National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 164
1979

# BIOASSAY OF N-NITROSODIPHENYLAMINE FOR POSSIBLE CARCINOGENICITY

**CAS No. 86-30-6**

**NCI-CG-TR-164**

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

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NIH Publication No. 79-1720

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FOREWORD: This report presents the results of the bioassay of N-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. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. A positive result demonstrates that a test chemical is carcinogenic for animals under the conditions of the test and indicates that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic in animals requires a wider analysis.

CONTRIBUTORS: This bioassay of N-nitrosodiphenylamine was conducted at the NCI Frederick Cancer Research Center (FCRC) (1), Frederick, Maryland, operated for NCI (2) by Litton Bionetics, Inc.

The manager of the bioassay at FCRC was Dr. B. Ulland, the toxicologist was Dr. E. Gordon, and Drs. R. Cardy and D. Creasia compiled the data. Ms. S. Toms was responsible for management of data, Mr. D. Cameron for management of histopathology, Mr. L. Callahan for management of the computer branch, and Mr. R. Cypher for management of the facilities. Mr. A. Butler performed the computer services. Histopathologic evaluations for rats and mice were performed by Dr. P. K. Hildebrandt (3). The diagnoses included in this report represent his interpretations.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (4). Statistical analyses were performed by Dr. J. R. Joiner (5) and Ms. P. L. Yong (5), using methods selected for the bioassay program by Dr. J. J. Gart (6). The chemicals used in this bioassay were analyzed at FCRC by Dr. W. Zielinsky (1). The chemical narrative and analyses were reviewed and approved by Dr. W. Lijinsky (1).

This report was prepared at Tracor Jitco (5) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. C. R. Angel, Acting Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. J. F. Robens, toxicologist; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Owen, Ms. M. S. King, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley and Ms. P. J. Graboske.

The following scientists at NCI were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. Morton H. Levitt, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. A. R. Patel, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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(1) Frederick Cancer Research Center, P.O. Box B, Frederick, Maryland.

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

(3) Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland.

(4) EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

(5) Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.

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

## SUMMARY

A bioassay of N-nitrosodiphenylamine for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F1 mice.

Groups of 50 rats of each sex were administered N-nitrosodiphenylamine at one of two doses, either 1,000 or 4,000 ppm, for 100 weeks. Matched controls consisted of 20 untreated rats of each sex. All surviving rats were killed at the end of administration of the test chemcial.

Groups of 50 male mice were administered N-nitrosodiphenylamine at one of two doses, either 10,000 or 20,000 ppm, for 101 weeks. Groups of 50 female mice were administered the test chemical at one of two doses, initially 5,000 or 10,000 ppm, for 38 weeks. Because of excessive depression in the amount of mean body weight gained in the dosed groups, the doses for the females were then reduced to 1,000 and 4,000 ppm, respectively, and administration at the lowered doses was continued for 60 weeks. The time-weighted average doses for the female mice were either 2,315 or 5,741 ppm. Matched controls consisted of 20 untreated mice of each sex. All surviving mice were killed at the end of administration of the test chemical.

Mean body weights of dosed rats and mice of each sex were lower than those of corresponding controls, and were dose related throughout the bioassay, except for those of female rats during the first part of the bioassay. Mortality was dose related in the female rats, but was not affected when the test chemical was administered to the male rats or the male or female mice. Survival at the end of the bioassay was 64% or greater in the dosed and control groups of rats and mice of each sex, and sufficient numbers of animals were at risk in all groups for the development of late-appearing tumors.

Transitional-cell carcinomas of the urinary bladder occurred at incidences that were dose related (P less than 0.001) in both male and female rats, and in direct comparisons the incidences of these tumors in the high-dose groups of each sex were significantly higher (P less than or equal to 0.001) than those in the corresponding controls (males: controls 0/19, low-dose 0/46, high-dose 16/45; females: controls 0/18, low-dose 0/48, high-dose 40/49). The possible mechanism by which these tumors were induced, such as calculi formation in the bladder or nitrosation of amines present in feed to a carcinogenic nitrosoamine, is unknown.

Fibromas of the integumentary system occurred in male rats at incidences that were dose related (P = 0.003), although in direct comparisons the incidences of these tumors in the individual dosed groups were not significantly higher than those in the control group (controls 1/20, or 5%; low-dose 1/50, or 2%; high-dose 10/50, or 20%). The incidence of fibromas of the integumentary system in historical-control male F344 rats at this laboratory is 6/285, or 2%. These results suggest an association of the fibromas in the male rats with the administration of the test chemical.

No tumors occurred in the mice of either sex at incidences that were significantly higher in the dosed groups than in the corresponding control groups. The only changes related to compound administration were chronic inflammatory lesions in the urinary bladders of dosed mice.

It is concluded that under the conditions of this bioassay, N-nitrosodiphenylamine was carcinogenic for both sexes of F344 rats, inducing transitional-cell carcinomas of the urinary bladder, but was not carcinogenic for B6C3F1 mice of either sex.

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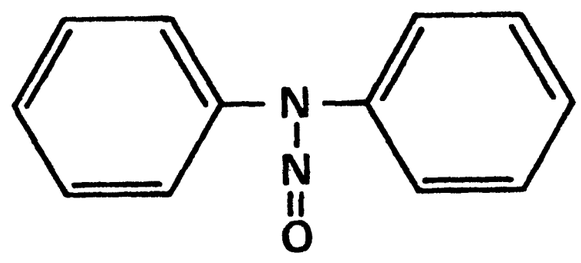
# I. INTRODUCTION

N-Nitrosodiphenylamine (CAS 86-30-6; NCI CO2880) is a nitrosoamine which is used as a vulcanization retarder in curing natural rubber and the synthetic elastomers styrenebutadiene and nitrile-butadiene (Del Gatto, 1968; Stern, 1967). U.S. production in 1976 was 1.3 million pounds (United States International Trade Commission, 1977).



N-nitrosodiphenylamine

The acute oral $LD_{50}$ for N-nitrosodiphenylamine in BD rats is estimated to be 3,000 mg/kg (Druckrey et al., 1967). The $LD_{50}$ of this compound in white mice (sex and strain not specified) when administered by intragastric intubation is 3,850 mg/kg (Zhilova and Kasparov, 1966).

Approximately 100 N-nitroso compounds have been demonstrated to be carcinogenic in animal systems (Magee et al., 1976) since the original work of Magee and Barnes (1956) which demonstrated the hepatocarcinogenicity of dimethylnitrosamine. Nitrosoamines have

been shown to cause cancer of the liver, lungs, esophagus, nasal cavities, bladder, and kidney in either rats or mice (Weisburger, 1975). Carcinogenic effects have also been observed in dogs, pigs, hamsters, fish, and primates (Weisburger, 1975; Magee et al., 1976).

There is strong evidence that bladder cancer rates are higher among men employed in the rubber industry than among the general population (Boyland et al., 1968; Case and Hosker, 1954), and there is a specific association between bladder cancer and the handling of chemicals by employees in shipping and receiving in the rubber industry, as well as those involved in compounding, mixing, and calendering rubber (McMichael et al., 1976).

N-nitrosodiphenylamine was tested by Innes et al. (1969) in a large-scale screen of industrial compounds for carcinogenic activity. Since the results of this preliminary bioassay in mice did not clearly associate the incidence of any tumor with administration of the test chemical, N-nitrosodiphenylamine was selected for further testing in the Carcinogenesis Testing Program.

# II. MATERIALS AND METHODS

## A. Chemical

Redax® (N-nitrosodiphenylamine) was obtained from R. T. Vanderbilt as a dark-brown solid. Its purity was estimated by high-pressure liquid chromatography to be 98%, with two impurities. Its melting point was 64.9°C, as compared with its reported melting point range of 63 to 66°C and its infrared spectrum was consistent with the chemical structure. Mass spectral analysis gave a molecular ion at 198 m/e and a base peak at 168 m/e. Elemental analysis showed 72.4% carbon, 5.2% hydrogen, and 13.6% nitrogen (theoretical 72.7%, 5.1%, and 14.1%).

## B. Dietary Preparation

Test diets containing N-nitrosodiphenylamine were prepared approximately weekly in 6- to 12-kg batches at appropriate doses. A known weight of the chemical was first mixed with an equal weight of autoclaved Wayne® Sterilizable Lab Meal with 4% fat (Allied Mills, Inc., Chicago, Ill.), using a mortar and pestle. The mixing was continued with second and third additions

of feed, and final mixing was performed with the remaining quantity of feed for a minimum of 15 minutes in a Patterson-Kelly twin-shell blender with an intensifier bar. The diets were routinely stored at 7°C until used.

## C. Animals

Male and female F344 (Fischer) rats and B6C3F1 mice were obtained as 4-week-old weanlings, all within 3 days of the same age, from the NCI Frederick Cancer Research Center animal farm (Frederick, Md.). The animals were housed within the test facility for 2 weeks and then were assigned four rats to a cage and five mice to a cage on a weight basis for each cage of animals of a given species and sex. For use in the chronic study, the male rats were required to weigh 90 to 105 g, averaging at least 100 g; the female rats, 80 to 95 g, averaging at least 90 g; the male mice, 18 to 22 g, averaging at least 19.5 g; and the female mice, 17 to 21 g, averaging at least 18.5 g. Individual animals were identified by ear punch.

D. Animal Maintenance

The animals were housed in polycarbonate cages (Lab Products Inc., Garfield, N.J.), 19 x 10-1/2 x 8 inches for the rats and 11-1/2 x 7-1/2 x 5 inches for the mice, which were suspended from aluminum racks (Scientific Cages, Inc., Bryan, Tex.) and were covered by nonwoven polyester-fiber 12-mil-thick filter paper (Hoeltge, Inc., Cincinnati, Ohio). The bedding used was Absorb-dri® hardwood chips (Northeastern Products, Inc., Warrenburg, N.Y.). The feed supplied was presterilized Wayne® Sterilizable Lab Meal provided ad libitum in suspended stainless steel hoppers and replenished at least three times per week. Water, acidified to pH 2.5, was supplied ad libitum from glass bottles. Sipper tubes (Lab Products, Inc.) were suspended through the tops of the cages.

The contaminated bedding was disposed of through an enclosed vacuum line that led to a holding tank from which the bedding was fed periodically into an incinerator. The cages were sanitized twice per week and the feed hoppers twice per month at 82 to 88°C in a tunnel-type cagewasher (Industrial Washing Machine Corp., Mataway, N. J.), using the detergents, Clout® (Pharmacal Research Laboratories, Greenwich, Conn.) or Oxford D'Chlor (Oxford Chemicals, Atlanta, Ga.).

The glass bottles and sipper tubes were sanitized at 82 to 88°C in a tunnel-type bottle washer (Consolidated Equipment Supply Co., Mercersburg, Pa.) three times per week, using a Calgen Commercial Division detergent (St. Louis, Mo.). The racks for the cages were changed and sanitized at or above 82°C in a rack washer (Consolidated Equipment Supply Co.) once per month, using the Calgen Commercial Division detergent, and the filter paper was changed at the same time.

The air in the animal rooms was maintained at 22 to 24°C and at 45 to 55% relative humidity. Fresh air was passed through a filter of 65% efficiency and a bag filter of 95% efficiency at the intake and through a "Z"-type roughing filter of 30% efficiency and a bag system of 90 to 95% efficiency at the exhaust (American Air Filters, Louisville, Ky.; Mine Safety Appliances, Pittsburgh, Pa.); the air was not recirculated. Room air was changed 15 times per hour. The air pressure was maintained negative to a clean hallway and positive to a return hallway. Fluorescent lighting was provided automatically on a 12-hour-per-day cycle.

Rats administered N-nitrosodiphenylamine and their corresponding controls were housed in the same room as rats on feeding studies of the following chemicals:

(CAS 156-62-7) calcium cyanamide
(CAS 3165-93-3) 4-chloro-o-toluidine hydrochloride

Mice administered N-nitrosodiphenylamine and their corresponding controls were housed in the same room as mice on feeding studies of the following chemicals:

(CAS 156-62-7) calcium cyanamide
(CAS 99-81-5) (2-chloroethyl)trimethylammonium chloride (CCC)
(CAS 95-80-7) 2,4-diaminotoluene
(CAS 19010-66-3) lead dimethyldithiocarbamate
(CAS 88-96-0) phthalamide
(CAS 120-62-7) piperonyl sulfoxide
(CAS 137-170-7) 2,4,5-trimethylaniline

## E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses (MTD's) of N-nitrosodiphenylamine, on the basis of which two concentrations (referred to in this report as "low" and "high" doses) were selected for administration in the chronic studies. Groups of five rats and five mice of each sex were fed diets containing N-nitrosodiphenylamine at one of several doses, while groups of five control animals of each species and sex were administered basal diet only. The length of the study in male rats was 11 weeks, while in female rats and male and female mice it was 8 weeks. Each animal was weighed twice per week. Tables 1 and 2 show the number of animals in each dosed group that survived to the end of the study, the week on study when the last

Table 1. N-Nitrosodiphenylamine Subchronic Feeding Studies in Rats

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| Dose (ppm) | Survival (a) | Week on Study When Last Death Occurred | Mean Weight at Week 11 as % of Control | Survival (a) | Week on Study When Last Death Occurred | Mean Weight at Week 7 as % of Control |
| First Study | | | | | | |
| 1,000 | 5/5 | | 92 | | | |
| 2,000 | 5/5 | | 94 | | | |
| 3,000 | 5/5 | | 92 | | | |
| 4,000 | 5/5 | | 88 | | | |
| 6,000 | 5/5 | | 87 | | | |
| 8,000 | 5/5 | | 81 | | | |
| 10,000 | 5/5 | | 84 | | | |
| Second Study | | | | | | |
| 4,000 | | | | 5/5 | | 86 |
| 8,000 | | | | 5/5 | | 86 |
| 16,000 | | | | 2/5 | 5 | 63 |
| 24,000 | | | | 0/5 | 4 | |
| 32,000 | | | | 0/5 | 4 | |
| 46,000 | | | | 0/5 | 2 | |

(a) Number surviving/number in group.

Table 2. N-Nitrosodiphenylamine Subchronic Feeding Studies in Mice

| Dose (ppm) | Male | | Female | |
|---|---|---|---|---|
| | Survival (a) | Mean Weight at Week 7 as % of Control | Survival (a) | Mean Weight at Week 7 as % of Control |
| First Study | | | | |
| 3,160 | 5/5 | 104 | 5/5 | 95 |
| 4,640 | 5/5 | 106 | 5/5 | 88 |
| 6,800 | 5/5 | 107 | 5/5 | 96 |
| 10,000 | 5/5 | 108 | 5/5 | 97 |
| 14,700 | 5/5 | 104 | 5/5 | 93 |
| Second Study | | | | |
| 4,250 | 5/5 | 97 | | |
| 7,500 | 5/5 | 92 | | |
| 8,500 | 5/5 | 97 | | |
| 9,500 | 5/5 | 86 | | |
| 11,000 | 5/5 | 90 | | |
| 15,000 | 5/5 | 88 | | |
| 22,000 | 5/5 | 86 | 5/5 | 94 |
| 32,000 | | | 5/5 | 94 |
| 46,000 | | | 5/5 | 86 |

(a) Number surviving/number in group.

death occurred, and the mean body weights of each dosed group at week 11 for the male rats and at week 7 for the female rats and the male and female mice, expressed as percentages of mean body weights of corresponding controls. Weights at week 7 rather than week 8 are included, since they were used for the MTD determination.

At the end of the subchronic studies, all animals were killed using $CO_2$ and necropsied. The only histopathologic lesions observed were trace amounts of pigmentation of Kupffer's cells in hepatic sinusoids of male mice dosed at 46,000 ppm.

A 10% depression in mean body weight was the major criterion for estimation of MTD's. The doses required to produce this response were determined by the following procedure: first, least squares regressions of mean body weights versus days on study were used to estimate mean body weights of each of the dosed groups at day 49. Next, probits of the percent weights of dosed groups at day 49 relative to weights of corresponding control groups were plotted against the logarithms of the doses, and least squares regressions fitted to the data were used to estimate the doses required to induce 10% depression in weight.

Based on the data thus obtained, the low and high doses for

chronic studies using rats were set at 1,000 ppm and 4,000 ppm; using male mice, 10,000 and 20,000 ppm, and using female mice, 5,000 and 10,000 ppm.

F. Chronic Studies

The test groups, doses administered, and durations of the chronic feeding studies are shown in tables 3 and 4. Due to excessive weight depression in the dosed female mice, doses for the low- and high-dose groups of the females were reduced to 1,000 and 4,000 ppm, respectively, after 38 weeks.

G. Clinical and Pathologic Examinations

All animals were observed twice daily, and the occurrences of sick, tumor-bearing, and moribund animals were recorded. Clinical examination and palpation for masses were performed each month, and the animals were generally weighed at least once per month, except for the period of week 42 through week 64, when weights were not recorded for the rats. Moribund animals and animals that survived to the end of the bioassay were killed using $CO_2$ and necropsied.

Table 3. N-Nitrosodiphenylamine Chronic Feeding Studies in Rats

| Sex and Test Group | Initial No. of Animals(a) | N-Nitroso-diphenylamine in Diet(b) (ppm) | Time on Study (weeks) |
|---|---|---|---|
| Male | | | |
| Matched-Control | 20 | 0 | 100 |
| Low-Dose | 50 | 1,000 | 100 |
| High-Dose | 50 | 4,000 | 100 |
| Female | | | |
| Matched-Control | 20 | 0 | 100 |
| Low-Dose | 50 | 1,000 | 100 |
| High-Dose | 50 | 4,000 | 100 |

(a) All animals were 6 weeks of age when placed on study.

(b) Test and control diets were provided ad libitum 7 days per week.

Table 4. N-Nitrosodiphenylamine Chronic Feeding Studies in Mice

| Sex and Test Group | Initial No. of Animals(a) | N-Nitroso-diphenylamine in Diet(b) (ppm) | Time on Study (weeks) | Time-Weighted Average Dose (c) (ppm) |
|---|---|---|---|---|
| Male | | | | |
| Matched-Control | 20 | 0 | 101 | |
| Low-Dose | 50 | 10,000 | 101 | |
| High-Dose | 50 | 20,000 | 101 | |
| Female | | | | |
| Matched-Control | 20 | 0 | 101 | |
| Low-Dose(d) | 50 | 5,000 | 38 | |
| | | 1,000 | 60 | 2,315 |
| High-Dose(d) | 50 | 10,000 | 38 | |
| | | 4,000 | 60 | 5,741 |

(a) All animals were 6 weeks of age when placed on study.

(b) Test and control diets were provided ad libitum 7 days per week.

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

(d) Because the dosed female mice failed to gain in weight comparable to the controls, the administration of doses to the females was stopped after 38 weeks and was started again at the lower doses indicated after 41 weeks.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions. The tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone marrow (femur), spleen, lymph nodes (mesenteric and submandibular), thymus, heart, salivary glands (parotid, sublingual, and submaxillary), liver, pancreas, esophagus, stomach (glandular and nonglandular), small and large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, mammary gland, uterus, ovary, brain (cerebrum and cerebellum), and all tissue masses. Peripheral blood smears also were made for all animals, whenever possible.

Necropsies were also performed on all animals found dead, unless precluded in whole or in part by autolysis or cannibalization. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group.

## H. Data Recording and Statistical Analyses

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

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox

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

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

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

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

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

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively

on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

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

The approximate 95 percent confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as $p_t/p_c$ where $p_t$ is the true binomial probability of the incidence of a specific type of tumor

in a dosed group of animals and $p_c$ is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a dosed group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the dosed group than in the control.

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

# III. RESULTS - RATS

## A. Body Weights and Clinical Signs (Rats)

Mean body weights of dosed male and female rats were lower than those of corresponding controls, and were dose related throughout the bioassay for the males, but only sometime following week 40 for the females (figure 1). Some fluctuation in the growth curves may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to greater variation. No data were recorded for the period from weeks 38 to 68. Corneal opacity occurred at higher incidences in high-dose males (15/50) and low-dose females (16/50) than in corresponding control males (0/20) and control females (1/20) and may have been related to the administration of the test chemical.

## B. Survival (Rats)

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

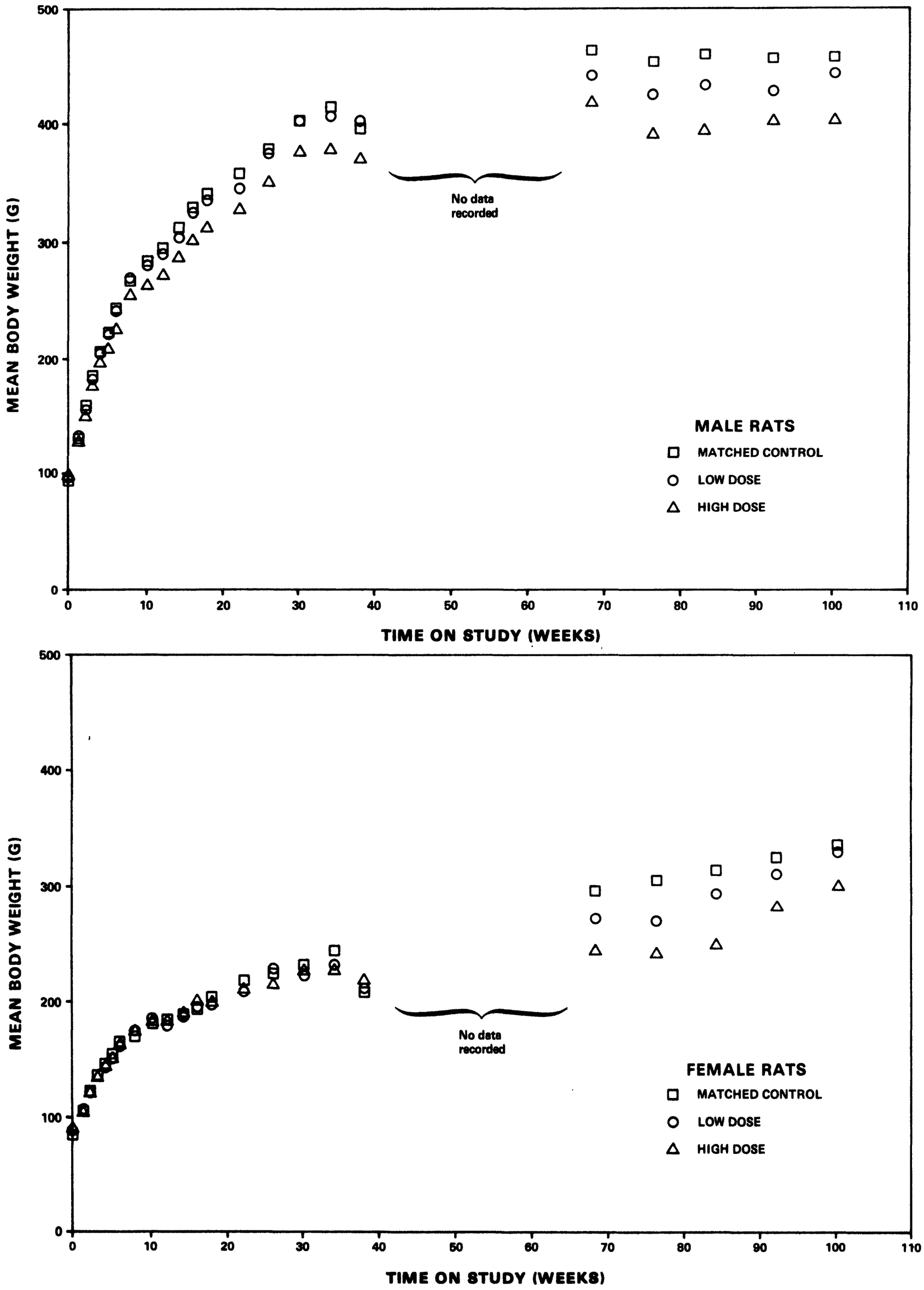



**Figure 1. Growth Curves for Rats Administered N-Nitrosodiphenylamine in the Diet**

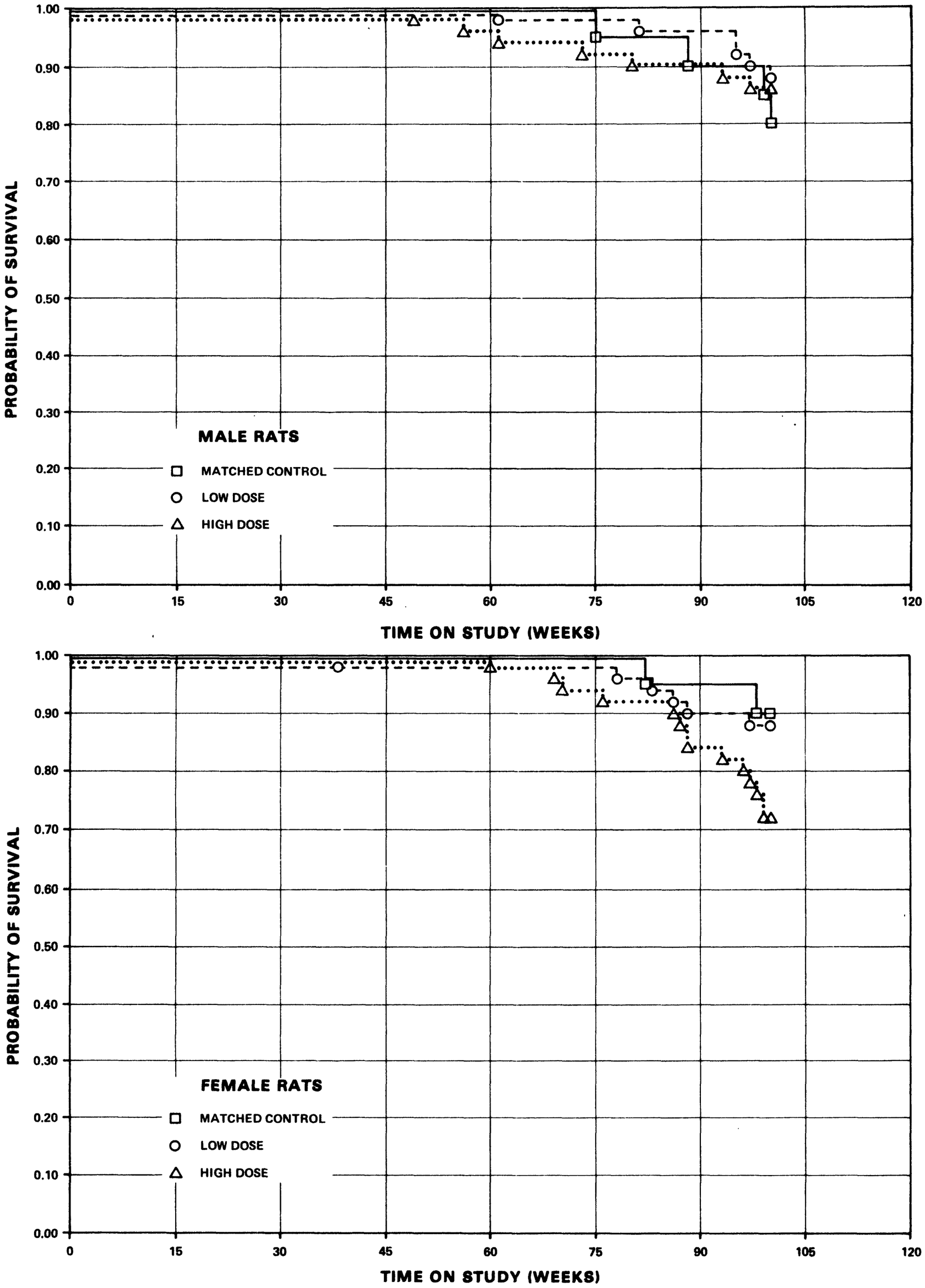



**Figure 2. Survival Curves for Rats Administered N-Nitrosodiphenylamine in the Diet**

result of the Tarone test for dose-related trend in mortality is not significant in male rats. In females, the result of the Tarone test is significant (P = 0.024).

In male rats, 43/50 (86%) of the high-dose group, 44/50 (88%) of the low-dose group, and 16/20 (80%) of the control group lived to the end of the study. In females, 35/50 (70%) of the high-dose group, 44/50 (88%) of the low-dose group, and 18/20 (90%) of the control group lived to the end of the study.

Sufficient numbers of rats of each sex were at risk for the development of late-appearing tumors.

## C. Pathology (Rats)

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

A variety of neoplasms are represented in both dosed and control groups of rats. Most of the types of tumors represented have been encountered previously in control aging F344 rats. There was a high incidence of tumors of the urinary bladder in the

high-dose groups of each sex. The incidence of these tumors, along with bladder hyperplasia, are shown in the following tabulation:

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | Control | Low Dose | High Dose | Control | Low Dose | High Dose |
| Number of Tissues Examined | 19 | 46 | 45 | 18 | 48 | 49 |
| Transitional-cell Carcinoma | | 16(36%) | | | 40(82%) | |
| Transitional-cell Carcinoma with Squamous Metaplasia | | 1(2%) | | | 2(4%) | |
| Epithelial Hyperplasia | 2(4%) | 6(13%) | | 4(8%) | 7(14%) | |

In high-dose groups of each sex, the entire spectrum from transitional-cell hyperplasia to transitional-cell carcinoma was observed in the urinary bladder. The hyperplastic foci consisted of enlarged transitional cells and the epithelium was several (7 to 10) layers in thickness. In some of the hyperplastic foci there was a tendency for the cells in the basilar layer to compartmentalize and form circular, almost acinar-like structures. Mitotic figures were often noted in these basilar cells. As the epithelium increased in thickness, fibrous tissue strands began to appear, forming a connective tissue stroma for

the proliferating epithelium. These lesions were diagnosed as transitional-cell neoplasms. The epithelium covering these fibrous strands was several (7 to 10) layers thick and mitotic figures were quite numerous in most cases. Many of the tumors were similar to papillomas; however, the thickness and activity of the epithelium was consistent with papillary transitional-cell carcinoma. Many of the tumors had less fibrous stroma, the mass consisted of solid sheets of epithelial cells, or was occasionally arranged in cords. In three cases there was squamous metaplasia. The base of the tumor was narrow in many cases, but was also rather broad in many others. The degree of infiltration into deeper layers of the bladder wall was also variable. There appeared to be a tendency for the tumor mass to remain rather superficial until the mass was quite large in size and the tumor cells were more anaplastic and active. At this time there was infiltration into the deeper layers; however, in only one case was there invasion through the entire wall and beyond the serosa. In none of these animals was a transitional-cell metastatic focus seen in another organ.

A second type of tumor, fibroma of the subcutis and skin, was observed at a higher frequency in the male high-dose group (10/45) than in the male low-dose group (1/46), in the male controls (1/19), or in any of the female groups.

The fibromas were composed of well-differentiated, dense, well-circumscribed areas of fibrous tissue.

A variety of nonneoplastic lesions were represented among both control and dosed groups of animals. Such lesions have been encountered previously in untreated aging F344 rats and are not considered to be compound related.

Based on the histopathologic examination, the high incidence of bladder tumors in both male and female high-dose groups indicates that N-nitrosodiphenylamine was carcinogenic for F344 rats under the conditions of this bioassay.

## D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In both sexes of rats, transitional-cell carcinomas of the urinary bladder occurred exclusively in the high-dose group. The result of the Cochran-Armitage test indicates a significant

positive trend (P less than 0.001) in each sex. An indicated departure from linear trend (P = 0.042) is observed in the females, due to the steep increase in the incidence of these tumors in the high-dose group. The Fisher exact test shows that the incidence in the high-dose group is significantly higher (P less than or equal to 0.001) than that in the control group in each sex. The statistical conclusion is that the incidence of transitional-cell carcinoma of the urinary bladder in rats is related to the administration of N-nitrosodiphenylamine.

In male rats, the result of the Cochran-Armitage test for positive dose-related trend in the incidence of fibroma of the integumentary system is significant (P = 0.003), but the results of the Fisher exact test are not significant. The historical records at this laboratory show an incidence of these tumors of ó/285 (2%), compared with 10/50 (20%) in the high-dose male rats of this bioassay.

Significant results in the negative direction are observed in the incidences of pituitary and adrenal tumors in male rats and in the incidence of hematopoietic tumors in female rats. In female rats, the significance in the negative direction may be accounted for by the difference in survival among the dosed and control groups.

# IV. RESULTS - MICE

## A. Body Weights and Clinical Signs (Mice)

Mean body weights of dosed male and female mice were lower than those of corresponding controls, especially for the females, and were dose related throughout the bioassay (figure 3). Some fluctuation in the growth curves may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to greater variation. Tissue masses occurred at low incidences in both control and dosed groups of mice.

## B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered N-nitrosodiphenylamine in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 4. The result of the Tarone test for dose-related trend in mortality is not significant in either sex.

In male mice, 41/50 (82%) of the high-dose group, 46/50 (92%) of

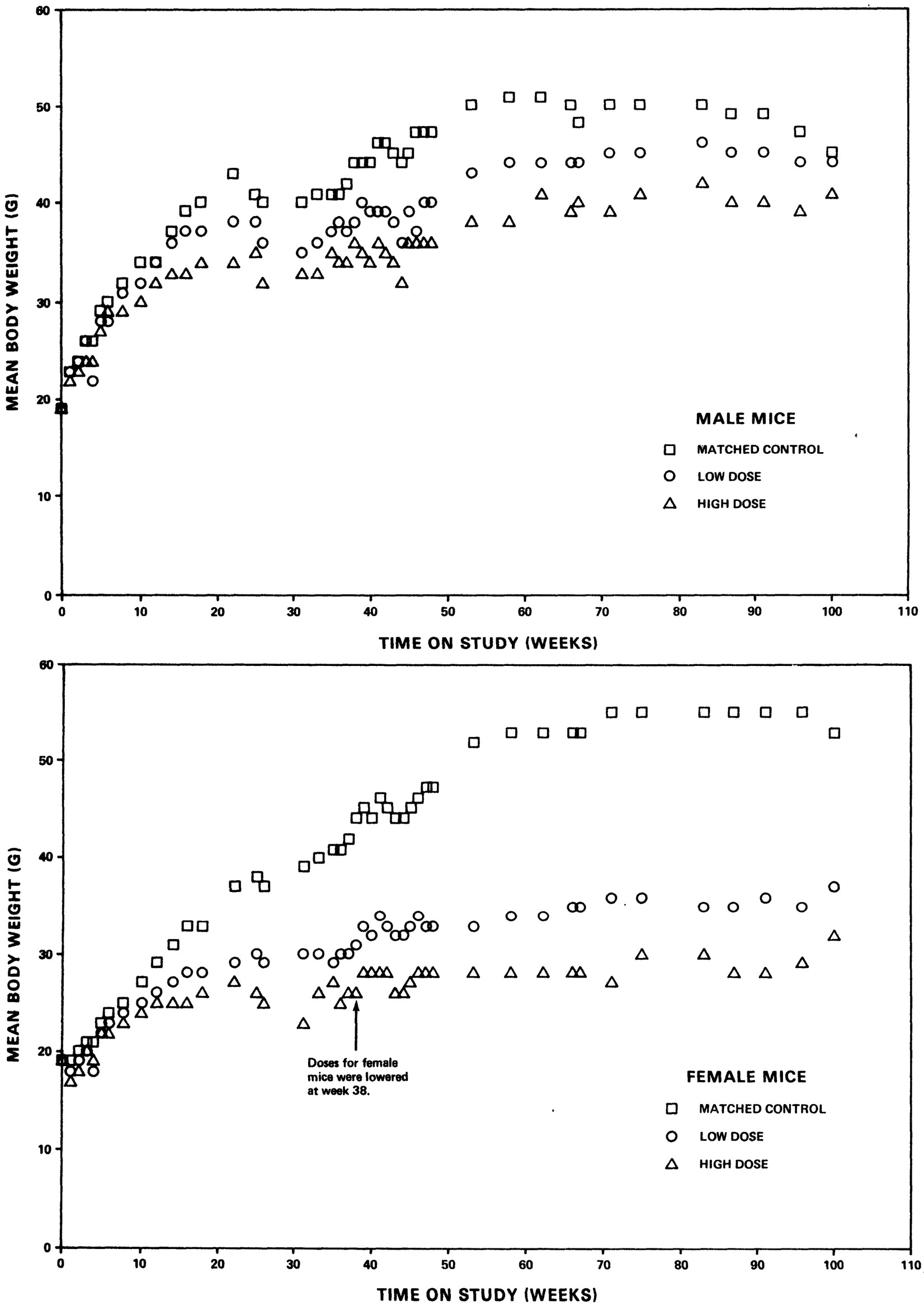



**Figure 3. Growth Curves for Mice Administered N-Nitrosodiphenylamine in the Diet**

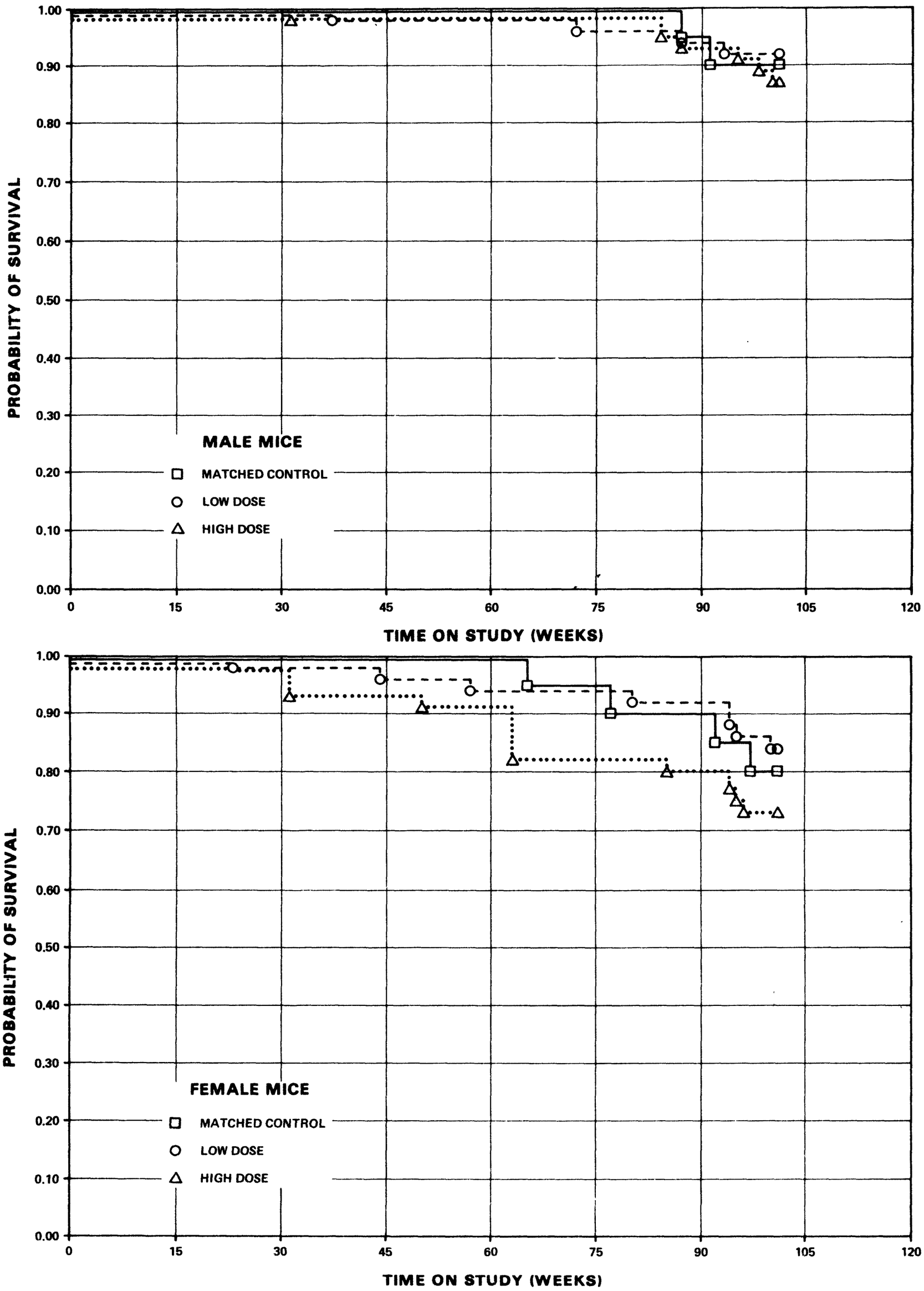



**Figure 4. Survival Curves for Mice Administered N-Nitrosodiphenylamine in the Diet**

the low-dose group, and 18/20 (90%) of the control group lived to the end of the study. In females, 31/50 (62%) of the high-dose group, 42/50 (84%) of the low-dose group, and 16/20 (80%) of the control group survived to the end of the study.

Sufficient numbers of mice of each sex were at risk for the development of late-appearing tumors.

C. Pathology (Mice)

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

There were similar incidences and types of tumors in control and dosed mice, and none appeared to be related to administration of the test chemical. However, there was a high incidence of bladder lesions in the dosed mice, as shown in the following tabulation:

| | Male | | | Female | | |
|---|---|---|---|---|---|---|
| | Control | Low Dose | High Dose | Control | Low Dose | High Dose |
| Number of Tissues Examined | 18 | 49 | 46 | 18 | 47 | 38 |
| Transitional-cell Carcinoma | | 1(2%) | | | 1(2%) | |
| Transitional-cell Papilloma or Papilloma, NOS | | 1(2%) | 1(2%) | | | |
| Hemangioma | | | | | 1(2%) | |
| Epithelial Hyperplasia | 2(4%) | 7(15%) | | 3(6%) | 6(16%) | |
| Inflammation, Chronic Submucosal | 12(24%) | 31(67%) | | 31(66%) | 30(79%) | |

The lesion most frequently observed in the bladders of the mice was chronic submucosal inflammation. No such lesion was observed in any control animal. An occasional focus of lymphocytes and an occasional blood vessel cuffed with lymphocytes, which are not an uncommon finding in the submucosa of the urinary bladder of normal mice, were observed in the control animals in this study. In the dosed animals diagnosed as having chronic submucosal inflammation of the bladder, there was an increase in the number of lymphocytes which was manifested by increased size and number of lymphocytic foci, infiltration of lymphocytes between collagen fibers, and more numerous blood vessels cuffed with lymphocytes. These lymphocytic cuffs were also much greater in thickness.

A degeneration of the collagen fibers of the submucosa was observed only in dosed mice. The degeneration was characterized by shrinking and curling of collagen bundles, and they had a more hyalinized appearance. This change appeared to occur first near, or immediately beneath, the basement membrane of the epithelium and extended to varying depths in the submucosa. Deeper in the submucosa the collagen bundles were more plump, but also more hyalinized. Blood vessels themselves also had a change which was more evident in small arterioles. This change was a thickening of the media with hyalinization of the muscle fibers. In the majority of cases this was not a severe change, but it was indeed observable. In two cases (high-dose females) this change was accompanied by acute and chronic inflammatory foci in the vessel wall and in still another case, again a high-dose female, there was fibrinoid necrosis of the vessel wall. Overall, the submucosa of the bladder seemed somewhat thickened and in two cases was considered to be edematous.

Although a few changes were observed in the epithelium of the bladder, it is somewhat suprising that more were not seen in view of the changes in the submucosa. The hyperplasia of the epithelium usually occurred as focal areas; however, in one case it occurred as diffuse hyperplasia. Two transitional-cell

carcinomas of the bladder were encountered, one of which occurred in a low-dose male, the other in a low-dose female. One transitional-cell papilloma also was seen, in a high-dose male.

A slight perivascular lymphocytic cuffing in the kidney is a normal finding in B6C3F1 mice. There were a few animals, both control and dosed, in which the degree of cuffing was considered to be greater than usual; these were diagnosed as having chronic inflammation. There was no correlation between these kidney lesions and changes in the urinary bladder.

In addition to the bladder lesions, a large number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the dosed and control groups. Again no correlation could be made between incidences of lesions and administration of the test chemical.

Because the incidence of bladder neoplasms encountered in the dosed mice of this study was very low, it does not appear that N-nitrosodiphenylamine was carcinogenic for B6C3F1 mice under the conditions of this bioassay. The compound does, however, produce a nonsuppurative inflammatory response associated with a connective tissue degeneration in the submucosa of the urinary bladder in B6C3F1 mice.

D. Statistical Analyses of Results (Mice)

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

The result of the Cochran-Armitage test for dose-related trend in the incidence of tumors, and the results of the Fisher exact test comparing the incidences of tumors in the dosed groups with that in the control group are not significant.

In each of the 95% confidence intervals for relative risk, shown in the tables, the value of one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by N-nitrosodiphenylamine, which could not be detected under the conditions of this test.

# V. DISCUSSION

Mean body weights of dosed rats and mice of each sex were lower than those of corresponding controls, and were dose related throughout the bioassay, except for female rats during the first part of the bioassay. Mortality was dose related in the female rats, but was not affected when the test chemical was administered to the male rats or the male or female mice. Survival at the end of the bioassay was 64% or greater in all dosed and control groups of rats and mice of each sex, and sufficient numbers of animals were at risk in all groups for the development of late-appearing tumors.

In both male and female rats, transitional-cell carcinomas of the urinary bladder occurred at incidences that were dose related (P less than 0.001), and in direct comparisons the incidences of these tumors in the high-dose groups of each sex were significantly higher (P less than or equal to 0.001) than those in the corresponding controls (males: controls 0/19, low-dose 0/46, high-dose 16/45; females: controls 0/18, low-dose 0/48, high-dose 40/49). Epithelial hyperplasia of the urinary bladder occurred in both the high- and low-dose groups of each sex, and squamous metaplasia of the bladder occurred in the high-dose

groups; neither of these lesions occurred in the corresponding control groups.

Fibromas of the integumentary system occurred in male rats at incidences that were dose related (P = 0.003), although in direct comparisons the incidences of these tumors in the individual dosed groups were not significantly higher than those in the control group (controls 1/20, or 5%; low-dose 1/50, or 2%; high-dose 10/50, or 20%). The incidence of fibromas of the integumentary system in historical-control male F344 rats at this laboratory is 6/285, or 2%. These results suggest an association of the fibromas in male rats with the administration of the test chemical.

No tumors occurred in the mice of either sex at incidences that were significantly higher in the dosed groups than in the corresponding control groups. However, submucosal inflammation of the urinary bladder occurred at high incidences in the dosed groups of mice of each sex, and epithelial hyperplasia of the bladder occurred at low incidences; neither lesion occurred in the corresponding control groups.

Previous reports on tests of the possible carcinogenicity of N-nitrosodiphenylamine have indicated that the compound was not

carcinogenic under the conditions tested. When 25 male Wistar rats were administered N-nitrosodiphenylamine by stomach tube 5 days per week for 45 weeks at doses of 1.07 mg suspended in 1 ml of 1% aqueous methylcellulose and the animals were killed at week 53, no tumors were observed on histopathologic examination of the liver, spleen, kidneys, lung, or any organs having macroscopic changes (Argus and Hoch-Ligeti, 1961). Neither were tumors observed when 20 BD rats were administered the test chemical in the diet 7 days per week for 100 weeks at doses of 120 mg/kg, or when 24 male CB rats were administered the test chemical by intraperitoneal injection in polyethylene glycol 400 solution once per week for 6 months at doses of 2.5 mg/wk and the tests were terminated after 2 years (Boyland et al., 1968). When 18 male and 18 female mice of each of two hybrids (C57BL/6 x C3H/Anf and C57BL/6 x AKR) were administered the test chemical by stomach tube daily for 3 weeks at 1,000 mg/kg body weight, then in the diet at 3,769 ppm for 18 months, no significant incidences of tumors were observed; however, reticulum-cell sarcomas were observed ($P = 0.05$) when the chemical was administered by subcutaneous injection (NTIS, 1968; Innes et al., 1969). The occurrence of statistically significant incidences of transitional-cell carcinomas of the urinary bladder in the rats of the present bioassay in contrast to the apparent lack of carcinogenicity in previous studies using oral administration may

have been due to the difference in route, dose, duration of administration of the test chemical, or in the total period of observation of the dosed animals. It must be recognized that the actual mechanism by which bladder tumors were induced, such as calculi formation or nitrosation of amines present in feed to a carcinogenic nitrosamine, is unknown.

It is concluded that under the conditions of this bioassay, N-nitrosodiphenylamine was carcinogenic for both sexes of F344 rats, inducing transitional-cell carcinomas of the urinary bladder, but was not carcinogenic for B6C3F1 mice of either sex.

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# APPENDIX A

## SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS

## ADMINISTERED N-NITROSODIPHENYLAMINE IN THE DIET

## TABLE A1.

## SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED N-NITROSODIPHENYLAMINE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 20 | 50 | 50 |
| ANIMALS NECROPSIED | 20 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 20 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (20) | (50) | (50) |
| BASAL-CELL TUMOR | 1 (5%) | | |
| SEBACEOUS ADENOMA | | | 1 (2%) |
| FIBROMA | | | 1 (2%) |
| *SUBCUT TISSUE | (20) | (50) | (50) |
| BASAL-CELL TUMOR | | 5 (10%) | 3 (6%) |
| SWEAT GLAND CARCINOMA | | | 1 (2%) |
| FIBROMA | 1 (5%) | 1 (2%) | 9 (18%) |
| HEMANGIOMA | | | 1 (2%) |
| OSTEOMA | | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (20) | (49) | (49) |
| ALVEOLAR/BRONCHIOLAR ADENOMA | | | 1 (2%) |
| OSTEOSARCOMA, INVASIVE | | 1 (2%) | |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (20) | (50) | (50) |
| ERYTHROCYTIC LEUKEMIA | | 1 (2%) | |
| GRANULOCYTIC LEUKEMIA | 2 (10%) | 1 (2%) | 2 (4%) |
| #SPLEEN | (19) | (50) | (48) |
| HEMANGIOSARCOMA | | | 1 (2%) |
| GRANULOCYTIC LEUKEMIA | 1 (5%) | | |
| #THYMUS | (14) | (46) | |
| OSTEOSARCOMA, INVASIVE | | 1 (2%) | |

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

## TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| CIRCULATORY SYSTEM | | | |
| #HEART | (20) | (50) | (49) |
| SARCOMA, NOS | | | 1 (2%) |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (20) | (50) | (50) |
| HEPATOCELLULAR ADENOMA | | | 1 (2%) |
| HEMANGIOSARCOMA, METASTATIC | | | 1 (2%) |
| #PANCREAS | (18) | (45) | (36) |
| ACINAR-CELL ADENOMA | 1 (6%) | 1 (2%) | |
| URINARY SYSTEM | | | |
| #URINARY BLADDER | (19) | (46) | (45) |
| TRANSITIONAL-CELL CARCINOMA | | | 16 (36%) |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (18) | (47) | (47) |
| CHROMOPHOBE ADENOMA | 9 (50%) | 9 (19%) | 7 (15%) |
| ACIDOPHIL ADENOMA | | | 1 (2%) |
| #ADRENAL | (19) | (46) | (49) |
| PHEOCHROMOCYTOMA | 3 (16%) | 6 (13%) | 1 (2%) |
| #THYROID | (19) | (49) | (48) |
| FOLLICULAR-CELL ADENOMA | | 2 (4%) | |
| FOLLICULAR-CELL CARCINOMA | | 1 (2%) | |
| C-CELL ADENOMA | 3 (16%) | 1 (2%) | 2 (4%) |
| #PARATHYROID | (17) | (40) | (38) |
| ADENOMA, NOS | | | 1 (3%) |
| #PANCREATIC ISLETS | (18) | (45) | (36) |
| ISLET-CELL ADENOMA | 2 (11%) | 1 (2%) | 2 (6%) |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (20) | (50) | (50) |
| ADENOMA, NOS | | 1 (2%) | |

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

## TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| #TESTIS | (19) | (50) | (49) |
| INTERSTITIAL-CELL TUMOR | 15 (79%) | 38 (76%) | 38 (78%) |
| NERVOUS SYSTEM | | | |
| #BRAIN | (19) | (47) | (46) |
| ASTROCYTOMA | | 1 (2%) | |
| #CEREBELLUM | (19) | (47) | (46) |
| ASTROCYTOMA | | 1 (2%) | |
| SPECIAL SENSE ORGANS | | | |
| *EYE | (20) | (50) | (50) |
| SQUAMOUS CELL CARCINOMA | | | 1 (2%) |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| NONE | | | |
| ALL OTHER SYSTEMS | | | |
| THORAX | | | |
| OSTEOSARCOMA | | 1 | |
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 20 | 50 | 50 |
| NATURAL DEATH@ | 4 | 2 | 7 |
| MORIBUND SACRIFICE | | 4 | |
| SCHEDULED SACRIFICE | | | |
| ACCIDENTALLY KILLED | | | |
| TERMINAL SACRIFICE | 16 | 44 | 43 |
| ANIMAL MISSING | | | |

@ INCLUDES AUTOLYZED ANIMALS

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

## TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 19 | 48 | 47 |
| TOTAL PRIMARY TUMORS | 38 | 72 | 91 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 19 | 47 | 45 |
| TOTAL BENIGN TUMORS | 35 | 66 | 69 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 3 | 5 | 21 |
| TOTAL MALIGNANT TUMORS | 3 | 6 | 22 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | | 1 | 1 |
| TOTAL SECONDARY TUMORS | | 2 | 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 A2.

## SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED N-NITROSODIPHENYLAMINE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 20 | 50 | 50 |
| ANIMALS MISSING | | | 1 |
| ANIMALS NECROPSIED | 20 | 50 | 49 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 20 | 50 | 49 |
| INTEGUMENTARY SYSTEM | | | |
| *SUBCUT TISSUE | (20) | (50) | (49) |
| EPITHELIAL TUMOR, NOS, BENIGN | | | 1 (2%) |
| BASAL-CELL TUMOR | 1 (5%) | 1 (2%) | 3 (6%) |
| FIBROMA | | 1 (2%) | 3 (6%) |
| FIBROSARCOMA | | | 1 (2%) |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (19) | (50) | (46) |
| ALVEOLAR/BRONCHIOLAR CARCINOMA | | | 1 (2%) |
| C-CELL CARCINOMA, METASTATIC | | | 1 (2%) |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (20) | (50) | (49) |
| GRANULOCYTIC LEUKEMIA | 2 (10%) | | |
| #SPLEEN | (20) | (48) | (49) |
| MESENCHYMOMA, BENIGN | 1 (5%) | | |
| GRANULOCYTIC LEUKEMIA | 1 (5%) | | |
| CIRCULATORY SYSTEM | | | |
| NONE | | | |
| DIGESTIVE SYSTEM | | | |
| NONE | | | |

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

## TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| URINARY SYSTEM | | | |
| #URINARY BLADDER | (18) | (48) | (49) |
| TRANSITIONAL-CELL CARCINOMA | | | 40 (82%) |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (19) | (49) | (48) |
| CHROMOPHOBE ADENOMA | 8 (42%) | 13 (27%) | 12 (25%) |
| #ADRENAL | (19) | (50) | (48) |
| PHEOCHROMOCYTOMA | | 1 (2%) | |
| #THYROID | (20) | (49) | (48) |
| FOLLICULAR-CELL ADENOMA | | 1 (2%) | 1 (2%) |
| FOLLICULAR-CELL CARCINOMA | 1 (5%) | | |
| C-CELL ADENOMA | 1 (5%) | 5 (10%) | 2 (4%) |
| C-CELL CARCINOMA | | | 1 (2%) |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (20) | (50) | (49) |
| BASAL-CELL TUMOR | 1 (5%) | | |
| ADENOMA, NOS | | 1 (2%) | |
| FIBROADENOMA | 4 (20%) | 6 (12%) | 6 (12%) |
| #UTERUS | (20) | (50) | (46) |
| HEMANGIOMA | 1 (5%) | | |
| #CERVIX UTERI | (20) | (50) | (46) |
| FIBROMA | | 1 (2%) | |
| NERVOUS SYSTEM | | | |
| #CEREBELLUM | (19) | (50) | (49) |
| MENINGIOMA | | | 1 (2%) |
| SPECIAL SENSE ORGANS | | | |
| NONE | | | |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |

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

## TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| BODY CAVITIES | | | |
| *ABDOMINAL CAVITY | (20) | (50) | (49) |
| SARCOMA, NOS | | 1 (2%) | |
| ALL OTHER SYSTEMS | | | |
| NONE | | | |
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 20 | 50 | 50 |
| NATURAL DEATH@ | | 4 | 10 |
| MORIBUND SACRIFICE | 2 | 2 | 4 |
| SCHEDULED SACRIFICE | | | |
| ACCIDENTALLY KILLED | | | |
| TERMINAL SACRIFICE | 18 | 44 | 35 |
| ANIMAL MISSING | | | 1 |
| @ INCLUDES AUTOLYZED ANIMALS | | | |
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 13 | 23 | 43 |
| TOTAL PRIMARY TUMORS | 21 | 31 | 72 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 13 | 22 | 22 |
| TOTAL BENIGN TUMORS | 17 | 30 | 29 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 4 | 1 | 40 |
| TOTAL MALIGNANT TUMORS | 4 | 1 | 43 |
| 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

# APPENDIX B

## SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADIMINISTERED N-NITROSODIPHENYLAMINE IN THE DIET

## TABLE B1.

## SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED N-NITROSODIPHENYLAMINE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 20 | 50 | 50 |
| ANIMALS MISSING | | | 2 |
| ANIMALS NECROPSIED | 20 | 49 | 48 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 20 | 49 | 48 |
| **INTEGUMENTARY SYSTEM** | | | |
| *SKIN | (20) | (49) | (48) |
| SEBACEOUS ADENOMA | 1 (5%) | | |
| HEMANGIOMA | 1 (5%) | | |
| **RESPIRATORY SYSTEM** | | | |
| #LUNG | (20) | (48) | (48) |
| CARCINOMA,NOS | | | 1 (2%) |
| HEPATOCELLULAR CARCINOMA, METAST | | 1 (2%) | |
| ALVEOLAR/BRONCHIOLAR ADENOMA | 4 (20%) | 9 (19%) | 7 (15%) |
| **HEMATOPOIETIC SYSTEM** | | | |
| *MULTIPLE ORGANS | (20) | (49) | (48) |
| MALIG.LYMPHOMA, HISTIOCYTIC TYPE | | | 1 (2%) |
| GRANULOCYTIC LEUKEMIA | 1 (5%) | 1 (2%) | 1 (2%) |
| *ABDOMINAL CAVITY | (20) | (49) | (48) |
| MALIG.LYMPHOMA, HISTIOCYTIC TYPE | | | 1 (2%) |
| *BLOOD | (20) | (49) | (48) |
| LYMPHOCYTIC LEUKEMIA | | | 1 (2%) |
| #SPLEEN | (20) | (49) | (48) |
| HEMANGIOMA | | | 2 (4%) |
| HEMANGIOSARCOMA | | 2 (4%) | 2 (4%) |
| #SMALL INTESTINE | (20) | (47) | (46) |
| MALIG.LYMPHOMA, UNDIFFER-TYPE | | 1 (2%) | |
| **CIRCULATORY SYSTEM** | | | |
| NONE | | | |

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

## TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| DIGESTIVE SYSTEM | | | |
| #SALIVARY GLAND | (20) | (48) | (45) |
| NEOPLASM, NOS, MALIGNANT | | 1 (2%) | |
| #LIVER | (20) | (49) | (48) |
| NEOPLASM, NOS, METASTATIC | | 1 (2%) | |
| HEPATOCELLULAR ADENOMA | 6 (30%) | 11 (22%) | 7 (15%) |
| HEPATOCELLULAR CARCINOMA | | 1 (2%) | |
| HEMANGIOMA | | 3 (6%) | |
| HEMANGIOSARCOMA | | 1 (2%) | |
| HEMANGIOSARCOMA, METASTATIC | | 2 (4%) | |
| #STOMACH | (20) | (48) | (46) |
| PAPILLARY ADENOMA | 1 (5%) | | |
| URINARY SYSTEM | | | |
| #KIDNEY | (20) | (49) | (48) |
| TUBULAR-CELL ADENOMA | | | 1 (2%) |
| #URINARY BLADDER | (18) | (49) | (46) |
| PAPILLOMA, NOS | | 1 (2%) | |
| TRANSITIONAL-CELL PAPILLOMA | | | 1 (2%) |
| TRANSITIONAL-CELL CARCINOMA | | 1 (2%) | |
| ENDOCRINE SYSTEM | | | |
| #ADRENAL | (18) | (49) | (48) |
| PHEOCHPOMOCYTOMA | | 1 (2%) | |
| REPRODUCTIVE SYSTEM | | | |
| NONE | | | |
| NERVOUS SYSTEM | | | |
| NONE | | | |
| SPECIAL SENSE ORGANS | | | |
| *EYE/LACRIMAL GLAND | (20) | (49) | (48) |
| ADENOMA, NOS | | 1 (2%) | 1 (2%) |

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

## TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| CYSTADENOMA, NOS | | 1 (2%) | |
| **MUSCULOSKELETAL SYSTEM** | | | |
| NONE | | | |
| **BODY CAVITIES** | | | |
| NONE | | | |
| **ALL OTHER SYSTEMS** | | | |
| *MULTIPLE ORGANS | (20) | (49) | (48) |
| NEOPLASM, NOS, MALIGNANT | | 1 (2%) | |
| HEMANGIOMA | | | 1 (2%) |
| HEMANGIOSARCOMA | | 1 (2%) | |
| **ANIMAL DISPOSITION SUMMARY** | | | |
| ANIMALS INITIALLY IN STUDY | 20 | 50 | 50 |
| NATURAL DEATH@ | 2 | 4 | 6 |
| MORIBUND SACRIFICE | | | |
| SCHEDULED SACRIFICE | | | |
| ACCIDENTALLY KILLED | | | 1 |
| TERMINAL SACRIFICE | 18 | 46 | 41 |
| ANIMAL MISSING | | | 2 |

@ INCLUDES AUTOLYZED ANIMALS

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

## TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 10 | 29 | 22 |
| TOTAL PRIMARY TUMORS | 14 | 37 | 27 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 9 | 23 | 18 |
| TOTAL BENIGN TUMORS | 13 | 27 | 20 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 1 | 9 | 6 |
| TOTAL MALIGNANT TUMORS | 1 | 10 | 7 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | | 4 | |
| TOTAL SECONDARY TUMORS | | 4 | |
| 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 ADMINISTERED N-NITROSODIPHENYLAMINE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 20 | 50 | 50 |
| ANIMALS MISSING | | | 7 |
| ANIMALS NECROPSIED | 20 | 50 | 41 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 20 | 50 | 40 |
| INTEGUMENTARY SYSTEM | | | |
| *SUBCUT TISSUE | (20) | (50) | (41) |
| BASAL-CELL TUMOR | | | 1 (2%) |
| RHABDOMYOSARCOMA | | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (20) | (47) | (38) |
| CARCINOMA,NOS | | 1 (2%) | |
| ADENOCARCINOMA, NOS, METASTATIC | | 1 (2%) | |
| ALVEOLAR/BRONCHIOLAR ADENOMA | 3 (15%) | 9 (19%) | 5 (13%) |
| ALVEOLAR/BRONCHIOLAR CARCINOMA | | 2 (4%) | |
| OSTEOSARCOMA, METASTATIC | 1 (5%) | | |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (20) | (50) | (41) |
| MALIG.LYMPHOMA, UNDIFFER-TYPE | | 1 (2%) | |
| MALIG.LYMPHOMA, HISTIOCYTIC TYPE | | 1 (2%) | |
| PLASMA-CELL TUMOR | | 1 (2%) | |
| LYMPHOCYTIC LEUKEMIA | | 1 (2%) | |
| GRANULOCYTIC LEUKEMIA | | | 1 (2%) |
| *ABDOMINAL CAVITY | (20) | (50) | (41) |
| MALIG.LYMPHOMA, HISTIOCYTIC TYPE | | | 1 (2%) |
| #SPLEEN | (20) | (48) | (38) |
| SARCOMA, NOS | 1 (5%) | | |
| HEMANGIOMA | | | 1 (3%) |
| MALIG.LYMPHOMA, UNDIFFER-TYPE | | | 1 (3%) |
| #SMALL INTESTINE | (19) | (48) | (39) |
| MALIG.LYMPHOMA, UNDIFFER-TYPE | | 1 (2%) | |

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

## TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| *MESENTERY | (20) | (50) | (41) |
| MALIG.LYMPHOMA, UNDIFFER-TYPE | | | 1 (2%) |
| #KIDNEY | (20) | (49) | (38) |
| MALIG.LYMPHOMA, HISTIOCYTIC TYPE | 1 (5%) | | |
| CIRCULATORY SYSTEM | | | |
| NONE | | | |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (20) | (49) | (38) |
| HEPATOCELLULAR ADENOMA | 3 (15%) | 7 (14%) | 4 (11%) |
| HEPATOCELLULAR CARCINOMA | 1 (5%) | | |
| HEMANGIOMA | | | 1 (3%) |
| URINARY SYSTEM | | | |
| #URINARY BLADDER | (18) | (47) | (38) |
| TRANSITIONAL-CELL CARCINOMA | | 1 (2%) | |
| #U.BLADDER/SUBMUCOSA | (18) | (47) | (38) |
| HEMANGIOMA | | 1 (2%) | |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (14) | (37) | (26) |
| CHROMOPHOBE ADENOMA | | 1 (3%) | |
| #ADRENAL | (20) | (45) | (37) |
| CORTICAL ADENOMA | 1 (5%) | | |
| REPRODUCTIVE SYSTEM | | | |
| #UTERUS | (20) | (50) | (37) |
| LEIOMYOMA | | 1 (2%) | |
| HEMANGIOMA | | | 1 (3%) |
| #OVARY/FOLLICLE | (20) | (48) | (38) |
| ADENOMA, NOS | | | 1 (3%) |

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

## TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| NERVOUS SYSTEM | | | |
| NONE | | | |
| SPECIAL SENSE ORGANS | | | |
| *EYE/LACRIMAL GLAND | (20) | (50) | (41) |
| ADENOMA, NOS | | | 1 (2%) |
| MUSCULOSKELETAL SYSTEM | | | |
| *SKULL | (20) | (50) | (41) |
| OSTEOSARCOMA | 1 (5%) | | |
| *SKELETAL MUSCLE | (20) | (50) | (41) |
| RHABDOMYOSARCOMA | | 1 (2%) | |
| BODY CAVITIES | | | |
| NONE | | | |
| ALL OTHER SYSTEMS | | | |
| NONE | | | |
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 20 | 50 | 50 |
| NATURAL DEATH@ | 4 | 7 | 11 |
| MORIBUND SACRIFICE | | 1 | 1 |
| SCHEDULED SACRIFICE | | | |
| ACCIDENTALLY KILLED | | | |
| TERMINAL SACRIFICE | 16 | 42 | 31 |
| ANIMAL MISSING | | | 7 |

@ INCLUDES AUTOLYZED ANIMALS

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

## TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 9 | 23 | 15 |
| TOTAL PRIMARY TUMORS | 11 | 30 | 19 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 7 | 17 | 13 |
| TOTAL BENIGN TUMORS | 7 | 19 | 15 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 3 | 10 | 4 |
| TOTAL MALIGNANT TUMORS | 4 | 10 | 4 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | 1 | 1 | |
| TOTAL SECONDARY TUMORS | 1 | 1 | |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT | | 1 | |
| TOTAL UNCERTAIN TUMORS | | 1 | |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC | | | |
| TOTAL UNCERTAIN TUMORS | | | |

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

# APPENDIX C

## SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS ADMINISTERED N-NITROSODIPHENYLAMINE IN THE DIET

## TABLE C1.

## SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED N-NITROSODIPHENYLAMINE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 20 | 50 | 50 |
| ANIMALS NECROPSIED | 20 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 20 | 50 | 50 |
| **INTEGUMENTARY SYSTEM** | | | |
| *SKIN | (20) | (50) | (50) |
| HYPERKERATOSIS | | 1 (2%) | |
| *SUBCUT TISSUE | (20) | (50) | (50) |
| INFLAMMATION, ACUTE | | | 1 (2%) |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| **RESPIRATORY SYSTEM** | | | |
| #LUNG | (20) | (49) | (49) |
| HYPERPLASIA, ADENOMATOUS | 1 (5%) | | 1 (2%) |
| **HEMATOPOIETIC SYSTEM** | | | |
| #MANDIBULAR L. NODE | (19) | (50) | (50) |
| HYPERPLASIA, NOS | | 1 (2%) | |
| **CIRCULATORY SYSTEM** | | | |
| #MYOCARDIUM | (20) | (50) | (49) |
| FIBROSIS | | | 1 (2%) |
| **DIGESTIVE SYSTEM** | | | |
| #LIVER | (20) | (50) | (50) |
| DEGENERATION, NOS | 2 (10%) | 5 (10%) | 1 (2%) |
| HYPERPLASIA, NODULAR | | | 1 (2%) |
| HYPERPLASIA, FOCAL | 1 (5%) | | 1 (2%) |
| #LIVER/CENTRILOBULAR | (20) | (50) | (50) |
| DEGENERATION, NOS | 1 (5%) | | |

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

## TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| NECROSIS, NOS | 1 (5%) | | |
| #BILE DUCT | (20) | (50) | (50) |
| HYPERPLASIA, NOS | | 1 (2%) | |
| #PANCREATIC ACINUS | (18) | (45) | (36) |
| HYPERPLASIA, NODULAR | 1 (6%) | | |
| URINARY SYSTEM | | | |
| #URINARY BLADDER | (19) | (46) | (45) |
| HYPERPLASIA, EPITHELIAL | | 2 (4%) | 6 (13%) |
| METAPLASIA, SQUAMOUS | | | 1 (2%) |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (18) | (47) | (47) |
| ANGIECTASIS | | | 1 (2%) |
| #ADRENAL | (19) | (46) | (49) |
| HEMORRHAGIC CYST | 1 (5%) | | |
| #ADRENAL CORTEX | (19) | (46) | (49) |
| HYPERPLASIA, FOCAL | | 1 (2%) | |
| #ADRENAL MEDULLA | (19) | (46) | (49) |
| HYPERPLASIA, NOS | | | 2 (4%) |
| HYPERPLASIA, FOCAL | 1 (5%) | 1 (2%) | |
| #THYROID | (19) | (49) | (48) |
| CYSTIC FOLLICLES | | 2 (4%) | |
| PIGMENTATION, NOS | | 1 (2%) | |
| HYPERPLASIA, C-CELL | 1 (5%) | 1 (2%) | 1 (2%) |
| #PANCREATIC ISLETS | (18) | (45) | (36) |
| HYPERPLASIA, NOS | | 1 (2%) | |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (20) | (50) | (50) |
| CYST, NOS | | 1 (2%) | |
| METAPLASIA, SQUAMOUS | | 1 (2%) | |

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

## TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| #TESTIS | (19) | (50) | (49) |
| HYPERPLASIA, INTERSTITIAL CELL | 1 (5%) | 1 (2%) | |
| NERVOUS SYSTEM | | | |
| NONE | | | |
| SPECIAL SENSE ORGANS | | | |
| NONE | | | |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| NONE | | | |
| ALL OTHER SYSTEMS | | | |
| NONE | | | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NO LESION REPORTED | 1 | 1 | 3 |

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

## TABLE C2.

## SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED N-NITROSODIPHENYLAMINE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 20 | 50 | 50 |
| ANIMALS MISSING | | | 1 |
| ANIMALS NECROPSIED | 20 | 50 | 49 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 20 | 50 | 49 |
| INTEGUMENTARY SYSTEM | | | |
| *SUBCUT TISSUE | (20) | (50) | (49) |
| NECROSIS, NOS | | | 1 (2%) |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (19) | (50) | (46) |
| PNEUMONIA, CHRONIC MURINE | 1 (5%) | | |
| HYPERPLASIA, ADENOMATOUS | 1 (5%) | | |
| HEMATOPOIETIC SYSTEM | | | |
| *BLOOD | (20) | (50) | (49) |
| POLYCHROMASIA | | 2 (4%) | |
| #SPLEEN | (20) | (48) | (49) |
| HEMATOPOIESIS | | 1 (2%) | |
| #THYMUS | (19) | (48) | (23) |
| HYPERTROPHY, NOS | | 1 (2%) | |
| CIRCULATORY SYSTEM | | | |
| NONE | | | |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (20) | (50) | (49) |
| INFLAMMATION, ACUTE | | | 1 (2%) |
| INFLAMMATION, FOCAL GRANULOMATOU | | 1 (2%) | |

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

## TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| DEGENERATION, NOS | 1 (5%) | 2 (4%) | |
| NECROSIS, COAGULATIVE | | | 1 (2%) |
| HYPERPLASIA, NODULAR | 1 (5%) | | |
| HYPERPLASTIC NODULE | | | 1 (2%) |
| HYPERPLASIA, FOCAL | | 3 (6%) | |
| **URINARY SYSTEM** | | | |
| #URINARY BLADDER | (18) | (48) | (49) |
| FIBPOSIS | | | 1 (2%) |
| HYPERPLASIA, EPITHELIAL | | 4 (8%) | 7 (14%) |
| METAPLASIA, SQUAMOUS | | | 2 (4%) |
| #U.BLADDER/SUBMUCOSA | (18) | (48) | (49) |
| INFLAMMATION, CHRONIC | | 1 (2%) | |
| **ENDOCRINE SYSTEM** | | | |
| #PITUITARY | (19) | (49) | (48) |
| HEMORRHAGE | | 1 (2%) | |
| HEMORRHAGIC CYST | | 1 (2%) | |
| ANGIECTASIS | | 1 (2%) | |
| #ADRENAL | (19) | (50) | (48) |
| LIPOIDOSIS | | 1 (2%) | |
| ANGIECTASIS | | 1 (2%) | |
| #ADRENAL CORTEX | (19) | (50) | (48) |
| HYPERPLASIA, FOCAL | | 1 (2%) | |
| #THYROID | (20) | (49) | (48) |
| CYSTIC FOLLICLES | | 3 (6%) | |
| HYPERPLASIA, C-CELL | | | 1 (2%) |
| **REPRODUCTIVE SYSTEM** | | | |
| *MAMMARY GLAND | (20) | (50) | (49) |
| DILATATION/DUCTS | 3 (15%) | 3 (6%) | 5 (10%) |
| FIBROSIS | | | 1 (2%) |
| LACTATION | | | 2 (4%) |
| #UTERUS | (20) | (50) | (46) |
| HYDROMETRA | | 1 (2%) | |

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

## TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| THROMBUS, ORGANIZED | 1 (5%) | | |
| FIBROSIS | | 1 (2%) | |
| NECROSIS, NOS | 1 (5%) | | |
| POLYP | 3 (15%) | 5 (10%) | 1 (2%) |
| #UTERUS/ENDOMETRIUM | (20) | (50) | (46) |
| INFLAMMATION, NECROTIZING | | | 1 (2%) |
| HYPERPLASIA, CYSTIC | | 1 (2%) | 1 (2%) |
| #OVARY | (20) | (50) | (49) |
| FOLLICULAR CYST, NOS | | 1 (2%) | 1 (2%) |
| NERVOUS SYSTEM | | | |
| NONE | | | |
| SPECIAL SENSE ORGANS | | | |
| NONE | | | |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| NONE | | | |
| ALL OTHER SYSTEMS | | | |
| *MULTIPLE ORGANS | (20) | (50) | (49) |
| POSTMORTEM CHANGE | | 1 (2%) | 1 (2%) |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NO LESION REPORTED | 6 | 13 | |
| ANIMAL MISSING/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 ADMINISTERED N-NITROSODIPHENYLAMINE IN THE DIET

## TABLE D1.

## SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED N-NITROSODIPHENYLAMINE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 20 | 50 | 50 |
| ANIMALS MISSING | | | 2 |
| ANIMALS NECROPSIED | 20 | 49 | 48 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 20 | 49 | 48 |
| INTEGUMENTARY SYSTEM | | | |
| *SUBCUT TISSUE | (20) | (49) | (48) |
| CYST, NOS | | | 1 (2%) |
| NECROSIS, FAT | | | 1 (2%) |
| RESPIRATORY SYSTEM | | | |
| NONE | | | |
| HEMATOPOIETIC SYSTEM | | | |
| #BONE MARROW | (18) | (49) | (48) |
| HYPERPLASIA, HEMATOPOIETIC | | 1 (2%) | |
| #SPLEEN | (20) | (49) | (48) |
| HEMATOPOIESIS | | | 1 (2%) |
| #LYMPH NODE | (20) | (48) | (46) |
| HYPERPLASIA, NOS | 1 (5%) | | |
| #MESENTERIC L. NODE | (20) | (48) | (46) |
| HEMORRHAGE | | 1 (2%) | |
| HYPERPLASIA, NOS | | | 1 (2%) |
| CIRCULATORY SYSTEM | | | |
| NONE | | | |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (20) | (49) | (48) |
| THROMBOSIS, NOS | | 2 (4%) | |

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

## TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| HEMORRHAGE | | 1 (2%) | |
| DEGENERATION, NOS | | | 1 (2%) |
| NECROSIS, NOS | | 2 (4%) | |
| METAMORPHOSIS FATTY | 2 (10%) | 3 (6%) | 1 (2%) |
| HYPERPLASIA, NODULAR | 1 (5%) | 1 (2%) | |
| HYPERPLASIA, FOCAL | 1 (5%) | | 3 (6%) |
| #HEPATIC CAPSULE | (20) | (49) | (48) |
| FIBROSIS | | 1 (2%) | |
| #SMALL INTESTINE | (20) | (47) | (46) |
| HYPERPLASIA, ADENOMATOUS | | 1 (2%) | |
| #DUODENUM | (20) | (47) | (46) |
| HYPERPLASIA, NOS | | | 1 (2%) |
| **URINARY SYSTEM** | | | |
| #KIDNEY | (20) | (49) | (48) |
| PYELONEPHRITIS, ACUTE | | | 1 (2%) |
| INFLAMMATION, CHRONIC | | 9 (18%) | 5 (10%) |
| PERIVASCULAR CUFFING | | 1 (2%) | |
| #URINARY BLADDER | (18) | (49) | (46) |
| INFLAMMATION, ACUTE | | | 1 (2%) |
| INFLAMMATION, CHRONIC | | 2 (4%) | |
| CYTOLOGIC DEGENERATION | | | 1 (2%) |
| HYPERPLASIA, EPITHELIAL | | 2 (4%) | 7 (15%) |
| #U.BLADDER/SUBMUCOSA | (18) | (49) | (46) |
| INFLAMMATION, NOS | | 1 (2%) | |
| INFLAMMATION, CHRONIC | | 12 (24%) | 31 (67%) |
| HYPERPLASIA, LYMPHOID | | | 1 (2%) |
| **ENDOCRINE SYSTEM** | | | |
| #THYROID | (19) | (47) | (45) |
| CYSTIC FOLLICLES | | | 1 (2%) |
| HEMORRHAGE | | | 1 (2%) |
| HYPERPLASIA, FOLLICULAR-CELL | | 1 (2%) | |
| #PANCREATIC ISLETS | (17) | (45) | (43) |
| HYPERPLASIA, NOS | | 1 (6%) | |

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

## TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| REPRODUCTIVE SYSTEM | | | |
| *SEMINAL VESICLE | (20) | (49) | (48) |
| DISTENTION | 1 (5%) | | |
| NERVOUS SYSTEM | | | |
| #MIDBRAIN | (19) | (49) | (48) |
| HEMORRHAGE | | | 1 (2%) |
| #MEDULLA OBLONGATA | (19) | (49) | (48) |
| HEMORRHAGE | | | 1 (2%) |
| SPECIAL SENSE ORGANS | | | |
| NONE | | | |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| *ABDOMINAL CAVITY | (20) | (49) | (48) |
| NECROSIS, FAT | | 1 (2%) | 1 (2%) |
| *MESENTERY | (20) | (49) | (48) |
| NECROSIS, FAT | 1 (5%) | | |
| ALL OTHER SYSTEMS | | | |
| NONE | | | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NO LESION REPORTED | 6 | 11 | 4 |
| ANIMAL MISSING/NO NECROPSY | | | 2 |

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

## TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| AUTO/NECROPSY/HISTO PERF | | | 1 |
| AUTOLYSIS/NO NECROPSY | | 1 | |

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

## TABLE D2.

## SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED N-NITROSODIPHENYLAMINE IN THE DIET

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| ANIMALS INITIALLY IN STUDY | 20 | 50 | 50 |
| ANIMALS MISSING | | | 7 |
| ANIMALS NECROPSIED | 20 | 50 | 41 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 20 | 50 | 40 |
| **INTEGUMENTARY SYSTEM** | | | |
| *SUBCUT TISSUE | (20) | (50) | (41) |
| THROMBOSIS, NOS | 1 (5%) | | |
| HEMORRHAGE | 2 (10%) | | |
| INFLAMMATION, ACUTE/CHRONIC | 2 (10%) | | |
| **RESPIRATORY SYSTEM** | | | |
| #LUNG | (20) | (47) | (38) |
| NECROSIS, CENTRAL | | 1 (2%) | |
| HYPERPLASIA, ADENOMATOUS | | | 1 (3%) |
| **HEMATOPOIETIC SYSTEM** | | | |
| #SPLEEN | (20) | (48) | (38) |
| THROMBOSIS, NOS | | | 1 (3%) |
| #MESENTERIC L. NODE | (20) | (47) | (38) |
| HEMORRHAGE | 1 (5%) | | |
| HYPERPLASIA, NOS | 1 (5%) | | |
| #THYMUS | (17) | (38) | (37) |
| CONGESTION, NOS | 1 (6%) | | |
| HEMORRHAGE | 1 (6%) | | |
| HYPERPLASIA, NOS | 1 (6%) | | |
| **CIRCULATORY SYSTEM** | | | |
| NONE | | | |
| **DIGESTIVE SYSTEM** | | | |
| #LIVER | (20) | (49) | (38) |
| THROMBOSIS, NOS | | | 1 (3%) |

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

## TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| INFLAMMATION, ACUTE FOCAL | | | 1 (3%) |
| DEGENERATION, NOS | 1 (5%) | | 1 (3%) |
| NECROSIS, NOS | | 1 (2%) | |
| NECROSIS, COAGULATIVE | | | 1 (3%) |
| INFARCT, NOS | | 1 (2%) | |
| #LIVER/CENTRILOBULAR | (20) | (49) | (38) |
| NECROSIS, NOS | | | 1 (3%) |
| #PANCREAS | (18) | (42) | (35) |
| INFLAMMATION, ACUTE | | | 1 (3%) |
| INFLAMMATION, ACUTE/CHRONIC | 1 (6%) | | |
| INFLAMMATION, CHRONIC FOCAL | | | 1 (3%) |
| #STOMACH | (19) | (49) | (39) |
| INFLAMMATION, ACUTE | | | 1 (3%) |
| INFLAMMATION, ACUTE FOCAL | 1 (5%) | | |
| INFLAMMATION, ACUTE/CHRONIC | | | 1 (3%) |
| HYPERPLASIA, EPITHELIAL | | | 1 (3%) |
| #GASTRIC SUBMUCOSA | (19) | (49) | (39) |
| INFLAMMATION, CHRONIC | | | 1 (3%) |
| #SMALL INTESTINE | (19) | (48) | (39) |
| HYPERPLASIA, ADENOMATOUS | | 1 (2%) | |
| **URINARY SYSTEM** | | | |
| #KIDNEY | (20) | (49) | (38) |
| INFLAMMATION, CHRONIC | 5 (25%) | | 14 (37%) |
| PERIVASCULAR CUFFING | | 5 (10%) | |
| #URINARY BLADDER | (18) | (47) | (38) |
| EDEMA, NOS | | | 1 (3%) |
| LYMPHOCYTIC INFLAMMATORY INFILTR | | 1 (2%) | |
| INFLAMMATION, CHRONIC | | | 5 (13%) |
| PERIVASCULITIS | | | 2 (5%) |
| DEGENERATION, NOS | | 1 (2%) | |
| NECROSIS, FIBRINOID | | | 1 (3%) |
| HYPERPLASIA, EPITHELIAL | | 3 (6%) | 5 (13%) |
| #U.BLADDER/SUBMUCOSA | (18) | (47) | (38) |
| EDEMA, NOS | | 1 (2%) | |
| LYMPHOCYTIC INFLAMMATORY INFILTR | 1 (6%) | | |

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

## TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| INFLAMMATION, CHRONIC | | 31 (66%) | 30 (79%) |
| HYPERPLASIA, EPITHELIAL | | | 1 (3%) |
| **ENDOCRINE SYSTEM** | | | |
| #THYROID | (19) | (47) | (37) |
| CYSTIC FOLLICLES | | | 1 (3%) |
| **REPRODUCTIVE SYSTEM** | | | |
| #UTERUS/ENDOMETRIUM | (20) | (50) | (37) |
| CYST, NOS | | 5 (10%) | |
| INFLAMMATION, NOS | 1 (5%) | | |
| INFLAMMATION, ACUTE | | 1 (2%) | |
| HYPERPLASIA, NOS | | 1 (2%) | |
| HYPERPLASIA, CYSTIC | 6 (30%) | 9 (18%) | 2 (5%) |
| #OVARY | (20) | (48) | (38) |
| FOLLICULAR CYST, NOS | | 2 (4%) | 3 (8%) |
| PAROVARIAN CYST | 1 (5%) | | |
| INFLAMMATION, ACUTE/CHRONIC | 1 (5%) | | |
| HYPERPLASIA, GRANULOSA-CELL | | 1 (2%) | |
| **NERVOUS SYSTEM** | | | |
| NONE | | | |
| **SPECIAL SENSE ORGANS** | | | |
| NONE | | | |
| **MUSCULOSKELETAL SYSTEM** | | | |
| NONE | | | |
| **BODY CAVITIES** | | | |
| NONE | | | |
| **ALL OTHER SYSTEMS** | | | |
| NONE | | | |

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

## TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | MATCHED CONTROL | LOW DOSE | HIGH DOSE |
|---|---|---|---|
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NO LESION REPORTED | 4 | 6 | 1 |
| ANIMAL MISSING/NO NECROPSY | | | 7 |
| NECROPSY PERF/NO HISTO PERFORMED | | | 1 |
| AUTO/NECROPSY/HISTO PERF | | 2 | 1 |
| AUTOLYSIS/NO NECROPSY | | | 2 |

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

# APPENDIX E

## ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED N-NITROSODIPHENYLAMINE IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered N-Nitrosodiphenylamine in the Diet (a)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Integumentary System: Basal-cell Tumor (b) | 1/20 (5) | 5/50 (10) | 3/50 (6) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | 2.000 | 1.200 |
| Lower Limit | | 0.249 | 0.106 |
| Upper Limit | | 92.596 | 61.724 |
| Weeks to First Observed Tumor | 100 | 81 | 61 |
| Integumentary System: Fibroma (b) | 1/20 (5) | 1/50 (2) | 10/50 (20) |
| P Values (c,d) | P = 0.003 | N.S. | N.S. |
| Relative Risk (f) | | 0.400 | 4.000 |
| Lower Limit | | 0.005 | 0.642 |
| Upper Limit | | 30.802 | 169.457 |
| Weeks to First Observed Tumor | 100 | 100 | 100 |

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered N-Nitrosodiphenylamine in the Diet (a)

(continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Hematopoietic System: | | | |
| Leukemia (b) | 3/20 (15) | 2/50 (4) | 2/50 (4) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | 0.267 | 0.267 |
| Lower Limit | | 0.024 | 0.024 |
| Upper Limit | | 2.190 | 2.190 |
| Weeks to First Observed Tumor | 88 | 95 | 93 |
| Urinary Bladder: Transitional-cell Carcinoma (b) | 0/19 (0) | 0/46 (0) | 16/45 (36) |
| P Values (c,d) | P less than 0.001 | -- | P = 0.001 |
| Relative Risk (f) | | -- | Infinite |
| Lower Limit | | -- | 2.239 |
| Upper Limit | | -- | Infinite |
| Weeks to First Observed Tumor | -- | -- | 97 |

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered N-Nitrosodiphenylamine in the Diet (a)

(continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Pituitary: Chromophobe Adenoma (b) | 9/18 (50) | 9/47 (19) | 7/47 (15) |
| P Values (c,d) | P = 0.020 (N) | P = 0.017 (N) | P = 0.006 (N) |
| Departure from Linear Trend (e) | P = 0.022 | | |
| Relative Risk (f) | | 0.383 | 0.298 |
| Lower Limit | | 0.175 | 0.122 |
| Upper Limit | | 0.930 | 0.772 |
| Weeks to First Observed Tumor | 100 | 100 | 100 |
| Adrenal: Pheochromocytoma (b) | 3/19 (16) | 6/46 (13) | 1/49 (2) |
| P Values (c,d) | P = 0.017 (N) | N.S. | N.S. |
| Relative Risk (f) | | 0.826 | 0.129 |
| Lower Limit | | 0.204 | 0.003 |
| Upper Limit | | 4.740 | 1.517 |
| Weeks to First Observed Tumor | 99 | 100 | 100 |

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered N-Nitrosodiphenylamine in the Diet (a)

(continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Thyroid: C-cell Adenoma (b) | 3/19 (16) | 1/49 (2) | 2/48 (4) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Departure from Linear Trend (e) | P = 0.030 (N) | | |
| Relative Risk (f) | | 0.129 | 0.264 |
| Lower Limit | | 0.003 | 0.024 |
| Upper Limit | | 1.517 | 2.160 |
| Weeks to First Observed Tumor | 100 | 100 | 100 |
| Thyroid: Follicular-cell Carcinoma or Adenoma (b) | 0/19 (0) | 3/49 (6) | 0/48 (0) |
| P Values (c,d) | N.S. | N.S. | -- |
| Relative Risk (f) | | Infinite | -- |
| Lower Limit | | 0.243 | -- |
| Upper Limit | | Infinite | -- |
| Weeks to First Observed Tumor | -- | 100 | -- |

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered N-Nitrosodiphenylamine in the Diet (a)

(continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Pancreatic Islet: Islet-cell Adenoma (b) | 2/18 (11) | 1/45 (2) | 2/36 (6) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | 0.200 | 0.500 |
| Lower Limit | | 0.004 | 0.040 |
| Upper Limit | | 3.663 | 6.508 |
| Weeks to First Observed Tumor | 100 | 100 | 100 |
| Testis: Interstitial-cell Tumor (b) | 15/19 (79) | 38/50 (76) | 38/49 (78) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | 0.963 | 0.982 |
| Lower Limit | | 0.756 | 0.772 |
| Upper Limit | | 1.401 | 1.419 |
| Weeks to First Observed Tumor | 88 | 95 | 100 |

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats
Administered N-Nitrosodiphenylamine in the Diet (a)

(continued)

(a) Dosed groups received 1,000 or 4,000 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

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

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered N-Nitrosodiphenylamine in the Diet (a)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Integumentary System: Basal-cell Tumor of the Subcutaneous Tissue (b) | 1/20 (5) | 1/50 (2) | 3/49 (6) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | 0.400 | 1.224 |
| Lower Limit | | 0.005 | 0.108 |
| Upper Limit | | 30.802 | 62.958 |
| Weeks to First Observed Tumor | 100 | 86 | 76 |
| Integumentary System: Fibroma of the Subcutaneous Tissue (b) | 0/20 (0) | 1/50 (2) | 3/49 (6) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | Infinite | Infinite |
| Lower Limit | | 0.022 | 0.255 |
| Upper Limit | | Infinite | Infinite |
| Weeks to First Observed Tumor | -- | 100 | 97 |

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered N-Nitrosodiphenylamine in the Diet (a)

(continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Hematopoietic System: | | | |
| Granulocytic Leukemia (b) | 3/20 (15) | 0/50 (0) | 0/49 (0) |
| P Values (c,d) | P = 0.022 (N) | P = 0.021 (N) | P = 0.022 (N) |
| Departure from Linear Trend (e) | P = 0.002 | | |
| Relative Risk (f) | | 0.000 | 0.000 |
| Lower Limit | | 0.000 | 0.000 |
| Upper Limit | | 0.659 | 0.673 |
| Weeks to First Observed Tumor | 82 | -- | -- |
| Urinary Bladder: | | | |
| Transitional-cell Carcinoma (b) | 0/18 (0) | 0/48 (0) | 40/49 (82) |
| P Values (c,d) | P less than 0.001 | -- | P less than 0.001 |
| Departure from Linear Trend (e) | P = 0.042 | | |
| Relative Risk (f) | | -- | Infinite |
| Lower Limit | | -- | 5.314 |
| Upper Limit | | -- | Infinite |
| Weeks to First Observed Tumor | -- | -- | 69 |

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered N-Nitrosodiphenylamine in the Diet (a)

(continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Pituitary: Chromophobe Adenoma (b) | 8/19 (42) | 13/49 (27) | 12/48 (25) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | 0.630 | 0.594 |
| Lower Limit | | 0.306 | 0.281 |
| Upper Limit | | 1.512 | 1.445 |
| Weeks to First Observed Tumor | 98 | 100 | 88 |
| Thyroid: C-cell Carcinoma or Adenoma (b) | 1/20 (5) | 5/49 (10) | 3/48 (6) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | 2.041 | 1.250 |
| Lower Limit | | 0.254 | 0.110 |
| Upper Limit | | 94.440 | 64.251 |
| Weeks to First Observed Tumor | 100 | 100 | 88 |

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered N-Nitrosodiphenylamine in the Diet (a)

(continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Mammary Gland: Fibroadenoma (b) | 4/20 (20) | 6/50 (12) | 6/49 (12) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk | | 0.600 | 0.612 |
| Lower Limit | | 0.164 | 0.168 |
| Upper Limit | | 2.659 | 2.710 |
| Weeks to First Observed Tumor | 82 | 88 | 100 |

(a) Dosed groups received 1,000 or 4,000 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

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

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

# APPENDIX F

## ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED N-NITROSODIPHENYLAMINE IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered N-Nitrosodiphenylamine in the Diet (a)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Lung: Alveolar/Bronchiolar Adenoma (b) | 4/20 (20) | 9/48 (19) | 7/48 (15) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | 0.938 | 0.729 |
| Lower Limit | | 0.307 | 0.216 |
| Upper Limit | | 3.804 | 3.112 |
| Weeks to First Observed Tumor | 101 | 101 | 87 |
| Hematopoietic System: Lymphoma or Leukemia (b) | 1/20 (5) | 2/49 (4) | 3/48 (6) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | 0.816 | 1.250 |
| Lower Limit | | 0.046 | 0.110 |
| Upper Limit | | 47.195 | 64.251 |
| Weeks to First Observed Tumor | 91 | 93 | 101 |

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered N-Nitrosodiphenylamine in the Diet (a)

(continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| All Sites: Hemangioma (b) | 1/20 (5) | 3/49 (6) | 3/48 (6) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | 1.224 | 1.250 |
| Lower Limit | | 0.108 | 0.110 |
| Upper Limit | | 62.958 | 64.251 |
| Weeks to First Observed Tumor | 101 | 87 | 98 |
| All Sites: Hemangiosarcoma (b) | 0/20 (0) | 4/49 (8) | 2/48 (4) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | Infinite | Infinite |
| Lower Limit | | 0.394 | 0.128 |
| Upper Limit | | Infinite | Infinite |
| Weeks to First Observed Tumor | -- | 101 | 101 |

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered N-Nitrosodiphenylamine in the Diet (a)

(continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| All Sites: Hemangioma or Hemangiosarcoma (b) | 1/20 (5) | 7/49 (14) | 5/48 (10) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | 2.857 | 2.083 |
| Lower Limit | | 0.411 | 0.259 |
| Upper Limit | | 125.833 | 96.358 |
| Weeks to First Observed Tumor | 101 | 87 | 98 |
| Liver: Hepatocellular Carcinoma or Adenoma (b) | 6/20 (30) | 12/49 (24) | 7/48 (15) |
| P Values (c,d) | N.S. | N.S | N.S. |
| Relative Risk (f) | | 0.816 | 0.486 |
| Lower Limit | | 0.343 | 0.167 |
| Upper Limit | | 2.350 | 1.567 |
| Weeks to First Observed Tumor | 101 | 72 | 101 |

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice
Administered N-Nitrosodiphenylamine in the Diet (a)
(continued)

(a) Dosed groups received 10,000 or 20,000 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

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

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered N-Nitrosodiphenylamine in the Diet (a)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b) | 3/20 (15) | 11/47 (23) | 5/38 (13) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | 1.560 | 0.877 |
| Lower Limit | | 0.480 | 0.195 |
| Upper Limit | | 8.051 | 5.213 |
| Weeks to First Observed Tumor | 101 | 80 | 101 |
| Hematopoietic System: Lymphoma or Leukemia (b) | 1/20 (5) | 4/50 (8) | 4/41 (10) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | 1.600 | 1.951 |
| Lower Limit | | 0.175 | 0.214 |
| Upper Limit | | 77.169 | 93.623 |
| Weeks to First Observed Tumor | 77 | 95 | 63 |

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered N-Nitrosodiphenylamine in the Diet (a)

(continued)

| Topography: Morphology | Matched Control | Low Dose | High Dose |
|---|---|---|---|
| All Sites: Hemangioma (b) | 0/20 (0) | 1/50 (2) | 3/41 (7) |
| P Values (c,d) | N.S. | N.S. | N.S. |
| Relative Risk (f) | | Infinite | Infinite |
| Lower Limit | | 0.022 | 0.305 |
| Upper Limit | | Infinite | Infinite |
| Weeks to First Observed Tumor | -- | 101 | 94 |
| Liver: Hepatocellular Carcinoma or Adenoma (b) | 3/20 (15) | 7/49 (14) | 4/38 (11) |
| P Values (c,d) | N.S. | N.S | N.S. |
| Relative Risk (f) | | 0.952 | 0.702 |
| Lower Limit | | 0.250 | 0.134 |
| Upper Limit | | 5.317 | 4.432 |
| Weeks to First Observed Tumor | 101 | 100 | 101 |

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered N-Nitrosodiphenylamine in the Diet (a)

(continued)

(a) Dosed groups received time-weighted average doses of 2,315 or 5,741 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

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

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

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

December 13, 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 on the Institute's bioassay program to identify and 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 N-Nitrosodiphenylamine.

The primary reviewer for the report on the bioassay of N-Nitrosodiphenylamine agreed with the conclusion that the compound was carcinogenic in treated rats but was not in treated mice, under the conditions of test. After a brief description of the experimental design, he said that there were no outstanding shortcomings worth noting. Based on the findings, he said that N-Nitrosodiphenylamine may be a potential human carcinogen.

The secondary reviewer raised a question as to whether the bladder tumors in the treated rats were related to the presence of calculi. NCI pathologists responded that no calculi were reported by the examining pathologist and, at this point, there was no way of determining if they were specifically looked for. Since a carcinogenic effect was demonstrated, one Subgroup member commented that the report should stand on its own even though the mechanism by which the bladder tumors were induced is unknown.

A Subgroup member said that N-Nitrosodiphenylamine is a classical, non-biologically active nitrosamine. He suggested that the test compound may have nitrosated an amine present in the food which resulted in the formation of a carcinogenic nitrosamine whose target organ was the bladder. Because of the possibility, this Subgroup member urged

great caution in the interpretation of the results of the study for man. He recommended that the compound be retested using a diet free of nitrosatable amines.

After discussion regarding the framing of an appropriate motion, it was moved that the report on the bioassay of N-Nitrosodiphenylamine be accepted with the addition of comments to the Report's Summary section concerning: 1) the unknown role of calculi in the etiology of the bladder cancer in treated rats, because of the lack of knowledge as to whether they were present and; 2) the uncertainty as to whether the test compound reacted with a nitrosatable amine(s) in the diet to form a carcinogenic nitrosamine responsible for the induction of the bladder tumors. The motion was seconded and approved unanimously.

Clearinghouse Members Present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center
Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Verald K. Rowe, Dow Chemical USA
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center
Kenneth Wilcox, Michigan State Health Department

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

*U.S. GOVERNMENT PRINTING OFFICE: 1979 281-217/3151 1-3

**DHEW Publication (NIH) No. 79-1720**