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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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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 phenoxybenzamine hydrochloride conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected environmental chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do 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 of phenoxybenzamine hydrochloride was conducted by Southern Research Institute, Birmingham, Alabama, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

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

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[4]. The statistical analyses were performed by Dr. J. R. Joiner[5], using methods selected for the bioassay program by Dr. J. J. Gart[6]. Chemicals used in this bioassay were analyzed under the direction of Dr. E. Murrill[7], and the analytical results were reviewed by Dr. C. W. Jameson[5].

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

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

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

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SUMMARY

A bioassay of phenoxybenzamine hydrochloride for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and B6C3F1 mice.

Groups of 35 rats of each sex were administered phenoxybenzamine hydrochloride 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 31 or 32 weeks. The vehicle used for most of the period of the bioassay was 6% propylene glycol in saline, although other vehicles, including 0.05% polysorbate 80 in saline, were used at the beginning. Controls consisted of groups of 10 vehicle controls and 10 untreated controls of each sex. All surviving rats were killed at 83-85 weeks.

Groups of 35 mice of each sex were administered phenoxybenzamine hydrochloride at one of two doses, either 12.5 or 25 mg/kg body weight, three times per week for 50 or 52 weeks, then observed for an additional 31-33 weeks. Controls consisted of groups of 15 males and 15 females which were administered the vehicle (vehicle controls), and groups of 14 males and 16 females which were untreated (untreated controls). All surviving mice were killed at 83-85 weeks.

Mean body weights of the low-dose male rats, low- and high-dose female rats, and low-dose male and female mice were comparable to those of the untreated and vehicle controls. The mean body weights of the high-dose male rats and the high-dose male and female mice, which died early, were lower than those of the controls.

Sarcoma of the abdominal cavity (peritoneum) was found in dosed animals of both species, but did not occur in either the untreated or vehicle controls. In male rats, this lesion occurred with a significant dose-related trend ($P < 0.001$), using vehicle controls, and also at significant incidences in direct comparisons of the dosed groups with the vehicle controls

(controls 0/10, low-dose 11/31, P = 0.027; high-dose 16/20, P < 0.001). In female rats, the lesion occurred at a significant incidence in the high-dose group compared with the vehicle controls (controls 0/9, high-dose 16/30, P = 0.004). None were observed among low-dose females.

In the mice, sarcoma of the abdominal cavity (peritoneum) occurred at a high and statistically significant incidence in the high-dose groups of each sex compared with vehicle controls (males: controls 0/15, high-dose 17/21, P < 0.001; females: controls 0/13, high-dose 16/20, P < 0.001). None were observed among low-dose groups. The morphology of the sarcoma was similar in the rats and the mice.

It is concluded that under the conditions of this bioassay, phenoxybenzamine hydrochloride was carcinogenic (sarcomagenic) for the peritoneum of both sexes of Sprague-Dawley rats and B6C3F1 mice.

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I. INTRODUCTION

Phenoxybenzamine hydrochloride (CAS 63-92-3; NCI C01661) is an antihypertensive agent that is used in controlling specific hypertensive crises such as those that result from high blood levels of sympathomimetic amines. Chemically, phenoxybenzamine undergoes cyclization and conversion to a reactive ethyleneimmonium intermediate. This intermediate binds almost irreversibly to alpha-adrenergic receptors in the autonomic nervous system, blocking further stimulation of these receptors by sympathetic nerves, or by sympathomimetic amines such as epinephrine or norepinephrine. Since these receptors control tension in vascular smooth muscle, peripheral blood pressure is reduced (Carrier, 1972; Goldstein et al., 1974; Nickerson and Collier, 1975).

Phenoxybenzamine hydrochloride is administered orally in doses of 20-200 mg/day, or by slow intravenous infusion at a dose of 1.0 mg/kg. It is used principally to manage hypertension associated with inoperable pheochromocytoma which causes excessive adrenal secretion of epinephrine and norepinephrine. This therapy may be extended for several years. Phenoxybenzamine hydrochloride is also recommended in the treatment of some peripheral vascular diseases such as Raynaud's syndrome, acrocyanosis, and frostbite sequelae (Nickerson and Collier, 1975; Smith, Kline, and French,

1977). Phenoxybenzamine hydrochloride was selected for testing as a part of the efforts of the Carcinogenesis Testing Program to investigate drugs that may be used for prolonged periods in humans.

II. MATERIALS AND METHODS

A. Chemical

Phenoxybenzamine hydrochloride (N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride) was obtained in two batches for the chronic studies (Lot Nos. 3-9DI-2 and 688-A-1-1DI) from the Smith, Kline, and French Laboratories, Philadelphia, Pennsylvania. The identity and purity of Lot No. 688-A-1-1DI was confirmed in analyses at the Midwest Research Institute. The melting point was 137-140°C, conforming to the published value of 138-140°C (Kerwin and Ullyot, 1952). Elemental analyses (C, H, N, Cl) were consistent with $C_{18}H_{23}Cl_2NO$, the molecular formula for phenoxybenzamine hydrochloride. Nuclear magnetic resonance, infrared, and ultraviolet spectra were in agreement with the structure and matched the spectra given in the literature (Sadtler Standard Spectra, 1966; Kracmar et al., 1966).

The chemical was stored at room temperature.

B. Dosage Preparation

Because of the difficulty encountered in maintaining the phenoxybenzamine hydrochloride in suspension, several different vehicles were used during the chronic studies. The procedure was to mix

the chemical with the vehicle in a Potter-Elvehjem tissue grinder for 20 seconds. Each concentration (0.125, 0.25, 0.2, and 0.4%) was prepared separately. The test suspensions were prepared daily and administered within 15 minutes of preparation. The suspensions of the test chemical administered to rats was contained in phosphate-buffered isotonic (pH 6.9) saline for one week, 0.05% polysorbate in phosphate-buffered saline (pH 6.9) for the next 13 weeks, saline only for one week, and 6% propylene glycol in saline for the remaining 37 weeks. Suspensions for mice were prepared with 0.05% polysorbate 80 in phosphate-buffered saline (pH 6.9) for the first 10 weeks and with 6% propylene glycol in saline for the remaining 42 weeks of the bioassay.

C. Animals

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

For the chronic studies, male and female Sprague-Dawley rats and B6C3F1 mice were obtained from Charles River Laboratories under a contract with the Division of Cancer Treatment, National Cancer Institute. On arrival at the laboratory, animals were 4-6 weeks of age. All animals were quarantined, rats for 7 days and mice

for approximately 14 days. 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%. 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 (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, beginning at week 14, cage tops were covered with disposable filter bonnets; mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N.Y.). Bedding was replaced once per week; cages, water bottles, and feeders were sanitized at 82°C once per week; 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 phenoxybenzamine hydrochloride were maintained in the same rooms as animals of the same species being administered the following chemicals:

RATS

Gavage Studies

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

Intraperitoneal Injection Studies

4'-(9-acridinylamino)methansulfon-m-aniside monohydrochloride (MAAM) (NSC 141549)
acronycine (CAS 7008-42-6)
5-azacytidine (CAS 320-67-2)
beta-2'-deoxy-6-thioguanosine monohydrate (beta-TGdR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3'-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] (CAS 3458-22-8)
+)-4,4'-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(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)

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)

Gavage Studies

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

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride (MAAM) (NSC 141549)
acronycine (CAS 7008-42-6)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGdR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride [IPD] (CAS 3458-22-8)
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N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)
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tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)

E. Subchronic Studies

Subchronic studies were conducted to estimate the maximum tolerated doses of phenoxybenzamine hydrochloride, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high doses") were determined for use in the chronic study. Phenoxybenzamine hydrochloride was administered in saline by intraperitoneal injection to female Sprague-Dawley rats at doses of 1.5, 3.75, 7.5, 15, or 30 mg/kg body weight and to male Swiss mice at doses of 2.5, 6.25, 12.5, 25, or 50 mg/kg. The animals were injected three times per week for 45 days, then observed for an additional 45 days. Five animals of each species were injected with the chemical at each dose, 10 received saline vehicle by the same route, and 10 were maintained as untreated controls.

In the rats, one death occurred after 10 weeks at 30 mg/kg. There were no effects on mean body weight gain, but all animals at this dose had abdominal distention, which occurred at week 5. At 15 mg/kg, 3/5 animals had abdominal distention, but there were no deaths or effects on mean weight gain. There were no deaths related to the administration of the chemical and no effects on mean weight gain at any of the lower doses. Gross necropsy of rats administered 30 mg/kg showed abdominal adhesions in 2/4

animals and small spleens in 2/4 animals. The low and high doses for rats were set at 5 and 10 mg/kg for the chronic studies.

All mice receiving 50 mg/kg died. There were no deaths in animals at any other dose tested, nor were mean body weight gains of animals at lower doses different from those of controls. No gross abnormalities were seen in any of the mice at necropsy. The low and high doses for mice were set at 12.5 and 25 mg/kg for the chronic studies.

F. Designs of Chronic Studies

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

No comparable vehicle-control groups from other studies were available to form pooled-control groups for statistical analysis.

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 that died prior to day 100, due, presumbly, to toxicity of the test chemical. Rats and mice were weighed individually every 2 weeks. 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

Table 1. Design of Chronic Studies of Phenoxybenzamine Hydrochloride in Rats

Sex and Test Group	Initial No. of Animals[a]	Phenoxybenzamine Hydrochloride Doses[b] (mg/kg)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male				
Untreated-Control	10	0		84
Vehicle-Control[c]	10	0	52	31-32
Low-Dose	35	5	52	31
High-Dose	35	10	52	6[d]
Female				
Untreated-Control	10	0		84-85
Vehicle-Control[c]	10	0	52	32
Low-Dose	35	5	52	32
High-Dose	35	10	52	31

[a]Males were 35 days of age and females were 42 days of age when placed on study.

[b]Phenoxybenzamine hydrochloride was administered intraperitoneally in a vehicle of phosphate-buffered saline for 1 week, 0.05% polysorbate 80 in phosphate-buffered saline for the next 13 weeks, saline only for 1 week, and 6% propylene glycol in saline for the remaining 37 weeks. The chemical was administered 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 vehicles, as described above, at a volume of 0.25 ml/100 g body weight.

[d]The observation period for the high-dose males terminated at the time indicated, due to the death of all animals.

Table 2. Design of Chronic Studies of Phenoxybenzamine Hydrochloride in Mice

Sex and Test Group	Initial No. of Animals[a]	Phenoxybenzamine Hydrochloride Doses[b] (mg/kg)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male				
Untreated-Control	14	0		84
Vehicle-Control[c]	15	0	52	32
Low-Dose	35	12.5	52	31
High-Dose	35	25	50[d]	
Female				
Untreated-Control	16	0		85
Vehicle-Control[c]	15	0	52	33
Low-Dose	35	12.5	52	32
High-Dose	35	25	52	2[e]

[a]Male mice were 41 days of age and females were 55 days of age when placed on study.

[b]Phenoxybenzamine hydrochloride was administered intraperitoneally in a vehicle of 0.05% polysorbate in phosphate-buffered saline for the first 10 weeks, then in a vehicle of 6% propylene glycol in saline for the remaining 42 weeks. The chemical was administered three times per week at a volume of 1 ml/100 g body weight. Doses were based on the mean weights of the animals in each cage.

[c]Vehicle-control groups received only the vehicles, as described above, at 1 ml/100 g body weight.

[d]The period of administration for the high-dose males terminated at the time indicated, due to the death of all animals.

[e]The observation period for the high-dose females terminated at the time indicated, due to the death of all animals.

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 narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

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

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

of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

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

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

group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the dosed group than in the control.

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

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of the low-dose male and the low- and high-dose female rats were comparable to those of the untreated and vehicle controls throughout the study (figure 1). Mean body weights of the high-dose males were consistently lower than those of the control groups from week 22 until the death of all animals in this group by week 58. Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. A few animals in the high-dose groups (3/35 males and 9/35 females) had distended abdomens, which was the only other sign of toxicity related to the administration of the chemical.

To control respiratory disease, oxytetracycline was administered in the drinking water at a dose of 0.6 mg/ml during weeks 14-18 and at a dose of 0.3 mg/ml during weeks 18-23.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered phenoxybenzamine hydrochloride by intraperitoneal injection at the doses of this bioassay, together with those of the vehicle and untreated controls, are shown in figure 2.

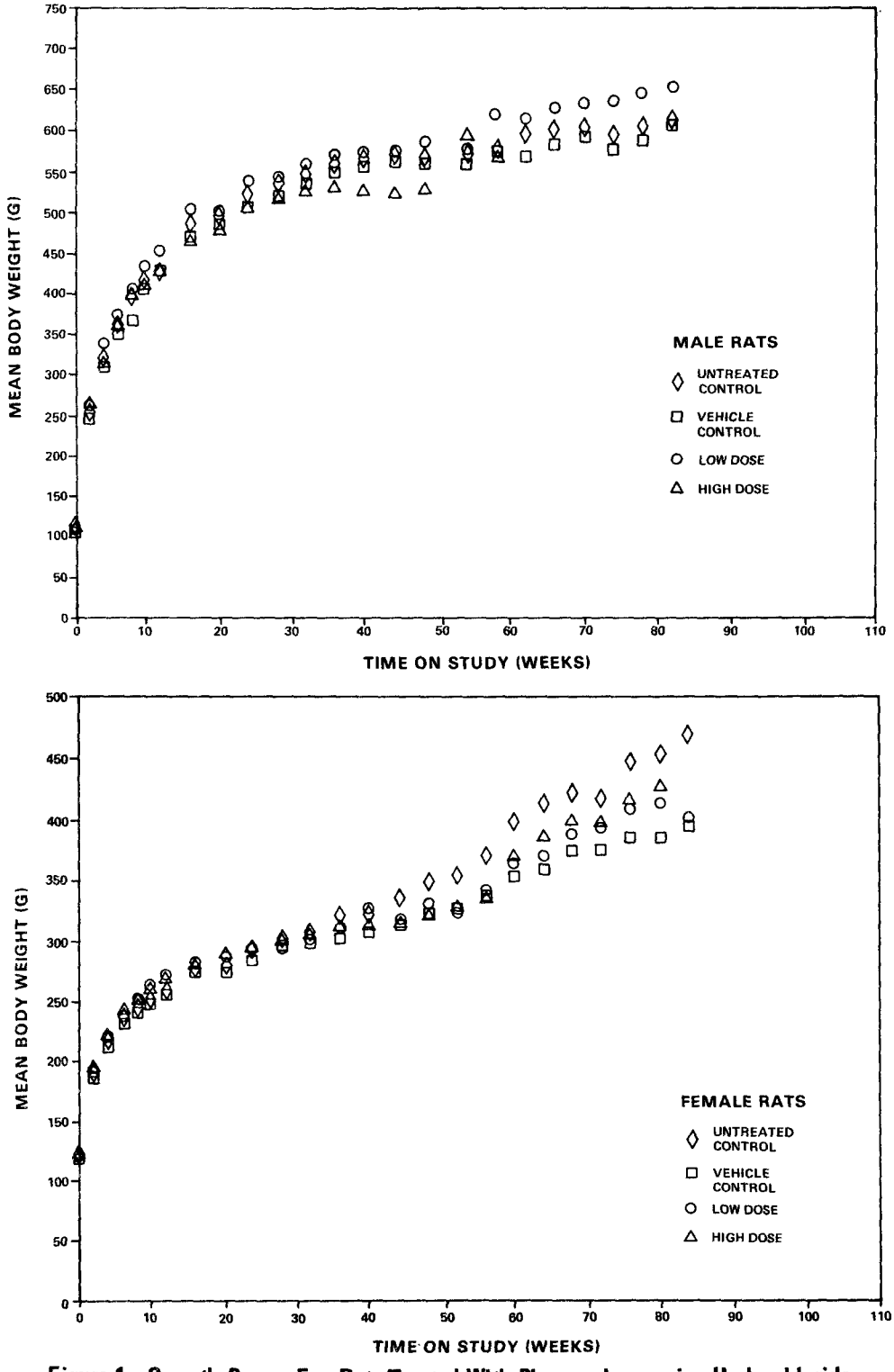


Figure 1. Growth Curves For Rats Treated With Phenoxybenzamine Hydrochloride

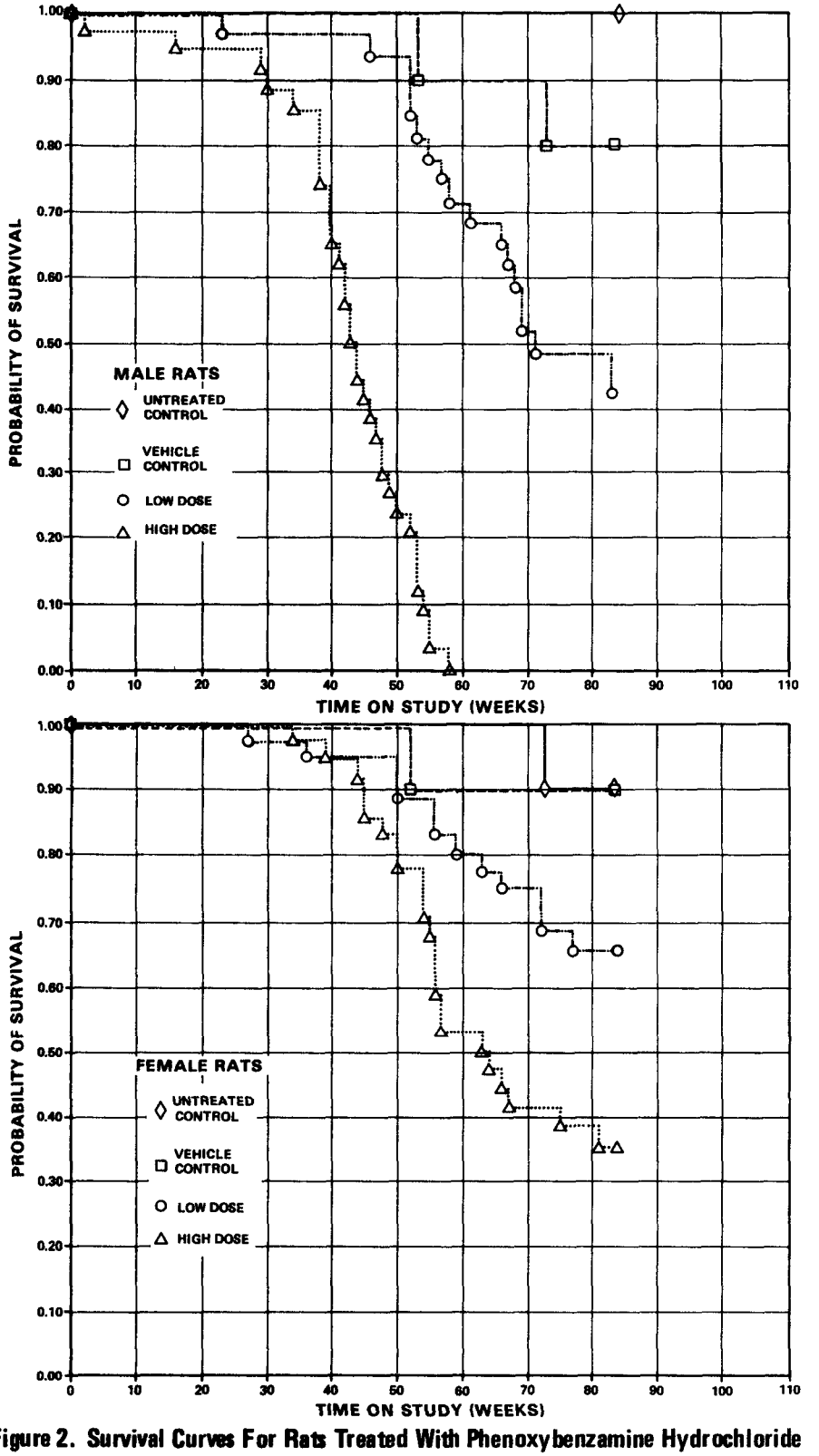


Figure 2. Survival Curves For Rats Treated With Phenoxybenzamine Hydrochloride

In each sex, the results of the Tarone test for positive dose-related trend are significant ($P < 0.001$), and an indicated departure from linear trend is observed in the male rats ($P < 0.001$), because of the steep decrease in survival in the high-dose male rats, of which none (0/35) lived to the end of the study. From the number started on study, 13/35 (37%) of the low-dose males and at least 80% of the vehicle- (8/10 [80%]) and untreated-control (10/10) males were alive at termination of the study. The median times on study of the high- and low-dose male rats were 43 weeks and 69 weeks, respectively. Of the high-dose male rats, 27/35 died before week 52 on study, and several tumors were observed before this time, one as early as week 30.

Of the number of female rats started on study, 12/35 (34%) of the high-dose group, 23/35 (66%) of the low-dose group, and 9/10 (90%) of both the vehicle- and the untreated-control groups lived to the end of the study. The median time on study of the high-dose female rats was 63 weeks. An adequate number of female rats was at risk for development of late-appearing tumors.

C. Pathology (Rats)

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

A variety of neoplasms occurred in both the control (untreated and vehicle) and dosed groups. Some types of neoplasms occurred only in rats of dosed groups, or with a greater frequency in dosed groups when compared with controls. Most of these tumors either occurred in insignificant numbers or were considered to be common in the Sprague-Dawley rat. However, the high incidence of peritoneal sarcomas in the dosed rats indicated that their presence was related to the intraperitoneal injection of phenoxybenzamine hydrochloride. The incidences of these tumors and of other possible drug-related lesions of the abdominal cavity were as follows:

MALES	Untreated Control	Vehicle Control	Low Dose	High Dose
Number of rats examined microscopically	(10)	(10)	(31)	(20)
Abdominal Cavity (Peritoneum)				
sarcoma, NOS*	0	0	11	16
chronic inflammation	0	0	4	1
granulomatous inflammation	0	0	0	1
osseous metaplasia	0	0	0	1
FEMALES				
Number of rats examined microscopically	(29)	(29)	(35)	(30)
Abdominal Cavity (Peritoneum)				
sarcoma, NOS*	0	0	0	16
chronic inflammation	0	0	2	1

*Not otherwise specified

The peritoneal sarcomas usually were multiple and firmly attached to the serosal surfaces of most abdominal organs. Histologically, the cells comprising these peritoneal sarcomas exhibited a wide range of cellular morphologies. The more highly differentiated cells were spindle shaped and had a large, open-faced, oval to elongated nucleus and an abundant eosinophilic cytoplasm. These spindle-shaped cells were usually associated with the deposition of a fibrous stroma, probably collagen. Other less differentiated cells were histiocytic in appearance. These cells had a large open-faced nucleus, a prominent eosinophilic nucleolus, and a moderate amount of eosinophilic cytoplasm. The cytoplasmic borders of these cells could not be delineated. The cells that exhibited the most poorly differentiated morphology had a large, oval to elongated nucleus that contained numerous fine clumps of chromatin. The chromatin gave the nuclei a stippled basophilic appearance. These cells had a moderate amount of eosinophilic cytoplasm, and the cytoplasmic borders were poorly defined. Giant cells were commonly observed in the tumors. The peritoneal tumors had often infiltrated the smooth muscle layers of the tubular abdominal organs. However, tumor metastases to the thorax were not observed.

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). Most of these nonneoplastic lesions are commonly seen in aged Sprague-Dawley rats. However, peritonitis and osseous metaplasia were observed only in dosed rats and were apparently related to the intraperitoneal injection of phenoxybenzamine hydrochloride.

The intraperitoneal administration of phenoxybenzamine hydrochloride to Sprague-Dawley rats at both doses of 5 and 10 mg/kg resulted in a high frequency of peritoneal sarcomas. In the judgment of the pathologists, phenoxybenzamine hydrochloride was carcinogenic to Sprague-Dawley rats under the conditions of this study.

D. Statistical Analyses of Results (Rats)

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

In each sex, the results of the Cochran-Armitage test for positive dose-related trend in the incidence of sarcoma of the

peritoneum are significant ($P < 0.001$), and an indicated departure from linear trend is observed ($P = 0.009$) in female rats, because of the steep increase in incidence in the high-dose group. The Fisher exact test shows that the incidences in the high-dose groups of each sex are significantly higher ($P < 0.005$) than those in the control groups. The Fisher exact comparison of this incidence of tumors between the low-dose males and the matched controls indicates a probability level of 0.027, which is above the 0.025 level required by the Bonferroni inequality criterion for multiple comparisons. The time-adjusted data on the incidence of this tumor in male rats, using only those animals that died at 30 weeks or more on study, are 0/10 in the vehicle-control group, 11/27 (41%) in the low-dose group, and 16/20 (80%) in the high-dose group. Using these incidences, the results of the Cochran-Armitage test are significant ($P < 0.001$) and those of the Fisher exact test are also significant when the incidences in both the low- and high-dose groups are compared with that in the controls ($P = 0.015$ and $P < 0.001$, respectively). The statistical conclusion is that the incidence of sarcoma of the peritoneum in rats is related to administration of the test chemical.

Significant results in the negative direction are observed in the incidences of pituitary tumors in each sex, where the incidences

in the controls exceed those in the dosed rats, probably because the dosed animals did not live as long as the controls, thus precluding the observation of the development of tumors in the dosed groups.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the low-dose mice of each sex were comparable to those of the vehicle controls for the duration of the study (figure 3); however, the mean body weights of the high-dose mice of each sex were lower than those of the vehicle controls, especially after week 20. The mean body weights of the untreated controls of each sex were higher than those of the corresponding vehicle controls throughout most of the study. Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. No other signs of toxicity related to the administration of the chemical were observed.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered phenoxybenzamine hydrochloride by intraperitoneal injection at the doses of this bioassay, together with those of the vehicle and untreated controls, are shown in figure 4.

In each sex, the results of the Tarone test for positive dose-related trend in mortality are significant ($P < 0.001$), and

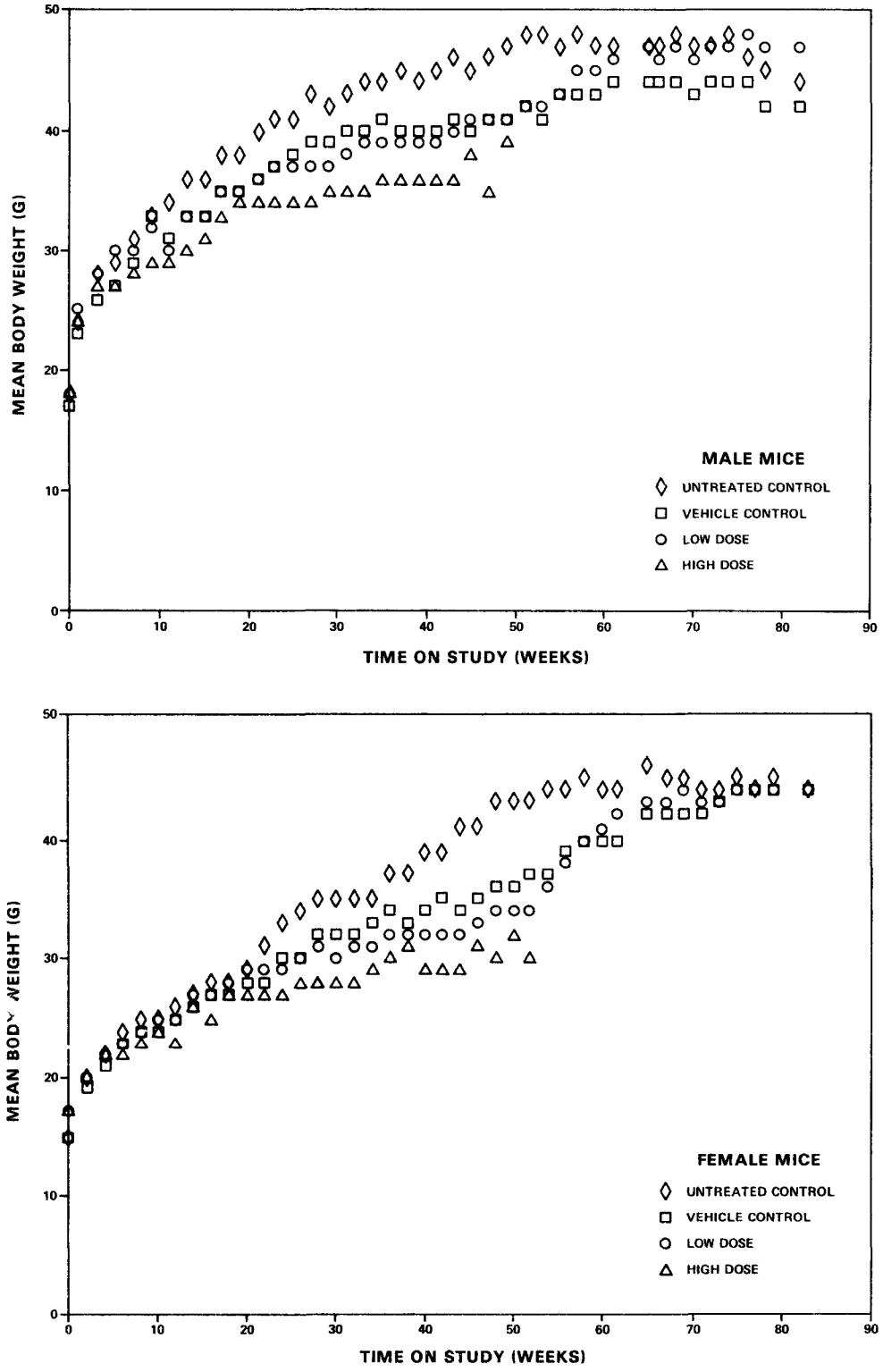
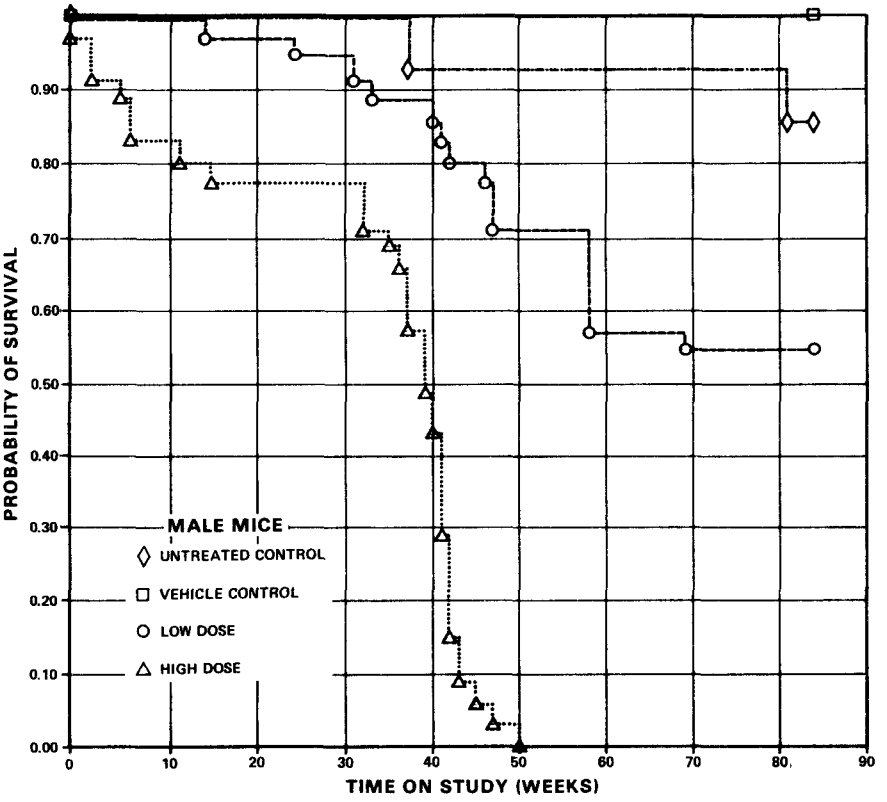


Figure 3. Growth Curves For Mice Treated With Phenoxybenzamine Hydrochloride



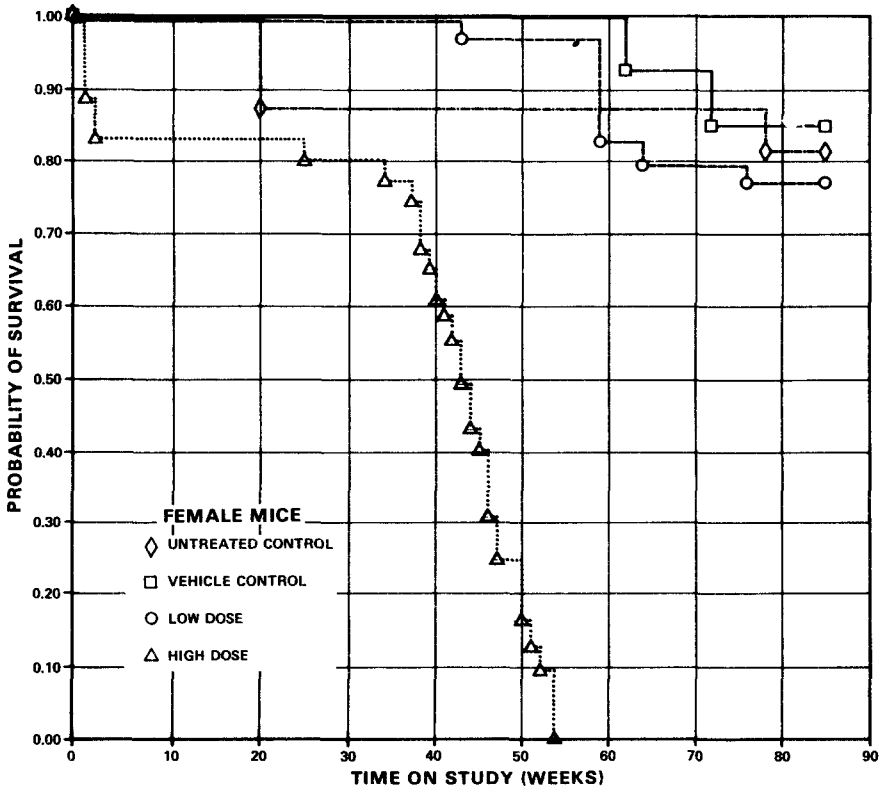


Figure 4. Survival Curves For Mice Treated With Phenoxybenzamine Hydrochloride

an indicated departure from linearity is observed (male mice $P = 0.001$, female mice $P < 0.001$), because of the steep decrease in survival in the high-dose animals. Of the male mice started in the bioassay, none (0/35) in the high-dose group, 19/35 (54%) in the low-dose group, all (15/15) in the vehicle-control group, and 12/14 (86%) in the untreated-control group lived to the end of the bioassay. The median time on study of the high-dose male mice was only 39 weeks. All the high-dose males died before 1 year on study, and tumors were observed as early as week 32 on study.

Of the female mice started in the bioassay, none (0/35) in the high-dose group, but at least 71% in the other three groups studied (25/35 [71%] in low-dose group, 11/15 [73%] in vehicle-control group, and 13/15 [86%] in untreated-control group), lived to the end of the bioassay. The median time on study of the high-dose female mice was 43 weeks. Of the high-dose female mice, 31/35 died before week 52, and a tumor was found as early as week 34.

C. Pathology (Mice)

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

A variety of neoplasms occurred in both untreated and vehicle controls and in dosed groups. Some types of neoplasms occurred only in mice of dosed groups, or with greater frequency in dosed groups when compared with controls. Most of these lesions either occurred in insignificant numbers or were considered to be a common finding in B6C3F1 mice. However, the peritoneal sarcomas observed at a high incidence in the dosed mice were considered to be related to the intraperitoneal injection of phenoxybenzamine hydrochloride. These tumors were observed in 17 high-dose male and 16 high-dose female mice, but in no low-dose, untreated- or vehicle-control mice.

The morphology and distribution of the peritoneal tumors in B6C3F1 mice were similar to those described for the rats.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the dosed and control groups (Appendix D). These nonneoplastic lesions are commonly seen in aged B6C3F1 mice.

The intraperitoneal administration of phenoxybenzamine hydrochloride to B6C3F1 mice at the high dose of 25 mg/kg resulted in a high frequency of peritoneal sarcomas. In the judgment of the pathologists, phenoxybenzamine hydrochloride was carcinogenic to B6C3F1 mice under the conditions of this study.

D. Statistical Analyses of Results (Mice)

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

In each sex, sarcomas of the peritoneum occurred exclusively in the high-dose group. The results of the Cochran-Armitage test for positive dose-related trend are significant ($P < 0.001$), and an indicated departure from linear trend is observed ($P < 0.001$) since there were no sarcomas occurring in the low-dose group. The Fisher exact test shows that the incidences in the high-dose groups are significantly higher ($P < 0.001$) than those in the controls. The statistical conclusion is that the incidence of sarcomas of the peritoneum is related to administration of the test chemical.

No other tumors appeared in a significantly higher proportion in any dosed group compared with that in the controls.

V. DISCUSSION

In this bioassay, mean body weights of the dosed groups that survived to the end of the bioassay were comparable to those of the untreated and vehicle controls, while those of the dosed groups that died early (high-dose male rats and high-dose male and female mice) were only slightly lower. None of the high-dose male rats or high-dose male and female mice lived to the end of the bioassay. Rates of survival of the high- and low-dose female rats were 34% and 66%, respectively, and those of the low-dose males were 37%. In the mice, 54% of the low-dose males and 71% of the low-dose females survived to the end of the study. It should also be noted that animals were administered the test chemical for only 52 weeks, which is a shorter period of time than used in current bioassays.

Sarcomas of the abdominal cavity (peritoneum) were found in dosed animals of both species, but did not occur in either the untreated or vehicle controls. In male rats, these lesions occurred with a significantly dose-related trend ($P < 0.001$) using vehicle controls and also at significant incidences in direct comparisons of the dosed groups with the controls (controls 0/10, low-dose 11/31, $P = 0.027$; high-dose 16/20, $P < 0.001$). In female rats, the lesions occurred at a significant incidence in the high-dose group compared with the

vehicle controls (controls 0/9, high-dose 16/30, $P = 0.004$). None were observed among low-dose females.

In the mice, sarcomas of the abdominal cavity (peritoneum) occurred at a high and statistically significant incidence in the high-dose groups of each sex compared with the vehicle controls (males: controls 0/15, high-dose 17/21, $P < 0.001$; females: controls 0/13, high-dose 16/20, $P < 0.001$). None were observed among low-dose groups. The morphology of the sarcomas found in the mice was similar to that of the sarcomas found in the rats.

Since the phenoxybenzamine hydrochloride was administered by intraperitoneal injection, and since the only tumors occurring at statistically significant incidences in either the dosed rats or the dosed mice were found in the peritoneum, the occurrence of these tumors appears to be related to the intraperitoneal injection of the test chemical.

The carcinogenic potential of phenoxybenzamine hydrochloride was examined by Stoner et al. (1973) in the strain A mouse pulmonary tumor test system. Four intraperitoneal injections into A/He mice of each sex of total doses of phenoxybenzamine hydrochloride of 200, 100, or 40 mg/kg body weight resulted in a greater incidence of lung tumors in the group administered the middle dose than in the controls by the time the study was terminated

(24 weeks). The investigators found the interpretation of these results difficult, however, because of the higher incidence at the middle dose than at the high dose.

It is concluded that under the conditions of this bioassay, phenoxybenzamine hydrochloride was carcinogenic for the peritoneum of both sexes of Sprague-Dawley rats and B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS GIVEN INTRAPERITONEAL INJECTIONS OF PHENOXYBENZAMINE HYDROCHLORIDE

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF PHENOXYBENZAMINE HYDROCHLORIDE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	10	31	20
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	31	20
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(10)	(10)	(31)	(20)
SQUAMOUS CELL CARCINOMA, METASTA			1 (3%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(10)	(10)	(31)	(20)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)	
#LYMPH NODE OF THORAX			(4)	(2)
CARCINOMA, NOS, METASTATIC			1	
SQUAMOUS CELL CARCINOMA, METASTA			1	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(10)	(10)	(31)	(20)
ADENOCARCINOMA, NOS, METASTATIC			1 (3%)	
#STOMACH	(10)	(10)	(31)	(19)
SQUAMOUS CELL CARCINOMA			1 (3%)	
ADENOCARCINOMA, NOS				1 (5%)
URINARY 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
ENDOCRINE SYSTEM				
#PITUITARY	(8)	(9)	(27)	(16)
CHROMOPHOBE ADENOMA	1 (13%)	3 (33%)		
CHROMOPHOBE CARCINOMA	1 (13%)			
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(10)	(10)	(31)	(20)
SQUAMOUS CELL CARCINOMA, METASTA			1 (3%)	
ADENOCARCINOMA, NOS			1 (3%)	
ADENOCARCINOMA, NOS, METASTATIC				1 (5%)
*PERITONEUM	(10)	(10)	(31)	(20)
SARCOMA, NOS			11 (35%)	16 (80%)
MESOTHELIOMA, NOS			4 (13%)	
ALL OTHER SYSTEMS				
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
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	35	35
NATURAL DEATH@			10	22
MORIBUND SACRIFICE		2	8	12
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED			4	1
TERMINAL SACRIFICE	10	8	13	
ANIMAL MISSING				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	3	15	16
TOTAL PRIMARY TUMORS	2	3	18	17
TOTAL ANIMALS WITH BENIGN TUMORS	1	3		
TOTAL BENIGN TUMORS	1	3		
TOTAL ANIMALS WITH MALIGNANT TUMORS	1		12	16
TOTAL MALIGNANT TUMORS	1		14	17
TOTAL ANIMALS WITH SECONDARY TUMORS#			3	1
TOTAL SECONDARY TUMORS			5	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			4	
TOTAL UNCERTAIN TUMORS			4	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

@ INCLUDES AUTOLYZED ANIMALS

* 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 GIVEN INTRAPERITONEAL INJECTIONS OF PHENOXYBENZAMINE HYDROCHLORIDE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	9	35	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	9	35	30
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(9)	(35)	(30)
CARCINOMA,NOS			1 (3%)	
*SUBCUT TISSUE	(10)	(9)	(35)	(30)
FIBROMA			2 (6%)	
RESPIRATORY SYSTEM				
#LUNG	(10)	(9)	(35)	(30)
HEMANGIOSARCOMA, METASTATIC			1 (3%)	
HEMATOPOIETIC SYSTEM				
NONE				
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(10)	(9)	(35)	(30)
HEMANGIOSARCOMA			1 (3%)	
URINARY SYSTEM				
NONE				

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
ENDOCRINE SYSTEM				
#PITUITARY	(6)	(7)	(32)	(29)
CHROMOPHOBE ADENOMA	1 (17%)	3 (43%)	11 (34%)	1 (3%)
CHROMOPHOBE CARCINOMA	1 (17%)		1 (3%)	
#ADRENAL	(10)	(9)	(35)	(30)
PHEOCHROMOCYTOMA, MALIGNANT			1 (3%)	
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(9)	(35)	(30)
ADENOMA, NOS				1 (3%)
ADENOCARCINOMA, NOS	1 (10%)		1 (3%)	
FIBROADENOMA	4 (40%)	2 (22%)	6 (17%)	3 (10%)
#UTERUS	(10)	(9)	(35)	(30)
LEIOMYOMA			1 (3%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(10)	(9)	(35)	(30)
ADENOCARCINOMA, NOS				1 (3%)
*PERITONEUM	(10)	(9)	(35)	(30)
SARCOMA, NOS				16 (53%)
MESOTHELIOMA, NOS				2 (7%)

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
MESOTHELIOMA, MALIGNANT			1 (3%)	
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	10	10	35	35
NATURAL DEATH@		1	5	9
MORIBUND SACRIFICE	1		7	13
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				1
TERMINAL SACRIFICE	9	9	23	12
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	5	20	20
TOTAL PRIMARY TUMORS	7	5	26	24
TOTAL ANIMALS WITH BENIGN TUMORS	5	5	15	4
TOTAL BENIGN TUMORS	5	5	20	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	2		6	16
TOTAL MALIGNANT TUMORS	2		6	17
TOTAL ANIMALS WITH SECONDARY TUMORS#			1	
TOTAL SECONDARY TUMORS			1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				2
TOTAL UNCERTAIN TUMORS				2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE GIVEN INTRAPERITONEAL INJECTIONS OF PHENOXYBENZAMINE HYDROCHLORIDE

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF PHENOXYBENZAMINE HYDROCHLORIDE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	14	15	35	35
ANIMALS NECROPSIED	14	15	30	21
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	15	30	21
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(14)	(15)	(30)	(21)
SARCOMA, NOS				1 (5%)
RESPIRATORY SYSTEM				
#LUNG	(14)	(15)	(30)	(21)
HEPATOCELLULAR CARCINOMA, METAST	1 (7%)	1 (7%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (7%)	2 (13%)	1 (3%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA			2 (7%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(14)	(15)	(30)	(21)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (7%)			
#LYMPH NODE OF THORAX	(1)	(3)	(3)	
HEPATOCELLULAR CARCINOMA, METAST		1		
#HILAR LYMPH NODE	(1)	(3)	(3)	
ADENOCARCINOMA, NOS, METASTATIC			1	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(14)	(14)	(30)	(21)
HEPATOCELLULAR ADENOMA	3 (21%)		6 (20%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA	2 (14%)	1 (7%)		
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(14)	(15)	(30)	(21)
ADENOCARCINOMA, NOS			1 (3%)	
SARCOMA, NOS				17 (81%)
*PLEURA	(14)	(15)	(30)	(21)
ADENOCARCINOMA, NOS			1 (3%)	
ALL OTHER SYSTEMS				
NONE				

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	14	15	35	35
NATURAL DEATH@	2		11	25
MORIBUND SACRIFICE			5	10
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	12	15	19	
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	3	7	18
TOTAL PRIMARY TUMORS	7	3	11	18
TOTAL ANIMALS WITH BENIGN TUMORS	4	2	6	
TOTAL BENIGN TUMORS	4	2	7	
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	1	2	18
TOTAL MALIGNANT TUMORS	3	1	4	18
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	1	
TOTAL SECONDARY TUMORS	1	2	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 FEMALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF PHENOXYBENZAMINE HYDROCHLORIDE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS MISSING		2	1	1
ANIMALS NECROPSIED	15	13	33	20
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	13	33	20
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(15)	(13)	(33)	(20)
ADENOCARCINOMA, NOS		1 (8%)		
RESPIRATORY SYSTEM				
#LUNG	(15)	(13)	(33)	(20)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (13%)	1 (8%)	1 (3%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (7%)			
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(13)	(33)	(20)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		2 (15%)	2 (6%)	
LYMPHOCYTIC LEUKEMIA			1 (3%)	
#MEDIASTINAL L.NODE	(2)	(1)		(1)
ADENOCARCINOMA, NOS, METASTATIC	1			
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(15)	(13)	(33)	(20)
HEPATOCELLULAR ADENOMA			1 (3%)	
HEPATOCELLULAR CARCINOMA		1 (8%)		
URINARY SYSTEM				
NONE				

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM				
#THYROID	(10)	(11)	(28)	(12)
PAPILLARY ADENOCARCINOMA	1 (10%)			
REPRODUCTIVE SYSTEM				
#UTERUS	(15)	(13)	(33)	(20)
ENDOMETRIAL STROMAL POLYP			1 (3%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(15)	(13)	(33)	(20)
SARCOMA, NOS				16 (80%)
*PLEURA	(15)	(13)	(33)	(20)
SARCOMA, NOS			1 (3%)	
ALL OTHER SYSTEMS				
NONE				

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	35	35
NATURAL DEATH@	2	2	3	18
MORIBUND SACRIFICE	1		5	15
SCHEDULED SACRIFICE			1	
ACCIDENTALLY KILLED				1
TERMINAL SACRIFICE	13	11	25	
ANIMAL MISSING		2	1	1
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	5	7	16
TOTAL PRIMARY TUMORS	4	5	7	16
TOTAL ANIMALS WITH BENIGN TUMORS	2	1	3	
TOTAL BENIGN TUMORS	2	1	3	
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	4	4	16
TOTAL MALIGNANT TUMORS	2	4	4	16
TOTAL ANIMALS WITH SECONDARY TUMORS#	1			
TOTAL SECONDARY TUMORS	1			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS GIVEN INTRAPERITONEAL INJECTIONS OF PHENOXYBENZAMINE HYDROCHLORIDE

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF PHENOXYBENZAMINE HYDROCHLORIDE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	10	31	20
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	31	20
INTEGUMENTARY SYSTEM				
*SKIN	(10)	(10)	(31)	(20)
EPIDERMAL INCLUSION CYST			1 (3%)	
HYPERKERATOSIS			1 (3%)	
*SUBCUT TISSUE	(10)	(10)	(31)	(20)
INFLAMMATION, CHRONIC	1 (10%)			
RESPIRATORY SYSTEM				
#LUNG/BRONCHIOLE	(10)	(10)	(31)	(20)
HYPERPLASIA, LYMPHOID	2 (20%)	3 (30%)		
#LUNG	(10)	(10)	(31)	(20)
BRONCHOPNEUMONIA SUPPURATIVE	1 (10%)			
BRONCHOPNEUMONIA CHRONIC SUPPURA	1 (10%)	2 (20%)	2 (6%)	
PIGMENTATION, NOS				1 (5%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(9)	(10)	(31)	(20)
ATROPHY, NOS	6 (67%)	5 (50%)	7 (23%)	
#SPLEEN	(10)	(10)	(31)	(19)
HEMATOPOIESIS			2 (6%)	1 (5%)
#MESENTERIC L. NODE			(4)	(2)
HYPERPLASIA, LYMPHOID			1 (25%)	
CIRCULATORY SYSTEM				
NONE				

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
DIGESTIVE SYSTEM				
#LIVER	(10)	(10)	(31)	(20)
HEMORRHAGE	1 (10%)			
INFLAMMATION, NECROTIZING			1 (3%)	
NECROSIS, COAGULATIVE			1 (3%)	2 (10%)
LIPOIDOSIS		1 (10%)		1 (5%)
#LIVER/CENTRILOBULAR	(10)	(10)	(31)	(20)
NECROSIS, COAGULATIVE			2 (6%)	2 (10%)
LIPOIDOSIS				1 (5%)
#PANCREAS	(10)	(10)	(31)	(19)
INFLAMMATION, ACUTE NECROTIZING				1 (5%)
#PANCREATIC ACINUS	(10)	(10)	(31)	(19)
ATROPHY, NOS			1 (3%)	
#STOMACH	(10)	(10)	(31)	(19)
CALCIFICATION, METASTATIC			1 (3%)	
URINARY SYSTEM				
#KIDNEY	(10)	(10)	(31)	(20)
INFLAMMATION, CHRONIC	6 (60%)	5 (50%)	13 (42%)	
ENDOCRINE SYSTEM				
#ADRENAL	(10)	(10)	(31)	(20)
ANGIECTASIS	1 (10%)			
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				

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
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(10)	(10)	(31)	(20)
INFLAMMATION, CHRONIC			4 (13%)	1 (5%)
INFLAMMATION, GRANULOMATOUS				1 (5%)
METAPLASIA, OSSEOUS				1 (5%)
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED		2	3	
ACCIDENTAL DEATH			1	1
AUTO/NECROPSY/HISTO PERF			1	
AUTOLYSIS/NO NECROPSY			3	14

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF PHENOXYBENZAMINE HYDROCHLORIDE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	35	35
ANIMALS NECROPSIED	10	9	35	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	9	35	30
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#TRACHEA	(10)	(9)	(35)	(30)
INFLAMMATION, SUPPURATIVE			2 (6%)	
INFLAMMATION, CHRONIC		1 (11%)		
#LUNG/BRONCHUS	(10)	(9)	(35)	(30)
INFLAMMATION, SUPPURATIVE			1 (3%)	
#LUNG/BRONCHIOLE	(10)	(9)	(35)	(30)
INFLAMMATION OBLITERATIVE			1 (3%)	
HYPERPLASIA, EPITHELIAL			1 (3%)	
HYPERPLASIA, LYMPHOID	2 (20%)			
#LUNG	(10)	(9)	(35)	(30)
BRONCHOPNEUMONIA SUPPURATIVE	1 (10%)		1 (3%)	
PNEUMONIA INTERSTITIAL CHRONIC	1 (10%)			
BRONCHOPNEUMONIA CHRONIC SUPPURA	2 (20%)		1 (3%)	
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(10)	(8)	(35)	(29)
ATROPHY, NOS	3 (30%)	3 (38%)	10 (29%)	5 (17%)
#SPLEEN	(10)	(9)	(35)	(30)
HEMATOPOIESIS			1 (3%)	2 (7%)
#MESENTERIC L. NODE			(1)	(1)
CONGESTION, NOS			1 (100%)	
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)	(9)	(35)	(30)
NECROSIS, FOCAL				1 (3%)
ANGIECTASIS				1 (3%)
#PANCREATIC ACINUS	(10)	(9)	(35)	(30)
ATROPHY, NOS			1 (3%)	
URINARY SYSTEM				
#KIDNEY	(10)	(9)	(35)	(30)
INFLAMMATION, NOS				1 (3%)
PYELONEPHRITIS SUPPURATIVE			1 (3%)	
INFLAMMATION, CHRONIC	1 (10%)	2 (22%)	7 (20%)	1 (3%)
ENDOCRINE SYSTEM				
#ADRENAL	(10)	(9)	(35)	(30)
ANGIECTASIS	4 (40%)	1 (11%)	3 (9%)	1 (3%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(10)	(9)	(35)	(30)
CYST, NOS	1 (10%)		8 (23%)	3 (10%)
*VAGINA	(10)	(9)	(35)	(30)
INFLAMMATION, SUPPURATIVE		1 (11%)		
#CERVIX UTERI	(10)	(9)	(35)	(30)
INFLAMMATION, ACUTE/CHRONIC		1 (11%)		
#UTERUS/ENDOMETRIUM	(10)	(9)	(35)	(30)
INFLAMMATION, SUPPURATIVE	3 (30%)	4 (44%)	5 (14%)	2 (7%)
INFLAMMATION, ACUTE/CHRONIC		1 (11%)		
INFLAMMATION, CHRONIC SUPPURATIV			2 (6%)	
HYPERPLASIA, CYSTIC				1 (3%)
METAPLASIA, SQUAMOUS		1 (11%)		
#OVARY	(10)	(9)	(33)	(30)
CYST, NOS	2 (20%)			1 (3%)

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
ABSCESS, NOS		1 (11%)		
INFLAMMATION, CHRONIC SUPPURATIV		1 (11%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(10)	(9)	(35)	(30)
STEATITIS			2 (6%)	
*PERITONEUM	(10)	(9)	(35)	(30)
INFLAMMATION, CHRONIC			1 (3%)	1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)	
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	1	1	6	1
AUTOLYSIS/NO NECROPSY		1		5

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE GIVEN INTRAPERITONEAL INJECTIONS OF PHENOXYBENZAMINE HYDROCHLORIDE

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF PHENOXYBENZAMINE HYDROCHLORIDE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	14	15	35	35
ANIMALS NECROPSIED	14	15	30	21
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	15	30	21
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(14)	(15)	(30)	(21)
BRONCHOPNEUMONIA SUPPURATIVE		1 (7%)		
PNEUMONIA INTERSTITIAL CHRONIC			1 (3%)	
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(14)	(14)	(30)	(18)
HYPERPLASIA, HEMATOPOIETIC	1 (7%)			
#SPLEEN	(14)	(15)	(30)	(21)
HYPERPLASIA, HEMATOPOIETIC	1 (7%)			
HEMATOPOIESIS	4 (29%)	1 (7%)	1 (3%)	2 (10%)
#MESENTERIC L. NODE	(1)	(3)	(3)	
CONGESTION, NOS		1 (33%)	1 (33%)	
INFLAMMATION, SUPPURATIVE		1 (33%)		
HYPERPLASIA, LYMPHOID			1 (33%)	
#INGUINAL LYMPH NODE	(1)	(3)	(3)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (33%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(14)	(14)	(30)	(21)
HYPERPLASIA, NODULAR		1 (7%)	1 (3%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, HEMATOPOIETIC	1 (7%)			
#LIVER CENTRILOBULAR	(14)	(14)	(30)	(21)
NECROSIS, NOS	1 (7%)			
URINARY SYSTEM				
#KIDNEY	(14)	(15)	(30)	(21)
HYDRONEPHROSIS	1 (7%)	1 (7%)	1 (3%)	
PYELONEPHRITIS SUPPURATIVE	1 (7%)			
#URINARY BLADDER	(14)	(15)	(30)	(20)
INFLAMMATION, CHRONIC			1 (3%)	
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
#PROSTATE	(14)	(15)	(30)	(19)
INFLAMMATION, SUPPURATIVE	1 (7%)			
*SEMINAL VESICLE	(14)	(15)	(30)	(21)
INFLAMMATION, SUPPURATIVE	1 (7%)	1 (7%)		
*EPIDIDYMIS	(14)	(15)	(30)	(21)
INFLAMMATION, SUPPURATIVE	1 (7%)			
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	3	6	20	3
NO NECROPSY PERFORMED				7
AUTOLYSIS/NO NECROPSY			5	7

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF PHENOXYBENZAMINE HYDROCHLORIDE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS MISSING		2	1	1
ANIMALS NECROPSIED	15	13	33	20
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	13	33	20
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(15)	(13)	(33)	(20)
HYPERPLASIA, LYMPHOID		1 (8%)		
HEMATOPOIETIC SYSTEM				
#SPLEEN	(15)	(13)	(33)	(20)
HEMATOPOIESIS	1 (7%)		1 (3%)	1 (5%)
#MESENTERIC L. NODE	(2)	(1)		(1)
HYPERPLASIA, LYMPHOID	1 (50%)			
#THYMUS	(15)	(13)	(33)	(19)
INFLAMMATION, SUPPURATIVE	1 (7%)			
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(15)	(13)	(33)	(20)
NECROSIS, COAGULATIVE			1 (3%)	
HYPERPLASIA, NODULAR			1 (3%)	
#PANCREATIC ACINUS	(15)	(12)	(33)	(19)
ATROPHY, NOS				1 (5%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#ILEUM	(15)	(13)	(33)	(19)
ULCER, FOCAL			1 (3%)	
URINARY SYSTEM				
#KIDNEY	(15)	(13)	(33)	(20)
INFLAMMATION, CHRONIC			1 (3%)	
HYPERPLASIA, LYMPHOID		1 (8%)		
#KIDNEY/GLOMERULUS	(15)	(13)	(33)	(20)
AMYLOIDOSIS	1 (7%)			
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
#UTERUS/ENDOMETRIUM	(15)	(13)	(33)	(20)
INFLAMMATION, SUPPURATIVE	1 (7%)			
HYPERPLASIA, CYSTIC	12 (80%)	10 (77%)	27 (82%)	2 (10%)
#OVARY	(15)	(13)	(33)	(20)
CYST, NOS			2 (6%)	
HEMORRHAGE				1 (5%)
HEMORRHAGIC CYST			1 (3%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES				
*ABDOMINAL CAVITY	(15)	(13)	(33)	(20)
STEATITIS	1 (7%)			
*PERITONEUM	(15)	(13)	(33)	(20)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)	
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	2		3	3
ANIMAL MISSING/NO NECROPSY		2	1	1
NO NECROPSY PERFORMED				6
AUTOLYSIS/NO NECROPSY	1		1	8

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS TREATED WITH PHENOXYBENZAMINE HYDROCHLORIDE

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Treated with Phenoxybenzamine Hydrochloride[a]

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma[b]	3/9 (33)	0/27 (0)	0/16 (0)
P Values[c,d]	P = 0.006(N)	P = 0.012(N)	P = 0.037(N)
Departure from Linear Trend[e]	P = 0.011		
Relative Risk (Vehicle Control)[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		0.527	0.863
Weeks to First Observed Tumor	73	--	--
Peritoneum: Sarcoma, NOS[b]	0/10 (0)	11/31 (35)	16/20 (80)
P Values[c,d]	P < 0.001	P = 0.027	P < 0.001
Relative Risk (Vehicle Control)[f]		Infinite	Infinite
Lower Limit		1.204	2.961
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	46	30

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Treated with Phenoxybenzamine Hydrochloride[a]

(continued)

Topography:	Morphology	Vehicle Control	Low Dose	High Dose
Peritoneum:	Mesothelioma[b]	0/10 (0)	4/31 (13)	0/20 (0)
P Values[c,d]		N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			Infinite	--
Lower Limit			0.334	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor		--	83	--

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

[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 when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

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

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

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

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Treated with Phenoxybenzamine Hydrochloride[a]

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Subcutaneous Tissue: Fibroma[b]	0/9 (0)	2/35 (6)	0/30 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		Infinite	--
Lower Limit		0.085	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	84	--
Pituitary: Chromophobe Adenoma or Carcinoma[b]	3/7 (43)	12/32 (38)	1/29 (3)
P Values[c,d]	P = 0.002(N)	N.S.	P = 0.018(N)
Relative Risk (Vehicle Control)[f]		0.875	0.080
Lower Limit		0.376	0.002
Upper Limit		4.030	0.874
Weeks to First Observed Tumor	84	56	83

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Treated with Phenoxybenzamine Hydrochloride[a]

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	2/9 (22)	6/35 (17)	3/30 (10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.771	0.450
Lower Limit		0.184	0.067
Upper Limit		7.155	4.926
Weeks to First Observed Tumor	84	50	56
Peritoneum: Sarcoma, NOS[b]	0/9 (0)	0/35 (0)	16/30 (53)
P Values[c,d]	P < 0.001	N.S.	P = 0.004
Departure from Linear Trend[e]	P = 0.009		
Relative Risk (Vehicle Control)[f]		--	Infinite
Lower Limit		--	1.740
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	45

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Treated with Phenoxybenzamine Hydrochloride[a]

(continued)

Topography:	Morphology	Vehicle Control	Low Dose	High Dose
Peritoneum:	Mesothelioma[b]	0/9 (0)	1/35 (3)	2/30 (7)
P Values[c,d]		N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]			Infinite	Infinite
Lower Limit			0.015	0.100
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor		--	56	57

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

[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 when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

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

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

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE TREATED WITH PHENOXYBENZAMINE HYDROCHLORIDE

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Treated with Phenoxybenzamine Hydrochloride[a]

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/15 (0)	2/30 (7)	0/21 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		Infinite	--
Lower Limit		0.156	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	69	--
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	2/15 (13)	3/30 (10)	0/21 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.750	0.000
Lower Limit		0.099	0.000
Upper Limit		8.343	2.319
Weeks to First Observed Tumor	84	69	--

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Treated with Phenoxybenzamine Hydrochloride[a]

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma[b]	0/14 (0)	6/30 (20)	0/21 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.006		
Relative Risk (Vehicle Control)[f]		Infinite	--
Lower Limit		0.806	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	58	--
Liver: Hepatocellular Adenoma or Carcinoma[b]	1/14 (7)	6/30 (20)	0/21 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.034		
Relative Risk (Vehicle Control)[f]		2.800	0.000
Lower Limit		0.399	0.000
Upper Limit		123.635	12.140
Weeks to First Observed Tumor	82	58	--

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Treated with Phenoxybenzamine Hydrochloride[a]

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Abdominal Cavity: Sarcoma, NOS[b]	0/15 (0)	0/30 (0)	17/21 (81)
P Values[c,d]	P < 0.001	N.S.	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Vehicle Control)[f]		--	Infinite
Lower Limit		--	4.345
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	32

[a]Dosed groups received 12.5 or 25 mg/kg by intraperitoneal injection.

[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 when P < 0.05; otherwise, not significant (N.S.) is indicated.

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

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

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

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Treated with Phenoxybenzamine Hydrochloride[a]

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	2/13 (15)	2/33 (6)	0/20 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.394	0.000
Lower Limit		0.033	0.000
Upper Limit		5.070	2.100
Weeks to First Observed Tumor	62	84	--
Hematopoietic System: Lymphoma or Leukemia[b]	2/13 (15)	3/33 (9)	0/20 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[f]		0.591	0.000
Lower Limit		0.080	0.000
Upper Limit		6.583	2.100
Weeks to First Observed Tumor	62	76	--

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Treated with Phenoxybenzamine Hydrochloride[a]

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Abdominal Cavity: Sarcoma, NOS[b]	0/13 (0)	0/33 (0)	16/20 (80)
P Values[c,d]	P < 0.001	N.S.	P < 0.001
Departure from Linear Trend[e]	P < 0.001		
Relative Risk (Vehicle Control)[f]		--	Infinite
Lower Limit		--	3.745
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	34

[a]Dosed groups received 12.5 or 25 mg/kg by intraperitoneal injection.

[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 when P < 0.05; otherwise, not significant (N.S.) is indicated.

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

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

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

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

March 7, 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 Phenoxybenzamine Hydrochloride for carcinogenicity.

The primary reviewer agreed with the conclusion that Phenoxybenzamine Hydrochloride produced sarcomas in rats and mice upon interperitoneal injection. Although other tumors were found among treated animals that were not observed in controls, none occurred in statistically significant numbers. He pointed out the bone marrow atrophy in both controls and treated animals. He also noted that the increased incidence of lung tumors, reported by Stoner et. al. in the Strain A mouse system, was in the "mid-zone" and is really equivalent to a negative. The primary reviewer said that the results did not indicate that Phenoxybenzamine Hydrochloride should be discontinued for the treatment of hypertensive crises, especially since the drug is administered intravenously in humans.

The secondary reviewer agreed with the conclusion given in the report. He pointed out the change in vehicle which occurred during the course of the chronic study. Given the usefulness of the drug, he said that it probably should continue to be used.

The primary reviewer moved that the report on the bioassay of Phenoxybenzamine Hydrochloride 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

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

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