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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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FOR POSSIBLE CARCINOGENICITY

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

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REPORT ON THE BIOASSAY OF 2-NITRO-p-PHENYLENEDIAMINE
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 2-nitro-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 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 2-nitro-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., 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.

Histopathologic examinations were performed by Drs. B. Cockrell (4), F. M. Garner (4), E. Georgacz (4), P. Hildebrandt (4), C. Montgomery (4), and N. J. Wosu (4) at Litton Bionetics, Inc., and reviewed by Dr. F. M. Garner (4); the pathology narratives were written by Dr. F. M. Garner (4). Dr. J. M. Ward (1) reviewed all of the slides of livers from female mice. The diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (6).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (7); the statistical analysis was performed by Mr. W. W. Belew (8,9), Mr. R. M. Helfand (8) and Dr. J. P. Dirkse, III (10), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (11).

This report was prepared at METREK, a Division of The MITRE Corporation (8) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (8), task leader Ms. P. Walker (8), senior biologist Mr. M. Morse (8), biochemist Mr. S. C. Drill (8), chemist Dr. N. Zimmerman (8), and technical editor Ms. P. A. Miller (8). 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 (1,12), Dr. S. F. Stinson (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay for the possible carcinogenicity of 2-nitro-p-phenylenediamine was conducted using Fischer 344 rats and B6C3F1 mice. 2-Nitro-p-phenylenediamine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of 2-nitro-p-phenylenediamine were, respectively, 1100 and 550 ppm for male rats, 2200 and 1100 ppm for female rats, and 4400 and 2200 ppm for mice of both sexes. The compound was administered in the diet for 78 weeks, followed by an observation period of 27 weeks for rats and 12 to 13 weeks for mice.

There were no significant positive associations between the dietary concentrations of 2-nitro-p-phenylenediamine 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. Mean body weight depression, relative to controls, was observed in dosed rats and mice of both sexes, indicating that the concentrations administered to these animals may have approximated the maximum tolerated dosages.

When the female mice in each group, having hepatocellular carcinoma or hepatocellular adenoma, were combined and the resulting incidences statistically analyzed, there was a significant positive association between concentration administered and the incidence of these tumors. This finding was supported by a significant high dose to control Fisher exact comparison. No tumors occurred in statistically significant increased incidences when dosed male or female rats or male mice were compared to their respective controls.

Under the conditions of this bioassay, dietary administration of 2-nitro-p-phenylenediamine was carcinogenic to female B6C3F1 mice, causing an increased incidence of hepatocellular neoplasms, primarily hepatocellular adenomas. There was no convincing evidence for the carcinogenicity of the compound in Fischer 344 rats or in male B6C3F1 mice.

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I. INTRODUCTION

2-Nitro-p-phenylenediamine (Figure 1) (NCI No. CO2222), a component of both semipermanent and permanent hair dye formulations, was selected for bioassay by the National Cancer Institute because of the increased incidence of bladder cancer observed among dye manufacturing industry workers (Wynder et al., 1963; Anthony and Thomas, 1970). Aromatic amines are one of several classes of organic chemicals thought to contribute to the increased cancer risk in this industry (Clayson and Garner, 1976). The widespread exposure to 2-nitro-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 this compound for testing.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 2-nitro-1,4-benzenediamine.* It is also known as diaminonitrobenzene; m-nitro-p-phenylenediamine; o-nitro-p-phenylenediamine; 2-nitro-1,4-diaminobenzene; 1,4-diamino-2-nitrobenzene; 2-NP; 2-NPPD; 2-N-p-PDA; Ursol Brown RR; Zoba Brown RR; Fourrine Brown 2R; Fourrine 36; Fouramine 2R; and C.I. (Colour Index) Oxidation Base 22 (C.I. 76070).

2-Nitro-p-phenylenediamine is a low molecular weight red dye which is able to penetrate into hair shafts; consequently, this compound is one of the most commonly used dyes in semipermanent hair colorants (Corbett and Menkart, 1973).

*The CAS registry number is 5307-14-2.

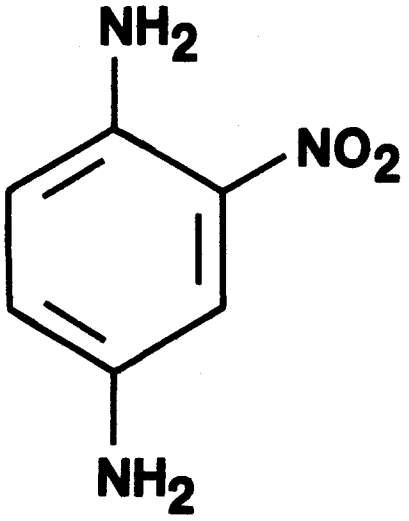

FIGURE 1
CHEMICAL STRUCTURE OF 2-NITRO-p-PHENYLENEDIAMINE

2-Nitro-p-phenylenediamine is also an ingredient in permanent hair dye formulations (Burnett et al., 1976; Markland, 1966). The active ingredients in these dyes react with each other and with hydrogen peroxide, within the hair shafts, to produce the permanent colors (Corbett and Menkart, 1973). 2-Nitro-p-phenylenediamine is used to produce light brown or reddish shades (Markland, 1966). In a similar process, 2-nitro-p-phenylenediamine is used in fur dyeing to produce a red-brown color or to add red shading when used in combination with other oxidation bases (Society of Dyers and Colourists, 1956).

Specific production data for 2-nitro-p-phenylenediamine are not available; however, this compound is produced in commercial quantities (in excess of 1000 pounds or $1000 in value annually) by two U.S. companies (Stanford Research Institute, 1977). Imports of 2-nitro-p-phenylenediamine through principal U.S. customs districts amounted to 3180 pounds in 1974 (U.S. International Trade Commission, 1976).

Exposure to 2-nitro-p-phenylenediamine via dermal contact at the scalp is unavoidable among persons whose hair is colored with dyes that contain this compound, and hairdressers who apply these dyes may also be exposed. It is estimated that 40 percent of U.S. women are regular users of hair dyes (Corbett and Menkart, 1973). Semipermanent dyes must be used more frequently than permanent dyes to maintain an artificial hair color, thus exposure to 2-nitro-p-phenylenediamine

would occur considerably more often among users of the semipermanent dyes than among users of the permanent dyes. Additionally, because the dyes in semipermanent hair colorants are not chemically altered during the dyeing process, exposure to 2-nitro-p-phenylenediamine may also occur between dyeings by leaching of the compound from the hair shafts and subsequent deposition on the hands and scalp.

A potential for exposure to 2-nitro-p-phenylenediamine also exists among workers in the chemical and dye manufacturing and fur dyeing industries.

2-Nitro-p-phenylenediamine displayed no teratogenic activity in two studies with rats and one with rabbits. Seven topical applications of 2 ml/kg of a hair dye formulation containing this compound at a concentration of 1.1 percent to 20 female Charles River CD rats during gestation produced no significant changes in the numbers of corpora lutea, implantation sites, and live fetuses over those of controls, and no differences were seen in the number of resorption sites between groups (Burnett et al., 1976). No teratologic effects were seen in two groups of 20 female CFE-S rats fed a diet incorporating either 1950 or 7800 ppm of a preparation containing 0.24 percent 2-nitro-p-phenylenediamine from day 6 through day 15 of gestation (Wernick et al., 1975). Similarly, no teratologic effects were observed in two groups of 12 female New Zealand white rabbits intubated with either 19.5 or 97.5 mg/kg/day of the same dye preparation on days 6 to 18 of gestation (Wernick et al., 1975).

2-Nitro-p-phenylenediamine was mutagenic in Salmonella typhimurium strain TA1538 (Ames et al., 1975; Searle et al., 1975) and weakly mutagenic in strain TA1537 (Searle et al., 1975), inducing frame shift reversions from a histidine requirement back to prototype. The compound was not mutagenic in S. typhimurium TA1535 and Escherichia coli WP2, WP2 uvrA, and WP2 exrA which revert by base-pair substitution (Searle et al., 1975).

In a forward mutational assay system which utilizes the thymidine kinase locus of L5178Y mouse lymphoma cells, 2-nitro-p-phenylenediamine was weakly mutagenic at concentrations of 25, 50, and 75 μg/ml (Palmer et al., 1977). However, 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). 2-Nitro-p-phenylenediamine also 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).

2-Nitro-p-phenylenediamine has been found to induce morphological transformations, or chromosomal aberrations in a variety of mammalian systems. 2-Nitro-p-phenylenediamine produced morphological transformation in mouse C3H/10T½2CL8 cells in doses from 1.53×10^{-1} mg/ml to 1.53×10^{-3} mg/ml, and produced a significant number of chromosome breaks in A(T_1)Cl-3 hamster cells in doses from 3.06×10^{-2} mg/ml to

1.53×10^{-3} mg/ml (Benedict, 1976). The compound produced a time-dependent increase in the number of chromosome aberrations following exposure of Chinese hamster prostate gland CHMP/E cells to 25 μg/ml (Kirkland and Venitt, 1976). 2-Nitro-p-phenylenediamine produced a considerable number of chromatid gaps and breaks in cultured human peripheral blood lymphocytes at concentrations between 50 μg/ml and 100 μg/ml (Searle et al., 1975).

II. MATERIALS AND METHODS

A. Chemicals

Commercial-grade 2-nitro-p-phenylenediamine was obtained from Ashland Chemical Company, Columbus, Ohio. Chemical analysis was performed by Litton Bionetics, Inc., Kensington, Maryland. The experimentally determined melting point range was 138° to 139°C. No literature value was found for comparison. Thin-layer chromatography was performed utilizing two solvent systems (i.e., diethyl ether:ethyl acetate:acetic acid and diethyl ether:acetic acid:hexane). Each plate was visualized with ultraviolet and visible light, iodine vapor and ferric chloride-potassium ferricyanide spray. In each case, only one spot was revealed. The results of infrared and nuclear magnetic resonance analyses were consistent with those expected on the basis of the structure of the compound. Ultraviolet/visible analysis revealed λ_{max} at 240 and 470 nm with respective molar extinction coefficients of 2.21×10^4 and 0.51×10^4.

Throughout this report, the term 2-nitro-p-phenylenediamine is used to represent this commercial-grade material.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® meal (Allied Mills, Inc., Chicago, Illinois). 2-Nitro-p-phenylenediamine was administered to the dosed animals as a component of the diet.

The chemical was removed from its container and a proper amount was blended with an aliquot of the ground 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 550 and 2200 ppm of 2-nitro-p-phenylenediamine were analyzed spectrophotometrically. The mean result immediately after preparation was 98 percent of theoretical (ranging from 95 to 100 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. All rats were supplied by A. R. Schmidt, Madison, Wisconsin, and Laboratory Supply Company, Inc., Indianapolis, Indiana. All mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

Rats and mice were approximately 4 weeks old when received. Upon receipt, animals were examined for visible signs of disease or parasites and 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

All animals were housed by species in temperature- and humidity-controlled rooms. The temperature range was 22° to 26°C and the relative humidity was maintained between 45 and 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.).

All rats were housed four per cage by sex and all 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 were provided twice weekly. Ab-sorb-dri® hardwood chip bedding (Wilner Wood Products Company, Norway, Maine) was used in polycarbonate cages for the entire bioassay.

Acidulated water (pH 2.5) was supplied to animals in water bottles filled by an automated metering device that was checked daily for diluting accuracy. Water bottles were changed and washed twice

weekly, and 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* 3-chloro-p-toluidine (95-74-9); 5-chloro-o-toluidine (95-74-4); and nitrofen (1836-75-5).

All dosed and control mice were housed in a room with mice receiving diets containing Michler's ketone (90-94-8); 4,4'-methylene-bis(N,N-dimethyl)benzenamine (101-61-1); p-chloroaniline (106-47-8); 5-chloro-o-toluidine (95-79-4); N-phenyl-p-phenylenediamine hydrochloride (2198-59-6); 1-phenyl-2-thiourea (103-85-5); trimethylthiourea (2489-77-2); dibutyltin diacetate (1067-33-0); and 3-chloro-p-toluidine (95-74-9).

E. Selection of Initial Concentrations

To establish the maximum tolerated concentrations of 2-nitro-p-phenylenediamine for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Rats were distributed among six groups, each consisting of five males and five females. 2-Nitro-p-phenylenediamine was incorporated into the basal laboratory diet and supplied ad libitum to five of the six rat groups in concentrations of 315, 680, 1465,

*CAS registry numbers are given in parentheses.

3155 and 6800 ppm. The remaining rat group served as a control group, receiving only the basal laboratory diet.

Mice were distributed among ten groups, each consisting of five males and five females. 2-Nitro-p-phenylenediamine was incorporated into the basal laboratory diet and supplied ad libitum to eight of the ten mouse groups in concentrations of 810, 1180, 1740, 2550, 3750, 5550, 8080, and 11,830 ppm. The two remaining mouse groups served as control groups, receiving only the basal laboratory diet.

The dosed dietary preparations were administered for a period of 4 weeks, followed by a 2-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 study all survivors were sacrificed and necropsied.

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

ppm	Mean Body Weight Gain (%)*		Survival	
	Males	Females	Males	Females
0	--	--	5/5	5/5
315	-23	-7	5/5	5/5
680	-13	-8	5/5	5/5
1465	-35	-9	5/5	5/5
3155	-37	-13	5/5	5/5
6800	-52	-24	4/5	5/5

*+ is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.

No abnormal clinical signs were recorded for any rat group. The high concentrations selected for administration to dosed rats in the chronic bioassay were 1100 and 2200 ppm for males and females, respectively.

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

	Mean Body Weight Gain (%)*		Survival	
ppm	Males	Females	Males	Females
0	--	--	5/5	5/5
810	+5	+1	5/5	5/5
1,180	+9	+19	5/5	5/5
1,740	+8	+6	5/5	5/5
2,550	0	+7	5/5	5/5
3,750	+4	+10	5/5	5/5
5,550	-2	+8	5/5	5/5
8,080	0	+5	5/5	5/5
11,830	+3	+6	2/5	2/5

No abnormal clinical signs were recorded for any mouse group. The high concentration selected for administration to dosed mice in the chronic bioassay was 4400 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 simultaneously. The dietary

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* + is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
2-NITRO-p-PHENYLENEDIAMINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2-NITRO-p-PHENYLENEDIAMINE CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	105
LOW DOSE	50	550	78	
		0		27
HIGH DOSE	50	1100	78	
		0		27
FEMALE				
CONTROL	20	0	0	105
LOW DOSE	50	1100	78	
		0		27
HIGH DOSE	50	2200	78	
		0		27

[a]Concentrations given in parts per million.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
2-NITRO-p-PHENYLENEDIAMINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2-NITRO-p-PHENYLENEDIAMINE CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	90
LOW DOSE	50	2200	78	
		0		12
HIGH DOSE	50	4400	78	
		0		12
FEMALE				
CONTROL	20	0	0	90
LOW DOSE	50	2200	78	
		0		12
HIGH DOSE	50	4400	78	
		0		13

[a]Concentrations given in parts per million.

concentrations of 2-nitro-p-phenylenediamine administered to male rats were 1100 and 550 ppm. Throughout this report those male 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. The dietary concentrations of 2-nitro-p-phenylenediamine administered to female rats were 2200 and 1100 ppm. Throughout this report those female 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. Dosed rats were supplied with feed containing 2-nitro-p-phenylenediamine for 78 weeks followed by a 27-week observation period.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test simultaneously. The dietary concentrations of 2-nitro-p-phenylenediamine administered were 4400 and 2200 ppm. 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. Dosed mice were supplied with feed containing 2-nitro-p-phenylenediamine for 78 weeks followed by a 12- to 13-week observation period.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment and body weights were recorded once a week for the first 6 weeks, every 2 weeks for the next 12 weeks, and at monthly intervals

thereafter. All animals were inspected twice daily for mortality. 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 by carbon dioxide asphyxiation, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of 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, pancreatic islets, 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

Dose-related mean body weight depression was apparent in male rats from week 12 until week 87. Female rats evidenced distinct and consistent dose-related mean body weight depression throughout the bioassay (Figure 2).

No other abnormal clinical signs were recorded.

B. Survival

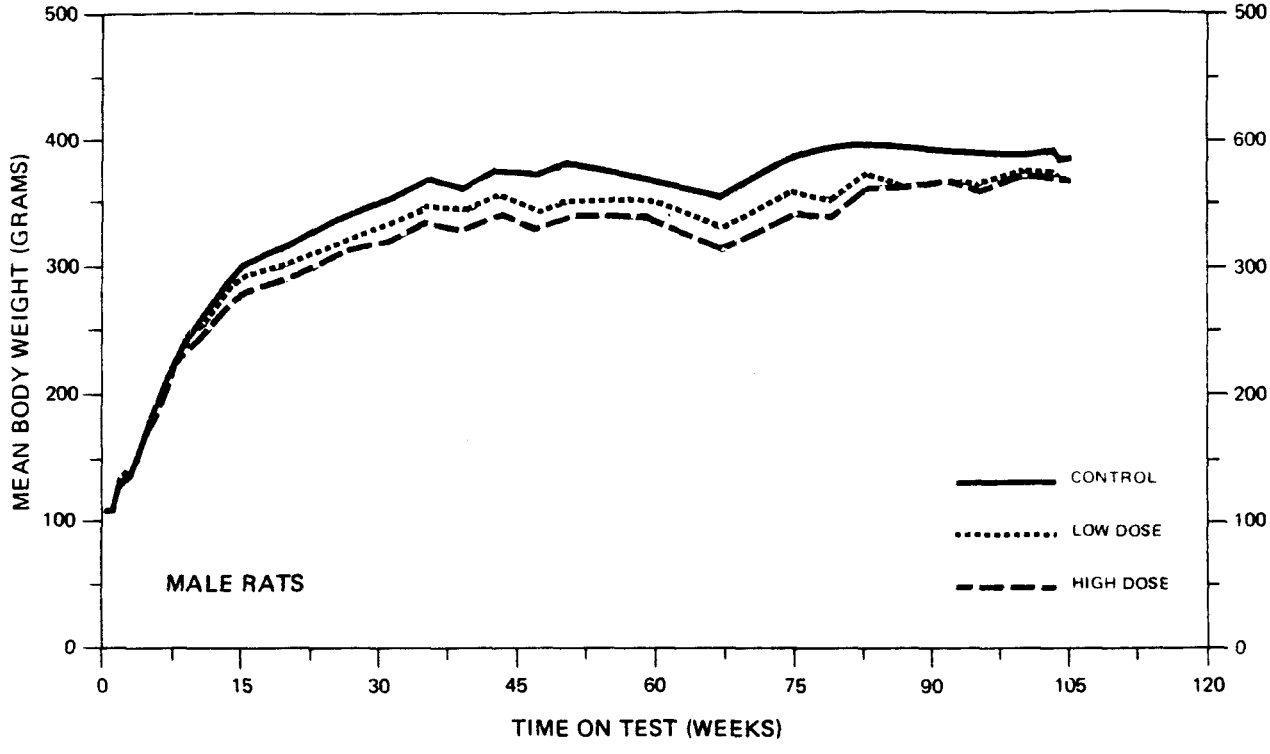
The estimated probabilities of survival for male and female rats in the control and 2-nitro-p-phenylenediamine-dosed groups are shown in Figure 3. The Tarone test for association between dosage and mortality was not significant for either males or females.

There were adequate numbers of male rats at risk from late-developing tumors as 94 percent (47/50) of the high dose, 92 percent (46/50) of the low dose, and 80 percent (16/20) of the controls survived on test until the termination of the study.

For females 76 percent (38/50) of the high dose, 90 percent (45/50) of the low dose, and 90 percent (18/20) of the controls survived on test until the termination of the study. Thus, there were adequate numbers of female rats 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).



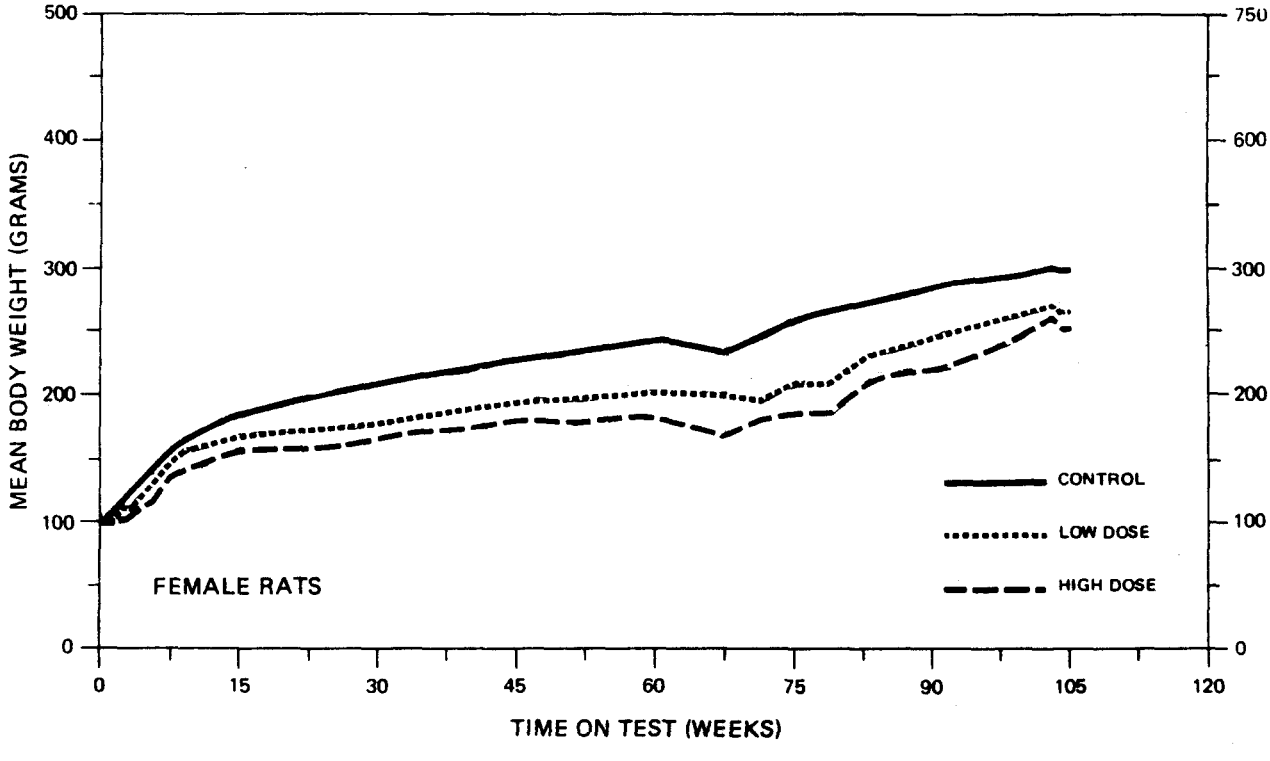
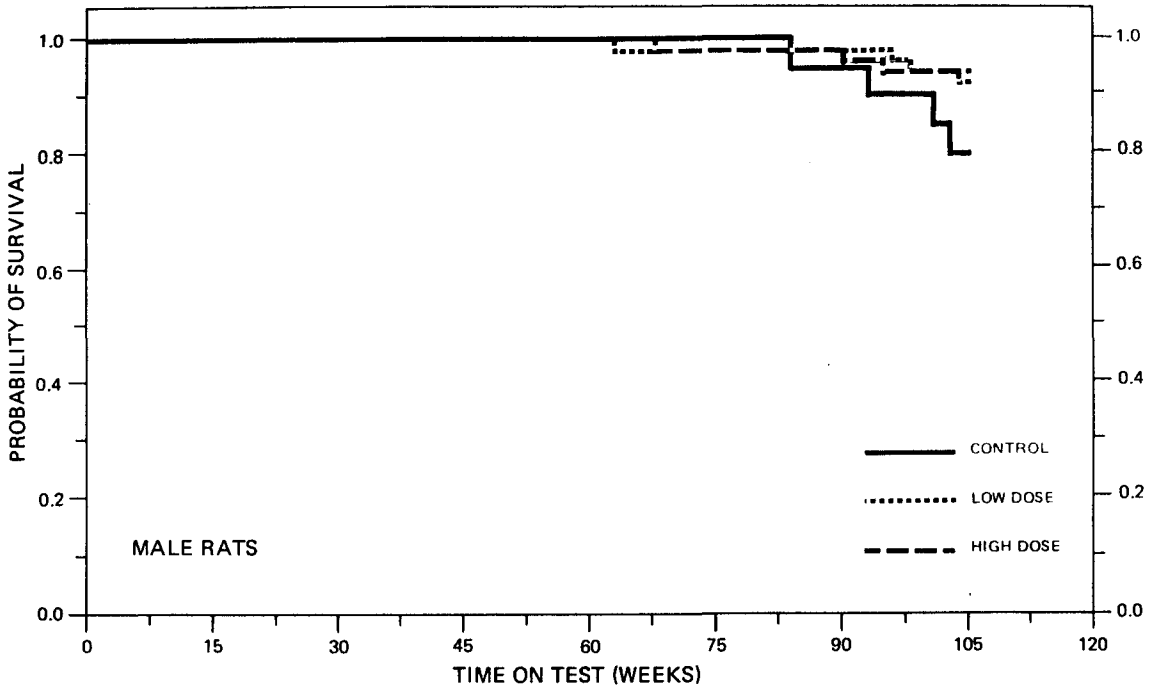


FIGURE 2
GROWTH CURVES FOR 2-NITRO-p-PHENYLENEDIAMINE CHRONIC STUDY RATS



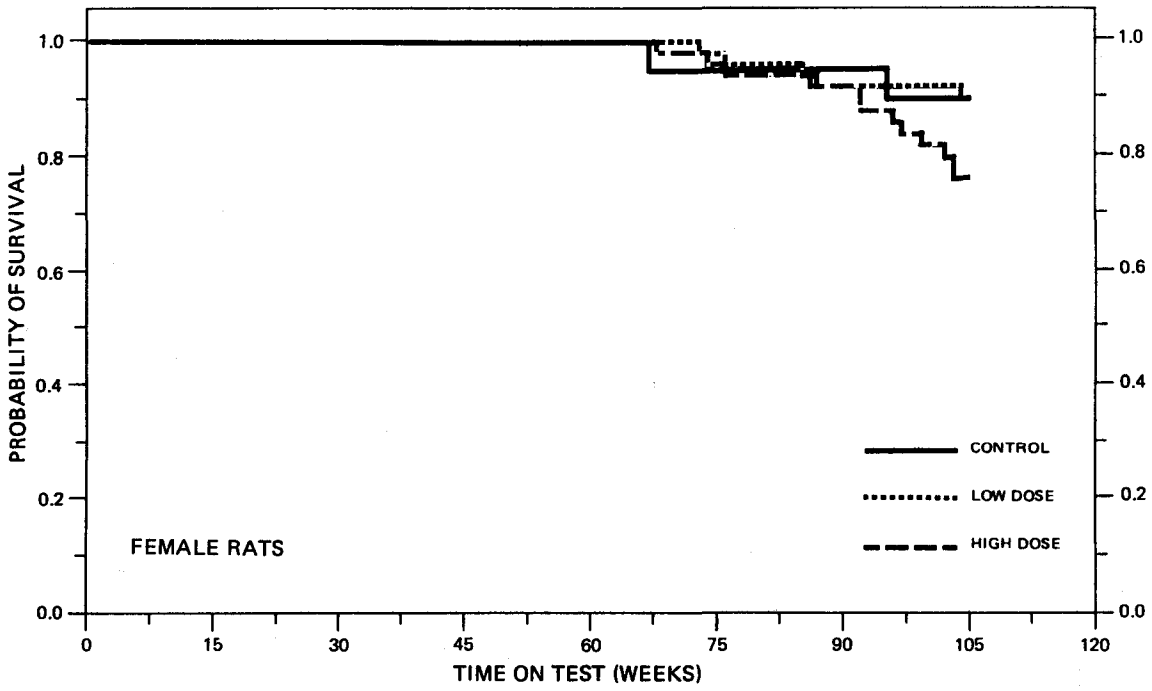


FIGURE 3
SURVIVAL COMPARISONS OF 2-NITRO-p-PHENYLENEDIAMINE CHRONIC STUDY RATS

A variety of neoplasms was seen in both control and dosed rats. Each type of tumor represented had been encountered previously as a spontaneous lesion in rats. There was also a variety of nonneoplastic lesions in both control and dosed animals. Such lesions have been encountered previously as spontaneous occurrences in laboratory rats and are considered to represent spontaneous lesions in these animals.

Based on the results of this pathologic examination, 2-nitro-p-phenylenediamine 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 2-nitro-p-phenylenediamine-dosed groups and where such tumors were observed in at least 5 percent of the group.

For male rats, the Cochran-Armitage test indicated a significant ($P = 0.017$) positive association between dose and the combined incidence of C-cell carcinomas or C-cell adenomas of the thyroid. However, the Fisher exact tests comparing high dose to control and low dose to control were not significant. Similarly, for female rats the Cochran-Armitage test indicated a significant ($P = 0.039$) positive association between dose and the combined incidence of leukemia or

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 2-NITRO-p-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	3/20(0.15)	1/50(0.02)	0/50(0.00)
P Values[c]	P = 0.007(N)	N.S.	P = 0.021(N)
Relative Risk (Control)[d]	---	0.133	0.000
Lower Limit	---	0.003	0.000
Upper Limit	---	1.568	0.659
Weeks to First Observed Tumor	101	96	---
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	0/20(0.00)	1/45(0.02)	6/43(0.14)
P Values[c]	P = 0.017	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.025	0.776
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	96	68
Pancreatic Islets: Islet-Cell Adenoma[b]	3/20(0.15)	7/50(0.14)	2/42(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.933	0.317
Lower Limit	---	0.245	0.029
Upper Limit	---	5.215	2.590
Weeks to First Observed Tumor	105	105	90

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Testis: Interstitial-Cell Tumor[b]	16/20(0.80)	47/50(0.94)	47/50(0.94)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.175	1.175
Lower Limit	---	0.953	0.953
Upper Limit	---	1.430	1.430
Weeks to First Observed Tumor	101	96	90

[a]Treated groups received doses of 550 or 1100 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 2-NITRO-p-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	0/20(0.00)	0/50(0.00)	4/50(0.08)
P Values[c]	P = 0.039	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.386
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	97
Pituitary: Chromophobe Adenoma[b]	2/19(0.11)	10/49(0.20)	5/44(0.11)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.939	1.080
Lower Limit	---	0.476	0.200
Upper Limit	---	17.231	10.742
Weeks to First Observed Tumor	105	105	105
Uterus: Endometrial Stromal Polyp[b]	6/20(0.30)	0/48(0.00)	1/48(0.02)
P Values[c]	P = 0.001(N)	P < 0.001(N)	P = 0.002(N)
Departure from Linear Trend[e]	P < 0.001	---	---
Relative Risk (Control)[d]	---	0.000	0.069
Lower Limit	---	0.000	0.002
Upper Limit	---	0.256	0.526
Weeks to First Observed Tumor	105	---	105

TABLE 4 (CONCLUDED)

[a]Treated groups received doses of 1100 or 2200 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 $P < 0.05$.

malignant lymphomas but again the Fisher exact tests were not significant.

No other statistical test for any site in either male or female rats indicated a significant positive association between dosage and tumor incidence. Thus, based upon these results there was no conclusive evidence that 2-nitro-p-phenylenediamine was a carcinogen in Fischer 344 rats under the conditions of this bioassay.

For male rats there was the possibility of a negative association between dose and the combined incidence of leukemia or malignant lymphoma as the Cochran-Armitage test and the Fisher exact tests indicated significant negative results. For females, the possibility of a negative association between dose and endometrial stromal polyps was noted with significant negative results from the Fisher exact tests and the Cochran-Armitage test. However, the control incidence may have been high since the historical incidence of endometrial stromal polyps in female Fischer 344 control rats from this same laboratory for the NCI Carcinogenesis Testing Program was 9 percent (29/319) as compared with 30 percent (6/20) in 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 many 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

many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by 2-nitro-p-phenylenediamine that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

Distinct and consistent dose-related mean body weight depression was apparent in both male and female mice throughout the bioassay (Figure 4).

No other abnormal clinical signs were recorded.

B. Survival

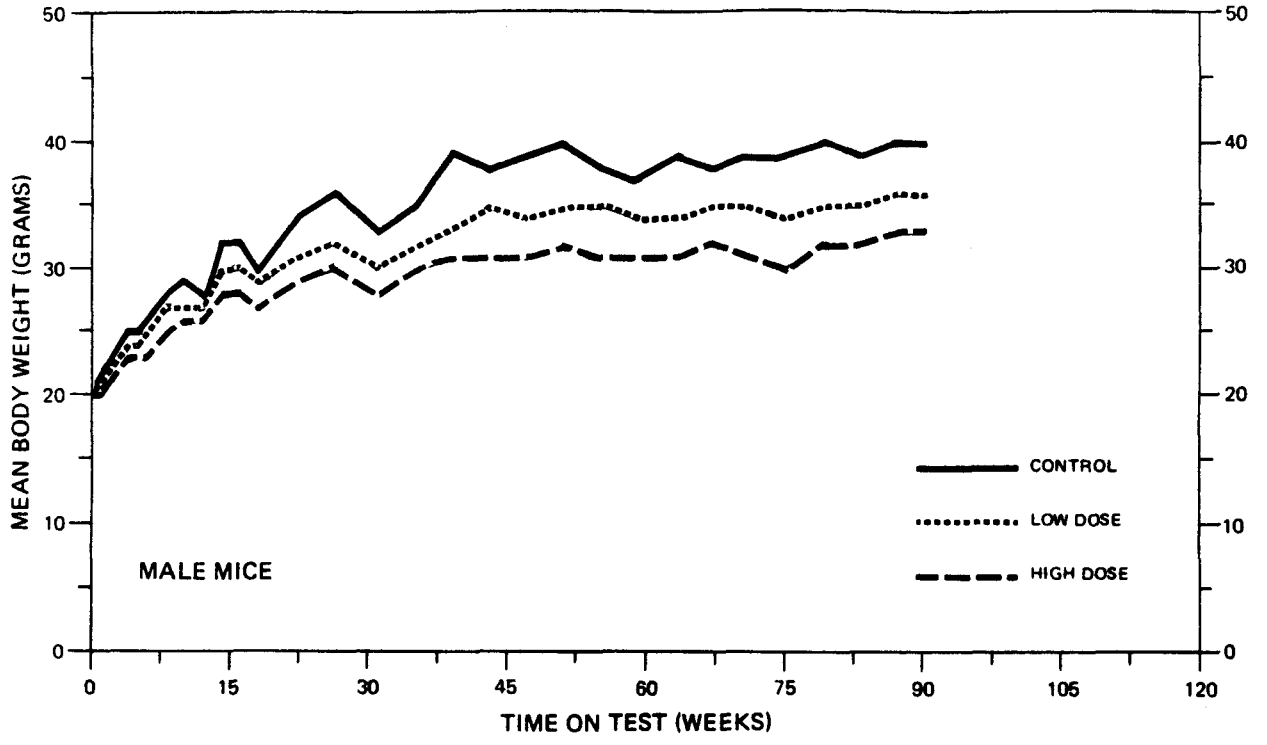
The estimated probabilities of survival for male and female mice in the control and 2-nitro-p-phenylenediamine-dosed groups are shown in Figure 5. The Tarone test for association between dosage and mortality was not significant for either male or female mice.

There were adequate numbers of male mice at risk from late-developing tumors, as 98 percent (49/50) of the high dose, 92 percent (46/50) of the low dose and 90 percent (18/20) of the controls survived on test until termination of the study.

There were adequate numbers of female mice at risk from late-developing tumors. Eighty-six percent (43/50) of the high dose, 90 percent (45/50) of the low dose and 100 percent (20/20) of the controls survived on test until the termination of the study.

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).



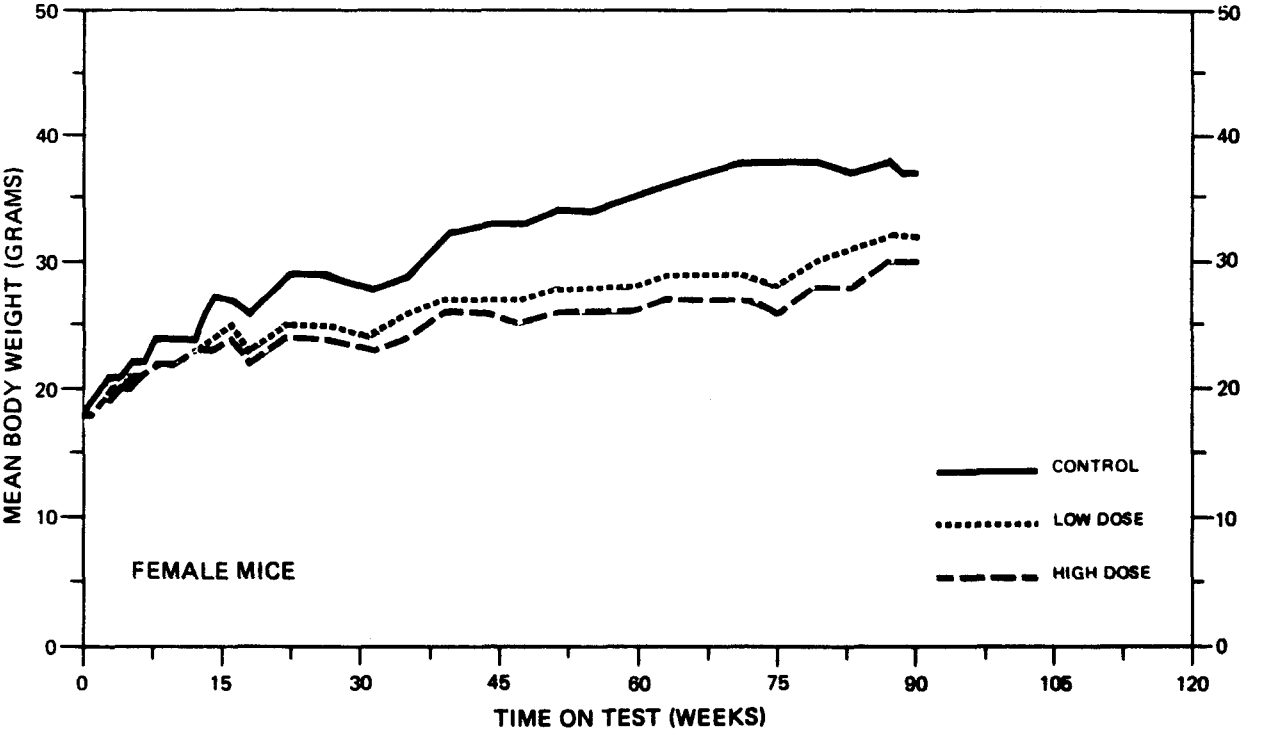
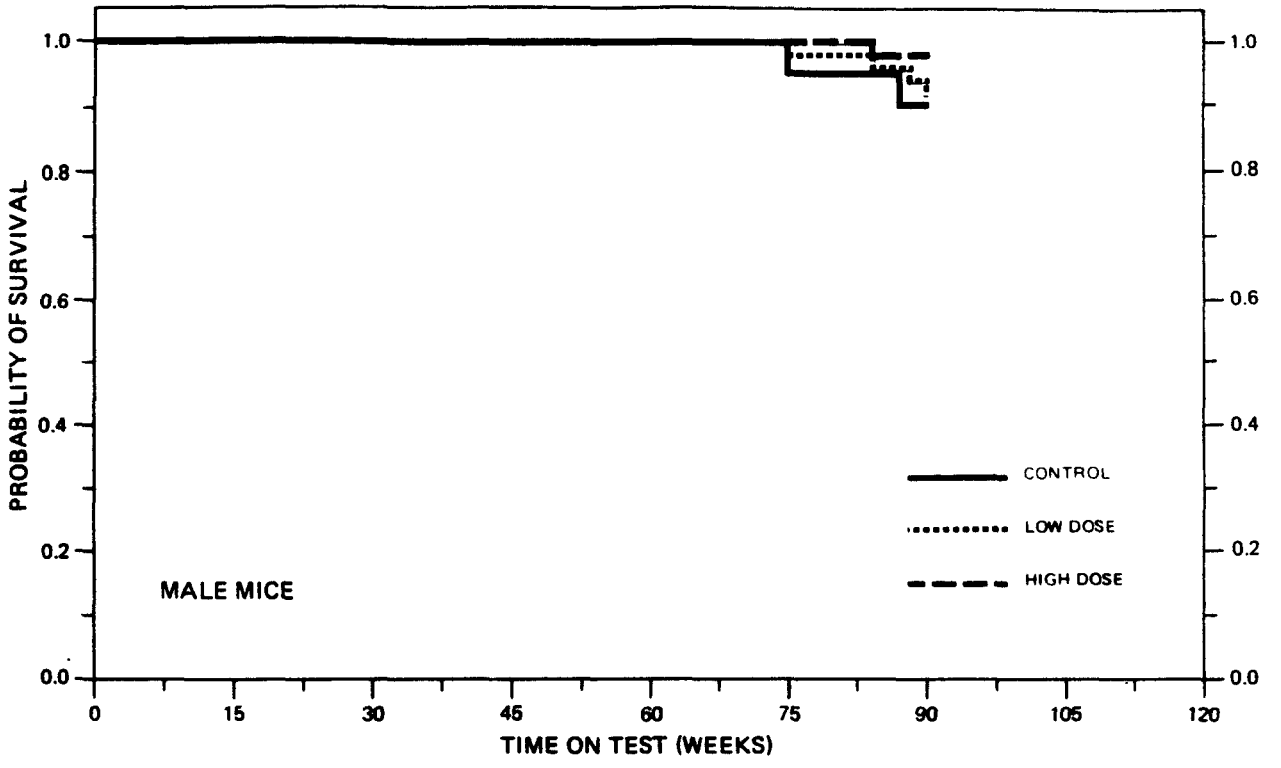


FIGURE 4
GROWTH CURVES FOR 2-NITRO-p-PHENYLENEDIAMINE CHRONIC STUDY MICE



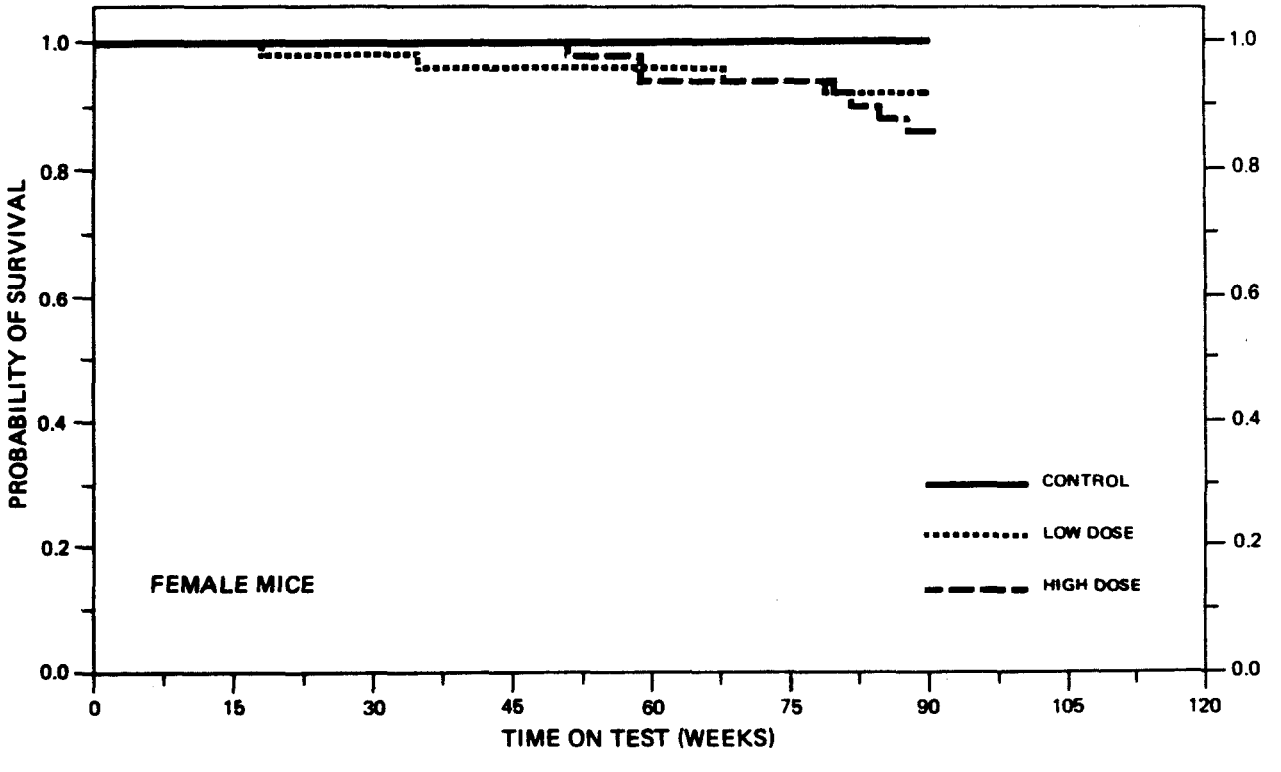


FIGURE 5
SURVIVAL COMPARISONS OF 2-NITRO-p-PHENYLENEDIAMINE CHRONIC STUDY MICE

Hepatocellular adenoma and hepatocellular carcinoma occurred in a dose-related distribution in female mice. The incidence of these liver tumors is shown below:

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Number of Animals with Livers Examined Histopathologically	(20)	(50)	(47)	(20)	(49)	(48)
Hepatocellular Adenoma	3	6	2	1	10	14
Hepatocellular Carcinoma	0	1	1	0	0	3

Liver tumors in the control and dosed males were of the usual types and in the usual incidences seen in aging B6C3F1 mice. The adenoma in the control female mouse was composed of small basophilic hepatocytes. Adenomas in dosed mice were composed of large eosinophilic hepatocytes forming solid patterns. Nuclear pleomorphism was prominent, and an occasional mitotic figure was seen. Carcinomas were composed of eosinophilic or basophilic cells forming trabecular patterns. Multiple tumors were seen in several dosed mice.

In addition to the hepatic adenomas and carcinomas, foci of cellular alteration occurred in 3/49 (6 percent) low dose and 3/48 (6 percent) high dose nontumor-bearing female mice. The foci contained large eosinophilic hepatocytes similar to those in adenomas.

A variety of nonneoplastic lesions was present in both control and dosed animals. Such lesions have been encountered previously in laboratory mice. They are considered to represent spontaneous lesions in these animals.

A generalized intracellular deposition of a golden-brown pigment was observed in the dosed animals of both species and sex. However, it seemed to be inert and no lesion was attributed to its presence.

This pathologic examination provided evidence for an association between the administration of 2-nitro-p-phenylenediamine and increased incidences of liver neoplasms in female mice.

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 2-nitro-p-phenylenediamine-dosed groups and where such tumors were observed in at least 5 percent of the group.

For female mice the combined incidences of hepatocellular carcinomas or hepatocellular adenomas were significant ($P = 0.005$) when comparing the dosed groups to the controls using the Cochran-Armitage test. These Cochran-Armitage test results were supported by a significant ($P = 0.007$) Fisher exact test result for the comparison of high dose to control. Historical data for untreated control female B6C3F1 mice compiled by this laboratory for the NCI Carcinogenesis Testing Program indicate that 9/319 (3 percent) of the females had liver neoplasms as compared with the 1/20 (5 percent) incidence in the control group of this study. Based upon these statistical results the administration of 2-nitro-p-phenylenediamine was associated with the increased combined incidence of hepatocellular

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH 2-NITRO-p-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	1/20(0.05)	8/50(0.16)	2/49(0.04)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.041	---	---
Relative Risk (Control)[d]	---	3.200	0.816
Lower Limit	---	0.482	0.046
Upper Limit	---	138.771	47.195
Weeks to First Observed Tumor	90	90	90
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	2/20(0.10)	3/50(0.06)	2/50(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.600	0.400
Lower Limit	---	0.076	0.032
Upper Limit	---	6.860	5.277
Weeks to First Observed Tumor	75	75	90
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	3/20(0.15)	7/50(0.14)	3/47(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.933	0.426
Lower Limit	---	0.245	0.063
Upper Limit	---	5.215	2.974
Weeks to First Observed Tumor	90	90	84

TABLE 5 (CONCLUDED)

[a]Treated groups received doses of 2200 or 4400 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 $P < 0.05$.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 2-NITRO-p-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	0/20(0.00)	5/47(0.11)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.559	0.255
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	90	90
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	2/20(0.10)	4/49(0.08)	6/50(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.816	1.200
Lower Limit	---	0.131	0.243
Upper Limit	---	8.603	11.574
Weeks to First Observed Tumor	90	90	80
Liver: Hepatocellular Carcinoma[b]	0/20(0.00)	0/49(0.00)	3/48(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.261
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	90

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	1/20(0.05)	10/49(0.20)	17/48(0.35)
P Values[c]	P = 0.005	N.S.	P = 0.007
Relative Risk (Control)[d]	---	4.082	7.083
Lower Limit	---	0.655	1.264
Upper Limit	---	172.772	286.807
Weeks to First Observed Tumor	90	90	90

[a]Treated groups received doses of 2200 or 4400 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.

carcinomas or hepatocellular adenomas under the conditions of this bioassay.

No statistical tests for tumor incidence at any site in male mice were significant.

V. DISCUSSION

There were no significant positive associations between the dietary concentration of 2-nitro-p-phenylenediamine 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. Mean body weight depression, relative to controls, was observed in dosed rats and mice of both sexes, indicating that the concentrations administered to these animals may have approximated the maximum tolerated dosages.

Among male rats there was a significant positive association between dosage and the combined incidences of thyroid tumors (i.e., C-cell carcinoma and C-cell adenoma), while among female rats there was a significant positive association between dosage and the combined incidences of leukemia and malignant lyphoma. There were no significant Fisher exact comparisons that supported these findings. No other statistical tests for the incidence of tumors at any site in male or female rats were positive and significant.

When the female mice in each group, having hepatocellular carcinoma or hepatocellular adenoma, were combined and the resulting incidences statistically analyzed, there was a significant positive association between concentration administered and the incidence of these tumors. This finding was supported by a significant high dose to control Fisher exact comparison. In addition, the historical data for untreated control female B6C3F1 mice compiled by this laboratory

for the NCI Carcinogenesis Testing Program indicate that only 9/319 (3 percent) of the females had liver neoplasms. No other statistical tests for tumor incidence at any site in male or female mice were positive and significant.

Under the conditions of this bioassay, dietary administration of 2-nitro-p-phenylenediamine was carcinogenic to female B6C3F1 mice, causing an increased incidence of hepatocellular neoplasms, primarily hepatocellular adenomas. There was no convincing evidence for the carcinogenicity of the compound in Fischer 344 rats or in male B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 2-NITRO-p-PHENYLENEDIAMINE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH 2-NITRO-p-PHENYLENEDIAMINE

	CONTROL (UNTR) 11-1105	LOW DOSE 11-1103	HIGH DOSE 11-1101
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA			1 (2%)
FIBROSARCOMA	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	2 (10%)		
LEUKEMIA,NOS	1 (5%)	1 (2%)	
#SPLEEN	(20)	(50)	(40)
SARCOMA, NOS			1 (3%)
FIBROMA			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(39)
NEOPLASTIC NODULE	1 (5%)		
#PANCREAS	(20)	(50)	(42)
ACINAR-CELL CARCINOMA			1 (2%)

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

TABLE A1 (CONTINUED)

	CONTROL (WATER) 11-1105	LOW DOSE 11-1103	HIGH DOSE 11-1101
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
LIPOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(48)	(48)
CHROMOPHOBE ADENOMA		2 (4%)	
#ADRENAL	(20)	(49)	(49)
PHEOCHROMOCYTOMA	1 (5%)	2 (4%)	
#THYROID	(20)	(45)	(43)
FOLLICULAR-CELL CARCINOMA	1 (5%)	2 (4%)	
C-CELL ADENOMA		1 (2%)	4 (9%)
C-CELL CARCINOMA			2 (5%)
#PANCREATIC ISLETS	(20)	(50)	(42)
ISLET-CELL ADENOMA	3 (15%)	7 (14%)	2 (5%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
FIBROADENOMA			1 (2%)
*PREPUTIAL GLAND	(20)	(50)	(50)
ADENOMA, NOS	1 (5%)		
#TESTIS	(20)	(50)	(50)
INTERSTITIAL-CELL TUMOR	16 (80%)	47 (94%)	47 (94%)
NERVOUS SYSTEM			
#BRAIN	(19)	(50)	(49)
GLIOMA, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 11-1105	LOW DOSE 11-1103	HIGH DOSE 11-1101
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	3	2
MORIBUND SACRIFICE	2	1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	46	47
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	19	48	49
TOTAL PRIMARY TUMORS	27	65	61
TOTAL ANIMALS WITH BENIGN TUMORS	17	48	49
TOTAL BENIGN TUMORS	21	61	57
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	3	4
TOTAL MALIGNANT TUMORS	5	4	4
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH 2-NITRO-p-PHENYLENEDIAMINE

	CONTROL (UNTR) 11-1106	LOW DOSE 11-1104	HIGH DOSE 11-1102
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%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA			2 (4%)
PAPILLARY ADENOCARCINOMA, METAST			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			2 (4%)
LEUKEMIA,NOS			1 (2%)
#KIDNEY	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(46)	(44)
ADENOMA, NOS			1 (2%)
#LIVER	(20)	(48)	(47)
HEPATOCELLULAR ADENOMA			1 (2%)

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1106	LOW DOSE 11-1104	HIGH DOSE 11-1102
NEOPLASTIC NODULE	1 (5%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(49)	(44)
CHROMOPHOBE ADENOMA	2 (11%)	10 (20%)	5 (11%)
#THYROID	(15)	(47)	(45)
FOLLICULAR-CELL ADENOMA			1 (2%)
C-CELL ADENOMA	1 (7%)	1 (2%)	2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
PAPILLARY ADENOMA	1 (5%)		
FIBROADENOMA		2 (4%)	
#UTERUS	(20)	(48)	(48)
ADENOMA, NOS	1 (5%)		
ENDOMETRIAL STROMAL POLYP	6 (30%)		1 (2%)
#OVARY	(20)	(48)	(48)
PAPILLARY ADENOCARCINOMA			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(49)
GLIOMA, NOS			1 (2%)
SPECIAL SENSE ORGANS			
*EAR CANAL	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 11-1106	LOW DOSE 11-1104	HIGH DOSE 11-1102
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	4	5
MORIBUND SACRIFICE	1	1	7
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	45	38
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	12	17
TOTAL PRIMARY TUMORS	12	13	21
TOTAL ANIMALS WITH BENIGN TUMORS	10	12	9
TOTAL BENIGN TUMORS	11	13	13
TOTAL ANIMALS WITH MALIGNANT TUMORS			8
TOTAL MALIGNANT TUMORS			8
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 2-NITRO-p-PHENYLENEDIAMINE**

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH 2-NITRO-p-PHENYLENEDIAMINE

	CONTROL (UNTR) 22-2105	LOW DOSE 22-2103	HIGH DOSE 22-2101
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	8 (16%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
LEUKEMIA, NOS	1 (5%)	2 (4%)	
LYMPHOCYTIC LEUKEMIA	1 (5%)		
#LYMPH NODE	(15)	(39)	(44)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#SMALL INTESTINE	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(47)
HEPATOCELLULAR ADENOMA	3 (15%)	6 (12%)	2 (4%)
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 22-2105	LOW DOSE 22-2103	HIGH DOSE 22-2101
HEMANGIOSARCOMA	1 (5%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(14)	(21)	(16)
FOLLICULAR-CELL ADENOMA	1 (7%)		
C-CELL CARCINOMA	1 (7%)		
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 B1 (CONCLUDED)

	CONTROL (UNTR) 22-2105	LOW DOSE 22-2103	HIGH DOSE 22-2101
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	3	1
MORIBUND SACRIFICE		1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	46	49
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	17	7
TOTAL PRIMARY TUMORS	9	18	7
TOTAL ANIMALS WITH BENIGN TUMORS	5	13	4
TOTAL BENIGN TUMORS	5	14	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	4	3
TOTAL MALIGNANT TUMORS	4	4	3
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 B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH 2-NITRO-p-PHENYLENEDIAMINE

	CONTROL (UNTR) 22-2106	LOW DOSE 22-2104	HIGH DOSE 22-2102
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(47)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA		4 (9%)	3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)	3 (6%)	5 (10%)
LEUKEMIA,NOS			1 (2%)
#SPLEEN	(19)	(43)	(45)
HEMANGIOSARCOMA			1 (2%)
#LYMPH NODE	(16)	(38)	(41)
MALIGNANT LYMPHOMA, NOS	1 (6%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(48)
HEPATOCELLULAR ADENOMA	1 (5%)	10 (20%)	14 (29%)

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

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2106	LOW DOSE 22-2104	HIGH DOSE 22-2102
HEPATOCELLULAR CARCINOMA			3 (6%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(17)	(36)	(36)
FOLLICULAR-CELL ADENOMA	1 (6%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(48)	(47)
LEIOMYOMA		1 (2%)	
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 (CONCLUDED)

	CONTROL (UNTR) 22-2106	LOW DOSE 22-2104	HIGH DOSE 22-2102
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@		4	7
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	20	45	43
ANIMAL MISSING		1	
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	18	27
TOTAL PRIMARY TUMORS	4	20	27
TOTAL ANIMALS WITH BENIGN TUMORS	2	13	17
TOTAL BENIGN TUMORS	2	15	17
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	5	10
TOTAL MALIGNANT TUMORS	2	5	10
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			

@ INCLUDES AUTOLYZED ANIMALS

* 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 2-NITRO-p-PHENYLENEDIAMINE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH 2-NITRO-p-PHENYLENEDIAMINE

	CONTROL (UNTR) 11-1105	LOW DOSE 11-1103	HIGH DOSE 11-1101
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
INFLAMMATION, INTERSTITIAL	1 (5%)		
BRONCHOPNEUMONIA ACUTE SUPPURATI			1 (2%)
ABSCESS, NOS	2 (10%)		2 (4%)
PNEUMONIA, CHRONIC MURINE	8 (40%)	26 (52%)	22 (44%)
ABSCESS, CHRONIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(50)	(40)
PIGMENTATION, NOS			1 (3%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(50)	(49)
INFLAMMATION, FOCAL	1 (5%)	1 (2%)	1 (2%)
INFLAMMATION, DIFFUSE	1 (5%)		
FIBROSIS	6 (30%)	7 (14%)	2 (4%)
DEGENERATION, NOS	1 (5%)		
*CORONARY ARTERY	(20)	(50)	(50)
HYPERTROPHY, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(39)
HEPATITIS, TOXIC	1 (5%)		

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-1105	LOW DOSE 11-1103	HIGH DOSE 11-1101
METAMORPHOSIS FATTY		3 (6%)	2 (5%)
#PANCREATIC ACINUS	(20)	(50)	(42)
ATROPHY, NOS		5 (10%)	5 (12%)
#SMALL INTESTINE	(20)	(50)	(50)
HYPERPLASIA, LYMPHOID	1 (5%)	3 (6%)	6 (12%)
#COLON	(20)	(50)	(50)
NEMATODIASIS	1 (5%)	10 (20%)	9 (18%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, CHRONIC	14 (70%)	41 (84%)	37 (74%)
NEPHROPATHY, TOXIC	1 (5%)		1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL	(20)	(49)	(49)
HEMORRHAGIC CYST		2 (4%)	
PIGMENTATION, NOS			1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	
#ADRENAL CORTEX	(20)	(49)	(49)
LIPOIDOSIS			1 (2%)
HYPERPLASIA, NODULAR			1 (2%)
HYPERPLASIA, NOS		2 (4%)	
#ADRENAL MEDULLA	(20)	(49)	(49)
HEMORRHAGIC CYST		2 (4%)	
HYPERPLASIA, NOS	1 (5%)		
#THYROID	(20)	(45)	(43)
PIGMENTATION, NOS		2 (4%)	21 (49%)
HYPERPLASIA, C-CELL	1 (5%)	4 (9%)	2 (5%)
HYPERPLASIA, FOLLICULAR-CELL			1 (2%)
REPRODUCTIVE SYSTEM			
#PROSTATE	(18)	(49)	(44)
DILATATION, NOS			2 (5%)

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 11-1105	LOW DOSE 11-1103	HIGH DOSE 11-1101
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, ACUTE FOCAL			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, CYSTIC	1 (6%)	1 (2%)	
#TESTIS	(20)	(50)	(50)
GRANULOMA, SPERMATIC		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
*MESENTERY	(20)	(50)	(50)
PERIARTERITIS	1 (5%)	1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
PIGMENTATION, NOS		1 (2%)	3 (6%)
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 TREATED WITH 2-NITRO-p-PHENYLENEDIAMINE

	CONTROL (UNTR) 11-1106	LOW DOSE 11-1104	HIGH DOSE 11-1102
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
ABSCESS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
PNEUMONIA, CHRONIC MURINE	13 (65%)	31 (62%)	27 (54%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(17)	(41)	(38)
MYELOFIBROSIS		1 (2%)	
HYPERPLASIA, GRANULOCYTIC			1 (3%)
HYPERPLASIA, RETICULUM CELL		1 (2%)	
#SPLEEN	(20)	(48)	(47)
HEMATOPOIESIS		1 (2%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(19)	(50)	(47)
INFLAMMATION, FOCAL		1 (2%)	
FIBROSIS		3 (6%)	2 (4%)
FIBROSIS, FOCAL	1 (5%)		
DEGENERATION, NOS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(47)
INFLAMMATION, GRANULOMATOUS			2 (4%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1106	LOW DOSE 11-1104	HIGH DOSE 11-1102
GRANULOMA, NOS			1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
NECROSIS, NOS			1 (2%)
METAMORPHOSIS FATTY			1 (2%)
#PANCREAS	(20)	(48)	(46)
GRANULOMA, NOS		1 (2%)	
#PANCREATIC ACINUS	(20)	(48)	(46)
ATROPHY, NOS	2 (10%)	1 (2%)	2 (4%)
#STOMACH	(19)	(48)	(49)
INFLAMMATION, CHRONIC			1 (2%)
PERIARTERITIS			1 (2%)
#SMALL INTESTINE	(20)	(49)	(50)
ABSCESS, NOS			1 (2%)
PERFORATION, INFLAMMATORY			1 (2%)
HYPERPLASIA, LYMPHOID			2 (4%)
#LARGE INTESTINE	(20)	(47)	(50)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
#COLON	(20)	(47)	(50)
NEMATODIASIS	2 (10%)	14 (30%)	8 (16%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
INFLAMMATION, CHRONIC	6 (30%)	15 (31%)	11 (22%)
CALCINOSIS, NOS		2 (4%)	2 (4%)
PIGMENTATION, NOS		1 (2%)	
#URINARY BLADDER	(19)	(46)	(44)
HEMORRHAGE	1 (5%)		
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(49)	(44)
CYST, NOS	1 (5%)	2 (4%)	1 (2%)
HEMORRHAGIC CYST	2 (11%)		
#ADRENAL	(19)	(49)	(50)
LIPOIDOSIS		1 (2%)	

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1106	LOW DOSE 11-1104	HIGH DOSE 11-1102
PIGMENTATION, NOS		2 (4%)	
#THYROID	(15)	(47)	(45)
PIGMENTATION, NOS		5 (11%)	
HYPERPLASIA, C-CELL	1 (7%)	2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(48)	(48)
HYDROMETRA	1 (5%)	1 (2%)	
INFLAMMATION, NOS	2 (10%)	3 (6%)	4 (8%)
PYOMETRA	5 (25%)	2 (4%)	2 (4%)
#UTERUS/ENDOMETRIUM	(20)	(48)	(48)
CYST, NOS			1 (2%)
INFLAMMATION, NOS	1 (5%)		
INFLAMMATION, FOCAL	1 (5%)		
INFLAMMATION, SUPPURATIVE		1 (2%)	2 (4%)
HYPERPLASIA, NOS	1 (5%)		1 (2%)
HYPERPLASIA, CYSTIC			1 (2%)
#OVARY	(20)	(48)	(48)
CYST, NOS	3 (15%)	3 (6%)	1 (2%)
PARAVARIAN CYST			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(49)
MINERALIZATION		1 (2%)	
ABSCESS, NOS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*EPICARDIUM	(20)	(50)	(50)
HEMORRHAGIC CYST			1 (2%)

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

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 11-1106	LOW DOSE 11-1104	HIGH DOSE 11-1102
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
PIGMENTATION, NOS		10 (20%)	32 (64%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	2	1
AUTO/NECROPSY/HISTO PERF			1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 2-NITRO-p-PHENYLENEDIAMINE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH 2-NITRO-p-PHENYLENEDIAMINE

	CONTROL (UNTR) 22-2105	LOW DOSE 22-2103	HIGH DOSE 22-2101
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(40)	(45)
HYPERPLASIA, LYMPHOID		1 (3%)	
#LYMPH NODE	(15)	(39)	(44)
HYPERPLASIA, LYMPHOID		1 (3%)	2 (5%)
#MESENTERIC L. NODE	(15)	(39)	(44)
HYPERPLASIA, LYMPHOID			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(47)
INFLAMMATION, ACUTE FOCAL	1 (5%)	1 (2%)	
ABSCESS, NOS			1 (2%)
NECROSIS, FOCAL		3 (6%)	
PIGMENTATION, NOS		3 (6%)	

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2105	LOW DOSE 22-2103	HIGH DOSE 22-2101
ANGIECTASIS		1 (2%)	1 (2%)
#LIVER/PERIPORTAL	(20)	(50)	(47)
INFLAMMATION, FOCAL		1 (2%)	
#STOMACH	(20)	(48)	(49)
INFLAMMATION, ACUTE			1 (2%)
#SMALL INTESTINE	(20)	(50)	(49)
INFLAMMATION, ACUTE			1 (2%)
HYPERPLASIA, LYMPHOID			2 (4%)
#PEYERS PATCH	(20)	(50)	(49)
HYPERPLASIA, NOS			1 (2%)
#COLON	(20)	(49)	(48)
NEMATODIASIS		3 (6%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(47)
INFLAMMATION, CHRONIC		2 (4%)	2 (4%)
CALCINOSIS, NOS			2 (4%)
METAPLASIA, OSSEOUS		1 (2%)	
#URINARY BLADDER	(18)	(44)	(46)
CALCULUS, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#THYROID	(14)	(41)	(46)
PIGMENTATION, NOS		14 (34%)	5 (11%)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(20)	(50)	(50)
MULTIPLE CYSTS	1 (5%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 22-2105	LOW DOSE 22-2103	HIGH DOSE 22-2101
MUSCULOSKELETAL SYSTEM			
*STERNUM	(20)	(50)	(50)
ANGIECTASIS		1 (2%)	
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
MULTIPLE SITES			
PIGMENTATION, NOS		1	
*MULTIPLE ORGANS	(20)	(50)	(50)
AMYLOIDOSIS	1 (5%)		1 (2%)
PIGMENTATION, NOS		14 (28%)	39 (78%)
HEMOSIDEROSIS			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	9	11	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 2-NITRO-p-PHENYLENEDIAMINE

	CONTROL (UNTR) 22-2106	LOW DOSE 22-2104	HIGH DOSE 22-2102
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(49)	(50)
HEMATOMA, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(47)	(49)
BRONCHOPNEUMONIA SUPPURATIVE		1 (2%)	
PNEUMONIA, CHRONIC MURINE	1 (5%)		3 (6%)
HYPERPLASIA, ADENOMATOUS			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(42)	(39)
MYELOFIBROSIS		1 (2%)	
#SPLEEN	(19)	(43)	(45)
PIGMENTATION, NOS		1 (2%)	2 (4%)
HYPERPLASIA, NODULAR	1 (5%)		
HYPERPLASIA, LYMPHOID	2 (11%)	2 (5%)	2 (4%)
#LYMPH NODE	(16)	(38)	(41)
HYPERPLASIA, LYMPHOID	2 (13%)	3 (8%)	1 (2%)
#MESENTERIC L. NODE	(16)	(38)	(41)
HYPERPLASIA, LYMPHOID		1 (3%)	
CIRCULATORY SYSTEM			
#HEART	(19)	(47)	(48)
DEGENERATION, NOS		2 (4%)	

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

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2106	LOW DOSE 22-2104	HIGH DOSE 22-2102
#MYOCARDIUM	(19)	(47)	(48)
INFLAMMATION, FOCAL		1 (2%)	
DEGENERATION, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(48)
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, ACUTE FOCAL	3 (15%)	2 (4%)	3 (6%)
INFLAMMATION, CHRONIC FOCAL	1 (5%)		
NECROSIS, FOCAL		1 (2%)	1 (2%)
METAMORPHOSIS FATTY		1 (2%)	
PIGMENTATION, NOS		1 (2%)	
FOCAL CELLULAR CHANGE		3 (6%)	3 (6%)
HYPERPLASIA, NODULAR		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (5%)		1 (2%)
#BILE DUCT	(20)	(49)	(48)
HYPERPLASIA, NOS		1 (2%)	
#PANCREAS	(20)	(45)	(46)
CYST, NOS			1 (2%)
CYSTIC DUCTS		1 (2%)	
ATROPHY, NOS		1 (2%)	
#SMALL INTESTINE	(20)	(49)	(44)
HYPERPLASIA, LYMPHOID		1 (2%)	
#COLON	(20)	(47)	(43)
NEMATODIASIS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(48)
INFLAMMATION, CHRONIC	1 (5%)	2 (4%)	3 (6%)
ENDOCRINE SYSTEM			
#ADRENAL	(15)	(36)	(33)
NECROSIS, NOS	1 (7%)		

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2106	LOW DOSE 22-2104	HIGH DOSE 22-2102
METAMORPHOSIS FATTY	1 (7%)		
#THYROID	(17)	(36)	(36)
PIGMENTATION, NOS		4 (11%)	9 (25%)
#THYROID FOLLICLE	(17)	(36)	(36)
PIGMENTATION, NOS		1 (3%)	
#PANCREATIC ISLETS	(20)	(45)	(46)
HYPERPLASIA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(48)	(47)
HYDROMETRA			1 (2%)
CYST, NOS			1 (2%)
INFLAMMATION, ACUTE		1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(48)	(47)
CYST, NOS	7 (35%)	14 (29%)	7 (15%)
INFLAMMATION, NOS	2 (10%)	1 (2%)	3 (6%)
INFLAMMATION, ACUTE	1 (5%)	4 (8%)	
INFLAMMATION, ACUTE SUPPURATIVE	1 (5%)		
#OVARY	(20)	(48)	(45)
FOLLICULAR CYST, NOS	2 (10%)	5 (10%)	1 (2%)
PAROVARIAN CYST			2 (4%)
INFLAMMATION, NOS	1 (5%)		
NERVOUS SYSTEM			
#BRAIN/MENINGES	(20)	(48)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 22-2106	LOW DOSE 22-2104	HIGH DOSE 22-2102
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(49)	(50)
PIGMENTATION, NOS		16 (33%)	24 (48%)
HYPERPLASIA, LYMPHOID		2 (4%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	5	2
ANIMAL MISSING/NO NECROPSY		1	
AUTO/NECROPSY/HISTO PERF		2	1
AUTO/NECROPSY/NO HISTO			1

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

Review of the Bioassay of 2-Nitro-p-Phenylenediamine* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

August 31, 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 2-Nitro-p-Phenylenediamine for carcinogenicity.

A representative of Clairol presented a public statement regarding the bioassay of 2-Nitro-p-Phenylenediamine. He noted that the compound has been commonly used in hair dyes since the early 1900's. He said that the only significant finding in the bioassay was an increased incidence of hepatomas among treated female mice and that early animal mortality was not associated with the tumors. The Clairol representative questioned if the metabolism of 4'-(Chloroacetyl)-Acetanilide would be the same when given orally, as in the bioassay, as when applied as a hair dye product in humans. He noted that the solubility of the material differs considerably in aqueous and acidic systems. He suggested that absorption of the compound would be facilitated in the body because of the acidity of the GI tract. He estimated that the dose used in the high dose group of mice was a 150,000- fold exaggeration of human exposure. He recommended that this fact be considered in interpreting the human risk posed by 2-Nitro-p-Phenylenediamine.

The primary reviewer said that the bioassay data indicated a positive association between the induction of liver tumors and treatment in female mice. After a brief description of the experimental design,

the primary reviewer said that the study was deficient in that the number of control mice used was too small. He said, however, that the shortcoming did not affect the interpretation of the results. Based on the bioassay and mutagenicity findings in Salmonella, he concluded that the compound may pose a risk to humans.

The secondary reviewer questioned the relevance of the route of exposure for assessing the human risk of 2-Nitro-p-Phenylenediamine. She noted that the histopathological description of the liver tumors indicated that they were composed of large eosinophilic hepatocytes and she wondered if this characteristic was sufficiently unusual as to add to the significance of the tumors. The secondary reviewer said that the questionable relevance of the route of exposure prevented a statement regarding the potential human risk posed by 2-Nitro-p-Phenylenediamine. She added that the mutagenicity data was of questionable significance because of the weak positive response observed.

A Program staff pathologist said that the liver tumors in treated female mice were morphologically different from ones observed in control animals. He added that the treatment relatedness of the liver tumors could be based on an increased incidence and morphological difference as compared to controls.

A Subgroup member questioned the significance of the results, given that a positive finding was observed only in female mice. He suggested that a true positive would not have been sex-linked. One Clearinghouse member noted that, in his experience, several nitrosamines induce tumors in one sex of rats and none in the opposite sex. Although hormonal imbalance may be a factor, its influence is generally unknown.

In reference to the appropriateness of the route of exposure, a Clearinghouse member commented that the accepted practice for testing compounds for carcinogenicity is to expose animals to the largest doses possible that are compatible with survival. It, therefore, is legitimate to use the oral route to increase the exposure level, even though humans may be primarily exposed through the skin. He noted that one reason for using high dose levels is to overcome the statistical insensitivity of the bioassay, resulting from the use of relatively small numbers of animals. Despite the difference in exposure routes, he said that the compound must be considered to pose some possible risk to humans. Since 2-Nitro-p-Phenylenediamine is an aromatic amine, another Clearinghouse member agreed that some statement is necessary regarding the possible human risk posed by the compound.

The secondary reviewer moved that the report on the bioassay of 2-Nitro-p-Phenylenediamine be accepted as written and that no statement be made assessing the human risk of the compound. The motion was seconded. In further discussion, she argued that the term "carcinogen" should not be used in this instance since 2-Nitro-p-Phenylenediamine induced primarily hepatocellular adenomas. She predicted that the hepatocellular carcinomas would not be statistically significant if they were evaluated independent of the adenomas. She also emphasized that the response was observed in only one sex and species. Based on these considerations, the secondary reviewer contended that no statement could be made regarding the human risk posed by 2-Nitro-p-Phenylenediamine. Another Subgroup member argued that it was appropriate to combine adenomas and carcinomas in evaluating the results. He considered the results significant and the study adequate and, therefore, he suggested that the compound could pose a possible human risk. One Clearinghouse member said that similar compounds have been demonstrated to be absorbed through the skin into the body fluids and excreted in the urine. After a lengthy discussion regarding various issues at contention, a Program staff member noted that a conclusionary statement had been inadvertently omitted from the report. He said that a statement should have been included that 2-Nitro-p-Phenylenediamine was considered to be carcinogenic for the female mouse. Based on the revised conclusion, the secondary reviewer withdrew her motion.

A Program staff member said that the conclusion in the report should have read: "Under the conditions of the bioassay, dietary administration of 2-Nitro-p-Phenylenediamine was carcinogenic to the female B6C3F1 mice, causing an increased incidence of hepatocellular neoplasms." He noted that the increased incidence was statistically significant at the high dose level by the Fischer Exact Test and that the conclusion was supported by a trend analysis and a comparison of the liver tumor incidence with historical control data. A Program staff pathologist said that it was appropriate to combine the benign and malignant liver tumors, since the adenomas are considered to be part of a spectrum leading to the carcinomas. He noted that this conclusion was based on experimental evidence, including transplantation studies. It was recommended that a statement be added to the report indicating that the adenomas were considered to be premalignant lesions.

It was moved that the report on the bioassay of 2-Nitro-p-Phenylenediamine be accepted with the modification provided by the Program staff member. It was further moved that the compound be considered to pose a potential human risk. The motion was seconded and approved with two abstentions.

Members present were:

Arnold Brown, University of Wisconsin School of Medicine
Joseph Highland, Environmental Defense Fund
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center

* 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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