY SYSTEM			
#KIDNEY	(15)	(33)	(32)
PYELONEPHRITIS, NOS	1 (7%)		
INFLAMMATION, CHRONIC	5 (33%)	3 (9%)	1 (3%)
NECROSIS, ISCHEMIC	1 (7%)		
INFARCT, HEALED		1 (3%)	
#URINARY BLADDER	(13)	(28)	(27)
HYPERPLASIA, EPITHELIAL	1 (8%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
METAPLASIA, SQUAMOUS	1 (8%)		
ENDOCRINE SYSTEM			
#ADRENAL	(14)	(33)	(31)
HYPERPLASIA, NOS			1 (3%)
#THYROID	(11)	(32)	(31)
HYPERPLASIA, C-CELL		2 (6%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(33)	(32)
DILATATION/DUCTS	1 (7%)		
#UTERUS	(15)	(33)	(32)
CYST, NOS	1 (7%)		
#UTERUS/ENDOMETRIUM	(15)	(33)	(32)
INFLAMMATION, SUPPURATIVE	2 (13%)	3 (9%)	6 (19%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)		
HYPERPLASIA, NOS	2 (13%)		
HYPERPLASIA, CYSTIC	1 (7%)	3 (9%)	
DECIDUAL ALTERATION, NOS	1 (7%)	1 (3%)	
#OVARY/OVIDUCT	(15)	(33)	(32)
INFLAMMATION, SUPPURATIVE	4 (27%)		1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)		
#OVARY	(13)	(33)	(32)
CYSTIC FOLLICLES		1 (3%)	
FOLLICULAR CYST, NOS		1 (3%)	2 (6%)
MULTIPLE CYSTS			1 (3%)
INFLAMMATION, SUPPURATIVE	1 (8%)	4 (12%)	2 (6%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(14)	(33)	(32)
INFLAMMATION, NOS			1 (3%)
SPECIAL SENSE ORGANS			
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
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			1
NO NECROPSY PERFORMED			2
AUTO/NECROPSY/HISTO PERF	1		
AUTOLYSIS/NO NECROPSY		1	1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED PHENAZOPYRIDINE HYDROCHLORIDE IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED PHENAZOPYRIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	35	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	35	34
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(15)	(35)	(34)
PNEUMONIA, LIPID	1 (7%)		
PNEUMONIA INTERSTITIAL CHRONIC		1 (3%)	1 (3%)
BRONCHOPNEUMONIA CHRONIC SUPPURA		1 (3%)	1 (3%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(15)	(35)	(34)
HEMATOPOIESIS	1 (7%)		2 (6%)
#MESENTERIC L. NODE	(13)	(31)	(31)
CONGESTION, NOS	1 (8%)	7 (23%)	
INFLAMMATION, SUPPURATIVE			1 (3%)
ATROPHY, NOS		3 (10%)	
HYPERPLASIA, LYMPHOID		1 (3%)	1 (3%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(15)	(35)	(34)
INFLAMMATION, SUPPURATIVE			1 (3%)
DIGESTIVE SYSTEM			
#LIVER	(15)	(35)	(33)
INFLAMMATION, NECROTIZING		1 (3%)	1 (3%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, COAGULATIVE		1 (3%)	1 (3%)
CYTOPLASMIC VACUOLIZATION		1 (3%)	1 (3%)
FOCAL CELLULAR CHANGE	1 (7%)		
ANGIECTASIS		2 (6%)	
HYPERPLASIA, LYMPHOID			1 (3%)
HEMATOPOIESIS	1 (7%)		1 (3%)
#LIVER/HEPATOCYTES	(15)	(35)	(33)
CYTOPLASMIC VACUOLIZATION		2 (6%)	
#PANCREAS	(15)	(35)	(33)
INFLAMMATION, INTERSTITIAL		1 (3%)	
#PANCREATIC ACINUS	(15)	(35)	(33)
ATROPHY, NOS		1 (3%)	
#COLON	(15)	(35)	(34)
NEMATODIASIS		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(15)	(35)	(34)
HYDRONEPHROSIS		1 (3%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
*TRIGEMINAL GANGLION	(15)	(35)	(34)
INFLAMMATION, CHRONIC	2 (13%)		
SPECIAL SENSE ORGANS			
*MIDDLE EAR	(15)	(35)	(34)
INFLAMMATION, SUPPURATIVE	1 (7%)		1 (3%)

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

TABLE D1. MALE MICE NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(15)	(35)	(34)
NECROSIS, FAT			1 (3%)
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	13	8
AUTOLYSIS/NO NECROPSY			1

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED PHENAZOPYRIDINE HYDROCHLORIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	34	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	34	32
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(15)	(34)	(32)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
#LUNG/BRONCHUS	(15)	(34)	(32)
INFLAMMATION, CHRONIC SUPPURATIV		1 (3%)	
#LUNG/BRONCHIOLE	(15)	(34)	(32)
HYPERPLASIA, LYMPHOID	1 (7%)		
#LUNG	(15)	(34)	(32)
PNEUMONIA INTERSTITIAL CHRONIC		1 (3%)	
BRONCHOPNEUMONIA CHRONIC SUPPURA	1 (7%)	3 (9%)	1 (3%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(15)	(34)	(32)
ANGIECTASIS			1 (3%)
HEMATOPOIESIS		3 (9%)	1 (3%)
#MANDIBULAR L. NODE	(13)	(33)	(29)
HYPERPLASIA, PLASMA CELL			1 (3%)
#PANCREATIC L.NODE	(13)	(33)	(29)
CONGESTION, NOS		1 (3%)	
HYPERPLASIA, LYMPHOID			1 (3%)
#MESENTERIC L. NODE	(13)	(33)	(29)
CONGESTION, NOS		3 (9%)	2 (7%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ATROPHY, NOS		2 (6%)	1 (3%)
HYPERPLASIA, LYMPHOID			1 (3%)
#INGUINAL LYMPH NODE	(13)	(33)	(29)
HYPERPLASIA, PLASMA CELL			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(15)	(34)	(32)
CYTOPLASMIC VACUOLIZATION	2 (13%)	1 (3%)	
HYPERPLASIA, NODULAR		1 (3%)	
ANGIECTASIS		1 (3%)	1 (3%)
HEMATOPOIESIS		1 (3%)	
#PANCREAS	(15)	(34)	(32)
HEMORRHAGE		1 (3%)	
NECROSIS, FAT		1 (3%)	
#PANCREATIC ACINUS	(15)	(34)	(32)
ATROPHY, NOS		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(15)	(34)	(32)
HYPERPLASIA, LYMPHOID		1 (3%)	1 (3%)
ENDOCRINE SYSTEM			
#THYROID	(14)	(30)	(29)
HYPERPLASIA, FOLLICULAR-CELL			1 (3%)
REPRODUCTIVE SYSTEM			
#UTERUS	(15)	(34)	(32)
HEMORRHAGE		1 (3%)	1 (3%)
#UTERUS/ENDOMETRIUM	(15)	(34)	(32)
INFLAMMATION, SUPPURATIVE		1 (3%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, CYSTIC	10 (67%)	31 (91%)	23 (72%)
#OVARY	(15)	(34)	(32)
CYST, NOS	2 (13%)	12 (35%)	5 (16%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(15)	(34)	(32)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1		
AUTO/NECROPSY/HISTO PERF			1
AUTOLYSIS/NO NECROPSY		1	3

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS FED PHENAZOPYRIDINE HYDROCHLORIDE IN THE DIET

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	0/12 (0)	3/33 (9)	0/35 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.048		
Relative Risk[f]		Infinite	--
Lower Limit		0.238	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	81	--
Hematopoietic System: Malignant Reticulosis[b]	0/13 (0)	2/33 (6)	0/35 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	--
Lower Limit		0.125	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	73	--

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Large Intestine (Colon or Rectum): Adenocarcinoma, NOS[b]	0/14 (0)	4/34 (12)	8/35 (23)
P Values[c,d]	P = 0.028	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.410	0.985
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	98
Large Intestine (Colon or Rectum): Adenocarcinoma, NOS or Adenoma, NOS[b]	0/14 (0)	4/34 (12)	9/35 (26)
P Values[c,d]	P = 0.015	N.S.	P = 0.034
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.410	1.134
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	98

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Pheochromocytoma[b]	2/13 (15)	1/34 (3)	1/34 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.191	0.191
Lower Limit		0.004	0.004
Upper Limit		3.442	3.442
Weeks to First Observed Tumor	105	105	104
Thyroid: C-cell Adenoma[b]	2/14 (14)	0/32 (0)	1/32 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.000	0.219
Lower Limit		0.000	0.004
Upper Limit		1.447	3.935
Weeks to First Observed Tumor	93	--	101

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor[b]	14/14 (100)	22/34 (65)	7/33 (21)
P Values[c,d]	P < 0.001(N)	P = 0.008(N)	P < 0.001(N)
Relative Risk[f]		0.647	0.212
Lower Limit		0.000	0.000
Upper Limit		0.868	0.349
Weeks to First Observed Tumor	92	81	101

[a]Dosed groups received 3,700 or 7,500 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 dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P < 0.05; otherwise, not significant (N.S.) is indicated.

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

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

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Colon: Adenocarcinoma, NOS[b]	0/14 (0)	3/33 (9)	5/32 (16)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.273	0.594
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	105
Pituitary: Chromophobe Adenoma[b]	2/9 (22)	1/30 (3)	1/25 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.150	0.180
Lower Limit		0.003	0.004
Upper Limit		2.655	3.156
Weeks to First Observed Tumor	97	105	105

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	2/15 (13)	0/33 (0)	0/32 (0)
P Values[c,d]	P = 0.032(N)	N.S.	N.S.
Relative Risk[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.506	1.551
Weeks to First Observed Tumor	97	--	--

[a]Dosed groups received 3,700 or 7,500 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 dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P < 0.05; otherwise, not significant (N.S.) is indicated.

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE FED PHENAZOPYRIDINE HYDROCHLORIDE IN THE DIET

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	1/15 (7)	3/35 (9)	4/34 (12)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.286	1.765
Lower Limit		0.117	0.200
Upper Limit		65.499	84.137
Weeks to First Observed Tumor	106	100	91
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	1/15 (7)	4/35 (11)	4/34 (12)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.714	1.765
Lower Limit		0.195	0.200
Upper Limit		81.831	84.137
Weeks to First Observed Tumor	106	100	91

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	1/15 (7)	0/35 (0)	2/34 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.000	0.882
Lower Limit		0.000	0.051
Upper Limit		7.949	50.523
Weeks to First Observed Tumor	106	--	105
Hematopoietic System: Lymphoma or Leukemia[b]	1/15 (7)	0/35 (0)	3/34 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.000	1.324
Lower Limit		0.000	0.121
Upper Limit		7.949	67.356
Weeks to First Observed Tumor	106	--	105

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	2/15 (13)	5/35 (14)	9/33 (27)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.071	2.045
Lower Limit		0.206	0.509
Upper Limit		10.495	17.921
Weeks to First Observed Tumor	90	100	82
Liver: Hepatocellular Adenoma or Carcinoma[b]	5/15 (33)	15/35 (43)	15/33 (45)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.286	1.364
Lower Limit		0.573	0.609
Upper Limit		3.811	4.002
Weeks to First Observed Tumor	90	64	82

[a]Dosed groups received 600 or 1,200 ppm.

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

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

(continued)

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

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Sarcoma, NOS, of the Subcutaneous Tissue[b]	0/15 (0)	0/34 (0)	2/32 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		--	Infinite
Lower Limit		--	0.147
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	88
Lung: Alveolar/Bronchiolar Adenoma[b]	1/15 (7)	1/34 (3)	2/32 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.441	0.938
Lower Limit		0.006	0.054
Upper Limit		33.649	53.563
Weeks to First Observed Tumor	106	106	90

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	4/15 (27)	6/34 (18)	2/32 (6)
P Values[c,d]	P = 0.044(N)	N.S.	N.S.
Relative Risk[f]		0.662	0.234
Lower Limit		0.193	0.024
Upper Limit		2.825	1.469
Weeks to First Observed Tumor	90	89	90
Liver: Hepatocellular Carcinoma[b]	2/15 (13)	6/34 (18)	14/32 (44)
P Values[c,d]	P = 0.010	N.S.	P = 0.039
Relative Risk[f]		1.324	3.281
Lower Limit		0.281	0.918
Upper Limit		12.462	26.847
Weeks to First Observed Tumor	90	90	94

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	2/15 (13)	11/34 (32)	19/32 (59)
P Values[c,d]	P = 0.002	N.S.	P = 0.003
Relative Risk[f]		2.426	4.453
Lower Limit		0.639	1.327
Upper Limit		20.695	34.479
Weeks to First Observed Tumor	90	90	94
Pituitary: Chromophobe Adenoma[b]	1/10 (10)	2/30 (7)	0/23 (0)
p Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.667	0.000
Lower Limit		0.041	0.000
Upper Limit		38.024	7.962
Weeks to First Observed Tumor	106	105	--

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Harderian Gland: Adenoma, NOS[b]	0/15 (0)	2/34 (6)	3/32 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.138	0.300
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	105

[a]Dosed groups received 600 or 1,200 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 dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

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

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

April 26, 1978

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

The primary reviewer noted that the compound induced colonic polyps in treated rats; the report did not indicate whether they were multiple or whether metastases were detected. An elevated but non-statistically significant incidence of hepatomas was observed in treated male mice. He suggested that the report could be strengthened if it indicated "the low nature of the response" and the questionable malignant character of the colonic polyps. Given Phenazopyridine Hydrochloride's use as a therapeutic agent, the primary reviewer said that the carcinogenic risk to man is minimal.

A Program staff pathologist commented that the colon tumors were identical to those induced by known colon carcinogens. He noted that some were polypoid and had a degree of invasion in the stalk when it was present. He

added that most colon carcinogens also induce liver tumors, as was the case in this bioassay.

A Subgroup member said that the data were adequate to classify Phenazopyridine Hydrochloride as a weak colon carcinogen. A Program staff member argued that tumor incidence alone should not be the determinant in classifying a carcinogen as weak or strong. A discussion ensued regarding the assessment of strong versus weak carcinogens.

A motion was approved unanimously that the report on the bioassay of Phenazopyridine Hydrochloride be accepted.

Members present were:

Michael Shimkin (Acting Chairman), University of California at San Diego
Joseph Highland, Environmental Defense Fund
George Roush, Jr., Monsanto Company
Louise Strong, University of Texas Health Sciences Center
John Weisburger, American Health Foundation

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

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