National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 7
January 1977

BIOASSAY OF PHENFORMIN FOR POSSIBLE CARCINOGENICITY

CAS No. 114-86-3

NCI-CG-TR-7

The Chemical Abstracts Service (CAS) Registry number used to track this bioassay is 834-28-6, which is determined to best define the material used in the conduct of this bioassay.

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

BIOASSAY OF

PHENFORMIN

FOR POSSIBLE CARCINOGENICITY

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DHEW Publication No. (NIH) 77-807

BIOASSAY OF

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Carcinogenesis Program
Division of Cancer Cause and Prevention
National Cancer Institute

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Compilation of individual animal survival and summary tables was performed by C. A. Dominick[1], and the pathology tables were compiled at Southern Research Institute. The statistical analyses were performed by Dr. J. R. Joiner[6], using methods selected for the Bioassay Program by Dr. J. J. Gart[7]. Chemicals used in this bioassay were analyzed under the direction of Dr. E.

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SUMMARY

A bioassay of the carcinogenicity of phenformin hydrochloride was conducted using Fischer 344 rats and B6C3F1 mice. The compound was administered in the diet for 78 weeks to groups of 35 animals of each species and sex, using concentrations of 15,000 and 30,000 ppm for rats and concentrations of 1,200 and 2,500 ppm for mice. Treatment was followed by a period of observation of 26 weeks. Control groups consisted of 15 untreated animals of each species and sex.

Average weights attained by treated groups of rats and mice were consistently lower than those of control groups in all tests except that for male rats, in which case the weights shown by treated and control animals were indistinguishable. Survival was apparently unaffected in both species by treatment with phenformin, but was poor in mice due to intercurrent disease.

Tumors appearing in treated rats and mice were similar in type and number to those in controls, and no pathologic or statistical evidence of induction of tumors in these species by phenformin was found.

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I. INTRODUCTION

Phenformin is a synthetic oral hypoglycemic agent used to control maturity-onset diabetes. Pharmacologically, phenformin acts to enhance anaerobic glycolysis, decrease gluconeogenesis, and inhibit intestinal absorption of glucose (Goodman and Gilman, 1975). This compound was selected for carcinogenicity testing since, in the treatment of diabetes, it is administered chronically.

II. MATERIALS AND METHODS

A. Chemicals

Phenformin hydrochloride (DBI®) is 1-phenethylbiguanide hydrochloride, manufactured by CIBA-GEIGY, Ardsley, N.Y. The stated purity of lot #22332 was 99.7% (spectrophotometric analysis). One trace impurity identified as biguanide (0.34%) and two other trace impurities were detected by thin-layer chromatography. Purity by perchloric acid titration was 100.9 ± 0.3%. The melting point of this sample was 177-178°C, which was comparable to the literature value of 175-178°C. The infrared and nuclear magnetic resonance spectra conformed to standard spectra for this compound. The phenformin used in this bioassay was stored in plastic containers at 5°C.

B. Dietary Preparation

Dosed feed mixtures were prepared by combining the total dose of the compound with a small amount of feed and then mixing this premix with the remaining quantity of animal feed for 10 minutes in a twin-shell blender. Mixtures were prepared every two weeks and stored at room temperature. Analyses to determine the concentration or stability of the compound in the feed or the homogeneity of the mix were not performed.

C. Animals

Fischer 344 rats were received from Charles River Breeding Laboratories, Wilmington, Mass., at 40 days of age. B6C3F1 mice were also obtained from Charles River, at 41 days of age. Following a two-week quarantine, those animals with a clinical appearance of health and adequate body weight were assigned to test groups and matched-control groups. Animals were individually identified within cages by ear marking.

D. Animal Maintenance

Rats and mice were housed five per cage in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). Rat cages were lined with Iso-Dri® hardwood chips (Carworth, Edison, N.J.), and covered with disposable filter bonnets; mouse cages were lined with Sterolit clay bedding (Englehard Mineral and Chemical Co., New York, N.Y.). Dosed feed mixtures and diets for the untreated controls were prepared with Wayne® Lab Blox Meal (Allied Mills, Chicago, Ill.). City tap water was used. Cages, water bottles, feeders, and racks were sanitized weekly. Bedding was replaced weekly. Animal rooms were illuminated for nine hours per day by fluorescent lighting and natural light. Room air underwent 15 changes per hour, and was maintained at 20-24° C and 38-42% relative humidity.

Rats and mice treated with phenformin were housed in rooms where, at one time or another, other chemicals were being tested. These chemicals were as follows:

R A T S

anthranilic acid
pyrazinecarboxamide
L-tryptophan
1-butyl-3-(p-tolylsulfonyl)urea; tolbutamide
4,4′-sulfonyldianiline
4,4′-thiodianiline
2,6 diamino-3-(phenylazo)pyridine hydrochloride
2-ethyl-4-pyridinecarbothioamide; ethionamide
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine; pyrimethamine
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide; chloropropamide
*N-9H-fluoren-2-ylacetamide; 2-AAF
4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide; acetohexamide
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea; tolazamide

M I C E

anthranilic acid
pyrazinecarboxamide
L-tryptophan
3,3′-iminodi-1-propanol dimethanesulfonate (ester) hydrochloride
5-azacytidine
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione; ICRF-159
beta-2′-deoxy-6-thioguanosine
1,4-butanediol dimethanesulfonate; busulfan
acronine
emetine dihydrochloride tetrahydrate
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide; isophosphamide
tris(1-aziridinyl)phosphine sulfide; thioTEPA
1-butyl-3-(p-tolylsulfonyl)urea; tolbutamide
4,4′-sulfonyldianiline
4,4′-thiodianiline
cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate; phenesterin
estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate); estradiol mustard

2,6 diamino-3-(phenylazo)pyridine hydrochloride
2-ethyl-4-pyridinecarbothioamide; ethionamide
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride; phenoxybenzamine hydrochloride
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine; pyrimethamine
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide; chloropropamide
*N-9H-fluoren-2-ylacetamide; 2-AAF
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride; procarbazine hydrochloride
4-aceyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide; acetohexamide
4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride; MAAM
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea; tolazamide
2,4,6-tris(dimethylamino)-s-triazine
adriamycin

*positive control

E. Subchronic Studies

Feeding studies were conducted to estimate the maximum tolerated dose (MTD) for administration in the chronic study. The low dose given in the chronic study is 1/2 of the high dose. In the subchronic studies, phenformin was administered in the diet to female Fischer 344 rats and B6C3F1 mice for 45 days at five dose concentrations. Five animals were tested at each dose. Following the treatment there was a 45-day rest period, after which all animals were killed and necropsied.

For rats doses of 300, 800, 1,500, 3,000, and 6,000 ppm were used. After 84 days the gain in body weight of the treated rats was 90% of that of the controls at 300 ppm; 89% at 800 ppm; 81%

at 1,500 ppm; 82% at 3,000 ppm; and 69% at 6,000 ppm. Two rats died at 6,000 ppm, but no animals died at lower doses. Based on these results, doses of 400 ppm and 800 ppm were selected for rats in the chronic study.

For mice doses of 500, 1,200, 2,500, 5,000, and 10,000 ppm were used. After 84 days the gain in body weight of the treated mice was similar to that of the controls at 500 ppm, 66% of that of the controls at 1,200 ppm, 66% at 2,500 ppm, and 58% at 5,000 ppm. All mice treated at 10,000 ppm died, but no mortality occurred in the animals treated at lower doses. No gross abnormalities were noted at necropsy. Based on these results, doses of 1,200 ppm and 2,500 ppm were selected for mice in the chronic study.

F. Design of the Chronic Study

The design of the chronic study, including both test and matched-control groups, is shown in table 1. The matched controls were housed in the same rooms as the test animals.

G. Clinical and Pathologic Examinations

All animals were observed for signs of toxicity twice daily and were palpated for masses at each weighing. Animals appearing moribund at the time of clinical examination were killed and necropsied. In the chronic study, the following tissues and

Table 1. Chronic Study of Phenformin

Group	No. of Animals	Dose (ppm)	Time on Study Treated (weeks)	Time on Study Untreated (weeks)
RATS				
Male				
Matched-Control	15	0	78	26
Low-Dose	35	400	78	26
High-Dose	35	800	78	26
Female				
Matched-Control	15	0	78	26
Low-Dose	35	400	78	26
High-Dose	35	800	78	26
MICE				
Male				
Matched-Control	15	0	78	26
Low-Dose	35	1,200	78	26
High-Dose	35	2,500	78	26
Female				
Matched-Control	15	0	78	26
Low-Dose	35	1,200	78	26
High-Dose	35	2,500	78	26

organs were taken from killed animals and, where possible, from animals found dead: brain, pituitary, lymph nodes (cervical and mesenteric), thyroid, parathyroid, salivary glands, lung, heart, diaphragm, stomach (pylorus and fundus), duodenum, jejunum or ileum, large intestine, pancreas, adrenal gland, kidney (longitudinal and transverse), liver, skin, entire gonads, urinary bladder, prostate or uterus, and femur with marrow. Tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, routinely stained with hematoxylin and eosin, and microscopically examined. An occasional section was subjected to special staining techniques for more definitive diagnosis.

Only positive histopathologic findings are tabulated. These are based primarily on hematoxylin and eosin-stained paraffin-process sections. Where possible all organs, tissues, and gross lesions were evaluated for every animal as specified in the current pathology protocol. A few tissues are missing from some animals, particularly from early deaths, which were necropsied according to earlier bioassay protocols. Also, occasional animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation and may not have been preserved for such purposes. Thus, the number of animals whose tissues were examined microscopically for any particular organ, tissue, or lesion varies and does not

necessarily represent the number of animals that were placed on experiment 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 included descriptive information on the chemicals, animals, experimental design, clinical observations, survival, animal 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.

Probabilities of survival were estimated by the product limit procedure of Kaplan and Meier (1958) and presented in this report in the form of graphs. Deaths due to accident or scheduled deaths are treated as censored observations and all other deaths are uncensored. Statistical tests of differences in survival between groups are compared using the method of Cox (1972) for two groups and an extension of this method by Tarone (1975) for more than two groups.

The number of animals with tumors was analyzed as a percentage of the number of animals pathologically examined. For specific

anatomic sites, the animal is not included in the denominator if that particular site was not histologically examined. For tumors which required gross detection, e.g. skin tumors, the denominator included all animals necropsied. For tumors that may appear at several sites, e.g. lymphoma, any animal that had at least one involved site histologically examined is entered in the denominator of the proportions given for that tumor.

Statistical analysis of the incidence of tumors was made using the Fisher exact test (Cox, 1970) to compare a control group to a group of treated animals at each dose level. In addition, the Armitage and Cochran test for linear trend in proportions, with continuity correction (Armitage, 1971), was used. This test, assuming a linear trend, determines if the slope of the dose-response curve is different from zero, at the 0.05 level of significance. The method also calculates the level of probability of a departure from linear trend.

A conservative adjustment, the Bonferroni inequality (Miller, 1966), was used for simultaneous comparison of several treated groups with a control group. For the comparison of results obtained with k different test doses with those for a control, this correction requires a level of significance less than or equal to 0.05/k for the overall comparison to be significant at the 0.05 level. This adjustment was not made in the tables where

the Fisher exact test results are shown but is discussed in the analysis when appropriate.

As an additional analysis, the exact 95% confidence interval for the odds ratio (Gart, 1970) between each of the dose groups and its control was calculated. The odds ratio is $p_t(1-p_c)/p_c(1-p_t)$ where p_t is the true binomial probability of tumor in a dosed animal and p_c is the true spontaneous tumor probability in the controls. The hypothesis of equality between the true proportion of a specific tumor in a dosed group and that in a control is expressed by an odds ratio of 1 (one). Values in excess of 1 (one) represent the condition of a larger proportion in the dosed group than in the control. The confidence interval entries in the statistical tables of this report represent the conversion of each odds ratio to the difference in probabilities, p_t-p_c, where $p_t-p_c = 0$ implies an odds ratio of 1 (one).

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

The average body weights of both high- and low-dose female rats were consistently lower than the controls during the treatment period, while body weights of the males were essentially unaffected by the compound (figure 1).

Due to signs of respiratory disease in several animals, oxytetracycline was administered in drinking water at 0.6 mg/ml from week 38 to 42 and at 0.3 mg/ml from week 42 to 44. Eye irritation was also reported in several treated animals. Masses were found by palpation in animals from all groups, beginning at week 77.

B. Survival (Rats)

There was no dose-related mortality apparent in the male rats, since the control and high-dose animals experienced comparable death rates and both groups had lower survival than the low-dose group (figure 2); however, 65% of the high-dose group survived to the end of the test. In the female rats, there were no significant differences between the mortality of the different groups, and 83% of the high-dose group survived to the end of the

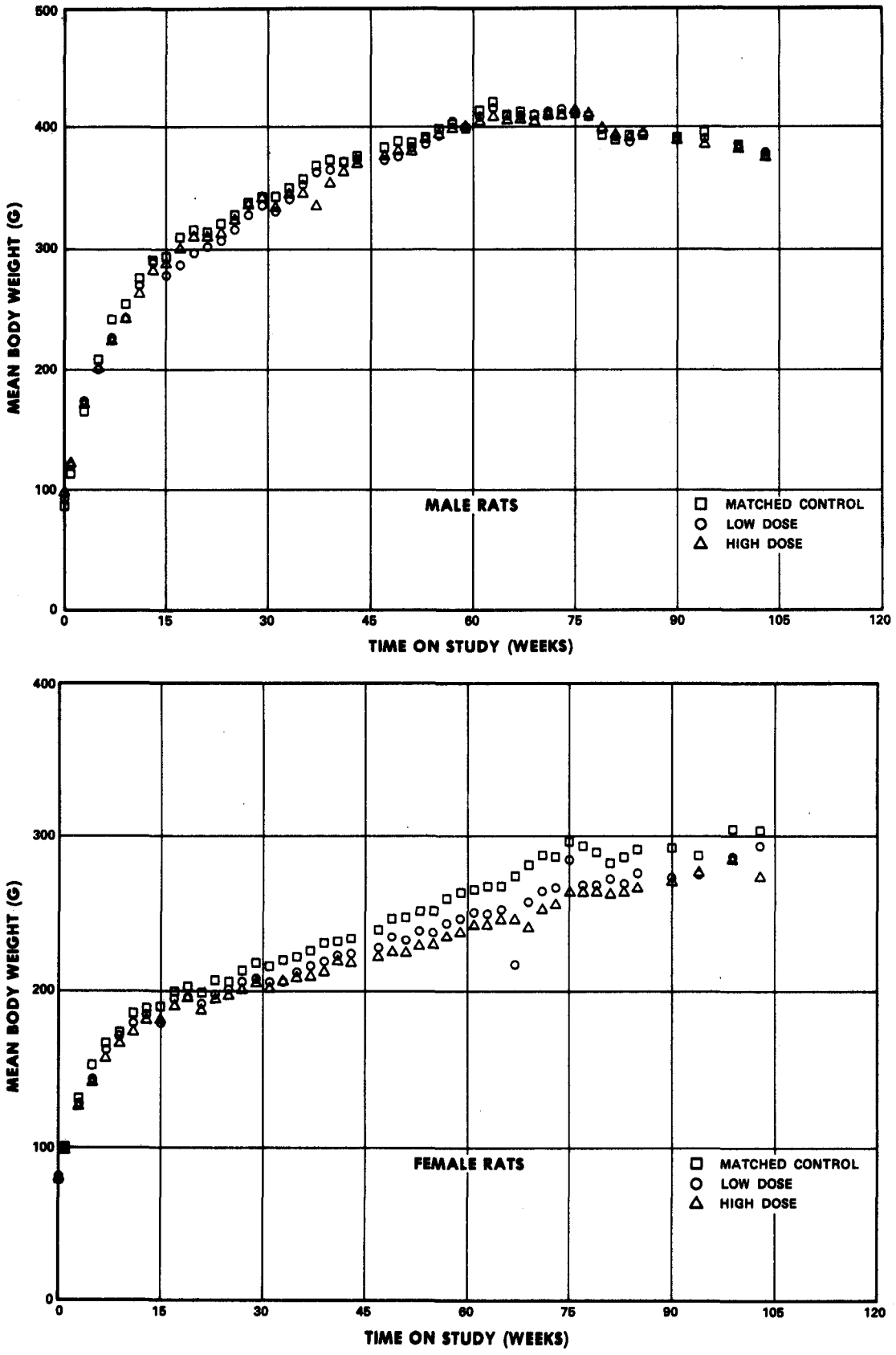

Figure 1. Growth Curves for Rats Fed Phenformin in the Diet

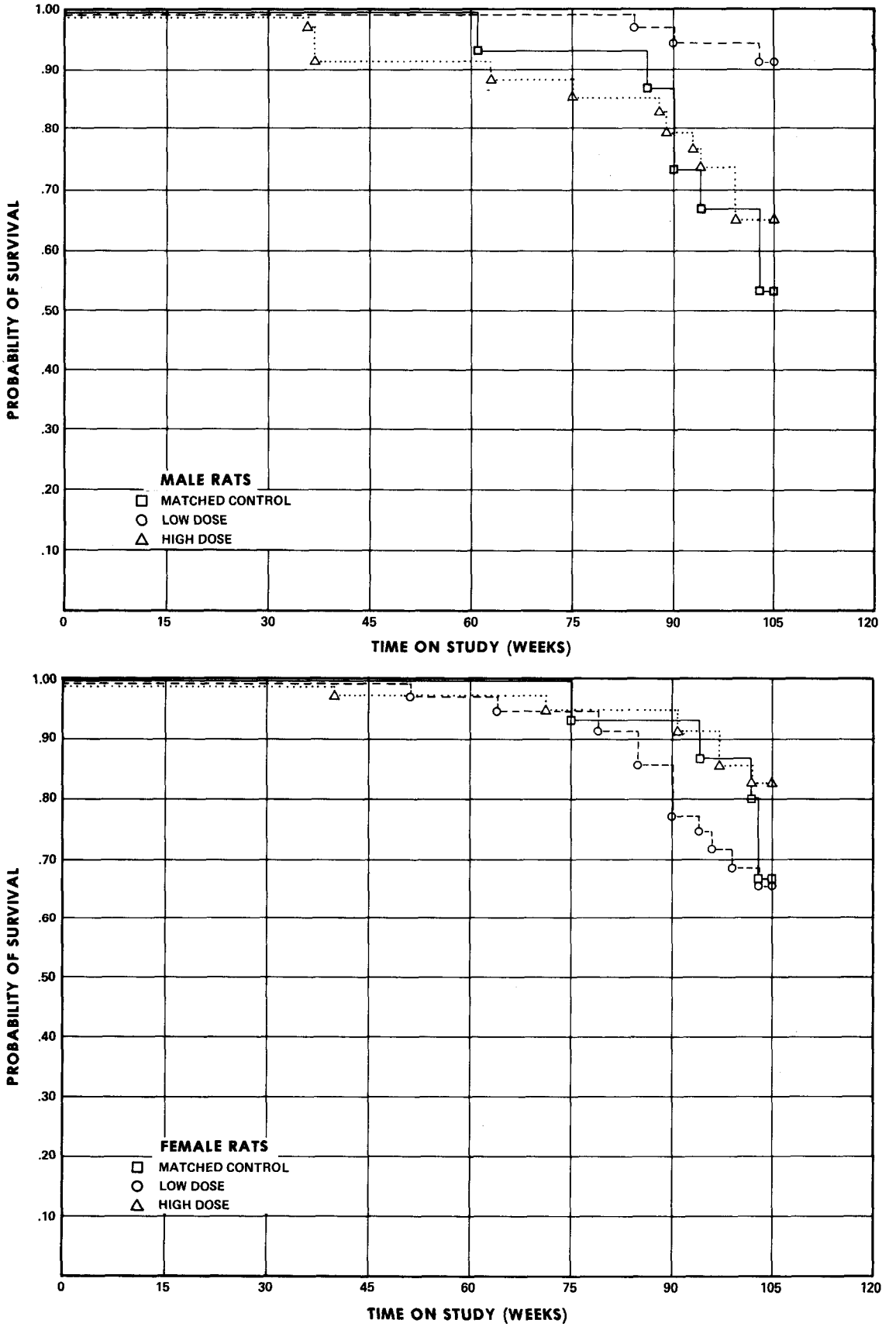

Figure 2. Survival Curves for Rats Fed Phenformin in the Diet

study. The survival in all groups appears adequate to provide meaningful statistical analysis.

C. Pathology (Rats)

Histopathologic findings are tabulated in Appendix A, tables A1-A6, covering neoplasms and other proliferative lesions, and in Appendix C, tables C1-C2, covering nontumor pathology.

Numerous inflammatory, degenerative, and proliferative lesions commonly observed in aged Fischer rats occurred with approximately equal frequency in drug-treated and control animals. These included chronic tracheitis and bronchiolitis; multifocal alveolar macrophage aggregates in the lung parenchyma; chronic nephritis with scarring, tubular dilatation and tubular regeneration; suppurative oophoritis, endometritis and cystic endometrial hyperplasia (table A2); testicular atrophy; biliary hyperplasia and focal hepatocytomegaly of the liver (table A6); and C-cell hyperplasia of the thyroid (table A1).

Other nonneoplastic proliferative lesions included follicular-cell hyperplasia of the thyroid, adrenal medullary hyperplasia, and islet-cell hyperplasia of the pancreas (table A1); endometrial stromal hyperplasia of the uterus (table A2); alveolar epithelial hyperplasia of the lung (table A3); epithelial hyperplasia of the renal pelvis, ureter, bladder,

and/or urethra (table A4); and focal hepatocyte hyperplasia (table A6).

Because of the current controversy regarding the nomenclature of proliferative hepatocyte lesions in rodents, the definition of the term "focal hepatocyte hyperplasia" used above requires clarification. The term was used in this study to indicate the presence in a liver section of one or more foci of hepatocytes with basophilic cytoplasm and a slight increase in the amount of nuclear chromatin. Many of these hepatocytes also had a slightly increased nuclear:cytoplasmic ratio when compared with adjacent normal hepatocytes, and infrequently mitotic figures or hepatocytes with double nuclei were observed. These foci of hyperbasophilic hepatocytes were thought to represent areas of hyperplasia and were coded as such in this study. They did not compress adjacent hepatic parenchyma and thus were not considered to represent "nodules" of hyperplasia. These lesions are similar morphologically to those described by Squire and Levitt (1975) as "basophilic foci".

The most frequently occurring neoplasm was the interstitial-cell tumor of the testis, which was observed in a very high percentage of sections of testes from both control and test rats (table A2). These tumors were very often bilateral, often occupied a large portion of the testis, and were usually accompanied by atrophy of

seminiferous epithelium. Pituitary adenomas were also very common, especially in females (table A1). Neoplasms of thyroid C-cells also occurred more frequently in females than males, especially C-cell adenomas (table A1). As in many endocrine neoplasms, differentiation between benign and malignant C-cell neoplasms was often difficult. C-cell lesions were classified as adenomas when the proliferating C-cells were present in nodular masses which widely separated thyroid follicles and distorted normal follicular architecture. In the larger, more discrete, nodular lesions, the proliferating C-cells sometimes were present as interlacing bundles of enlongated, spindling cells rather than the polyhedral to spherical shape characteristic of normal C-cells. When invasion of the thyroid capsule, adjacent tissues, or vessels was present, or when metastasis was detected, the lesion was classified as C-cell carcinoma. Metastases to the lung and the adrenal gland occurred in the C-cell carcinoma present in a high-dose female rat.

Follicular-cell neoplasms occurred much less frequently than C-cell neoplasms (table A1). Follicular-cell adenomas appeared microscopically as well circumscribed masses composed of enlarged follicles lined by hyperbasophilic follicular cells which were increased in number per unit area, either by papillary infolding of simple cuboidal or columnar epithelium into the follicular lumen, or stratification of follicular cells surrounding the

lumen. Distinct compression of adjacent normal thyroid parenchyma, usually with some evidence of fibrous encapsulation, was present. Follicular-cell lesions were classified as carcinoma, based upon the presence of anaplasia and histologic arrangement in disorderly nests and/or sheets. Areas with papillary patterns were also often present. Fibrous stroma often intermingled with, but did not encapsulate, the tumors. Evidence of invasion of the thyroid capsule was present in both of the lesions that were diagnosed as follicular-cell carcinoma.

Pheochromocytomas of adrenal medulla occurred in both test and control rats (table A1). Differentiation between pheochromocytoma and the previously mentioned adrenal medullary hyperplasia was difficult in some cases. The diagnosis of pheochromocytoma was made when the adrenal medullary lesion was present as a discrete hypercellular mass that compressed adjacent normal adrenal parenchyma.

One carcinoma and two adenomas of pancreatic islet cells were present, all occurring in high-dose male rats (table A1). The diagnosis of islet-cell carcinoma was based on the invasion of the capsule surrounding the neoplasm, the size of the neoplasm, and the anaplastic appearance of the neoplastic cells.

Two endometrial adenocarcinomas, two endometrial stromal sarcomas, and 10 endometrial stromal polyps were observed in

sections of uterus (table A2). The diagnosis of adenocarcinoma was based on cellular anaplasia and/or invasion of stroma and muscularis. Differentiation between endometrial stromal sarcoma and endometrial stromal polyp was based on cellular anaplasia, the mitotic index, the size of the lesion, and the invasion of adjacent tissues. Differentiation between stromal polyp and stromal hyperplasia was based on the degree of cellularity and the amount of discrete polypoid protrusion into the lumen of the uterus.

Fibroadenoma of the mammary gland was quite common in the female rats (table A2). Some of these neoplasms were multiple. All revealed a very distinct fibrous component that contained mature collagen, interspersed with a benign epithelial component. One mammary carcinoma was observed in a control female rat.

Malignant lymphomas occurred quite frequently in all groups of rats (table A5). Most of these neoplasms were composed of rather undifferentiated lymphoblastic cells; in some cases, autolysis prevented further classification of the neoplasm. Most involved multiple organs, and the organs most frequently involved were spleen, liver, thymus, and lymph nodes. In some cases, histologic evidence of leukemia was present.

A low incidence of various other neoplasms was present, including

the following: alveolar/bronchiolar carcinoma of the lung (table A3); nephroblastoma, hamartoma, and tubular adenoma of the kidney (table A4); Schwannoma, hemangioma, and fibroma of the subcutis (table A5); keratoacanthoma of the skin (table A5); carcinoma of the preputial gland (table A5); mesothelioma of the peritoneum (table A5); and adenocarcinoma of the small intestine (table A5). There were instances in this study, as noted above and in the summary tables, where neoplastic or hyperplastic lesions occurred only in test animals, or with increased frequency when compared to the control groups. In the judgment of the pathologist, the nature, incidence, and severity of the lesions observed provide no clear evidence of carcinogenic effect from exposure to phenformin.

D. Statistical Analyses of Results (Rats)

Tables 2 and 3 contain the statistical analyses of the proportions of tumors at specific sites, which occurred in more than 5% of a dosed group of rats. There were no statistically significant positive dose-related trends. The proportions of tumors in the matched controls were compared with the overall proportions observed to date for controls in the bioassay program at the laboratory in which the phenformin was tested (lab-historic controls). The proportion of thyroid tumors in the female matched controls (6/13, 46%) was significantly higher than

TABLE 2. Analyses of the Incidence of Primary Tumors at Specific Sites in Male Rats Fed Phenformin in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	4/15(0.27)	4/35(0.11)	7/34(0.21)
P Values[c]	N.S.		
95% Confidence Interval (matched)[d]		(-0.39,0.10)	(-0.36,0.18)
Weeks to First Observed Tumor	85	90	75
Reproductive System: All Tumors[b]	14/15(0.93)	35/35(1.00)	31/34(0.89)
P Values[c]	N.S.		
95% Confidence Interval (matched)[d]		(-0.03,1.00)	(-0.12,0.18)
Weeks to First Observed Tumor	85	84	63
Thyroid: C-cell Adenoma or Carcinoma[b]	1/15(0.07)	3/34(0.09)	2/32(0.06)
P Values[c]	N.S.		
95% Confidence Interval (matched)[d]		(-0.18,0.12)	(-0.16,0.09)
Weeks to First Observed Tumor	103	105	93

TABLE 2. Analyses of the Incidence of Primary Tumors at Specific Sites in Male Rats Fed Phenformin in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	0/14(0.00)	6/33(0.18)	5/29(0.17)
P Values[c]	N.S.		
95% Confidence Interval (matched)[d]		(-0.08,1.00)	(-0.09,1.00)
Weeks to First Observed Tumor	--	105	99
Adrenal: Pheochromocytoma[b]	1/15(0.07)	3/34(0.09)	1/33(0.03)
P Values[c]	N.S.		
95% Confidence Interval (matched)[d]		(-0.18,0.12)	(-0.13,0.06)
Weeks to First Observed Tumor	106	105	105

[a]Dosed groups received doses of 400 and 800 ppm in feed.

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

[c]Beneath the incidence of each of the control is the probability level for the Cochran-Armitage test for dose-related trend in proportions when it is below 0.10, otherwise, N.S. - not significant.
Departure from the linear trend is noted when it is below 0.05 for any comparison.
Beneath the dose group is the probability level for the Fisher exact (conditional) test for comparison of that dose group with the matched-control group when it is below 0.05.
(N) A negative trend results from lower incidence in dose group(s) than in matched controls.

[d]95% confidence interval of the difference in proportions of dose group and matched-control group.

TABLE 3. Analyses of the Incidence of Primary Tumors at Specific Sites in Female Rats Fed Phenformin in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	5/15(0.33)	5/34(0.15)	7/35(0.20)
P Values[c]	N.S.		
95% Confidence Interval (matched)[d]		(-0.45,0.09)	(-0.43,0.15)
Weeks to First Observed Tumor	75	79	71
Reproductive System: All Tumors[b]	7/15(0.47)	9/34(0.26)	6/35(0.17)
P Values[c]	P = 0.027(N)		
95% Confidence Interval (matched)[d]		(-0.51,0.12)	(-0.57,0.02)
Weeks to First Observed Tumor	75	51	101
Thyroid: C-cell Adenoma or Carcinoma[b]	5/13(0.38)	7/33(0.21)	7/31(0.23)
P Values[c]	N.S.		
95% Confidence Interval (matched)[d]		(-0.49,0.14)	(-0.48,0.16)
Weeks to First Observed Tumor	102	96	105

TABLE 3. Analyses of the Incidence of Primary Tumors at Specific Sites in Female Rats Fed Phenformin in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	7/12(0.58)	14/30(0.47)	19/26(0.73)
P Values[c]	N.S.		
95% Confidence Interval (matched)[d]		(-0.45,0.26)	(-0.20,0.50)
Weeks to First Observed Tumor	75	85	97
Adrenal: Pheochromocytoma[b]	0/13(0.00)	0/34(0.00)	3/32(0.09)
P Values[c]	P = 0.063		
95% Confidence Interval (matched)[d]		--	(-0.13,1.00)
Weeks to First Observed Tumor	--	--	105

[a]Dosed groups received doses of 400 and 800 ppm in feed.

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

[c]Beneath the incidence of each of the control is the probability level for the Cochran-Armitage test for dose-related trend in proportions when it is below 0.10, otherwise, N.S. - not significant.
Departure from the linear trend is noted when it is below 0.05 for any comparison.
Beneath the dose group is the probability level for the Fisher exact (conditional) test for comparison of that dose group with the matched-control group when it is below 0.05.
(N) A negative trend results from lower incidence in dose group(s) than in matched controls.

[d]95% confidence interval of the difference in proportions of dose group and matched-control group.

that in the female lab-historic controls (5/105, 5%), as also was the proportion of pituitary tumors in females (7/12, 58% vs. 20/105, 19%); otherwise the incidence of specific tumors in the matched controls was comparable to that of the lab-historic controls.

There is also no statistical evidence of the carcinogenicity of phenformin in rats at the individual dose concentrations administered.

As an additional statistic, the 95% confidence interval was calculated and entered in the tables. The implication of this interval is that in 95/100 (95%) of a large number of experiments, the true difference between the tumor rate for treated groups of animals and the rate for the control groups would be inside the interval calculated from the experiment. In each of the intervals shown in the tables, zero is included; this indicates the negative aspects of the results. It should also be noted that each of the intervals has a positive endpoint indicating the theoretical possibility of tumor induction by phenformin which could not be detected under the conditions of this test.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

The average body weights of both male and female mice were markedly lower than controls during the first 60-75 weeks of administration of compound (figure 3). Because of signs of respiratory disease in some mice, oxytetracycline (0.6 mg/ml during week 63 and 0.3 mg/ml during week 64) was added to the drinking water, and animals were exposed to a propylene glycol mist (weeks 63-64). However, between weeks 63 and 89 eight male and seven female control mice died and were diagnosed as having bronchopneumonia. The disease is believed to be associated with the sharp declines in the mean body weights of the females at 63 weeks and the males at 76 weeks.

B. Survival (Mice)

No significant positive dose-related trend was seen in the survival statistics of either the male or female mice. There were three matched-control mice missing at 8 weeks on study, and one high-dose female mouse was accidently killed at 15 weeks. In both sexes, the mortality in the matched controls was excessive after 75 weeks on study when compared with the dosed groups. The lowest overall survival was experienced in the low-dose male

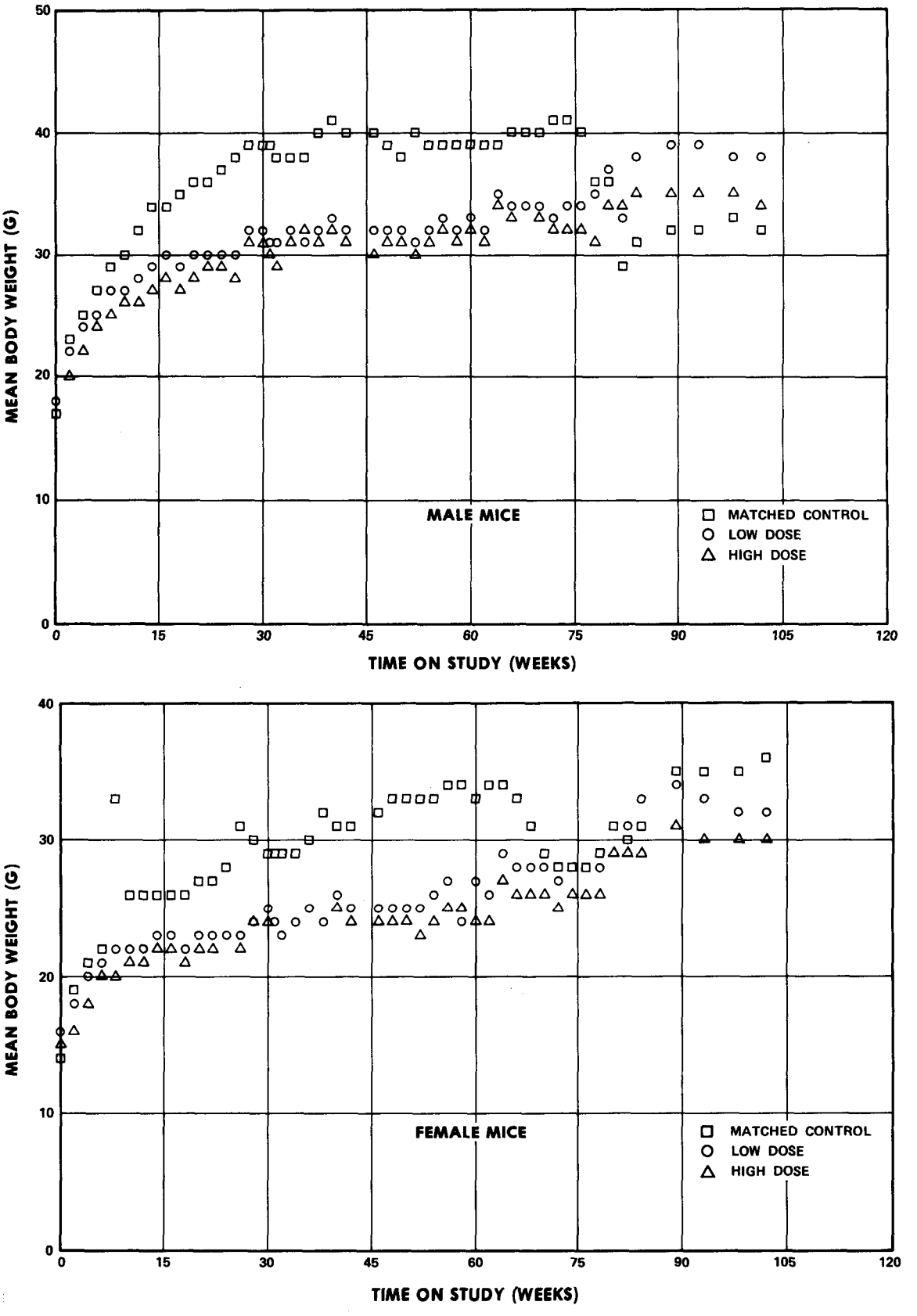

Figure 3. Growth Curves for Mice Fed Phenformin in the Diet

mouse group, in which 50% of the animals were dead by 74 weeks. Early deaths may increase the probability of making an error by the acceptance of the hypothesis of "no dose-related carcinogenicity" when the hypothesis is false; therefore, the test of the male mice may be inconclusive due to the limited survival (figure 4).

C. Pathology (Mice)

Histopathologic findings are tabulated in Appendix B, tables B1-B8, covering neoplasms and other proliferative lesions, and in Appendix D, tables D1-D2, covering nonneoplastic lesions.

Several chronic inflammatory, degenerative, and proliferative lesions frequently observed in aged laboratory mice occurred with approximately equal frequency and severity in test and control animals. These lesions included bronchopneumonia, suppurative oophoritis, endometritis, and cystic endometrial hyperplasia (table B5).

The incidence of proliferative lesions of the digestive tract, including the liver, is summarized in table B1. The term "hepatocellular carcinoma" was used for proliferative lesions of the livers in mice which, in the judgment of the pathologist, had the potential or the capacity for progressive growth, invasion, and metastasis and for causing the death of the host. This

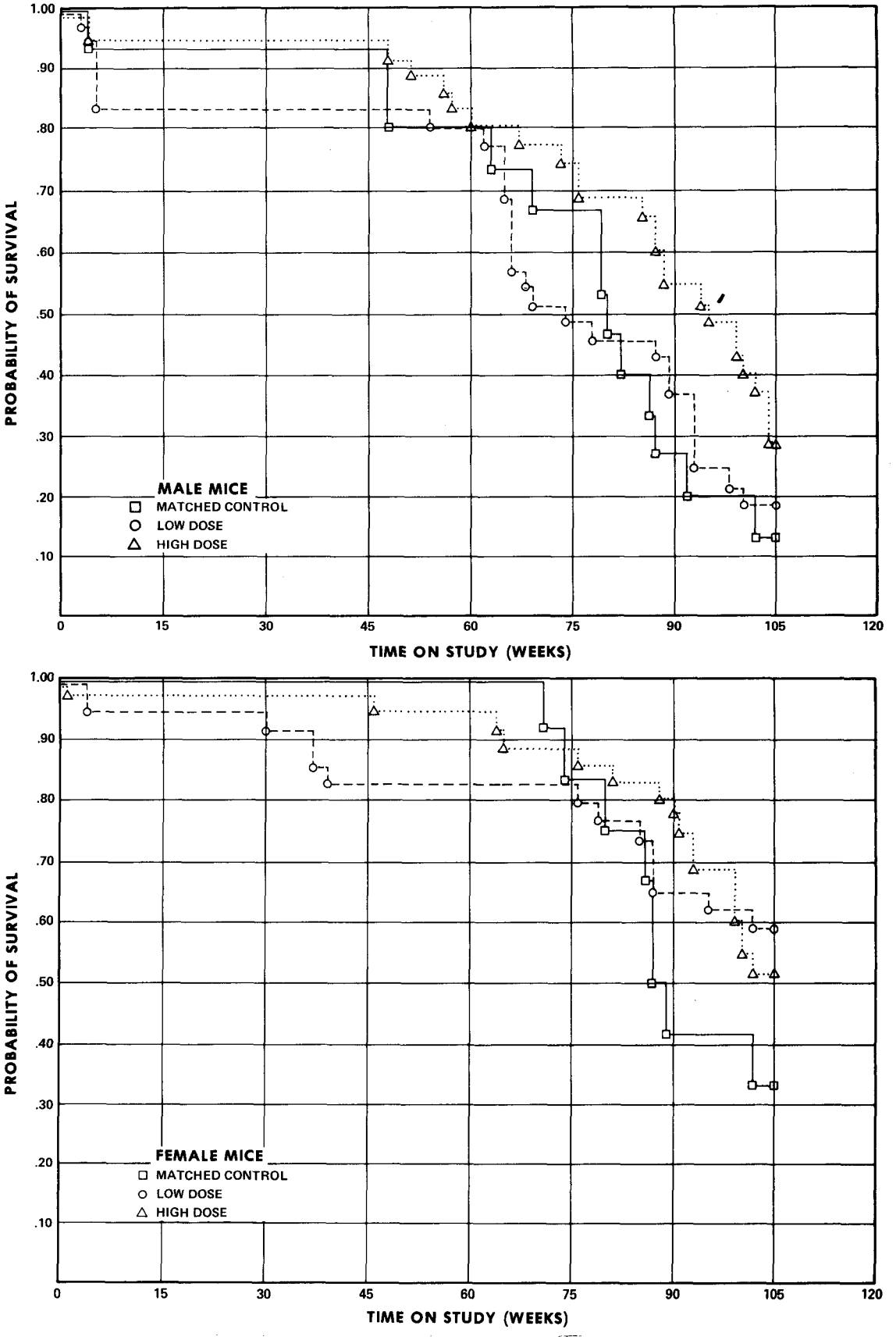

Figure 4. Survival Curves for Mice Fed Phenformin in the Diet

judgment was based upon the cytologic and histologic features of the neoplasms and the knowledge that lesions with the same morphologic characteristics have exhibited malignant biologic behavior. The hepatocellular carcinomas observed in the various test and control groups comprised the full spectrum of morphology of this entity. Microscopically, the neoplasms varied in appearance from nodules of hepatocytes with moderate deviation from normal hepatic architecture to large masses of anaplastic hepatocytes, with numerous mitotic figures and complete loss of normal hepatic architecture. Various types of hepatocellular carcinomas described in the literature were seen, including those with an orderly cord-like arrangement of neoplastic cells, those with a glandular pattern resembling adenocarcinoma, and those composed of sheets of highly anaplastic cells with little tendency to form a cord- or gland-like arrangement. Pulmonary metastasis of hepatocellular carcinoma occurred in two low-dose male mice.

Two proliferative hepatic lesions were classified as nodular hyperplasia, both from male mice in the high-dose group. Although both of these lesions compressed adjacent liver parenchyma, variations in cellular morphology and lobular architecture were not sufficient to warrant a diagnosis of neoplasia in either case.

Focal hepatocytomegaly was diagnosed in one male and one female mouse, both from the high-dose group. These lesions were composed of one or more foci of enlarged hepatocytes containing large amounts of finely vacuolated cytoplasm. Compression of adjacent hepatic parenchyma by these foci was minimal or absent. Many hepatocellular carcinomas had foci of these enlarged hepatocytes within the neoplasm.

One papilloma of squamous gastric epithelium was observed in a high-dose male mouse.

Malignant lymphomas were observed in all groups of mice (table B2). In summarizing the incidence of lymphomas, the data were divided into two categories, localized and generalized. Those neoplasms classified as generalized were observed in numerous organs and tissues; those classified as localized involved only two or three tissues. The organs most frequently involved with malignant lymphoma were the mesenteric lymph nodes, liver, spleen, thymus, and Peyer's patches. Several morphologic types of malignant lymphoma were observed, including the mixed (reticulum-cell sarcoma, type B, of Dunn [1954]), the histiocytic (reticulum-cell sarcoma, type A, of Dunn [1954]), and the undifferentiated. In some cases, autolysis prevented subclassification of malignant lymphomas; these were coded as malignant lymphoma, N.O.S.

Primary pulmonary neoplasms were observed only in male mice (table B3). Differentiation between adenoma and carcinoma was based on the degree of anaplasia, the mitotic index, the size of the neoplasm, and the microscopic evidence of invasion of adjacent pulmonary parenchyma (carcinoma), as opposed to the mere compression of adjacent parenchyma and thus a more discrete lesion (adenoma).

The incidence of neoplasms of the vasculature is summarized in table B4. All lesion classified as hemangiomas were cavernous in morphology, with little or no abnormal cytology of the endothelial cells. The hemangiosarcomas also had areas of cavernous spaces, but the endothelial cells lining these spaces often had plump, hyperchromatic nuclei, and there was a gradual transition to more densely cellular areas of endothelial-cell proliferation, with cellular atypia and an increased nuclear:cytoplasmic ratio.

Endometrial hyperplasia was seen frequently in all three groups of female mice (table B5), but only two neoplasms were present, a stromal polyp and a leiomyoma.

A very low incidence of proliferative lesions was present in the endocrine tissues, involving the thyroid, adrenal, and pituitary glands and the interstitial cells of the testis (table B6).

The incidence of proliferative lesions of the urinary bladder is summarized in table B7. In addition to the one cavernous hemangioma (see also table B4 and the preceding paragraph on neoplasms of the vasculature), proliferative lesions of the epithelium of the bladder were observed in five mice. These epithelial lesions had several similarities: (1) they all consisted of entirely intraluminal proliferation of the transitional cells with no evidence of invasion of the muscularis; (2) the epithelial cells protruding into the bladder lumen were very large, spherical to columnar in shape, and had a large amount of pale, eosinophilic cytoplasm and a large nucleus with a prominent nucleolus; (3) some cells appeared to have multiple nuclei; (4) the lesions were not focal, rather the entire epithelial surface appeared to be involved. In one high-dose male mouse the entire lumen of the bladder section was filled with proliferating transitional cells; in addition, numerous mitotic figures were evident. All five of these lesions were classified as epithelial hyperplasia with mild dysplasia.

Table B8 summarizes the incidence of subcutaneous neoplasms. In addition to the two vascular tumors already discussed, a malignant Schwannoma was present in the subcutis in the pelvic area of a high-dose female mouse. From the gross description at necropsy, this lesion apparently also involved peritoneal organs; although no section of this portion of the lesion was available,

the mass was described grossly as being located "intraperitoneally, below the bladder." Microscopically, this neoplasm was composed of oval to spindle cells in dense whorls and, to a lesser extent, in a loose, fibrillar pattern.

There were instances in this study, as noted above and in the summary tables, where neoplastic or hyperplastic lesions occurred only in test mice or with increased frequency when compared to control groups. In the judgment of the pathologist the nature, incidence, and severity of the lesions observed provide no clear evidence of carcinogenic effect from exposure to phenformin.

D. Statistical Analyses of Results (Mice)

Tables 4 and 5 contain the analyses of the incidence of tumors at any specific site where tumors were diagnosed in over 5% of any dosed group. There were no additional matched-control groups that had conditions suitably conformable to the matched-control group of phenformin, so no pooled-control group was formed. However, the proportions of particular tumors in matched-control mice were comparable to the proportions seen for the same tumors in all previous control mice used at the laboratory where phenformin was tested (lab historic controls), except that hematopoietic tumors were observed in 33% of the matched controls of both male and female mice, compared to only 1.5% hematopoietic tumors of the male and 5.4% of the female lab historic controls.

TABLE 4. Analyses of the Incidence of Primary Tumors at Specific Sites in Male Mice Fed Phenformin in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	1/14(0.07)	4/29(0.13)	1/33(0.03)
P Values[c]	N.S.		
Departure from Linear Trend	N.S.		
95% Confidence Interval (matched)[d]		(-0.19,0.17)	(-0.14,0.06)
Weeks to First Observed Tumor	82	105	104
Hematopoietic System: Lymphoma[b]	5/15(0.33)	1/35(0.03)	3/35(0.09)
P Values[c]	P = 0.048(N)	P = 0.007*(N)	P = 0.043*(N)
Departure from Linear Trend	P = 0.009		
95% Confidence Interval (matched)[d]		(-0.40,0.05)	(-0.46,0.02)
Weeks to First Observed Tumor	48	98	99

TABLE 4. Analyses of the Incidence of Primary Tumors at Specific Sites in Male Mice Fed Phenformin in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangioma or Hemangiosarcoma[b]	1/15(0.07)	1/35(0.03)	2/35(0.06)
P Values[c]	N.S.		
Departure from Linear Trend	N.S.		
95% Confidence Interval (matched)[d]		(-0.13,0.06)	(-0.16,0.08)
Weeks to First Observed Tumor	82	105	105
Liver: Hepatocellular Carcinoma[b]	1/14(0.07)	6/27(0.22)	4/33(0.12)
P Values[c]	N.S.		
Departure from Linear Trend	N.S.		
95% Confidence Interval (matched)[d]		(-0.15,0.26)	(-0.20,0.15)
Weeks to First Observed Tumor	87	78	104

[a]Dosed groups received 1,200 and 2,500 ppm in feed.

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

TABLE 4. Analyses of the Incidence of Primary Tumors at Specific Sites in Male Mice Fed Phenformin in the Diet[a]

(continued)

[c]Beneath the incidence of each of the controls is the probability level for the Cochran-Armitage test for dose-related trend in proportions when it is below 0.10, otherwise N.S. - not significant.
Departure from linear trend is noted when it is below 0.05 for any comparison. Beneath the dose-group incidence is the probability level for the Fisher exact (conditional) test for the comparison of that dose group with the matched-control group (*) when it is below 0.05.
(N) A negative trend results from lower incidence in dosed group(s) than in matched controls.

[d]95% confidence interval of the difference in proportions of dose group and matched-control group.

TABLE 5. Analyses of the Incidence of Primary Tumors at Specific Sites in Female Mice Fed Phenformin in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	4/12(0.33)	7/33(0.21)	3/33(0.09)
P Values[c]	P = 0.049(N)		
Departure from Linear Trend	N.S.		
95% Confidence Interval (matched)[d]		(-0.46,0.17)	(-0.48,0.04)
Weeks to First Observed Tumor	86	86	90
All Sites: Hemangioma or Hemangiosarcoma[b]	1/12(0.08)	3/33(0.09)	2/33(0.06)
P Values[c]	N.S.		
Departure from Linear Trend	N.S.		
95% Confidence Interval (matched)[d]		(-0.23,0.12)	(-0.21,0.08)
Weeks to First Observed Tumor	89	104	

TABLE 5. Analyses of the Incidence of Primary Tumors at Specific Sites in Female Mice Fed Phenformin in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	1/10(0.10)	3/29(0.10)	2/33(0.06)
P Values[c]	N.S.		
Departure from Linear Trend	N.S.		
95% Confidence Interval (matched)[d]		(-0.28,0.13)	(-0.26,0.09)
Weeks to First Observed Tumor	102	105	88

[a]Dosed groups received 1,200 and 2,500 ppm in feed.

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

[c]Beneath the incidence of each of the controls is the probability level for the Cochran-Armitage test for dose-related trend in proportions when it is below 0.10, otherwise N.S. - not significant.
Departure from linear trend is noted when it is below 0.05 for any comparison. Beneath the dose-group incidence is the probability level for the Fisher exact (conditional) test for the comparison of that dose group with the matched-control group (*) when it is below 0.05.
(N) A negative trend results from lower incidence in dosed group(s) than in matched controls.

[d]95% confidence interval of the difference in proportions of dose group and matched-control group.

The dosed groups did not exhibit as high proportions of hematopoietic tumors as those of the matched-control groups, with the result that the females showed a negative linear trend (P = 0.038) and the males showed a departure from negative linear trend (P = 0.009) with significant differences between each of the dosed groups and the matched control. There is no statistical evidence in the data that phenformin is carcinogenic to mice at the dose levels given.

As an additional statistical test, the 95% confidence intervals were calculated and are entered in the tables. The implication of these intervals is that in 95/100 (95%) of a large number of experiments the true difference between the treatment tumor rate and the control tumor rate would be inside the interval calculated from the experiment. In each of the intervals shown in the table, zero is included; this indicates the negative aspects of the results. It should be noted that each of the intervals has a positive endpoint indicating the theoretical possibility of tumor induction by phenformin which could not be detected by this test.

V. DISCUSSION

The phenformin used in this study was stored at 5°C, and there is no reason to suspect instability. However, no actual analysis of the dosed feed for concentration of phenformin was performed during the study. The doses of phenformin used in this study were slightly toxic, since comparisons of average group weights (figures 1 and 3) show that, except in male rats, the treated animals on either high or low doses of the compound gained less weight than the corresponding controls. Differences in weight gain between high- and low-dose animals were much less than differences between low-dose animals and controls. Survival was not dose-related for either sex of either species; however, because there were excessive numbers of deaths in mice, particularly males, these animals may not have been at risk long enough to develop any possible latent tumors. The possibility of an effect on tumor incidence of treatment of the mice with oxytetracycline at weeks 63-64 to control respiratory disease is not known; however, no mechanism is immediately apparent by means of which the antibiotic might be expected to exert an adverse effect on the reliability of the findings.

Various types of tumors were observed in the treated animals, but the incidences were not significantly different from those for

the same types seen in control animals. Thus, tumors appearing in hematopoietic systems, thyroids, and pituitaries of treated rats also appeared in untreated rats at the same sites and at insignificantly different rates of incidence. Similarly, tumors appearing in the respiratory systems, circulatory systems, and livers of treated mice also appeared in untreated mice at the same sites and at essentially the same incidences.

The observed failure of phenformin to induce tumors when administered to rats and mice extends the negative findings of an earlier report (Stoner et al., 1973) in which the compound was administered to mice at a level of 12.5-62.5 mg/kg/day by intraperitioneal injections three times per week for 8 weeks. In the present work, dosages for mice calculated from food consumption were 300-625 mg/kg/day administered in the diet five times per week for 78 weeks. In addition, Dil'man et al., (1974) reported that the incidence of DMBA-induced mammary tumors in 16 female rats that were force-fed phenformin (3 benign tumors; 9 highly differentiated tumors; 1 other type) was much less than that of 16 controls treated with DMBA but not phenformin (6 adenomas; 35 adenocarcinomas; 3 other types).

Thus, there is no evidence that under the conditions of this bioassay phenformin was carcinogenic. However, the stability of the test material in feed was not determined. Furthermore, the

tests performed in male mice may be inconclusive, since early deaths increase the probability of failing to discover carcinogenicity even when it exists. It should be noted that the confidence intervals for all tumor sites in rats and mice which were subjected to statistical analysis include a positive value indicating the theoretical possibility of tumor induction by phenformin which could not be detected under the conditions of this test.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS AND OTHER PROLIFERATIVE LESIONS IN RATS FED PHENFORMIN IN THE DIET

TABLE A1

PROLIFERATIVE LESIONS OF THE ENDOCRINE SYSTEM

	MALE RATS			FEMALE RATS		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
THYROID						
Follicular-cell Carcinoma	0/15	0/34	1/32 (3%)	0/13	0/33	1/31 (3%)
Follicular-cell Adenoma	0/15	0/35	1/32 (3%)	1/15 (7%)	0/33	0/34
C-cell Carcinoma	1/15 (7%)	0/35	1/32 (3%)	0/15	2/33 (6%)	1/34 (3%)
C-cell Adenoma	0/15	3/35 (8.6%)	1/32 (3%)	5/15 (33%)	5/33 (15%)	6/34 (18%)
Follicular-cell Hyperplasia	0/15	1/35 (3%)	2/32 (6%)	0/15	0/33	9/34 (3%)
C-cell Hyperplasia	4/15 (27%)	25/35 (71%)	14/32 (44%)	10/154 (67%)	16/33 (48%)	21/34 (62%)
ADRENAL						
Pheochromocytoma	1/15 (7%)	3/34 (8.6%)	1/33 (3%)	0/13	0/34	3/32 (9%)
Hyperplasia Adrenal Medulla	2/15 (13%)	6/35 (17%)	1/33 (3%)	2/15 (13%)	2/34 (6%)	0/35

TABLE A1

PROLIFERATIVE LESIONS OF THE ENDOCRINE SYSTEM

(continued)

	MALE RATS			FEMALE RATS		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
PITUITARY						
Adenoma	0/14	6/33 (18%)	7/29 (24%)	7/12 (58%)	14/30 (47%)	19/26 (73%)
PANCREAS						
Islet-cell Carcinoma	0/15	0/32	1/32	0/12	0/34	0/30
Islet-cell Adenoma	0/15	0/33	2/32 (6%)	0/14	0/34	0/33
Islet-cell Hyperplasia	1/15 (7%)	0/33	0/32	0/14	1/34 (3%)	0/33

TABLE A2

PROLIFERATIVE LESIONS OF THE
REPRODUCTIVE SYSTEM AND MAMMARY GLAND

	MALE RATS			FEMALE RATS		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
TESTIS						
Interstitial-cell Tumor	14/15 (93%)	34/34 (100%)	31/34 (91%)	---	---	---
UTERUS						
Adenocarcinoma	---	---	---	0/11	0/34	2/31 (6%)
Endometrial Stromal Sarcoma	---	---	---	1/13 (8%)	0/34	1/33 (3%)
Endometrial Stromal Polyp	---	---	---	4/13 (31%)	4/34 (12%)	2/33 (6%)
Endometrial Hyperplasia	---	---	---	3/13 (23%)	5/34 (15%)	5/33 (15%)
Endometrial Stromal Hyperplasia	---	---	---	0/13	2/34 (6%)	1/33 (3%)
MAMMARY GLAND						
Adenocarcinoma	0/15	0/32	0/28	1/13 (8%)	0/30	0/31
Fibroadenoma	0/15	0/32	1/28 (4%)	3/15 (20%)	5/34 (15%)	3/35 (9%)
Fibroma	0/15	0/32	0/28	0/15	0/34	1/35 (3%)

TABLE A3

PROLIFERATIVE LESIONS OF THE
RESPIRATORY TRACT

	MALE RATS			FEMALE RATS		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
LUNG						
Alveolar/Bronchiolar Carcinoma	0/15	0/34	0/34	1/13 (8%)	0/34	1/32 (3%)
Alveolar Epithelial Hyperplasia	1/15 (7%)	3/35 (9%)	0/33	0/15	0/34	0/35

TABLE A4

PROLIFERATIVE LESIONS OF THE
URINARY TRACT

	MALE RATS			FEMALE RATS		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
KIDNEY						
Nephroblastoma	0/15	0/34	0/34	0/12	1/34 (3%)	0/32
Hamartoma	1/15 (7%)	0/35	0/34	0/11	0/32	0/35
Tubular Adenoma	0/15	0/35	1/34 (3%)	0/11	0/32	0/35
Renal Pelvis/Ureter Hyperplasia	3/15 (20%)	6/35 (17%)	5/34 (15%)	2/11 (18%)	1/32 (3%)	0/35
BLADDER/Urethra						
Hyperplasia	0/13	1/31 (3%)	1/22 (5%)	1/10 (10%)	1/32 (3%)	1/29 (3%)

TABLE A5

MISCELLANEOUS PROLIFERATIVE LESIONS

	MALE RATS			FEMALE RATS		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM						
Malignant Lymphoma	4/15 (27%)	4/35 (11%)	7/34 (21%)	5/15 (33%)	5/34 (15%)	7/35 (20%)
SKIN/SUBCUTIS						
Preputial Gland Carcinoma	0/15	1/35 (3%)	1/34 (3%)	---	---	---
Schwannoma	1/15 (7%)	0/35	0/34	0/15	0/34	0/35
Hemangioma	0/15	0/35	2/34 (6%)	0/15	0/34	1/35 (3%)
Fibroma	0/15	1/35 (3%)	1/34 (3%)	0/15	0/34	0/35
Keratoacanthoma	1/15 (7%)	0/35	0/34	0/15	0/34	0/35
Carcinoma, N.O.S.	0/15	0/35	0/34	1/15 (7%)	0/34	0/35
Poorly Differentiated Adenocarcinoma	0/15	0/35	0/34	0/15	1/34 (3%)	0/35

TABLE A5

MISCELLANEOUS PROLIFERATIVE LESIONS

(continued)

	MALE RATS			FEMALE RATS		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
PERITONEUM						
Mesothelioma	0/15	0/35	1/34 (3%)	0/15	0/34	0/35
SMALL INTESTINE						
Adenocarcinoma	0/15	0/34	0/31	0/11	1/32 (3%)	0/32
HEART						
Sarcoma, N.O.S.	0/15	0/34	1/33 (3%)	0/13	0/33	0/32
MEDIASTINUM						
Osteogenic Sarcoma	0/15	0/35	0/34	0/15	1/34 (3%)	0/35

TABLE A6

PROLIFERATIVE LESIONS OF THE LIVER

	MALE RATS			FEMALE RATS		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
HEPATOCYTES						
Focal Hyperplasia	8/15 (53%)	3/35 (9%)	13/34 (38%)	8/15 (53%)	28/34 (82%)	14/35 (40%)
Hepatocytomegaly	3/15 (20%)	6/35 (17%)	3/34 (9%)	1/15 (7%)	1/34 (3%)	2/35 (6%)
BILE DUCTS						
Hyperplasia	12/15 (80%)	23/35 (66%)	11/34 (32%)	5/15 (33%)	8/34 (23.5%)	4/35 (11%)

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS AND OTHER PROLIFERATIVE LESIONS IN MICE FED PHENFORMIN IN THE DIET

TABLE B1

PROLIFERATIVE LESIONS OF THE DIGESTIVE SYSTEM

	MALE MICE			FEMALE MICE		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
LIVER						
Hepatocellular Carcinoma	1/14 (7%)	6/27 (22%)	4/33 (12%)	1/10 (10%)	3/29 (10%)	2/33 (6%)
Nodular Hyperplasia	0/14	0/27	2/33 (6%)	0/10	0/30	0/33
Focal Hepatocytomegally	0/14	0/27	1/33 (3%)	0/10	0/30	1/33 (3%)
Focal Angiectasis	1/14 (7%)	0/27	1/33 (3%)	0/10	0/30	0/33
STOMACH						
Papilloma, Squamous Epithelium	0/13	0/24	1/30 (3%)	0/11	0/30	0/33
ANUS						
Pseudoepitheliomatous Hyperplasia	0/14	0/29	0/33	0/12	0/33	1/33 (3%)

TABLE B2

NEOPLASMS OF THE HEMATOPOIETIC SYSTEM

	MALE MICE			FEMALE MICE		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Malignant Lymphoma, Generalized*	2/14 (14%)	1/29 (3%)	2/33 (6%)	3/12 (25%)	4/33 (12%)	1/33 (3%)
Malignant Lymphoma, Localized*	3/14 (21%)	0/29	1/33 (3%)	1/12 (8%)	3/33 (9%)	2/33 (6%)

TABLE B3

PRIMARY PULMONARY NEOPLASMS

	MALE MICE			FEMALE MICE		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
LUNG						
Alveolar/Bronchiolar Carcinoma	1/14 (7%)	1/29 (3%)	1/33 (3%)	0/12	0/30	0/32
Alveolar/Bronchiolar Adenoma	0/14	3/29 (10%)	0/33	0/12	0/31	0/32

*The tabulation of the incidence of this neoplasm is divided into those animals in which the neoplasm was generalized, i.e., involved numerous organs, and those in which the neoplasm was observed in only a few organs. The number of animals necropsied is used as the denominator.

TABLE B4

NEOPLASMS OF THE VASCULATURE

	MALE MICE			FEMALE MICE		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
SPLEEN						
Hemangiosarcoma	1/14 (7%)	1/27 (4%)	0/30	0/11	1/27 (4%)	0/32
SUBCUTIS						
Hemangiosarcoma	1/14 (7%)	0/29	0/33	0/12	0/33	0/33
Cavernous Hemangioma	0/14	0/29	0/33	1/12 (8%)	0/33	0/33
HEART						
Hemangiosarcoma	0/14	0/28	1/33 (3%)	0/11	0/31	0/32
MESENTERY						
Cavernous Hemangioma	0/14	0/29	0/33	0/12	1/33 (3%)	0/33

TABLE B4

NEOPLASMS OF THE VASCULATURE

(continued)

	MALE MICE			FEMALE MICE		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
STERNUM						
Cavernous Hemangioma	0/14	1/29 (3%)	0/33	0/12	0/33	0/33
BLADDER						
Cavernous Hemangioma	0/14	0/24	1/28 (4%)	0/10	0/27	0/30
PANCREAS						
Hemangioma	0/14	0/24	0/28	0/10	1/27 (4%)	0/30
Total Malignant Vascular Neoplasms	2/14 (14%)	1/29 (3%)	1/33 (3%)	0/12	1/33 (3%)	0/33
Total Benign Vascular Neoplasms	0/14	1/29 (3%)	1/33 (3%)	1/12 (8%)	2/33 (6%)	0/33

TABLE B5

PROLIFERATIVE LESIONS OF THE
REPRODUCTIVE SYSTEM

	MALE MICE			FEMALE MICE		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Teratoma, Ovary	---	---	---	0/11	1/28 (4%)	0/32
Endometrial Stromal Polyp	---	---	---	0/12	0/27	1/33 (3%)
Leiomyoma, Uterus	---	---	----	0/12	1/27 (4%)	0/33
Endometrial Hyperplasia	---	---	---	7/12 (58%)	9/27 (33%)	12/33 (36%)

TABLE B6

PROLIFERATIVE LESIONS OF THE ENDOCRINE SYSTEM

	MALE MICE			FEMALE MICE		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
THYROID						
Follicular-cell Adenoma	0/13	0/28	0/32	1/12 (8%)	0/30	2/33
Follicular-cell Hyperplasia	0/13	0/28	2/32 (6%)	1/12 (8%)	1/31 (3%)	1/33 (3%)
C-cell Hyperplasia	0/13	2/28 (7%)	4/32 (13%)	0/12	0/31	0/33
ADRENAL						
Focal Hyperplasia, Medulla	0/13	0/27	0/33	1/12 (8%)	0/30	0/31
PITUITARY						
Chromophobe Adenoma	0/6	0/9	0/19	1/9 (11%)	0/18	0/21
TESTIS						
Interstitial-cell Tumor	1/14 (7%)	0/28	1/33 (3%)	---	---	---

TABLE B7

PROLIFERATIVE LESIONS OF THE
URINARY BLADDER

	MALE MICE			FEMALE MICE		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Cavernous Hemangioma*	0/14	0/24	1/28 (4%)	0/10	0/27	0/30
Epithelial Hyperplasia and Dysplasia	1/14 (7%)	1/24 (4%)	1/28 (4%)	0/10	0/27	2/30 (7%)

*This lesion was also tabulated in Table B4, Neoplasms of the Vasculature.

TABLE B8

SUBCUTANEOUS NEOPLASMS

	MALE MICE			FEMALE MICE		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Hemangiosarcoma*	1/14 (7%)	0/29	0/33	0/12	0/33	0/33
Malignant Schwannoma	0/14	0/29	0/33	0/12	0/33	1/33 (3%)
Cavernous Hemangioma*	0/14	0/29	0/33	1/12 (8%)	0/33	0/33
Adenocarcinoma, N.O.S.	0/14	0/29	0/33	0/12	1/33 (3%)	0/33
Zymbal's Gland Carcinoma	0/14	0/29	0/33	0/12	1/33 (3%)	0/33

*This lesion was also tabulated in Table B4, Neoplasms of the Vasculature.

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS FED PHENFORMIN IN THE DIET

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED PHENFORMIN IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15 (100%)	35 (100%)	34 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	35	34
ANIMALS WITH NON-TUMOR PATHOLOGY	15 (100%)	35 (100%)	34 (100%)
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM	11 (73%)	29 (83%)	23 (68%)
TRACHEA	7	28	19
INFLAMMATION SUPPURATIVE	7	25	8
INFLAMMATION CHRONIC		3	11
LUNG/BRONCHUS	1	1	
BRONCHIECTASIS		1	
INFLAMMATION SUPPURATIVE	1		
METAPLASIA SQUAMOUS	1		
LUNG/BRONCHIOLE	2	2	4
INFLAMMATION SUPPURATIVE	2	1	4
HYPERPLASIA EPITHELIAL		1	
LUNG	7	9	6
CONGESTION	3	3	
HEMORRHAGE			1
BRONCHOPNEUMONIA	1		3
PIGMENTATION	1		
CYTOMEGALY	1		
ALVEOLAR MACROPHAGES	3	4	2
HYPERPLASIA ALVEOLAR-CELL	1	2	
LUNG/ALVEOLI		1	2
EMPHYSEMA		1	
INFLAMMATION SUPPURATIVE			2
HEMATOPOIETIC SYSTEM	2 (13%)	1 (3%)	3 (9%)
SPLEEN			2
LYMPHOID HYPERPLASIA			1

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIESIS			1
SPLENIC CAPSULE		1	
FIBROSIS FOCAL		1	
LYMPH NODE	1		1
DILATATION			1
LYMPHOID HYPERPLASIA	1		
MANDIBULAR L. NODE	1		
LYMPHOID HYPERPLASIA	1		
CIRCULATORY SYSTEM	9 (60%)	17 (49%)	8 (24%)
MYOCARDIUM	9	17	8
HEMORRHAGE	1		
INFLAMMATION SUPPURATIVE			1
FIBROSIS	8	17	7
METAPLASIA OSSEOUS	1		
ENDOCARDIUM			1
INFLAMMATION SUPPURATIVE			1
DIGESTIVE SYSTEM	12 (80%)	27 (77%)	24 (71%)
LIVER	9	7	15
NECROSIS FOCAL			1
HEPATOCYTOMEGALY	3	6	3
HYPERPLASIA FOCAL	8	3	13
ANGIECTASIS		1	
BILE DUCT	12	23	11
FIBROSIS	2	3	
HYPERPLASIA	12	23	11
PANCREAS	1	1	2
FIBROSIS			1
FIBROSIS FOCAL			1
FIBROSIS DIFFUSE	1		
PERIARTERITIS		1	
PANCREATIC ACINUS		2	
ATROPHY		1	
ATROPHY FOCAL		1	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ESOPHAGUS			1
INFLAMMATION SUPPURATIVE			1
STOMACH			1
INFLAMMATION ACUTE			1
GASTRIC MUCOSA		1	1
ABSCESS		1	
EROSIVE INFLAMMATION			1
SMALL INTESTINE			1
INFLAMMATION SUPPURATIVE			1
SMALL INTESTINAL SER			1
HEMORRHAGE			1
LARGE INTESTINE	1		
NEMATODIASIS	1		
URINARY SYSTEM	14 (93%)	35 (100%)	30 (88%)
KIDNEY	14	35	30
INFLAMMATION CHRONIC	14	35	30
HYPERPLASIA TUBULAR-CELL		1	
KIDNEY/PELVIS	3	6	5
HYPERPLASIA EPITHELIAL	3	6	5
URINARY BLADDER	1		
CONGESTION	1		
URETHRA		2	1
CALCULUS		1	
HYPERPLASIA EPITHELIAL		1	1
ENDOCRINE SYSTEM	6 (40%)	26 (74%)	21 (62%)
ADRENAL			1
METAMORPHOSIS FATTY			1
ADRENAL CORTEX			1
HYPERPLASIA FOCAL			1
ADRENAL MEDULLA	2	6	1
HYPERPLASIA	2	2	1

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA FOCAL		4	
THYROID	4	25	16
ULTIMOBRANCHIAL CYST			1
HYPERPLASIA C-CELL	4	25	14
HYPERPLASIA FOLLICULAR-CELL		1	2
PARATHYROID	3	1	4
HYPERPLASIA	3	1	4
PANCREATIC ISLETS	1		
HYPERPLASIA	1		
REPRODUCTIVE SYSTEM	12 (80%)	32 (91%)	30 (88%)
MAMMARY GLAND		3	1
GALACTOCELE		2	1
CYST		1	
PREPUTIAL GLAND		1	2
CYST			1
CYSTIC DUCTS		1	
ABSCESS			1
HYPERPLASIA		1	
PROSTATE	4	6	2
INFLAMMATION SUPPURATIVE	3	6	2
PERIARTERITIS	1		
TESTIS	11	30	30
ATROPHY	11	30	30
NERVOUS SYSTEM	2 (13%)	1 (3%)	3 (9%)
BRAIN/MENINGES		1	
FIBROSIS		1	
CEREBRUM			1
HEMORRHAGE			1
BRAIN	2		1
HEMORRHAGE	2		1
CEREBELLUM			1
NECROSIS FOCAL			1
SPECIAL SENSE ORGANS		2 (6%)	1 (3%)

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C1 MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
EYE/CRYSTALLINE LENS		2	
MINERALIZATION		2	
EYELID		1	
INFLAMMATION SUPPURATIVE		1	
EAR CANAL			1
INFLAMMATION SUPPURATIVE			1
HYPERKERATOSIS			1
MUSCULOSKELETAL SYSTEM	1 (7%)		
SKELETAL MUSCLE	1		
HEMORRHAGE	1		
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS		1 (3%)	
ADIPOSE TISSUE		1	
INFLAMMATION		1	
SPECIAL MORPHOLOGY SUMMARY			
NO NECROPSY PERFORMED			1

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED PHENFORMIN IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15(100%)	34(100%)	35(100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	34	35
ANIMALS WITH NON-TUMOR PATHOLOGY	14 (93%)	33 (97%)	32 (91%)
INTEGUMENTARY SYSTEM		1 (3%)	
SKIN		1	
ULCER FOCAL		1	
RESPIRATORY SYSTEM	12 (80%)	22 (65%)	16 (46%)
TRACHEA	10	18	15
INFLAMMATION SUPPURATIVE	6	11	12
INFLAMMATION CHRONIC	4	7	3
LUNG/BRONCHUS		1	1
BRONCHIECTASIS		1	1
LUNG/BRONCHIOLE	1	3	
INFLAMMATION SUPPURATIVE	1	3	
HYPERPLASIA EPITHELIAL		1	
LUNG	4	8	
CONGESTION	1	6	
HEMORRHAGE	2	1	
ALVEOLAR MACROPHAGES	1	2	
HEMATOPOIETIC SYSTEM	3 (20%)	3 (9%)	1 (3%)
BONE MARROW	1		
HYPERPLASIA HEMATOPOIETIC	1		
SPLEEN	3	3	1
FIBROSIS FOCAL			1
HEMATOPOIESIS	3	3	
CIRCULATORY SYSTEM	4 (27%)	6 (18%)	3 (9%)
MYOCARDIUM	4	6	3
INFLAMMATION		1	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
FIBROSIS	4	5	3
DIGESTIVE SYSTEM	13 (87%)	32 (94%)	19 (54%)
LIVER	10	29	16
GRANULOMA			1
NECROSIS FOCAL	1	1	
METAMORPHOSIS FATTY		2	
HEPATOCYTOMEGALY	1	1	2
HYPERPLASIA FOCAL	8	28	14
LIVER/CENTRILOBULAR	1		
NECROSIS FOCAL	1		
BILE DUCT	5	8	5
FIBROSIS			1
HYPERPLASIA	5	8	4
PANCREAS	1	4	3
FIBROSIS	1	4	3
PANCREATIC ACINUS	3		3
ATROPHY	3		3
STOMACH		2	
INFLAMMATION		1	
ULCER FOCAL		1	
URINARY SYSTEM	12 (80%)	29 (85%)	25 (71%)
KIDNEY	10	28	25
INFLAMMATION CHRONIC	10	28	25
KIDNEY/TUBULE			1
PIGMENTATION			1
KIDNEY/PELVIS	1	1	
HYPERPLASIA EPITHELIAL	1	1	
URETER	1		
HYPERPLASIA EPITHELIAL	1		
URINARY BLADDER	1	1	1
HYPERPLASIA EPITHELIAL	1	1	1

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
U. BLADDER/MUSCULARIS		1	
EDEMA		1	
ENDOCRINE SYSTEM	11 (73%)	17 (50%)	22 (63%)
ADRENAL CORTEX		1	
CYTOMEGALY		1	
ADRENAL MEDULLA	2	2	
HYPERPLASIA FOCAL	2	2	
THYROID	10	16	22
HYPERPLASIA C-CELL	10	16	22
HYPERPLASIA FOLLICULAR-CELL			1
PARATHYROID	1	1	
HYPERPLASIA	1	1	
PANCREATIC ISLETS		1	
HYPERPLASIA		1	
REPRODUCTIVE SYSTEM	8 (53%)	20 (59%)	17 (49%)
MAMMARY GLAND	2	3	2
GALACTOCELE	2	3	2
MAMMARY LOBULE		1	1
HYPERPLASIA		1	1
CLITORAL GLAND			1
CYSTIC DUCTS			1
HYPERPLASIA			1
UTERUS/ENDOMETRIUM	6	16	13
INFLAMMATION SUPPURATIVE	5	13	10
HYPERPLASIA			2
HYPERPLASIA CYSTIC	3	5	3
HYPERPLASIA STROMAL		2	1
METAPLASIA SQUAMOUS	2		1
OVARY	2	6	2
CYST	1		
INFLAMMATION SUPPURATIVE		4	2

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ABSCESS	1	1	
INFLAMMATION CHRONIC		1	
NERVOUS SYSTEM			1 (3%)
BRAIN			1
GLIOSIS			1
CORPORA AMYLACEA			1
SPECIAL SENSE ORGANS		1 (3%)	1 (3%)
EYE			1
INFLAMMATION GRANULOMATOUS			1
EYE/CORNEA		1	
INFLAMMATION CHRONIC FOCAL		1	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			1 (3%)
ADIPOSE TISSUE			1
INFLAMMATION GRANULOMATOUS			1
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY PERF/HISTO PERF			1
AUTOLYSIS/NO NECROPSY PERFORMED		1	

APPENDIX D

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

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED PHENFORMIN IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14 (100%)	29 (100%)	33 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	29	33
ANIMALS WITH NON-TUMOR PATHOLOGY	13 (93%)	26 (90%)	33 (100%)
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM	13 (93%)	10 (34%)	27 (82%)
TRACHEA	7	5	14
INFLAMMATION SUPPURATIVE	7	5	14
LUNG	12	10	25
BRONCHOPNEUMONIA	11	10	21
ALVEOLAR MACROPHAGES	4		5
LUNG/ALVEOLI			1
EMPHYSEMA			1
HEMATOPOIETIC SYSTEM		2 (7%)	3 (9%)
SPLEEN		1	1
AMYLOIDOSIS			1
HEMATOPOIESIS		1	
LYMPH NODE			1
HYPERPLASIA RETICULUM-CELL			1
BRONCHIAL LYMPH NODE		1	1
PHAGOCYTIC CELL			1
LYMPHOID HYPERPLASIA		1	
MESENTERIC LYMPHNODE			1
HYPERPLASIA RETICULUM-CELL			1
CIRCULATORY SYSTEM		7 (24%)	4 (12%)
MYOCARDIUM		7	4
INFLAMMATION INTERSTITIAL		1	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE D1 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION SUPPURATIVE		4	1
FIBROSIS		4	3
FIBROSIS FOCAL			1
CALCIFICATION			1
AORTA		1	
THROMBOSIS		1	
DIGESTIVE SYSTEM	3 (21%)	1 (3%)	7 (21%)
LIVER	2	1	3
NECROSIS FOCAL		1	
METAMORPHOSIS FATTY			1
HEPATOCYTOMEGALY			1
HYPERPLASIA NODULAR			2
ANGIECTASIS	1		1
HEMATOPOIESIS	1		
PANCREATIC ACINUS			2
ATROPHY			2
ESOPHAGUS	1		
INFLAMMATION SUPPURATIVE	1		
LARGE INTESTINE	1		2
NEMATODIASIS	1		2
URINARY SYSTEM	3 (21%)	10 (34%)	19 (58%)
KIDNEY	2	10	18
INFLAMMATION CHRONIC	2	10	18
AMYLOIDOSIS	1		
URINARY BLADDER	1	1	1
HYPERPLASIA EPITHELIAL	1	1	1
DYSPLASIA EPITHELIAL	1	1	1
ENDOCRINE SYSTEM	1 (7%)	3 (10%)	6 (18%)
ADRENAL CORTEX	1		
HYPERPLASIA	1		
THYROID		3	6
INFLAMMATION FOCAL GRANULOMATOUS		1	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE D1 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA C-CELL		2	4
HYPERPLASIA FOLLICULAR-CELL			2
REPRODUCTIVE SYSTEM	1 (7%)		1 (3%)
PREPUTIAL GLAND	1		1
INFLAMMATION CHRONIC	1		1
NERVOUS SYSTEM	1 (7%)	9 (31%)	10 (30%)
BRAIN	1	9	10
HEMORRHAGE		1	
CORPORA AMYLACEA	1	8	10
SPECIAL SENSE ORGANS			1 (3%)
MIDDLE EAR			1
INFLAMMATION SUPPURATIVE			1
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS	1 (7%)	1 (3%)	
MULTIPLE SITES	1		
LYMPHOID HYPERPLASIA	1		
MULTIPLE ORGANS	1	1	
PERIARTERITIS		1	
HYPERPLASIA RETICULUM-CELL	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	1	
NO NECROPSY PERFORMED	1	6	2

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE D1 MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
AUTO/NECROPSY PERF/HISTO PERF		1	

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED PHENFORMIN IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING	3		
ANIMALS NECROPSIED	12(100%)	33(100%)	33(100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	12	33	33
ANIMALS WITH NON-TUMOR PATHOLOGY	12 (100%)	25 (76%)	30 (91%)
INTEGUMENTARY SYSTEM			1 (3%)
SKIN			1
METAPLASIA SQUAMOUS			1
RESPIRATORY SYSTEM	10 (83%)	16 (48%)	21 (64%)
TRACHEA	3	4	8
INFLAMMATION SUPPURATIVE	3	4	8
LUNG	10	15	21
HEMORRHAGE			1
BRONCHOPNEUMONIA	9	6	11
BRONCHOPNEUMONIA FOCAL		1	
INFLAM SUPPURATIVE GRANULAMATOUS		1	4
ALVEOLAR MACROPHAGES	1	7	6
HYPERPLASIA ALVEOLAR-CELL			1
HEMATOPOIETIC SYSTEM	1 (8%)		2 (6%)
SPLEEN	1		1
HEMATOPOIESIS	1		1
LYMPH NODE	1		
LYMPHOID HYPERPLASIA	1		
MESENTERIC LYMPHNODE			1
HYPERPLASIA RETICULUM-CELL			1
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM		6 (18%)	6 (18%)

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE D2 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
LIVER		2	2
NECROSIS FOCAL		1	
HEPATOCYTOMEGALY			1
HEMATOPOIESIS		1	1
PANCREAS		2	3
CYSTIC DUCTS		2	1
INFLAMMATION ACUTE FOCAL			1
ABSCESS			1
INFLAMMATION CHRONIC			1
PANCREATIC ACINUS		2	1
ATROPHY		2	1
LARGE INTESTINE		3	
NEMATODIASIS		3	
RECTUM			1
INFLAMMATION CHRONIC			1
ANUS			1
HYPERPLASIA PSEUDOEPITHELIOMATOU			1
URINARY SYSTEM	5 (42%)	7 (21%)	9 (27%)
KIDNEY	5	7	8
INFLAMMATION CHRONIC	5	7	8
URINARY BLADDER			2
HYPERPLASIA EPITHELIAL			2
DYSPLASIA EPITHELIAL			2
ENDOCRINE SYSTEM	2 (17%)	1 (3%)	1 (3%)
ADRENAL MEDULLA	1		
HYPERPLASIA FOCAL	1		
THYROID	1	1	1
HYPERPLASIA FOLLICULAR-CELL	1	1	1
REPRODUCTIVE SYSTEM	8 (67%)	10 (30%)	18 (55%)
UTERUS/ENDOMETRIUM	7	9	14
INFLAMMATION SUPPURATIVE	2		3

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE D2 FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA	1		
HYPERPLASIA CYSTIC	6	9	12
HYPERPLASIA STROMAL		1	
METAPLASIA SQUAMOUS	1		
OVARY	3	4	4
CYST		4	3
HEMORRHAGE			1
INFLAMMATION SUPPURATIVE	1	1	
ABSCESS	2		
NERVOUS SYSTEM		2 (6%)	5 (15%)
BRAIN		2	5
HEMORRHAGE		1	
CORPORA AMYLACEA		1	5
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			1 (3%)
PERITONEUM			1
ABSCESS			1
ALL OTHER SYSTEMS		1 (3%)	
MESENTERY		1	
ABSCESS		1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		6	1
ANIMAL MISSING/NO NECROPSY PERF	3		
NO NECROPSY PERFORMED		2	1
AUTO/NECROPSY PERF/HISTO PERF		1	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE D2 FEMALE MICE. NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
AUTOLYSIS/NO NECROPSY PERFORMED			1

☆ U. S. GOVERNMENT PRINTING OFFICE : 1977 241-161/3052

DHEW Publication No. (NIH) 77-807