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PHENESTERIN

FOR POSSIBLE CARCINOGENICITY

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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
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Bethesda, Maryland 20014

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FOREWORD: This report presents the results of the bioassay of phenesterin 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: The bioassay was conducted by Southern Research Institute, Birmingham, Alabama, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

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

Animal pathology tables and survival tables were compiled by EG&G

Mason Research Institute[4]. The statistical analyses were performed by Dr. J. R. Joiner[5], using methods selected for the bioassay program by Dr. J. J. Gart[6]. Chemicals used in this bioassay were analyzed by Mr. E. Brunson[7] and Ms. N. Gross[7], and the analytical results were reviewed by Dr. C. W. Jameson[5].

This report was prepared at Tracor Jitco[5] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg, Director of the Bioassay Program; Dr. L. A. Campbell, Deputy Director for Science; 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.

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

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

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SUMMARY

A bioassay of phenesterin for possible carcinogenicity was conducted by administering the chemical by gavage to Sprague-Dawley rats and B6C3F1 mice.

Groups of 35 rats of each sex were administered phenesterin at one of two doses, either 5 or 10 mg/kg body weight, three times per week for 52 weeks, then observed for an additional 32 or 33 weeks. The vehicle used was 0.05% polysorbate 80 in buffered saline. Controls consisted of groups of 10 rats of each sex which received the vehicle (vehicle control) and 10 rats of each sex which were untreated (untreated control). All surviving rats were killed at 84 or 85 weeks.

Groups of 35 mice of each sex were administered the chemical at one of two doses, either 15 or 30 mg/kg body weight, three times per week for 52 weeks. The males receiving 15 mg/kg were observed for an additional period of 29 weeks, and those surviving to this time were then killed; the animals of the remaining groups were observed for additional periods of only 10-22 weeks, due to early deaths. Seventy-seven weeks after the foregoing groups were started, additional groups of 40 mice of each sex were started and were administered the chemical at 7 mg/kg body weight three times per week; administration of the chemical terminated at week 102 for the males and at week 88 for the females, due to deaths of all females at this time. Controls for the low-dose (7 mg/kg) groups of mice consisted of groups of 20 mice of each sex which received the vehicle (vehicle control) and 20 mice of each sex which were untreated (untreated control); controls for the mid-dose (15 mg/kg) and the high-dose (30 mg/kg) controls consisted of groups of 15 mice of each sex similarly receiving the vehicle or untreated. All surviving low-dose controls were killed at 104 weeks, and all surviving mid- and high-dose controls were killed at 81-84 weeks.

Phenesterin was toxic to rats and mice at the doses used, as shown by reduced mean body weights and survival. Time-adjusted analyses were used for evaluation of incidences of tumors in the female mice.

In female rats, a dose-related trend ($P = 0.019$) was present in adenocarcinoma of the mammary gland, using the pooled controls, and the incidences of the tumor in the individual dosed groups were significant ($P \leq 0.009$) when compared with those in the pooled controls (controls 1/18, low-dose 12/29, high-dose 12/30).

In male mice, the incidence of alveolar/bronchiolar carcinomas or combined alveolar/bronchiolar adenomas and carcinomas in the low-dose group (18/40) was significantly higher ($P \leq 0.020$) than that in the low-dose vehicle-control group (0/16). In female mice, seven low-dose animals had alveolar/bronchiolar adenomas and eight other low-dose animals had alveolar/bronchiolar carcinomas. When these tumors were combined, their time-adjusted incidence was significant ($P = 0.004$) when compared with that in the low-dose vehicle controls (controls 1/18, low-dose 15/35). The lower and nonsignificant incidences of these tumors observed in the mid- and high-dose groups may be due to the earlier mortality in these groups compared with the low-dose groups.

In each sex of mid- and high-dose mice, incidences of lymphoma and leukemia were dose related ($P \leq 0.005$), using vehicle controls; they were also significant ($P \leq 0.018$) in direct comparisons of mid- and high-dose groups of both sexes with respective vehicle controls (males: controls 0/14, mid-dose 9/29, high-dose 11/25; females, time-adjusted: controls 0/15, mid-dose 14/18, high-dose 17/19). The significance of the incidence of lymphoma and leukemia in the mid- and high-dose groups of males was increased ($P \leq 0.001$) when the pooled-control group was used, both in the test for dose-related trend and in tests for direct comparisons of dosed groups with the controls.

In each sex of mice, sarcomas of the myocardium were found in all groups of dosed animals, but in no control animals (males: low-dose 5/40, mid-dose 7/29, high-dose 2/25; females: low-dose 8/34, mid-dose 2/7, high-dose 3/7). In males, the incidence in the mid-dose group was significant when compared with that in the pooled controls ($P = 0.006$); in females, the incidences in the low- and high-dose groups were significant ($P \leq 0.023$).

It is concluded that under the conditions of this bioassay, phenesterin was carcinogenic in female Sprague-Dawley rats, producing adenocarcinomas of the mammary gland, and in both sexes of B6C3F1 mice, producing alveolar/bronchiolar carcinomas, hematopoietic tumors, and myocardial sarcomas.

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I. INTRODUCTION

Phenesterin (CAS 3546-10-9; NCI C01558), an experimental anticancer agent, is a steroidal alkylating agent composed of the carboxylic acid ester of cholesterol and an aryl nitrogen mustard (Wall et al., 1969). The linkage to cholesterol was selected to improve the lipophilicity of the compound, and thereby, to facilitate its transport across cell membranes. Phenesterin has shown antitumor activity against a number of rat mammary tumors and leukemias and has been tested in clinical trials (Ansfield et al., 1971). Phenesterin was selected for testing for carcinogenic activity because of the possibility that, as an anticancer agent, it would be used on a chronic basis.

II. MATERIALS AND METHODS

A. Chemical

The phenesterin (cholesteryl p-bis(2-chloroethyl)aminophenylacetate) used in the chronic study was obtained from the Upjohn Company, North Haven, Connecticut, as Lot Nos. 10328-BDA-8 and 10328-BDA-612. The identity and purity of Lot No. 10328-BDA-8 was confirmed in analyses at the University of Tennessee College of Pharmacy. The melting point for this lot was 89-92°C, which was consistent with the reported value of 90-90.5°C (Merck Index, 1976). Four trace impurities were detected by thin-layer chromatography; no attempt was made to identify or quantitate these impurities. Infrared and nuclear magnetic resonance spectra were in agreement with the structure.

The bulk chemical was stored in the presence of a desiccant (Drierite®) at 5°C.

B. Dosage Preparation

The dosage mixtures of the phenesterin were prepared fresh for each administration. The chemical was suspended in a buffered saline vehicle by mixing in a Potter-Elvehjem tissue grinder. The buffered saline vehicle (pH 6.9) contained 0.85% NaCl, 0.40% NaH_2PO_4, 0.65% Na_2HPO_4, and 0.05% polysorbate 80.

No concentration or stability analyses of the chemical in the buffered saline vehicle were performed.

C. Animals

Male Sprague-Dawley rats and male Swiss mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, for use in the subchronic studies.

For the chronic studies, male and female Sprague-Dawley rats and male and female B6C3F1 mice were obtained from Charles River Breeding Laboratories under a contract with the Division of Cancer Treatment, National Cancer Institute. Male rats were 30 days of age, female rats were 37 days of age, and male and female mice were 31 days of age on arrival at the laboratory. Animals were quarantined (rats for 5 days, mice for 12 days) prior to the start of the chronic studies. B6C3F1 mice that were started later in the study were received from Litton Bionetics, Inc., at 29 days of age and were quarantined for 12 days. At the end of the quarantine period, all animals with no visible signs of disease were 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%. The room air was changed 15 times per hour and passed through both intake and exhaust fiberglass roughing filters. In addition to natural light, illumination was provided by fluorescent light for 9 hours per day. Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) and water were supplied daily and were available ad libitum.

Rats were housed five per cage and mice seven per cage in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). The rat cages were provided with Iso-Dri® hardwood chip bedding (Carworth, Edison, N.J.), and cage tops were covered with disposable filter bonnets beginning at week 17; mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N.Y.). Low-dose mice and the vehicle controls were housed similarly, except for the final 4 months of the study, during which time they were housed in cages provided with a hardwood chip bedding (Betta-Chip, Northeastern Products Corp., Warrenton, N.Y.). Bedding was replaced once or twice per week; cages, water bottles, and feeders were sanitized at 82°C once per week; racks were cleaned once per week.

The rats and mice were housed in separate rooms. Control animals were housed with respective dosed animals. Animals administered

phenesterin were maintained in the same rooms as animals of the same species being dosed with the following chemicals:

RATS

Gavage Studies

estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate) (estradiol mustard) (CAS 22966-79-6)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride (MAAM) (NSC 141549)
acronycine (CAS 7008-42-6)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGdR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride (phenoxybenzamine hydrochloride) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)

MICE

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide (acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide (chlorpropamide) (CAS 94-20-2)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (pyrimethamine) (CAS 58-14-0)

2,6-diamino-3-(phenylazo)pyridine hydrochloride (phenazopyridine hydrochloride) (CAS 136-40-3)
L-tryptophan (CAS 73-22-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea (tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
4,4′-thiodianiline (CAS 139-65-1)
ethionamide (CAS 536-33-4)
reserpine (CAS 50-55-5)

Gavage Studies

estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate) (estradiol mustard) (CAS 22966-79-6)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride (MAAM) (NSC 141549)
acronycine (CAS 7008-42-6)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGdR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride (phenoxybenzamine hydrochloride) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)
adriamycin (CAS 23214-92-8)

E. Subchronic Studies

Subchronic studies were conducted with male Sprague-Dawley rats

and male Swiss mice to estimate the maximum tolerated doses of phenesterin, on the basis of which "low" doses and "high" doses were determined for the chronic studies. Phenesterin was administered by gavage in a vehicle of 0.05% polysorbate 80 in saline. Rats received doses of 1.0, 2.5, 5.0, 10.0, or 20.0 mg/kg and mice received doses of 1.7, 4.25, 8.5, 17.0, 34.0, 68.0, or 136.0 mg/kg. Animals were administered the chemical three times per week for 45 days, and were observed for 45 days following the administration of the chemical. Five animals of each species were tested at each dose (except for 10 animals tested at 34.0 mg/kg), 10 rats and 5 mice were maintained as untreated controls, and 10 rats and 10 mice received the vehicle only.

In rats, one animal administered 20 mg/kg died during week 10 of the study; there were no deaths at lower doses. Mean body weight gains were depressed 10% at 2.5 mg/kg, 11% at 5.0 mg/kg, 20% at 10 mg/kg, and 34% at 20 mg/kg by the end of the period of administration of the chemical. Mean weight gains remained depressed during the observation period. No gross abnormalities were seen at necropsy. The low and high doses for the chronic studies using rats were set at 5 and 10 mg/kg.

Mean weight gains in dosed mice did not show any trends and were similar to those of controls in all except the highest dosed

group (136 mg/kg) where weight gain during the period of administration of the chemical was depressed 45%. Except for accidental deaths, there were no other deaths at any of the doses tested. No gross abnormalities were observed at necropsy. Because the toxic effects of this drug were known to be cumulative, the high dose was set lower than would otherwise be predicted from these data. The low and high doses for the chronic studies using mice were set at 15 and 30 mg/kg.

F. Designs of Chronic Studies

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

Since the numbers of animals in the untreated- and vehicle-control groups of rats and in the mid- and high-dose untreated- and vehicle-control groups of mice were small, pooled vehicle-control groups of rats and mice also were used for statistical comparisons. The groups of 10 vehicle-control rats of each sex from the bioassay of phenesterin were combined with corresponding groups of 10 vehicle-control rats of each sex from a similar bioassay of estradiol mustard. The pooled controls for statistical tests using rats thus consisted of 20 males and 20 females. The groups of 15 mid- and high-dose vehicle-control mice of each sex from the bioassay of phenesterin were combined with the corresponding groups of 15 mid- and high-dose vehicle-control

Table 1. Design of Chronic Studies of Phenesterin in Rats

Sex and Test Group	Initial No. of Animals[a]	Phenesterin Dose[b] (mg/kg)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male				
Untreated-Control	10	0		85
Vehicle-Control	10	0[c]	52	33
Low-Dose	35	5	52	32-33
High-Dose	35	10	52	32
Female				
Untreated-Control	10	0		85
Vehicle-Control	10	0[c]	52	33
Low-Dose	35	5	52	33
High-Dose	35	10	52	32

[a]Male rats were 35 days of age and female rats were 42 days of age when placed on study.

[b]Phenesterin was administered by gavage in a vehicle of 0.05% polysorbate 80 in buffered saline three times per week at a volume of 0.25 ml/100 g body weight. Doses were based on individual weights.

[c]Vehicle-control groups received only the vehicle of 0.05% polysorbate 80 in buffered saline at the same volume and on the same schedule as dosed rats.

Table 2. Design of Chronic Studies of Phenesterin in Mice

Sex and Test Group	Initial No. of Animals[a]	Phenesterin Dose[b] (mg/kg)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male				
Mid- and High-Dose Untreated-Control	15	0		83-84
Mid- and High-Dose Vehicle-Control	15	0[c]	52	29-30
Low-Dose Untreated-Control[d]	20	0		104
Low-Dose Vehicle-Control[d]	20	0[c]	103	1
Low-Dose[d]	40	7	102[e]	
Mid-Dose	35	15	52	29
High-Dose	35	30	52	22[f]
Female				
Mid- and High-Dose Untreated-Control	15	0		84
Mid- and High-Dose Vehicle-Control	15	0[c]	52	30-31
Low-Dose Untreated-Control[d]	20	0		104
Low-Dose Vehicle-Control[d]	20	0[c]	103	1
Low-Dose[d]	40	7	88[e]	
Mid-Dose	35	15	52	10[f]
High-Dose	35	30	52	10[f]

[a]High- and mid-dose mice and their controls were 43 days of age when placed on study; low-dose mice and their controls were 41 days of age.

[b]Phenesterin was administered by gavage in the vehicle of 0.05% polysorbate 80 in buffered saline three times per week at a volume of 0.1 ml/10 g body weight. Doses were based on the mean weight of the animals in each cage.

[c]Vehicle-control groups received only the vehicle of 0.05% polysorbate 80 in buffered saline at the same volume and on the same schedule as dosed mice.

Table 2. Design of Chronic Studies of Phenesterin in Mice

(continued)

[d]The low-dose groups and their controls were started 77 weeks after the mid- and high-dose groups, because of deaths in these groups.

[e]Administration of the chemical terminated at the time indicated, due to death of all animals.

[f]Observation terminated at the time indicated, due to death of all animals.

mice of each sex from a similar bioassay of estradiol mustard to give pooled-control groups of 30 mid- and high-dose vehicle-control mice of each sex. The vehicle-control groups of rats and the vehicle-control groups of mice that were used in the respective pooled-control groups were each of the same strain, obtained from the same supplier, and examined by the same pathologists. Further, the different control groups were placed on study at starting times differing by no more than 3 months.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and animals that were moribund were killed and necropsied, except for those dying prior to day 100, due, presumably, to toxicity of the test chemical. Rats were weighed once per week for 4 weeks and once every 2 weeks thereafter; mid- and high-dose mice were weighed every 2 weeks throughout the study; low-dose mice were weighed every 2 weeks for 34 weeks and once per month thereafter. Palpation for masses was carried out at each weighing.

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

thymus, heart, salivary gland, liver, gallbladder and bile duct (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, brain, and sensory organs. Peripheral blood smears were prepared from each animal whenever possible. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

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

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data

System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of 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 narr-

ative 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)

The mean body weights of the dosed male and female rats were lower than those of the untreated or vehicle controls throughout most of the study, especially in the high-dose groups (figure 1). Fluctuations in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. No other signs of toxicity related to the administration of the chemical were recorded.

Because of respiratory disease in the colony, oxytetracycline was administered in drinking water at 0.6 mg/ml during weeks 17-23 and at 0.3 mg/ml during weeks 23-27.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered phenesterin by gavage at the doses of this bioassay, together with those of the matched controls, are shown in figure 2.

In each sex, the Tarone test result for positive dose-related trend in mortality is significant ($P < 0.001$), using the high-dose, low-dose, and vehicle-control groups. In the males, 4/35 (11%) of the high-dose group, 8/35 (23%) of the low-dose

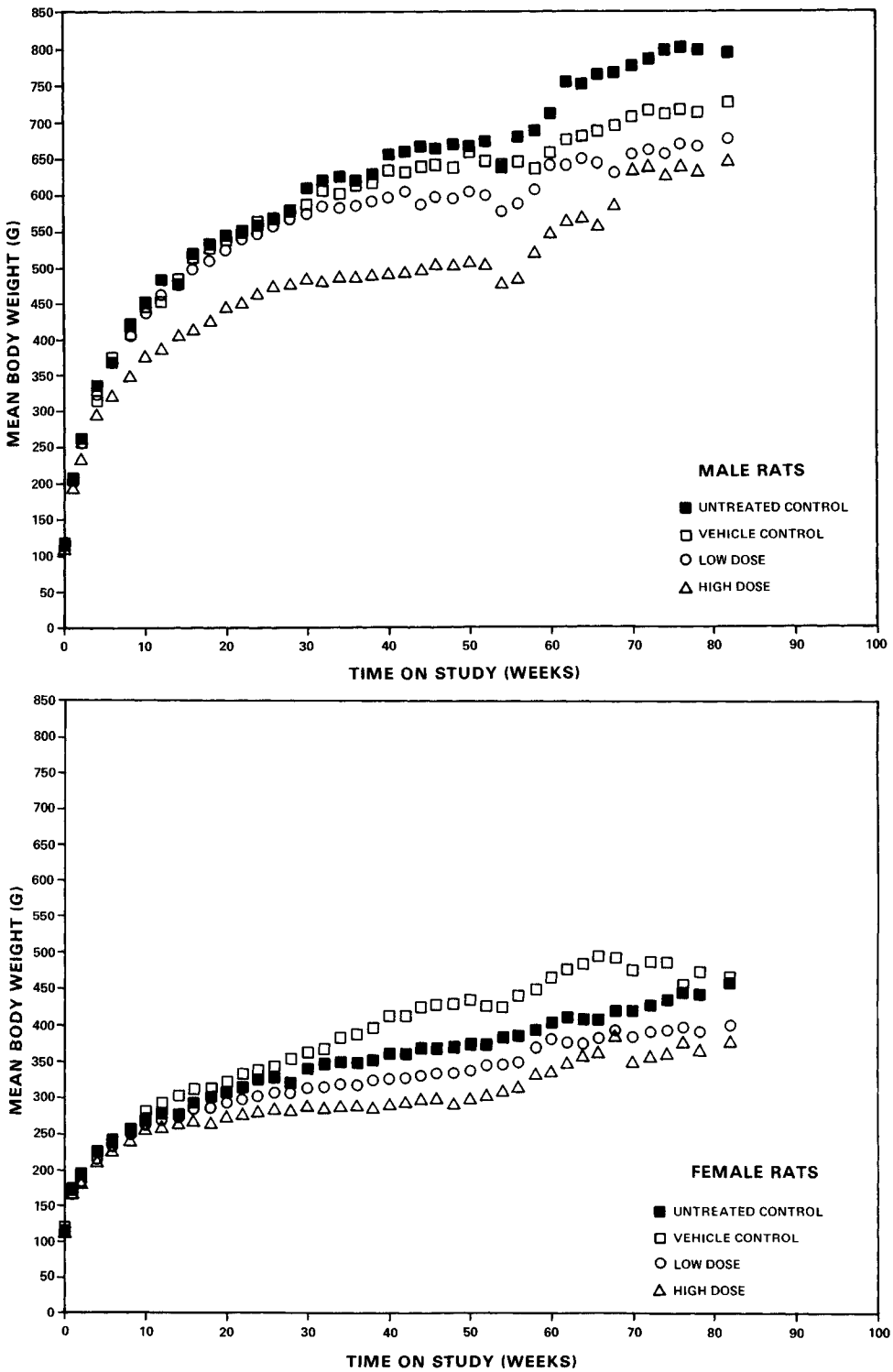

Figure 1. Growth Curves For Rats Administered Phenesterin by Gavage

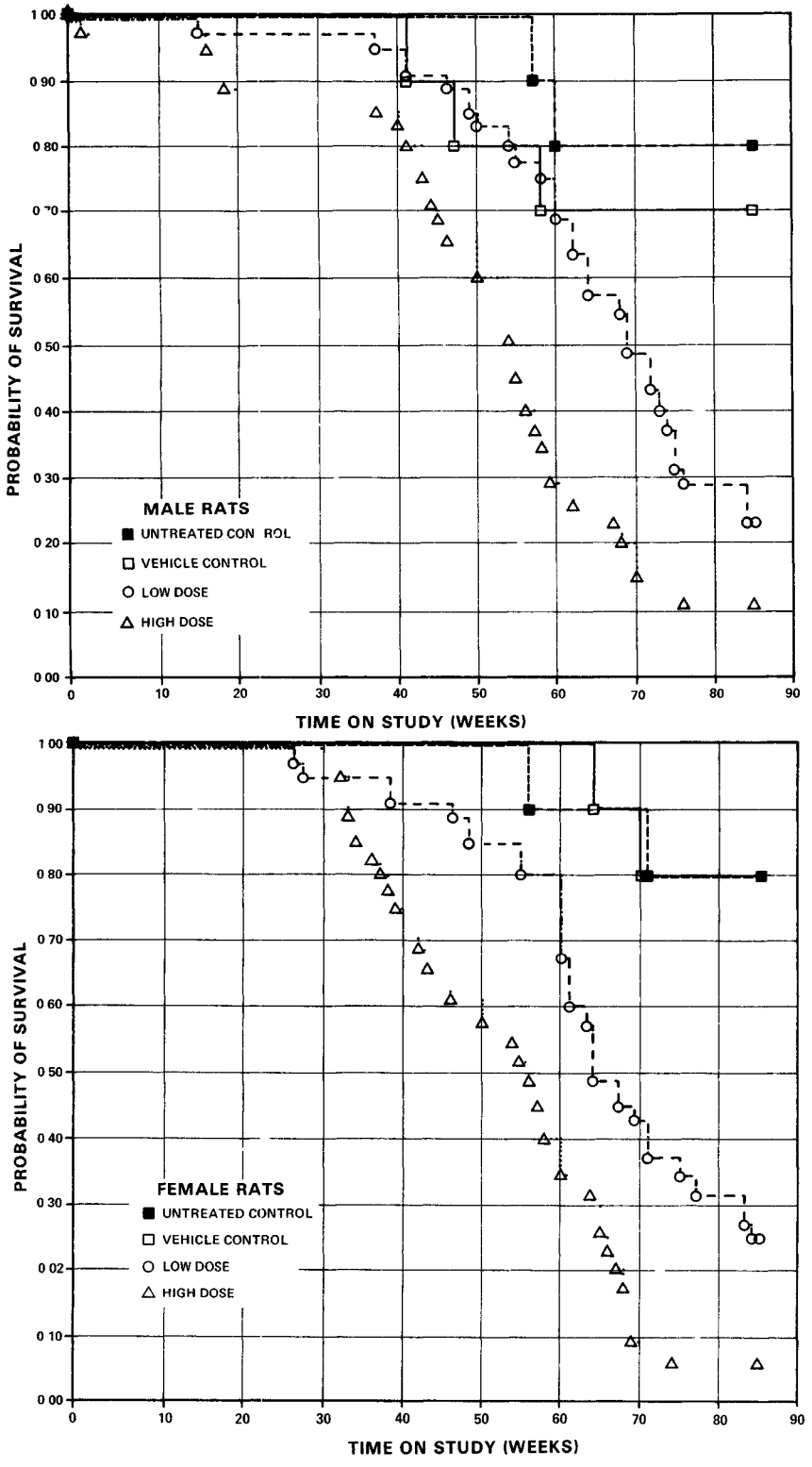

Figure 2. Survival Curves for Rats Administered Phenesterin by Gavage

group, and 7/10 (70%) of the controls were alive at the end of the bioassay. In females, 2/35 (6%) of the high-dose group, 9/35 (26%) of the low-dose group, and 8/10 (80%) of the controls were alive at the end of the bioassay. Over 50% of each group of males or females survived at least 52 weeks. The untreated-control groups are not used in these comparisons, since the test conditions of the vehicle controls more closely resembled those of the dosed rats; however, the Kaplan and Meier curves of the untreated-control groups are shown in figure 2.

C. Pathology (Rats)

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

A variety of neoplasms occurred in both the control (untreated and vehicle) and dosed groups. These lesions, however, are not uncommon in this strain of rat independent of any treatment. The following sarcomas occurred primarily in the dosed groups:

	RATS			
	MALE		FEMALE	
	Low Dose	High Dose	Low Dose	High Dose
Number of Animals Necropsied	(32)	(30)	(29)	(30)
Subcutis				
-sarcoma, NOS*	2	1	0	1
-fibrosarcoma	1	0	1	0
Bone				
-osteosarcoma	1	1	0	0
Abdominal cavity				
-sarcoma, NOS	1	2	1	0
-hemangiosarcoma	1	0	0	0
Multiple organs, other				
-sarcoma, NOS	1	0	0	0
-sarcoma, NOS, metastatic (primary unknown)	0	0	0	1
Mammary gland				
-sarcoma, NOS	0	0	0	1
Cranial Cavity				
-sarcoma, NOS	0	0	0	1
Number of Animals With Tumors	7	4	2	4

*Not otherwise specified

These sarcomas included fibrosarcomas, osteosarcomas, hemangiosarcomas, and undifferentiated sarcomas, and they occurred more frequently in males than in females. Metastases to the lungs occurred in two high-dose males. Only one control animal, an untreated male, had a sarcoma (abdominal cavity).

Adenocarcinomas and fibroadenomas of the mammary gland were the

most frequent tumors in female rats. The incidence was as follows:

	RATS			
	Untreated Control	Vehicle Control	Low Dose	High Dose
FEMALE				
Number of Animals Necropsied	(10)	(10)	(29)	(30)
Mammary Gland				
-adenocarcinoma	0	1	12	12
-fibroadenoma	6	4	21	11
Number of Animals With Tumors	6	5	26	19

Metastases to the lung occurred in one high-dose animal.

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

Based on this histopathologic examination, phenesterin administered by gavage to Sprague-Dawley rats at doses of 5 or 10 mg/kg was associated with an increased frequency of tumors of the mammary gland, especially adenocarcinomas, in dosed females.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and with an incidence of at least 5% in one or more than one group. Vehicle-control groups and pooled vehicle-control groups are used in the statistical analysis. The untreated controls are not included in the tables and analyses, since the test conditions of the vehicle controls more closely resemble those of the dosed rats.

In male rats, the results of the Cochran-Armitage test for positive dose-related trend in the incidence of animals with lymphoma or leukemia are significant ($P = 0.037$) using the pooled controls, but the results of the Fisher exact test are not significant. Statistical test results on this incidence of tumors in female rats are not significant.

In female rats, the results of the Cochran-Armitage test on the incidence of adenocarcinomas of the mammary gland are significant ($P = 0.019$) when the pooled vehicle-control group is used. The Fisher exact tests show that the incidences in both of the dosed groups are significantly higher ($P < 0.010$) than the incidence in the pooled controls. These tumors of the mammary gland were first observed as early as 26 weeks in the low-dose group and 33

weeks in the high-dose group. The statistical conclusion is that this incidence of tumors in female rats is dose associated.

The results of the Cochran-Armitage test and the Fisher exact test on the incidence of fibroadenomas of the mammary gland in female rats are not significant. Significant results in the negative direction are observed in the incidence of chromophobe adenomas of the pituitary in female rats, where the incidences in the control groups exceed those in the dosed groups. Time-adjusted analysis, eliminating animals that died before 52 weeks on study, indicated similar levels of significance in the negative direction. The time-adjusted data are 10/16 (63%) of the pooled controls, 6/10 (60%) of the vehicle controls, 4/24 (17%) of the low-dose group, and 0/16 of the high-dose group. Survival in the dosed groups was shorter than in the controls, and this shortened survival may account for the low incidence of the pituitary tumors in the dosed groups.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

The mean body weights of the low-dose groups of dosed male and female mice were unaffected during the first 20-30 weeks, but for the males, were lower than those of the vehicle controls, and for the females, were lower than those of both the untreated and vehicle controls, for the remaining period of the bioassay (figures 3 and 4). Progressive weight loss was recorded in several individual animals of each sex. The mean body weights of the mid- and high-dose groups of each sex were lower than those of the controls throughout most of the study. Fluctuations in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. No other signs of toxicity related to the administration of the chemical were recorded.

Some of the animals in the low-dose groups, together with corresponding controls, had signs of respiratory disease. These groups were administered oxytetracycline in the drinking water at 0.6 mg/ml during weeks 14-15 and at 0.3 mg/ml during week 15. To reduce the transmission of airborne microorganisms, propylene glycol was vaporized in the mouse room during weeks 14-25.

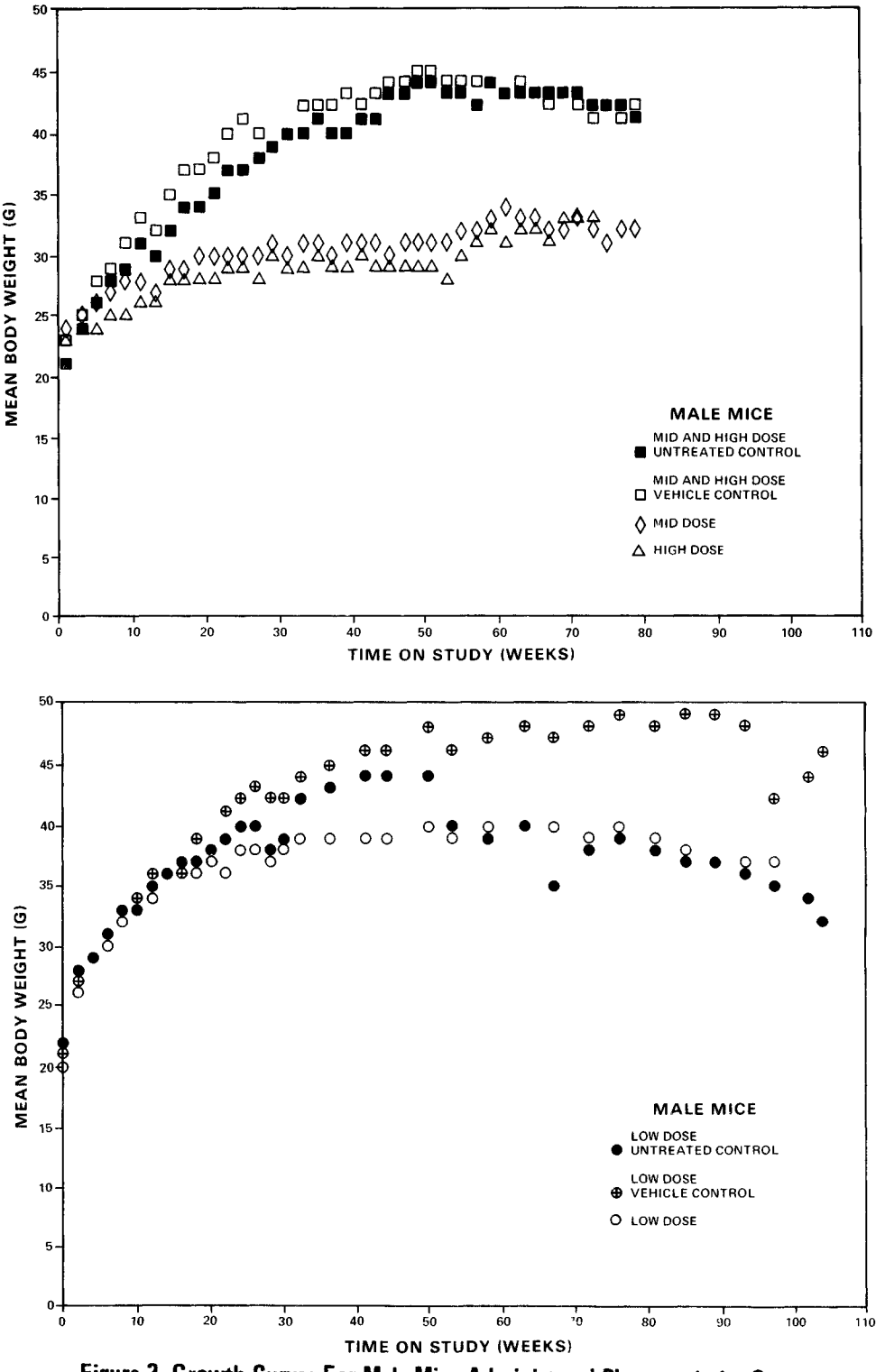

Figure 3. Growth Curves For Male Mice Administered Phenesterin by Gavage

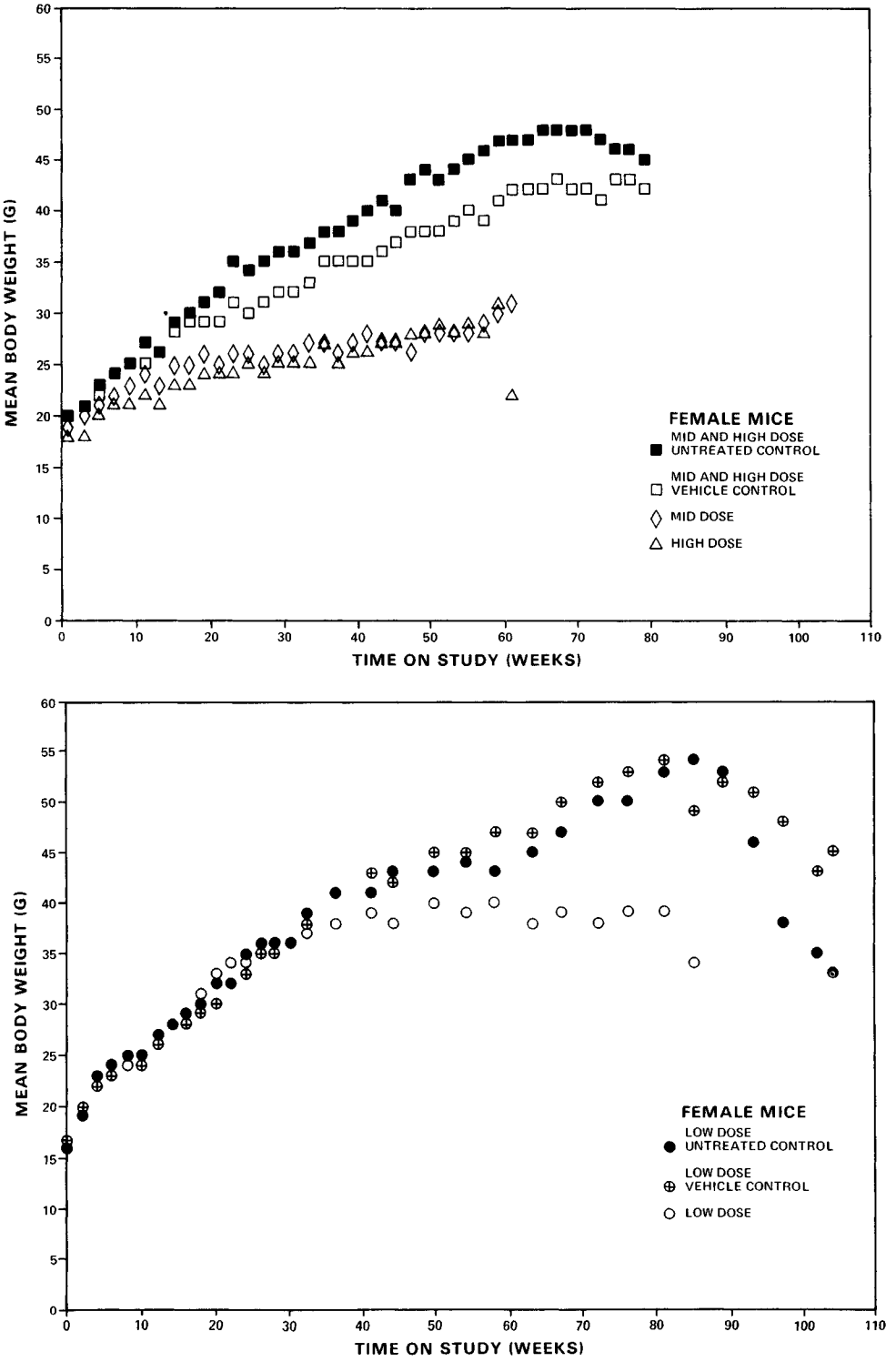

Figure 4. Growth Curves For Female Mice Administered Phenesterin by Gavage

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered phenesterin by gavage at the doses of this bioassay, together with those of the matched controls, are shown in figures 5 and 6.

In each sex, the Tarone test result for positive dose-related trend in mortality is significant ($P < 0.001$), using the high-dose, mid-dose, and corresponding vehicle-control groups. Departures from linear trend are observed ($P < 0.001$ in males and $P = 0.021$ in females), because of the sharp decrease in survival in the dosed mice. The result of the Cox test comparing the low-dose vehicle-control group and the low-dose group is not significant in male mice, whereas in female mice, it is significant ($P < 0.001$). The untreated-control groups are not used for comparison, since the test conditions of the vehicle controls more closely resembled those of the dosed mice; however, the Kaplan and Meier curves of the untreated-control groups are included in figures 5 and 6.

In male mice, none of the dosed animals were alive at the end of the study, but over 55% of them lived at least as long as 52 weeks on study (20/35 [57%] in the high-dose group, 26/35 [74%] in the mid-dose group, and 33/40 [83%] in the low-dose group).

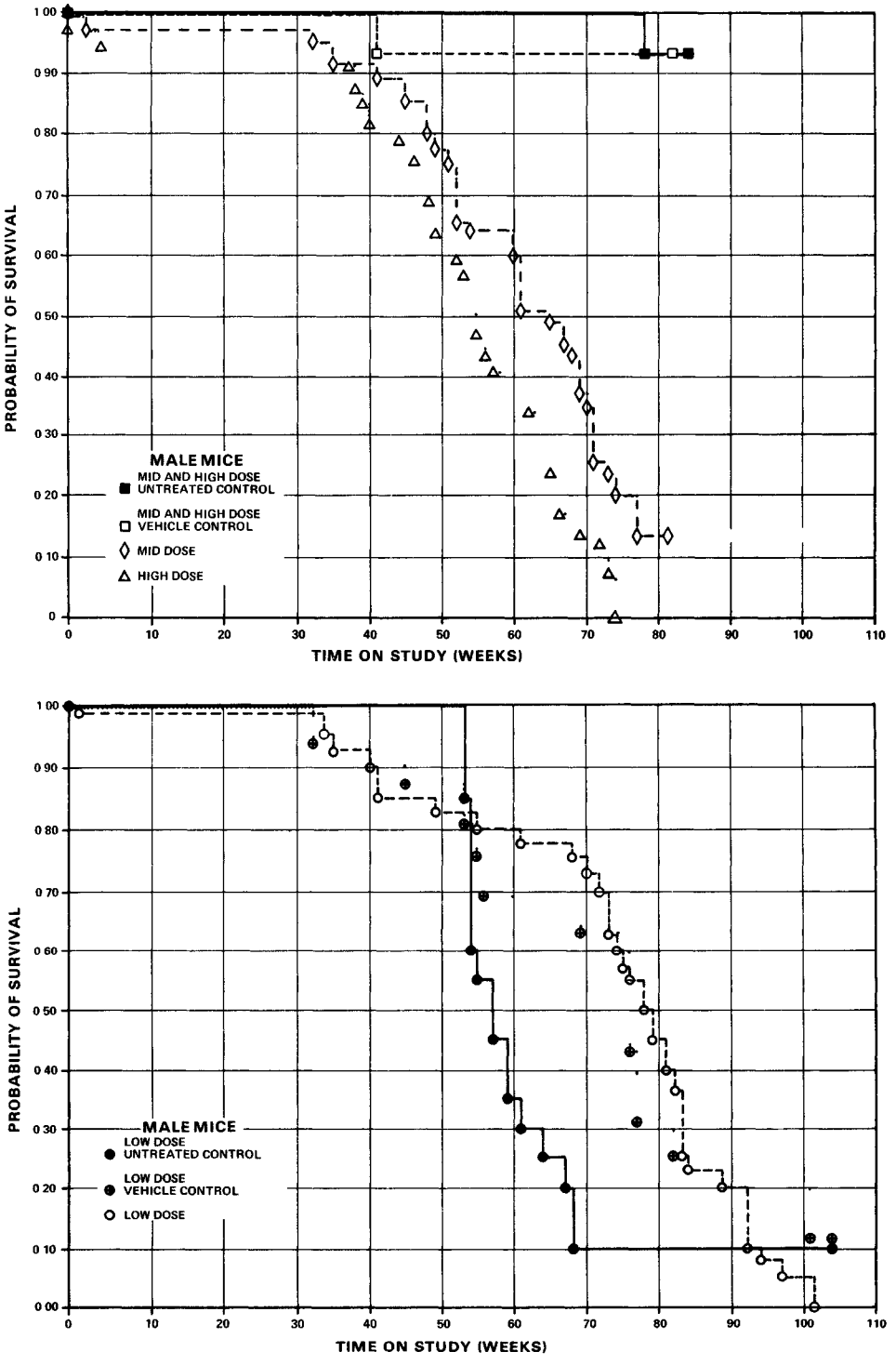

Figure 5. Survival Curves For Male Mice Administered Phenesterin by Gavage

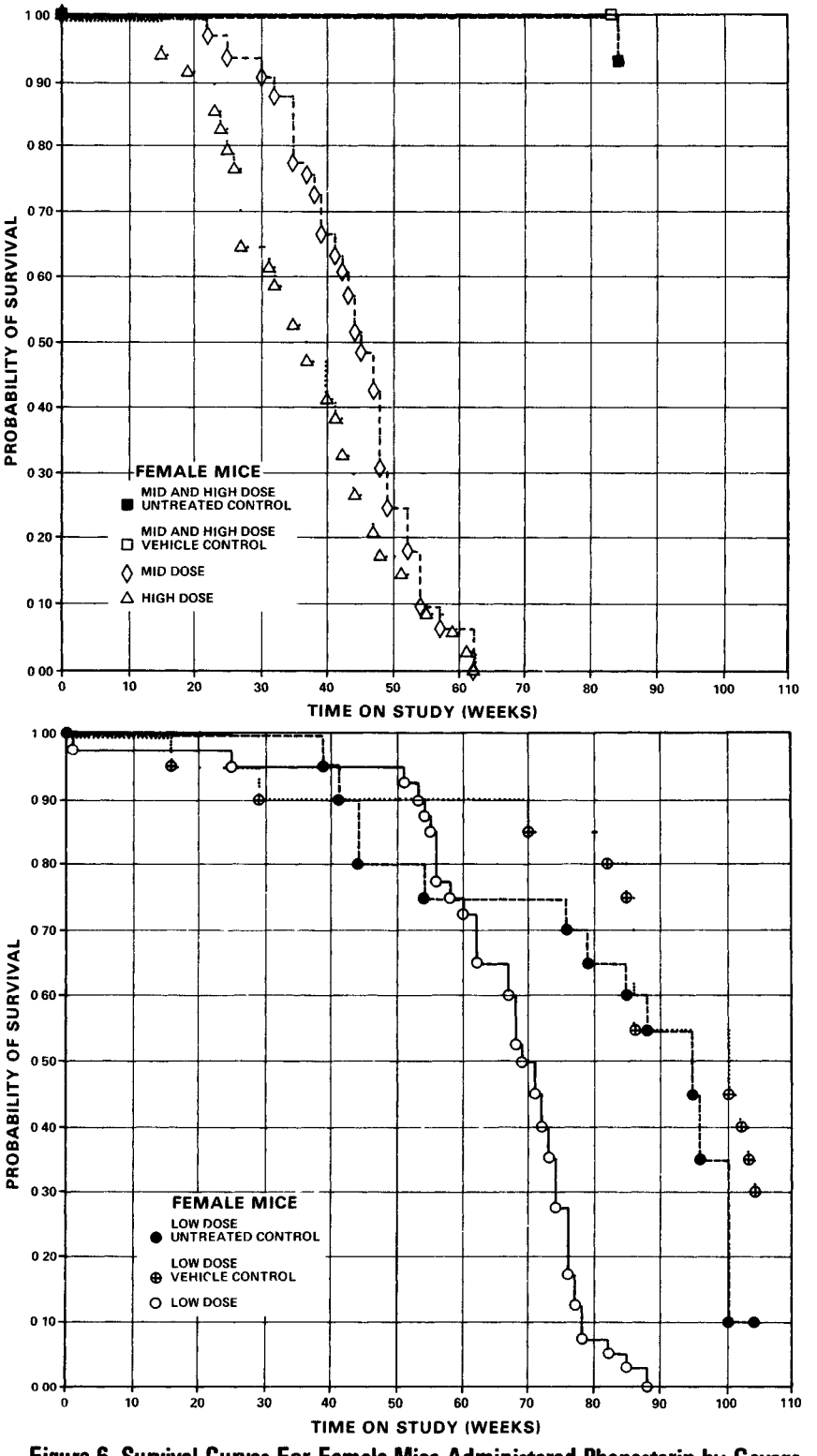

Figure 6. Survival Curves For Female Mice Administered Phenesterin by Gavage

The proportions of animals alive at week 75 are 0/35 in the high-dose group, 7/35 (20%) in the mid-dose group, and 24/40 (60%) in the low-dose group.

In females, no dosed mice lived to termination of the study. Although 37/40 (93%) of the low-dose group were alive at week 52, only 5/35 (14%) of the high-dose group and 8/35 (23%) of the mid-dose group were alive at least as long as week 52; therefore, time-adjusted analyses were performed. At week 75 all of the animals in the mid- and high-dose groups were dead and only 11/40 (28%) in the low-dose group were alive.

C. Pathology (Mice)

Histopathologic findings on neoplasms in mice are summarized in Appendix B, tables B1-B4; findings on nonneoplastic lesions are summarized in Appendix D, tables D1-D4.

A variety of neoplasms occurred both in control groups (untreated and vehicle) and in dosed groups. Some types of neoplasms occurred only in mice of dosed groups, or with a greater frequency in dosed groups when compared with controls. These lesions are not uncommon in this strain of mouse independent of any treatment. However, many of the tumors appeared to be related to the administration of the chemical. These tumors were all malignant, many had metastasized to one or more locations,

and, except for the malignant lymphomas and mammary gland adenocarcinomas, were observed only in dosed mice.

Alveolar-cell adenocarcinomas were present in 14/40 (35%) low-dose males and 8/38 (21%) low-dose females. Alveolar-cell adenomas occurred in a few animals in all dosed groups as well as in three control males and one control female. These neoplasms consisted of cuboidal to columnar cells aligned along the alveolar septa. Often the cells projected into the alveolar spaces, resulting in the formation of numerous papillary structures. Neoplastic cell nuclei ranged from small and darkly basophilic to large and vesicular. The large open-faced nuclei often contained a prominent nucleolus. Pulmonary tumors had metastasized to the liver in 1/39 (3%) males and to the mediastinal lymphatics in 1/36 (3%) females. In some of the mice, the alveolar-cell adenocarcinomas were multiple in origin.

The hematopoietic neoplasms were observed in 11/25 (44%) high-dose, 9/29 (31%) mid-dose, and 11/40 (28%) low-dose males; and in 17/32 (53%) high-dose, 14/27 (48%) mid-dose, and 12/38 (32%) low-dose females. Among controls these neoplasms were observed in 1/35 (3%) untreated-control and 1/30 (3%) vehicle-control males, and in 5/35 (14%) vehicle-control females, but in no other control groups. The malignant lymphomas were classified as lymphocytic, histiocytic, and mixed type. The lymphocytic type

was comprised of cells having a small, darkly basophilic to large, lightly basophilic, vesicular nucleus and a rim of eosinophilic cytoplasm. Malignant lymphomas composed of lymphoblastic (undifferentiated) cells were included in the lymphocytic type. The histiocytic type was comprised primarily of cells with a large open-faced nucleus, distinct eosinophilic nucleolus, and abundant eosinophilic cytoplasm. However, some histiocytic tumors contained many cells having a smaller, pleomorphic, often elliptical or indented, nucleus. The mixed-cell type was a combination of the lymphocytic and histiocytic types. The malignant lymphomas had cellular distortion which prevented further classification. Lymphocytic leukemia was differentiated from malignant lymphoma by the diffuse infiltration of the neoplastic cells within the involved organs, especially the liver. In lymphoma, the neoplastic cells were more solid in arrangement.

The eosinophilic leukemia was characterized by marked infiltration of spleen and liver sinusoids with cells having a segmented nucleus and numerous eosinophilic cytoplasmic granules.

Myocardial sarcomas were observed in 5/40 (13%) low-dose, 7/29 (24%) mid-dose, and 2/25 (8%) high-dose males; and in 8/36 (22%) low-dose, 2/27 (7%) mid-dose, and 3/31 (10%) high-dose females. The neoplastic cells were pleomorphic and varied from those with

a small round, basophilic nucleus to those with a large, vesicular nucleus. The larger nuclei contained one or more distinct nucleoli. All cells had an abundant cytoplasm. The marked interstitial proliferation of neoplastic cells resulted in compression atrophy and necrosis of adjacent myocardial fibers. The origin of the neoplastic cells was not determined, but they appeared to arise from the perimysial connective tissue surrounding myocardial fibers or from Antischkow cells. In some of the mice, the neoplastic cells had invaded through the endocardium and existed as large tumor thrombi within the ventricles. The myocardial sarcomas had metastasized to the lungs in 3/40 (8%) low-dose, 1/29 (3%) mid-dose, and 2/25 (8%) high-dose males; and in 6/38 (16%) low-dose, 2/27 (7%) mid-dose, and 2/31 (6%) high-dose females.

Adenocarcinomas of the mammary gland were present in 6/38 (16%) low-dose and in 2/20 (10%) untreated-control females. The morphology of the mammary tumors varied considerably. The lobular type consisted of multiple compact foci of cells having a large, open-faced nucleus, prominent nucleolus, and a moderate amount of cytoplasm. The foci of cells were separated by a fine fibrovascular stroma. The acinar type was comprised of cuboidal to columnar cells aligned along a basement membrane. The lining of the acini was often several cells thick, and the center of the

acinus often contained numerous concentric layers of keratin. In 1/38 (3%) low-dose mice, the mammary adenocarcinoma had metastasized to the lungs.

Hemangiosarcomas involved the subcutaneous tissue, liver, and abdominal cavity of dosed males and females; and the lungs, spleen, and bone of dosed females only. The incidence of the vascular tumor in any one organ in a group of dosed mice was low; however, when the incidences in the various organs were added together, the combined incidence was too significant to overlook.

Hemangiosarcomas involving the liver were present in 4/39 (10%) low-dose males and 1/37 (3%) low-dose females. The endothelial lining of many hepatic sinusoids was markedly thickened, with cells having a large, open-faced nucleus, one or more nucleoli, and abundant cytoplasm. The neoplastic cells were locally invasive, resulting in compression atrophy and necrosis of hepatocytes in the centrolobular zone. The sinusoidal spaces were observed to be greatly distended.

Hemangiosarcomas involving the other organs were similar in morphology. The neoplastic cells were often large and contained an open-faced nucleus, prominent nucleolus, and abundant cytoplasm. The neoplastic cells were associated with various-sized vascular spaces, and appeared to radiate out from these

spaces. In 1/27 (4%) mid-dose females, a peritoneal hemangiosarcoma had invaded the liver and one of the kidneys.

Based on this histopathologic examination, phenesterin administered by gavage to B6C3F1 mice at doses of 7, 15, and 30 mg/kg was associated with an increase in both epithelial and nonepithelial malignant tumors. These tumors included pulmonary alveolar-cell adenocarcinomas, mammary gland adenocarcinomas, hemangiosarcomas of several organs, malignant lymphomas and leukemias, and sarcomas of the heart.

D. Statistical Analyses of Results (Mice)

Tables F1-F6 in Appendix F contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and with an incidence of at least 5% in one or more than one group. Mid- and high-dose vehicle-control groups and mid- and high-dose pooled vehicle-control groups are used in the statistical analyses. No pooled low-dose control group is used, since there are no appropriate controls to be combined. The untreated controls are not included in the tables and analyses, since the test conditions of the vehicle controls more closely resemble those of the dosed mice. Due to poor survival of the dosed female mice, time-adjusted analyses are performed, eliminating animals that died before 52 weeks on

study, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons are based exclusively on animals that survived at least as long as the animal in which the first tumor was found.

In the following paragraphs, the statistical narrative on female mice is based on time-adjusted data only.

In male mice, the Fisher exact test shows that the incidence of alveolar/bronchiolar carcinomas or combined alveolar/bronchiolar adenomas and carcinomas in the low-dose group is significantly ($P \leq 0.020$) higher than that in the low-dose vehicle controls, but this positive result is not confirmed by the incidences in the mid- or high-dose groups when compared with their controls.

In female mice, the Fisher exact comparison of the incidence of alveolar/bronchiolar adenomas and carcinomas between the low-dose group and the low-dose vehicle-control group shows a probability level of 0.004. The incidences in the mid- and high-dose groups are not significant, nor are the occurrences of carcinomas alone in any group. The lower proportions of these tumors observed in the mid- and high-dose groups of either sex might be attributed to the earlier mortality in these groups compared with the low-dose group. For example, the first tumor to be observed in the low-dose groups of either sex was at week 68, and at that point

in the bioassay, 60/70 (83%) of the mid- and high-dosed males were dead and all of the female mid- and high-dose group were dead.

In each sex of mid- and high-dose mice, the results of the Fisher exact test indicate that the incidences of lymphomas or leukemias are significantly higher in each of the dosed groups than in their respective controls. The results of the Cochran-Armitage test for positive dose-related trend are significant ($P \leq 0.005$). An indicated departure from linear trend is observed in the females ($P = 0.016$), due to the steep increase of these tumors in the dosed animals. In the low-dose groups of each sex, an increased incidence of these tumors is observed when compared with the respective control group, (males: controls 1/16 [6%], low-dose 11/40 [28%]; females: controls 5/18 [28%], low-dose 12/36 [33%]). These tumors were observed as early as 32 weeks in both the low-dose and the low-dose vehicle-control groups of male mice and as early as 15 and 22 weeks in the dosed groups of female mice. While the comparisons in the low-dose groups are not statistically significant, the incidences are in the same direction as those of the statistically significant mid- and high-dose.

The analysis of the incidence of sarcomas of the myocardium is shown in the tables. In male mice, this tumor is seen in signifi-

cant incidences only in the mid-dose group when compared with the pooled controls (P = 0.006). In females, the high- and low-dose groups were observed to have this tumor in significant incidences ($P \leq 0.023$). These data suggest an association of this tumor with administration of the chemical.

Although none of the results of the Fisher exact test of the incidence of hemangiosarcomas were statistically significant, it should be observed that each of the dosed groups of either sex had a higher incidence than the respective control group. No such tumor was reported in any control group, while dosed groups had incidences ranging from 3% to 33%.

The Fisher exact test shows that the incidence of tubular adenomas of the ovary in the low-dose females is significantly higher (P = 0.024) than that in the low-dose vehicle controls, but no such tumor was observed in the mid- and high-dose groups.

Significant results in the negative direction were observed in the incidence of hepatocellular adenomas in male mice, where the incidences in the mid- and high-dose groups are lower than those in the controls, probably due to the early mortality of the dosed animals.

In summary, while early mortality in the dosed groups may have curtailed the number of animals at risk for the development of

late-appearing tumors, the data indicate an association between the administration of the chemical and tumors of the lung, hematopoietic system, and myocardium.

V. DISCUSSION

Under the conditions of this bioassay, phenesterin was toxic to rats and mice at the doses employed, as shown by reduced mean body weights and survival. Survival was sufficient, however, for the development of significant incidences of tumors in female rats and in both sexes of mice. Time-adjusted analyses were used for evaluation of the incidences of tumors in the female mice.

In male rats, a variety of sarcomas occurred in different organs (pooled controls 1/18, vehicle controls 0/9, low-dose 7/32, high-dose 4/30). The incidences in the individual dosed groups were not statistically significant, however, when compared with either the pooled or vehicle controls. Similar tumors occurred among the female rats (pooled controls 0/18, vehicle controls 0/10, low-dose 2/29, high-dose 4/30), but also at incidences that were not statistically significant. A dose-related trend ($P = 0.037$) was present in lymphoma and leukemia in male rats, using the pooled controls; however, the incidences of these neoplasms in the individual dosed groups were not significant when compared with incidences in either the pooled or vehicle controls (pooled controls 0/18, vehicle controls 0/9, low-dose 2/32, high-dose 5/30).

In female rats, a dose-related trend ($P = 0.019$) was present in

adenocarcinoma of the mammary gland, using the pooled controls, and the incidences of the tumor in the individual dosed groups were significant ($P \leq 0.009$) when compared with those in the pooled controls (controls 1/18, low-dose 12/29, high-dose 12/30). Metastases to the lung occurred in one high-dose animal.

In male mice, the incidence of alveolar/bronchiolar carcinomas or combined alveolar/bronchiolar adenomas and carcinomas in the low-dose group (14/40) was significantly higher ($P \leq 0.020$) than that in the low-dose vehicle-control group (0/16). Alveolar/bronchiolar adenomas also occurred in the mid-dose (6/29) and high-dose (2/25) groups, but these incidences were not significant when compared with either the respective pooled or vehicle controls. In female mice, seven low-dose animals had alveolar/bronchiolar adenomas and eight other low-dose animals had alveolar/bronchiolar carcinomas. When these tumors were combined, their time-adjusted incidence was significant ($P = 0.004$) when compared with that in the low-dose vehicle controls (controls 1/18, low-dose 15/35). Only two animals in the mid- and high-dose females had alveolar/bronchiolar neoplasms. The lower incidences of these tumors observed in the mid- and high-dose groups may be due to the earlier mortality in these groups compared with the low-dose groups.

In each sex of mid- and high-dose mice, incidences of lymphomas

and leukemias were dose related ($P \leq 0.005$) using vehicle controls; they were also significant ($P \leq 0.018$) in direct comparisons of mid- and high-dose groups of each sex with respective vehicle controls (males: controls 0/14, mid-dose 9/29, high-dose 11/25; females, time-adjusted: controls 0/15, mid-dose 14/18, high-dose 17/19). The significance of the incidence of lymphomas and leukemias in the mid- and high-dose groups of males was increased ($P \leq 0.001$) when a pooled-control group was used, both in the test for dose-related trend and in tests for direct comparisons of dosed groups with the controls. In the low-dose groups of each sex, an increased incidence of these tumors was observed when compared with the respective controls (males: controls 1/16, low-dose 11/40; females: controls 5/18, low-dose 12/36). These incidences in the low-dose groups were not statistically significant.

In each sex of mice, sarcomas of the myocardium were found in all groups of dosed animals, but in no control animals (males: low-dose 5/40, mid-dose 7/29, high-dose 2/25; females: low-dose 8/34, mid-dose 2/7, high-dose 3/7). In males, the incidence in the mid-dose group was significant when compared with that in the pooled controls ($P = 0.006$); in females, the incidences in the low- and high-dose groups were significant ($P \leq 0.023$). In some of the mice, the neoplastic cells had invaded the myocardium and

formed large thrombi within the ventricles. The myocardial sarcomas had metastasized to the lungs in several animals in each dosed group. No sarcomas of the myocardium have occurred in over 500 male and 500 female historical-control mice of this strain at the laboratory.

Hemangiosarcomas were observed in mice at the following incidences (males: low-dose vehicle controls 0/20, low-dose 6/40, mid- and high-dose vehicle controls 0/14, mid-dose 1/29, high-dose 3/25; females: low-dose vehicle controls 0/18, low-dose 5/35, mid- and high-dose vehicle controls 0/15, mid-dose 1/7, high-dose 2/6). Although none of the direct comparisons of the dosed groups with controls were statistically significant, hemangiosarcomas occurred in each of the dosed groups of either sex, but in no control animals. Thus, the occurrence of this tumor may be related to administration of the test chemical.

In low-dose female mice, adenocarcinomas of the mammary gland were observed at an incidence of 6/35 and tubular adenomas of the ovary at an incidence of 8/34. Neither tumor was found in the vehicle controls or in the mid- or high-dose groups of female mice; however, there were two untreated-control mice with adenocarcinomas of the mammary gland. The different response of the low-dose mice may have resulted from their longer survival or

because they were started on study 75 weeks after the other groups studied.

Previous work showed that the toxicity of phenesterin administered by subcutaneous injection is relatively low, with an LD_{50} for rats of 2.0 g/kg for a single injection (Larionov et al., 1962); this toxicity is less than that of some of the nitrogen mustards in general use (Wall et al., 1969). Chronic administration to rats, dogs, and monkeys (Vollmer et al., 1973) resulted, however, in cumulative toxicity, manifested by weight loss and myelosuppression. Chronic treatment of cancer patients (Ansfield et al., 1971) also resulted in myelosuppression. The carcinogenicity of phenesterin has been tested in a pulmonary tumor test system in strain A mice administered the chemical intraperitoneally three times per week for 8 weeks at total doses of 2,400, 6,000, or 12,000 mg/kg (Stoner et al., 1973). Under these conditions, phenesterin induced significant incidences ($P = 0.001$) of tumors of the lung in the mice at all doses tested.

It is concluded that under the conditions of this bioassay, phenesterin was carcinogenic in female Sprague-Dawley rats, producing adenocarcinomas of the mammary gland, and in both sexes of B6C3F1 mice, producing alveolar/bronchiolar carcinomas, hematopoietic tumors, and myocardial sarcomas.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED PHENESTERIN BY GAVAGE

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED PHENESTERIN BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	9	32	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	9	32	30
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(9)	(32)	(30)
SQUAMOUS CELL PAPILLOMA				1 (3%)
*SUBCUT TISSUE	(10)	(9)	(32)	(30)
SEBACEOUS ADENOMA			1 (3%)	
SARCOMA, NOS			2 (6%)	1 (3%)
FIBROMA			1 (3%)	
FIBROSARCOMA			1 (3%)	
LIPOMA	1 (10%)			
CHONDROMA			1 (3%)	
RESPIRATORY SYSTEM				
#LUNG	(10)	(9)	(32)	(30)
SARCOMA, NOS, METASTATIC				1 (3%)
OSTEOSARCOMA, METASTATIC				1 (3%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(10)	(9)	(32)	(30)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)	
LYMPHOCYTIC LEUKEMIA			1 (3%)	3 (10%)
GRANULOCYTIC LEUKEMIA	1 (10%)			2 (7%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
NONE				

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM				
#KIDNEY	(10)	(9)	(32)	(30)
LIPOMA			1 (3%)	
ENDOCRINE SYSTEM				
#PITUITARY	(10)	(9)	(29)	(29)
CHROMOPHOBE ADENOMA			2 (7%)	
CHROMOPHOBE CARCINOMA		1 (11%)	1 (3%)	
#ADRENAL	(10)	(9)	(32)	(30)
PHEOCHROMOCYTOMA			1 (3%)	
#PANCREATIC ISLETS	(10)	(9)	(32)	(30)
ISLET-CELL ADENOMA			1 (3%)	
REPRODUCTIVE SYSTEM				
#TESTIS	(10)	(9)	(32)	(30)
INTERSTITIAL-CELL TUMOR	1 (10%)			
NERVOUS SYSTEM				
#BRAIN	(10)	(9)	(30)	(30)
ASTROCYTOMA			1 (3%)	1 (3%)
SPECIAL SENSE ORGANS				
*EAR CANAL	(10)	(9)	(32)	(30)
SQUAMOUS CELL PAPILLOMA			2 (6%)	
MUSCULOSKELETAL SYSTEM				
*BONE	(10)	(9)	(32)	(30)
OSTEOSARCOMA			1 (3%)	1 (3%)
BODY CAVITIES				
*ABDOMINAL CAVITY	(10)	(9)	(32)	(30)
SARCOMA, NOS	1 (10%)		1 (3%)	2 (7%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HEMANGIOSARCOMA			1 (3%)	
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(10)	(9)	(32)	(30)
SARCOMA, NOS			1 (3%)	
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	35	35
NATURAL DEATH@	2	3	15	10
MORIBUND SACRIFICE			12	21
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	8	7	8	4
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	1	18	11
TOTAL PRIMARY TUMORS	4	1	21	11
TOTAL ANIMALS WITH BENIGN TUMORS	2		9	1
TOTAL BENIGN TUMORS	2		10	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	1	11	10
TOTAL MALIGNANT TUMORS	2	1	11	10
TOTAL ANIMALS WITH SECONDARY TUMORS#				2
TOTAL SECONDARY TUMORS				2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED PHENESTERIN BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	10	29	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	29	30
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(10)	(10)	(29)	(30)
SARCOMA, NOS				1 (3%)
FIBROSARCOMA			1 (3%)	
RESPIRATORY SYSTEM				
#LUNG	(10)	(10)	(28)	(30)
ADENOCARCINOMA, NOS, METASTATIC				1 (3%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (4%)	1 (3%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(10)	(10)	(29)	(30)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				1 (3%)
GRANULOCYTIC LEUKEMIA			2 (7%)	1 (3%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
NONE				
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(10)	(10)	(27)	(30)
CHROMOPHOBE ADENOMA	1 (10%)	6 (60%)	4 (15%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC ISLETS	(10)	(10)	(29)	(30)
ISLET-CELL ADENOMA		1 (10%)		
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(10)	(29)	(30)
ADENOCARCINOMA, NOS		1 (10%)	12 (41%)	12 (40%)
SARCOMA, NOS				1 (3%)
FIBROADENOMA	6 (60%)	4 (40%)	21 (72%)	11 (37%)
#UTERUS	(10)	(10)	(29)	(30)
ADENOCARCINOMA, NOS			1 (3%)	1 (3%)
ENDOMETRIAL STROMAL POLYP		1 (10%)	5 (17%)	2 (7%)
#CERVIX UTERI	(10)	(10)	(29)	(30)
SQUAMOUS CELL CARCINOMA			1 (3%)	1 (3%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EYE/RETINA	(10)	(10)	(29)	(30)
NEUROBLASTOMA				1 (3%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(10)	(10)	(29)	(30)
ADENOCARCINOMA, NOS	1 (10%)			
SARCOMA, NOS			1 (3%)	
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(10)	(10)	(29)	(30)
ADENOCARCINOMA, NOS, METASTATIC	1 (10%)		1 (3%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
SARCOMA, NOS, METASTATIC				1 (3%)
CRANIAL CAVITY				
SARCOMA, NOS				1
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	35	35
NATURAL DEATH@		1	14	9
MORIBUND SACRIFICE	2	1	12	24
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	8	8	9	2
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	7	28	22
TOTAL PRIMARY TUMORS	8	13	49	34
TOTAL ANIMALS WITH BENIGN TUMORS	6	7	23	13
TOTAL BENIGN TUMORS	7	12	30	13
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	1	16	16
TOTAL MALIGNANT TUMORS	1	1	19	21
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		1	2
TOTAL SECONDARY TUMORS	1		1	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED PHENESTERIN BY GAVAGE

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED PHENESTERIN BY GAVAGE (CONTROL GROUPS)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	15	20	15	20
ANIMALS NECROPSIED	15	20	14	16
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	20	14	16
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(15)	(20)	(14)	(16)
HEPATOCELLULAR CARCINOMA, METAST	1 (7%)			
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (7%)			2 (13%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(20)	(14)	(16)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (7%)			
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				1 (6%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(15)	(19)	(14)	(16)
HEPATOCELLULAR ADENOMA	1 (7%)	2 (11%)	4 (29%)	3 (19%)
HEPATOCELLULAR CARCINOMA	2 (13%)	2 (11%)		
HEMANGIOMA				1 (6%)
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
NONE				

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

TABLE B1. MALE MICE (CONTROL GROUPS): NEOPLASMS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(15)	(20)	(14)	(16)
ADENOMA, NOS				1 (6%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(15)	(20)	(14)	(16)
SARCOMA, NOS	1 (7%)			
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	20	15	20
NATURAL DEATH@	1	7	1	1
MORIBUND SACRIFICE		11		13
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				4
TERMINAL SACRIFICE	14	2	14	2
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE (CONTROL GROUPS): NEOPLASMS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	4	4	7
TOTAL PRIMARY TUMORS	6	4	4	8
TOTAL ANIMALS WITH BENIGN TUMORS	2	2	4	6
TOTAL BENIGN TUMORS	2	2	4	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	2		1
TOTAL MALIGNANT TUMORS	4	2		1
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 B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED PHENESTERIN BY GAVAGE (TREATED GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	40	35	35
ANIMALS MISSING			2
ANIMALS NECROPSIED	40	29	25
ANIMALS EXAMINED HISTOPATHOLOGICALLY	40	29	25
INTEGUMENTARY SYSTEM			
*SKIN	(40)	(29)	(25)
CARCINOMA,NOS	1 (3%)		
KERATOACANTHOMA	1 (3%)		
*SUBCUT TISSUE	(40)	(29)	(25)
FIBROSARCOMA	2 (5%)		
HEMANGIOSARCOMA		1 (3%)	3 (12%)
RESPIRATORY SYSTEM			
#LUNG	(40)	(29)	(25)
ALVEOLAR/BRONCHIOLAR ADENOMA	4 (10%)	6 (21%)	2 (8%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	14 (35%)		
TUBULAR-CELL ADENOCARCINOMA, MET		1 (3%)	
SARCOMA, NOS, METASTATIC	3 (8%)	1 (3%)	2 (8%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(40)	(29)	(25)
MALIGNANT LYMPHOMA, NOS			1 (4%)
MALIG.LYMPHOMA, UNDIFFER-TYPE	2 (5%)		
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		7 (24%)	9 (36%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	5 (13%)		1 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (3%)		
LYMPHOCYTIC LEUKEMIA	1 (3%)	2 (7%)	
EOSINOPHILIC LEUKEMIA	1 (3%)		
#MESENTERIC L. NODE	(35)	(4)	
MALIC.LYMPHOMA, HISTIOCYTIC TYPE	1 (3%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(40)	(29)	(25)
SARCOMA, NOS	5 (13%)	7 (24%)	2 (8%)

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

TABLE B2. MALE MICE (TREATED GROUPS): NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(39)	(29)	(25)
HEPATOCELLULAR ADENOMA	7 (18%)	2 (7%)	
HEPATOCELLULAR CARCINOMA	3 (8%)		
ALVEOLAR/BRONCHIOLAR CA, METASTA	1 (3%)		
HEMANGIOSARCOMA	4 (10%)		
#STOMACH	(40)	(29)	(24)
SQUAMOUS CELL PAPILLOMA	1 (3%)		
URINARY SYSTEM			
#KIDNEY	(40)	(29)	(25)
TUBULAR-CELL ADENOCARCINOMA		1 (3%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(40)	(29)	(25)
ADENOMA, NOS	2 (5%)		1 (4%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(40)	(29)	(25)
SARCOMA, NOS	1 (3%)		

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

TABLE B2. MALE MICE (TREATED GROUPS): NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
HEMANGIOSARCOMA	2 (5%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(40)	(29)	(25)
SARCOMA, NOS, METASTATIC	1 (3%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	40	35	35
NATURAL DEATH@	9	19	18
MORIBUND SACRIFICE	31	11	13
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			2
TERMINAL SACRIFICE		5	
ANIMAL MISSING			2
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	29	20	17
TOTAL PRIMARY TUMORS	58	26	19
TOTAL ANIMALS WITH BENIGN TUMORS	11	7	2
TOTAL BENIGN TUMORS	15	8	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	27	18	16
TOTAL MALIGNANT TUMORS	43	18	16
TOTAL ANIMALS WITH SECONDARY TUMORS#	5	2	2
TOTAL SECONDARY TUMORS	5	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

TABLE B3.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED PHENESTERIN BY GAVAGE (CONTROL GROUPS)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	15	20	15	20
ANIMALS NECROPSIED	15	20	15	20
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	20	15	20
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(15)	(20)	(15)	(20)
HEMANGIOMA				2 (10%)
RESPIRATORY SYSTEM				
#LUNG	(15)	(20)	(15)	(20)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (7%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA				1 (5%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(20)	(15)	(20)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE				1 (5%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				3 (15%)
MALIGNANT LYMPHOMA, MIXED TYPE				1 (5%)
#SPLEEN	(15)	(20)	(15)	(19)
HEMANGIOMA	1 (7%)			
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(15)	(19)	(15)	(20)
HEPATOCELLULAR ADENOMA	2 (13%)			2 (10%)
HEPATOCELLULAR CARCINOMA				1 (5%)
URINARY SYSTEM				
NONE				

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

TABLE B3. FEMALE MICE (CONTROL GROUPS): NEOPLASMS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
ENDOCRINE SYSTEM				
#PITUITARY	(14)	(17)	(15)	(14)
CHROMOPHOBE ADENOMA				1 (7%)
#THYROID	(14)	(20)	(14)	(16)
FOLLICULAR-CELL ADENOMA			1 (7%)	
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(15)	(20)	(15)	(20)
ADENOCARCINOMA, NOS		2 (10%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
*BONE	(15)	(20)	(15)	(20)
OSTEOSARCOMA		1 (5%)		
BODY CAVITIES				
*PERITONEUM	(15)	(20)	(15)	(20)
LIPOMA		1 (5%)		
ALL OTHER SYSTEMS				
NONE				

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

TABLE B3. FEMALE MICE (CONTROL GROUPS): NEOPLASMS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	20	15	20
NATURAL DEATH@	1	3		5
MORIBUND SACRIFICE		15		9
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	14	2	15	6
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	3	4	2	9
TOTAL PRIMARY TUMORS	3	4	2	12
TOTAL ANIMALS WITH BENIGN TUMORS	3	1	2	4
TOTAL BENIGN TUMORS	3	1	2	5
TOTAL ANIMALS WITH MALIGNANT TUMORS		3		7
TOTAL MALIGNANT TUMORS		3		7
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 B4.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED PHENESTERIN BY GAVAGE (TREATED GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	40	35	35
ANIMALS NECROPSIED	38	27	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	38	27	31
INTEGUMENTARY SYSTEM			
*SKIN	(38)	(27)	(32)
SQUAMOUS CELL PAPILLOMA	1 (3%)		
*SUBCUT TISSUE	(38)	(27)	(32)
CARCINOMA,NOS		1 (4%)	
HEMANGIOSARCOMA	2 (5%)		1 (3%)
RESPIRATORY SYSTEM			
#LUNG	(38)	(27)	(31)
ADENOCARCINOMA, NOS, METASTATIC	1 (3%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	7 (18%)	1 (4%)	1 (3%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	8 (21%)		
SARCOMA, NOS, METASTATIC	6 (16%)	2 (7%)	2 (6%)
HEMANGIOSARCOMA			1 (3%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(38)	(27)	(32)
MALIGNANT LYMPHOMA, NOS			2 (6%)
MALIG.LYMPHOMA, UNDIFFER-TYPE	4 (11%)		
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (5%)	12 (44%)	14 (44%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	4 (11%)		
LYMPHOCYTIC LEUKEMIA	1 (3%)	2 (7%)	
#SPLEEN	(38)	(27)	(30)
HEMANGIOSARCOMA	1 (3%)		
#MEDIASTINAL L.NODE	(36)	(4)	(2)
ALVEOLAR/BRONCHIOLAR CA, METASTA	1 (3%)		
#THYMUS	(35)	(27)	(28)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (3%)		

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

TABLE B4. FEMALE MICE (TREATED GROUPS): NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (4%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(36)	(27)	(31)
SARCOMA, NOS	8 (22%)	2 (7%)	3 (10%)
DIGESTIVE SYSTEM			
#LIVER	(37)	(27)	(30)
HEPATOCELLULAR CARCINOMA	1 (3%)		
HEMANGIOSARCOMA	1 (3%)		
#STOMACH	(36)	(26)	(29)
SQUAMOUS CELL CARCINOMA	1 (3%)	1 (4%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(38)	(27)	(32)
ADENOCARCINOMA, NOS	6 (16%)		
#UTERUS	(38)	(27)	(30)
ENDOMETRIAL STROMAL SARCOMA	1 (3%)		
#OVARY	(37)	(27)	(30)
CARCINOMA,NOS	1 (3%)		
ADENOMA, NOS	1 (3%)		
TUBULAR ADENOMA	8 (22%)		
NERVOUS SYSTEM			
NONE			

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

TABLE B4. FEMALE MICE (TREATED GROUPS): NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(38)	(27)	(32)
ADENOMA, NOS	1 (3%)		
MUSCULOSKELETAL SYSTEM			
*BONE	(38)	(27)	(32)
HEMANGIOSARCOMA	1 (3%)		
BODY CAVITIES			
*PERITONEUM	(38)	(27)	(32)
HEMANGIOSARCOMA		1 (4%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(38)	(27)	(32)
SQUAMOUS CELL CARCINOMA, METASTA		1 (4%)	
FIBROUS HISTIOCYTOMA, MALIGNANT	1 (3%)		
HEMANGIOSARCOMA, METASTATIC		1 (4%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	40	35	35
NATURAL DEATH@	13	13	18
MORIBUND SACRIFICE	27	20	16
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		2	1
TERMINAL SACRIFICE			
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B4. FEMALE MICE (TREATED GROUPS): NEOPLASMS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	33	17	21
TOTAL PRIMARY TUMORS	62	20	23
TOTAL ANIMALS WITH BENIGN TUMORS	15	1	1
TOTAL BENIGN TUMORS	18	1	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	30	17	21
TOTAL MALIGNANT TUMORS	44	19	22
TOTAL ANIMALS WITH SECONDARY TUMORS#	8	4	2
TOTAL SECONDARY TUMORS	8	4	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 ADMINISTERED PHENESTERIN BY GAVAGE

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED PHENESTERIN BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	9	32	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	9	32	30
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(9)	(32)	(30)
EPIDERMAL INCLUSION CYST				1 (3%)
INFLAMMATION, SUPPURATIVE			1 (3%)	
INFLAMMATION, NECROTIZING			1 (3%)	
INFLAMMATION, CHRONIC			2 (6%)	
HYPERKERATOSIS			2 (6%)	
*SUBCUT TISSUE	(10)	(9)	(32)	(30)
GRANULATION, TISSUE		2 (6%)	1 (3%)	
RESPIRATORY SYSTEM				
#TRACHEA	(10)	(9)	(32)	(30)
INFLAMMATION, SUPPURATIVE	1 (10%)		1 (3%)	1 (3%)
#LUNG/BRONCHIOLE	(10)	(9)	(32)	(30)
HYPERPLASIA, LYMPHOID			2 (6%)	
#LUNG	(10)	(9)	(32)	(30)
INFLAMMATION, INTERSTITIAL			4 (13%)	2 (7%)
PNEUMONIA, LIPID			1 (3%)	
BRONCHOPNEUMONIA SUPPURATIVE			1 (3%)	1 (3%)
ABSCESS, NOS			1 (3%)	
BRONCHOPNEUMONIA CHRONIC SUPPURA			2 (6%)	3 (10%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(9)	(9)	(32)	(26)
ATROPHY, NOS	8 (89%)	8 (89%)	18 (56%)	14 (54%)
#SPLEEN	(10)	(9)	(32)	(29)
HEMORRHAGE	1 (10%)			

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION WITH FIBROSIS	1 (10%)			
HEMATOPOIESIS			3 (9%)	1 (3%)
#MESENTERIC L. NODE			(1)	(4)
CONGESTION, NOS				1 (25%)
CIRCULATORY SYSTEM				
#MYOCARDIUM	(10)	(9)	(32)	(30)
INFLAMMATION, INTERSTITIAL				1 (3%)
INFLAMMATION, CHRONIC			1 (3%)	
CALCIFICATION, METASTATIC			1 (3%)	
DIGESTIVE SYSTEM				
#LIVER	(10)	(9)	(32)	(30)
LIPOIDOSIS				1 (3%)
#LIVER/CENTRILOBULAR	(10)	(9)	(32)	(30)
NECROSIS, NOS			2 (6%)	1 (3%)
#PANCREAS	(10)	(9)	(32)	(30)
ANEURYSM			1 (3%)	
#PANCREATIC ACINUS	(10)	(9)	(32)	(30)
ATROPHY, NOS		1 (11%)		
URINARY SYSTEM				
#KIDNEY	(10)	(9)	(32)	(30)
INFLAMMATION, INTERSTITIAL	1 (10%)		1 (3%)	
INFLAMMATION, CHRONIC	4 (40%)	5 (56%)	18 (56%)	16 (53%)
INFLAMMATION WITH FIBROSIS			1 (3%)	
ENDOCRINE SYSTEM				
#ADRENAL	(10)	(9)	(32)	(30)
ANGIECTASIS	1 (10%)			
#THYROID	(10)	(8)	(28)	(27)
INFLAMMATION, INTERSTITIAL				1 (4%)
INFLAMMATION, CHRONIC			1 (4%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM				
#PROSTATE	(10)	(9)	(32)	(29)
INFLAMMATION, SUPPURATIVE				2 (7%)
NERVOUS SYSTEM				
#BRAIN/MENINGES	(10)	(9)	(30)	(30)
INFLAMMATION, NOS			1 (3%)	
#BRAIN/EPENDYMA	(10)	(9)	(30)	(30)
INFLAMMATION, NOS			1 (3%)	
#BRAIN	(10)	(9)	(30)	(30)
PERIARTERITIS			1 (3%)	
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(10)	(9)	(32)	(30)
INFLAMMATION, FIBRINOUS			1 (3%)	
INFLAMMATION, CHRONIC				1 (3%)
INFLAMMATION, CHRONIC NECROTIZIN	1 (10%)			
*PLEURA	(10)	(9)	(32)	(30)
INFLAMMATION, CHRONIC				1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV		1 (11%)		
ALL OTHER SYSTEMS				
ADIPOSE TISSUE				
INFLAMMATION, CHRONIC		2		1

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	1			
AUTOLYSIS/NO NECROPSY		1	3	5

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED PHENESTERIN BY GAVAGE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	10	29	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	29	30
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(10)	(10)	(29)	(30)
INFLAMMATION, CHRONIC FOCAL				1 (3%)
RESPIRATORY SYSTEM				
#TRACHEA	(10)	(10)	(28)	(30)
INFLAMMATION, CHRONIC				1 (3%)
#LUNG/BRONCHIOLE	(10)	(10)	(28)	(30)
METAPLASIA, SQUAMOUS				1 (3%)
#LUNG	(10)	(10)	(28)	(30)
INFLAMMATION, INTERSTITIAL		2 (20%)	2 (7%)	
BRONCHOPNEUMONIA SUPPURATIVE	1 (10%)			2 (7%)
ABSCESS, NOS			1 (4%)	1 (3%)
BRONCHOPNEUMONIA, CHRONIC		1 (10%)		
BRONCHOPNEUMONIA CHRONIC SUPPURA	1 (10%)		3 (11%)	3 (10%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(9)	(10)	(29)	(28)
ATROPHY, NOS	3 (33%)	7 (70%)	7 (24%)	9 (32%)
#SPLEEN	(10)	(10)	(29)	(30)
HEMATOPOIESIS			4 (14%)	11 (37%)
#MANDIBULAR L. NODE	(1)		(2)	
HYPERPLASIA, LYMPHOID			1 (50%)	
#MESENTERIC L. NODE	(1)		(2)	
CONGESTION, NOS			1 (50%)	
CIRCULATORY SYSTEM				
NONE				

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM				
#LIVER	(10)	(10)	(28)	(30)
HEMORRHAGE			1 (4%)	
FIBROSIS			1 (4%)	
LIPOIDOSIS		2 (20%)		
HYPERPLASIA, GRANULOCYTIC			1 (4%)	
#LIVER/CENTRILOBULAR	(10)	(10)	(28)	(30)
NECROSIS, NOS		1 (10%)		1 (3%)
#PANCREATIC ACINUS	(10)	(10)	(29)	(30)
ATROPHY, NOS				1 (3%)
URINARY SYSTEM				
#KIDNEY	(10)	(10)	(29)	(30)
INFLAMMATION, CHRONIC	1 (10%)	3 (30%)	5 (17%)	3 (10%)
ENDOCRINE SYSTEM				
#ADRENAL	(10)	(10)	(29)	(30)
ANGIECTASIS			3 (10%)	2 (7%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(10)	(29)	(30)
CYST, NOS	3 (30%)	4 (40%)	2 (7%)	4 (13%)
#UTERUS	(10)	(10)	(29)	(30)
ABSCESS, NOS				1 (3%)
METAPLASIA, SQUAMOUS				1 (3%)
#UTERUS/ENDOMETRIUM	(10)	(10)	(29)	(30)
INFLAMMATION, SUPPURATIVE	1 (10%)	1 (10%)	7 (24%)	1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (10%)		5 (17%)	2 (7%)
HYPERPLASIA, CYSTIC			1 (3%)	
#OVARY	(10)	(10)	(29)	(30)
CYST, NOS		1 (10%)		
INFLAMMATION, CHRONIC SUPPURATIV	1 (10%)			
NERVOUS SYSTEM				
NONE				

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS				
*EYE/CORNEA	(10)	(10)	(29)	(30)
INFLAMMATION, CHRONIC				1 (3%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
ADIPOSE TISSUE				
INFLAMMATION, CHRONIC		1		1
NECROSIS, FAT				1
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	1			1
AUTOLYSIS/NO NECROPSY			6	5

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED PHENESTERIN BY GAVAGE

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED PHENESTERIN BY GAVAGE (CONTROL GROUPS)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	15	20	15	20
ANIMALS NECROPSIED	15	20	14	16
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	20	14	16
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#TRACHEA	(15)	(19)	(14)	(16)
INFLAMMATION, SUPPURATIVE		4 (21%)		3 (19%)
INFLAMMATION, CHRONIC		1 (5%)		
INFLAMMATION, CHRONIC SUPPURATIV		1 (5%)		
#LUNG/BRONCHIOLE	(15)	(20)	(14)	(16)
HYPERPLASIA, PLASMA CELL		1 (5%)		2 (13%)
#LUNG	(15)	(20)	(14)	(16)
INFLAMMATION, INTERSTITIAL			2 (14%)	
BRONCHOPNEUMONIA SUPPURATIVE		10 (50%)		10 (63%)
BRONCHOPNEUMONIA CHRONIC SUPPURA		5 (25%)		
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(14)	(20)	(14)	(16)
ATROPHY, NOS			1 (7%)	
#SPLEEN	(15)	(19)	(14)	(16)
HYPERPLASIA, LYMPHOID	1 (7%)			
HEMATOPOIESIS	3 (20%)		3 (21%)	
#LYMPH NODE	(6)	(18)	(3)	(16)
HYPERPLASIA, LYMPHOID				1 (6%)
#MESENTERIC L. NODE	(6)	(18)	(3)	(16)
CONGESTION, NOS			1 (33%)	
INFLAMMATION, ACUTE/CHRONIC			1 (33%)	

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

TABLE D1. MALE MICE (CONTROL GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
HYPERPLASIA, LYMPHOID	1 (17%)			
HEMATOPOIESIS	1 (17%)			
#INGUINAL LYMPH NODE	(6)	(18)	(3)	(16)
INFLAMMATION, CHRONIC	1 (17%)			
HYPERPLASIA, LYMPHOID	1 (17%)			
CIRCULATORY SYSTEM				
#MYOCARDIUM	(15)	(20)	(14)	(16)
MINERALIZATION		1 (5%)		
INFLAMMATION, SUPPURATIVE		3 (15%)		1 (6%)
INFLAMMATION, CHRONIC DIFFUSE		1 (5%)		
INFLAMMATION, CHRONIC SUPPURATIV		2 (10%)		
DEGENERATION, GRANULAR		4 (20%)		1 (6%)
DIGESTIVE SYSTEM				
#LIVER	(15)	(19)	(14)	(16)
CYTOLOGIC DEGENERATION		6 (32%)		2 (13%)
HYPERPLASIA, NODULAR		3 (16%)		
HYPERPLASIA, LYMPHOID				1 (6%)
HEMATOPOIESIS			1 (7%)	
#LIVER/CENTRILOBULAR	(15)	(19)	(14)	(16)
NECROSIS, NOS	1 (7%)			
URINARY SYSTEM				
#KIDNEY	(15)	(19)	(14)	(16)
HYDRONEPHROSIS	2 (13%)			
INFLAMMATION, CHRONIC		1 (5%)		
INFLAMMATION, CHRONIC FOCAL				1 (6%)
#KIDNEY/MEDULLA	(15)	(19)	(14)	(16)
ATROPHY, NOS	1 (7%)			
#URINARY BLADDER	(15)	(19)	(14)	(16)
INFLAMMATION, CHRONIC			1 (7%)	
ENDOCRINE SYSTEM				
#PANCREATIC ISLETS	(15)	(18)	(14)	(16)
HYPERPLASIA, DIFFUSE		1 (6%)		

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

TABLE D1. MALE MICE (CONTROL GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*MIDDLE EAR	(15)	(20)	(14)	(16)
INFLAMMATION, CHRONIC SUPPURATIV		1 (5%)		2 (13%)
MUSCULOSKELETAL SYSTEM				
*JOINT	(15)	(20)	(14)	(16)
EXOSTOSIS		1 (5%)		
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(15)	(20)	(14)	(16)
HYPERPLASIA, LYMPHOID			1 (7%)	
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	5		5	2
ACCIDENTAL DEATH				4
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 MALE MICE ADMINISTERED PHENESTERIN BY GAVAGE (CONTROL GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	40	35	35
ANIMALS MISSING			2
ANIMALS NECROPSIED	40	29	25
ANIMALS EXAMINED HISTOPATHOLOGICALLY	40	29	25
INTEGUMENTARY SYSTEM			
*SKIN	(40)	(29)	(25)
INFLAMMATION, FIBRINOUS	1 (3%)		
ULCER, CHRONIC	1 (3%)		
HYPERKERATOSIS		1 (3%)	
ACANTHOSIS		1 (3%)	
*SUBCUT TISSUE	(40)	(29)	(25)
HEMORRHAGE		1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV	3 (8%)		
RESPIRATORY SYSTEM			
#TRACHEA	(39)	(29)	(25)
INFLAMMATION, SUPPURATIVE	3 (8%)		
INFLAMMATION, CHRONIC SUPPURATIV	1 (3%)		
#LUNG/BRONCHIOLE	(40)	(29)	(25)
FIBROSIS		1 (3%)	
HYPERPLASIA, LYMPHOID		1 (3%)	
#LUNG	(40)	(29)	(25)
INFLAMMATION, INTERSTITIAL		2 (7%)	
PNEUMONIA, LIPID			1 (4%)
BRONCHOPNEUMONIA SUPPURATIVE	10 (25%)		
BRONCHOPNEUMONIA CHRONIC SUPPURA	1 (3%)	1 (3%)	
CHOLESTEROL DEPOSIT			1 (4%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(39)	(28)	(24)
CONGESTION, NOS			2 (8%)
ATROPHY, NOS			7 (29%)

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

TABLE D2. MALE MICE (TREATED GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
HYPERPLASIA, NEUTROPHILIC			1 (4%)
#SPLEEN	(40)	(29)	(25)
HYPERPLASIA, NEUTROPHILIC			1 (4%)
HEMATOPOIESIS		5 (17%)	5 (20%)
#MESENTERIC L. NODE	(35)	(4)	
INFLAMMATION, SUPPURATIVE	1 (3%)		
HYPERPLASIA, LYMPHOID		2 (50%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(40)	(29)	(25)
INFLAMMATION, SUPPURATIVE	1 (3%)		
INFLAMMATION, CHRONIC SUPPURATIV	1 (3%)		
NECROSIS, COAGULATIVE	1 (3%)		
DIGESTIVE SYSTEM			
#LIVER	(39)	(29)	(25)
HEMORRHAGE	1 (3%)		
NECROSIS, NOS		2 (7%)	
NECROSIS, COAGULATIVE	4 (10%)		
ANGIECTASIS	1 (3%)	1 (3%)	
HEMATOPOIESIS			1 (4%)
#LIVER/CENTRILOBULAR	(39)	(29)	(25)
NECROSIS, NOS		1 (3%)	
NECROSIS, COAGULATIVE	1 (3%)		
URINARY SYSTEM			
#KIDNEY	(40)	(29)	(25)
INFLAMMATION, CHRONIC	1 (3%)		
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			

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

TABLE D2. MALE MICE (TREATED GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(40)	(29)	(25)
INFLAMMATION, HEMORRHAGIC			1 (4%)
INFLAMMATION, CHRONIC SUPPURATIV	2 (5%)		
NECROSIS, FAT	1 (3%)		
*PLEURA	(40)	(29)	(25)
INFLAMMATION, CHRONIC SUPPURATIV	1 (3%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		4	2
ANIMAL MISSING/NO NECROPSY			2
ACCIDENTAL DEATH			2
NO NECROPSY PERFORMED		1	1
AUTO/NECROPSY/HISTO PERF		1	
AUTOLYSIS/NO NECROPSY		5	5

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

TABLE D3.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED PHENESTERIN BY GAVAGE (CONTROL GROUPS)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
ANIMALS INITIALLY IN STUDY	15	20	15	20
ANIMALS NECROPSIED	15	20	15	20
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	20	15	20
INTEGUMENTARY SYSTEM				
*SKIN	(15)	(20)	(15)	(20)
ULCER, CHRONIC		1 (5%)		
RESPIRATORY SYSTEM				
#TRACHEA	(15)	(20)	(15)	(20)
INFLAMMATION, SUPPURATIVE		3 (15%)		2 (10%)
HYPERPLASIA, PLASMA CELL		1 (5%)		1 (5%)
#LUNG/BRONCHUS	(15)	(20)	(15)	(20)
INFLAMMATION, SUPPURATIVE		1 (5%)		
#LUNG/BRONCHIOLE	(15)	(20)	(15)	(20)
HYPERPLASIA, PLASMA CELL		9 (45%)		3 (15%)
HYPERPLASIA, LYMPHOID	1 (7%)		1 (7%)	2 (10%)
#LUNG	(15)	(20)	(15)	(20)
INFLAMMATION, INTERSTITIAL	1 (7%)		1 (7%)	1 (5%)
PNEUMONIA, ASPIRATION		1 (5%)		
BRONCHOPNEUMONIA SUPPURATIVE		15 (75%)	1 (7%)	9 (45%)
BRONCHOPNEUMONIA CHRONIC SUPPURA				1 (5%)
HYPERPLASIA, PLASMA CELL				1 (5%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(13)	(20)	(14)	(18)
ATROPHY, NOS	3 (23%)		3 (21%)	
#SPLEEN	(15)	(20)	(15)	(19)
HYPERPLASIA, LYMPHOID			1 (7%)	
HEMATOPOIESIS	1 (7%)			
CIRCULATORY SYSTEM				
NONE				

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

TABLE D3. FEMALE MICE (CONTROL GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
DIGESTIVE SYSTEM				
#LIVER	(15)	(19)	(15)	(20)
HEMORRHAGE				1 (5%)
NECROSIS, COAGULATIVE				1 (5%)
HYPERPLASIA, LYMPHOID		1 (5%)		1 (5%)
URINARY SYSTEM				
#KIDNEY	(15)	(20)	(15)	(19)
HYPERPLASIA, LYMPHOID			1 (7%)	
ENDOCRINE SYSTEM				
#PITUITARY	(14)	(17)	(15)	(14)
HEMORRHAGE				1 (7%)
#ADRENAL	(15)	(20)	(15)	(20)
HEMORRHAGE				1 (5%)
REPRODUCTIVE SYSTEM				
#UTERUS/ENDOMETRIUM	(15)	(20)	(15)	(20)
INFLAMMATION, SUPPURATIVE		1 (5%)		
HYPERPLASIA, CYSTIC	13 (87%)	1 (5%)	14 (93%)	
#OVARY	(15)	(20)	(15)	(20)
CYST, NOS			1 (7%)	
NERVOUS SYSTEM				
#BRAIN	(15)	(20)	(15)	(19)
EPIDERMAL INCLUSION CYST		1 (5%)		
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				

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

TABLE D3. FEMALE MICE (CONTROL GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	MID & HIGH DOSE UNTREATED CONTROL	LOW DOSE UNTREATED CONTROL	MID & HIGH DOSE VEHICLE CONTROL	LOW DOSE VEHICLE CONTROL
BODY CAVITIES				
*PERITONEUM	(15)	(20)	(15)	(20)
INFLAMMATION, CHRONIC		1 (5%)		
INFLAMMATION, CHRONIC DIFFUSE				1 (5%)
NECROSIS, FAT	1 (7%)			1 (5%)
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(15)	(20)	(15)	(20)
HYPERPLASIA, PLASMA CELL		1 (5%)		
HYPERPLASIA, RETICULUM CELL		1 (5%)		
HYPERPLASIA, LYMPHOID		1 (5%)		
ADIPOSE TISSUE				
INFLAMMATION, CHRONIC FOCAL		1		2
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	1	1		3

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

TABLE D4.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED PHENESTERIN BY GAVAGE (TREATED GROUPS)

	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	40	35	35
ANIMALS NECROPSIED	38	27	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	38	27	31
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(38)	(27)	(32)
HEMATOMA, NOS	1 (3%)		
RESPIRATORY SYSTEM			
#TRACHEA	(38)	(27)	(30)
INFLAMMATION, SUPPURATIVE	3 (8%)		
PLASMA-CELL INFILTRATE	1 (3%)		
#LUNG/BRONCHIOLE	(38)	(27)	(31)
HYPERPLASIA, PLASMA CELL	3 (8%)		
#LUNG	(38)	(27)	(31)
CONGESTION, NOS		1 (4%)	
HEMORRHAGE		1 (4%)	
INFLAMMATION, INTERSTITIAL		1 (4%)	
PNEUMONIA, LIPID			1 (3%)
BRONCHOPNEUMONIA SUPPURATIVE	5 (13%)		
PNEUMONIA INTERSTITIAL CHRONIC	1 (3%)		
BRONCHOPNEUMONIA CHRONIC SUPPURA		8 (30%)	
CHOLESTEROL DEPOSIT			1 (3%)
ALVEOLAR MACROPHAGES		1 (4%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(38)	(27)	(28)
CONGESTION, NOS			2 (7%)
ATROPHY, NOS		6 (22%)	14 (50%)
HYPERPLASIA, NEUTROPHILIC		2 (7%)	
#SPLEEN	(38)	(27)	(30)
CONTRACTURE			1 (3%)

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

TABLE D4. FEMALE MICE (TREATED GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
HYPERPLASIA, NEUTROPHILIC		2 (7%)	
HEMATOPOIESIS		6 (22%)	3 (10%)
#SPLENIC FOLLICLES	(38)	(27)	(30)
ATROPHY, NOS			1 (3%)
#MESENTERIC L. NODE	(36)	(4)	(2)
HYPERPLASIA, NEUTROPHILIC		1 (25%)	
HYPERPLASIA, LYMPHOID	1 (3%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(37)	(27)	(30)
HEMORRHAGE	1 (3%)		
INFLAMMATION, CHRONIC SUPPURATIV	1 (3%)		
NECROSIS, NOS	1 (3%)		
NECROSIS, MIDZONAL			1 (3%)
CYTOLOGIC DEGENERATION	1 (3%)		
HYPERPLASIA, NODULAR	1 (3%)		
ANGIECTASIS			1 (3%)
#LIVER/CENTRILOBULAR	(37)	(27)	(30)
NECROSIS, NOS			1 (3%)
NECROSIS, COAGULATIVE	3 (8%)		
URINARY SYSTEM			
#KIDNEY	(38)	(27)	(30)
INFLAMMATION, CHRONIC	1 (3%)		
AMYLOIDOSIS	1 (3%)		
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#UTERUS	(38)	(27)	(30)
CONGESTION, NOS			1 (3%)

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

TABLE D4. FEMALE MICE (TREATED GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
#OVARY	(37)	(27)	(30)
CYST, NOS			1 (3%)
CONGESTION, NOS			1 (3%)
HEMORRHAGE	9 (24%)		2 (7%)
INFLAMMATION, SUPPURATIVE		1 (4%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(38)	(27)	(32)
INFLAMMATION, SUPPURATIVE	1 (3%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(38)	(27)	(32)
HEMORRHAGE	1 (3%)		
INFLAMMATION, SUPPURATIVE	1 (3%)		
INFLAMMATION, CHRONIC			1 (3%)
NECROSIS, FAT			1 (3%)
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC			1
INFLAMMATION, CHRONIC SUPPURATIV	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			3
ACCIDENTAL DEATH		2	1
NECROPSY PERF/NO HISTO PERFORMED			1

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

TABLE D4. FEMALE MICE (TREATED GROUPS): NONNEOPLASTIC LESIONS (CONTINUED)

	LOW DOSE	MID DOSE	HIGH DOSE
NC NECROPSY PERFORMED	1		
AUTOLYSIS/NC NECROPSY	1	6	2

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED PHENESTERIN BY GAVAGE

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Phenesterin by Gavage[a]

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Granulocytic Leukemia[b]	0/18 (0)	0/9 (0)	0/32 (0)	2/30 (7)
P Value[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]				Infinite
Lower Limit				0.186
Upper Limit				Infinite
Relative Risk (Vehicle Control)[f]				Infinite
Lower Limit				0.100
Upper Limit				Infinite
Weeks to First Observed Tumor		--		37
Hematopoietic System: Malignant Lymphoma or Lymphocytic Leukemia[b]	0/18 (0)	0/9 (0)	2/32 (6)	3/30 (10)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.174	0.379
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.093	0.204
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	75	50

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia[b]	0/18 (0)	0/9 (0)	2/32 (6)	5/30 (17)
P Values[c,d]	P = 0.037	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.174	0.798
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.093	0.430
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	75	37
Pituitary: Chromophobe Adenoma[b]	0/18 (5)	0/9 (0)	2/29 (7)	0/29 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			Infinite	--
Lower Limit			0.192	--
Upper Limit			Infinite	--
Relative Risk (Vehicle Control)[f]			Infinite	--
Lower Limit			0.103	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor		--	73	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma[b]	1/18 (6)	1/9 (11)	3/29 (10)	0/29 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			1.862	0.000
Lower Limit			0.167	0.000
Upper Limit			94.142	11.440
Relative Risk (Vehicle Control)[f]			0.931	0.000
Lower Limit			0.093	0.000
Upper Limit			47.120	5.736
Weeks to First Observed Tumor		85	73	--
Ear Canal: Squamous-cell Papilloma[b]	0/18 (0)	0/9 (0)	2/32 (6)	0/30 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			Infinite	--
Lower Limit			0.174	--
Upper Limit			Infinite	--
Relative Risk (Vehicle Control)[f]			Infinite	--
Lower Limit			0.093	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor		--	49	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
All Sites: Sarcoma[b]	1/18 (6)	0/9 (0)	7/32 (22)	4/30 (13)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			3.938	2.400
Lower Limit			0.579	0.268
Upper Limit			170.622	113.825
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.621	0.315
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	50	41

[a]Dosed groups received 5 or 10 mg/kg.

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

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

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Phenesterin by Gavage[a]

(continued)

[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 percent confidence interval of the relative risk between each dosed group and the specified control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Phenesterin by Gavage[a]

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Granulocytic Leukemia[b]	0/18 (0)	0/10 (0)	2/29 (7)	1/30 (3)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.192	0.033
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.113	0.019
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	46	50
Hematopoietic System: Lymphoma or Leukemia[b]	0/18 (0)	0/10 (0)	2/29 (7)	2/30 (7)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.192	0.186
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.113	0.109
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	46	50

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	10/16 (63)	6/10 (60)	4/27 (15)	0/30 (0)
P Values[c,d]	P < 0.001(N)	P < 0.001(N)	P = 0.012*(N) P = 0.002**(N)	P < 0.001*(N) P < 0.001**(N)
Relative Risk (Pooled Control)[f]			0.237	0.000
Lower Limit			0.076	0.000
Upper Limit			0.673	0.169
Relative Risk (Vehicle Control)[f]			0.247	0.000
Lower Limit			0.083	0.000
Upper Limit			0.843	0.194
Weeks to First Observed Tumor		64	71	--
Mammary Gland: Adenocarcinoma, NOS[b]	1/18 (6)	1/10 (10)	12/29 (41)	12/30 (40)
P Values[c,d]	P = 0.019	N.S.	P = 0.007**	P = 0.009**
Relative Risk (Pooled Control)[f]			7.448	7.200
Lower Limit			1.284	1.239
Upper Limit			300.235	291.162
Relative Risk (Vehicle Control)[f]			4.138	4.000
Lower Limit			0.780	0.753
Upper Limit			166.921	161.888
Weeks to First Observed Tumor		84	26	33

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	8/18 (44)	4/10 (40)	21/29 (72)	11/30 (37)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.008	P = 0.009		
Relative Risk (Pooled Control)[f]			1.629	0.825
Lower Limit			0.923	0.392
Upper Limit			3.090	1.942
Relative Risk (Vehicle Control)[f]			1.810	0.917
Lower Limit			0.882	0.387
Upper Limit			5.317	3.277
Weeks to First Observed Tumor		64	60	42
Uterus: Endometrial Stromal Polyp[b]	1/17 (6)	1/10 (10)	5/29 (17)	2/30 (7)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			2.931	1.133
Lower Limit			0.375	0.065
Upper Limit			133.079	64.588
Relative Risk (Vehicle Control)[f]			1.724	0.667
Lower Limit			0.240	0.041
Upper Limit			78.324	38.024
Weeks to First Observed Tumor		85	67	69

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Pooled Control	Vehicle Control	Low Dose	High Dose
All Sites: Sarcoma[b]	0/18 (0)	0/10 (0)	2/29 (7)	4/30 (13)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.192	0.585
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.113	0.345
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	60	34

[a]Dosed groups received 5 or 10 mg/kg.

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

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

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Phenesterin by Gavage[a]

(continued)

[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 percent confidence interval of the relative risk between each dosed group and the specified control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED PHENESTERIN BY GAVAGE

Table F1. Analyses of the Incidence of Primary Tumors in Low-Dose Male Mice Administered Phenesterin by Gavage[a]

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/16 (0)	14/40 (35)
P Values[c,d]		P = 0.004
Relative Risk (Low-Dose Control)[e]		Infinite
Lower Limit		1.857
Upper Limit		Infinite
Weeks to First Observed Tumor	--	68
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	2/16 (13)	18/40 (45)
P Values[c,d]		P = 0.020
Relative Risk (Low-Dose Control)[e]		3.600
Lower Limit		1.037
Upper Limit		29.336
Weeks to First Observed Tumor	77	68

Table F1. Analyses of the Incidence of Primary Tumors in Low-Dose Male Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
Hematopoietic System: Malignant Lymphoma or Lymphocytic Leukemia[b]	1/16 (6)	10/40 (25)
P Values[c,d]		N.S.
Relative Risk (Low-Dose Control)[e]		4.000
Lower Limit		0.662
Upper Limit		168.059
Weeks to First Observed Tumor	32	49
Hematopoietic System: Lymphoma or Leukemia[b]	1/16 (6)	11/40 (28)
P Values[c,d]		N.S.
Relative Risk (Low-Dose Control)[e]		4.400
Lower Limit		0.745
Upper Limit		183.149
Weeks to First Observed Tumor	32	49

Table F1. Analyses of the Incidence of Primary Tumors in Low-Dose Male Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
Liver: Hepatocellular Adenoma[b]	3/16 (19)	7/39 (18)
P Values[c,d]		N.S.
Relative Risk (Low-Dose Control)[e]		0.957
Lower Limit		0.261
Upper Limit		5.237
Weeks to First Observed Tumor	56	76
Liver: Hepatocellular Carcinoma[b]	0/16 (0)	3/39 (8)
P Values[c,d]		N.S.
Relative Risk (Low-Dose Control)[e]		Infinite
Lower Limit		0.261
Upper Limit		Infinite
Weeks to First Observed Tumor	--	73

Table F1. Analyses of the Incidence of Primary Tumors in Low-Dose Male Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	3/16 (19)	10/39 (26)
P Values[c,d]		N.S.
Relative Risk (Low-Dose Control)[e]		1.368
Lower Limit		0.425
Upper Limit		7.013
Weeks to First Observed Tumor	56	73
Myocardium: Sarcoma, NOS[b]	0/16 (0)	5/40 (13)
P Values[c,d]		N.S.
Relative Risk (Low-Dose Control)[e]		Infinite
Lower Limit		0.535
Upper Limit		Infinite
Weeks to First Observed Tumor	--	78

Table F1. Analyses of the Incidence of Primary Tumors in Low-Dose Male Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
All Sites: Hemangiosarcoma[b]	0/20 (0)	6/40 (15)
P Values[c,d]		N.S.
Relative Risk (Low-Dose Control)[e]		Infinite
Lower Limit		0.835
Upper Limit		Infinite
Weeks to First Observed Tumor	--	78

[a]Dosed group received 7 mg/kg.

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

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

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

[e]The 95 percent confidence interval of the relative risk between the dosed group and the control group.

Table F2. Analyses of the Incidence of Primary Tumors in Mid- and High-Dose Male Mice Administered Phenesterin by Gavage[a]

Topography: Morphology	Mid- and High-Dose Pooled Control	Mid- and High-Dose Vehicle Control	Mid Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	2/28 (7)	0/14 (0)	6/29 (21)	2/25 (8)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]		P = 0.038		
Relative Risk (Pooled Control)[f]			2.897	1.120
Lower Limit			0.575	0.087
Upper Limit			27.279	14.392
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.833	0.177
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	71	73

Table F2. Analyses of the Incidence of Primary Tumors in Mid- and High-Dose Male Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Mid- and High-Dose Pooled Control	Mid- and High-Dose Vehicle Control	Mid Dose	High Dose
Hematopoietic System:				
Lymphoma or Leukemia[b]	0/28 (0)	0/14 (0)	9/29 (31)	11/25 (44)
P Values[c,d]	P < 0.001	P = 0.005	P = 0.018* P = 0.001**	P = 0.003* P < 0.001**
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			2.616	3.859
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			1.372	2.023
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	32	49

Table F2. Analyses of the Incidence of Primary Tumors in Mid- and High-Dose Male Mice Administered Phenesterin by Gavage[a]

(continued)

Topography:	Morphology	Mid- and High-Dose Pooled Control	Mid- and High-Dose Vehicle Control	Mid Dose	High Dose
Myocardium:	Sarcoma, NOS[b]	0/28 (0)	0/14 (0)	7/29 (24)	2/25 (8)
P Value[c,d]		N.S.	N.S.	P = 0.048* P = 0.006**	N.S.
Relative Risk (Pooled Control)[f]				Infinite	Infinite
	Lower Limit			1.928	0.339
	Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]				Infinite	Infinite
	Lower Limit			1.012	0.177
	Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	--	48	57
All Sites:	Hemangiosarcoma[b]	0/28 (0)	0/14 (0)	1/29 (3)	3/25 (12)
P Values[c,d]		P = 0.046	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]				Infinite	Infinite
	Lower Limit			0.053	0.691
	Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]				Infinite	Infinite
	Lower Limit			0.027	0.362
	Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor			--	74	66

Table F2. Analyses of the Incidence of Primary Tumors in Mid- and High-Dose Male Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Mid- and High-Dose Pooled Control	Mid- and High-Dose Vehicle Control	Mid Dose	High Dose
Liver: Hepatocellular Adenoma[b]	6/28 (21)	4/14 (29)	2/29 (7)	0/25 (0)
P Values[c,d]	P = 0.008 (N)	P = 0.005 (N)	N.S.	P = 0.012* (N) P = 0.016**(N)
Relative Risk (Pooled Control)[f]			0.322	0.000
Lower Limit			0.034	0.000
Upper Limit			1.623	0.677
Relative Risk (Vehicle Control)[f]			0.241	0.000
Lower Limit			0.026	0.000
Upper Limit			1.498	0.577
Weeks to First Observed Tumor		81	67	--

[a]Dosed groups received 15 or 30 mg/kg.

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

Table F2. Analyses of the Incidence of Primary Tumors in Mid- and High-Dose Male Mice Administered Phenesterin by Gavage[a]

(continued)

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

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

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

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

Table F3. Analyses of the Incidence of Primary Tumors in Low-Dose Female Mice Administered Phenesterin by Gavage[a]

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	1/20 (5)	8/38 (21)
P Values[c,d]		N.S.
Relative Risk (Low-Dose Control)[e]		4.211
Lower Limit		0.638
Upper Limit		180.874
Weeks to First Observed Tumor	82	71
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	1/20 (5)	15/38 (39)
P Values[c,d]		P = 0.004
Relative Risk (Low-Dose Control)[e]		7.895
Lower Limit		1.391
Upper Limit		318.390
Weeks to First Observed Tumor	82	68

Table F3. Analyses of the Incidence of Primary Tumors in Low-Dose Female Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
Hematopoietic System: Lymphoma or Leukemia[b]	5/20 (25)	12/38 (32)
P Values[c,d]		N.S.
Relative Risk (Low-Dose Control)[e]		1.263
Lower Limit		0.499
Upper Limit		4.019
Weeks to First Observed Tumor	86	51
Liver: Hepatocellular Carcinoma[b]	1/20 (5)	1/37 (3)
P Values[c,d]		N.S.
Relative Risk (Low-Dose Control)[e]		0.541
Lower Limit		0.007
Upper Limit		41.317
Weeks to First Observed Tumor	104	54

Table F3. Analyses of the Incidence of Primary Tumors in Low-Dose Female Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	3/20 (15)	1/37 (3)
P Values[c,d]		N.S.
Relative Risk (Low-Dose Control)[e]		0.180
Lower Limit		0.004
Upper Limit		2.095
Weeks to First Observed Tumor	82	54
Myocardium: Sarcoma, NOS[b]	0/20 (0)	8/36 (22)
P Values[c,d]		P = 0.021
Relative Risk (Low-Dose Control)[e]		Infinite
Lower Limit		1.327
Upper Limit		Infinite
Weeks to First Observed Tumor	--	62

Table F3. Analyses of the Incidence of Primary Tumors in Low-Dose Female Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
All Sites: Hemangiosarcoma[b]	0/20 (0)	5/38 (13)
P Values[c,d]		N.S.
Relative Risk (Low-Dose Control)[e]		Infinite
Lower Limit		0.693
Upper Limit		Infinite
Weeks to First Observed Tumor	--	62
Mammary Gland: Adenocarcinoma, NOS[b]	0/20 (0)	6/38 (16)
P Values[c,d]		N.S.
Relative Risk (Low-Dose Control)[e]		Infinite
Lower Limit		0.879
Upper Limit		Infinite
Weeks to First Observed Tumor	--	54

Table F3. Analyses of the Incidence of Primary Tumors in Low-Dose Female Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
Ovary: Tubular Adenoma[b]	0/20 (0)	8/37 (22)
P Values[c,d]		P = 0.023
Relative Risk (Low-Dose Control)[e]		Infinite
Lower Limit		1.291
Upper Limit		Infinite
Weeks to First Observed Tumor	--	67

[a]Dosed group received 7 mg/kg.

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

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

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

[e]The 95 percent confidence interval of the relative risk between the dosed group and the control group.

Table F4. Analyses of the Incidence of Primary Tumors in Mid- and High-Dose Female Mice Administered Phenesterin by Gavage[a]

Topography: Morphology	Mid- and High-Dose Pooled Control	Mid- and High-Dose Vehicle Control	Mid Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	1/31 (3)	1/15 (7)	1/27 (4)	1/31 (3)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			1.148	1.000
Lower Limit			0.015	0.013
Upper Limit			86.774	75.984
Relative Risk (Vehicle Control)[f]			0.556	0.484
Lower Limit			0.008	0.007
Upper Limit			42.017	36.791
Weeks to First Observed Tumor		83	39	61

Table F4. Analyses of the Incidence of Primary Tumors in Mid- and High-Dose Female Mice Administered Pheaesterin by Gavage[a]

(continued)

Topography: Morphology	Mid- and High-Dose Pooled Control	Mid- and High-Dose Vehicle Control	Mid Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia[b]	0/31 (0)	0/15 (0)	14/27 (52)	17/32 (53)
P Values[c,d]	P < 0.001	P = 0.002	P < 0.001* P < 0.001**	P < 0.001* P < 0.001**
Departure from Linear Trend[e]	P = 0.018	P = 0.036		
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			5.181	5.399
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			2.624	2.735
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	22	15

Table F4. Analyses of the Incidence of Primary Tumors in Mid- and High-Dose Female Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Mid- and High-Dose Pooled Control	Mid- and High-Dose Vehicle Control	Mid Dose	High Dose
All Sites: Hemangiosarcoma[b]	0/31 (0)	0/15 (0)	1/27 (4)	2/32 (6)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.062	0.291
Upper Limit			Infinite	Infinite
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.031	0.147
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	57	51

[a]Dosed groups received 15 or 30 mg/kg.

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

Table F4. Analyses of the Incidence of Primary Tumors in Mid- and High-Dose Female Mice Administered Phenesterin by Gavage[a]

(continued)

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

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

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

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

Table F5. Time-Adjusted Analyses of the Incidence of Primary Tumors in Low-Dose Female Mice Administered Phenesterin by Gavage[a]

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
Lung: Alveolar/Bronchiolar Carcinoma[c]	1/18 (6)	8/35 (23)
P Values[d,e]		N.S.
Relative Risk (Low-Dose Control)[f]		4.114
Lower Limit		0.631
Upper Limit		176.029
Weeks to First Observed Tumor	82	71
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[c]	1/18 (6)	15/35 (43)
P Values[d,e]		P = 0.004
Relative Risk (Low-Dose Control)[f]		7.714
Lower Limit		1.380
Upper Limit		309.076
Weeks to First Observed Tumor	82	68

Table F5. Time-Adjusted Analyses of the Incidence of Primary Tumors in Low-Dose Female Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
Hematopoietic System: Lymphoma or Leukemia[b]	5/18 (28)	12/36 (33)
P Values[d,e]		N.S.
Relative Risk (Low-Dose Control)[f]		1.200
Lower Limit		0.484
Upper Limit		3.772
Weeks to First Observed Tumor	86	51
Liver: Hepatocellular Carcinoma[c]	1/18 (6)	1/34 (3)
P Values[d,e]		N.S.
Relative Risk (Low-Dose Control)[f]		0.529
Lower Limit		0.007
Upper Limit		40.372
Weeks to First Observed Tumor	104	54

Table F5. Time-Adjusted Analyses of the Incidence of Primary Tumors in Low-Dose Female Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
Liver: Hepatocellular Adenoma or Carcinoma[c]	3/18 (17)	1/34 (3)
P Values[d,e]		N.S.
Relative Risk (Low-Dose Control)[f]		0.176
Lower Limit		0.004
Upper Limit		2.038
Weeks to First Observed Tumor	82	54
Myocardium: Sarcoma, NOS[c]	0/18 (0)	8/34 (24)
P Values[d,e]		P = 0.024
Relative Risk (Low-Dose Control)[f]		Infinite
Lower Limit		1.276
Upper Limit		Infinite
Weeks to First Observed Tumor	--	62

Table F5. Time-Adjusted Analyses of the Incidence of Primary Tumors in Low-Dose Female Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
All Sites: Hemangiosarcoma[c]	0/18 (0)	5/35 (14)
P Values[d,e]		N.S.
Relative Risk (Low-Dose Control)[f]		Infinite
Lower Limit		0.682
Upper Limit		Infinite
Weeks to First Observed Tumor	--	62
Mammary Gland: Adenocarcinoma, NOS[c]	0/18 (0)	6/35 (17)
P Values[d,e]		N.S.
Relative Risk (Low-Dose Control)[f]		Infinite
Lower Limit		0.866
Upper Limit		Infinite
Weeks to First Observed Tumor	--	54

Table F5. Time-Adjusted Analyses of the Incidence of Primary Tumors in Low-Dose Female Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Low-Dose Vehicle Control	Low Dose
Ovary: Tubular Adenoma[c]	0/18 (0)	8/34 (24)
P Values[d,e]		P = 0.024
Relative Risk (Low-Dose Control)[f]		Infinite
Lower Limit		1.276
Upper Limit		Infinite
Weeks to First Observed Tumor	--	67

[a]Dosed group received 7 mg/kg.

[b]Number of tumor-bearing animals/number of animals examined at site (percent) which survived at least 51 weeks of the study.

[c]Number of tumor-bearing animals/number of animals examined at site (percent) which survived at least 52 weeks of the study.

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

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

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

Table F6. Time-Adjusted Analyses of the Incidence of Primary Tumors in Mid- and High-Dose Female Mice Administered Phenesterin by Gavage[a]

Topography: Morphology	Mid- and High-Dose Vehicle Control	Mid Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[c]	1/15 (7)	1/18 (6)	1/5 (20)
P Values[e,g]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[i]		0.833	3.000
Lower Limit		0.011	0.041
Upper Limit		61.743	178.689
Weeks to First Observed Tumor	83	39	61
Hematopoietic System: Lymphoma or Leukemia[b]	0/15 (0)	14/18 (78)	17/19 (89)
P Values[f,g]	P < 0.001	P < 0.001	P < 0.001
Departure from Linear Trend[h]	P = 0.016		
Relative Risk (Vehicle Control)[i]		Infinite	Infinite
Lower Limit		4.089	4.966
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	22	15

Table F6. Time-Adjusted Analyses of the Incidence of Primary Tumors in Mid- and High-Dose Female Mice Administered Phenesterin by Gavage[a]

(continued)

Topography: Morphology	Mid- and High-Dose Vehicle Control	Mid Dose	High Dose
Myocardium: Sarcoma, NOS[d]	0/15 (0)	2/7 (29)	3/7 (43)
P Values[f,g]	P = 0.012	N.S.	P = 0.023
Relative Risk (Vehicle Control)[i]		Infinite	Infinite
Lower Limit		0.685	1.421
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	62	42
All Sites: Hemangiosarcoma[e]	0/15 (0)	1/7 (14)	2/6 (33)
P Values[e,f]	P = 0.033	N.S.	N.S.
Relative Risk (Vehicle Control)[i]		Infinite	Infinite
Lower Limit		0.119	0.802
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	57	51

[a]Dosed groups received 15 or 30 mg/kg.

[b]Number of tumor-bearing animals/number of animals examined at site (percent) which survived at least 15 weeks of the study.

[c]Number of tumor-bearing animals/number of animals examined at site (percent) which survived at least 39 weeks of the study.

Table F6. Time-Adjusted Analyses of the Incidence of Primary Tumors in Mid- and High-Dose Female Mice Administered Phenesterin by Gavage[a]

(continued)

[d]Number of tumor-bearing animals/number of animals examined at site (percent) which survived at least 42 weeks of the study.

[e]Number of tumor-bearing animals/number of animals examined at site (percent) which survived at least 51 weeks of study.

[f]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.

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

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

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

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

March 6, 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 Phenestrin for carcinogenicity.

In a written review submitted by a Subgroup member, the reviewer agreed with the conclusion in the report that, under the conditions of test, Phenesterin was carcinogenic in female rats and in both sexes of mice. He briefly described the experimental design and the conditions under which Phenesterin was tested. In his critique, he noted the following points: the inadequate size of the treatment and control groups; the use of excessively high dose levels; and animals were housed in the same room in which other chemicals were under test. The reviewer said that these shortcomings detracted from the value of the study. He added that extrapolation of the results was made difficult by the use of excessive doses and other conditions under which Phenesterin was tested.

The secondary reviewer noted the poor selection of dose levels. He agreed that it would be difficult to extrapolate to lower doses for purposes of assessing human risk. He added that human risk is a secondary consideration

since Phenesterin is an anti-cancer agent. One Subgroup member said he would agree if Phenesterin is an effective anti-cancer agent. Another Subgroup member noted that anti-cancer agents also may be used to treat other diseases.

Another reviewer commented on the excessive amount of toxicity resulting from the high treatment doses administered, He felt, however, that an adequate number of animals survived to demonstrate the carcinogenicity of Phenesterin.

It was moved that the report on Phenesterin be accepted as written. The motion was seconded and approved unanimously,

Members present were

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology
Arnold L. Brown, Mayo Clinic
Lawrence Garfinkel, American Cancer Society
E. Cuyler Hammond, American Cancer Society
Joseph Highland, Environmental Defense Fund
Henry Pitot, University of Wisconsin Medical Center
George Roush, Jr., Monsanto Company
Sheldon Samuels, Industrial Union Department, AFL-CIO
Michael Shimkin, University of California at San Diego
John Weisburger, American Health Foundation
Sidney Wolfe, Health Research Group

* 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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