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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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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 4-NITRO-o-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 4-nitro-o-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 4-nitro-o-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. Chemical analysis was performed by Midwest Research Institute (6) and the analytical results were reviewed by Dr. N. Zimmerman (7).

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

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (9); the statistical analysis was performed by Mr. R. M. Helfand (7) 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 (7) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (7), task leader Ms. P. Walker (7), senior biologist Mr. M. Morse (7), biochemist Mr. S. C. Drill (7), and technical editor Ms. P. A. Miller (7). The final report was reviewed by members of the participating organizations.

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

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SUMMARY

A bioassay for the possible carcinogenicity of 4-nitro-o-phenylenediamine was conducted using Fischer 344 rats and B6C3F1 mice. 4-Nitro-o-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 4-nitro-o-phenylenediamine were, respectively, 75 0 and 375 ppm for rats and 7500 and 3750 ppm for mice. The compound was administered for 103 weeks to rats and for 102 weeks to mice. The period of compound administration was followed by an observation period of 2 weeks for rats and mice.

There were no significant positive associations between the concentrations of 4-nitro-o-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. Distinct dose-related mean body weight depression was observed in mice, indicating that the concentrations of 4-nitro-o-phenylenediamine administered to these animals in this bioassay may have approximated the maximum tolerated concentrations, Since no distinct mean body weight depression relative to controls, no significantly accelerated mortality, and no other manifestations of chronic toxicity were associated with administration of 4-nitro-o-phenylenediamine to male or female rats, it is possible that these animals may have been able to tolerate a higher dietary concentration.

None of the statistical tests for any site in rats or in mice of either sex indicated a significant positive association between compound administration and tumor incidence.

Under the conditions of this bioassay, dietary administration of 4-nitro-o-phenylenediamine was not carcinogenic in Fischer 344 rats or B6C3F1 mice.

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I. INTRODUCTION

4-Nitro-o-phenylenediamine (Figure 1) (NCI No. C03941), a component of both semipermanent and permanent hair dye formulations, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer reported among workers in the dye manufacturing industry (Wynder et al., 1963; Anthony and Thomas, 1970). Occupational exposure to several classes of chemicals, including aromatic nitro- and amino- compounds, is thought to contribute to the increased cancer risk in this industry (Clayson and Garner, 1976). The widespread exposure to 4-nitro-o-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 4-nitro-1,2-benzenediamine.* It is also called 4-nitro-1,2-phenylenediamine; 4-nitro-1,2-diaminobenzene; 1,2-diamino-4-nitrobenzene; 2-amino-4-nitroaniline; 4-NO; 4-NOP; 4-NOPD; 4-N-o-PDA; and C.I. (Colour Index) 76020.

4-Nitro-o-phenylenediamine is a low molecular weight yellow dye which is capable of penetrating into the cortex of a hair shaft; as such, it is one of the most commonly used dyes in semipermanent hair colorants (Corbett and Menkart, 1973).

*The CAS registry number is 99-56-9.

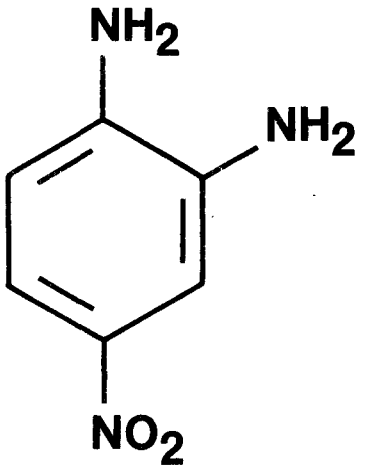


FIGURE 1
CHEMICAL STRUCTURE OF 4-NITRO-o-PHENYLENEDIAMINE

4-Nitro-o-phenylenediamine is also an ingredient in permanent hair dye formulations (Burnett et al., 1976; Markland, 1966). The active ingredients in these hair colorants react with each other and with hydrogen peroxide, within the hair shafts, to produce the permanent colors (Corbett and Menkart, 1973). 4-Nitro-o-phenylenediamine is used to produce reddish shades (Markland, 1966). In a similar process, 4-nitro-o-phenylenediamine is used as an oxidation base for fur dyeing (Society of Dyers and Colourists, 1956).

4-Nitro-o-phenylenediamine dihydrochloride (C.I. Oxidation Base 9A) is also used in fur dyeing and may be used in some permanent hair dye formulations in place of the free base (Society of Dyers and Colourists, 1956).

4-Nitro-o-phenylenediamine is used as a reagent for the detection and determination of -keto acids (e.g., pyruvic acid and -ketoglutaric acid) in blood and urine (Hockenhull and Floodgate, 1952; Taylor and Smith, 1955).

Specific production data for 4-nitro-o-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).

Exposure to 4-nitro-o-phenylenediamine via dermal contact 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

hair dye users (Corbett and Menkart, 1973). As semipermanent dyes must be used more often than permanent ones to maintain an artificial hair color, exposure to 4-nitro-o-phenylenediamine would occur with considerably greater frequency among users of the former type of dye. Additionally, because the ingredients of semipermanent dyes are not chemically altered during the dyeing process, exposure to 4-nitro-o-phenylenediamine may also occur between dyeings by leaching of the compound from the hair and subsequent deposition on the hands and scalp. Exposure to this compound may also result from unreacted or nonabsorbed portions of hair dyes reaching rivers and streams via domestic wastewater.

A potential for exposure to 4-nitro-o-phenylenediamine also exists among workers in the chemical and dye manufacturing, and fur dyeing industries, and among laboratory personnel.

4-Nitro-o-phenylenediamine showed no teratogenic activity in two studies with rats and one with rabbits. Seven topical applications of 2 ml/kg of a preparation containing the compound at a concentration of 0.25 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 in the number of resorption sites between groups (Burnett et al., 1976). The compound demonstrated no teratogenicity in two groups of 20 female CFE-S rats fed a diet including either 1950 or

7800 ppm of a hair dye preparation containing 0.16 percent 4-nitro-o-phenylenediamine from day 6 through day 15 of gestation (Wernick et al., 1975). Similarly, no teratogenic 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 dyė preparation on days 6 to 18 of gestation (Wernick et al., 1975).

4-Nitro-o-phenylenediamine was mutagenic in Salmonella typhimurium strain TA 1538 (Ames et al., 1975; Searle et al., 1975; Mohn and de Serres, 1976) and weakly mutagenic in strain TA 1537 (Searle et al., 1975), inducing frame shift reversions from a histidine requirement back to prototype. The compound was not mutagenic in S. typhimurium TA 1535 and Escherichia coli WP2, WP2 uvrA, and WP2exrA which revert by base-pair substitution (Searle et al., 1975), but was mutagenic in E. coli 343/113 (Mohn and deSerres, 1976). In a recessive lethal assay using Drosophila melanogaster, 4-nitro-o-phenylenediamine demonstrated weak mutagenic activity (Blijleven, 1977).

4-Nitro-o-phenylenediamine has been found to be mutagenic or to produce morphological transformation or chromosomal aberrations in a variety of mammalian systems. In a forward mutational assay system which utilizes the thymidine kinase locus of L5178Y mouse lymphoma cells, 4-nitro-o-phenylenediamine was weakly mutagenic at concentrations of 50, 100, and 200 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). 4-Nitro-o-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).

4-Nitro-o-phenylenediamine produced morphological transformation in mouse C3H/10T½CL8 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 4.59×10^{-2} mg/ml to 1.53×10^{-3} mg/ml (Benedict, 1976). The compound also 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 in cultured human peripheral blood lymphocytes (Searle et al., 1975).

II. MATERIALS AND METHODS

A. Chemicals

4-Nitro-o-phenylenediamine was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. Chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri. According to the manufacturer, the chemical was 98.07 percent 4-nitro-o-phenylenediamine with a total iron concentration of 15 ppm and a range in melting point of 203° to 205°C.

Nonaqueous titration of the amine function with perchloric acid revealed a purity of 98.6 $\pm$ 0.3 percent. Thin-layer chromatography was performed utilizing two solvent systems (i.e., acetone:benzene:ammonium hydroxide and ethyl acetate:ammonium hydroxide). Each plate, visualized with 254 and 367 nm light and furfural, revealed one major spot and one trace impurity. Vapor-phase chromatography revealed one homogeneous peak. The results of elemental analysis were within 2 percent of that expected on the basis of the molecular formula of 4-nitro-o-phenylenediamine, $C_6H_7N_3O_2$. The experimentally determined melting point range was 204° to 205°C. The results of infrared analysis were consistent with those reported in the technical literature (Sadtler Standard Spectra). Although no values were found for comparison, the results of nuclear magnetic resonance analysis were consistent with the structure of the compound. Ultraviolet/visible analysis revealed λ_{max} at 274 and 408 nm with molar extinction coefficients of approximately 76 x 10^2 and 92.7 x 10^2,

respectively. These were compared with literature values (Corbett, 1967) of λ_{max} at 226, 275, and 408 nm with molar extinction coefficients of (shoulder), 74 x 10^2, and 79 x 10^2, respectively.

Throughout this report, the term 4-nitro-o-phenylenediamine is used to represent this 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). 4-Nitro-o-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 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 7500 and 375 ppm of 4-nitro-o-phenylenediamine were analyzed spectrophotometrically. The mean result immediately after preparation was 104 percent of theoretical (ranging from 102 to 107 percent). After 10 days at ambient room temperature, the mean result was 91 percent of theoretical (ranging from 79 to 98 percent).

C. Animals

The two animal species, Fischer 344 rats and B6C3F1 mice, used in the carcinogenicity bioassay were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Rats and mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

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

D. Animal Maintenance

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

Rats were housed four per cage by sex and mice were housed five per cage by sex. Throughout the study dosed and control animals of both species were housed in polycarbonate cages (Lab Products, Inc.,

Garfield, New Jersey) suspended from aluminum racks. Racks were fitted with a continuous piece of stainless steel mesh over which a sheet of filter paper was firmly secured. Filter paper was changed at 2-week intervals, when the racks were sanitized. Clean cages and bedding (Ab-sorb-dri® hardwood chip bedding [Wilner Wood Products Company, Norway, Maine]) were provided twice weekly.

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

Dosed and control rats were housed in a room with other rats receiving diets containing* N,N'-diethylthiourea (105-55-5) and 1-phenyl-3-methyl-5-pyrazolone (89-25-8); and with other rats intubated with 3-(chloromethyl)pyridine hydrochloride (3099-31-8).

Dosed and control mice were housed in a room with mice receiving diets containing 4'-(chloroacetyl)acetanilide (140-49-8); nithiazide (139-94-6); 1-phenyl-3-methyl-5-pyrazolone (89-25-8); 2,4-dimethoxyaniline hydrochloride (54150-69-5); and p-phenylenediamine dihydrochloride (624-18-0); and other mice intubated with trimethylphosphate (512-56-1); 2-(chloromethyl)pyridine hydrocholoride (6959-47-3);

*CAS registry numbers are given in parentheses.

3-(chloromethyl)pyridine hydrochloride (3099-31-8); and pivalolactone (1955-45-9).

E. Selection of Initial Concentrations

To establish the concentrations of 4-nitro-o-phenylenediamine for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among ten groups, each consisting of five males and five females. 4-Nitro-o-phenylenediamine was incorporated into the basal laboratory diet and supplied ad libitum to eight of the ten rat groups in concentrations of 681, 1000, 1430, 2160, 3150, 4600, 6800 and 10,000 ppm and to eight of the ten mouse groups in concentrations of 1470, 2160, 3150, 4600, 6800, 10,000, 14,700 and 21,500 ppm. The two remaining groups of each species served as control groups, receiving only the basal laboratory diet.

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

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

RAT SUBCHRONIC STUDY RESULTS

	Mean Body Weight Gain (%)*		Survival**		Observation of Arched Backs and Rough Coats**	
ppm	Males	Females	Males	Females	Males	Females
10,000	-77	-30	4/5	5/5	5/5	5/5
6,800	-54	- 9	5/5	5/5	5/5	5/5
4,600	-38	-12	5/5	5/5	0/5	0/5
3,150	-20	- 3	5/5	5/5	0/5	0/5
2,160	-20	- 8	5/5	5/5	0/5	0/5
1,430	-18	-12	5/5	5/5	0/5	0/5
1,000	-17	-12	5/5	5/5	0/5	0/5
681	-11	+ 1	5/5	5/5	0/5	0/5
0	—	—	5/5	5/5	0/5	0/5

The high concentration selected for administration to dosed rats in the chronic bioassay was 750 ppm.

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

MOUSE SUBCHRONIC STUDY RESULTS

	Mean Body Weight Gain (%)*		Survival**		Observation of Orange-Colored Fur**	
ppm	Males	Females	Males	Females	Males	Females
21,500	-25	-13	5/5	5/5	5/5	5/5
14,700	-23	-13	5/5	5/5	5/5	5/5
10,000	-12	-16	5/5	5/5	0/5	0/5
6,800	- 2	- 3	5/5	5/5	0/5	0/5
4,600	+ 1	+ 3	5/5	5/5	0/5	0/5
3,150	+ 8	-18	5/5	5/5	0/5	0/5
2,160	-19	-13	5/5	5/5	0/5	0/5
1,470	-29	+ 3	5/5	5/5	0/5	0/5
0	—	—	5/5	4/5	0/5	0/5

———————————

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

The high concentration selected for administration to dosed mice. in the chronic bioassay was 7500 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 Ir; Table-1 and 2.

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed rats were supplied with diets containing 750 and 375 ppm 4-nitro-o-phenylene-diamine for 103 weeks followed by a 2-week observation period, when no test chemicals were used. Throughout this report those rats receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed mice were supplied with diets containing 7500 and 3750 ppm 4-nitro-o-phenylene-diamine for 102 weeks followed by a 2-week observation period, when no test chemicals were used. Throughout this report those mice receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups.

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
4-NITRO-o-PHENYLENEDIAMINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	4-NITRO-o-PHENYLENEDIAMINE CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	105
LOW DOSE	50	375	103	
		0		2
HIGH DOSE	50	750	103	
		0		2
FEMALE				
CONTROL	20	0	0	105
LOW DOSE	50	375	103	
		0		2
HIGH DOSE	50	750	103	
		0		2

[a]Concentrations given in parts per million.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
4-NITRO-o-PHENYLENEDIAMINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	4-NITRO-o-PHENYLENEDIAMINE CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	104
LOW DOSE	50	3750	102	
		0		2
HIGH DOSE	50	7500	102	
		0		2
FEMALE				
CONTROL	20	0	0	104
LOW DOSE	50	3750	102	
		0		2
HIGH DOSE	50	7500	102	
		0		2

[a]Concentrations given in parts per million.

G. Clinical and Histopathologic Examinations

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

All moribund animals or those which developed large palpable masses that jeopardized their health, or survived to the end of the bioassay were euthanized with carbon dioxide. A necropsy was performed immediately on each killed animal as well as those found dead. Gross and microscopic examinations were performed on all major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

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

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

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing,

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

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically

censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure

an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test determined if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

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

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

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

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses.

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

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

Slight dose-related mean body weight depression was apparent in male rats. Dosed female rats had slight, although not dose-related, mean body weight depression in comparison with their controls after week 50 (Figure 2).

No other clinical signs were recorded.

B. Survival

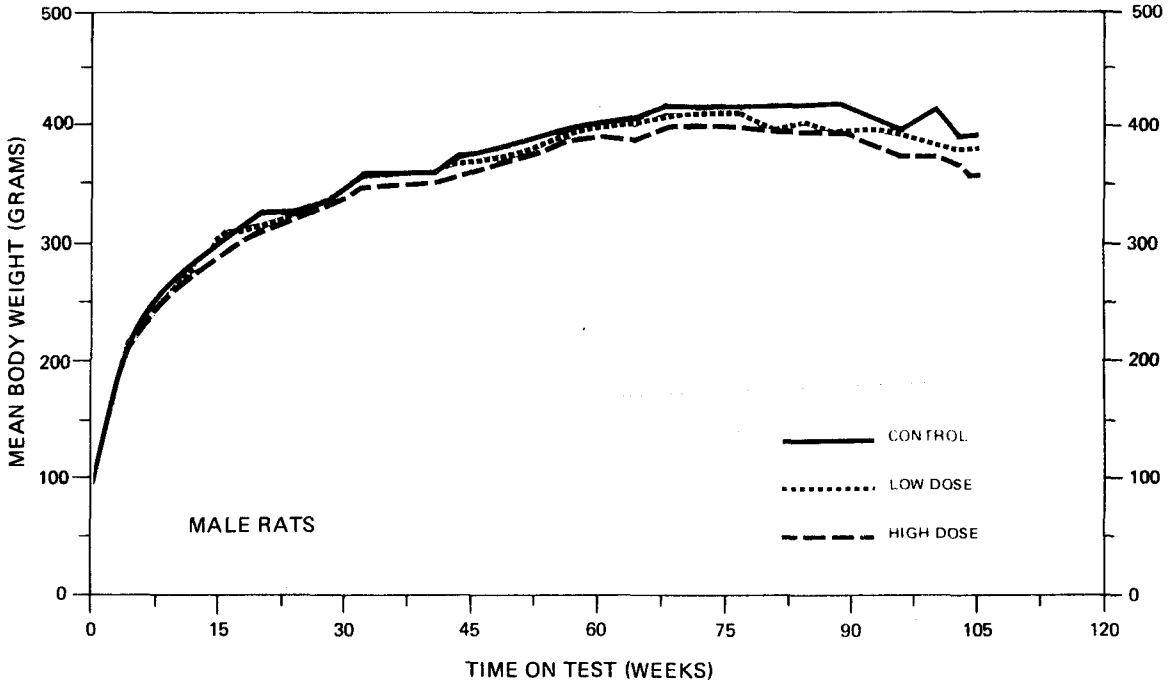
The estimated probabilities of survival for male and female rats in the control and 4-nitro-o-phenylenediamine-dosed groups are shown in Figure 3. The Tarone test for association between dosage and mortality was not significant for either males or females.

There were adequate numbers of male rats at risk from late-developing tumors as 88 percent (44/50) of the high dose, 82 percent (41/50) of the low dose, and 80 percent (16/20) of the controls survived on test until the termination of the study.

There were adequate numbers of female rats at risk from late-developing tumors, as 86 percent (43/50) of the high dose, 84 percent (42/50) of the low dose, and 85 percent (17/20) of the controls survived on test until the termination of the study. Two low dose females were missing in week 12.

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).



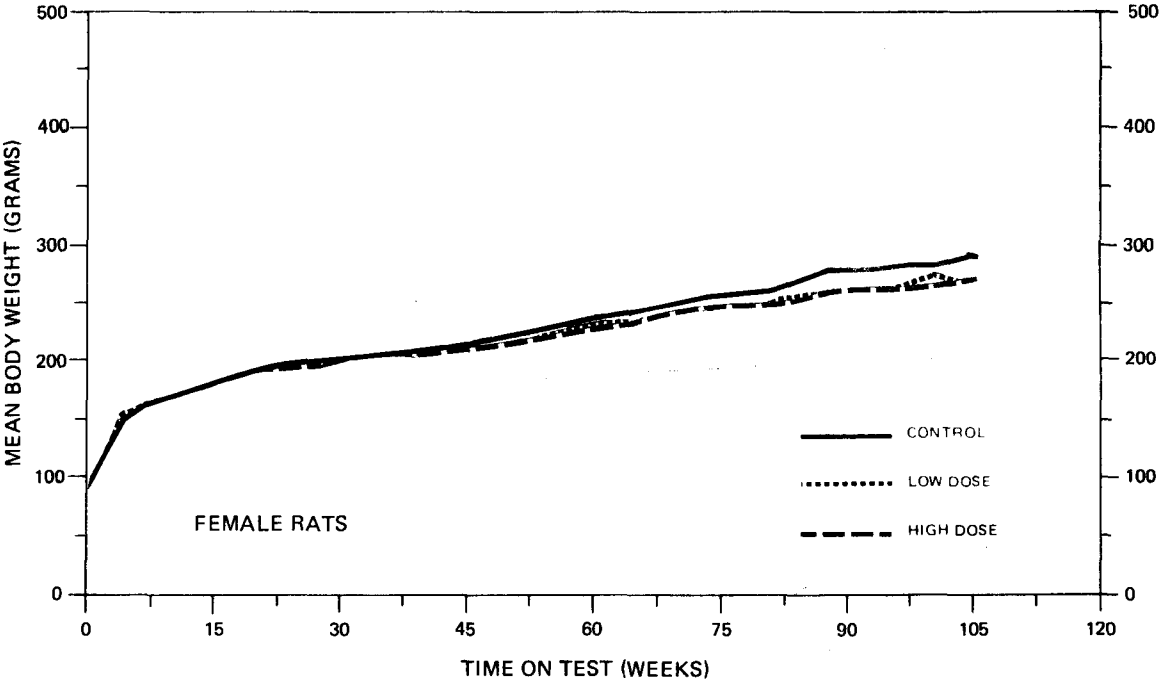
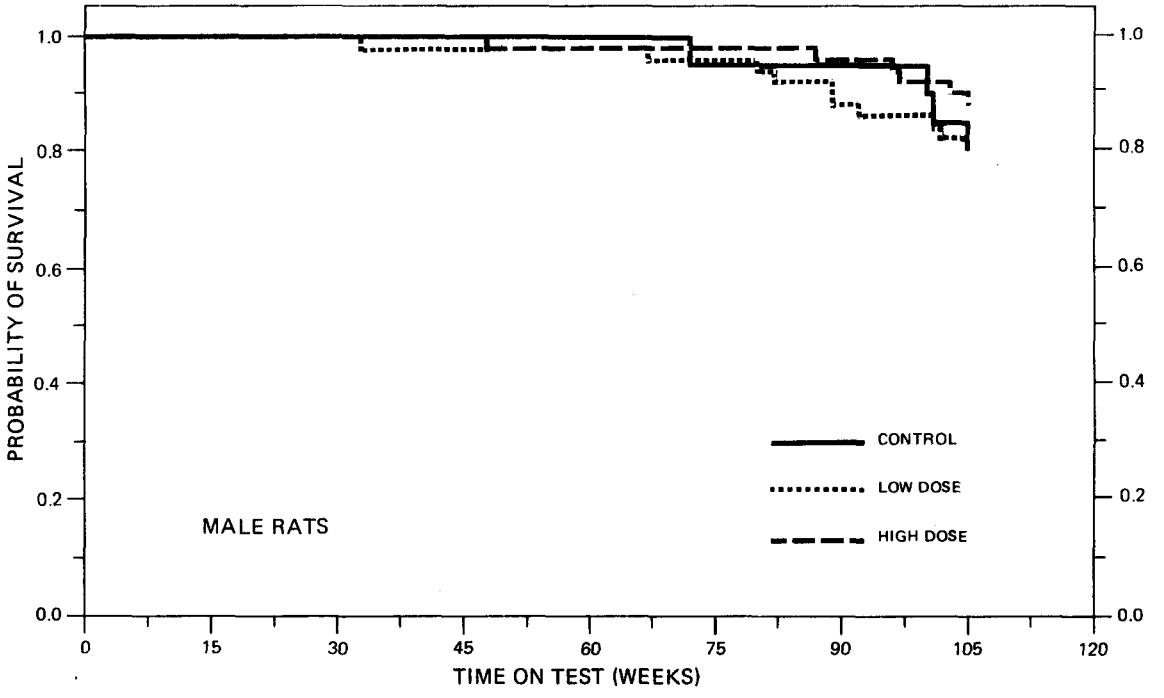


FIGURE 2
GROWTH CURVES FOR 4-NITRO-o-PHENYLENEDIAMINE CHRONIC STUDY RATS



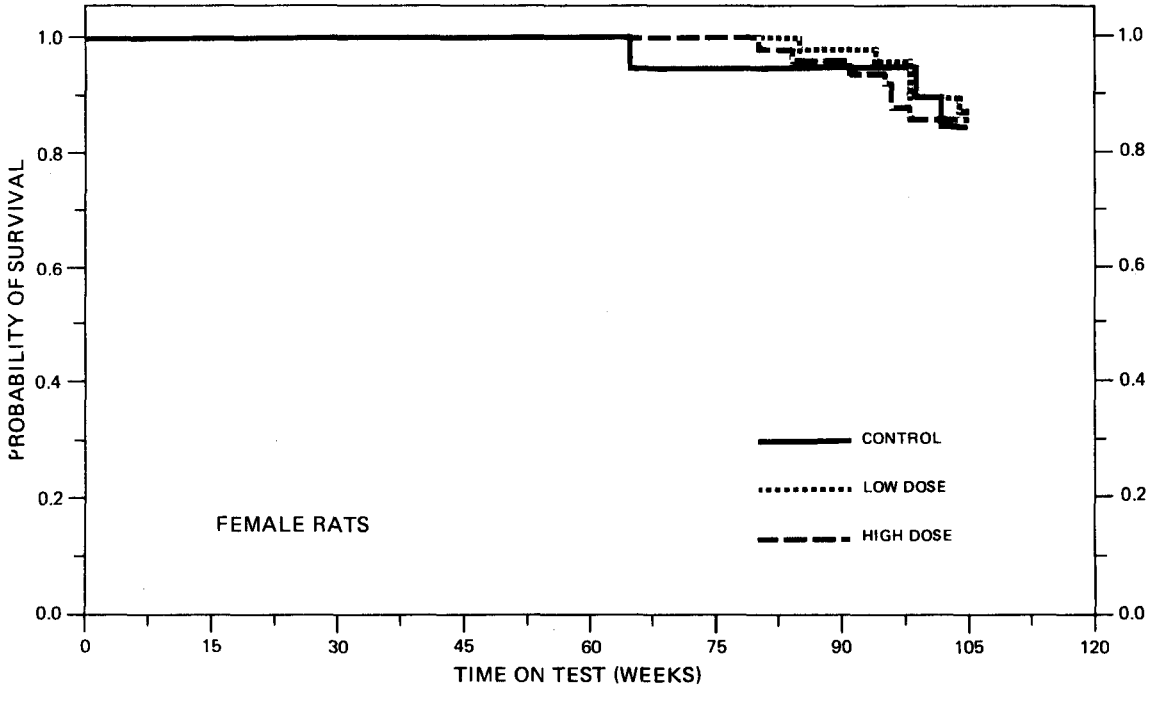


FIGURE 3
SURVIVAL COMPARISONS OF 4-NITRO-o-PHENYLENEDIAMINE CHRONIC STUDY RATS

The types of neoplasms present in both the dosed and control groups have been encountered previously as spontaneous lesions in aging rats of this strain.

There was a greater incidence of neoplasms of the adrenal gland in dosed male rats and the hematopoietic system of both male and female rats when compared with control rats. These neoplasms are frequently seen in aged Fischer 344 rats and the incidence observed in this study is similar to the incidence of spontaneous occurrence of these neoplasms in this strain of rat.

A variety of proliferative, inflammatory and degenerative nonneoplastic lesions was present in both dosed and control rats. These lesions had a spontaneous and random distribution and are commonly observed in aged Fischer 344 rats.

Based on the results of this pathology examination, 4-nitro-o-phenylenediamine, at the dosage levels used, did not induce neoplastic or nonneoplastic lesions in 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 4-nitro-o-phenylenediamine-dosed groups and where such tumors were observed in at least 5 percent of the group.

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 4-NITRO-o-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma or Fibroadenoma[b]	0/20(0.00)	3/50(0.06)	0/50(0.00)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.047	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.250	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	82	---
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	0/20(0.00)	5/50(0.10)	7/50(0.14)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.525	0.809
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	67	87
Pituitary: Chromophobe Adenoma[b]	2/18(0.11)	8/47(0.17)	4/46(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.532	0.783
Lower Limit	---	0.353	0.127
Upper Limit	---	14.008	8.213
Weeks to First Observed Tumor	100	89	105

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Adrenal: Pheochromocytoma[b]	2/20(0.10)	8/49(0.16)	7/50(0.14)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.633	1.400
Lower Limit	---	0.371	0.303
Upper Limit	---	14.987	13.138
Weeks to First Observed Tumor	105	102	105
Pancreatic Islets: Islet-Cell Adenoma[b]	1/19(0.05)	0/49(0.00)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	1.163
Lower Limit	---	0.000	0.103
Upper Limit	---	7.244	59.809
Weeks to First Observed Tumor	105	---	103
Testis: Interstitial-Cell Tumor or Malignant Interstitial-Cell Tumor	18/19(0.95)	43/50(0.86)	47/50(0.94)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.908	0.992
Lower Limit	---	0.842	0.922
Upper Limit	---	1.163	1.179
Weeks to First Observed Tumor	100	82	87

TABLE 3 (CONCLUDED)

[a]Treated groups received doses of 375 or 750 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 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 4-NITRO-o-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	0/20(0.00)	3/48(0.06)	5/50(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.261	0.525
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	104	91
Pituitary: Chromophobe Carcinoma or Chromophobe Adenoma[b]	7/19(0.37)	20/47(0.43)	15/45(0.33)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.155	0.905
Lower Limit	---	0.589	0.434
Upper Limit	---	2.759	2.259
Weeks to First Observed Tumor	99	85	84
Mammary Gland: Adenoma NOS, Cystadenoma NOS, Papillary Cystadenoma NOS, or Fibroadenoma[b]	2/20(0.10)	3/48(0.06)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.625	0.600
Lower Limit	---	0.079	0.076
Upper Limit	---	7.137	6.860
Weeks to First Observed Tumor	99	105	95

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Uterus: Endometrial Stromal Polyp[b]	6/20(0.30)	11/48(0.23)	12/50(0.24)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.764	0.800
Lower Limit	---	0.313	0.336
Upper Limit	---	2.231	2.306
Weeks to First Observed Tumor	105	98	96

[a]Treated groups received doses of 375 or 750 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.

None of the statistical tests for any site in rats of either sex indicated a significant positive association between the administration of 4-nitro-o-phenylenediamine and an increased tumor incidence. Thus there was no evidence to indicate that 4-nitro-o-phenylenediamine was a carcinogen in Fischer 344 rats at the dose levels used and under the conditions of this bioassay.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In 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 4-nitro-o-phenylenediamine that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

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

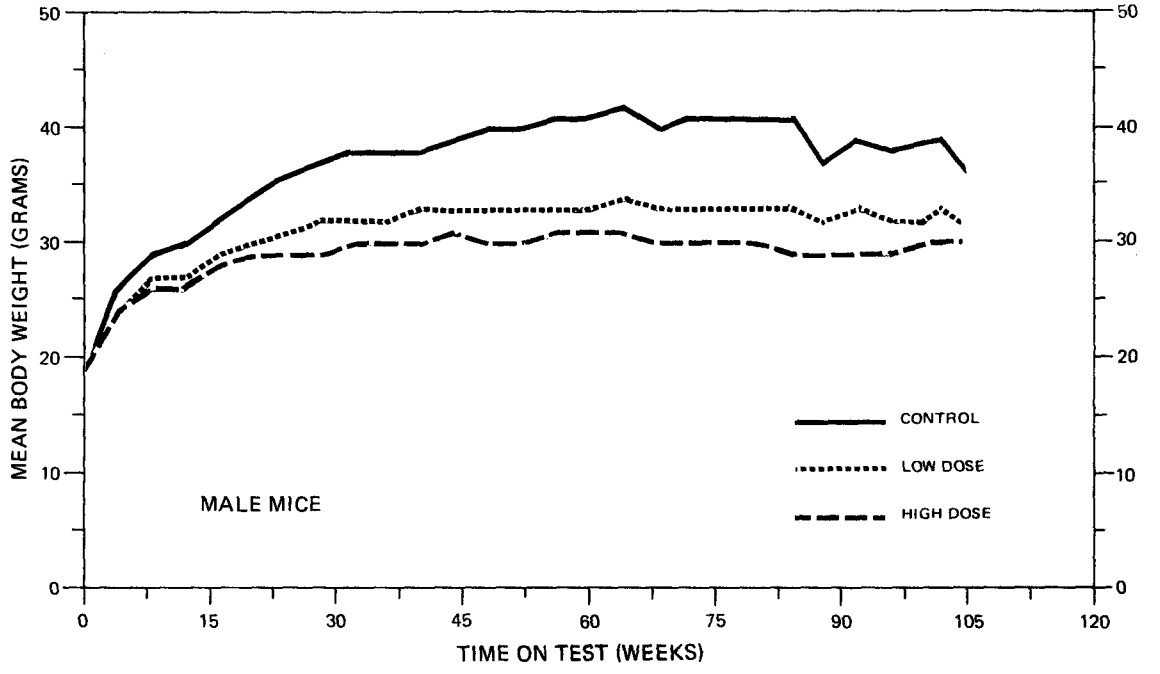
No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 4-nitro-o-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. For females, the Cox test indicated a significant negative association when comparing the high dose to control ($P = 0.032$) and the low dose to control ($P = 0.002$). The percentages of males and females surviving on test are shown in Figure 6.

Five high dose, six low dose, and one control male were missing in weeks 11 through 102 and one additional control male mouse was accidentally killed in week 96. Nevertheless, there were adequate numbers of male mice at risk from late-developing tumors, as 76 percent (38/50) of the high dose, 76 percent (38/50) of the low dose and 60 percent (12/20) of the controls survived on test until the termination of the study.

Although five high dose, two low dose, and three control females were missing by week 18, and one low dose female was accidentally killed in week 95, there were adequate numbers of female mice at risk



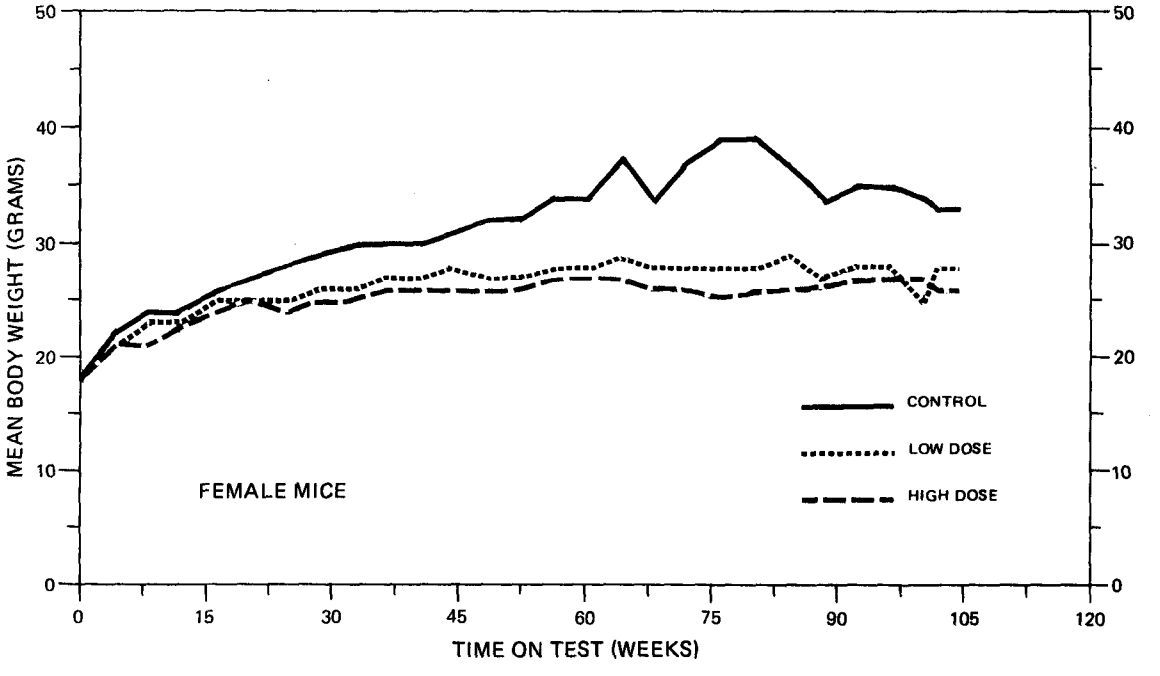


FIGURE 4
GROWTH CURVES FOR 4-NITRO-o-PHENYLENEDIAMINE CHRONIC STUDY MICE

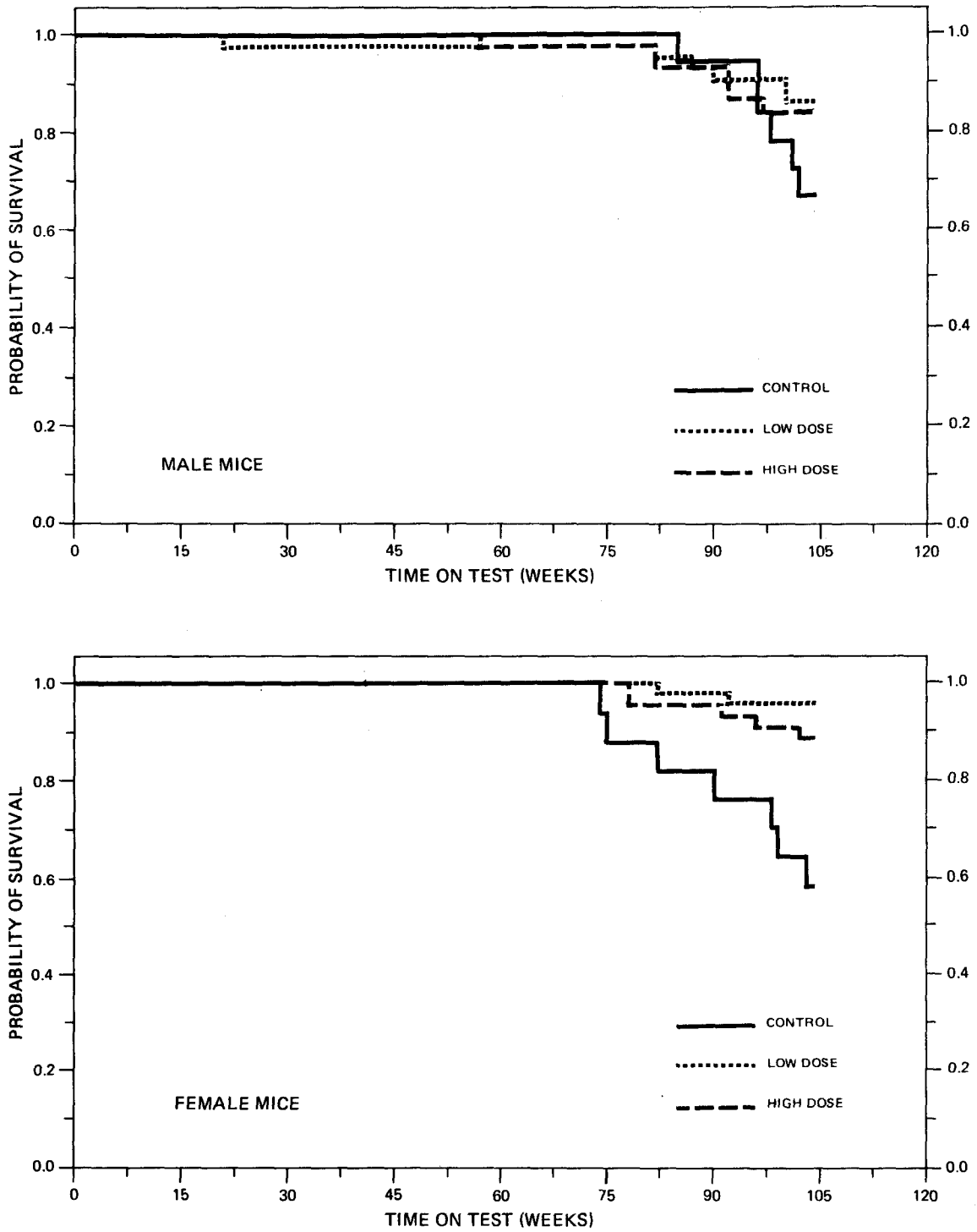


FIGURE 5
SURVIVAL COMPARISONS OF 4-NITRO-o-PHENYLENEDIAMINE CHRONIC STUDY MICE

from late-developing tumors. Eighty percent (40/50) of the high dose, 90 percent (45/50) of the low dose and 50 percent (10/20) 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).

The types of neoplasms present in both the dosed and control groups have been encountered previously in aging B6C3F1 mice.

In the low dose females there was an increase in hepatocellular adenomas (9/47 [19 percent]) as compared with controls (1/17 [6 percent]). The neoplasms were nodular masses of well-differentiated cells which compressed but did not infiltrate adjacent hepatic parenchyma. Neoplastic cells resembled normal hepatocytes and were arranged in a lobular pattern similar to that of normal liver.

The neoplasms observed in the lung and thyroid occurred as spontaneous neoplasms in aged B6C3F1 hybrid mice. Although there was a slight increased incidence of these neoplasms in individual groups of dosed mice, the incidence was within the range of spontaneous occurrence of these neoplasms in this strain of mouse.

In the dosed group most tissues contained a deposition of iron-negative brown pigment that was particularly prominent in the thyroid and large neurons of the pons. The pigment was apparently inert, and

did not elicit a microscopic tissue response. This pigment was not chemically characterized.

A variety of proliferative, inflammatory and degenerative non-neoplastic lesions was present in both dosed and control mice. These lesions were interpreted as spontaneous and are commonly observed in aged B6C3F1 mice.

Therefore, based on the results of this pathology examination, feeding of 4-nitro-o-phenylenediamine was associated with an increased incidence of hepatocellular adenomas in female B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 4-nitro-o-phenylenediamine-dosed groups and where such tumors were observed in at least 5 percent of the group.

None of the statistical tests for any site in mice of either sex indicated a significant positive association between the administration of 4-nitro-o-phenylenediamine and an increased tumor incidence. Thus, at the dose levels used in this experiment, there was not sufficient evidence that 4-nitro-o-phenylenediamine was a carcinogen in B6C3F1 mice.

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH 4-NITRO-o-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	1/18(0.06)	1/41(0.02)	2/38(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.439	0.947
Lower Limit	---	0.006	0.054
Upper Limit	---	33.654	54.413
Weeks to First Observed Tumor	104	104	104
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	3/18(0.17)	13/41(0.32)	6/38(0.16)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.902	0.947
Lower Limit	---	0.623	0.236
Upper Limit	---	9.457	5.372
Weeks to First Observed Tumor	96	104	104
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	2/19(0.11)	5/44(0.11)	2/45(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.080	0.422
Lower Limit	---	0.200	0.033
Upper Limit	---	10.742	5.547
Weeks to First Observed Tumor	101	87	82

TABLE 5 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	2/18(0.11)	1/44(0.02)	2/45(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.205	0.400
Lower Limit	---	0.004	0.032
Upper Limit	---	3.744	5.250
Weeks to First Observed Tumor	96	104	104
Liver: Hepatocellular Carcinoma, Hepatocellular Adenoma or Mixed Hepato/Cholangio Carcinoma[b]	4/18(0.22)	6/44(0.14)	4/45(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.614	0.400
Lower Limit	---	0.172	0.086
Upper Limit	---	2.689	1.965
Weeks to First Observed Tumor	85	104	104
Thyroid: Follicular-Cell Carcinoma or Follicular-Cell Adenoma[b]	0/14(0.00)	1/36(0.03)	3/35(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.022	0.258
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	104	104

TABLE 5 (CONCLUDED)

[a]Treated groups received doses of 3750 or 7500 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 greated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

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

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 4-NITRO-o-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	3/15(0.20)	5/41(0.12)	4/41(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.610	0.488
Lower Limit	---	0.142	0.098
Upper Limit	---	3.599	3.060
Weeks to First Observed Tumor	74	104	104
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	4/17(0.24)	3/48(0.06)	3/45(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.266	0.283
Lower Limit	---	0.045	0.048
Upper Limit	---	1.441	1.533
Weeks to First Observed Tumor	90	92	96
Liver: Hepatocellular Adenoma[b]	1/17(0.06)	9/47(0.19)	3/45(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	3.255	1.133
Lower Limit	---	0.518	0.101
Upper Limit	---	139.091	58.167
Weeks to First Observed Tumor	104	104	104

TABLE 6 (CONCLUDED)

[a]Treated groups received doses of 3750 or 7500 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.

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 5 and 6, 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 mice by 4-nitro-o-phenylenediamine that could not be established under the conditions of this test.

V. DISCUSSION

Semipermanent properties of hair colorants containing 4-nitro-o-phenylenediamine are induced with hydrogen peroxide during the application process. The 4-nitro-o-phenylenediamine used in this bioassay was not treated with hydrogen peroxide and hydrogen peroxide was not incorporated into diets containing the test dye.

There were no significant positive associations between the concentrations of 4-nitro-o-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. Distinct dose-related mean body weight depression was observed in mice, indicating that the concentrations of 4-nitro-o-phenylenediamine administered to these animals in this bioassay may have approximated the maximum tolerated concentrations. Since no distinct mean body weight despression relative to controls, no significantly accelerated mortality, and no other manifestations of chronic toxicity were associated with administration of 4-nitro-o-phenylenediamine to male or female rats, it is possible that these animals may have been able to tolerate a higher dietary concentration.

None of the statistical tests for any site in rats or in mice of either sex indicated a significant positive association between compound administration and tumor incidence. Although increased

incidences of hepatocellular adenomas were observed in dosed female mice when compared to controls (i.e., 1/17, 9/47 and 3/45 in the control, low dose, and high dose, respectively), the tumors were observed primarily among the low dose group, and they did not occur in statistically significant incidences.

Under the conditions of this bioassay, dietary administration of 4-nitro-o-phenylenediamine was not carcinogenic in Fischer 344 rats or B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 4-NITRO-o-PHENYLENEDIAMINE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH 4-NITRO-o-PHENYLENEDIAMINE

	CONTROL (UNTR) 11-1465	LOW DOSE 11-1463	HIGH DOSE 11-1461
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		2 (4%)	
SQUAMOUS CELL CARCINOMA		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA		2 (4%)	
CARCINOSARCOMA	1 (5%)		
FIBROADENOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
INTERSTITIAL-CELL TUMOR, METASTA	1 (5%)		
HEMATOPOIETIC SYSTEM			
#BRAIN	(20)	(50)	(50)
MALIGNANT RETICULOSIS		1 (2%)	
*MULTIPLE ORGANS	(20)	(50)	(50)
LYMPHOCYTIC LEUKEMIA		5 (10%)	6 (12%)
GRANULOCYTIC LEUKEMIA			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(50)	(50)
SARCOMA, NOS		1 (2%)	

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1465	LOW DOSE 11-1463	HIGH DOSE 11-1461
#LIVER	(19)	(50)	(49)
NEOPLASTIC NODULE	1 (5%)	2 (4%)	2 (4%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(47)	(46)
CHROMOPHOBE ADENOMA	2 (11%)	8 (17%)	4 (9%)
#ADRENAL	(20)	(49)	(50)
PHEOCHROMOCYTOMA	2 (10%)	8 (16%)	7 (14%)
#THYROID	(16)	(43)	(37)
FOLLICULAR-CELL ADENOMA		2 (5%)	1 (3%)
C-CELL ADENOMA	1 (6%)	1 (2%)	
#PANCREATIC ISLETS	(19)	(49)	(49)
ISLET-CELL ADENOMA	1 (5%)		3 (6%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
PAPILLARY ADENOCARCINOMA		1 (2%)	
#TESTIS	(19)	(50)	(50)
INTERSTITIAL-CELL TUMOR	17 (89%)	43 (86%)	47 (94%)
INTERSTITIAL-CELL TUMOR, MALIGNA	1 (5%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A1 (CONCLUDED)

	CONTROL(UNTR) 11-1465	LOW DOSE 11-1463	HIGH DOSE 11-1461
BODY CAVITIES			
*TUNICA VAGINALIS	(20)	(50)	(50)
MESOTHELIOMA, NOS		2 (4%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	3	5
MORIBUND SACRIFICE	2	6	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	41	44
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	48	48
TOTAL PRIMARY TUMORS	26	80	71
TOTAL ANIMALS WITH BENIGN TUMORS	18	47	48
TOTAL BENIGN TUMORS	23	67	62
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	9	7
TOTAL MALIGNANT TUMORS	2	9	7
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		
TOTAL SECONDARY TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	4	2
TOTAL UNCERTAIN TUMORS	1	4	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH 4-NITRO-o-PHENYLENEDIAMINE

	CONTROL(UNTR) 11-1466	LOW DOSE 11-1464	HIGH DOSE 11-1462
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		2	
ANIMALS NECROPSIED	20	48	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	48	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(48)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
SEBACEOUS ADENOMA			1 (2%)
*SUBCUT TISSUE	(20)	(48)	(50)
CARCINOMA,NOS	1 (5%)		
FIBROADENOMA		2 (4%)	2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)		1 (2%)
HEMATOPOIETIC SYSTEM			
#BRAIN	(20)	(47)	(50)
MALIGNANT RETICULOSIS		1 (2%)	
*MULTIPLE ORGANS	(20)	(48)	(50)
LEUKEMIA,NOS		1 (2%)	
LYMPHOCYTIC LEUKEMIA			5 (10%)
GRANULOCYTIC LEUKEMIA		2 (4%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(47)	(49)
ADENOMA, NOS			1 (2%)

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1466	LOW DOSE 11-1464	HIGH DOSE 11-1462
#LIVER	(20)	(47)	(50)
HEPATOCELLULAR ADENOMA		2 (4%)	
URINARY SYSTEM			
#KIDNEY	(20)	(47)	(50)
HAMARTOMA+		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(47)	(45)
CHROMOPHOBE ADENOMA	7 (37%)	19 (40%)	15 (33%)
CHROMOPHOBE CARCINOMA		1 (2%)	
#ADRENAL	(20)	(46)	(49)
CORTICAL ADENOMA			1 (2%)
PHEOCHROMOCYTOMA		1 (2%)	2 (4%)
#THYROID	(13)	(30)	(42)
FOLLICULAR-CELL ADENOMA			1 (2%)
FOLLICULAR-CELL CARCINOMA		1 (3%)	
C-CELL ADENOMA	1 (8%)	1 (3%)	2 (5%)
#PANCREATIC ISLETS	(19)	(47)	(50)
ISLET-CELL ADENOMA		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(48)	(50)
ADENOMA, NOS	1 (5%)		
CYSTADENOMA, NOS		2 (4%)	
PAPILLARY CYSTADENOMA, NOS	1 (5%)		
FIBROADENOMA		1 (2%)	3 (6%)
#UTERUS	(20)	(48)	(50)
ADENOCARCINOMA, NOS			2 (4%)
ENDOMETRIAL STROMAL POLYP	6 (30%)	11 (23%)	12 (24%)
NERVOUS SYSTEM			
NONE			

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
\+ THIS IS CONSIDERED TO BE A BENIGN FORM OF THE MIXED TUMOR OF THE KIDNEY AND CONSISTS OF PROLIFERATIVE LIPOCYTES, TUBULAR STRUCTURES, FIBROBLASTS, AND VASCULAR SPACES IN VARYING PROPORTIONS.

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1466	LOW DOSE 11-1464	HIGH DOSE 11-1462
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(20)	(48)	(50)
BASAL-CELL TUMOR			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*BODY CAVITIES	(20)	(48)	(50)
MESOTHELIOMA, MALIGNANT			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	1	3
MORIBUND SACRIFICE	2	5	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	42	43
ANIMAL MISSING		2	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 11-1466	LOW DOSE 11-1464	HIGH DOSE 11-1462
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	15	31	33
TOTAL PRIMARY TUMORS	18	48	51
TOTAL ANIMALS WITH BENIGN TUMORS	14	30	30
TOTAL BENIGN TUMORS	17	41	43
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	7	7
TOTAL MALIGNANT TUMORS	1	7	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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 4-NITRO-o-PHENYLENEDIAMINE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH 4-NITRO-o-PHENYLENEDIAMINE

	CONTROL (UNTR) 22-2465	LOW DOSE 22-2463	HIGH DOSE 22-2461
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	6	5
ANIMALS NECROPSIED	19	44	45
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	19	44	45
INTEGUMENTARY SYSTEM			
*SKIN	(19)	(44)	(45)
FIBROMA	1 (5%)		
LEIOMYOSARCOMA	1 (5%)		
*SUBCUT TISSUE	(19)	(44)	(45)
FIBROSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(18)	(41)	(38)
HEPATOCELLULAR CARCINOMA, METAST	1 (6%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (11%)	12 (29%)	4 (11%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (6%)	1 (2%)	2 (5%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(19)	(44)	(45)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (5%)	1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		2 (5%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	1 (2%)	1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	
GRANULOCYTIC LEUKEMIA			1 (2%)
#MESENTERIC L. NODE	(17)	(43)	(43)
HEMANGIOSARCOMA	1 (6%)		
CIRCULATORY SYSTEM			
NONE			

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

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 22-2465	LOW DOSE 22-2463	HIGH DOSE 22-2461
DIGESTIVE SYSTEM			
#LIVER	(18)	(44)	(45)
HEPATOCELLULAR ADENOMA	2 (11%)	4 (9%)	2 (4%)
HEPATOCELLULAR CARCINOMA	2 (11%)	1 (2%)	2 (4%)
MIXED HEPATO/CHOLANGIO CARCINOMA		1 (2%)	
HEMANGIOSARCOMA		1 (2%)	
HEMANGIOSARCOMA, METASTATIC	1 (6%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(14)	(36)	(35)
FOLLICULAR-CELL ADENOMA			3 (9%)
FOLLICULAR-CELL CARCINOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
#TESTIS	(18)	(44)	(44)
INTERSTITIAL-CELL TUMOR		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(19)	(44)	(45)
FIBROSARCOMA	1 (5%)		
RHABDOMYOSARCOMA		1 (2%)	

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

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 22-2465	LOW DOSE 22-2463	HIGH DOSE 22-2461
BODY CAVITIES			
*ABDOMINAL CAVITY	(19)	(44)	(45)
LEIOMYOSARCOMA	1 (5%)		
*MESENTERY	(19)	(44)	(45)
HEMANGIOMA	1 (5%)		
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	5	5	4
MORIBUND SACRIFICE	1	1	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED	1		
TERMINAL SACRIFICE	12	38	38
ANIMAL MISSING	1	6	5
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	25	12
TOTAL PRIMARY TUMORS	15	29	15
TOTAL ANIMALS WITH BENIGN TUMORS	5	16	7
TOTAL BENIGN TUMORS	6	17	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	12	6
TOTAL MALIGNANT TUMORS	9	12	6
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		
TOTAL SECONDARY TUMORS	2		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH 4-NITRO-o-PHENYLENEDIAMINE

	CONTROL (UNTR) 22-2466	LOW DOSE 22-2464	HIGH DOSE 22-2462
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	3	2	5
ANIMALS NECROPSIED	17	48	45
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	17	48	45
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(15)	(41)	(41)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (13%)	5 (12%)	4 (10%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (7%)		
FIBROSARCOMA, METASTATIC	1 (7%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(17)	(48)	(45)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (6%)	1 (2%)	
UNDIFFERENTIATED LEUKEMIA	1 (6%)		
LYMPHOCYTIC LEUKEMIA			1 (2%)
GRANULOCYTIC LEUKEMIA			1 (2%)
MONOCYTIC LEUKEMIA	1 (6%)		
#MESENTERIC L. NODE	(16)	(46)	(42)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (6%)		
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	
#AXILLARY LYMPH NODE	(16)	(46)	(42)
BASAL-CELL CARCINOMA, METASTATIC	1 (6%)		
CIRCULATORY SYSTEM			
NONE			

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2466	LOW DOSE 22-2464	HIGH DOSE 22-2462
DIGESTIVE SYSTEM			
#LIVER	(17)	(47)	(45)
HEPATOCELLULAR ADENOMA	1 (6%)	9 (19%)	3 (7%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(7)	(34)	(30)
FOLLICULAR-CELL ADENOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(17)	(48)	(45)
CYSTOSARCOMA PHYLLODES, BENIGN		1 (2%)	
#UTERUS	(17)	(44)	(45)
ENDOMETRIAL STROMAL SARCOMA		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(17)	(48)	(45)
FIBROSARCOMA	1 (6%)		
BODY CAVITIES			
*PERITONEUM	(17)	(48)	(45)
MESOTHELIOMA, NOS			1 (2%)

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 22-2466	LOW DOSE 22-2464	HIGH DOSE 22-2462
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	5	2	2
MORIBUND SACRIFICE	2		3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	10	45	40
ANIMAL MISSING	3	2	5
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	18	11
TOTAL PRIMARY TUMORS	9	20	11
TOTAL ANIMALS WITH BENIGN TUMORS	2	15	7
TOTAL BENIGN TUMORS	3	16	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	4	3
TOTAL MALIGNANT TUMORS	6	4	3
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		
TOTAL SECONDARY TUMORS	2		
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 C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 4-NITRO-o-PHENYLENEDIAMINE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH 4-NITRO-o-PHENYLENEDIAMINE

	CONTROL (UNTR) 11-1465	LOW DOSE 11-1463	HIGH DOSE 11-1461
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, NECROTIZING			1 (2%)
RESPIRATORY SYSTEM			
#TRACHEA	(19)	(49)	(50)
INFLAMMATION, NOS	2 (11%)	9 (18%)	14 (28%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, CHRONIC	1 (5%)	1 (2%)	1 (2%)
#LUNG	(20)	(50)	(50)
CONGESTION, NOS	1 (5%)	1 (2%)	
EDEMA, NOS	1 (5%)	1 (2%)	
PNEUMONIA, CHRONIC MURINE	3 (15%)	12 (24%)	18 (36%)
FIBROSIS, FOCAL	1 (5%)		
HYPERPLASIA, ADENOMATOUS		1 (2%)	2 (4%)
HISTIOCYTOSIS	1 (5%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(50)	(50)
HEMOSIDEROSIS		1 (2%)	
#MESENTERIC L. NODE	(20)	(49)	(50)
DILATATION, NOS	1 (5%)		
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(50)
ENDOCARDITIS, BACTERIAL			1 (2%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-1465	LOW DOSE 11-1463	HIGH DOSE 11-1461
FIBROSIS, FOCAL		1 (2%)	1 (2%)
#MYOCARDIUM	(20)	(50)	(50)
INFLAMMATION, FOCAL		2 (4%)	
FIBROSIS	11 (55%)	24 (48%)	26 (52%)
FIBROSIS, FOCAL			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(19)	(50)	(49)
DILATATION, NOS			1 (2%)
HEMORRHAGE		1 (2%)	
CHOLANGIOFIBROSIS			2 (4%)
DEGENERATION, GRANULAR		1 (2%)	
METAMORPHOSIS FATTY	3 (16%)	2 (4%)	
FOCAL CELLULAR CHANGE	4 (21%)	4 (8%)	2 (4%)
CYTOLOGIC DEGENERATION	1 (5%)		
#BILE DUCT	(19)	(50)	(49)
HYPERPLASIA, NOS	2 (11%)		1 (2%)
#PANCREAS	(19)	(49)	(49)
ATROPHY, NOS			2 (4%)
ATROPHY, FOCAL	2 (11%)		2 (4%)
#PANCREATIC ACINUS	(19)	(49)	(49)
ATROPHY, NOS		2 (4%)	
#ESOPHAGUS	(20)	(48)	(50)
INFLAMMATION, NOS			1 (2%)
#STOMACH	(20)	(50)	(50)
INFLAMMATION, NOS		2 (4%)	
INFLAMMATION, CHRONIC	1 (5%)		
FIBROSIS	2 (10%)		
#LARGE INTESTINE	(20)	(48)	(50)
NEMATODIASIS	6 (30%)	27 (56%)	25 (50%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
CYST, NOS		2 (4%)	

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-1465	LOW DOSE 11-1463	HIGH DOSE 11-1461
MULTIPLE CYSTS		1 (2%)	
INFLAMMATION, CHRONIC	12 (60%)	30 (60%)	29 (58%)
SCAR	1 (5%)		
HEMOSIDEROSIS		1 (2%)	
#URINARY BLADDER	(15)	(48)	(42)
CALCULUS, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(47)	(46)
CYST, NOS		1 (2%)	1 (2%)
HEMORRHAGIC CYST			1 (2%)
#ADRENAL	(20)	(49)	(50)
ECTOPIA			1 (2%)
CYTOLOGIC DEGENERATION	1 (5%)		
#ADRENAL CORTEX	(20)	(49)	(50)
METAMORPHOSIS FATTY			1 (2%)
LIPOIDOSIS		2 (4%)	1 (2%)
CYTOLOGIC DEGENERATION		3 (6%)	
#THYROID	(16)	(43)	(37)
INFLAMMATION, NOS			1 (3%)
HYPERPLASIA, C-CELL	1 (6%)		
#PANCREATIC ISLETS	(19)	(49)	(49)
HYPERPLASIA, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
#TESTIS	(19)	(50)	(50)
MINERALIZATION	1 (5%)		
ATROPHY, NOS	3 (16%)	2 (4%)	3 (6%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(50)
HYDROCEPHALUS, NOS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 11-1465	LOW DOSE 11-1463	HIGH DOSE 11-1461
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
PERIARTERITIS		1 (2%)	
NECROSIS, FAT	1 (5%)		1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	

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 4-NITRO-o-PHENYLENEDIAMINE

	CONTROL (UNTR) 11-1466	LOW DOSE 11-1464	HIGH DOSE 11-1462
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		2	
ANIMALS NECROPSIED	20	48	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	48	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(48)	(50)
GRANULOMA, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#TRACHEA	(17)	(43)	(49)
INFLAMMATION, NOS		8 (19%)	7 (14%)
#LUNG	(20)	(48)	(50)
CONGESTION, NOS			1 (2%)
EDEMA, NOS			1 (2%)
BRONCHOPNEUMONIA, NOS		1 (2%)	
INFLAMMATION, INTERSTITIAL			2 (4%)
PNEUMONIA, CHRONIC MURINE		7 (15%)	2 (4%)
HYPERPLASIA, ADENOMATOUS			1 (2%)
HISTIOCYTOSIS		1 (2%)	
LEUKOCYTOSIS, NOS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(18)	(46)	(49)
OSTEOSCLEROSIS		1 (2%)	
#SPLEEN	(20)	(46)	(50)
NECROSIS, FOCAL		1 (2%)	
HEMOSIDEROSIS	1 (5%)	2 (4%)	
HYPERPLASIA, NOS			1 (2%)
HEMATOPOIESIS	1 (5%)	2 (4%)	1 (2%)
#LYMPH NODE	(20)	(47)	(49)
NECROSIS, NOS		1 (2%)	

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1466	LOW DOSE 11-1464	HIGH DOSE 11-1462
CIRCULATORY SYSTEM			
#HEART	(20)	(47)	(50)
ENDOCARDITIS, BACTERIAL			1 (2%)
#MYOCARDIUM	(20)	(47)	(50)
INFLAMMATION, FOCAL			2 (4%)
INFLAMMATION, CHRONIC		1 (2%)	
FIBROSIS	5 (25%)	9 (19%)	6 (12%)
FIBROSIS, FOCAL		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(47)	(50)
INFLAMMATION, NOS		2 (4%)	
INFLAMMATION, FOCAL		1 (2%)	1 (2%)
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY	2 (10%)	1 (2%)	2 (4%)
FOCAL CELLULAR CHANGE	12 (60%)	10 (21%)	13 (26%)
ANGIECTASIS		1 (2%)	
#LIVER/CENTRILOBULAR	(20)	(47)	(50)
NECROSIS, NOS			1 (2%)
#BILE DUCT	(20)	(47)	(50)
HYPERPLASIA, NOS			2 (4%)
#PANCREATIC ACINUS	(19)	(47)	(50)
ATROPHY, NOS	1 (5%)	1 (2%)	
#STOMACH	(20)	(46)	(50)
ULCER, FOCAL			1 (2%)
#LARGE INTESTINE	(20)	(48)	(50)
NEMATODIASIS	8 (40%)	18 (38%)	19 (38%)
#COLON	(20)	(48)	(50)
NEMATODIASIS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(47)	(50)
HYDRONEPHROSIS		1 (2%)	1 (2%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1466	LOW DOSE 11-1464	HIGH DOSE 11-1462
CYST, NOS			1 (2%)
INFLAMMATION, CHRONIC	5 (25%)	4 (9%)	5 (10%)
NEPHROPATHY		1 (2%)	
HEMOSIDEROSIS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(47)	(45)
CYST, NOS		1 (2%)	
HEMORRHAGE	1 (5%)		
HEMORRHAGIC CYST	1 (5%)	1 (2%)	1 (2%)
#ADRENAL	(20)	(46)	(49)
HEMORRHAGIC CYST	1 (5%)		
#ADRENAL CORTEX	(20)	(46)	(49)
METAMORPHOSIS FATTY	1 (5%)	1 (2%)	
LIPOIDOSIS		1 (2%)	
HYPERPLASIA, NOS			2 (4%)
#PANCREATIC ISLETS	(19)	(47)	(50)
HYPERPLASIA, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(48)	(50)
CYST, NOS	1 (5%)	2 (4%)	3 (6%)
PYOMETRA			1 (2%)
ABSCESS, NOS		1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(48)	(50)
CYST, NOS	1 (5%)	2 (4%)	
INFLAMMATION, NOS		6 (13%)	2 (4%)
FIBROSIS		1 (2%)	
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, CYSTIC	2 (10%)	4 (8%)	4 (8%)
#OVARY/OVIDUCT	(20)	(48)	(50)
INFLAMMATION, NOS		1 (2%)	
#OVARY	(20)	(46)	(48)
CYST, NOS	1 (5%)	3 (7%)	4 (8%)
PAROVARIAN CYST		1 (2%)	3 (6%)

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

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 11-1466	LOW DOSE 11-1464	HIGH DOSE 11-1462
INFLAMMATION, NOS		2 (4%)	1 (2%)
NERVOUS SYSTEM			
*BRAIN	(20)	(47)	(50)
HYDROCEPHALUS, NOS		1 (2%)	1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(48)	(50)
INFLAMMATION, CHRONIC			1 (2%)
*MESENTERY	(20)	(48)	(50)
MINERALIZATION			1 (2%)
NECROSIS, FAT	1 (5%)		2 (4%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	
ANIMAL MISSING/NO NECROPSY		2	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 4-NITRO-o-PHENYLENEDIAMINE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH 4-NITRO-o-PHENYLENEDIAMINE

	CONTROL (UNTR) 22-2465	LOW DOSE 22-2463	HIGH DOSE 22-2461
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	6	5
ANIMALS NECROPSIED	19	44	45
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	19	44	45
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(18)	(41)	(38)
INFLAMMATION, INTERSTITIAL	1 (6%)		
INFLAMMATION, CHRONIC FOCAL			1 (3%)
PERIVASCULAR CUFFING		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(17)	(40)	(44)
HYPERPLASIA, NODULAR		1 (3%)	
HYPERPLASIA, LYMPHOID	1 (6%)		
#MESENTERIC L. NODS	(17)	(43)	(43)
HEMORRHAGE	2 (12%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(17)	(41)	(40)
SCAR		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(18)	(44)	(45)
INFLAMMATION, DIFFUSE	1 (6%)		
NECROSIS, FOCAL			1 (2%)
BASOPHILIC CYTO CHANGE	1 (6%)		

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2465	LOW DOSE 22-2463	HIGH DOSE 22-2461
#PANCREAS	(16)	(42)	(43)
INFLAMMATION, ACUTE			1 (2%)
ATROPHY, NOS		1 (2%)	
#JEJUNUM	(18)	(43)	(44)
NEMATODIASIS			1 (2%)
#LARGE INTESTINE	(19)	(44)	(43)
NEMATODIASIS	1 (5%)		
#COLON	(19)	(44)	(43)
NEMATODIASIS	8 (42%)	15 (34%)	22 (51%)
URINARY SYSTEM			
#KIDNEY	(18)	(44)	(45)
INFARCT, NOS			1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(14)	(36)	(35)
CYST, NOS	1 (7%)		
PIGMENTATION, NOS		1 (3%)	
HYPERPLASIA, FOLLICULAR-CELL		1 (3%)	
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(19)	(44)	(45)
DILATATION, NOS	1 (5%)		
#TESTIS	(18)	(44)	(44)
ATROPHY, NOS		2 (5%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 22-2465	LOW DOSE 22-2463	HIGH DOSE 22-2461
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(19)	(44)	(45)
PIGMENTATION, NOS		42 (95%)	44 (98%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2		
ANIMAL MISSING/NO NECROPSY	1	6	5
AUTO/NECROPSY/HISTO PERF		1	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 4-NITRO-o-PHENYLENEDIAMINE

	CONTROL(UNTR) 22-2466	LOW DOSE 22-2464	HIGH DOSE 22-2462
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	3	2	5
ANIMALS NECROPSIED	17	48	45
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	17	48	45
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(15)	(41)	(41)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, INTERSTITIAL		1 (2%)	
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(17)	(47)	(45)
NECROSIS, NOS			1 (2%)
BASOPHILIC CYTO CHANGE		1 (2%)	
#PANCREAS	(16)	(47)	(44)
EMBRYONAL DUCT CYST	1 (6%)		
ATROPHY, NOS		1 (2%)	
#PEYERS PATCH	(16)	(46)	(44)
HYPERPLASIA, NOS	1 (6%)		
#COLON	(16)	(47)	(45)
NEMATODIASIS	1 (6%)	12 (26%)	12 (27%)

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

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2466	LOW DOSE 22-2464	HIGH DOSE 22-2462
HYPERPLASIA, LYMPHOID	1 (6%)		
URINARY SYSTEM			
#KIDNEY	(17)	(48)	(45)
PYELONEPHRITIS, CHRONIC			1 (2%)
AMYLOIDOSIS	1 (6%)		
HYPERPLASIA, LYMPHOID		1 (2%)	
ENDOCRINE SYSTEM			
#THYROID	(7)	(34)	(30)
HYPERPLASIA, FOCAL			1 (3%)
REPRODUCTIVE SYSTEM			
#UTERUS	(17)	(44)	(45)
HYDROMETRA		2 (5%)	4 (9%)
PYOMETRA		1 (2%)	
#UTERUS/ENDOMETRIUM	(17)	(44)	(45)
CYST, NOS		1 (2%)	
INFLAMMATION, NOS		2 (5%)	
INFLAMMATION, SUPPURATIVE			1 (2%)
HYPERPLASIA, CYSTIC	5 (29%)	8 (18%)	1 (2%)
#OVARY	(11)	(31)	(39)
CYST, NOS	1 (9%)	9 (29%)	3 (8%)
NERVOUS SYSTEM			
#BRAIN	(16)	(48)	(45)
HYDROCEPHALUS, NOS	1 (6%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 22-2466	LOW DOSE 22-2464	HIGH DOSE 22-2462
BODY CAVITIES			
*PERITONEUM	(17)	(48)	(45)
INFLAMMATION, CHRONIC	1 (6%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(17)	(48)	(45)
AMYLOIDOSIS		1 (2%)	
PIGMENTATION, NOS		47 (98%)	45 (100%)
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY	3	2	5
AUTO/NECROPSY/HISTO PERF	1		

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

Review of the Bioassay of 4-Nitro-*o*-Phenylenediamine* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

October 25, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, and State health officials. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 4-Nitro-*o*-Phenylenediamine for carcinogenicity.

The reviewer said that 4-Nitro-*o*-Phenylenediamine was not carcinogenic in rats or mice, under the conditions of test. After briefly describing the experimental design, he said that there were no unusual highlights and that the study was well-conducted and adequate. Based on the results of the study, the reviewer said that 4-Nitro-*o*-Phenylenediamine would not appear to pose a carcinogenic risk to humans.

A Program staff member pointed out that 4-Nitro-*o*-Phenylenediamine was found to be mutagenic and that chemicals of similar structure were shown to induce liver tumors in mice. He said that these findings may suggest that further studies are needed on the carcinogenicity of 4-Nitro-*o*-Phenylenediamine.

There was no objection to a motion that the bioassay of 4-Nitro-*o*-Phenylenediamine be accepted as written.

Clearinghouse Members Present:

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

* 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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