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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
National Cancer Institute
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Bethesda, Maryland 20014

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

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

The experimental design and doses were chosen by Drs. E. K. Weisburger[1], J. H. Weisburger[1,2], N. P. Page[1,3], F. M. Garner[4] and B. M. Ulland[4,5]. Mr. R. Cypher[4], Mr. H. D. Thornett[4], and Mr. D. J. Howard[4] were responsible for administration of test chemical and for care and observation of animals.

Histopathologic examinations were performed by Drs. B. Cockrell[4], A. dePaoli[4], F. M. Garner[4], E. Gorgas[4], C. Montgomery[4], and N.

Wosu[4] for the rat study, and by Drs. Cockrell, dePaoli, Garner, Montgomery, and Wosu for the mouse study. Histologic sections of all tumors and hyperplasia were reexamined by Dr. R. A. Montali[4], who also reviewed all diagnoses and prepared the initial interpretive pathology summary.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[6]. The statistical analyses were performed by Dr. J. R. Joiner[7], using methods selected for the bioassay program by Dr. J. J. Gart[8]. Chemicals used in this bioassay were analyzed under the direction of Mr. H. Paulin[4], and the results of these analyses were reviewed by Dr. S. S. Olin[7]. The structural formula was supplied by NCI[1].

This report was prepared at Tracor Jitco[7] 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 and Ms. P. J. Graboske.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of NCI[8]: Dr. John J. Gart, Mr. Jun-mo Nam, Dr. Hugh M. Pettigrew, and Dr. Robert E. Tarone.

The following other scientists at the NCI[1] were responsible for evaluating the bioassay, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Dawn G. Goodman, Dr. Richard A. Griesemer, Dr. Harry Milman, Dr. Thomas W. Orme, Dr. Robert A. Squire[9], and Dr. Jerrold M. Ward.

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SUMMARY

A bioassay of N-phenyl-p-phenylenediamine for possible carcinogenicity was conducted by administering the test chemical in the diet to Fischer 344 rats and B6C3F1 mice.

Groups of 50 rats of each sex were administered N-phenyl-p-phenylenediamine at one of two doses, either 600 or 1,200 ppm, for 78 weeks and were then observed for 26 additional weeks. Matched controls consisted of groups of 20 untreated rats of each sex. All surviving rats were killed at 104 weeks.

Groups of 50 mice of each sex were initially administered N-phenyl-p-phenylenediamine at one of the following doses, either 2,500 or 5,000 ppm for the males and either 5,000 or 10,000 ppm for the females, for 31 weeks. Because of toxicity of the chemical, the doses were lowered at that time and terminated at 48 weeks. The animals were then observed for 43 additional weeks. Time-weighted average doses during the period of administration were 2,057 or 4,114 ppm for the males and 3,672 or 8,170 ppm for the females. Matched controls consisted of groups of 20 untreated mice of each sex. All surviving mice were killed at 91 weeks.

Mean body weights of the dosed rats were only slightly lower than those of the matched controls during the bioassay. Mean body weights of the dosed mice were appreciably lower than those of the matched controls, and mortality was high in the dosed groups prior to reduction of the doses, particularly in the females. Sufficient numbers of rats and mice of each sex were at risk for the development of late-appearing tumors; however, the shortened period used for administering N-phenyl-p-phenylenediamine to the mice may not have been adequate for determining the carcinogenic potential of the test chemical in this species.

In the male and female rats, the incidences of neoplasms in the

groups receiving the test chemical were not significantly different from those in the corresponding control groups.

In the male mice, the incidence of combined hepatocellular adenomas and carcinomas was significantly higher (P = 0.022) in the low-dose group than in the controls, but there was no significant dose-related trend (controls 2/20, low-dose 18/49, high-dose 10/50). Furthermore, since at this laboratory the overall historical incidences of these combined lesions in male mice have been 53/340 (15.6%) and have been as high as 7/20 (35%), these neoplasms could not be established as being compound related. Unusually extensive hepatic inflammation occurred in large numbers of the dosed males (controls 0/20, low-dose 23/49, high-dose 24/50) and in lesser numbers of the dosed females (controls 1/20, low-dose 8/49, high-dose 2/48).

It is concluded that under the conditions of this bioassay, N-phenyl-p-phenylenediamine was not carcinogenic for Fischer 344 rats or for B6C3F1 mice.

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I. INTRODUCTION

N-phenyl-p-phenylenediamine (CAS 101-54-2; NCI C02233) is an industrial intermediate that is used in the production of several different chemical products. It is an intermediate for photographic chemicals, pharmaceuticals, microbicides, and other organics (Uniroyal Chemicals, 1976); it is used in the manufacture of dyes and dye reagents (Colour Index, 1956); and it reacts with ketones to form derivatives of p-phenylenediamine which are used as antiozonants in rubber (Shaver, 1968).

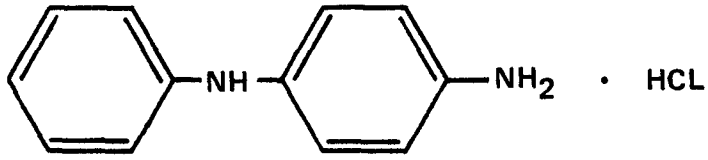
N-phenyl-p-phenylenediamine is listed as a suggested ingredient of oxidation-type hair dyes (Wall, 1972) and is, or has been, used in 39 diferent hair dye products, according to voluntary reports from the cosmetics industry to the Food and Drug Administration (Greif, 1977).

Because of the increasing concern for occupational safety and health in the chemical industry and because N-phenyl-p-phenylenediamine is an aromatic amine, it was selected for testing in the Carcinogenesis Testing Program.

II. MATERIALS AND METHODS

A. Chemical

N-PHENYL-p-PHENYLENEDIAMINE



The chemical used in the bioassay was technical-grade N-phenyl-p-phenylenediamine, supplied in a single batch (Lot No. 0100700 GB) by Uniroyal Chemical, Division of Uniroyal, Inc., Naugatuck, Connecticut. Analyses at Litton Bionetics, Inc., confirmed its identity. Infrared and nuclear magnetic resonance (nmr) spectra were consistent with the structure. The purity was estimated to be approximately 90%, as determined by thin-layer chromatography (tlc), column chromatography, and nmr. No attempt was made to identify two impurities that were detected. The melting range was 64.5-68.0°C, similar to the published value of 66°C (Weast, 1974) and the manufacturer's value of 69°C.

The chemical was reanalyzed by tlc at 6-month intervals. No change in the tlc pattern was detected.

The chemical was stored at 4°C in the original container.

B. Dietary Preparation

A 6-kilogram batch of feed was prepared twice per week for mice and three times per week for rats. To obtain each dietary concentration of N-phenyl-p-phenylenediamine, the appropriate weight of the chemical was mixed with a small portion of Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) using a mortar and pestle. This premix was then added to the remaining weight of feed and mixed in a twin-shell blender for at least 15 minutes. Feed preparations containing the test chemical were stored at 1°C for no longer than 1 week.

The stability of the chemical mixed with feed was determined at concentrations of 600 and 10,000 ppm. After 10 days at room temperature, no change in concentration was detected at either level.

Ground Wayne® Lab Blox animal meal not containing added test chemical (basal diet) was used as the diet for the control groups of animals.

C. Animals

Fischer 344 rats were obtained from A. R. Schmidt, Madison, Wisconsin, and B6C3F1 mice from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, at 28 days of age. These laboratories were under contract with the Division of Cancer Treatment, National Cancer Institute, to provide the animals used for testing. On arrival at the laboratory, the animals were quarantined for 14 days. The animals were considered acceptable for testing if they had no clinical signs of disease and were within a weight range of 19-22 g for mice and 85-110 g for rats at the end of the period of quarantine. Cage assignments were made by grouping the animals by weight and choosing one animal from each group so that the total weight of animals in each cage was approximately the same.

D. Animal Maintenance

Animals were housed in temperature- and humidity-controlled rooms. The temperature was maintained at 21-25°C and the relative humidity at 45-55%. Room air was changed 15 times per hour and passed through both intake and exhaust HEPA (High Efficiency Particulate Air) filters. The animal rooms were positively pressurized with respect to the exit hall and negatively pressurized with respect to the entrance hall. Cool white

fluorescent lighting was provided 8 hours per day. Test and control diets were available ad libitum; feed hoppers were replenished three times per week. Tap water, acidified to pH 2.5, was also available ad libitum.

Rats were housed four per cage and mice five per cage in solid polycarbonate cages. Each cage was covered with a wire mesh screen and a sheet of filter paper. Heat-treated hardwood chip bedding (Absorb-Dri®, Lab Products, Garfield, N.J.) was used in the cages. Cages and water bottles were sanitized twice per week and feed hoppers once per week at approximately 82°C; bedding was replaced twice per week.

Rats and mice were housed in separate rooms. Control animals and dosed animals were housed in the same room. Animals fed N-phenyl-p-phenylenediamine were housed in the same room as animals of the same species being fed the following chemicals:

RATS

N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
4,4′-diisocyanato-3,3′-dimethoxy-1,1′-biphenyl (CAS 91-93-0)
N,N′-bis(carboxymethyl)glycine (NTA) (CAS 139-13-9)

MICE

4,4′-methylenebis(N,N′-dimethyl)benzenamine (CAS 101-61-1)
4,4′-bis(dimethylamino)benzophenone (CAS 90-94-8)
phenylthiourea (CAS 103-85-5)
bis(acetyloxy)dibutylstannane (CAS 1067-33-0)
N,N,N′-trimethylthiourea (CAS 2489-77-2)
4-chlorobenzenamine (CAS 106-47-8)

3-chloro-4-methylbenzenamine (CAS 95-74-9)
5-chloro-o-toluidine (CAS 95-79-4)
2-nitro-1,4-benzenediamine (CAS 5307-14-2)

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses of N-phenyl-p-phenylenediamine, 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 studies. In the subchronic studies, N-phenyl-p-phenylenediamine was administered to rats at concentrations in the feed of 2,200, 3,200, 4,600, 6,800, or 10,000 ppm, and to mice at concentrations of 3,000, 4,400, 6,500, 9,500, 14,700, 21,600, or 31,500 ppm. Feed containing the test chemical was provided to dosed groups of five male and five female animals of each species, and feed not containing the test chemical was similarly provided to control groups of five male and five female animals of each species. Dosed animals received the test diets for 7 weeks and basal diets for the final week of the studies. All animals were killed and necropsied at week 8.

At 2,200 ppm, the mean body weight gain in the male rats after 7 weeks was 72% of that of the controls, and in the females, 50% of that of the controls. Mean body weight gains were progressively lower at higher doses. Deaths occurred in three males and two

females at 6,800 ppm and in four males and four females at 10,000 ppm. The low and high doses for the chronic studies using rats were set at 600 and 1,200 ppm.

Mice administered the chemical at doses of 3,000 to 9,500 ppm did not show any effects. When groups were restarted at doses of 14,700 21,600, or 31,500 ppm, the male mice still showed no effects; however, mean body weight gains were depressed in the female mice. The low and high doses for the chronic studies using male mice were set at 2,500 and 5,000 ppm; using females, they were set at 5,000 and 10,000 ppm.

F. Designs of Chronic Studies

The designs of the chronic studies are shown in tables 1 and 2. By week 31 of the study, signs of central nervous system toxicity occurred in the male and female mice administered either the low or high initial dose. Consequently, the doses were reduced by 1/2 in the low- and high-dose male mice, discontinued in the high-dose females, and reduced by 1/4 in the low-dose females. At week 39, the high-dose females were given diets containing 2,500 ppm. At week 48, all mice were placed on basal diets until termination of the period of observation at week 91.

Table 1. Design of Chronic Studies of N-phenyl-p-phenylenediamine in Rats

Sex and Test Group	Initial No. of Animals[a]	N-phenyl-p-phenylene-diamine in Diet (ppm)[b]	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Control	20	0		104
Low-Dose	50	600	78	26
High-Dose	50	1,200	78	26
Female				
Control	20	0		104
Low-Dose	50	600	78	26
High-Dose	50	1,200	78	26

[a]All animals were approximately 42 days of age when placed on study.

[b]Test and control diets were available *ad libitum* 7 days per week.

Table 2. Design of Chronic Studies of N-phenyl-p-phenylenediamine in Mice

Sex and Test Group	Initial No. of Animals[a]	N-phenyl-p-phenylene-diamine in Diet (ppm)[b]	Time on Study Dosed (weeks)	Time on Study Observed (weeks)	Time-Weighted Average Dose[c] (ppm)
Male					
Control	20	0		91	
Low-Dose	50	2,500	31		
		1,250	17		
		0		43	2,057
High-Dose	50	5,000	31		
		2,500	17		
		0		43	4,114
Female					
Control	20	0		91	
Low-Dose	50	5,000	31		
		1,250	17		
		0		43	3,672
High-Dose	50	10,000	31		
		0	7		
		2,500	10		
		0		43	8,170

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

[b]Test and control diets were available *ad libitum* 7 days per week.

[c]Time-weighted average dose $= \frac{\Sigma(\text{dose in ppm x no. of weeks at that dose})}{\Sigma(\text{no. of weeks receiving each dose})}$

G. Clinical and Pathological Examinations

All animals were observed twice daily for signs of toxicity, and animals that were moribund were killed and necropsied. Rats and mice were weighed individually at regular intervals. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross and microscopic examination of all major tissues, organs, or gross lesions from killed animals and from animals found dead. The following tissues and organs were routinely subjected to microscopic examination: skin, lymph nodes, mammary gland, salivary gland, bone marrow, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, small intestine, large intestine, liver, gallbladder (mice), pancreas, spleen, kidney, adrenal, urinary bladder, prostate or uterus, testis or ovary, brain, and pituitary. The tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

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

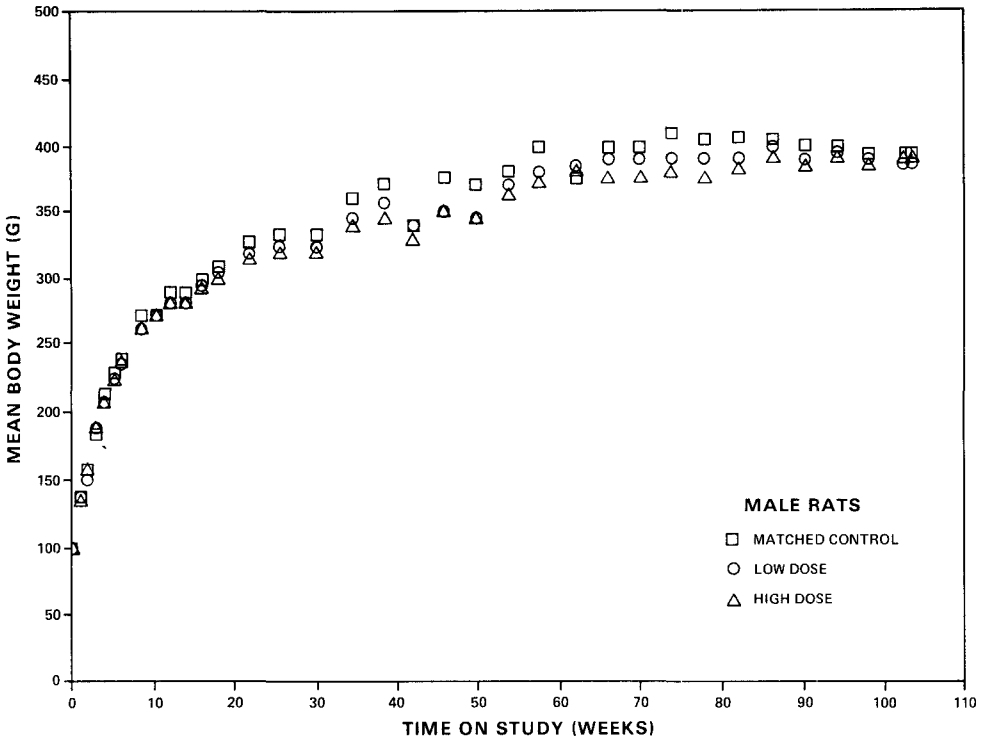
A. Body Weights and Clinical Signs (Rats)

The mean body weights of the dosed male and female rats were slighty lower than those of the corresponding matched controls (figure 1). Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. No other clinical signs were recorded.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats fed N-phenyl-p-phenylenediamine in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2.

The result of the Tarone test for positive dose-related trend in mortality is not significant in either sex. At least 84% of the males (42/50 of the high-dose group, 42/50 of the low-dose group, and 18/20 of the control group) and over 85% of the females (46/50 of the high-dose group, 43/50 of the low-dose group, and 18/20 of the control group) were alive at week 104. Sufficient numbers of rats of each sex were at risk for development of late-appearing tumors.



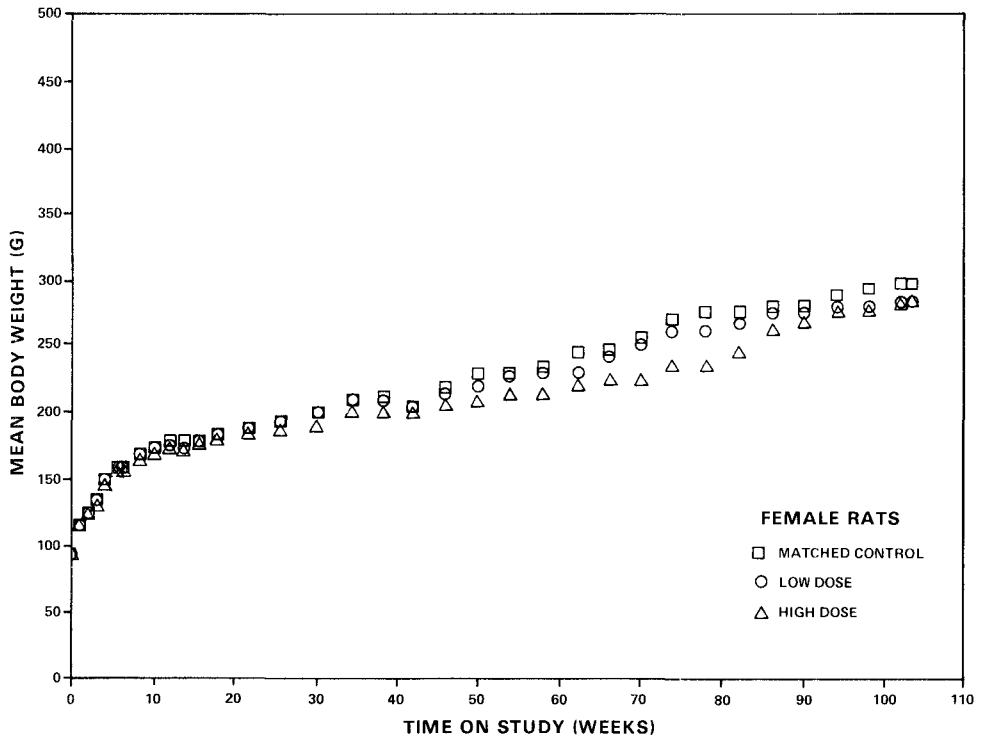


Figure 1. Growth Curves For Rats Fed N-Phenyl-p-Phenylenediamine in The Diet

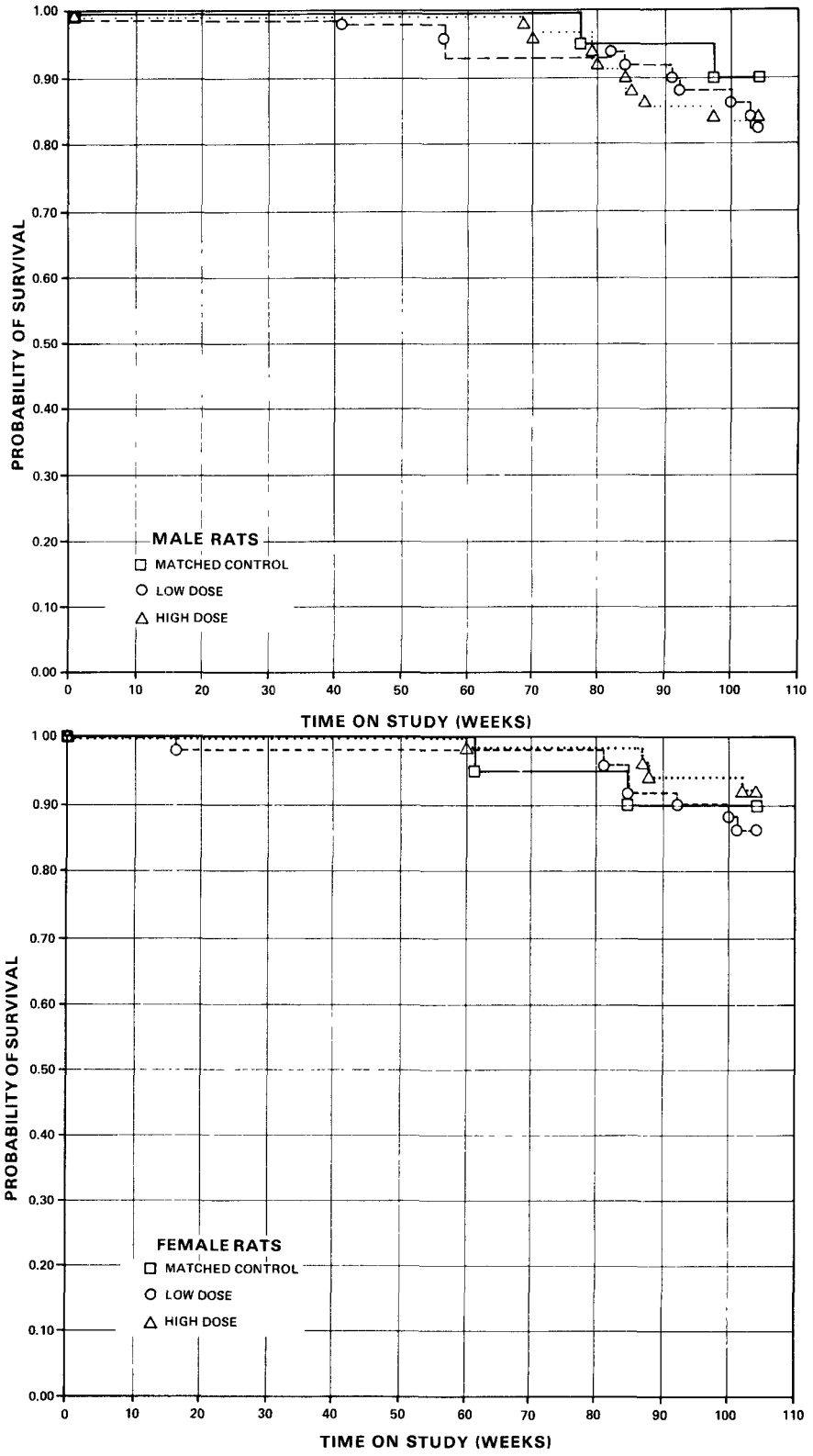


Figure 2. Survival Curves For Rats Fed N-Phenyl-p-Phenylenediamine in The Diet

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 tumors of the integumentary system were observed in the dosed rats, but none were observed in the controls. These and the remaining tumors that were observed in the dosed and control rats were considered to be spontaneous; most of them occurred at incidences that were to be expected for this age group of the Fischer 344 rat. They included testicular interstitial-cell tumors in the males and pituitary chromophobe adenomas in the females. Although no chromophobe adenomas or C-cell tumors of the thyroid glands occurred in the control male rats, the numbers of these relatively common tumors for aging rats that were in the dosed animals did not exceed expected incidences. There was a relatively low incidence of leukemias in both male and female rats. Most were compatible with leukemia (undifferentiated) as usually observed in the Fischer 344 rat, or were unclassifiable and occurred with approximately equal frequency in dosed and control groups.

A variety of degenerative and inflammatory lesions of the type usually encountered in aged Fischer 344 rats were observed, but

none was considered to be attributable to administration of the test chemical.

Based on the histopathologic examination, there was no evidence for the carcinogenicity of N-phenyl-p-phenylenediamine in rats under the conditions of this bioassay.

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 results of the Cochran-Armitage test for positive dose-related trend and of the Fisher exact test for direct comparison of the incidences in dosed and control groups are not significant in either sex. In the females, a significant trend in the negative direction is observed in the incidence of fibroadenoma of the mammary gland; however, this negative significance cannot be accounted for by differences in survival in the dosed and control groups.

In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one is included; this indicates the absence of significant positive results. It should also be noted

that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by N-phenyl-p-phenylenediamine, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

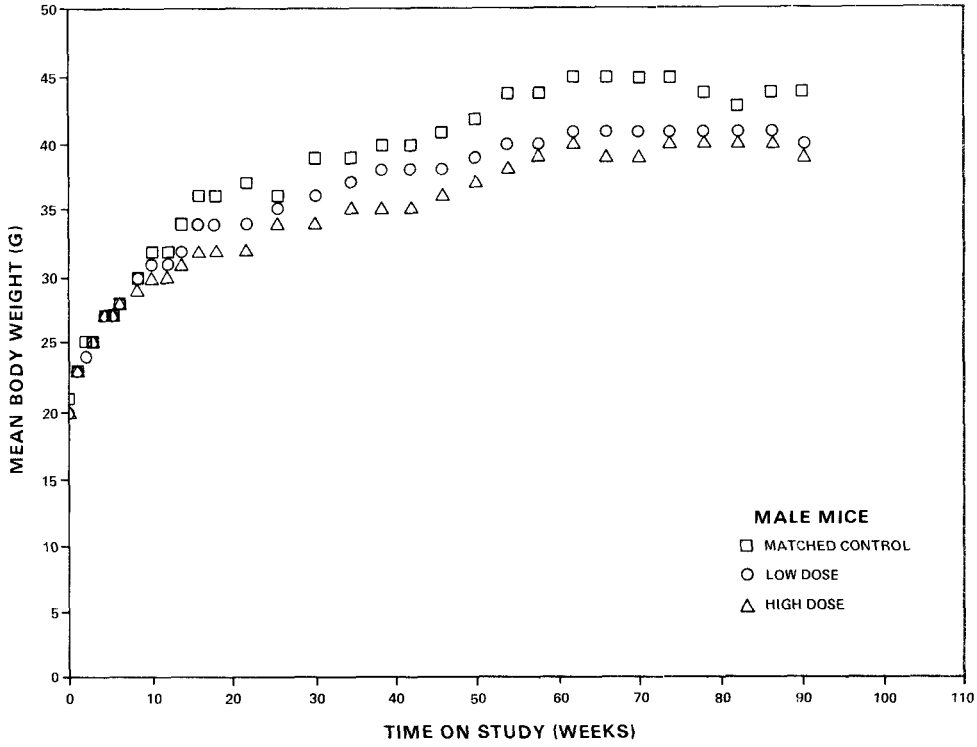
A. Body Weights and Clinical Signs (Mice)

The mean body weights of the dosed male mice were lower than those of the matched controls throughout the bioassay, and those of the high-dose animals were slightly lower than those of the low-dose animals (figure 3). The mean body weights of the dosed female mice also were lower than those of the matched controls, but those of the low- and high-dose groups did not differ from one another. Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. Clinical signs of effects on the central nervous system were reported in the dosed mice; these signs were not, however, further described.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice fed N-phenyl-p-phenylenediamine in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 4.

In the male mice, the result of the Tarone test for positive dose-related trend in mortality is not significant. At least 85% of the males from each group (44/50 of the high-dose group, 46/49



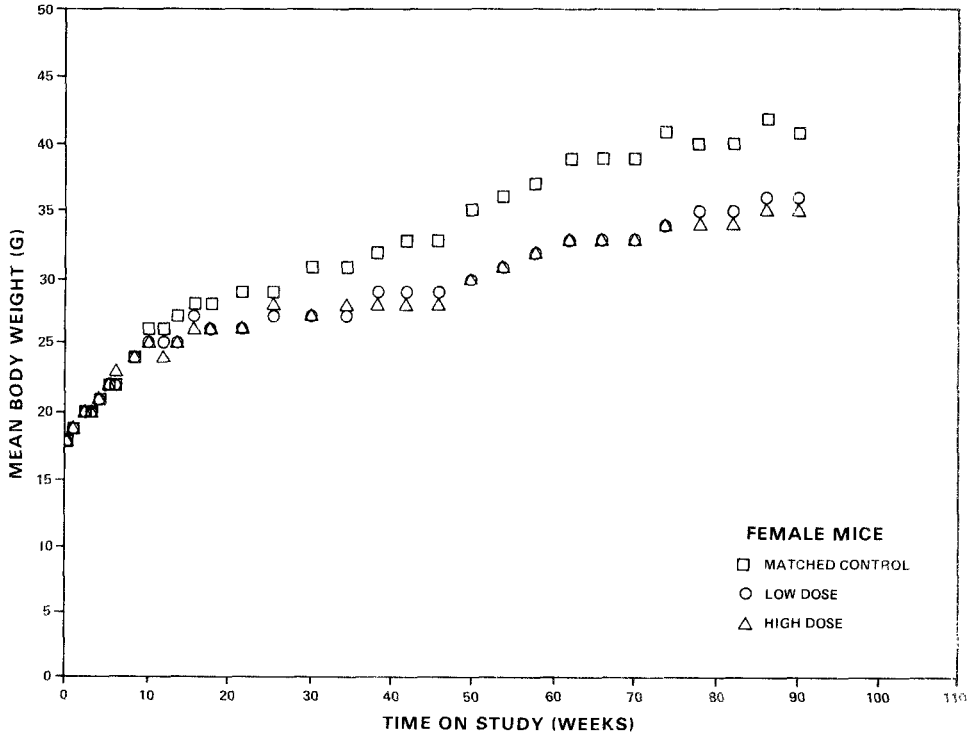


Figure 3. Growth Curves For Mice Fed N-Phenyl-p-Phenylenediamine in The Diet

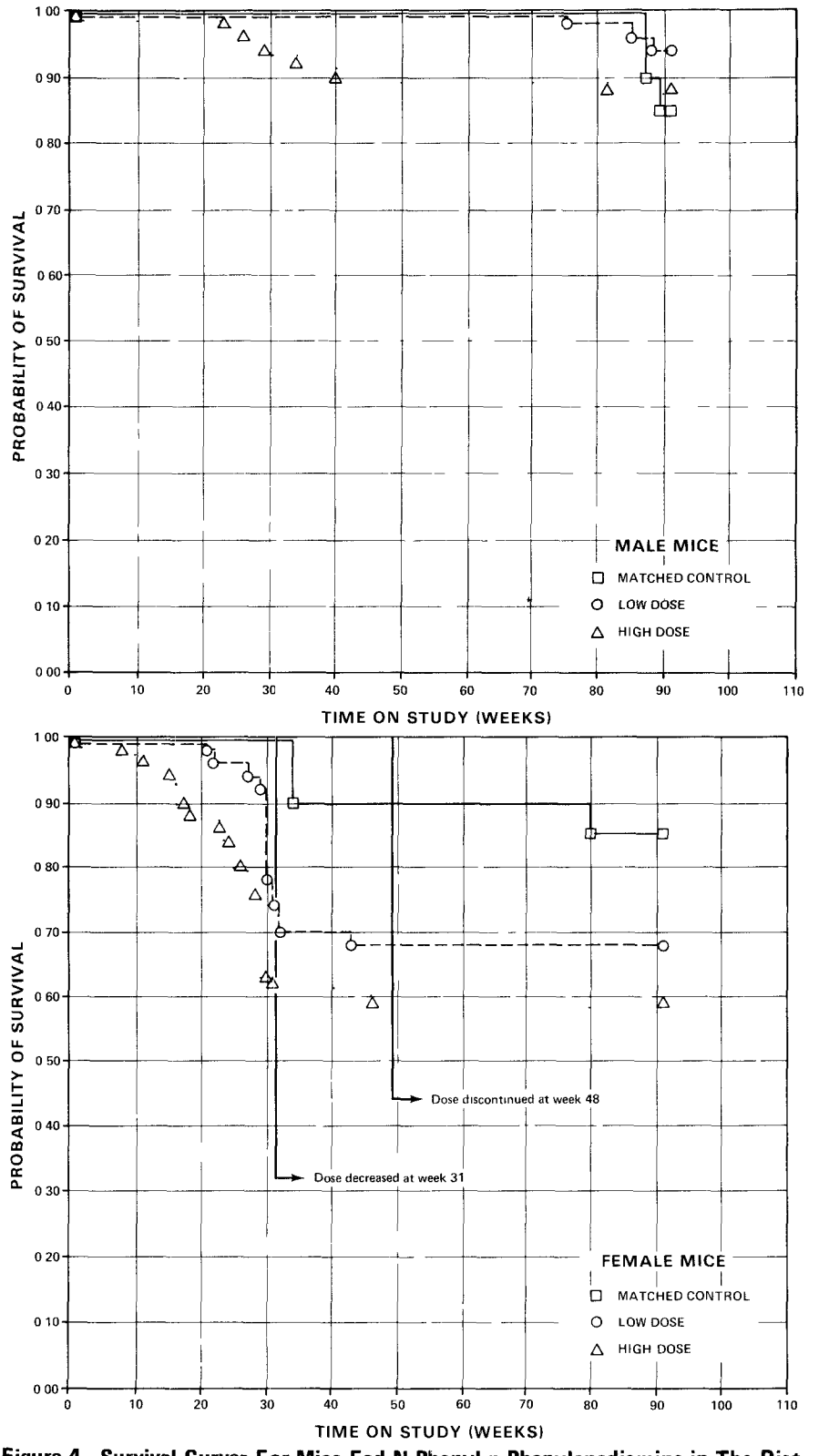


Figure 4. Survival Curves For Mice Fed N-Phenyl-p-Phenylenediamine in The Diet

of the low-dose group, and 17/20 of the control group) lived to the end of the study. In the females, the result of the Tarone test is significant (P = 0.021), with 29/50 (58%) of the high-dose group, 34/50 (68%) of the low-dose group, and 17/20 (85%) of the matched controls surviving to the termination of the study. Approximately 25% of the low-dose and 40% of the high-dose female mice died with signs indicative of a central nervous system (CNS) disturbance. Deaths occurred from week 8 to 46, with the majority occurring at about 30 weeks after the start of the study. Sufficient numbers of mice of each sex were at risk for development of late-appearing tumors.

C. Pathology (Mice)

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

A significant morbidity and mortality occurred in the dosed female mice; this was attributed to administration of the N-phenyl-p-phenylenediamine. No pathological changes were evident, however, in the CNS or elsewhere to account for the toxic manifestations.

The incidence of hepatocellular neoplasms in the dosed groups of

male mice exceeded that in the controls, as shown in the following table:

	MICE					
	Male			Female		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Number of animals with tissues examined microscopically	20	49	50	20	49	48
Hepatocellular carcinoma	2(10%)	6(12%)	5(10%)	0(0%)	0(0%)	0(0%)
Hepatocellular adenoma or carcinoma	2(10%)	18(37%)	10(20%)	1(5%)	2(4%)	1(2%)

One unusual feature was the number of tumors interpreted as adenomas. These usually occurred as single, discrete nodules of enlarged cells that were either vacuolated or contained densely eosinophilic cytoplasm. The cells that made up the nodules formed sheets or fairly regular plates which compressed surrounding normal hepatic parenchyma. The hepatic tumors interpreted as hepatocellular carcinomas were mostly larger versions of the adenomas and were adjudged carcinomas mainly on the basis of their size, although several had irregular trabecular structures and glandular patterns.

Inflammatory and other lesions that were considered to be significant and possibly related to administration of the test

chemical occurred in the livers of the dosed male mice, as shown in the following table:

	Male Mice		
	Matched Control	Low Dose	High Dose
Number of animals with tissues examined microscopically	20	49	50
Inflammation, focal	0(0%)	23(47%)	24(48%)
Hepatocellular hyperplasia	0(0%)	0(0%)	2(4%)
Bile duct hyperplasia	0(0%)	2(4%)	3(6%)
Hepatocytomegaly	0(0%)	0(0%)	4(8%)

Although it is not unusual to see some mild chronic inflammation in the livers of aged B6C3F1 mice, the inflammatory changes in these animals were often extensive and consisted of mononuclear cells occurring as foci within the parenchyma and infiltrates in portal triads. These were accompanied by a proliferation of Kupffer's cells and increased numbers of megalocytes. A few of these livers had hyperplastic bile ducts. The changes occurred in many of the livers that also had hepatocellular neoplasms. The hepatocellular hyperplasia noted in the two high-dose male mice were lobular proliferations of hepatocytes that did not form true nodules. Hepatocellular giant cells were multinucleated and often contained from 10 to 20 small nuclei.

Based on the histopathologic examination, there was no conclusive

evidence for the carcinogenicity of N-phenyl-p-phenylenediamine in mice under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

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

In male mice, the result of the Cochran-Armitage test for positive dose-related trend in the combined incidence of hepatocellular adenomas or carcinomas is not significant; however, an indicated departure from linear trend is observed (P = 0.010), because the incidence in the low-dose group is higher than that in the high-dose group. The results of the Fisher exact test show that the incidence in the low-dose group is significantly higher (P = 0.022) than that in the matched controls, but a significant incidence is not indicated in the high-dose group.

In female mice, a significant incidence of lymphoma is observed in the negative direction; this may be accounted for by the higher mortality in the dosed groups of animals than in the control group.

In each of the 95% confidence intervals of relative risk, shown

in the tables, except that for hepatocellular adenoma or carcinoma of the liver in the low-dose male mice, the value of one or less than one is included; this indicates the absence of significant results. It should also be noted that each of the intervals (except that for the incidence of lymphoma in the low-dose female mice) has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by N-phenyl-p-phenylenediamine, which could not be detected under the conditions of this test.

V. DISCUSSION

Under the conditions of this bioassay, N-phenyl-p-phenylenediamine had no appreciable toxic effect on Fischer 344 rats, but it was quite toxic to the mice at the doses administered. The doses for the mice in the chronic studies were initially set higher than those for the rats, because little toxicity was observed in the mice in the subchronic studies. In the chronic studies, however, the original doses of N-phenyl-p-phenylenediamine were toxic in the mice; therefore, the doses used in the chronic studies were lowered during the course of the bioassay, and administration of the test chemical was terminated at 48 weeks. Sufficient numbers of rats and mice of each sex were at risk for the development of late-appearing tumors; however, the shortened period used for administering N-phenyl-p-phenylenediamine to the mice may not have been adequate for determining the carcinogenic potential of the test chemical in this species.

In the male and female rats, the incidences of neoplasms in the dosed groups were not significantly different from those in the corresponding control groups.

In the male mice, the incidence of combined hepatocellular adenomas and carcinomas was significantly higher ($P = 0.022$) in the low-dose group than in the controls, but there was no

significant dose-related trend, since the incidence in the high-dose group was lower than that in the low-dose group (controls 2/20, low-dose 18/49, high-dose 10/50). Furthermore, since at this laboratory the overall historical incidences of these combined lesions in male mice have been 53/340 (15.6%) and have been as high as 7/20 (35%), these neoplasms could not be established as being compound related. No hepatocellular carcinomas were observed in the dosed or control female mice, and there were only two incidences of hepatocellular adenomas in the low-dose females and one in the high-dose females, compared with one in the controls. Unusually extensive hepatic inflammation occurred in large numbers of the dosed males (controls 0/20, low-dose 23/49, high-dose 24/50) and in lesser numbers of the dosed females (controls 1/20, low-dose 8/49, high-dose 2/48). The inflammation occurred in many of the livers in which hepatocellular neoplasms were also observed.

N-phenyl-p-phenylenediamine has been reported to have a low acute oral LD_{50} of 464 mg/kg body weight for rats (Naugatuck Chemicals, 1976). The chemical was not carcinogenic when administered to strain A mice by subcutaneous injection for 14 or 16 months (Shear and Stewart, 1941) or when administered orally to dogs for 6 years (Deichmann and Lampe, 1967). It was also negative for mutagenicity in the Salmonella/microsome test (McCann et al.,

1975). Skin irritation and sensitization have been reported in persons exposed to the chemical (Naugatuck Chemicals, 1976; Greenberg and Lester, 1954).

It is concluded that under the conditions of this bioassay, N-phenyl-p-phenylenediamine was not carcinogenic for Fischer 344 rats or for B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS FED N-PHENYL-p-PHENYLENEDIAMINE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED N-PHENYL-p-PHENYLENEDIAMINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
PAPILLOMA, NOS	1 (5%)		
SQUAMOUS CELL CARCINOMA		1 (2%)	
BASAL-CELL TUMOR		1 (2%)	
FIBROMA			1 (2%)
HEMANGIOSARCOMA		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA			2 (4%)
BASAL-CELL TUMOR			1 (2%)
FIBROMA			3 (6%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)		3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
FIBROMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
LEUKEMIA,NOS	2 (10%)	1 (2%)	2 (4%)
UNDIFFERENTIATED LEUKEMIA			1 (2%)
LYMPHOCYTIC LEUKEMIA			1 (2%)
GRANULOCYTIC LEUKEMIA	1 (5%)	1 (2%)	
CIRCULATORY SYSTEM			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(17)	(48)	(47)
ADENOMA, NOS			1 (2%)
#SMALL INTESTINE	(20)	(50)	(50)
LEIOMYOMA		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(46)	(48)
CHROMOPHOBE ADENOMA		3 (7%)	3 (6%)
#ADRENAL	(20)	(50)	(50)
PHEOCHROMOCYTOMA	1 (5%)	2 (4%)	3 (6%)
#THYROID	(20)	(50)	(50)
C-CELL ADENOMA		1 (2%)	3 (6%)
C-CELL CARCINOMA		1 (2%)	
#PANCREATIC ISLETS	(20)	(48)	(49)
ISLET-CELL ADENOMA		3 (6%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
FIBROADENOMA			1 (2%)
*PREPUTIAL GLAND	(20)	(50)	(50)
ADENOMA, NOS		1 (2%)	
#TESTIS	(20)	(50)	(49)
INTERSTITIAL-CELL TUMOR	19 (95%)	46 (92%)	46 (94%)
NERVOUS SYSTEM			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EAR	(20)	(50)	(50)
SEBACEOUS ADENOCARCINOMA		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	3	4
MORIBUND SACRIFICE		6	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	41	42
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	48	49
TOTAL PRIMARY TUMORS	26	65	73
TOTAL ANIMALS WITH BENIGN TUMORS	20	46	48
TOTAL BENIGN TUMORS	23	59	65
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	6	8
TOTAL MALIGNANT TUMORS	3	6	8
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED N-PHENYL-p-PHENYLENEDIAMINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROADENOMA	1 (5%)	1 (2%)	2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA			2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKEMIA,NOS		2 (4%)	1 (2%)
UNDIFFERENTIATED LEUKEMIA	3 (15%)		2 (4%)
LYMPHOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(20)	(50)	(49)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(47)	(46)
CHROMOPHOBE ADENOMA	7 (35%)	14 (30%)	9 (20%)
CHROMOPHOBE CARCINOMA		1 (2%)	
#ADRENAL	(20)	(48)	(50)
PHEOCHROMOCYTOMA	1 (5%)	2 (4%)	1 (2%)
#THYROID	(19)	(46)	(49)
ADENOCARCINOMA, NOS		1 (2%)	
C-CELL ADENOMA	2 (11%)	1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
LEIOMYOSARCOMA		1 (2%)	
FIBROADENOMA	2 (10%)		
*PREPUTIAL GLAND	(20)	(50)	(50)
ADENOMA, NOS		1 (2%)	2 (4%)
#UTERUS	(20)	(49)	(50)
ADENOCARCINOMA, NOS			1 (2%)
FIBROMA	1 (5%)		
ENDOMETRIAL STROMAL POLYP	2 (10%)	4 (8%)	4 (8%)
#CERVIX UTERI	(20)	(49)	(50)
LEIOMYOSARCOMA		1 (2%)	
#OVARY	(20)	(49)	(50)
LUTEOMA		1 (2%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(20)	(50)	(48)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	2	3
MORIBUND SACRIFICE		5	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	43	46
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	26	24
TOTAL PRIMARY TUMORS	19	31	28
TOTAL ANIMALS WITH BENIGN TUMORS	12	22	19
TOTAL BENIGN TUMORS	16	24	21
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	6	7
TOTAL MALIGNANT TUMORS	3	7	7
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	
TOTAL SECONDARY TUMORS		2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE FED N-PHENYL-p-PHENYLENEDIAMINE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED N-PHENYL-p-PHENYLENEDIAMINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	@50	50
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (5%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	4 (20%)	4 (8%)	5 (10%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)		1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)		
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	
#LYMPH NODE	(19)	(48)	(47)
MALIGNANT LYMPHOMA, NOS	1 (5%)		1 (2%)
#MESENTERIC L. NODE	(19)	(48)	(47)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
#SMALL INTESTINE	(20)	(48)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
HEPATOCELLULAR ADENOMA		12 (24%)	5 (10%)

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

@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS DELETED WHEN FOUND TO BE A FEMALE IN A MALE GROUP.

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA	2 (10%)	6 (12%)	5 (10%)
HEMANGIOSARCOMA	1 (5%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#TESTIS	(19)	(48)	(50)
INTERSTITIAL-CELL TUMOR		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(20)	(49)	(50)
FIBROSARCOMA		1 (2%)	
*ABDOMINAL CAVITY	(20)	(49)	(50)
LIPOMA	1 (5%)		
ALL OTHER SYSTEMS			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	3	5
MORIBUND SACRIFICE			1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	46	44
ANIMAL MISSING			
ANIMAL DELETED(WRONG SEX)		1	
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	10	25	15
TOTAL PRIMARY TUMORS	11	26	18
TOTAL ANIMALS WITH BENIGN TUMORS	5	17	9
TOTAL BENIGN TUMORS	5	17	10
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	9	8
TOTAL MALIGNANT TUMORS	6	9	8
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		
TOTAL SECONDARY TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED N-PHENYL-p-PHENYLENEDIAMINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(49)
SARCOMA, NOS		1 (2%)	
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
#SPLEEN	(19)	(43)	(47)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)		
#LIVER	(20)	(49)	(48)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
#SMALL INTESTINE	(20)	(50)	(42)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
#KIDNEY	(20)	(50)	(48)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(48)
HEPATOCELLULAR ADENOMA	1 (5%)	2 (4%)	1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMANGIOSARCOMA		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	14	20
MORIBUND SACRIFICE	1	2	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	34	29
ANIMAL MISSING			1
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	5	4	2
TOTAL PRIMARY TUMORS	5	4	3
TOTAL ANIMALS WITH BENIGN TUMORS	1	2	1
TOTAL BENIGN TUMORS	1	2	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	2	2
TOTAL MALIGNANT TUMORS	4	2	2
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS FED N-PHENYL-p-PHENYLENEDIAMINE IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED N-PHENYL-p-PHENYLENEDIAMINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
HYPERKERATOSIS			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
ABSCESS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(20)	(50)	(49)
INFLAMMATION, CHRONIC		3 (6%)	
#LUNG	(20)	(50)	(49)
CONGESTION, NOS	2 (10%)	1 (2%)	1 (2%)
HEMORRHAGE	1 (5%)	1 (2%)	
PNEUMONIA, CHRONIC MURINE	5 (25%)	12 (24%)	16 (33%)
PNEUMONIA INTERSTITIAL CHRONIC	1 (5%)		
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
FOAM-CELL	1 (5%)		
HYPERPLASIA, LYMPHOID		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(17)	(45)	(48)
MYELOSCLEROSIS		1 (2%)	
HYPERPLASIA, GRANULOCYTIC	2 (12%)	1 (2%)	
#SPLEEN	(20)	(50)	(50)
CONGESTION, NOS			1 (2%)
FIBROSIS, FOCAL			1 (2%)
FIBROSIS, MULTIFOCAL		1 (2%)	
HEMOSIDEROSIS	1 (5%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIESIS	1 (5%)		
#LYMPH NODE	(19)	(49)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	
#MANDIBULAR L. NODE	(19)	(49)	(49)
INFLAMMATION, CHRONIC	1 (5%)		
HYPERPLASIA, PLASMA CELL			2 (4%)
#MESENTERIC L. NODE	(19)	(49)	(49)
CONGESTION, CHRONIC		1 (2%)	
EDEMA, NOS			1 (2%)
HYPERPLASIA, RETICULUM CELL		1 (2%)	1 (2%)
HYPERPLASIA, LYMPHOID	1 (5%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(19)	(48)	(49)
FIBROSIS	2 (11%)	2 (4%)	6 (12%)
DEGENERATION, NOS	2 (11%)	2 (4%)	1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(47)
INFLAMMATION, CHRONIC		1 (2%)	
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY		1 (2%)	1 (2%)
BASOPHILIC CYTO CHANGE	2 (10%)	2 (4%)	
HYPERPLASIA, NODULAR		1 (2%)	
HYPERPLASIA, FOCAL	1 (5%)		1 (2%)
#LIVER/CENTRILOBULAR	(20)	(50)	(47)
DEGENERATION, NOS		1 (2%)	
*BILE DUCT	(20)	(50)	(50)
HYPERPLASIA, NOS	2 (10%)	2 (4%)	2 (4%)
#PANCREAS	(20)	(48)	(49)
ATROPHY, FOCAL	1 (5%)		
#STOMACH	(20)	(49)	(50)
GRANULOMA, NOS		1 (2%)	
#SMALL INTESTINE	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE		2 (4%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, LYMPHOID		1 (2%)	
#ILEUM	(20)	(50)	(50)
HYPERPLASIA, LYMPHOID	1 (5%)	1 (2%)	
#LARGE INTESTINE	(19)	(48)	(50)
NEMATODIASIS	8 (42%)	19 (40%)	15 (30%)
#COLON	(19)	(48)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
CYST, NOS			1 (2%)
PYELONEPHRITIS, NOS		1 (2%)	
INFLAMMATION, CHRONIC	13 (65%)	19 (38%)	32 (64%)
#KIDNEY/CORTEX	(20)	(50)	(50)
CYST, NOS			1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL	(20)	(50)	(50)
HEMORRHAGIC CYST		2 (4%)	
LIPOIDOSIS	1 (5%)		
#ADRENAL CORTEX	(20)	(50)	(50)
HYPERPLASIA, NODULAR		1 (2%)	
#THYROID	(20)	(50)	(50)
HYPERPLASIA, C-CELL	1 (5%)	1 (2%)	1 (2%)
#PANCREATIC ISLETS	(20)	(48)	(49)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(20)	(50)	(50)
ABSCESS, NOS		1 (2%)	
#TESTIS	(20)	(50)	(49)
HYPERPLASIA, INTERSTITIAL CELL			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(20)	(48)	(50)
HYDROCEPHALUS, INTERNAL	1 (5%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(50)
INFLAMMATION, FOCAL			1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED N-PHENYL-p-PHENYLENEDIAMINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
CYST, NOS			2 (4%)
EPIDERMAL INCLUSION CYST		1 (2%)	
INFLAMMATION, CHRONIC	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(50)
CYST, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(19)	(50)	(50)
INFLAMMATION, CHRONIC			1 (2%)
#LUNG	(19)	(50)	(50)
HEMORRHAGE	1 (5%)	1 (2%)	
PNEUMONIA, CHRONIC MURINE	7 (37%)	13 (26%)	12 (24%)
INFLAMMATION, CHRONIC SUPPURATIV		1 (2%)	
GRANULOMA, NOS	1 (5%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(50)	(49)
HYPERPLASIA, LYMPHOID	1 (5%)		
HEMATOPOIESIS			1 (2%)
#MANDIBULAR L. NODE	(20)	(47)	(48)
HYPERPLASIA, RETICULUM CELL	1 (5%)	1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	
#MESENTERIC L. NODE	(20)	(47)	(48)
LYMPHANGIECTASIS		1 (2%)	

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

TABLE C2. FEMALE RATS : NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
EDEMA, NOS		1 (2%)	
DEGENERATION, CYSTIC		1 (2%)	
HYPERPLASIA, RETICULUM CELL	1 (5%)	1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(45)	(47)
FIBROSIS, DIFFUSE	1 (5%)		1 (2%)
DEGENERATION, NOS	1 (5%)	2 (4%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(47)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
GRANULOMA, NOS		1 (2%)	
METAMORPHOSIS FATTY		2 (4%)	1 (2%)
CYTOPLASMIC VACUOLIZATION		1 (2%)	
BASOPHILIC CYTO CHANGE	3 (15%)	8 (16%)	2 (4%)
HEPATOCYTOMEGALY		1 (2%)	
HYPERPLASIA, NODULAR	3 (15%)	6 (12%)	
HYPERPLASIA, FOCAL	2 (10%)	1 (2%)	2 (4%)
HYPERPLASIA, DIFFUSE			1 (2%)
*BILE DUCT	(20)	(50)	(50)
HYPERPLASIA, NOS	2 (10%)	2 (4%)	2 (4%)
#PANCREAS	(20)	(50)	(48)
INFLAMMATION, NOS	1 (5%)		
#PANCREATIC ACINUS	(20)	(50)	(48)
ATROPHY, NOS		1 (2%)	
#SMALL INTESTINE	(20)	(48)	(50)
HYPERPLASIA, LYMPHOID	1 (5%)		
#PEYERS PATCH	(20)	(48)	(50)
HYPERPLASIA, LYMPHOID	1 (5%)	1 (2%)	
#ILEUM	(20)	(48)	(50)
HYPERPLASIA, LYMPHOID	1 (5%)	2 (4%)	
#LARGE INTESTINE	(20)	(47)	(50)
ULCER, NOS			1 (2%)

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

TABLE C2. FEMALE RATS : NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NEMATODIASIS	7 (35%)	13 (28%)	15 (30%)
#COLON	(20)	(47)	(50)
HYPERPLASIA, LYMPHOID	1 (5%)		
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
CYST, NOS		1 (2%)	
INFLAMMATION, INTERSTITIAL			1 (2%)
INFLAMMATION, CHRONIC	6 (30%)	18 (36%)	11 (22%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(47)	(46)
CYST, NOS	1 (5%)	2 (4%)	1 (2%)
HEMORRHAGIC CYST	1 (5%)	1 (2%)	3 (7%)
#ADRENAL	(20)	(48)	(50)
HEMORRHAGIC CYST	2 (10%)	1 (2%)	
METAMORPHOSIS FATTY		2 (4%)	
#ADRENAL CORTEX	(20)	(48)	(50)
NECROSIS, NOS			1 (2%)
#THYROID	(19)	(46)	(49)
HYPERPLASIA, C-CELL		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
DILATATION/DUCTS	1 (5%)	1 (2%)	1 (2%)
CYSTIC DUCTS	1 (5%)		
#UTERUS	(20)	(49)	(50)
HYDROMETRA			1 (2%)
INFLAMMATION, SUPPURATIVE	1 (5%)		2 (4%)
#CERVIX UTERI	(20)	(49)	(50)
INFLAMMATION, NOS	1 (5%)		
ABSCESS, NOS	1 (5%)		
#UTERUS/ENDOMETRIUM	(20)	(49)	(50)
INFLAMMATION, NOS			1 (2%)

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

TABLE C2. FEMALE RATS : NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, SUPPURATIVE		1 (2%)	1 (2%)
HYPERPLASIA, CYSTIC	1 (5%)		1 (2%)
#OVARY/OVIDUCT	(20)	(49)	(50)
INFLAMMATION, NOS			3 (6%)
#OVARY	(20)	(49)	(50)
CYST, NOS	5 (25%)	10 (20%)	4 (8%)
INFLAMMATION, NOS		1 (2%)	1 (2%)
INFLAMMATION, SUPPURATIVE		1 (2%)	1 (2%)
INFLAMMATION, NECROTIZING			1 (2%)
INFLAMMATION, CHRONIC SUPPURATIV		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(48)
HYDROCEPHALUS, NOS			1 (2%)
HYDROCEPHALUS, INTERNAL	1 (5%)	1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(20)	(50)	(50)
INFLAMMATION, CHRONIC			1 (2%)
*EPICARDIUM	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
PERIVASCULITIS		1 (2%)	

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

TABLE C2. FEMALE RATS : NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			7

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED N-PHENYL-p-PHENYLENEDIAMINE IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED N-PHENYL-p-PHENYLENEDIAMINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	@50	50
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(49)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(20)	(49)	(50)
HYPERPLASIA, FOCAL	1 (5%)		
#LUNG	(20)	(49)	(50)
PNEUMONIA, ASPIRATION			1 (2%)
PNEUMONIA, CHRONIC MURINE	1 (5%)	2 (4%)	5 (10%)
HYPERPLASIA, FOCAL		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(43)	(46)
INFARCT, NOS	1 (5%)		
#LYMPH NODE	(19)	(48)	(47)
HYPERPLASIA, LYMPHOID		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(18)	(44)	(47)
PERIVASCULITIS		1 (2%)	
#MYOCARDIUM	(18)	(44)	(47)
INFLAMMATION, CHRONIC		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
INFLAMMATION, FOCAL		23 (47%)	24 (48%)

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

@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS DELETED WHEN FOUND TO BE A FEMALE IN A MALE GROUP.

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
GRANULOMA, NOS			1 (2%)
INFARCT, NOS	1 (5%)		
HEPATOCYTOMEGALY			4 (8%)
CYTOLOGIC DEGENERATION		1 (2%)	
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL			1 (2%)
LIPOMATOSIS		1 (2%)	
#LIVER/CENTRILOBULAR	(20)	(49)	(50)
CYTOLOGIC DEGENERATION			1 (2%)
*BILE DUCT	(20)	(49)	(50)
HYPERPLASIA, NOS		2 (4%)	3 (6%)
#PANCREAS	(19)	(45)	(42)
DILATATICN/DUCTS		1 (2%)	
HEMORRHAGIC CYST		1 (2%)	
#PANCREATIC ACINUS	(19)	(45)	(42)
ATRCPHY, NOS		1 (2%)	
#IARGE INTESTINE	(18)	(49)	(48)
NEMATODIASIS	5 (28%)	6 (12%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(49)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, INTERSTITIAL		1 (2%)	1 (2%)
CALCINOSIS, NOS			1 (2%)
ENDCCRINE SYSTEM			
NONE			
REPRCDUCTIVE SYSTEM			
NONE			
NERVCUS SYSTEM			
#BRAIN	(20)	(49)	(48)
HYDROCEPHALUS, INTERNAL			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CORPORA AMYLACEA	6 (30%)	15 (31%)	17 (35%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, NECROTIZING	1		
INFLAMMATION, GRANULOMATOUS	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	4	6
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 FED N-PHENYL-p-PHENYLENEDIAMINE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(49)
CONGESTION, NOS		1 (2%)	1 (2%)
HEMORRHAGE		3 (6%)	1 (2%)
INFLAMMATION, FOCAL	1 (5%)		
INFLAMMATION, MULTIFOCAL			1 (2%)
INFLAMMATION, INTERSTITIAL			2 (4%)
PNEUMONIA, ASPIRATION		1 (2%)	
PNEUMONIA, CHRONIC MURINE	1 (5%)	2 (4%)	6 (12%)
HYPERPLASIA, ADENOMATOUS	1 (5%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(43)	(47)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (5%)		
#THYMUS	(1)	(2)	(2)
DEGENERATION, HYALINE			1 (50%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(17)	(36)	(38)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (3%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(20)	(49)	(48)
MINERALIZATION			1 (2%)
CYST, NOS			1 (2%)
INFLAMMATION, FOCAL	1 (5%)	8 (16%)	2 (4%)
#LIVER/PERIPORTAL	(20)	(49)	(48)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
#LARGE INTESTINE	(19)	(49)	(41)
NEMATODIASIS		1 (2%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(48)
NEPHROSIS, NOS		1 (2%)	
#PERIRENAL TISSUE	(20)	(50)	(48)
HEMATOMA, ORGANIZED			1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(16)	(36)	(28)
HYPERPLASIA, FOLLICULAR-CELL		1 (3%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(48)	(46)
CYST, NOS	2 (10%)	9 (19%)	1 (2%)
HEMORRHAGE			1 (2%)
INFLAMMATION, SUPPURATIVE			2 (4%)
#UTERUS/ENDOMETRIUM	(20)	(48)	(46)
CYST, NOS	7 (35%)	5 (10%)	5 (11%)
INFLAMMATION, NOS			1 (2%)
#OVARY	(18)	(38)	(38)
CYST, NOS	4 (22%)	4 (11%)	5 (13%)
NERVOUS SYSTEM			
#BRAIN	(19)	(49)	(48)
CORPORA AMYLACEA	3 (16%)	7 (14%)	4 (8%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(50)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	15	17
ANIMAL MISSING/NO NECROPSY			1
AUTO/NECROPSY/HISTO PERF		1	5

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS FED N-PHENYL-p-PHENYLENEDIAMINE IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed N-Phenyl-p-Phenylenediamine in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Subcutaneous Tissue: Fibroma[b]	0/20 (0)	0/50 (0)	3/50 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		--	Infinite
Lower Limit		--	0.250
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	80
Subcutaneous Tissue: Squamous-cell Carcinoma[b]	0/20 (0)	0/50 (0)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		--	Infinite
Lower Limit		--	0.123
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	70

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed N-Phenyl-p-Phenylenediamine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	2/20 (10)	0/50 (0)	4/49 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.032		
Relative Risk[f]		0.000	0.816
Lower Limit		0.000	0.131
Upper Limit		1.345	8.603
Weeks to First Observed Tumor	104	--	104
Hematopoietic System: Lymphoma, Leukemia, NOS, Undifferentiated Leukemia, or Lymphocytic Leukemia[b]	2/20 (10)	1/50 (2)	5/50 (10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.200	1.000
Lower Limit		0.004	0.184
Upper Limit		3.681	10.007
Weeks to First Observed Tumor	77	91	79

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed N-Phenyl-p-Phenylenediamine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma or Leukemia[b]	3/20 (15)	2/50 (4)	5/50 (10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.267	0.667
Lower Limit		0.024	0.147
Upper Limit		2.190	4.014
Weeks to First Observed Tumor	77	91	79
Pituitary: Chromophobe Adenoma[b]	0/20 (0)	3/46 (7)	3/48 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.272	0.261
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	103	104

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed N-Phenyl-p-Phenylenediamine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Pheochromocytoma[b]	1/20 (5)	2/50 (4)	3/50 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.800	1.200
Lower Limit		0.045	0.106
Upper Limit		46.273	61.724
Weeks to First Observed Tumor	104	104	84
Thyroid: C-cell Adenoma or Carcinoma[b]	0/20 (0)	2/50 (4)	3/50 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.123	0.250
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	104	104

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed N-Phenyl-p-Phenylenediamine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pancreatic Islets: Islet-cell Adenoma[b]	0/20 (0)	3/48 (6)	0/49 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.043		
Relative Risk[f]		Infinite	--
Lower Limit		0.261	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	104	--
Testis: Interstitial-cell Tumor[b]	19/20 (95)	46/50 (92)	46/49 (94)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.968	0.988
Lower Limit		0.902	0.920
Upper Limit		1.174	1.168
Weeks to First Observed Tumor	77	82	79

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed N-Phenyl-p-Phenylenediamine in the Diet[a]

(continued)

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

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

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

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

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

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

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed N-Phenyl-p-Phenylenediamine in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	0/19 (0)	0/50 (0)	3/50 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		--	Infinite
Lower Limit		--	0.238
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	104
Hematopoietic System: Lymphoma or Leukemia[b]	3/20 (15)	2/50 (4)	5/50 (10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.267	0.667
Lower Limit		0.024	0.147
Upper Limit		2.190	4.014
Weeks to First Observed Tumor	61	16	87

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed N-Phenyl-p-Phenylenediamine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma[b]	7/20 (35)	15/47 (32)	9/46 (20)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.912	0.559
Lower Limit		0.431	0.226
Upper Limit		2.295	1.553
Weeks to First Observed Tumor	104	81	104
Thyroid: C-cell Adenoma[b]	2/19 (11)	1/46 (2)	1/49 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.207	0.194
Lower Limit		0.004	0.003
Upper Limit		3.789	3.563
Weeks to First Observed Tumor	104	104	104

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed N-Phenyl-p-Phenylenediamine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	2/20 (10)	0/50 (0)	0/50 (0)
P Values[c,d]	P = 0.024(N)	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.042		
Relative Risk[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.345	1.345
Weeks to First Observed Tumor	104	--	--
Uterus: Endometrial Stromal Polyp[b]	2/20 (10)	4/49 (8)	4/50 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.816	0.800
Lower Limit		0.131	0.128
Upper Limit		8.603	8.436
Weeks to First Observed Tumor	104	104	104

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed N-Phenyl-p-Phenylenediamine in the Diet[a]

(continued)

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

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

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

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

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

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE FED N-PHENYL-p-PHENYLENEDIAMINE IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed N-Phenyl-p-Phenylenediamine in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	4/20 (20)	4/49 (8)	5/50 (10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.408	0.500
Lower Limit		0.086	0.124
Upper Limit		2.022	2.322
Weeks to First Observed Tumor	91	91	91
Hematopoietic System: Lymphoma[b]	3/20 (15)	2/49 (4)	3/50 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.272	0.400
Lower Limit		0.025	0.060
Upper Limit		2.233	2.802
Weeks to First Observed Tumor	87	91	91

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed N-Phenyl-p-Phenylenediamine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	2/20 (10)	6/49 (12)	5/50 (10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.224	1.000
Lower Limit		0.248	0.184
Upper Limit		11.802	10.007
Weeks to First Observed Tumor	91	91	82
Liver: Hepatocellular Adenoma or Carcinoma[b]	2/20 (10)	18/49 (37)	10/50 (20)
P Values[c,d]	N.S.	P = 0.022	N.S.
Departure from Linear Trend[e]	P = 0.010		
Relative Risk[f]		3.673	2.000
Lower Limit		1.019	0.488
Upper Limit		30.643	17.808
Weeks to First Observed Tumor	91	85	82

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed N-Phenyl-p-Phenylenediamie in the Diet[a]

(continued)

[a]Dosed groups received time-weighted average doses of 2,057 or 4,114 ppm.

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

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

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

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

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

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed N-Phenyl-p-Phenylenediamine in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	4/20 (20)	0/50 (0)	2/49 (4)
P values[c,d]	N.S.	P = 0.005(N)	N.S.
Departure from Linear Trend[e]	P = 0.003		
Relative Risk[f]		0.000	0.204
Lower Limit		0.000	0.020
Upper Limit		0.427	1.323
Weeks to First Observed Tumor	34	--	91
Liver: Hepatocellular Adenoma[b]	1/20 (5)	2/49 (4)	1/48 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.816	0.417
Lower Limit		0.046	0.006
Upper Limit		47.195	32.058
Weeks to First Observed Tumor	91	91	91

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed N-Phenyl-p-Phenylenediamine in the Diet[a]

(continued)

[a]Dosed groups received time-weighted average doses of 3,672 or 8,170 ppm.

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

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

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

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

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

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

March 6, 1978

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

The primary reviewer briefly described the experimental design under which N-Phenyl-p-phenylenediamine was tested and the results of the study. Although some liver tumors were found among the treated mice, they were not statistically significant when compared to the historical control animals. He agreed with the conclusion that N-Phenyl-p-phenylenediamine was not carcinogenic in rats or mice, under the conditions of test.

The secondary reviewer pointed out the higher incidence of total tumors in treated mice as compared with matched controls. He also noted the staff's conclusion that the shortened treatment period may not have been long enough to evaluate the carcinogenicity of N-Phenyl-p-phenylenediamine in the mice. The secondary reviewer recommended that the chemical be considered for retest.

The primary reviewer commented that the treated and control animals should be compared on an organ site basis, since total tumor incidence may be misleading. A discussion ensued on the differences in carcinogenicity displayed by N-Phenyl-p-phenylenediamine and its structural relatives, such as 4-Chloro-o-phenylenediamine and N-Phenyl-o-phenylenediamine.

A subgroup member questioned the general practice of considering a high incidence of liver tumors in the treated animals as not statistically significant based on a single case in which a similar increase was seen in a laboratory control group. He cited the 37% incidence of liver tumors in the treated low dose male mice as an example of such a case. Another Subgroup member added that it was important to evaluate an increased incidence with respect to the overall experience in a given laboratory. A Program staff member responded that the high control incidence of liver tumors was not unusual in this particular laboratory. He continued that the liver tumor incidence in the treated mice was within the usual control values. It was suggested that average tumor incidences be given for comparative purposes in instances of this nature.

A motion was made that the report on the bioassay of N-Phenyl-p-phenylenediamine be accepted as written. The motion was seconded and passed with Dr. Pitot, Dr. Weisburger, Dr. Shimkin, Dr. Roush, Dr. Brown, Dr. Wogan, and Mr. Garfinkel in favor and Dr. Highland, Dr. Wolfe, and Mr. Samuels opposed.

A minority opinion was voiced by Dr. Wolfe that there appeared to be a statistically significant increase in liver tumors (adenomas plus carcinomas) among the low dose male mice. He noted that benign and malignant tumors have been combined in the past for purposes of statistical analyses. Mr. Samuels said he voted against the motion because the study was too inadequate to make any judgment on the carcinogenicity of N-Phenyl-p-phenylenediamine.

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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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