National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 190
1979

BIOASSAY OF p-NITROSODIPHENYLAMINE FOR POSSIBLE CARCINOGENICITY

CAS No. 156-10-5

NCI-CG-TR-190

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

BIOASSAY OF

p-NITROSODIPHENYLAMINE

FOR POSSIBLE CARCINOGENICITY

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

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

DHEW Publication No. (NIH) 79-1746

REPORT ON THE BIOASSAY OF p-NITROSODIPHENYLAMINE
FOR POSSIBLE CARCINOGENICITY

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

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

CONTRIBUTORS: This bioassay of p-nitrosodiphenylamine 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 at Litton Bionetics, Inc. (4) and the results for rats were reviewed by Dr. D. A. Willigan (4); the rat liver lesions were further reviewed by Dr. J. M. Ward (1). The pathology narrative for rats was written by Dr. D. A. Willigan (4), and the mouse pathology narrative was written by Dr. J. C. Peckham (4). 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. R. M. Helfand (8) and Dr J. P. Dirkse, III (9) using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (10).

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. A. R. Patel (1), Dr. S. F. Stinson (1), and Dr. C. E. Whitmire (1).

---

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

2. Now with the U.S. Environmental Protection Agency, 401 M Street S.W., Washington, D.C.

3. Now with the Naylor Dana Institute for Disease Prevention, American Health Foundation, Hammon House Road, Valhalla, New York.

4. Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland.

5. Now with Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike, Vienna, Virginia.

6. Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.

7. EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

8. The MITRE Corporation, METREK Division, 1820 Dolley Madison Boulevard, McLean, Virginia.

9. Consultant to The MITRE Corporation, currently a professor in the Department of Statistics at The George Washington University, 2100 Eye Street, N.W., Washington, D.C.

10. Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

SUMMARY

A bioassay for the possible carcinogenicity of p-nitrosodiphenylamine was conducted using Fischer 344 rats and B6C3F1 mice. p-Nitrosodiphenylamine 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 p-nitrosodiphenylamine were, respectively, 5000 and 2500 ppm for rats. The high and low time-weighted average concentrations for mice were 9000 and 4254 ppm, respectively. The compound was administered for 78 weeks to rats, for 50 weeks to high dose mice and for 57 weeks to low dose mice. The period of compound administration was followed by an observation period of 27 weeks for rats and 35 weeks for mice.

There were significant positive associations between the concentrations of p-nitrosodiphenylamine administered and mortality among male and female mice, but not for rats of either sex. Although 19/50 high dose male mice and 21/50 high dose female mice died before week 52, adequate numbers of mice and rats survived sufficiently long to be at risk from late-developing tumors. The toxicity observed in mice and the dose-related mean body weight depression apparent in male and female rats indicated that the concentrations of p-nitrosodiphenylamine administered to these animals in this bioassay may have approached or exceeded the maximum tolerated concentrations.

In male rats, there was a significant positive association between concentration administered and the incidence of a combination of hepatocellular carcinomas and neoplastic nodules. In addition, both the high dose to control and the low dose to control Fisher exact comparisons were significant. There was also a significant positive association between concentration administered and the incidence of alveolar/bronchiolar adenomas in male rats; however, neither of the Fisher exact comparisons were significant. There were no positive, significant statistical tests for tumor incidence at any site in female rats.

Due to the large number of early deaths among high dose mice of both sexes, the statistical conclusion concerning carcinogenicity was based on comparisons between the low dose and control groups. The incidence of hepatocellular carcinomas was significantly higher among the low dose males than among their controls. Although hepatocellular neoplasms were observed in dosed females, there were no tumors occurring with a significantly increased incidence when low dose females were compared to their controls.

Under the conditions of this bioassay, p-nitrosodiphenylamine was carcinogenic when administered in the diet to male B6C3F1 mice, causing hepatocellular carcinomas. The chemical was also carcinogenic in male Fischer 344 rats, causing liver neoplasms. No evidence was provided for the carcinogenicity of p-nitrosodiphenylamine in female B6C3F1 mice or in female Fischer 344 rats.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF TABLES (Concluded)

I. INTRODUCTION

p-Nitrosodiphenylamine (Figure 1) (NCI No. C02244), a vulcanization accelerator and chemical intermediate, was selected for bioassay by the National Cancer Institute because of the structural similarity of this compound to p-nitrosodimethylbenzenamine, a carcinogen in rats (Weisburger, 1975). The apparent relationship between exposure to aromatic dyestuff intermediates, particularly amines, and the increased incidence of bladder cancer among workers in the dye manufacturing industry (Wynder et al., 1963; Clayson and Garner, 1976) was an additional factor in the selection of this compound for testing.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 4-nitroso-N-phenylbenzenamine.* It is also called 4-nitrosodiphenylamine, p-nitroso-N-phenylaniline, and TKB.

p-Nitrosodiphenylamine is used to accelerate the vulcanization of rubber (Windholz, 1976). It is also used as an intermediate in the manufacture of dyes and pharmaceutical compounds and as an inhibitor of polymerization during the production of vinyl monomers such as styrene (Naugatuck® Chemicals, undated).

Specific production data for p-nitrosodiphenylamine are not available; however, this compound is produced in commercial quantities (in excess of 1000 pounds or $1000 in value annually) by one U.S. company (U.S. International Trade Commission, 1977).

*The CAS registry number is 156-10-5.

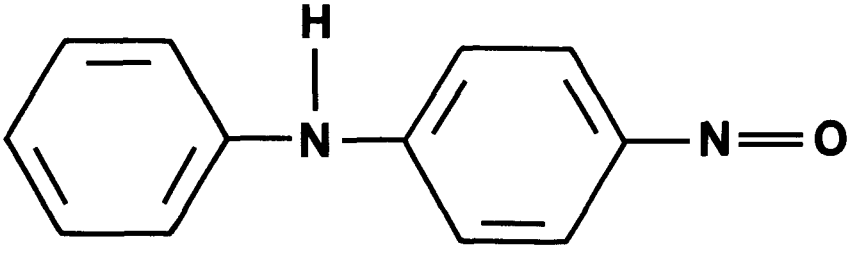


FIGURE 1
CHEMICAL STRUCTURE OF p-NITROSODIPHENYLAMINE

The potential for exposure to p-nitrosodiphenylamine is greatest for workers in elastomer, dye, pharmaceutical, and vinyl monomer manufacturing facilities.

p-Nitrosodiphenylamine showed no evidence of potential carcinogenicity in an *in vivo-in vitro* combination bioassay. Pregnant Syrian golden hamsters were given an intraperitoneal injection of 0.5 ml of a solution containing 0.5 to 2 mg of the chemical per 100 gm maternal weight on day 10 or 11 of gestation. Embryos were excised at day 13, and cells from these embryos were cultured and scored for transformation. No transformed cells were observed. Subcutaneous injection of the cultured cells into weanling, male hamsters produced no tumors (DiPaolo et al., 1973).

II. MATERIALS AND METHODS

A. Chemicals

Technical-grade p-nitrosodiphenylamine was purchased from Uniroyal Chemical, Naugatuck, Connecticut. Chemical analysis was performed by Litton Bionetics, Inc., Kensington, Maryland. The experimentally determined melting point range was 139.5° to 142.5°C. Ultraviolet/visible analysis revealed λ_{max} at 268 and 420 nm with respective molar extinction coefficients of 1.02×10^4 and 3.63×10^4. Thin-layer chromatography was performed utilizing two solvent systems (i.e., chloroform:ammonium hydroxide and benzene:methanol). Each plate, visualized with ultraviolet and visible light, iodine vapor, and ferric chloride-potassium ferricyanide spray, revealed one single spot. Gravimetric analysis of the water content of the compound indicated 25.4 percent. The results of nuclear magnetic resonance analysis indicated that the technical-grade material contained 73 percent p-nitrosodiphenylamine. The results of infrared analysis were consistent with those expected based on the structure of the compound.

Throughout this report, the term p-nitrosodiphenylamine is used to represent this technical-grade material, and dose levels are expressed in terms of this techical-grade product.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® meal (Allied Mills, Inc., Chicago,

Illinois). p-Nitrosodiphenylamine was administered to the dosed animals as a component of the diet.

The chemical 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 5000 and 2500 ppm of p-nitrosodiphenylamine were analyzed spectrophotometrically. The mean result immediately after preparation was 92 percent of theoretical (ranging from 84 to 116 percent). After 11 days at ambient room temperature, the mean result was 90 percent of theoretical (ranging from 80 to 97 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 were supplied by A. R. Schmidt, Madison, Wisconsin, and Laboratory Supply Company, Inc., Indianapolis, Indiana. Mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

Rats and mice, approximately 4 weeks old when received, were examined and any obviously ill or runted animals were killed. After

2 weeks quarantine, animals which did not manifest clinical signs of disease were placed on test. 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 maintained at a temperature of 22° to 26°C and a 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 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 hardwood chip bedding (Ab-sorb-dri®, 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* Michler's ketone (90-94-8); trimethylthiourea (2489-77-2); and p-chloroaniline (106-47-8).

Dosed and control mice were housed in a room with mice receiving diets containing nitrofen (1836-75-5); acetylaminofluorene (53-96-3); amitrole (61-82-5); NTA trisodium salt (5064-31-3); nitrilotriacetic acid (139-13-9); and other mice intubated with styrene (100-42-5) and β-nitrostyrene (102-96-5)

E. Selection of Initial Concentrations

To establish the concentrations of p-nitrosodiphenylamine 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 six groups, each consisting of five males and five females. p-Nitrosodiphenylamine was incorporated into the basal laboratory diet and supplied ad libitum to five of the six rat groups in concentrations of 6800, 10,000, 14,670 21,560 and 31,530 ppm and to five of the six mouse groups in concentrations of 1180, 2550, 5500, 13,900 and 25,520 ppm. The remaining group of each species served as a control group, receiving only the basal laboratory diet.

*CAS registry numbers are given in parentheses.

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 euthanized and necropsied.

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

RAT SUBCHRONIC STUDY RESULTS

ppm	Mean Body Weight Gain (%)[a]		Survival[b]		Observation of Clinical Signs[b]	
	Males	Females	Males	Females	Males	Females
31,530	--	--	0/5	0/5	5/5[c]	5/5[c]
21,560	-53	--	1/5	0/5	5/5[c]	5/5[c]
14,670	-47	-20	1/5	3/5	5/5[c]	5/5[c]
10,000	-78	-58	5/5	5/5	5/5[d]	5/5[d]
6,800	-11	-18	5/5	5/5	5/5[d]	5/5[d]
0	--	--	5/5	5/5	0/5	0/5

The high concentration selected for administration to dosed rats in the chronic bioassay was 5000 ppm.

[a] - is indicative of mean body weight gain less than that of controls.
[b] Number of animals observed/number of animals originally in group.
[c] These rats had rough, yellow-stained fur and arched backs.
[d] These rats had yellow-stained bodies.

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

MOUSE SUBCHRONIC STUDY RESULTS

ppm	Mean Body Weight Gain (%)*		Survival**		Observation of Yellow-Stained Fur**	
	Males	Females	Males	Females	Males	Females
25,520	- 7	0	5/5	5/5	5/5	5/5
13,900	- 1	- 6	5/5	5/5	5/5	5/5
5,500	- 1	- 1	5/5	5/5	0/5	0/5
2,550	- 2	- 3	5/5	5/5	0/5	0/5
1,180	+ 2	- 2	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 mice in the chronic bioassay was 10,000 ppm.

F. Experimental Design

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

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed rats were supplied with diets containing 5000 and 2500 ppm p-nitrosodiphenylamine for 78 weeks followed by a 27-week observation period, when no

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

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
p-NITROSODIPHENYLAMINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	p-NITROSODI-PHENYLAMINE CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	105
LOW DOSE	50	2500	78	
		0		27
HIGH DOSE	50	5000	78	
		0		27
FEMALE				
CONTROL	20	0	0	105
LOW DOSE	50	2500	78	
		0		27
HIGH DOSE	50	5000	78	
		0		27

[a]Concentrations given in parts per million.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
p-NITROSODIPHENYLAMINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	p-NITROSODI-PHENYLAMINE CONCENTRATION[a]	OBSERVATION PERIOD		TIME-WEIGHTED AVERAGE CONCENTRATION[b]
			TREATED (WEEKS)	UNTREATED (WEEKS)	
MALE					
CONTROL	20	0	0	92	
LOW DOSE	50	5,000	40		4254
		2,500	17		
		0		35	
HIGH DOSE	50	10,000	40		9000
		0		7	
		5,000	10		
		0		35	
FEMALE					
CONTROL	20	0	0	92	
LOW DOSE	50	5,000	40		4254
		2,500	17		
		0		35	
HIGH DOSE	50	10,000	40		9000
		0		7	
		5,000	10		
		0		35	

[a]Concentrations given in parts per million.

[b]Time-weighted average concentration = $\frac{\Sigma(\text{concentration X weeks received})}{\Sigma(\text{weeks receiving chemical})}$

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. The initial dietary concentrations of p-nitrosodiphenylamine administered were 10,000 and 5000 ppm. Throughout this report those mice initially receiving the former concentration are referred to as the high dose groups and those initially receiving the latter concentration are referred to as the low dose groups. In week 41, compound administration to the high dose mice was terminated and the dietary concentration administered to the low dose mice was reduced to 2500 ppm. Low dose mice continued to receive this concentration for 17 weeks. In week 48, dietary administration of the compound to the high dose groups resumed at a concentration of 5000 ppm and was continued for 10 weeks. A 35-week observation period followed, when no test chemicals were used.

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. Food consumption

data were collected at monthly intervals from 20 percent of the animals in each group.

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

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

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

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

animals that were recorded in each group at the time that the test was initiated.

H. Data Recording and Statistical Analyses

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

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported

for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was

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

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

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

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week

during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

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

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would

be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

Dose-related mean body weight depression was apparent in rats of both sexes beginning in week 20 (Figure 2).

No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female rats in the control and p-nitrosodiphenylamine-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 92 percent (46/50) of the high dose, 86 percent (43/50) of the low dose, and 90 percent (18/20) of the controls survived on test for at least 105 weeks.

There were adequate numbers of female rats at risk from late-developing tumors, as 92 percent (46/50) of the high dose, 84 percent (42/50) of the low dose, and 85 percent (17/20) of the controls survived on test for at least 105 weeks.

C. Pathology

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

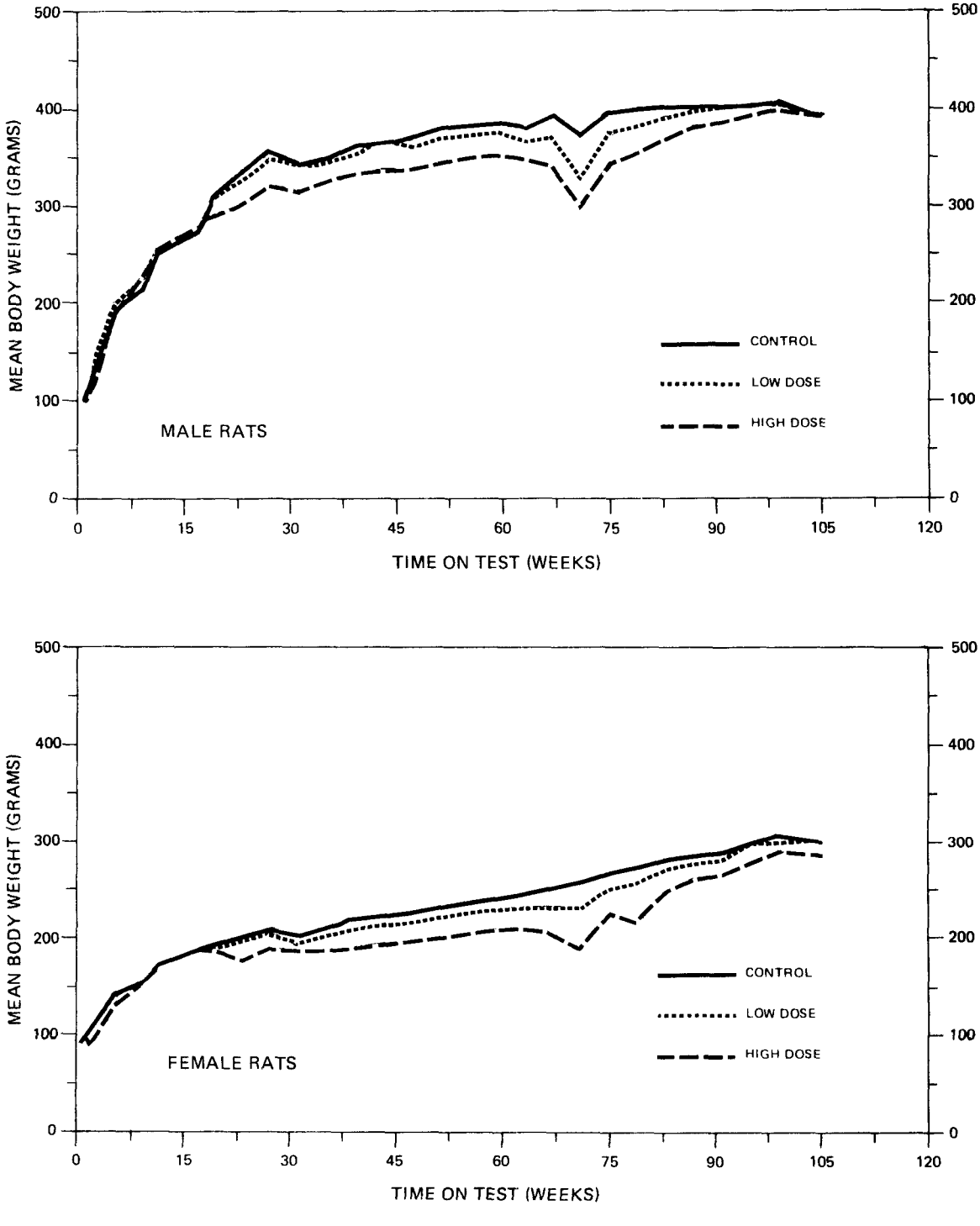


FIGURE 2
GROWTH CURVES FOR p-NITROSODIPHENYLAMINE CHRONIC STUDY RATS

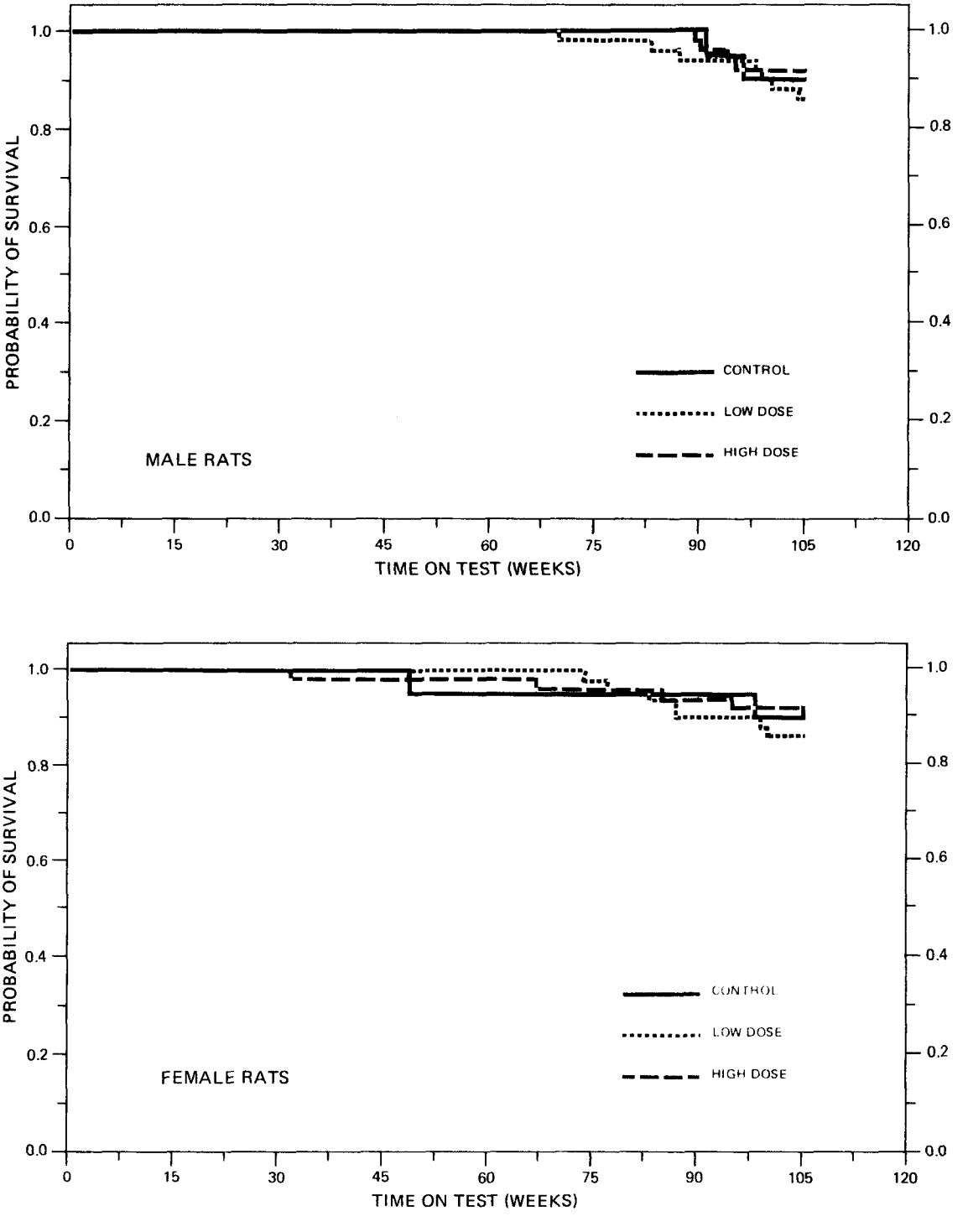


FIGURE 3
SURVIVAL COMPARISONS OF p-NITROSODIPHENYLAMINE CHRONIC STUDY RATS

The types of neoplasms observed in various tissues have been encountered previously in aging Fischer 344 rats. The incidence and type of neoplasms were not attributable to chemical exposure, except for those of the liver and lung.

The incidences of neoplastic nodules of the liver were 0/20 control males, 8/49 low dose males, 18/50 high dose males, 0/19 control females, 2/50 low dose females, and 5/48 high dose females. Hepatocellular carcinoma was seen in 0/20 control males, 2/49 low dose males and 1/50 high dose males. The neoplastic nodules were usually composed of eosinophilic hepatocytes forming cords one cell thick. Some livers had multiple nodules. Dilatation and sinusoids was a common finding within the nodules and adjacent hepatic parenchyma. Foci of cellular change (basophilic and eosinophilic) were seen in many control and dosed rats.

Lung neoplasms occurred in increased incidences in dosed rats. Alveolar/bronchiolar adenomas were seen in 1/19 control males, 0/49 low dose males, 9/50 high dose males, 2/20 control females, 2/49 low dose females, and 10/49 high dose females. They were usually singular and small tumors.

A variety of nonneoplastic lesions was observed among both control and dosed animals. However, all appeared to be unrelated by incidence and severity to test exposure and were not unlike those normally encountered in aging Fischer 344 rats.

Based on the results of this pathology examination, p-nitrosodiphenylamine was carcinogenic in Fischer 344 rats, causing a dose-related increased incidence of neoplastic nodules of the liver in male rats under the conditions of this bioassay.

D. Statistical Analysis of Results

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

In male rats the Cochran-Armitage test indicated a significant ($P = 0.001$) positive association between dose and the combined incidence of hepatocellular carcinomas or neoplastic nodules. This was supported by both a significant ($P < 0.001$) positive high dose to control Fisher exact comparison and by a significant ($P = 0.024$) positive low dose to control Fisher exact comparison. The Cochran-Armitage test also indicated a significant ($P = 0.013$) positive association between dose and the incidence of alveolar/bronchiolar adenomas, but this was not supported by either of the Fisher exact comparisons. The test for departure from linear trend was also significant for the alveolar/bronchiolar adenomas and for pheochromocytomas of the adrenal.

TABLE 3

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

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	1/19(0.05)	0/49(0.00)	9/50(0.18)
P Values[c]	P = 0.013	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.032	---	---
Relative Risk (Control)[d]	---	0.000	3.420
Lower Limit	---	0.000	0.536
Upper Limit	---	7.244	146.437
Weeks to First Observed Tumor	105	---	105
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	0/20(0.00)	3/50(0.06)	1/50(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.250	0.022
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	87	90
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	0/20(0.00)	10/49(0.20)	19/50(0.38)
P Values[c]	P = 0.001	P = 0.024	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	1.266	2.548
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	99	105

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	4/19(0.21)	5/45(0.11)	4/44(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.528	0.432
Lower Limit	---	0.132	0.092
Upper Limit	---	2.434	2.125
Weeks to First Observed Tumor	105	105	105
Adrenal: Pheochromocytoma[b]	2/20(0.10)	15/48(0.31)	4/49(0.08)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.004	---	---
Relative Risk (Control)[d]	---	3.125	0.816
Lower Limit	---	0.840	0.131
Upper Limit	---	26.507	8.603
Weeks to First Observed Tumor	105	104	105
Thyroid: C-Cell Carcinoma[b]	1/20(0.05)	0/46(0.00)	2/39(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	1.026
Lower Limit	---	0.000	0.058
Upper Limit	---	8.111	58.951
Weeks to First Observed Tumor	105	---	105

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	3/20(0.15)	5/46(0.11)	3/39(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.725	0.513
Lower Limit	---	0.160	0.077
Upper Limit	---	4.348	3.556
Weeks to First Observed Tumor	105	105	105
Pancreatic Islets: Islet-Cell Adenoma[b]	2/19(0.11)	6/47(0.13)	2/48(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.213	0.396
Lower Limit	---	0.247	0.031
Upper Limit	---	11.660	5.211
Weeks to First Observed Tumor	105	105	105
Testis: Interstitial-Cell Tumor or Interstitial-Cell Tumor, Malignant[b]	18/20(0.90)	47/49(0.96)	45/50(0.90)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.066	1.000
Lower Limit	---	0.937	0.877
Upper Limit	---	1.220	1.257
Weeks to First Observed Tumor	91	98	94

TABLE 3 (CONCLUDED)

[a]Treated groups received doses of 2500 or 5000 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 p-NITROSODIPHENYLAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	2/20(0.10)	2/49(0.04)	10/49(0.20)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.408	2.041
Lower Limit	---	0.032	0.498
Upper Limit	---	5.381	18.154
Weeks to First Observed Tumor	105	105	105
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	2/20(0.10)	4/50(0.08)	1/49(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.800	0.204
Lower Limit	---	0.128	0.004
Upper Limit	---	8.436	3.754
Weeks to First Observed Tumor	104	83	95
Liver: Neoplastic Nodule[b]	0/19(0.00)	2/50(0.04)	5/48(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.117	0.522
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	77	105

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	8/18(0.44)	24/43(0.56)	14/49(0.29)
P Values[c]	P = 0.044(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	1.256	0.643
Lower Limit	---	0.710	0.323
Upper Limit	---	2.637	1.513
Weeks to First Observed Tumor	98	104	95
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	2/18(0.11)	5/47(0.11)	1/41(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.957	0.220
Lower Limit	---	0.179	0.004
Upper Limit	---	9.541	4.008
Weeks to First Observed Tumor	105	87	105
Mammary Gland: Fibroadenoma[b]	2/20(0.10)	8/50(0.16)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.600	0.612
Lower Limit	---	0.364	0.078
Upper Limit	---	14.699	6.996
Weeks to First Observed Tumor	105	104	105

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma or Adenocarcinoma NOS[b]	3/20(0.15)	8/50(0.16)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.067	0.408
Lower Limit	---	0.295	0.061
Upper Limit	---	5.813	2.857
Weeks to First Observed Tumor	49	104	105
Uterus: Endometrial Stromal Polyp[b]	2/20(0.10)	6/50(0.12)	5/48(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.200	1.042
Lower Limit	---	0.243	0.192
Upper Limit	---	11.574	10.410
Weeks to First Observed Tumor	105	77	105

[a]Treated groups received doses of 2500 or 5000 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.

Based on these statistical results, p-nitrosodiphenylamine was carcinogenic to male Fischer 344 rats, inducing an elevated incidence of hepatocellular carcinomas or neoplastic nodules of the liver.

In female rats, none of the statistical tests indicated a significant positive association between dose and tumor incidence at any site. The Cochran-Armitage test did indicate a significant negative association between dose and the incidence of chromophobe adenomas of the pituitary gland.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

The mean body weights for both male and female high dose groups were consistently lower than those for controls after week 25 (Figure 4). Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variations.

No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the control and p-nitrosodiphenylamine-dosed groups are shown in Figure 5.

Of the 50 high dose male and female mice on test, 19 and 21, respectively, died before week 52 due to toxicity. The Tarone test for association between dosage and mortality was significant for both males ($P = 0.001$) and females ($P < 0.001$). The test for departure from linear trend for females was also significant. The Cox test comparing the high dose group to the control group was significant ($P = 0.0109$) for female mice.

There were adequate numbers of male mice at risk from late-developing tumors, as 60 percent (30/50) of the high dose, 88 percent (44/50) of the low dose and 85 percent (17/20) of the controls survived on test until the termination of the study.

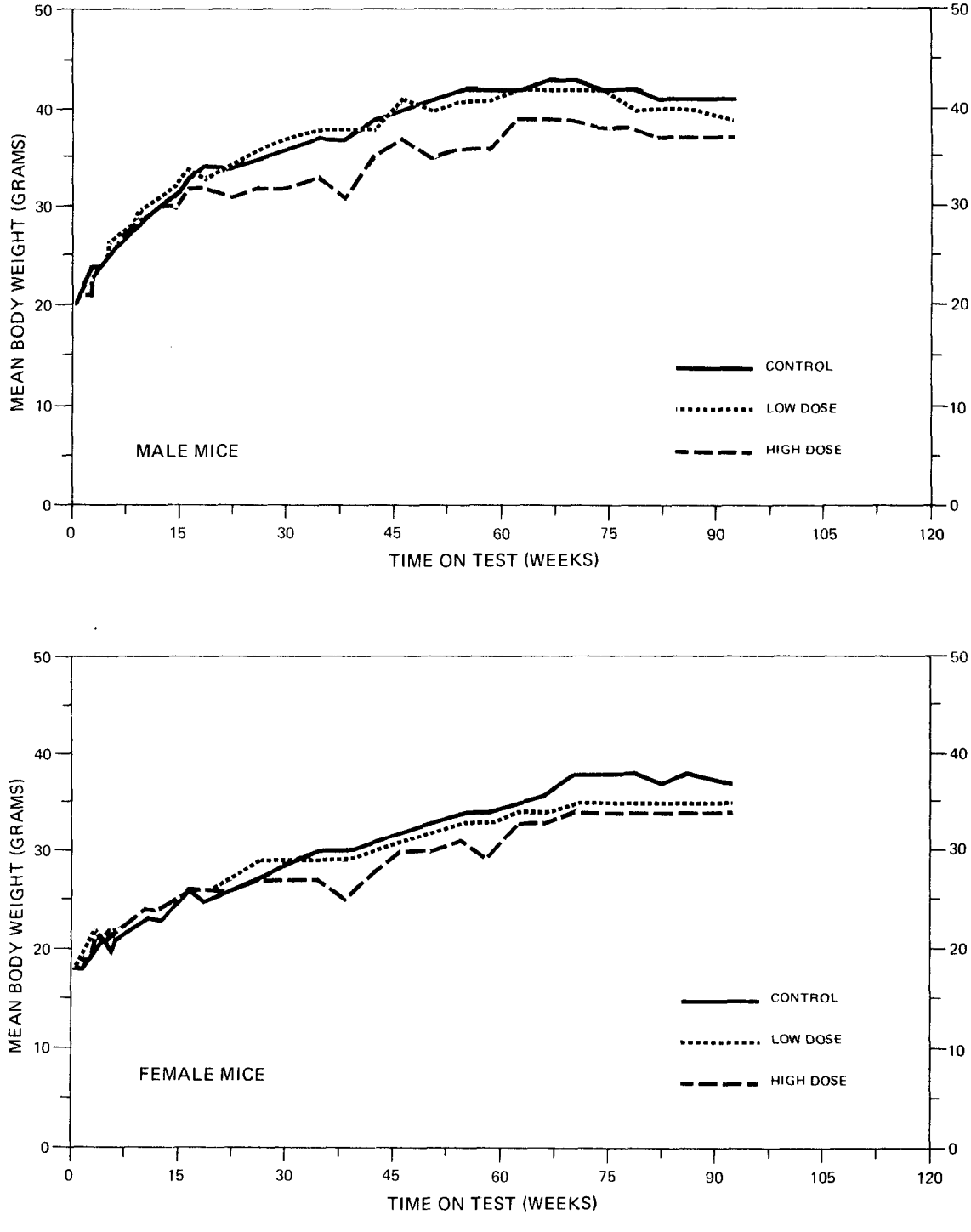
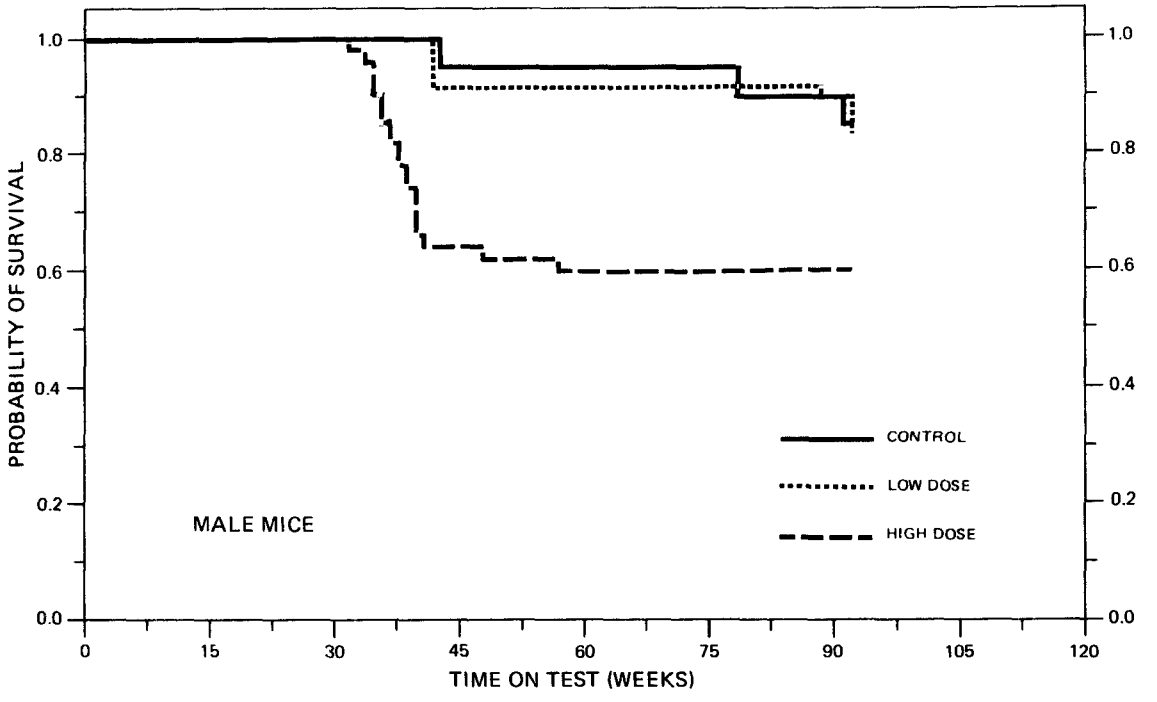


FIGURE 4
GROWTH CURVES FOR p-NITROSODIPHENYLAMINE CHRONIC STUDY MICE



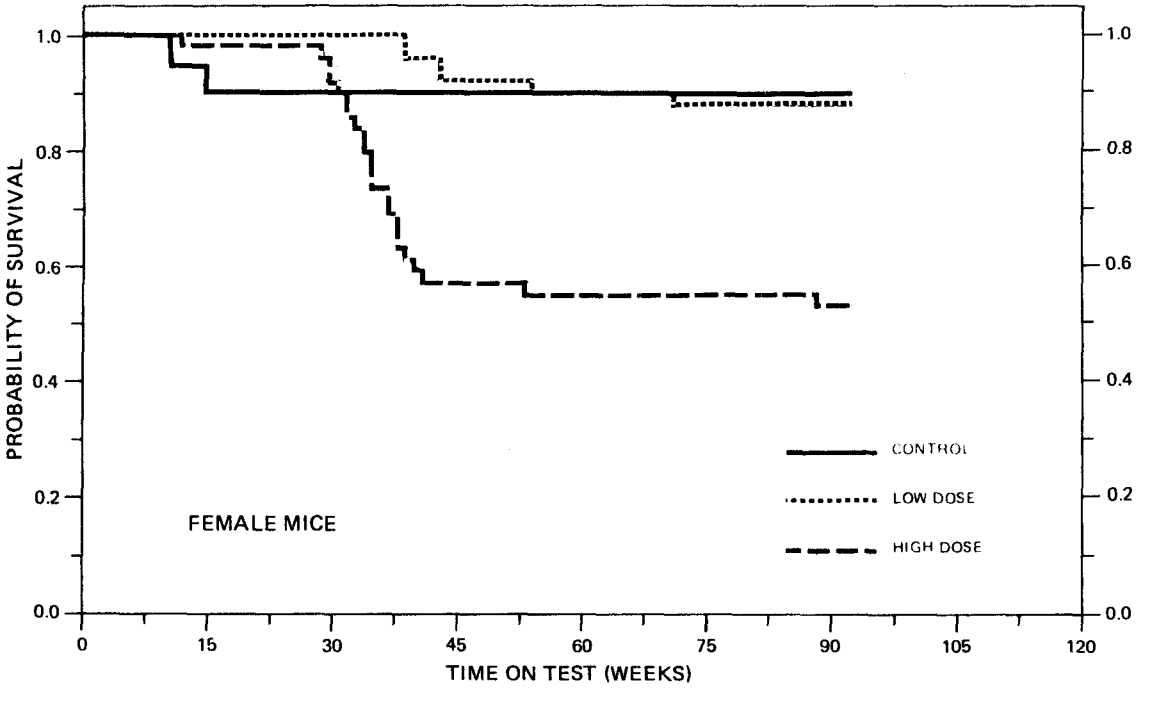


FIGURE 5
SURVIVAL COMPARISONS OF p-NITROSODIPHENYLAMINE CHRONIC STUDY MICE

There were adequate numbers of female mice at risk from late-developing tumors, as 52 percent (26/50) of the high dose, 84 percent (42/50) of the low dose and 90 percent (18/20) 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).

A variety of neoplasms occurred in approximately the same incidences in the control and dosed groups of mice. Most of these neoplasms are common in aging B6C3F1 mice independent of any treatment.

A few neoplasms occurred in higher frequency in dosed groups. The prevalent lesions were observed in the liver. Hepatocellular neoplasms occurred in 2/19 (11 percent) control males, 27/47 (57 percent) low dose males, 12/50 (24 percent) high dose males, 0/20 control females, 5/48 (10 percent) low dose females and 3/43 (7 percent) high dose females. In addition to the hepatocellular tumors, several mice had hepatocellular hyperplasia. All nodular hyperplasias and most hyperplasias NOS (cytomegaly) were seen in mice with a diagnosis of hepatocellular adenoma or hepatocellular carcinoma.

An unusual hepatocellular hyperplasia which was characterized by clusters of hepatocytic nuclei was observed in several mice. The cytoplasmic boundaries were unclear; however, the histologic appearance suggested that multinucleated or syncytial hepatocytes were present.

Many mice had various degrees of portal fibrosis and chronic inflammation of the liver. The distribution and types of hepatocellular proliferative and neoplastic lesions were:

	Control	Low Dose	High Dose
NUMBER OF MALES WITH LIVERS EXAMINED HISTOPATHOLOGICALLY	(19)	(47)	(50)
Number with Hepatocellular Proliferation and Neoplasia	2	30	22
Number with Hepatocellular Tumors	2	22	12
Hyperplasia NOS	0	20	1
Hyperplasia, Diffuse	0	0	8
Hyperplasia, Nodular	0	0	7
Hepatoblastoma	0	2	0
Hepatocellular Adenoma	2	17	11
Hepatocellular Carcinoma	0	10	1
Hepatocellular Carcinoma, Metastatic to Lung	0	1	0
NUMBER OF FEMALES WITH LIVERS EXAMINED HISTOPATHOLOGICALLY	(20)	(48)	(43)
Number with Hepatocellular Tumors	0	5	2
Hyperplasia, Nodular	1	3	1
Hepatocellular Adenoma	0	4	2
Hepatocellular Carcinoma	0	1	1

The hepatocellular proliferative and neoplastic lesions varied in morphology and arrangement from well-differentiated cells having an orderly plate arrangement to anaplastic cells having little resemblance to normal hepatic architecture. The hyperplastic hepatocytes resembled normal hepatocytes with enlarged nuclei and increased cytoplasm. The borders of the hyperplastic areas blended into adjacent

normal hepatic tissue. Hepatocellular adenomas also were composed of well-differentiated hepatocytes resembling normal hepatocytes; however, the arrangement was less orderly and a compression of adjacent hepatic tissue was apparent. Frequently the adenomas had hepatocytic degeneration and were of large size (1.0 to 2.5 cm). The carcinomas characteristically had a trabecular pattern and one had metastasized to the lungs. Two mice had hepatoblastomas which were highly cellular tumors with small basophilic nuclei, scanty cytoplasm, poorly defined cytoplasm, and little resemblance to hepatocytes.

Incidences of alveolar/bronchiolar adenomas were 1/20 (5 percent) in control males, 10/46 (22 percent) in low dose males, 4/49 (8 percent) in high dose males, 0/20 in control females, 2/49 (4 percent) in low dose females, and 1/45 (2 percent) in high dose females.

The pulmonary tumors were all well-differentiated alveolar/bronchiolar adenomas comprised of cuboidal to columnar cells aligned along the alveolar septa. In larger tumors, the cells projected into the alveolar spaces, resulting in the formation of numerous papillary structures. None of the tumors were classified as malignant. Alveolar/bronchiolar tumors are common in several strains of mice independent of treatment and vary in frequency from study to study.

Three low dose males (3/49, 6 percent) had a highly cellular neoplastic proliferation of the interstitial tissue in the testicles.

They were classified as interstitial-cell tumors, one benign and two malignant. None of these tumors were observed in the control (0/19) and high dose (0/48) males.

In addition to the neoplastic lesions, a few degenerative, proliferative, and inflammatory lesions were also encountered in animals of the dosed and control groups. Most of these lesions were types commonly encountered in aged B6C3F1 mice.

Although a few deaths were associated with inflammatory lesions, the cause of death for a majority of the mice that died prior to terminal sacrifice could not be determined. Several of these deaths were associated with convulsions and no significant microscopic changes were observed.

Based on the results of this pathology examination, p-nitrosodiphenylamine was carcinogenic in B6C3F1 mice, inducing neoplastic and associated nonneoplastic liver lesions under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or p-nitrosodiphenylamine-dosed groups and where such tumors were observed in at least 5 percent of the group. These analyses have been based upon those mice surviving at least 52 weeks.

TABLE 5

TIME-ADJUSTED ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH p-NITROSODIPHENYLAMINE[a,f]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	1/19(0.05)	10/41(0.24)	4/31(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	4.634	2.452
Lower Limit	---	0.751	0.272
Upper Limit	---	194.874	116.423
Weeks to First Observed Tumor	92	92	92
Hematopoietic System: Malignant Lymphoma[b]	2/19(0.11)	0/45(0.00)	2/31(0.06)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.044	---	---
Relative Risk (Control)[d]	---	0.000	0.613
Lower Limit	---	0.000	0.049
Upper Limit	---	1.416	7.930
Weeks to First Observed Tumor	92	---	92
Liver: Hepatocellular Carcinoma[b]	0/18(0.00)	10/42(0.24)	1/31(0.03)
P Values[c]	N.S.	P = 0.020	N.S.
Departure from Linear Trend[e]	P = 0.002	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	1.342	0.032
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	92	92

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	2/18(0.11)	22/42(0.52)	12/31(0.39)
P Values[c]	N.S.	P = 0.002	P = 0.038
Departure from Linear Trend[e]	P = 0.011	---	---
Relative Risk (Control)[d]	---	4.714	3.484
Lower Limit	---	1.378	0.918
Upper Limit	---	37.768	29.171
Weeks to First Observed Tumor	92	92	92
Testis: Interstitial-Cell Tumor or Interstitial-Cell Tumor, Malignant[b]	0/18(0.00)	3/44(0.07)	0/31(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.258	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	92	---

[a]Treated groups received time-weighted average doses of 4254 or 9000 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 $P < 0.05$.

[f]These analyses were based solely upon animals surviving at least 52 weeks.

TABLE 6

TIME-ADJUSTED ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE MICE TREATED WITH p-NITROSODIPHENYLAMINE[a,e]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Malignant Lymphoma[b]	2/18(0.11)	4/45(0.09)	4/28(0.14)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.800	1.286
Lower Limit	---	0.130	0.210
Upper Limit	---	8.389	13.164
Weeks to First Observed Tumor	92	92	88
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	0/18(0.00)	5/44(0.11)	2/26(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.542	0.214
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	92	92
Uterus: Endometrial Stromal Polyp[b]	0/18(0.00)	3/45(0.07)	0/27(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.252	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	92	---

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Ovary: Cystadenoma[b]	0/12(0.00)	2/30(0.07)	0/14(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.128	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	92	---

[a]Treated groups received time-weighted average doses of 4254 or 9000 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]These analyses were based solely upon animals surviving at least 52 weeks.

Due to the large number of early deaths in high dose mice of both sexes, the statistical conclusion of carcinogenicity was based on low dose to control Fisher exact comparisons.

In male mice the Fisher exact test comparing low dose to control indicated a significant (P = 0.020) positive association between dose and the incidence of hepatocellular carcinomas. The test for departure from linear trend was also significant. The Fisher exact test comparing low dose to control further indicated a significant (P = 0.002) positive association between dose and the combined incidence of hepatocellular carcinomas or hepatocellular adenomas.

Based on these statistical results, p-nitrosodiphenylamine was carcinogenic to male B6C3F1 mice, inducing an elevated incidence of hepatocellular carcinomas and an increased combined incidence of hepatocellular carcinomas or hepatocellular adenomas.

None of the statistical tests were significant at any site for female mice.

V. DISCUSSION

There were significant positive associations between the concentrations of p-nitrosodiphenylamine administered and mortality among male and female mice, but not for rats of either sex. In mice, dosing was altered after 40 weeks because of toxicity. Low dose mice were placed on diets containing half the initial concentration of the test chemical; dosing was discontinued for 7 weeks for the high dose mice and resumed at half the initial level for 10 additional weeks. Although 19/50 high dose male mice and 21/50 high dose female mice died before week 52, adequate numbers of mice and rats in all groups survived sufficiently long to be at risk from late-developing tumors. The toxicity observed in mice and the dose-related mean body weight depression apparent in male and female rats indicated that the concentrations of p-nitrosodiphenylamine administered to these animals in this bioassay may have approached or exceeded the maximum tolerated concentrations.

In male rats, there was a significant positive association between concentration administered and the incidence of a combination of hepatocellular carcinomas and neoplastic nodules. In addition, both the high dose to control and the low dose to control Fisher exact comparisons were significant. There was also a significant positive association between concentration administered and the incidence of alveolar/bronchiolar adenomas in male rats; however, neither of

the Fisher exact comparisons were significant. There were no positive, significant statistical tests for tumor incidence at any site in female rats.

Due to the large number of early deaths among high dose mice of both sexes, the statistical conclusion concerning carcinogenicity was based on comparisons between the low dose and control groups. The incidence of hepatocellular carcinomas was significantly higher among the low dose males than among their controls. Although hepatocellular neoplasms were observed in dosed females, there were no tumors occurring with a significantly increased incidence when low dose females were compared to their controls.

Under the conditions of this bioassay, p-nitrosodiphenylamine was carcinogenic when administered in the diet to male B6C3F1 mice, causing hepatocellular carcinomas. The chemical was also carcinogenic in male Fischer 344 rats, causing liver neoplasms. No evidence was provided for the carcinogenicity of p-nitrosodiphenylamine in female B6C3F1 mice or in female Fischer 344 rats.

VI. BIBLIOGRAPHY

Armitage, P., Statistical Methods in Medical Research, Chapter 14. J. Wiley & Sons, New York, 1971.

Berenblum, I., editor, Carcinogenicity Testing. International Union Against Cancer, Technical Report Series, Vol. 2. International Union Against Cancer, Geneva, 1969.

Chemical Abstracts Service, The Chemical Abstracts Service (CAS) Ninth Collective Index, Volumes 76-85, 1972-1976. American Chemical Society, Washington, D.C., 1977.

Clayson, D.B. and R.C. Garner, "Carcinogenic Aromatic Amines and Related Compounds." Chapter 8 in Carcinogenic Aromatic Amines, C.E. Searle, editor. American Chamical Society Monograph 173, Washington, D.C., 1976.

Cox, D.R., Analysis of Binary Data, Chapters 4 and 5. Methuen and Co., Ltd., London, 1970.

Cox, D.R., "Regression Models and Life-Tables." Journal of the Royal Statistical Society, Series "B" 34:187-220, 1972.

DiPaolo, J.A., R.L. Nelson, P.J. Donovan, and C.H. Evans, "Host-Mediated in Vivo-in Vitro Assay for Chemical Carcinogenesis." Archives of Pathology 95:380-385, 1973.

Gart, J.J., "The Comparison of Proportions: A Review of Significance Tests, Confidence Limits, and Adjustments for Stratification." International Statistical Institute Review 39:148-169, 1971.

Kaplan, E.L., and P. Meier, "Nonparametric Estimation from Incomplete Observations." Journal of the American Statistical Association 53:457-481, 1958.

Linhart, M.S., J.A. Cooper, R.L. Martin, N.P. Page, and J.A. Peters, "Carcinogenesis Bioassay Data System." Computers and Biomedical Research 7:230-248, 1974.

Miller, R.G., Simultaneous Statistical Inference. McGraw-Hill Book Co., New York, 1966.

Naugatuck® Chemicals, "TKB." Uniroyal Chemical, Uniroyal, Inc., Naugatuck, Connecticut, undated.

Saffiotti, U., R. Montesano, A.R. Sellakumar, F. Cefis, and D.G. Kaufman, "Respiratory Tract Carcinogenesis in Hamsters Induced by Different Numbers of Administration of Benzo (a) Pyrene and Ferric Oxide." Cancer Research 32:1073-1079, 1972.

Tarone, R.E., "Tests for Trend in Life-Table Analysis." Biometrika 62:679-682, 1975.

U.S. International Trade Commission, Synthetic Organic Chemicals: United States Production and Sales, 1975. USITC Publication 804, U.S. Government Printing Office, Washington, D.C., 1977.

Weisburger, E.K., "Industrial Cancer Risks." Dangerous Properties of Industrial Materials, 4th edition. N.I. Sax, editor. Van Nostrand Reinhold Company, New York, 1975.

Windholz, M., editor. The Merck Index: An Encyclopedia of Chemicals and Drugs, 9th edition. Merck and Co., Inc., Rahway, New Jersey, 1976.

Wynder, E.L., J. Onderdonk, and N. Mantel, "An Epidemiological Investigation of Cancer of the Bladder." Cancer 16:1388-1407, 1963.

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

October 25, 1978

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

The reviewer said that *p*-Nitrosodiphenylamine was judged to be carcinogenic in male mice and rats, under the conditions of test, inducing liver neoplasms in both species. After briefly describing the experimental design, he said that, despite a number of experimental shortcomings, the study was still acceptable. The reviewer concluded, based on the results of the study, that *p*-Nitrosodiphenylamine should be considered a potential human carcinogen.

There was no objection to a recommendation that the report on the bioassay of *p*-Nitrosodiphenylamine 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.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH p-NITROSODIPHENYLAMINE

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

	CONTROL(UNTR) 11-1165	LOW DOSE 11-1163	HIGH DOSE 11-1161
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SEBACEOUS ADENOCARCINOMA			1 (2%)
KERATOACANTHOMA			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
FIBROMA		1 (2%)	1 (2%)
HEMANGIOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)		9 (18%)
CARCINOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		2 (4%)	
GRANULOCYTIC LEUKEMIA		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
NEOPLASTIC NODULE		8 (16%)	18 (36%)

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1165	LOW DOSE 11-1163	HIGH DOSE 11-1161
HEPATOCELLULAR CARCINOMA		2 (4%)	1 (2%)
#COLON	(20)	(47)	(49)
ADENOCARCINOMA, NOS			1 (2%)
#COLONIC MUCOUS MEMBR	(20)	(47)	(49)
ADENOMA, NOS			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(45)	(44)
CHROMOPHOBE ADENOMA	4 (21%)	5 (11%)	4 (9%)
#ADRENAL	(20)	(48)	(49)
CORTICAL ADENOMA	1 (5%)	1 (2%)	
PHEOCHROMOCYTOMA	2 (10%)	15 (31%)	4 (8%)
GANGLIONEUROMA			1 (2%)
#THYROID	(20)	(46)	(39)
FOLLICULAR-CELL ADENOMA			1 (3%)
FOLLICULAR-CELL CARCINOMA	1 (5%)		
C-CELL ADENOMA	2 (10%)	5 (11%)	1 (3%)
C-CELL CARCINOMA	1 (5%)		2 (5%)
#PANCREATIC ISLETS	(19)	(47)	(48)
ISLET-CELL ADENOMA	2 (11%)	6 (13%)	2 (4%)
REPRODUCTIVE SYSTEM			
#MAMMARY GLAND	(20)	(50)	(50)
FIBROADENOMA		1 (2%)	
#PROSTATE	(19)	(47)	(48)
PAPILLARY ADENOMA			1 (2%)
ACINAR-CELL ADENOMA		1 (2%)	
#TESTIS	(20)	(49)	(50)
INTERSTITIAL-CELL TUMOR		5 (10%)	6 (12%)

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1165	LOW DOSE 11-1163	HIGH DOSE 11-1161
INTERSTITIAL-CELL TUMOR, MALIGNA	18 (90%)	42 (86%)	39 (78%)
NERVOUS SYSTEM			
*BRAIN	(20)	(49)	(48)
GLIOMA, NOS	1 (5%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*MUSCLE OF THORAX	(20)	(50)	(50)
CARCINOSARCOMA		1 (2%)	
BODY CAVITIES			
*TUNICA VAGINALIS	(20)	(50)	(50)
MESOTHELIOMA, NOS	1 (5%)		1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	4	4
MORIBUND SACRIFICE	1	3	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	43	46
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 11-1165	LOW DOSE 11-1163	HIGH DOSE 11-1161
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	49	50
TOTAL PRIMARY TUMORS	34	98	96
TOTAL ANIMALS WITH BENIGN TUMORS	10	29	23
TOTAL BENIGN TUMORS	12	40	32
TOTAL ANIMALS WITH MALIGNANT TUMORS	19	44	41
TOTAL MALIGNANT TUMORS	21	50	45
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	8	19
TOTAL UNCERTAIN TUMORS	1	8	19
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 p-NITROSODIPHENYLAMINE

	CONTROL (UNIT) 11-1166	LOW DOSE 11-1164	HIGH DOSE 11-1162
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(49)
PAPILLOMA, NOS			1 (2%)
KERATOACANTHOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)	2 (4%)	10 (20%)
C-CELL CARCINOMA, METASTATIC		2 (4%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS	2 (10%)	2 (4%)	1 (2%)
GRANULOCYTIC LEUKEMIA		2 (4%)	
*SKIN OF EAR	(20)	(50)	(49)
PLASMA-CELL TUMOR		1 (2%)	
#LYMPH NODE	(13)	(38)	(34)
C-CELL CARCINOMA, METASTATIC		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(50)	(48)
NEOPLASTIC NODULE		2 (4%)	5 (10%)

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1166	LOW DOSE 11-1164	HIGH DOSE 11-1162
*STOMACH	(19)	(49)	(48)
FIBROSARCOMA	1 (5%)		
URINARY SYSTEM			
*KIDNEY	(20)	(49)	(49)
ADENOCARCINOMA, NOS			1 (2%)
ENDOCRINE SYSTEM			
*PITUITARY	(18)	(43)	(49)
ADENOCARCINOMA, NOS	1 (6%)		
CHROMOPHOBE ADENOMA	8 (44%)	24 (56%)	14 (29%)
*ADRENAL	(19)	(49)	(49)
CORTICAL ADENOMA		1 (2%)	1 (2%)
PHEOCHROMOCYTOMA	1 (5%)	2 (4%)	
*THYROID	(18)	(47)	(41)
FOLLICULAR-CELL ADENOMA		1 (2%)	
C-CELL ADENOMA	2 (11%)	3 (6%)	
C-CELL CARCINOMA		2 (4%)	1 (2%)
*PANCREATIC ISLETS	(19)	(48)	(47)
ISLET-CELL ADENOMA		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
ADENOCARCINOMA, NOS	1 (5%)		
FIBROADENOMA	2 (10%)	8 (16%)	3 (6%)
*UTERUS	(20)	(50)	(48)
ADENOCARCINOMA, NOS	1 (5%)	1 (2%)	1 (2%)
ENDOMETRIAL STROMAL POLYP	2 (10%)	6 (12%)	5 (10%)
NERVOUS SYSTEM			
*BRAIN	(20)	(48)	(49)
GLIOMA, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1166	LOW DOSE 11-1164	HIGH DOSE 11-1162
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@		6	4
MORIBUND SACRIFICE	2	1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	43	45
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL(UNTR) 11-1166	LOW DOSE 11-1164	HIGH DOSE 11-1162
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	36	28
TOTAL PRIMARY TUMORS	23	60	44
TOTAL ANIMALS WITH BENIGN TUMORS	9	32	25
TOTAL BENIGN TUMORS	17	49	35
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	8	4
TOTAL MALIGNANT TUMORS	6	8	4
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	
TOTAL SECONDARY TUMORS		3	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		3	5
TOTAL UNCERTAIN TUMORS		3	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH p-NITROSODIPHENYLAMINE

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

	CONTROL (UNTR) 22-2165	LOW DOSE 22-2163	HIGH DOSE 22-2161
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)	(46)	(49)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	10 (22%)	4 (8%)
HEMATOPOIETIC SYSTEM			
*MEDIASTINAL L.NODE	(9)	(31)	(30)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (3%)
*MESENTERIC L. NODE	(9)	(31)	(30)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (11%)		
*PEYERS PATCH	(19)	(47)	(46)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*LIVER	(19)	(47)	(50)
HEPATOCELLULAR ADENOMA	2 (11%)	17 (36%)	11 (22%)
HEPATOCELLULAR CARCINOMA		10 (21%)	1 (2%)

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 22-2165	LOW DOSE 22-2163	HIGH DOSE 22-2161
HEPATOBLASTOMA		2 (4%)	
HEMANGIOMA		1 (2%)	1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(10)	(42)	(33)
PHEOCHROMOCYTOMA			1 (3%)
#THYROID	(14)	(41)	(34)
FOLLICULAR-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
#TESTIS	(19)	(49)	(48)
INTERSTITIAL-CELL TUMOR		1 (2%)	
INTERSTITIAL-CELL TUMOR, MALIGNA		2 (4%)	
HEMANGIOMA		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(20)	(50)	(50)
PAPILLARY ADENOMA			1 (2%)
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) 22-2165	LOW DOSE 22-2163	HIGH DOSE 22-2161
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	8	20
MORIBUND SACRIFICE	1		
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	17	41	30
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	30	17
TOTAL PRIMARY TUMORS	5	45	21
TOTAL ANIMALS WITH BENIGN TUMORS	3	24	15
TOTAL BENIGN TUMORS	3	31	18
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	13	3
TOTAL MALIGNANT TUMORS	2	14	3
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

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

	CONTROL (UNTR) 22-2166	LOW DOSE 22-2164	HIGH DOSE 22-2162
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	1
ANIMALS NECROPSIED	20	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(45)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(49)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	2 (4%)	
MALIGNANT LYMPHOMA, MIXED TYPE			2 (4%)
#SPLEEN	(19)	(45)	(42)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#ILEUM	(17)	(46)	(35)
MALIGNANT LYMPHOMA, NOS			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(18)	(41)	(28)
ADENOMA, NOS			1 (4%)

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2166	LOW DOSE 22-2164	HIGH DOSE 22-2162
#LIVER	(20)	(48)	(43)
HEPATOCELLULAR ADENOMA		4 (8%)	2 (5%)
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)
HEMANGIOMA		1 (2%)	1 (2%)
#PANCREAS	(20)	(43)	(40)
ADENOMA, NOS			1 (3%)
#ESOPHAGUS	(15)	(43)	(38)
SQUAMOUS CELL PAPILLOMA	1 (7%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(38)	(21)
CHROMOPHOBE ADENOMA	1 (6%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(49)	(43)
ENDOMETRIAL STROMAL POLYP		3 (6%)	
#OVARY	(14)	(32)	(28)
CYSTADENOMA, NOS		2 (6%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 22-2166	LOW DOSE 22-2164	HIGH DOSE 22-2162
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	4	23
MORIBUND SACRIFICE		2	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	18	42	26
ANIMAL MISSING		1	1
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	16	9
TOTAL PRIMARY TUMORS	4	17	11
TOTAL ANIMALS WITH BENIGN TUMORS	2	12	5
TOTAL BENIGN TUMORS	2	12	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	5	5
TOTAL MALIGNANT TUMORS	2	5	5
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH p-NITROSODIPHENYLAMINE

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

	CONTROL(UNTR) 11-1165	LOW DOSE 11-1163	HIGH DOSE 11-1161
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, CHRONIC		1 (2%)	
RESPIRATORY SYSTEM			
*NASAL MUCOSA	(20)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)
HYPERPLASIA, EPITHELIAL			1 (2%)
KERATIN-PEARL FORMATION			1 (2%)
*TRACHEA	(20)	(46)	(47)
INFLAMMATION, CHRONIC	16 (80%)	23 (50%)	28 (60%)
*LUNG/BRONCHUS	(19)	(49)	(50)
BRONCHIECTASIS			1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE		1 (2%)	
*LUNG	(19)	(49)	(50)
CONGESTION, NOS			2 (4%)
HEMORRHAGE	2 (11%)		1 (2%)
BRONCHOPNEUMONIA, NOS			1 (2%)
PNEUMONIA, CHRONIC MURINE	7 (37%)	8 (16%)	8 (16%)
HEMATOPOIETIC SYSTEM			
*SPLEEN	(20)	(49)	(50)
HEMORRHAGE			1 (2%)
*LYMPH NODE	(16)	(38)	(39)
LYMPHANGIECTASIS	1 (6%)	2 (5%)	1 (3%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-1165	LOW DOSE 11-1163	HIGH DOSE 11-1161
HYPERPLASIA, LYMPHOID	1 (6%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(49)	(50)
INFLAMMATION, CHRONIC	16 (80%)	36 (73%)	24 (48%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
CYST, NOS			1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
DEGENERATION, HYDROPIC		1 (2%)	
NECROSIS, FOCAL		1 (2%)	
LIPOIDOSIS	2 (10%)	5 (10%)	3 (6%)
BASOPHILIC CYTO CHANGE	13 (65%)	14 (29%)	2 (4%)
FOCAL CELLULAR CHANGE		9 (18%)	28 (56%)
GLYCOGENIC CELL		1 (2%)	
HYPERPLASIA, NOS	1 (5%)	2 (4%)	
ANGIECTASIS		21 (43%)	35 (70%)
#BILE DUCT	(20)	(49)	(50)
INFLAMMATION, CHRONIC	7 (35%)		
HYPERPLASIA, NOS	16 (80%)	1 (2%)	
#PANCREAS	(19)	(47)	(48)
PERIARTERITIS	1 (5%)		
#PANCREATIC ACINUS	(19)	(47)	(48)
ATROPHY, NOS		1 (2%)	3 (6%)
#COLON	(20)	(47)	(49)
ULCER, ACUTE	1 (5%)		
NEMATODIASIS		12 (26%)	9 (18%)
#COLONIC SEROSA	(20)	(47)	(49)
INFLAMMATION, CHRONIC	1 (5%)		
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
INFLAMMATION, CHRONIC	18 (90%)	26 (53%)	42 (84%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-1165	LOW DOSE 11-1163	HIGH DOSE 11-1161
ENDOCRINE SYSTEM			
#ADRENAL	(20)	(48)	(49)
NECROSIS, MEDULLARY		1 (2%)	
ANGIECTASIS	1 (5%)		3 (6%)
#ADRENAL CORTEX	(20)	(48)	(49)
LIPOIDOSIS	1 (5%)		
ATROPHY, NOS			1 (2%)
HYPERTROPHY, NOS			1 (2%)
HYPERPLASIA, NOS		1 (2%)	
#THYROID	(20)	(46)	(39)
FOLLICULAR CYST, NOS		1 (2%)	
HEMORRHAGIC CYST		1 (2%)	
HYPERPLASIA, C-CELL		2 (4%)	
REPRODUCTIVE SYSTEM			
#PROSTATE	(19)	(47)	(48)
INFLAMMATION, ACUTE	1 (5%)	6 (13%)	5 (10%)
#TESTIS	(20)	(49)	(50)
HEMORRHAGE		1 (2%)	
ATROPHY, NOS	18 (90%)	44 (90%)	39 (78%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE C1 (CONCLUDED)

	CONTROL(UNTR) 11-1165	LOW DOSE 11-1163	HIGH DOSE 11-1161
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/HISTO PERF		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 p-NITROSODIPHENYLAMINE

	CONTROL (UNTR) 11-1166	LOW DOSE 11-1164	HIGH DOSE 11-1162
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(49)
INFLAMMATION, ACUTE/CHRONIC	1 (5%)		
RESPIRATORY SYSTEM			
#TRACHEA	(18)	(49)	(47)
INFLAMMATION, CHRONIC	11 (61%)	34 (69%)	26 (55%)
*LUNG	(20)	(49)	(49)
CONGESTION, NOS			2 (4%)
EDEMA, NOS			1 (2%)
BRONCHOPNEUMONIA, NOS		1 (2%)	
BRONCHOPNEUMONIA, ACUTE			1 (2%)
PNEUMONIA, CHRONIC MURINE	7 (35%)	15 (31%)	12 (24%)
ABSCESS, CHRONIC		1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM			3 (6%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(12)	(39)	(43)
MYELOFIBROSIS	1 (8%)		
#SPLEEN	(19)	(50)	(47)
HEMATOPOIESIS	1 (5%)	3 (6%)	
#LYMPH NODE	(13)	(38)	(34)
LYMPHANGIECTASIS	1 (8%)	1 (3%)	
HEMORRHAGE		1 (3%)	
#CERVICAL LYMPH NODE	(13)	(38)	(34)
HEMOSIDEROSIS			1 (3%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1166	LOW DOSE 11-1164	HIGH DOSE 11-1162
*MISENTERIC L. NODE	(13)	(38)	(34)
LYMPHANGIECTASIS		2 (5%)	
HYPERPLASIA, RETICULUM CELL			1 (3%)
CIRCULATORY SYSTEM			
*MYOCARDIUM	(20)	(49)	(49)
INFLAMMATION, CHRONIC	7 (35%)	11 (22%)	9 (18%)
DIGESTIVE SYSTEM			
*LIVER	(19)	(50)	(48)
HEMORRHAGIC CYST			1 (2%)
NECROSIS, FOCAL		1 (2%)	
LIPOIDOSIS	1 (5%)	3 (6%)	1 (2%)
BASOPHILIC CYTO CHANGE	17 (89%)	37 (74%)	14 (29%)
FOCAL CELLULAR CHANGE		1 (2%)	9 (19%)
HYPERPLASIA, NOS	2 (11%)	2 (4%)	1 (2%)
ANGIECTASIS	1 (5%)	2 (4%)	4 (8%)
*BILE DUCT	(19)	(50)	(48)
INFLAMMATION, CHRONIC	4 (21%)	2 (4%)	1 (2%)
HYPERPLASIA, NOS	6 (32%)	12 (24%)	5 (10%)
*PANCREAS	(19)	(48)	(47)
INFLAMMATION, ACUTE/CHRONIC	1 (5%)		
*PANCREATIC ACINUS	(19)	(48)	(47)
ATROPHY, NOS	3 (16%)	4 (8%)	2 (4%)
*STOMACH	(19)	(49)	(48)
ULCER, ACUTE			1 (2%)
*COLON	(20)	(49)	(47)
ULCER, ACUTE		1 (2%)	
NEMATODIASIS	3 (15%)	5 (10%)	13 (28%)
URINARY SYSTEM			
*KIDNEY	(20)	(49)	(49)
INFLAMMATION, CHRONIC	4 (20%)	6 (12%)	5 (10%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1166	LOW DOSE 11-1164	HIGH DOSE 11-1162
#URINARY BLADDER	(18)	(44)	(44)
HYPERPLASIA, EPITHELIAL			3 (7%)
#U.BLADDER/SUBMUCOSA	(18)	(44)	(44)
HEMORRHAGE			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(43)	(49)
CYST, NOS	4 (22%)	9 (21%)	10 (20%)
HEMORRHAGIC CYST	1 (6%)	1 (2%)	3 (6%)
#ADRENAL	(19)	(49)	(49)
LIPOIDOSIS			1 (2%)
ANGIECTASIS	2 (11%)	1 (2%)	1 (2%)
#ADRENAL CORTEX	(19)	(49)	(49)
HYPERPLASIA, NOS			2 (4%)
#THYROID	(18)	(47)	(41)
HYPERPLASIA, C-CELL		2 (4%)	
REPRODUCTIVE SYSTEM			
#MAMMARY GLAND	(20)	(50)	(49)
GALACTOCELE	2 (10%)	2 (4%)	2 (4%)
CYST, NOS		1 (2%)	
#UTERUS	(20)	(50)	(48)
HYDROMETRA	3 (15%)	3 (6%)	4 (8%)
HEMOSIDEROSIS		1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(50)	(48)
CYST, NOS	2 (10%)	5 (10%)	3 (6%)
INFLAMMATION, ACUTE	4 (20%)	4 (8%)	3 (6%)
INFLAMMATION, ACUTE SUPPURATIVE	3 (15%)	9 (18%)	6 (13%)
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, CYSTIC	1 (5%)	1 (2%)	1 (2%)
#OVARY	(19)	(49)	(44)
CYST, NOS	5 (26%)	5 (10%)	8 (18%)
FOLLICULAR CYST, NOS	2 (11%)	3 (6%)	2 (5%)

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

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 11-1166	LOW DOSE 11-1164	HIGH DOSE 11-1162
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE	1 (5%)		
ABSCESS, CHRONIC			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(48)	(49)
HEMORRHAGE		1 (2%)	
ATROPHY, PRESSURE	2 (10%)	1 (2%)	4 (8%)
#CEREBELLUM	(20)	(48)	(49)
HEMORRHAGE			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
SOLE OF FOOT			
INFLAMMATION, CHRONIC			1
ADIPOSE TISSUE			
LIPOGRANULOMA	1	1	
SPECIAL MORPHOLOGY SUMMARY			
AUTOLYSIS/NO NECROPSY			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 p-NITROSODIPHENYLAMINE

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

	CONTROL (UNTR) 22-2165	LOW DOSE 22-2163	HIGH DOSE 22-2161
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
ABSCESS, CHRONIC		1 (2%)	
RESPIRATORY SYSTEM			
*LUNG	(20)	(46)	(49)
INFLAMMATION, INTERSTITIAL			5 (10%)
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	3 (6%)
HEMATOPOIETIC SYSTEM			
*SPLEEN	(17)	(47)	(48)
HYPERPLASIA, LYMPHOID		1 (2%)	
*BRONCHIAL LYMPH NODE	(9)	(31)	(30)
HEMORRHAGE	1 (11%)		
HYPERPLASIA, LYMPHOID			1 (3%)
*MESENTERIC L. NODE	(9)	(31)	(30)
INFLAMMATION, HEMORRHAGIC	1 (11%)		
HYPERPLASIA, LYMPHOID	1 (11%)	1 (3%)	
*THYMUS	(5)	(6)	(10)
INFLAMMATION, GRANULOMATOUS			1 (10%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2165	LOW DOSE 22-2163	HIGH DOSE 22-2161
DIGESTIVE SYSTEM			
#LIVER	(19)	(47)	(50)
INFLAMMATION, NECROTIZING			2 (4%)
NECROSIS, COAGULATIVE		1 (2%)	
CYTOPLASMIC VACUOLIZATION		1 (2%)	
CLEAR-CELL CHANGE		1 (2%)	
HYPERPLASIA, NODULAR			7 (14%)
HYPERPLASIA, NOS		20 (43%)	1 (2%)
HYPERPLASIA, DIFFUSE			8 (16%)
ANGIECTASIS		1 (2%)	
#ILEUM	(19)	(47)	(46)
INFLAMMATION, PYOGRANULOMATOUS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(19)	(48)	(49)
HYDRONEPHROSIS		1 (2%)	2 (4%)
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL			1 (2%)
HYPERPLASIA, LYMPHOID			1 (2%)
#KIDNEY/CORTEX	(19)	(48)	(49)
ATROPHY, CYSTIC			1 (2%)
#KIDNEY/MEDULLA	(19)	(48)	(49)
NECROSIS, COAGULATIVE			1 (2%)
#URINARY BLADDER	(18)	(44)	(46)
INFLAMMATION, CHRONIC			2 (4%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(20)	(50)	(50)
CYST, NOS			2 (4%)
*SEMINAL VESICLE	(20)	(50)	(50)
POLYP, INFLAMMATORY			1 (2%)

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

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 22-2165	LOW DOSE 22-2163	HIGH DOSE 22-2161
#TESTIS/TUBULE	(19)	(49)	(48)
HYPOSPERMATOGENESIS			3 (6%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(20)	(50)	(50)
INFLAMMATION, CHRONIC	1 (5%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(20)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
INFLAMMATION, CHRONIC	1 (5%)		
INFLAMMATION, CHRONIC SUPPURATIV			1 (2%)
HYPERPLASIA, LYMPHOID			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	15	10	16
AUTO/NECROPSY/HISTO PERF		1	

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

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

	CONTROL (UNTR) 22-2166	LOW DOSE 22-2164	HIGH DOSE 22-2162
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	1
ANIMALS NECROPSIED	20	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(49)	(49)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(45)
HEMORRHAGE	1 (5%)		
PNEUMONIA INTERSTITIAL CHRONIC			3 (7%)
INFARCT, HEALED	1 (5%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(45)	(42)
HYPERPLASIA, HEMATOPOIETIC			1 (2%)
HYPERPLASIA, LYMPHOID			1 (2%)
#MANDIBULAR L. NODE	(14)	(33)	(21)
HYPERPLASIA, LYMPHOID	1 (7%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(43)
CYST, NOS		1 (2%)	
INFLAMMATION, NECROTIZING			1 (2%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2166	LOW DOSE 22-2164	HIGH DOSE 22-2162
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION, NECRO GRAN		1 (2%)	
INFARCT, HEALED	3 (15%)		
HYPERPLASIA, NODULAR	1 (5%)	3 (6%)	1 (2%)
#PANCREAS	(20)	(43)	(40)
CYSTIC DUCTS		2 (5%)	
#PANCREATIC ACINUS	(20)	(43)	(40)
ATROPHY, FOCAL		1 (2%)	
#PEYERS PATCH	(17)	(46)	(35)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (3%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(44)
HYDRONEPHROSIS		4 (8%)	1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (5%)	1 (2%)	
#KIDNEY/CORTEX	(20)	(49)	(44)
CYST, NOS		1 (2%)	
#URINARY BLADDER	(16)	(39)	(35)
HYPERPLASIA, LYMPHOID			1 (3%)
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(38)	(21)
HYPERPLASIA, CHROMOPHOBE-CELL	1 (6%)		
REPRODUCTIVE SYSTEM			
#UTERUS/ENDOMETRIUM	(20)	(49)	(43)
CYST, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE	2 (10%)	3 (6%)	3 (7%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (5%)		
HYPERPLASIA, CYSTIC	7 (35%)	31 (63%)	21 (49%)
#OVARY	(14)	(32)	(28)
CYST, NOS	1 (7%)	8 (25%)	3 (11%)

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

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 22-2166	LOW DOSE 22-2164	HIGH DOSE 22-2162
INFLAMMATION, SUPPURATIVE	3 (21%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*STERNUM	(20)	(49)	(49)
DYSPLASIA, NOS	7 (35%)	17 (35%)	4 (8%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(49)	(49)
NECROSIS, FAT	1 (5%)		
*MESENTERY	(20)	(49)	(49)
PERIARTERITIS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(49)	(49)
HYPERPLASIA, LYMPHOID		4 (8%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	6	16
ANIMAL MISSING/NO NECROPSY		1	1
NECROPSY PERF/NO HISTO PERFORMED			1
AUTO/NECROPSY/HISTO PERF			5

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

DHEW Publication No. (NIH) 79-1746