National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 174
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FOR POSSIBLE CARCINOGENICITY

CAS No. 624-18-0

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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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DHEW Publication No. (NIH) 79-1730

REPORT ON THE BIOASSAY OF p-PHENYLENEDIAMINE DIHYDROCHLORIDE FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of p-phenylenediamine dihydrochloride 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 significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because 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 a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of p-phenylenediamine dihydrochloride was conducted by Litton Bionetics, Inc., Kensington, Maryland, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. N. P. Page (1,2), Dr. E. K. Weisburger (1) and Dr. J. H. Weisburger (1,3). The principal investigators for the contract were Dr. F. M. Garner (4) and Dr. B. M. Ulland (4,5). Mr. S. Johnson (4) was the coprincipal investigator for the contract. Animal treatment and observation were supervised by Mr. R. Cypher (4), Mr. D. S. Howard (4) and Mr. H. D. Thornett (4); Mr. H. Paulin (4) analyzed dosed feed mixtures. Ms. J. Blalock (4) was responsible for data collection and assembly. Chemical analysis was performed by Midwest Research Institute (6) and the analytical results were reviewed by Dr. N. Zimmerman (7).

Histopathologic examinations were performed by Dr. N. J. Wosu (4) at Litton Bionetics, Inc., the pathology narratives were written by Dr. N. J. Wosu (4), and the diagnoses included in this report represent the interpretation of this pathologist. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (8).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (9); the statistical analysis was performed by Mr. W. W. Belew (7,10), Mr. R. M. Helfand (7) and Dr. J. P. Dirkse, III (11), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (12).

This report was prepared at METREK, a Division of The MITRE Corporation (7) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (7), task leader Ms. P. Walker (7), senior biologist Mr. M. Morse (7), biochemist Mr. S. C. Drill (7), and technical editor Ms. P. A. Miller (7). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. R. A. Griesemer (1), Dr. T. E. Hamm (1), Dr. W. V. Hartwell (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,13), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay of p-phenylenediamine dihydrochloride for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F1 mice. p-Phenylenediamine dihydrochloride was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low concentrations of p-phenylenediamine dihydrochloride were, respectively, 1250 and 625 ppm for both rats and mice. After a 103-week period of compound administration, there were additional observation periods of 2 weeks for rats and 1 week for mice. Twenty animals of each sex and species were placed on test as controls.

There were no significant positive associations between the concentrations of p-phenylenediamine dihydrochloride administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Slight dose-related mean body weight depression was observed in female rats and the mean body weights among high dose male rats and dosed female mice were slightly depressed in relation to their respective controls, indicating that the concentrations of p-phenylenediamine dihydrochloride administered to these animals in this bioassay may have approximated the maximum tolerated concentrations. Since no distinct mean body weight depression relative to controls, no significant accelerated mortality, and no other signs of toxicity were associated with administration of p-phenylenediamine dihydrochloride to male mice, it is possible that these animals may have been able to tolerate a higher dietary concentration.

None of the statistical tests for any site in rats or mice of either sex, including time to leukemia or malignant lymphoma analysis in female mice, indicated a significant positive association between compound administration and tumor incidence.

Under the conditions of this bioassay, there was no convincing evidence that dietary administration of p-phenylenediamine dihydrochloride was carcinogenic in Fischer 344 rats or B6C3F1 mice.

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I. INTRODUCTION

p-Phenylenediamine dihydrochloride (Figure 1) (NCI No. C03930), a hydrochloride salt of p-phenylenediamine, the major component of many oxidation hair dyes (Corbett and Menkart, 1973), was selected for bioassay by the National Cancer Institute because of the increased incidence of bladder cancer reported among dye manufacturing industry workers (Wynder et al., 1963; Anthony and Thomas, 1970). Aromatic amines are one of several classes of chemicals thought to contribute to this increased cancer risk (Wynder et al., 1963). The widespread exposure to p-phenylenediamine among the general population and the possibility of an increased cancer risk among hairdressers (Anthony and Thomas, 1970) were additional factors in the selection of p-phenylenediamine dihydrochloride for testing.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 1,4-benzenediamine dihydrochloride.* It is also called p-PDA HCl; p-PD HCl; p-phenylenediamine di HCl; p-phenylenediamine HCl; Durafur Black RC; Fourrine DS; Fourrine 64; Pelagol Grey CD; and C.I. (Colour Index) Oxidation Base 10A (C.I. 76061).

p-Phenylenediamine is the most widely used primary intermediate in permanent hair dye formulations (Corbett and Menkart, 1973). p-Phenylenediamine is oxidized within the hair shaft by an oxidant, generally hydrogen peroxide, to produce a highly reactive di-imine.

*The CAS registry number is 624-18-0.

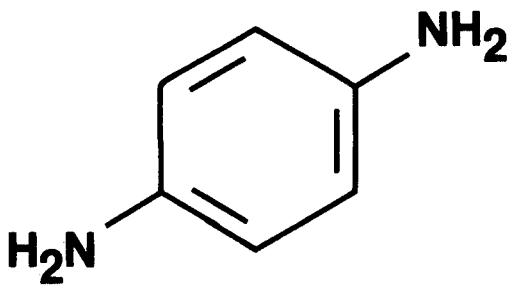


FIGURE 1
CHEMICAL STRUCTURE OF p-PHENYLENEDIAMINE (DIHYDROCHLORIDE)

This di-imine rapidly reacts with a coupler and/or an unoxidized p-phenylenediamine molecule to produce a stable indo dye. In the absence of a coupler, p-phenylenediamine produces a dark brown color, but by introducing various couplers into the dye formula, a wide range of brown, purple, and blue shades can be produced. The maximum concentration of p-phenylenediamine in commercial formulations is 3 percent (Corbett and Menkart, 1973). p-Phenylenediamine dihydrochloride may also be used to some extent as a primary intermediate in permanent hair dyes (Markland, 1966), and both this compound and the free base are used as oxidation bases in the dyeing of furs (Society of Dyers and Colourists, 1956; Windholz, 1976).

p-Phenylenediamine is a direct or indirect intermediate in the synthesis of at least 79 dyes, 7 of which are produced in commercially significant quantities in the United States: C.I. Disperse Black 2, C.I. Direct Green 28, C.I. Acid Violet 3, C.I. Direct Black 9, C.I. Direct Black 2, C.I. Direct Black 80, and C.I. Direct Black 19 (Society of Dyers and Colourists, 1956).

p-Phenylenediamine dihydrochloride is used as a reagent for blood, hydrogen sulfide, and amyl alcohol, and in the testing of milk (Windholz, 1976). p-Phenylenediamine is used as a photographic developing agent, in photochemical measurement processes, and as an intermediate in the manufacture of azo dyes, and antoxidants and accelerators for rubber vulcanization (Hawley, 1971).

Specific production data for p-phenylenediamine dihydrochloride and p-phenylenediamine are not available; however, inclusion of the latter compound in the 1977 Directory of Chemical Producers, U.S.A. (Stanford Research Institute, 1977) implies that p-phenylenediamine is produced in commercial quantities (greater than 1000 pounds or $1000 in value annually) in the United States. Commercial production of p-phenylenediamine dihydrochloride was reported in 1975 (U.S. International Trade Commission, 1977). Imports of p-phenylenediamine through principal U.S. customs districts amounted to 64,376 pounds in 1974 (U.S. International Trade Commission, 1976). Sales of C.I. Acid Violet 3 in 1975 amounted to 29,000 pounds (U.S. International Trade Commission, 1977).

Exposure to p-phenylenediamine dihydrochloride or p-phenylenediamine via dermal contact at the scalp is unavoidable among persons whose hair is colored with dyes that contain either of these substances. Hairdressers who apply these dyes may also be exposed. About 40 percent of U.S. women are regular users of hair dyes, and approximately three out of every four dollars spent on hair coloring is spent on the permanent type of dye (Corbett and Menkart, 1973). Additionally, exposure to these compounds may result from unreacted portions reaching rivers and streams via domestic wastewater.

Exposure to p-phenylenediamine and p-phenylenediamine dihydrochloride may occur at chemical and dye production facilities, among workers in the fur dyeing industry, and among laboratory personnel.

Exposure to the free base may occur via contact with photographic chemicals.

p-Phenylenediamine may cause skin irritation and is also responsible for asthmatic and other respiratory symptoms of workers in the fur dyeing industry. p-Phenylenediamine causes keratoconjunctivitis, swollen conjunctiva, and eczema of the eyelids (Sax, 1975).

p-Phenylenediamine was tumorigenic after subdermal injection in one study (species unspecified) (Hossack and Richardson, 1977), but no indication of carcinogenicity was found after up to 18 months of topical applications of hair dye preparations containing p-phenylenediamine to 600 random-bred Swiss Webster mice of both sexes (Burnett et al., 1975). Seven topical applications of preparations containing p-phenylenediamine to 80 female Charles River CD rats on days 1, 4, 7, 10, 13, 16 and 19 of gestation produced no teratogenic effects (Burnett et al., 1976). p-Phenylenediamine was topically administered twice weekly for life to Swiss mice and resulted in no significant decrease in lifespan and no statistically significant increased tumor incidence when dosed animals were compared to controls (Stenbäck et al., 1978).

p-Phenylenediamine failed to induce frame shift reversions from a histidine-requiring mutant back to prototype in Salmonella typhimurium strains TA97, TA98, TA100, and TA1538; however, the products of hydrogen peroxide oxidation of this compound were strongly mutagenic in TA1538 in the presence of rat liver microsomes (Ames et al., 1975). p-Phenylenediamine mutagenicity was indicated by marked difference

of inhibition for growth (DIG) in the DNA repair test in Escherichia coli strains B/r WP2 (trp^-) and WP100 (trp^-, $uvrA^-$, $recA^-$) (Nishioka, 1976). In a recessive lethal assay using Drosophila melanogaster, p-phenylenediamine demonstrated weak mutagenic activity (Blijleven, 1977).

Two mammalian mutagenicity studies have been conducted in vivo. p-Phenylenediamine showed no clear mutagenicity in the micronucleus test (increase in micronucleated erythrocytes) in CFY rats of both sexes after oral dosing (Hossack and Richardson, 1977). The compound was not mutagenic to germ cells in a dominant lethal study of Charles River CD rats following intraperitoneal administration of 20 mg/kg three times weekly for 8 weeks to 20 males (Burnett et al., 1977).

The previously mentioned known toxicity of p-phenylenediamine to humans and the suggestion of its tumorigenicity after subdermal injection in animals and mutagenicity in bacteria provided impetus for this bioassay of p-phenylenediamine dihydrochloride for possible carcinogenesis.

II. MATERIALS AND METHODS

A. Chemicals

Technical-grade p-phenylenediamine dihydrochloride was purchased in two batches from Aldrich Chemical Company, Milwaukee, Wisconsin. Chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri. Thin-layer chromatography (TLC) utilizing two solvent systems (i.e., methanol and dioxane) indicated one and three impurities, respectively. Infrared (IR) analysis indicated extraneous peaks in addition to those present in the reference spectra (Pouchert, 1975). Ultraviolet (UV) analysis indicated λ_{max} of 242 and 294 nm with respective molar extinction coefficients (ϵ) of 110 x 10^2 and 20 x 10^2. Although the ϵ values approximate the literature values (i.e., λ_{max} = 242 with ϵ = 100 x 10^2 and λ_{max} = 306 with ϵ = 21 x 10^2) (Barabashova et al., 1970), it is difficult to explain the shift of the second peak from 306 to 294 nm. Elemental analysis was consistent with the molecular formula for p-phenylenediamine dihydrochloride. Vapor-phase chromatography showed one homogeneous peak.

The second batch was purchased three months later from the same supplier. One nonmotile impurity was observed on the TLC plates, each developed with a different solvent system (i.e., methanol:ammonium hydroxide and n-butanol:H_2O:acetic acid). IR analysis again showed the presence of extraneous peaks. The results of UV analysis revealed λ_{max} of 239 and 292 nm with respective ϵ values of 122 x 10^2 and 19 x 10^2.

Throughout this report the term p-phenylenediamine dihydrochloride is used to represent this material. (There is no indication which of the two batches was utilized for the chronic bioassay.)

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® meal (Allied Mills, Inc., Chicago, Illinois). p-Phenylenediamine dihydrochloride was administered to the dosed animals as a component of the diet.

Fresh dietary mixtures were prepared each week. The chemical was removed from its container and a proper amount was blended with an aliquot of the feed using a mortar and pestle. Once visual homogeneity was attained, the mixture was placed in a 6 kg capacity Patterson-Kelley standard model twin-shell stainless steel V-blender along with the remainder of the feed to be prepared. After 20 minutes of blending, the mixtures were placed in double plastic bags and stored in the dark at 4°C. The mixture was prepared once weekly.

Dosed feed preparations containing 1250 and 625 ppm of p-phenylenediamine dihydrochloride were analyzed spectrophotometrically. The mean result immediately after preparation was 104 percent of theoretical (ranging from 92 to 110 percent).

C. Animals

The two animal species, Fischer 344 rats and B6C3F1 mice, used in the carcinogenicity bioassay were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Rats

and mice were supplied by the Frederick Cancer Research Center, Frederick, Maryland.

Rats and mice were approximately 4 weeks old when received. Upon receipt, animals were examined and any obviously ill or runted animals were killed. The remaining animals were quarantined for 2 weeks prior to initiation of test. Animals which did not manifest clinical signs of disease were placed on test at this time. Animals were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenance

Animals were housed by species in rooms with a temperature range of 22° to 26°C and a range in relative humidity of 45 to 55 percent. Incoming air was filtered through HEPA filters (Flanders Filters, McLean, Virginia) at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided 8 hours per day (9:00 a.m. to 5:00 p.m.).

Rats were housed four per cage by sex and mice were housed five per cage by sex. Throughout the study dosed and control animals of both species were housed in polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) suspended from aluminum racks. Racks were fitted with a continuous piece of stainless steel mesh over which a sheet of filter paper was firmly secured. Filter paper was changed

at 2-week intervals, when the racks were sanitized. Clean cages and bedding (Ab-sorb-dri® hardwood chip bedding [Wilner Wood Products Company, Norway, Maine]) were provided twice weekly.

Acidulated water (pH 2.5) was supplied to animals in water bottles which were changed and washed twice weekly. Sipper tubes were washed at weekly intervals. During the period of chemical administration, dosed and control animals received treated or untreated Wayne Lab-Blox® meal as appropriate. The feed was supplied in hanging stainless steel hoppers which were refilled three times per week and sanitized weekly. Food and water were available ad libitum for both species.

All dosed and control rats were housed in a room with other rats receiving diets containing* 2-(chloromethyl)pyridine hydrochloride (6959-47-3); and 4-amino-2-nitrophenol (119-34-6).

All dosed and control mice were housed in a room with mice receiving diets containing 1-phenyl-3-methyl-5-pyrazolone (89-25-8); 2,4-dimethoxyaniline hydrochloride (54150-69-5); 4'-(chloroacetyl)-acetanilide (140-49-8); nithiazide (139-94-6); and 4-nitro-o-phenylenediamine (99-56-9); and with other mice intubated with dosed solutions of trimethylphosphate (512-56-1); 2-(chloromethyl)pyridine hydrochloride (6959-47-3); 3-(chloromethyl)pyridine hydrochloride (3099-31-8); and pivalolactone (1955-45-9).

*CAS registry numbers are given in parentheses.

E. Selection of Initial Concentrations

To establish the concentrations of p-phenylenediamine dihydrochloride for administration to dosed animals in the chronic study, subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among six groups, each consisting of five males and five females. p-Phenylenediamine dihydrochloride was incorporated into the basal laboratory diet and supplied ad libitum to five of the six rat groups in concentrations of 681, 1000, 1470, 2150 and 3160 ppm and to five of the six mouse groups in concentrations of 1000, 1470, 2150, 3160, and 4640 ppm. The remaining group of each species served as a control group, receiving only the basal laboratory diet.

The dosed dietary preparations were administered for a period of 7 weeks, followed by a 1-week observation period during which all animals were fed the basal laboratory diet. Individual body weights and food consumption data were recorded twice weekly throughout the study. Upon termination of the observation period, all survivors were sacrificed and necropsied.

The following table indicates the mean body weight gain, relative to controls, survival, and incidence of arched backs and rough coats observed in each of the rat groups at the end of the subchronic test.

RAT SUBCHRONIC STUDY RESULTS

ppm	Mean Body Weight Gain (%)*		Survival**		Observation of Arched Backs and Rough Coats**	
	Males	Females	Males	Females	Males	Females
3160	-35	-34	5/5	5/5	5/5	5/5
2150	-28	-41	5/5	5/5	0/5	0/5
1470	- 1	-37	5/5	5/5	0/5	0/5
1000	-10	-13	5/5	5/5	0/5	0/5
681	+ 7	+21	5/5	5/5	0/5	0/5
464	+40	- 5	5/5	5/5	0/5	0/5
316	+41	+ 9	5/5	5/5	0/5	0/5
215	+28	+10	5/5	5/5	0/5	0/5
147	+24	+11	5/5	5/5	0/5	0/5
100	+12	+12	5/5	5/5	0/5	0/5
68	+48	+ 9	5/5	5/5	0/5	0/5
0	---	---	5/5	5/5	0/5	0/5

The high concentration selected for administration to dosed rats in the chronic bioassay was 1250 ppm.

The following table indicates the mean body weight gain, relative to controls, and survival observed in each of the mouse groups at the end of the subchronic test.

*- is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.
**number of animals observed/number of animals originally in group.

MOUSE SUBCHRONIC STUDY RESULTS

ppm	Mean Body Weight Gain (%)* Males	Females	Survival** Males	Females
4640	-18	-11	5/5	5/5
3160	-11	- 8	5/5	5/5
2150	- 9	-13	5/5	5/5
1470	-17	- 1	5/5	5/5
1000	- 7	+ 7	5/5	5/5
681	- 3	+ 9	5/5	5/5
464	0	+ 1	5/5	5/5
316	- 6	+ 6	5/5	5/5
215	-11	+10	5/5	5/5
147	0	+ 6	5/5	5/5
100	- 7	+ 4	5/5	5/5
0	---	---	5/5	5/5

No other clinical abnormalities which could be attributed to administration of the compound were observed. The high concentration selected for administration to dosed mice in the chronic bioassay was 1250 ppm.

F. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, concentrations administered, and duration of treated and untreated observation periods) are summarized in Tables 1 and 2.

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed rats were supplied with diets containing 1250 and 625 ppm p-phenylenediamine dihydrochloride for 103 weeks followed by a 2-week observation

*+ is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.
**number of animals observed/number of animals originally in group.

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
p-PHENYLENEDIAMINE DIHYDROCHLORIDE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	p-PHENYLENEDIAMINE DIHYDROCHLORIDE CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	105
LOW DOSE	50	625	103	
		0		2
HIGH DOSE	50	1250	103	
		0		2
FEMALE				
CONTROL	20	0	0	105
LOW DOSE	50	625	103	
		0		2
HIGH DOSE	50	1250	103	
		0		2

[a]Concentrations given in parts per million.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
p-PHENYLENEDIAMINE DIHYDROCHLORIDE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	p-PHENYLENEDIAMINE DIHYDROCHLORIDE CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	104
LOW DOSE	50	625	103	
		0		1
HIGH DOSE	50	1250	103	
		0		1
FEMALE				
CONTROL	20	0	0	104
LOW DOSE	50	625	103	
		0		1
HIGH DOSE	50	1250	103	
		0		1

[a]Concentrations given in parts per million.

period, when no test chemicals were used. Throughout this report those rats receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed mice were supplied with diets containing 1250 and 625 ppm p-phenylenediamine dihydrochloride for 103 weeks followed by a 1-week observation period, when no test chemicals were used. Throughout this report those mice receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment and body weights were recorded once monthly throughout the bioassay. All animals were inspected twice daily. Food consumption data were collected at monthly intervals from 20 percent of the animals in each group.

All moribund animals or animals that developed large, palpable masses that jeopardized their health were sacrificed. A necropsy was performed on each animal regardless of whether it died, was sacrificed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized using carbon dioxide, and were immediately necropsied. Gross and microscopic examinations were performed

on all major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in a 10 percent neutral buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, uterus, mammary gland, and ovary.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were recorded in each group at the time that the test was initiated.

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) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing 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 was 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, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated 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, pp. 6-10) 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, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test determined 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 was 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 animals died naturally or were 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 to 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 treated 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 treated 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 treated 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 percent of a large number of identical experiments, the true ratio of the risk in a treated 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 (a $P < 0.025$ 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. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

Slight mean body weight depression was associated with compound administration in high dose male rats and slight dose-related mean body weight depression was evident in the females (Figure 2).

No other clinical signs were recorded.

B. Survival

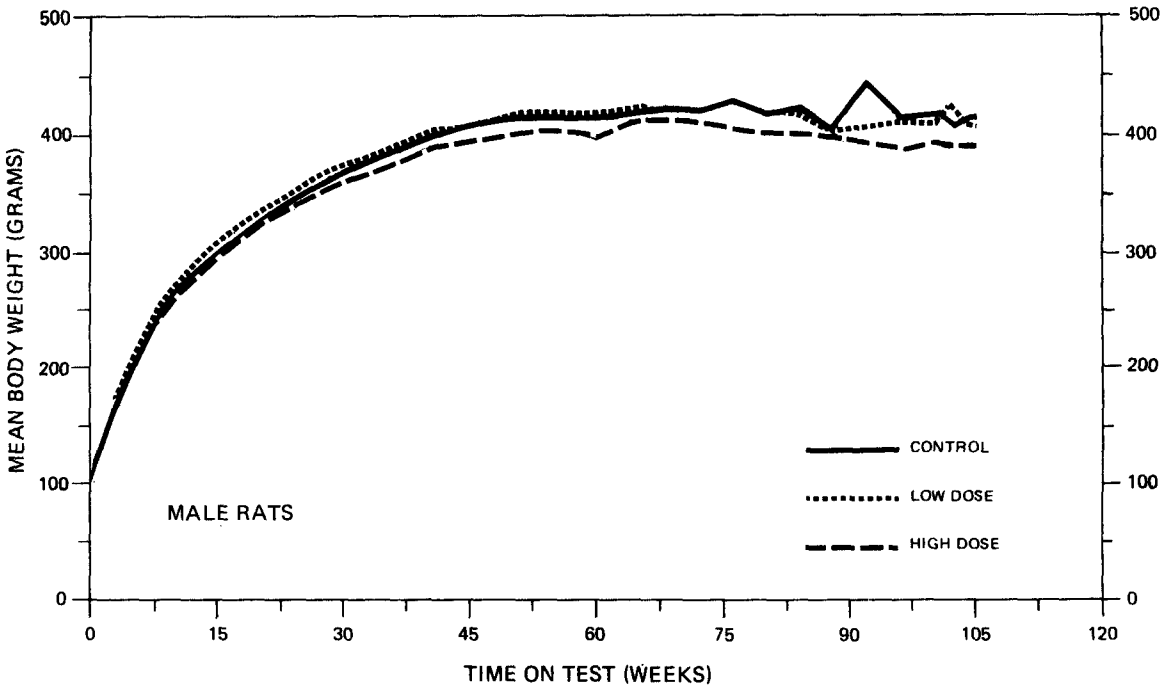
The estimated probabilities of survival for male and female rats in the control and p-phenylenediamine dihydrochloride-dosed groups are shown in Figure 3. For both male and female rats, the Tarone test for association between dosage and mortality was not significant.

There were adequate numbers of males at risk from late-developing tumors, as 68 percent (34/50) of the high dose, 76 percent (38/50) of the low dose and 65 percent (13/20) of the control group survived on test until termination of the study.

For females, with 78 percent (39/50) of the high dose, 78 percent (39/50) of the low dose, and 70 percent (14/20) of the control group surviving on test until the termination of the study, there were adequate numbers at risk from late-developing tumors.

C. Pathology

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).



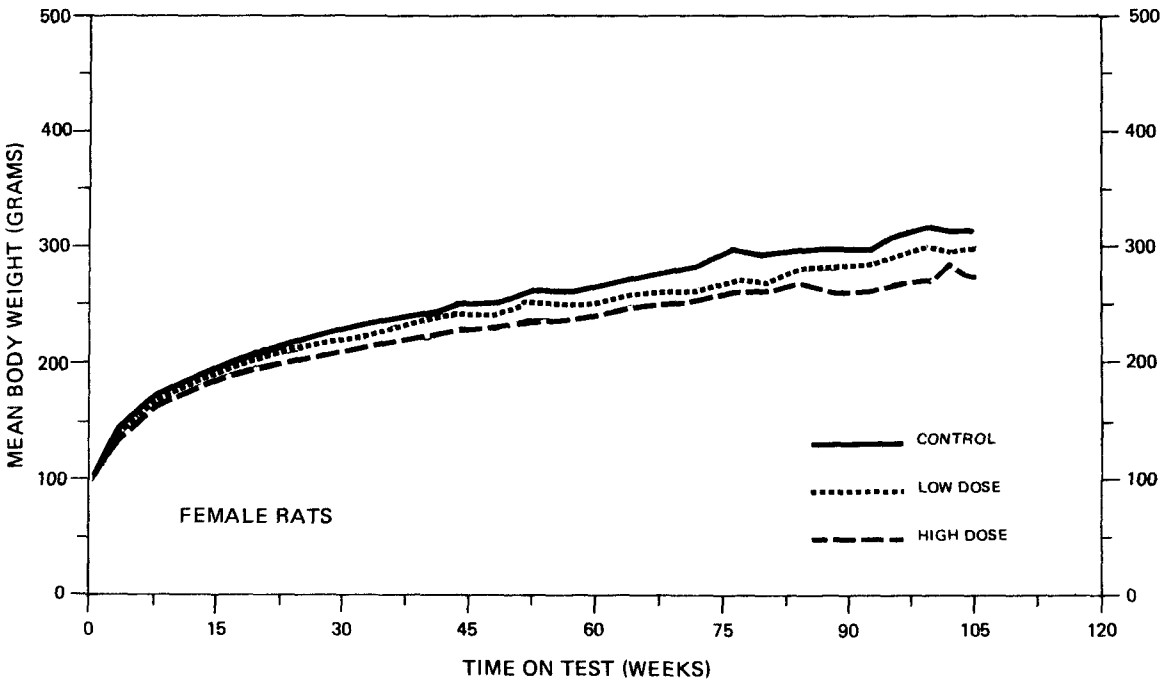
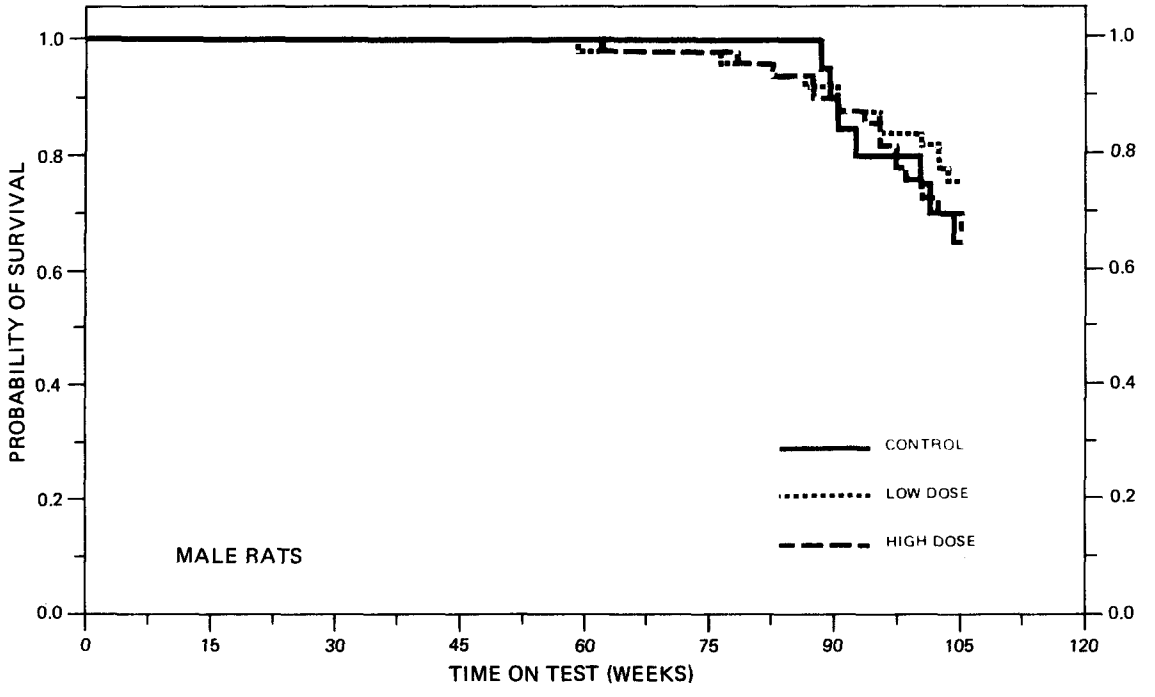


FIGURE 2
GROWTH CURVES FOR p-PHENYLENEDIAMINE DIHYDROCHLORIDE CHRONIC STUDY RATS



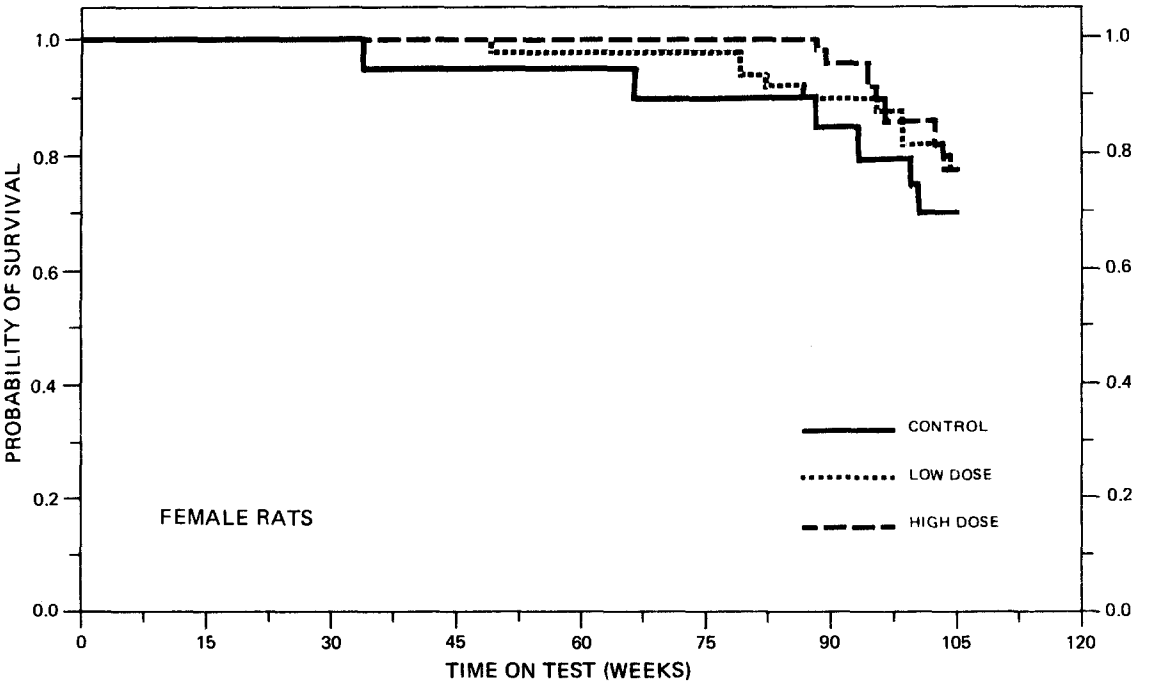


FIGURE 3
SURVIVAL COMPARISONS OF p-PHENYLENEDIAMINE DIHYDROCHLORIDE
CHRONIC STUDY RATS

A variety of neoplasms occurred in both dosed and control rats. These tumors were all distributed nearly equally among control and dosed animals and for each sex appeared to be within the incidence commonly encountered in aging Fischer 344 rats. A few neoplasms that occurred in various organs were encountered only in dosed animals. However, the incidence was very low, precluding association of these neoplasms with the administration of p-phenylenediamine dihydrochloride.

A number of degenerative, proliferative and inflammatory changes were encountered with similar incidence and severity in dosed and control animals, and were considered to be spontaneous.

Based upon the results of this pathology examination, p-phenylenediamine dihydrochloride was not carcinogenic to Fischer 344 rats under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or p-phenylenediamine dihydrochloride dosed groups and where such tumors were observed in at least 5 percent of the group.

None of the statistical tests for any site in rats of either sex indicated a significant positive association between the administration of p-phenylenediamine dihydrochloride and an increased tumor

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE RATS TREATED WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Skin and Subcutaneous Tissue: Fibroma[b]	2/20(0.10)	1/50(0.02)	1/50(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.200	0.200
Lower Limit	---	0.004	0.004
Upper Limit	---	3.681	3.681
Weeks to First Observed Tumor	88	102	105
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	1/18(0.06)	3/47(0.06)	0/46(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.149	0.000
Lower Limit	---	0.102	0.000
Upper Limit	---	59.033	7.302
Weeks to First Observed Tumor	92	90	---
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	4/20(0.20)	12/50(0.24)	13/50(0.26)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.200	1.300
Lower Limit	---	0.429	0.474
Upper Limit	---	4.650	4.977
Weeks to First Observed Tumor	90	59	82

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	5/16(0.31)	14/46(0.30)	4/39(0.10)
P Values[c]	P = 0.027(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.974	0.328
Lower Limit	---	0.416	0.079
Upper Limit	---	3.027	1.358
Weeks to First Observed Tumor	90	76	98
Adrenal: Pheochromocytoma[b]	3/19(0.16)	6/50(0.12)	3/46(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.760	0.413
Lower Limit	---	0.187	0.062
Upper Limit	---	4.377	2.880
Weeks to First Observed Tumor	90	86	95
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	0/19(0.00)	2/48(0.04)	3/44(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.122	0.271
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	100	105

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Testis: Interstitial-Cell Tumor[b]	18/20(0.90)	42/50(0.84)	38/47(0.81)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.933	0.898
Lower Limit	---	0.816	0.782
Upper Limit	---	1.226	1.198
Weeks to First Observed Tumor	89	90	87

[a]Treated groups received doses of 625 or 1250 ppm in feed.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE RATS TREATED WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	0/20(0.00)	1/49(0.02)	4/48(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.023	0.402
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	105
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	3/20(0.15)	15/50(0.30)	7/50(0.14)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.048	---	---
Relative Risk (Control)[d]	---	2.000	0.933
Lower Limit	---	0.662	0.245
Upper Limit	---	9.943	5.215
Weeks to First Observed Tumor	88	49	95
Pituitary: Chromophobe Carcinoma or Chromophobe Adenoma[b]	7/17(0.41)	19/44(0.43)	25/46(0.54)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.049	1.320
Lower Limit	---	0.545	0.719
Upper Limit	---	2.482	2.991
Weeks to First Observed Tumor	100	82	94

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma[b]	1/20(0.05)	5/50(0.10)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.000	1.200
Lower Limit	---	0.249	0.106
Upper Limit	---	92.596	61.724
Weeks to First Observed Tumor	100	98	103
Uterus: Endometrial Stromal Polyp[b]	1/20(0.05)	4/47(0.09)	4/47(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.702	1.702
Lower Limit	---	0.186	0.186
Upper Limit	---	81.978	81.978
Weeks to First Observed Tumor	105	105	96

[a]Treated groups received doses of 625 or 1250 ppm in feed.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

incidence. Based upon these statistical results, there was insufficient evidence that p-phenylenediamine dihydrochloride was a carcinogen in Fischer 344 rats under the conditions of this bioassay.

For male rats the Cochran-Armitage test did indicate a significant negative association between dosage and the incidence of chromophobe adenomas of the pituitary.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In all of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should also be noted that all of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by p-phenylenediamine dihydrochloride that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

There was no evidence of dose-related mean body weight depression in male mice. Mean body weights among dosed female mice were slightly lower than that among the controls (Figure 4).

No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the control and p-phenylenediamine dihydrochloride-dosed groups are shown in Figure 5. For both male and female mice the Tarone test for association between dosage and mortality was not significant.

There were adequate numbers of male mice at risk from late-developing tumors as 84 percent (42/50) of the high dose, 76 percent (38/50) of the low dose, and 80 percent (16/20) of the control group survived on test until the termination of the study.

For females, with 84 percent (41/49) of the high dose, 88 percent (44/50) of the low dose, and 85 percent (17/20) of the control group surviving on test until termination of the study, there were adequate numbers at risk from late-developing tumors.

C. Pathology

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).

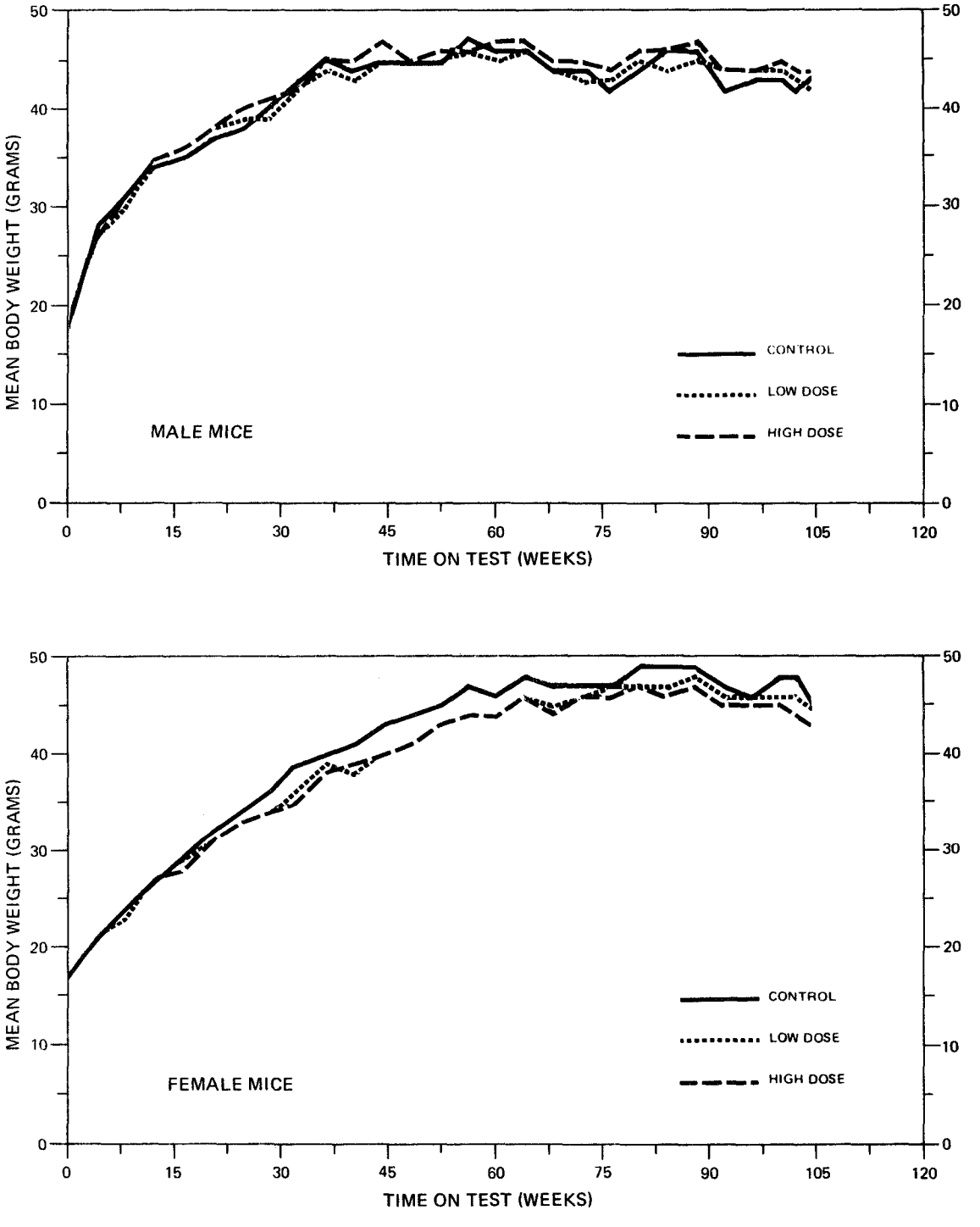
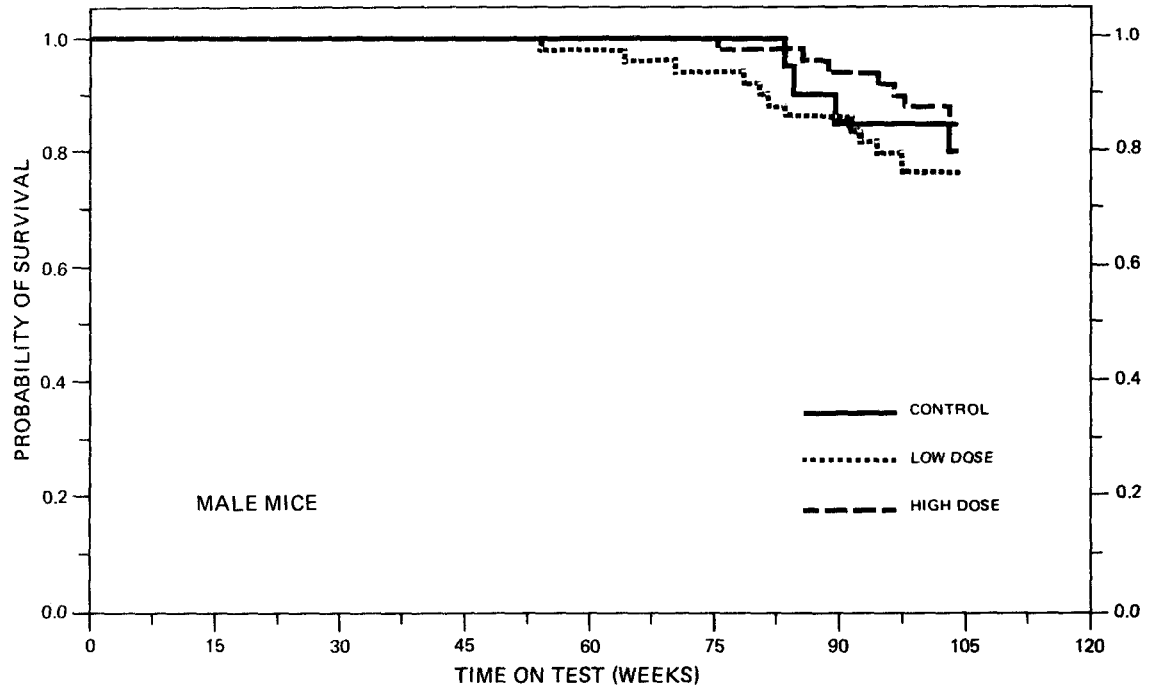


FIGURE 4
GROWTH CURVES FOR p-PHENYLENEDIAMINE DIHYDROCHLORIDE CHRONIC STUDY MICE



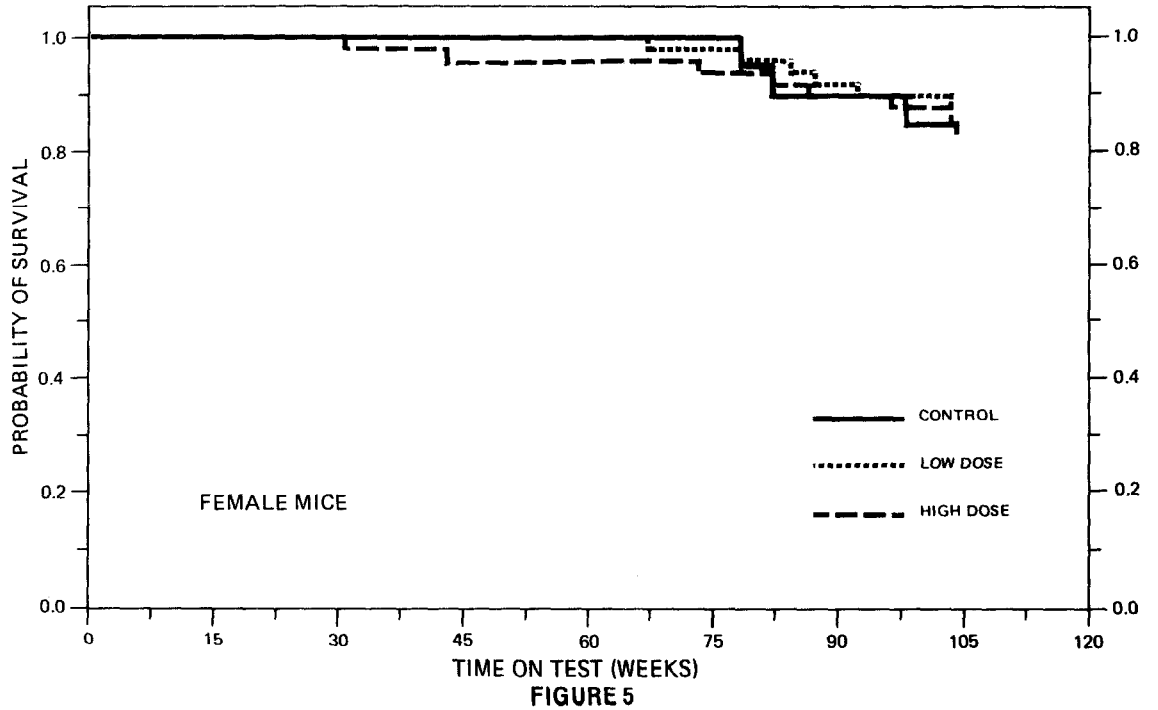


FIGURE 5
SURVIVAL COMPARISONS OF p-PHENYLENEDIAMINE DIHYDROCHLORIDE CHRONIC STUDY MICE

A variety of tumors occurred both in the control and dosed groups. A few neoplasms occurred only in the dosed groups or with greater frequency in dosed groups as compared with controls. The neoplasms which were observed have all been reported to occur spontaneously in this strain of mice. No neoplasms were considered to be compound-related.

A number of degenerative, proliferative and inflammatory changes was encountered in dosed and control mice. The incidence and severity of these nonneoplastic lesions are not unusual for aging B6C3F1 mice.

Based upon the results of this pathology examination, p-phenylenediamine dihydrochloride was not carcinogenic to B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or p-phenylenediamine dihydrochloride-dosed groups and where such tumors were observed in at least 5 percent of the group.

The number of weeks to first observed leukemia or malignant lymphoma in female mice decreased from 98 weeks in the control group to 67 weeks in the low dose group and then to 31 weeks in the high dose group, while the incidences in the low dose and high dose group, although not significantly different, were higher than those found in

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	1/20(0.05)	6/49(0.12)	6/50(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.449	2.400
Lower Limit	---	0.332	0.325
Upper Limit	---	110.166	108.021
Weeks to First Observed Tumor	104	80	104
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	4/20(0.20)	10/49(0.20)	8/50(0.16)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.020	0.800
Lower Limit	---	0.346	0.250
Upper Limit	---	4.068	3.327
Weeks to First Observed Tumor	104	70	96
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	3/20(0.15)	7/50(0.14)	1/50(0.02)
P Values[c]	P = 0.032(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.933	0.133
Lower Limit	---	0.245	0.003
Upper Limit	---	5.215	1.568
Weeks to First Observed Tumor	83	64	104

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	4/20(0.20)	9/49(0.18)	7/50(0.14)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.918	0.700
Lower Limit	---	0.300	0.207
Upper Limit	---	3.730	2.994
Weeks to First Observed Tumor	84	81	75
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	9/20(0.45)	18/49(0.37)	21/50(0.42)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.816	0.933
Lower Limit	---	0.443	0.522
Upper Limit	---	1.750	1.953
Weeks to First Observed Tumor	84	81	75

[a]Treated groups received doses of 625 or 1250 ppm in feed.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE MICE TREATED WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	0/20(0.00)	2/48(0.04)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.128	0.255
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	104	103
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	2/20(0.10)	10/50(0.20)	10/49(0.20)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.000	2.041
Lower Limit	---	0.488	0.498
Upper Limit	---	17.808	18.154
Weeks to First Observed Tumor	98	67	31
Liver: Hepatocellular Carcinoma[b]	1/20(0.05)	3/48(0.06)	2/45(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.250	0.889
Lower Limit	---	0.110	0.050
Upper Limit	---	64.251	51.294
Weeks to First Observed Tumor	104	87	104

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	2/20(0.10)	6/48(0.13)	8/45(0.18)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.250	1.778
Lower Limit	---	0.253	0.405
Upper Limit	---	12.039	16.259
Weeks to First Observed Tumor	104	87	86

[a]Treated groups received doses of 625 or 1250 ppm in feed.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

the control group. To further examine these results an additional, life-table analysis was performed. Figure 6 shows the probability of survival without a known leukemia or malignant lymphoma for female mice. The Tarone test did not indicate any significant differences between dosed and control groups.

No other statistical test in mice of either sex indicated a significant positive association between the administration of p-phenylenediamine dihydrochloride and an increased tumor incidence at any site. Based on these statistical results, there was insufficient evidence that p-phenylenediamine dihydrochloride was a carcinogen in B6C3F1 mice under the conditions of this bioassay.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In all of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that all of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by p-phenylenediamine dihydrochloride that could not be established under the conditions of this test.

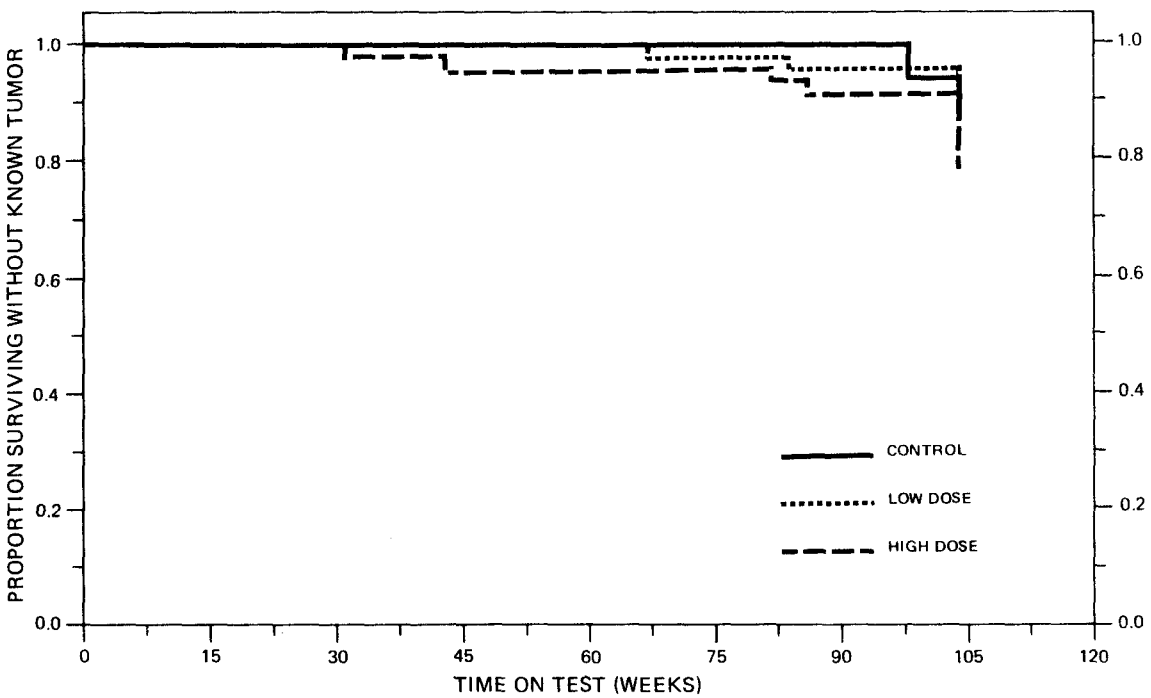


FIGURE 6
COMPARISIONS OF p-PHENYLENEDIAMINE DIHYDROCHLORIDE CHRONIC STUDY FEMALE MICE SURVIVING WITHOUT OBSERVED LEUKEMIAS OR MALIGNANT LYMPHOMAS

V. DISCUSSION

There were no significant positive associations between the concentrations of p-phenylenediamine dihydrochloride administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Slight dose-related mean body weight depression was observed in female rats and the mean body weights among high dose male rats and dosed female mice were slightly depressed in relation to their respective controls, indicating that the concentrations of p-phenylenediamine dihydrochloride administered to these animals in this bioassay may have approximated the maximum tolerated concentrations. Since no distinct mean body weight depression relative to controls, no significant accelerated mortality, and no other signs of toxicity were associated with administration of p-phenylenediamine dihydrochloride to male mice, it is possible that these animals may have been able to tolerate a higher dietary concentration.

None of the statistical tests for any site in rats or mice of either sex, including time to leukemia or malignant lymphoma analysis in female mice, indicated a significant positive association between compound administration and tumor incidence.

Under the conditions of this bioassay, there was no convincing evidence that dietary administration of p-phenylenediamine dihydrochloride was carcinogenic in Fischer 344 rats or B6C3F1 mice.

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Review of the Bioassay of *p*-Phenylenediamine Dihydrochloride*
for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

October 25, 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, and State health officials. 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 *p*-Phenylenediamine dihydrochloride for carcinogenicity.

The reviewer said that there was no convincing evidence that, under the conditions of test, *p*-Phenylenediamine dihydrochloride was carcinogenic in either treated rats or mice. After briefly describing the experimental design, he said that there were no unusual highlights or concerns worthy of special comment. Based on the results of the study, the reviewer said that *p*-Phenylenediamine dihydrochloride would not appear to pose a carcinogenic risk for humans. A motion to accept the report as written was seconded and approved unanimously.

Clearinghouse Members present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center
Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Kenneth Wilcox, Michigan State Health Department

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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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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED
WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL (UNTR) 11-1455	LOW DOSE 11-1453	HIGH DOSE 11-1451
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	48
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SEBACEOUS ADENOCARCINOMA		1 (2%)	
KERATOACANTHOMA		1 (2%)	
FIBROMA	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA	1 (5%)	1 (2%)	1 (2%)
FIBROSARCOMA	1 (5%)		1 (2%)
NEUROFIBROMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(18)	(47)	(46)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (6%)	2 (4%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
SEBACEOUS ADENOCARCINOMA, METAST		1 (2%)	
FIBROSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (5%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
LEUKEMIA,NOS	2 (10%)	11 (22%)	9 (18%)
UNDIFFERENTIATED LEUKEMIA			1 (2%)
GRANULOCYTIC LEUKEMIA	1 (5%)	1 (2%)	1 (2%)
MONOCYTIC LEUKEMIA			1 (2%)
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1455	LOW DOSE 11-1453	HIGH DOSE 11-1451
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(50)	(45)
ADENOCARCINOMA, NOS		1 (2%)	1 (2%)
#LIVER	(20)	(49)	(47)
NEOPLASTIC NODULE			1 (2%)
#PANCREAS	(19)	(50)	(44)
ACINAR-CELL ADENOMA	1 (5%)		1 (2%)
#SMALL INTESTINE	(19)	(50)	(43)
LEIOMYOSARCOMA			1 (2%)
#COLON	(19)	(50)	(44)
ADENOMATOUS POLYP, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(19)	(50)	(47)
TUBULAR-CELL ADENOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(46)	(39)
CHROMOPHOBE ADENOMA	5 (31%)	14 (30%)	4 (10%)
#ADRENAL	(19)	(50)	(46)
CORTICAL ADENOMA	1 (5%)		
PHEOCHROMOCYTOMA	3 (16%)	6 (12%)	3 (7%)
#THYROID	(19)	(48)	(44)
C-CELL ADENOMA			1 (2%)
C-CELL CARCINOMA		2 (4%)	2 (5%)
#PARATHYROID	(15)	(28)	(29)
ADENOMA, NOS			1 (3%)
#PANCREATIC ISLETS	(19)	(50)	(44)
ISLET-CELL ADENOMA	1 (5%)	1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(20)	(50)	(50)
ADENOMA, NOS	1 (5%)		2 (4%)

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1455	LOW DOSE 11-1453	HIGH DOSE 11-1451
#TESTIS	(20)	(50)	(47)
INTERSTITIAL-CELL TUMOR	18 (90%)	42 (84%)	38 (81%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(50)
MESOTHELIOMA, NOS	1 (5%)		1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
ADENOCARCINOMA, NOS, UNC PRIM OR			1 (2%)
OMENTUM			
SARCOMA, NOS			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	3	11
MORIBUND SACRIFICE	4	9	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	38	34
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 11-1455	LOW DOSE 11-1453	HIGH DOSE 11-1451
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	19	49	48
TOTAL PRIMARY TUMORS	39	86	75
TOTAL ANIMALS WITH BENIGN TUMORS	19	47	42
TOTAL BENIGN TUMORS	33	69	53
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	16	19
TOTAL MALIGNANT TUMORS	5	17	19
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1		2
TOTAL UNCERTAIN TUMORS	1		2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			1
TOTAL UNCERTAIN TUMORS			1

* 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 TREATED
WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL (UNTR) 11-1456	LOW DOSE 11-1454	HIGH DOSE 11-1452
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
BASAL-CELL TUMOR		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKEMIA,NOS	3 (15%)	14 (28%)	7 (14%)
GRANULOCYTIC LEUKEMIA		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(48)	(46)
ADENOCARCINOMA, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(48)
TUBULAR-CELL ADENOMA		1 (2%)	

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1456	LOW DOSE 11-1454	HIGH DOSE 11-1452
#URINARY BLADDER	(17)	(41)	(42)
TRANSITIONAL-CELL PAPILLOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(44)	(46)
CHROMOPHOBE ADENOMA	7 (41%)	19 (43%)	24 (52%)
CHROMOPHOBE CARCINOMA			1 (2%)
#ADRENAL	(18)	(45)	(46)
CORTICAL ADENOMA			1 (2%)
CORTICAL CARCINOMA			1 (2%)
PHEOCHROMOCYTOMA		1 (2%)	1 (2%)
#THYROID	(20)	(48)	(43)
FOLLICULAR-CELL CARCINOMA		1 (2%)	
C-CELL ADENOMA		2 (4%)	
#PANCREATIC ISLETS	(18)	(46)	(48)
ISLET-CELL ADENOMA		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
FIBROADENOMA	1 (5%)	5 (10%)	3 (6%)
*MAMMARY DUCT	(20)	(50)	(50)
ADENOCARCINOMA, NOS			1 (2%)
#UTERUS	(20)	(47)	(47)
ENDOMETRIAL STROMAL POLYP	1 (5%)	4 (9%)	4 (9%)
#CERVIX UTERI	(20)	(47)	(47)
SQUAMOUS CELL CARCINOMA			1 (2%)
#OVARY	(20)	(48)	(47)
GRANULOSA-CELL TUMOR		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(19)	(48)	(47)
CHROMOPHOBE CARCINOMA, INVASIVE			1 (2%)

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1456	LOW DOSE 11-1454	HIGH DOSE 11-1452
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	5	5	3
MORIBUND SACRIFICE	1	6	8
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	14	39	39
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 11-1456	LOW DOSE 11-1454	HIGH DOSE 11-1452
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	10	37	36
TOTAL PRIMARY TUMORS	12	53	50
TOTAL ANIMALS WITH BENIGN TUMORS	7	28	32
TOTAL BENIGN TUMORS	9	35	38
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	17	11
TOTAL MALIGNANT TUMORS	3	17	12
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED
WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL (UNTR) 22-2455	LOW DOSE 22-2453	HIGH DOSE 22-2451
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SARCOMA, NOS		1 (2%)	
FIBROMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	2 (4%)
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (15%)	4 (8%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)	6 (12%)	6 (12%)
OSTEOSARCOMA, METASTATIC	1 (5%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)	1 (2%)	
MALIG. LYMPHOMA, UNDIFFER-TYPE		1 (2%)	
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)		
MALIG. LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	1 (2%)	
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	
LEUKEMIA, NOS		1 (2%)	
#SPLEEN	(20)	(48)	(48)
HEMANGIOSARCOMA			1 (2%)
#MESENTERIC L. NODE	(20)	(47)	(45)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	
#PEYERS PATCH	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 22-2455	LOW DOSE 22-2453	HIGH DOSE 22-2451
*MESENTERY	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
HEPATOCELLULAR ADENOMA	5 (25%)	9 (18%)	14 (28%)
HEPATOCELLULAR CARCINOMA	4 (20%)	9 (18%)	7 (14%)
URINARY SYSTEM			
#KIDNEY/CORTEX	(20)	(48)	(50)
ADENOMA, NOS	1 (5%)		
#URINARY BLADDER	(15)	(40)	(43)
LEIOMYOSARCOMA			1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL	(20)	(47)	(47)
CARCINOMA,NOS	1 (5%)		
PHEOCHROMOCYTOMA			1 (2%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 22-2455	LOW DOSE 22-2453	HIGH DOSE 22-2451
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(50)
LIPOMA		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
SARCOMA, NOS	1 (5%)		1 (2%)
HEMANGIOSARCOMA		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	9	8
MORIBUND SACRIFICE		3	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	38	42
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 22-2455	LOW DOSE 22-2453	HIGH DOSE 22-2451
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	15	35	31
TOTAL PRIMARY TUMORS	19	39	35
TOTAL ANIMALS WITH BENIGN TUMORS	8	14	18
TOTAL BENIGN TUMORS	9	14	18
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	22	16
TOTAL MALIGNANT TUMORS	10	25	17
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

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL (UNTR) 22-2456	LOW DOSE 22-2454	HIGH DOSE 22-2452
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(49)
ADENOCARCINOMA, NOS, METASTATIC	1 (5%)		
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	3 (6%)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		4 (8%)	2 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (5%)	1 (2%)	1 (2%)
LEUKEMIA,NOS	1 (5%)	3 (6%)	1 (2%)
LYMPHOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(20)	(47)	(46)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#MESENTERIC L. NODE	(19)	(46)	(41)
SARCOMA, NOS, UNC PRIM OR META	1 (5%)		
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2456	LOW DOSE 22-2454	HIGH DOSE 22-2452
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(45)
HEPATOCELLULAR ADENOMA	1 (5%)	3 (6%)	6 (13%)
HEPATOCELLULAR CARCINOMA	1 (5%)	3 (6%)	2 (4%)
HEMANGIOSARCOMA	1 (5%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(43)	(34)
CHROMOPHOBE ADENOMA		1 (2%)	1 (3%)
#ADRENAL	(19)	(47)	(44)
CORTICAL ADENOMA			1 (2%)
PHEOCHROMOCYTOMA	1 (5%)	1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
ADENOMA, NOS	1 (5%)	1 (2%)	
#UTERUS	(19)	(47)	(48)
LEIOMYOSARCOMA		1 (2%)	
ENDOMETRIAL STROMAL POLYP			1 (2%)
HEMANGIOMA			1 (2%)
#CERVIX UTERI	(19)	(47)	(48)
SARCOMA, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2456	LOW DOSE 22-2454	HIGH DOSE 22-2452
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(49)
LIPOMA			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(49)
SARCOMA, NOS	1 (5%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	6	8
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	44	41
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 22-2456	LOW DOSE 22-2454	HIGH DOSE 22-2452
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	17	21
TOTAL PRIMARY TUMORS	9	22	28
TOTAL ANIMALS WITH BENIGN TUMORS	2	7	12
TOTAL BENIGN TUMORS	3	7	14
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	14	13
TOTAL MALIGNANT TUMORS	5	15	14
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	1		
TOTAL UNCERTAIN TUMORS	1		

* 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 TREATED WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL (UNTR) 11-1455	LOW DOSE 11-1453	HIGH DOSE 11-1451
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	48
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
CYST, NOS		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
DERMAL INCLUSION CYST		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(18)	(47)	(46)
ATELECTASIS	2 (11%)		2 (4%)
THROMBOSIS, NOS			1 (2%)
CONGESTION, NOS	2 (11%)	3 (6%)	4 (9%)
HEMORRHAGE			1 (2%)
INFLAMMATION, INTERSTITIAL		1 (2%)	1 (2%)
PNEUMONIA, CHRONIC MURINE	11 (61%)	24 (51%)	21 (46%)
FIBROSIS	1 (6%)		
HYPERPLASIA, ADENOMATOUS	1 (6%)	2 (4%)	3 (7%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(48)	(45)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	1 (2%)
#MEDIASTINAL L.NODE	(20)	(46)	(44)
CONGESTION, NOS	1 (5%)		
HEMORRHAGE	1 (5%)		
CIRCULATORY SYSTEM			
#HEART	(19)	(46)	(47)
INFLAMMATION, CHRONIC			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-1455	LOW DOSE 11-1453	HIGH DOSE 11-1451
#HEART/ATRIUM	(19)	(46)	(47)
THROMBOSIS, NOS	1 (5%)	1 (2%)	1 (2%)
#MYOCARDIUM	(19)	(46)	(47)
INFLAMMATION, NOS		1 (2%)	1 (2%)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, CHRONIC FOCAL		3 (7%)	1 (2%)
FIBROSIS	10 (53%)	29 (63%)	30 (64%)
FIBROSIS, FOCAL		1 (2%)	
FIBROSIS, DIFFUSE		1 (2%)	
DEGENERATION, NOS	1 (5%)		
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(50)	(45)
ATROPHY, NOS			1 (2%)
ATROPHY, DIFFUSE	1 (5%)		1 (2%)
HYPERPLASIA, EPITHELIAL		2 (4%)	
METAPLASIA, SQUAMOUS		3 (6%)	
#LIVER	(20)	(49)	(47)
CHOLANGIOFIBROSIS			1 (2%)
DEGENERATION, NOS	1 (5%)	2 (4%)	3 (6%)
NECROSIS, NOS	1 (5%)		
METAMORPHOSIS FATTY	1 (5%)	4 (8%)	4 (9%)
CYTOPLASMIC VACUOLIZATION			1 (2%)
BASOPHILIC CYTO CHANGE	1 (5%)		
HYPERPLASIA, FOCAL	7 (35%)	2 (4%)	2 (4%)
HYPERPLASIA, DIFFUSE		1 (2%)	
ADENOFIBROSIS			1 (2%)
#LIVER/CENTRILOBULAR	(20)	(49)	(47)
CONGESTION, NOS	1 (5%)		
NECROSIS, NOS		1 (2%)	1 (2%)
METAMORPHOSIS FATTY	1 (5%)		
#BILE DUCT	(20)	(49)	(47)
HYPERPLASIA, NOS	9 (45%)	19 (39%)	10 (21%)
#PANCREAS	(19)	(50)	(44)
HEMORRHAGE			1 (2%)
FIBROSIS, FOCAL			1 (2%)
ATROPHY, FOCAL	3 (16%)	5 (10%)	5 (11%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-1455	LOW DOSE 11-1453	HIGH DOSE 11-1451
#PANCREATIC ACINUS	(19)	(50)	(44)
ATROPHY, FOCAL	1 (5%)		
#STOMACH	(20)	(50)	(47)
INFLAMMATION, NOS		1 (2%)	
ULCER, NOS		1 (2%)	
INFLAMMATION, ACUTE	1 (5%)		
#GASTRIC SEROSA	(20)	(50)	(47)
INFLAMMATION, CHRONIC			1 (2%)
#PEYERS PATCH	(19)	(50)	(43)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
#COLON	(19)	(50)	(44)
INFLAMMATION, ACUTE	1 (5%)		
PARASITISM	6 (32%)	10 (20%)	3 (7%)
URINARY SYSTEM			
#KIDNEY	(19)	(50)	(47)
HYDRONEPHROSIS			1 (2%)
INFLAMMATION, CHRONIC	11 (58%)	29 (58%)	30 (64%)
NEPHROPATHY, TOXIC	3 (16%)	7 (14%)	5 (11%)
#URINARY BLADDER	(18)	(40)	(40)
CALCULUS, NOS	1 (6%)		
HYPERPLASIA, EPITHELIAL			1 (3%)
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(46)	(39)
CYST, NOS		1 (2%)	1 (3%)
HEMORRHAGE		1 (2%)	
ATYPIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL	1 (6%)	1 (2%)	1 (3%)
#ADRENAL	(19)	(50)	(46)
LIPOIDOSIS		2 (4%)	1 (2%)
CYTOPLASMIC VACUOLIZATION	1 (5%)	1 (2%)	
#ADRENAL MEDULLA	(19)	(50)	(46)
HYPERPLASIA, NOS			1 (2%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-1455	LOW DOSE 11-1453	HIGH DOSE 11-1451
HYPERPLASIA, FOCAL			1 (2%)
#THYROID	(19)	(48)	(44)
FOLLICULAR CYST, NOS			1 (2%)
HYPERPLASIA, C-CELL			2 (5%)
#THYROID FOLLICLE	(19)	(48)	(44)
HYPERPLASIA, CYSTIC	1 (5%)	1 (2%)	4 (9%)
#PANCREATIC ISLETS	(19)	(50)	(44)
HYPERPLASIA, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
#PROSTATE	(17)	(45)	(44)
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, NECROTIZING		1 (2%)	
INFLAMMATION, ACUTE FOCAL		1 (2%)	
INFLAMMATION, CHRONIC	1 (6%)		
*SEMINAL VESICLE	(20)	(50)	(50)
INFLAMMATION, NECROTIZING		1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)
#TESTIS	(20)	(50)	(47)
ATROPHY, NOS	1 (5%)	1 (2%)	1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(48)	(46)
THROMBOSIS, NOS			1 (2%)
HEMORRHAGE			1 (2%)
HEMATOMA, NOS			1 (2%)
DEMYELINIZATION		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 11-1455	LOW DOSE 11-1453	HIGH DOSE 11-1451
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, PYOGRANULOMATOUS			1
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/NO HISTO			2

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

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
TREATED WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL (UNTR) 11-1456	LOW DOSE 11-1454	HIGH DOSE 11-1452
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(20)	(49)	(48)
INFLAMMATION, ACUTE			1 (2%)
#LUNG	(20)	(49)	(48)
CONGESTION, NOS		1 (2%)	
EDEMA, NOS		1 (2%)	
PNEUMONIA, CHRONIC MURINE	7 (35%)	25 (51%)	22 (46%)
FIBROSIS		2 (4%)	
HYPERPLASIA, ADENOMATOUS		3 (6%)	2 (4%)
HISTIOCYTOSIS	1 (5%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(43)	(43)
MYELOFIBROSIS			1 (2%)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
#SPLEEN	(19)	(49)	(48)
INFARCT, NOS		1 (2%)	
HEMOSIDEROSIS		1 (2%)	
HEMATOPOIESIS			1 (2%)
CIRCULATORY SYSTEM			
#HEART/ATRIUM	(20)	(49)	(48)
THROMBOSIS, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1456	LOW DOSE 11-1454	HIGH DOSE 11-1452
#MYOCARDIUM	(20)	(49)	(48)
INFLAMMATION, FOCAL	2 (10%)		3 (6%)
INFLAMMATION, CHRONIC FOCAL			4 (8%)
FIBROSIS	8 (40%)	24 (49%)	17 (35%)
DEGENERATION, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(48)	(46)
ATROPHY, DIFFUSE			1 (2%)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, EPITHELIAL		1 (2%)	1 (2%)
METAPLASIA, SQUAMOUS		1 (2%)	
#LIVER	(20)	(49)	(48)
GRANULOMA, NOS		1 (2%)	
DEGENERATION, NOS		3 (6%)	3 (6%)
METAMORPHOSIS FATTY	2 (10%)	4 (8%)	3 (6%)
BASOPHILIC CYTO CHANGE	1 (5%)	3 (6%)	
FOCAL CELLULAR CHANGE		1 (2%)	
HYPERPLASIA, FOCAL	7 (35%)	18 (37%)	11 (23%)
#LIVER/CENTRILOBULAR	(20)	(49)	(48)
METAMORPHOSIS FATTY			1 (2%)
#BILE DUCT	(20)	(49)	(48)
HYPERPLASIA, NOS	6 (30%)	9 (18%)	6 (13%)
#PANCREAS	(18)	(46)	(48)
INFLAMMATION, CHRONIC	1 (6%)		
ATROPHY, NOS			1 (2%)
ATROPHY, FOCAL	1 (6%)	6 (13%)	5 (10%)
#PANCREATIC ACINUS	(18)	(46)	(48)
ATROPHY, FOCAL	1 (6%)		1 (2%)
ATROPHY, DIFFUSE	1 (6%)		
#STOMACH	(20)	(47)	(48)
EDEMA, NOS			1 (2%)
INFLAMMATION, ACUTE			1 (2%)
HYPERKERATOSIS			1 (2%)
#PEYERS PATCH	(18)	(47)	(47)
HYPERPLASIA, LYMPHOID		1 (2%)	

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1456	LOW DOSE 11-1454	HIGH DOSE 11-1452
#COLON	(19)	(47)	(47)
PARASITISM	2 (11%)	8 (17%)	2 (4%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(48)
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, CHRONIC	9 (45%)	29 (59%)	21 (44%)
NEPHROPATHY, TOXIC		2 (4%)	6 (13%)
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(44)	(46)
CYST, NOS	3 (18%)	6 (14%)	3 (7%)
HEMORRHAGE			1 (2%)
HEMORRHAGIC CYST	1 (6%)	2 (5%)	
#ADRENAL	(18)	(45)	(46)
LIPOIDOSIS	1 (6%)		
CYTOPLASMIC VACUOLIZATION			1 (2%)
#THYROID	(20)	(48)	(43)
ULTIMOBRANCHIAL CYST		1 (2%)	
CYSTIC FOLLICLES			1 (2%)
HYPERPLASIA, C-CELL		3 (6%)	
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	
#THYROID FOLLICLE	(20)	(48)	(43)
HYPERPLASIA, CYSTIC			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
DILATATION/DUCTS		2 (4%)	
#UTERUS	(20)	(47)	(47)
ABSCESS, NOS			1 (2%)
POLYPOID HYPERPLASIA	1 (5%)		
#UTERUS/ENDOMETRIUM	(20)	(47)	(47)
CONGESTION, NOS		1 (2%)	

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1456	LOW DOSE 11-1454	HIGH DOSE 11-1452
FIBROSIS			1 (2%)
HYPERPLASIA, NOS	1 (5%)	3 (6%)	1 (2%)
HYPERPLASIA, CYSTIC		4 (9%)	4 (9%)
#OVARY	(20)	(48)	(47)
CYST, NOS			2 (4%)
FOLLICULAR CYST, NOS	1 (5%)	3 (6%)	
#MESOVARIUM	(20)	(48)	(47)
PAROVARIAN CYST	1 (5%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
*MESENTERY	(20)	(50)	(50)
STEATITIS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2		5

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

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 11-1456	LOW DOSE 11-1454	HIGH DOSE 11-1452
AUTO/NECROPSY/HISTO PERF	2		
AUTO/NECROPSY/NO HISTO		1	2

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL (UNTR) 22-2455	LOW DOSE 22-2453	HIGH DOSE 22-2451
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED **	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
ULCER, NOS	1 (5%)		
GRANULOMA, NOS			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
ABSCESS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
INFLAMMATION, MULTIFOCAL			1 (2%)
PNEUMONIA, ASPIRATION			1 (2%)
PNEUMONIA, CHRONIC MURINE	4 (20%)	4 (8%)	5 (10%)
INFLAMMATION, CHRONIC	2 (10%)	1 (2%)	3 (6%)
INFLAMMATION, CHRONIC FOCAL	3 (15%)	3 (6%)	3 (6%)
HYPERPLASIA, ADENOMATOUS			2 (4%)
#LUNG/ALVEOLI	(20)	(49)	(50)
HISTIOCYTOSIS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(45)	(44)
HYPERPLASIA, HEMATOPOIETIC	1 (5%)		
#SPLEEN	(20)	(48)	(48)
HYPERPLASIA, RETICULUM CELL			1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#MESENTERIC L. NODE	(20)	(47)	(45)
LYMPHANGIECTASIS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2455	LOW DOSE 22-2453	HIGH DOSE 22-2451
HEMORRHAGE	1 (5%)		1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(20)	(48)	(50)
ENDOCARDIOSIS		1 (2%)	
#MYOCARDIUM	(20)	(48)	(50)
INFLAMMATION, FOCAL	1 (5%)	1 (2%)	
*AORTA	(20)	(50)	(50)
INFLAMMATION, FOCAL			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
INFLAMMATION, MULTIFOCAL	1 (5%)		
LIPOGRANULOMA		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
NECROSIS, HEMORRHAGIC			1 (2%)
METAMORPHOSIS FATTY		1 (2%)	1 (2%)
CYTOPLASMIC VACUOLIZATION		2 (4%)	1 (2%)
FOCAL CELLULAR CHANGE			2 (4%)
ATYPIA, NOS			1 (2%)
MULTINUCLEATE GIANT-CELL		1 (2%)	1 (2%)
HYPERPLASIA, FOCAL		3 (6%)	4 (8%)
HYPERPLASIA, DIFFUSE			1 (2%)
#LIVER/PERIPORTAL	(20)	(49)	(50)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	1 (2%)
#BILE DUCT	(20)	(49)	(50)
CYST, NOS	1 (5%)		
#PANCREAS	(20)	(49)	(48)
CYST, NOS			1 (2%)
CYSTIC DUCTS		1 (2%)	
#PANCREATIC ACINUS	(20)	(49)	(48)
ATROPHY, NOS		1 (2%)	
#STOMACH	(20)	(50)	(48)
INFLAMMATION, FOCAL		1 (2%)	1 (2%)

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2455	LOW DOSE 22-2453	HIGH DOSE 22-2451
INFLAMMATION, ACUTE FOCAL			1 (2%)
#LARGE INTESTINE	(20)	(48)	(50)
NEMATODIASIS		2 (4%)	1 (2%)
#COLON	(20)	(48)	(50)
INFLAMMATION, FOCAL	1 (5%)		
PARASITISM	2 (10%)	9 (19%)	8 (16%)
URINARY SYSTEM			
#KIDNEY	(20)	(48)	(50)
MINERALIZATION		2 (4%)	2 (4%)
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, CHRONIC	3 (15%)	3 (6%)	
NEPHROPATHY	1 (5%)	1 (2%)	
#URINARY BLADDER	(15)	(40)	(43)
ATROPHY, NOS			1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(20)	(46)	(48)
CYTOPLASMIC VACUOLIZATION			1 (2%)
#PANCREATIC ISLETS	(20)	(49)	(48)
HYPERPLASIA, NOS		2 (4%)	
REPRODUCTIVE SYSTEM			
#PROSTATE	(20)	(48)	(48)
INFLAMMATION, ACUTE			1 (2%)
*SEMINAL VESICLE	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(19)	(50)	(50)
HYDROCEPHALUS, NOS			1 (2%)
CORPORA AMYLACEA	7 (37%)	15 (30%)	13 (26%)

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

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 22-2455	LOW DOSE 22-2453	HIGH DOSE 22-2451
CALCIFICATION, FOCAL	1 (5%)	1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
STEATITIS		2 (4%)	1 (2%)
INFLAMMATION, GRANULOMATOUS		2 (4%)	
NECROSIS, NOS			1 (2%)
NECROSIS, FAT		5 (10%)	6 (12%)
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, GRANULOMATOUS			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		3	2
AUTO/NECROPSY/HISTO PERF			1

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

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
TREATED WITH p-PHENYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL (UNTR) 22-2456	LOW DOSE 22-2454	HIGH DOSE 22-2452
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(49)
INFLAMMATION, INTERSTITIAL		1 (2%)	
BRONCHOPNEUMONIA, ACUTE		1 (2%)	
PNEUMONIA, CHRONIC MURINE	1 (5%)	10 (21%)	
INFLAMMATION, CHRONIC		1 (2%)	2 (4%)
INFLAMMATION, CHRONIC FOCAL	1 (5%)	1 (2%)	2 (4%)
PERIARTERITIS		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(47)	(46)
HYPERPLASIA, HEMATOPOIETIC			1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
HEMATOPOIESIS		1 (2%)	1 (2%)
#LYMPH NODE	(19)	(46)	(41)
INFLAMMATION, NOS			1 (2%)
#MESENTERIC L. NODE	(19)	(46)	(41)
CYST, NOS		1 (2%)	
INFLAMMATION, NOS		1 (2%)	
CIRCULATORY SYSTEM			
#HEART/ATRIUM	(20)	(47)	(48)
THROMBUS, MURAL	1 (5%)		1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2456	LOW DOSE 22-2454	HIGH DOSE 22-2452
*CORONARY ARTERY	(20)	(50)	(49)
FIBROSIS		1 (2%)	
*PULMONARY ARTERY	(20)	(50)	(49)
HYPERTROPHY, NOS		2 (4%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(45)
THROMBOSIS, NOS		1 (2%)	
INFLAMMATION, CHRONIC FOCAL			1 (2%)
NECROSIS, FOCAL		1 (2%)	
INFARCT, NOS			1 (2%)
METAMORPHOSIS FATTY			1 (2%)
CYTOPLASMIC VACUOLIZATION	1 (5%)	2 (4%)	
HYPERPLASIA, FOCAL			1 (2%)
ANGIECTASIS		1 (2%)	
HEMATOPOIESIS			1 (2%)
#LIVER/PERIPORTAL	(20)	(48)	(45)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
#PANCREAS	(20)	(47)	(45)
CYSTIC DUCTS		2 (4%)	2 (4%)
#STOMACH	(20)	(49)	(47)
ULCER, NOS		1 (2%)	
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, CHRONIC		2 (4%)	1 (2%)
#COLON	(20)	(49)	(46)
PARASITISM		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(46)
HYDRONEPHROSIS		1 (2%)	2 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
#URINARY BLADDER	(15)	(43)	(36)
INFLAMMATION, CHRONIC			1 (3%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2456	LOW DOSE 22-2454	HIGH DOSE 22-2452
PERIARTERITIS			1 (3%)
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(43)	(34)
HEMORRHAGIC CYST		1 (2%)	1 (3%)
#ADRENAL	(19)	(47)	(44)
AMYLOIDOSIS		1 (2%)	
#ADRENAL MEDULLA	(19)	(47)	(44)
ATROPHY, FOCAL			1 (2%)
#THYROID	(18)	(47)	(41)
LIPOIDOSIS			1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(19)	(47)	(48)
HYDROMETRA	1 (5%)		
CYST, NOS	1 (5%)		1 (2%)
EDEMA, NOS	1 (5%)		
#UTERUS/ENDOMETRIUM	(19)	(47)	(48)
CYST, NOS	5 (26%)	15 (32%)	7 (15%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
HYPERPLASIA, DIFFUSE			1 (2%)
HYPERPLASIA, CYSTIC	5 (26%)	4 (9%)	
#OVARY	(16)	(42)	(40)
CYST, NOS	2 (13%)	4 (10%)	2 (5%)
PAROVARIAN CYST	1 (6%)	1 (2%)	1 (3%)
NERVOUS SYSTEM			
#BRAIN	(20)	(48)	(47)
PERIVASCULAR CUFFING		1 (2%)	
CORPORA AMYLACEA	8 (40%)	19 (40%)	8 (17%)
CALCIFICATION, FOCAL	1 (5%)		3 (6%)
SPECIAL SENSE ORGANS			
NONE			

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

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 22-2456	LOW DOSE 22-2454	HIGH DOSE 22-2452
MUSCULOSKELETAL SYSTEM			
*BONE	(20)	(50)	(49)
FIBROUS OSTEODYSTROPHY	2 (10%)		1 (2%)
BODY CAVITIES			
*MESENTERY	(20)	(50)	(49)
STEATITIS			1 (2%)
NECROSIS, FAT	1 (5%)	4 (8%)	2 (4%)
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, GRANULOMATOUS			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	4	7
AUTO/NECROPSY/HISTO PERF		1	1

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

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