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BIOASSAY OF N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE FOR POSSIBLE CARCINOGENICITY

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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BIOASSAY OF

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

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REPORT ON THE BIOASSAY OF N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE FOR POSSIBLE CARCINOGENICITY

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

FOREWORD: This report presents the results of the bioassay of N-(1-naphthyl)ethylenediamine dihydrochloride conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of N-(1-naphthyl)ethylenediamine dihydrochloride was conducted by Mason Research Institute, Worcester, Massachusetts, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. E. Smith (3) and Dr. A. Handler (3). Animal treatment and observation were supervised by Mr. G. Wade (3) and Ms. E. Zepp (3). Chemical analysis was performed by Midwest Research Institute (4) and the analytical results were reviewed by Dr. N. Zimmerman (5).

Histopathologic examinations were performed by Dr. A. B. Russfield (3) at the Mason Research Institute, and the diagnoses included in this report represent the interpretation of this pathologist. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (6).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (7); the statistical analysis was performed by Mr. R. M. Helfand (5) and Dr. J. P. Dirkse, III (8), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (9).

This report was prepared at METREK, a Division of The MITRE Corporation (5) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (5), task leader Ms. P. Walker (5), senior biologist Mr. M. Morse (5), biochemist Mr. S. C. Drill (5), and technical editor Ms. P. A. Miller (5). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1,10), Dr. R. A. Griesemer (1), Dr. T. E. Hamm (1), Dr. W. V. Hartwell (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,11), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay for the possible carcinogenicity of N-(1-naphthyl) ethylenediamine dihydrochloride was conducted using Fischer 344 rats and B6C3F1 mice. N-(1-Naphthyl)ethylenediamine dihydrochloride was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty-five rats of each sex and 50 mice of each sex were placed on test as controls. The high and low dietary concentrations of N-(1-naphthyl)ethylene-diamine dihydrochloride administered to rats and male mice were 0.1 and 0.05 percent, respectively. The high and low time-weighted average concentrations administered to female mice were, respectively, 0.3 and 0.2 percent. The compound was administered in the diet for 104 weeks, followed by an observation period of 4 weeks for high dose rats, 3 weeks for low dose rats, low dose female mice, and high dose female mice, and 1 week for high dose male mice.

There were no significant positive associations between the concentrations of N-(1-naphthyl)ethylenediamine dihydrochloride administered and mortality in rats of either sex or in male mice. There was a significant positive association between concentration and mortality in female mice. In all groups, except for high dose females, adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors. Mean body weight depression, in relation to controls, was apparent for both sexes of rats and mice, indicating that higher concentrations of the test chemical would not have been tolerated by these animals.

In rats or mice of either sex, there were no statisticaly significant positive associations between the concentration of N-(1-naphthyl) ethylenediamine dihydrochloride and tumor incidence.

Under the conditions of this bioassay, dietary administration of N-(1-naphthyl)ethylenediamine dihydrochloride was not carcinogenic in Fischer 344 rats or B6C3F1 mice.

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I. INTRODUCTION

N-(1-Naphthyl)ethylenediamine dihydrochloride (Figure 1) (NCI No. C03281), a diagnostic reagent derived from 1-naphthylamine, was selected for bioassay by the National Cancer Institute because of the suspected carcinogenicity of its parent compound, and the confirmed bladder carcinogenicity of the related compound 2-naphthylamine in humans (International Agency for Research on Cancer, 1974). The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is N-1-naphthalenyl-1,2-ethanediamine dihydrochloride.* It is also called N-1-naphthylethylenediamine dihydrochloride.

The use of N-(1-naphthyl)ethylenediamine dihydrochloride as a quantitative reagent for the colorimetric determination of sulfanilimide in blood, urine, and other body fluids was first described by Bratton and Marshall (1939). Use of the compound in this capacity has persisted and N-(1-naphthyl)ethylenediamine dihydrochloride is now used to determine the concentrations of potassium, nitrites, and sulfates, as well as sulfanilamide (Windholz, 1976).

Specific production data for N-(1-naphthyl)ethylenediamine dihydrochloride are not available; however, the inclusion of this compound in the 1977 Directory of Chemical Producers, U.S.A. (Stanford Research Institute, 1977) implies a total annual U.S. production in excess of 1000 pounds or $1000 in value.

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*The CAS Registry number is 1465-25-4.

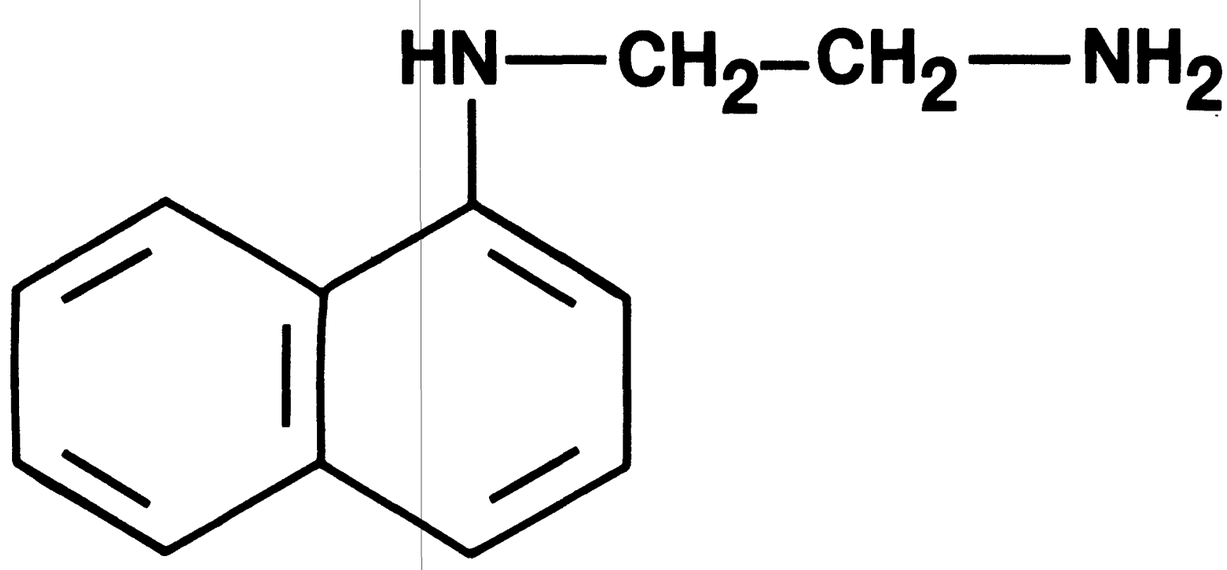


FIGURE 1
CHEMICAL STRUCTURE OF N-(1-NAPHTHYL)ETHYLENEDIAMINE(DIHYDROCHLORIDE)

Since the sole commercial application of N-(1-naphthyl)ethylenediamine dihydrochloride appears to be its use as a diagnostic reagent, the potential for exposure is presumably limited to medical and veterinary workers and employees of production facilities which manufacture the compound.

II. MATERIALS AND METHODS

A. Chemicals

N-(1-Naphthyl)ethylenediamine dihydrochloride was purchased from Schwarz/Mann, Orangeburg, New York. Chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri. The experimentally determined melting point range of 183° to 186°C was in general agreement with the value reported in the literature (i.e., 182° to 188°C) (Naletskaya et al., 1967). Thin-layer chromatography was performed utilizing two solvent systems (i.e., ethyl acetate:ammonium hydroxide:methanol and benzene:dioxane:ammonium hydroxide), and each plate was visualized with ultraviolet light and iodine vapor. Multiple trace impurities were revealed; it was concluded that these were not due to decomposition of the compound in the solvent systems. Elemental analysis was consistent with that expected for $C_{12}H_{16}N_2Cl_2$, the molecular formula for N-(1-naphthyl)ethylenediamine dihydrochloride. One major peak and four minor peaks were separated by vapor-phase chromatography. The largest impurity peak accounted for approximately 1.1 percent of the area of the major peak. The results of infrared, ultraviolet/visible, and nuclear magnetic resonance analyses were consistent with those expected based on the structure of the compound.

Throughout this report the term N-(1-naphthyl)ethylenediamine dihydrochloride is used to represent this material.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® (Allied Mills, Inc., Chicago, Illinois). N-(1-Naphthyl)ethylenediamine dihydrochloride was administered to the dosed animals as a component of the diet. Proper amounts of the chemical were removed from the stock bottle and blended in an aluminum bowl with an aliquot of the ground feed under an exhaust hood. The mixture was then placed into a 6 kg capacity Patterson-Kelley twin shell stainless steel V-blender along with the remainder of the meal. After 20 minutes of blending, the mixtures were placed in double plastic bags, and stored in the dark at 4°C. The mixture was prepared once weekly.

C. Animals

The two animal species, Fischer 344 rats and B6C3F1 mice, used in the carcinogenicity bioassay were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. All animals were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Dosed and control animals for each species were received in separate shipments.

Upon arrival, a sample of animals was examined for parasites and other signs of disease. All sampled animals had parasites. Consequently, all animals were administered 3.0 gm piperazine adipate per liter of drinking water, ad libitum, for 3 days, followed by 3 days of tap water and 3 more days of piperazine adipate. Animals to be

used in the chronic study were quarantined by species for 2 weeks prior to initiation of test. Animals were assigned to groups and distributed among cages so that average body weight per cage was approximately equal for a given sex and species.

D. Animal Maintenance

All animals were housed by species in rooms having a temperature range of 23° to 34°C. Incoming air was filtered through Tri-Dek® 15/40 denier Dacron® filters (Tri-Dim Filter Corporation, Hawthorne, New Jersey) providing six changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

Rats were housed five per cage by sex. During quarantine and for the first 11 months of study, rats were kept in galvanized- or stainless-steel wire-mesh cages (Fenco Cage Products, Boston, Massachusetts) suspended above newspapers. Newspapers under cages were replaced daily and cages and racks washed weekly. For the remainder of the study, rats were kept in suspended polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) equipped with disposable nonwoven fiber filter sheets. Clean bedding and cages were provided twice weekly. Aspen hardwood chip bedding (American Excelsior Company, Baltimore, Maryland) was used in polycarbonate cages.

Mice were housed by sex in polycarbonate shoe box type cages. Cages were fitted with perforated stainless steel lids (Lab Products, Inc.) and nonwoven fiber filter bonnets. Control mice were housed ten per cage for the first month of study and five per cage thereafter.

Dosed mice were housed five per cage for the entire study. Clean cages, lids, and bedding were provided three times per week for cage populations of ten, and twice per week for cage populations of five. SAN-I-CEL® corncob bedding (Paxton Processing Company, Paxton, Illinois) and Bed-o-cobs® (The Andersons Cob Division, Maumee, Ohio) were used for the first 17 months of study. Aspen hardwood chip bedding was used for the remainder of the study. Reusable filter bonnets and pipe racks were sanitized every 2 weeks throughout the study.

Tap water was available from 250 ml water bottles equipped with rubber stoppers and stainless steel sipper tubes. Bottles were replaced twice weekly and, for rats only, water was supplied as needed between changes. Food and water were available ad libitum.

During the period of chemical administration, all animals were fed Wayne Lab-Blox® meal containing the appropriate concentration of N-(1-naphthyl)ethylenediamine dihydrochloride. Control animals had untreated meal available ad libitum. For the first 11 months of study, meal was supplied to rats and mice from Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc., Woburn, Massachusetts) containing stainless steel baffles. All animals were fed from stainless steel gangstyle hoppers (Scientific Cages, Inc., Bryan, Texas) for the remainder of the study. Food hoppers were changed on the same schedule as were cages. Food was replenished daily in Alpine® feed cups.

Dosed and control rats were housed in a room with other rats receiving diets containing* n-butylurea (592-31-4); N,N-dimethyl-p-nitrosoaniline (138-89-6); 2,5-toluenediamine sulfate (6369-59-1); 2,4-dinitrotoluene (121-14-2); 1,5-naphthalenediamine (2243-62-1); 2-chloro-p-phenylenediamine sulfate (61702-44-1); aniline hydrochloride (142-04-1); and p-anisidine hydrochloride (20265-97-8).

All control mice and dosed male mice were housed in a room with other mice receiving diets containing hydrazobenzene (530-50-7); 2,3,5,6-tetrachloro-4-nitroanisole (2438-88-2); tris(2,3-dibromopropyl)phosphate (126-72-7); 2-chloro-p-phenylenediamine sulfate (61702-44-1); and aniline hydrochloride (142-04-1). Dosed female mice were housed in a room with mice intubated with m-cresidine (102-50-1); and with other mice receiving diets containing 1,5-naphthalenediamine (2243-62-1) and 1H-benzotriazole (95-14-7).

E. Selection of Initial Concentrations

To establish the maximum tolerated concentrations of N-(1-naphthyl)ethylenediamine dihydrochloride for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among six groups, each consisting of five males and five females. N-(1-Naphthyl)ethylenediamine dihydrochloride was incorporated into the laboratory diet and supplied ad libitum to five of the six rat groups in concentrations of 0.03, 0.1, 0.3, 1.0, and

*CAS registry numbers are given in parentheses.

3.0 percent and to five of the six mouse groups in concentrations of 0.003, 0.01, 0.03, 0.1 and 0.3 percent. The sixth group of each species served as a control group, receiving only the basal laboratory diet. The dosed dietary preparations were administered for 8 weeks.

Individual body weights were recorded weekly throughout the study. Food consumption data were recorded during weeks 1, 4 and 7. All survivors were sacrificed at the end of the subchronic test, and gross necropsies were performed.

The highest concentration causing no deaths, no compound-related gross abnormalities, and no mean body weight depression in excess of 20 percent relative to controls during the 8-week subchronic test was selected as the high concentration utilized for the rat and mouse chronic bioassays.

At the end of the subchronic test, the only rats surviving at a dietary concentration of 0.3 percent or greater were one male and five females receiving 0.3 percent and one female receiving 1.0 percent. No deaths were recorded in any rat group receiving less than 0.3 percent. No gross abnormalities were recorded at any concentration for male or female rats. Mean body weight depression, relative to controls, was 29.3, 16.2 and 19.9 percent for male rats receiving dietary concentrations of 0.3, 0.1 and 0.03 percent, respectively. Mean body weight depressions observed in female rats were 37.6, 20.3, 13.3 and 7.4 percent at dietary concentrations of 1.0, 0.3, 0.1 and

0.03 percent, respectively. The high concentration selected for administration to rats in the chronic bioassay was 0.1 percent.

At the end of the subchronic test, all mice except one male receiving a dietary concentration of 0.3 percent survived. No gross abnormalities were noted at any concentration for male or female mice. Mean body weight depression, relative to controls, was observed in only 2 of the dosed groups, males receiving 0.1 and 0.3 percent. Mean body weight depression in both cases was 6.4 percent. The high concentrations selected for administration to mice in the chronic bioassay were 0.1 and 0.4 percent for males and females, respectively.

F. Experimental Design

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

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test simultaneously. The dietary concentrations of N-(1-naphthyl)ethylenediamine dihydrochloride administered to rats were 0.1 and 0.05 percent. Throughout this report those rats receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups. The dosed rats were supplied with feed containing N-(1-naphthyl)ethylenediamine dihydrochloride for a total of

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE FEEDING EXPERIMENT

INITIAL GROUP SIZE	N-(1-NAPHTHYL) ETHYLENEDIAMINE DIHYDROCHLORIDE CONCENTRATION (PERCENT)	OBSERVATION PERIOD	
		TREATED (WEEKS)	UNTREATED (WEEKS)
MALE			
CONTROL 25	0	0	110
LOW DOSE 50	0.05	104	
	0		3
HIGH DOSE 50	0.1	104	
	0		4
FEMALE			
CONTROL 25	0	0	110
LOW DOSE 50	0.05	104	
	0		3
HIGH DOSE 50	0.1	104	
	0		4

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	N-(1-NAPHTHYL) ETHYLENEDIAMINE DIHYDROCHLORIDE CONCENTRATION (PERCENT)	OBSERVATION PERIOD		TIME-WEIGHTED AVERAGE CONCENTRATION[a]
			TREATED (WEEKS)	UNTREATED (WEEKS)	
MALE					
CONTROL	50	0	0	110	0
LOW DOSE	50	0.05	104		0.05
		0		0	
HIGH DOSE	50	0.1	104		0.1
		0		1	
FEMALE					
CONTROL	50	0	0	109	0
LOW DOSE	50	0.2	104		0.2
		0		3	
HIGH DOSE	50	0.4	73		0.3
		0.1	31		
		0		3	

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

104 weeks followed by an observation period of 4 weeks for the high dose groups and 3 weeks for the low dose groups.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test simultaneously. The dietary concentrations of N-(1-naphthyl)ethylenediamine dihydrochloride administered to male mice were 0.10 and 0.05 percent. Throughout this report those male mice receiving the 0.10 percent concentration are referred to as the high dose male mice and those receiving the 0.05 percent concentration are referred to as the low dose male mice. These concentrations were administered for 104 weeks followed by an observation period of 1 week for the high dose group.

The initial dietary concentrations administered to female mice were 0.4 and 0.2 percent. Due to excessive mortality in the group receiving 0.4 percent, this dosage was lowered to 0.1 percent in week 74, for the remaining 31 weeks of chemical administration. Throughout this report those female mice initially receiving a concentration of 0.4 percent are referred to as the high dose female mice and those receiving 0.2 percent are referred to as the low dose female mice. Dosed female mice were observed for 3 weeks after compound administration ceased.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. Body weights were recorded twice weekly for the first 12 weeks of the study and at monthly intervals thereafter. All

animals were inspected twice daily for mortality. Food consumption, for two cages from each group, was monitored for seven consecutive days once a month for the first nine months of the bioassay and for three consecutive days each month thereafter. The presence of tissue masses and lesions was determined by monthly observation and palpation of each animal.

A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized by carbon dioxide inhalation, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in 10 percent buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination. An occasional section was subjected to special staining techniques for more definitive diagnosis.

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

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

H. Data Recording and Statistical Analyses

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

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be

missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

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

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

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

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

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

procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

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

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

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

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

High dose male rats evidenced mean body weight depression relative to the control group. Distinct and consistent dose-related mean body weight depression was apparent in female rats throughout the bioassay (Figure 2).

Although isolated clinical observations were recorded, there were none that were consistently related to administration of the compound.

B. Survival

The estimated probabilities of survival for male and female rats in the control and N-(1-naphthyl)ethylenediamine dihydrochloride-dosed groups are shown in Figure 3.

For both male and female rats the Tarone test showed no positive association between dosage and mortality. For male rats the Tarone test did show a significant negative association; however, the Cox tests comparing both high dose to control and low dose to control were not significant. For female rats the Tarone test also showed a significant negative association between dosage and mortality as did the Cox test comparing high dose to control.

Adequate numbers of male rats were at risk from late-developing tumors, as 88 percent (44/50) of the high dose, 84 percent (42/50) of the low dose, and 68 percent (17/25) of the control group survived on test for at least 107 weeks. Four males in the control group died

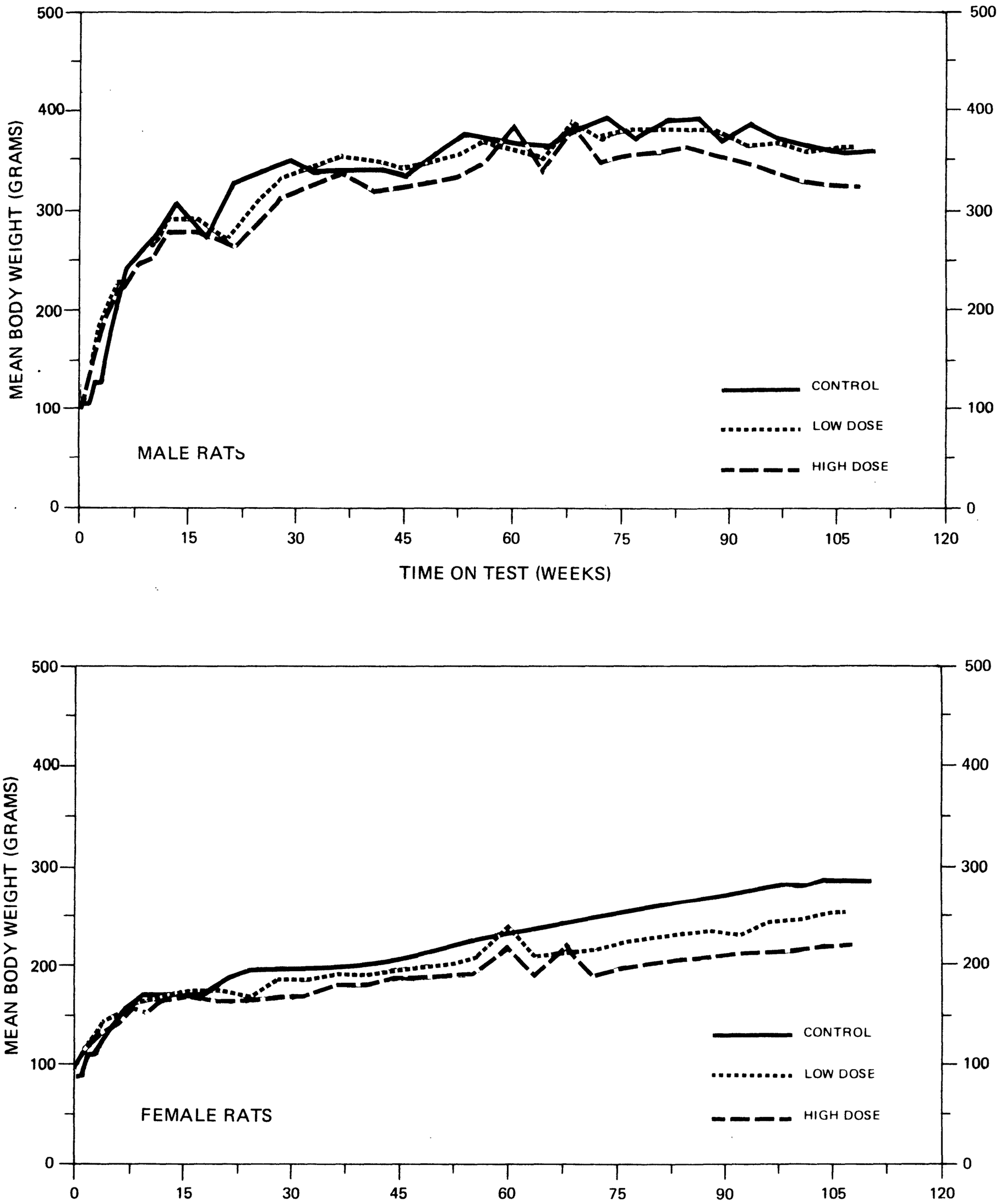


FIGURE 2
GROWTH CURVES FOR
N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE
CHRONIC STUDY RATS

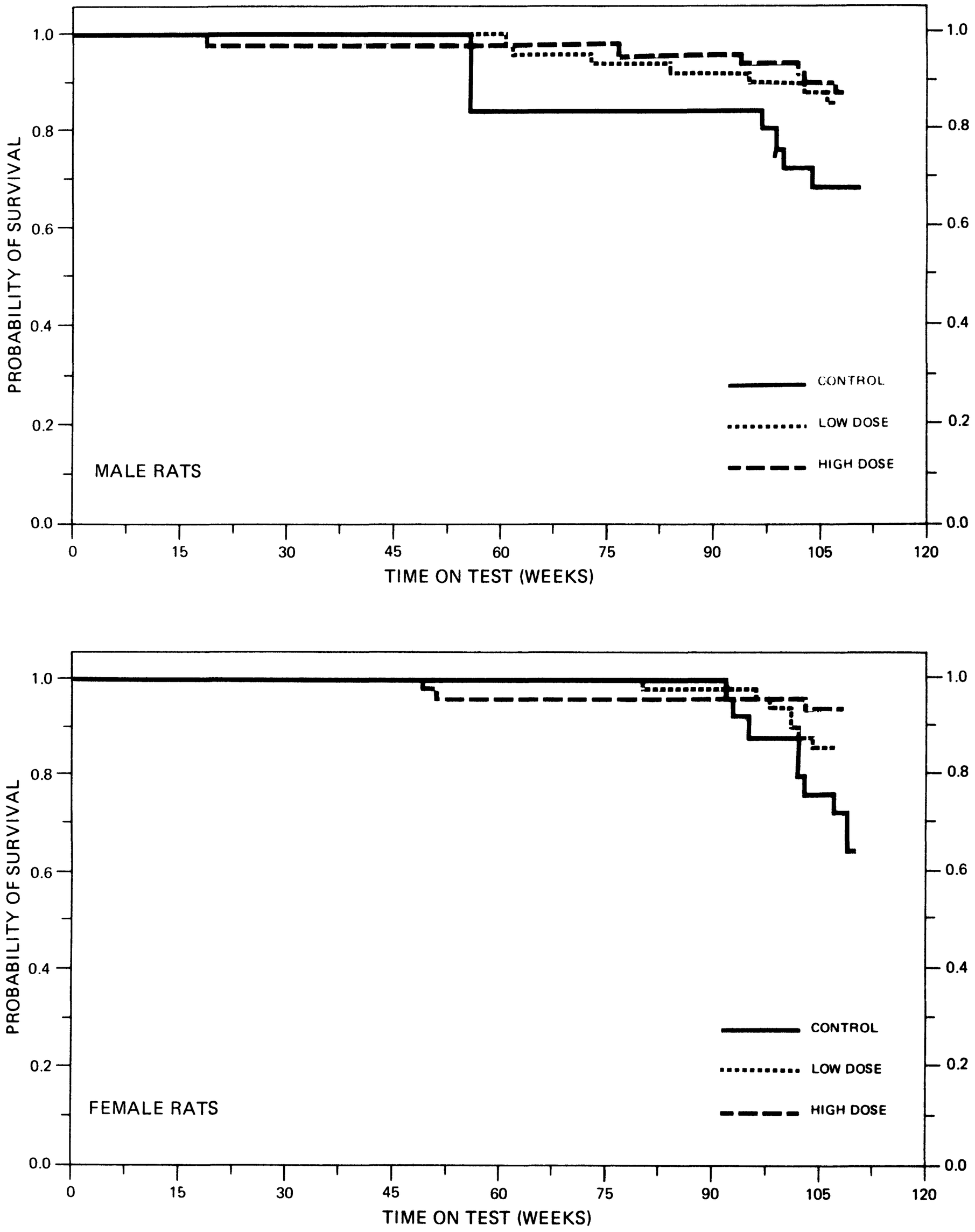


FIGURE 3
SURVIVAL COMPARISONS OF
N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE
CHRONIC STUDY RATS

in week 56; no tumors or other clinical signs were noted for these four rats.

For female rats, 94 percent (47/50) of the high dose, 86 percent (43/50) of the low dose, and 76 percent (19/25) of the control group survived on test for at least 107 weeks. Thus there were adequate numbers of female rats at risk from late-developing tumors.

C. Pathology

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

A variety of neoplasms occurred with similar frequencies in control and dosed rats except for those of the urinary tract and liver.

A dose-related change was observed in the renal pelvis of the male rats. The incidences of renal and bladder lesions were as follows:

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
URINARY SYSTEM						
Kidney						
(Number of Animals with Tissues Examined Histopathologically)	(25)	(48)	(49)	(24)	(50)	(50)
Tubular-Cell Adenoma	0	1	0	0	0	0
Hamartoma*	0	0	1	0	0	0
Kidney/Pelvis						
(Number of Animals with Tissues Examined Histopathologically)	(25)	(48)	(49)	(24)	(50)	(50)
Epithelial Hyperplasia	0	1	7	0	0	0
Urinary Bladder						
(Number of Animals with Tissues Examined Histopathologically)	(25)	(48)	(49)	(24)	(50)	(49)
Transitional-Cell Papilloma	0	1	0	1	1	0
Transitional-Cell Carcinoma	0	0	0	0	1	0
Epithelial Hyperplasia	0	0	1	0	0	0

Epithelial hyperplasia of the renal pelvis was a papillary and often multifocal proliferation of the transitional-cell epithelium. The transitional cells comprising these lesions were arranged in orderly cords and had an apparently intact basement membrane. Mitotic figures were seen, and nuclei showed moderate variation in size and shape with occasional parachromatin clearing. There was very little

*This is considered to be a benign form of the mixed tumor of the kidney and consists of proliferative lipocytes, tubular structures, fibroblasts, and vascular spaces in varying proportions.

stroma in the papillary formations and blood vessels were not prominent. Adjacent epithelium was often hyperplastic. The lesions were classified as papillomas.

There were two tumors of the renal parenchyma, a tubular-cell adenoma in a low dose male and a hamartoma in a high dose male. A papilloma and a noninvasive papillary carcinoma were seen in the transitional-cell epithelium of the bladder of low dose females. A papilloma also occurred in the bladder of one control female. There were liver neoplasms observed in the low dose female rats that may have been related to administration of the test chemical.

A variety of other nonneoplastic lesions which are commonly seen in aged Fischer 344 rats was observed with approximately equal frequency in the dosed and control groups.

Based on the results of this pathologic examination, the carcinogenicity of N-(1-naphthyl)ethylenediamine dihydrochloride for Fischer 344 rats was not established under the conditions of this experiment. Proliferative lesions of the renal pelvis which occurred only in dosed rats may be related to the dietary administration of N-(1-naphthyl)ethylenediamine dihydrochloride.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or N-(1-naphthyl)

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Skin: Squamous-Cell Papilloma[b]	2/25(0.08)	1/48(0.02)	0/50(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.260	0.000
Lower Limit	---	0.005	0.000
Upper Limit	---	4.803	1.685
Weeks to First Observed Tumor[f]	110	107	---
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	0/25(0.00)	1/48(0.02)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.028	0.315
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor[f]	---	107	107
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	1/25(0.04)	10/48(0.21)	4/50(0.08)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.017	---	---
Relative Risk (Control)[d]	---	5.208	2.000
Lower Limit	---	0.819	0.215
Upper Limit	---	220.262	96.452
Weeks to First Observed Tumor[f]	110	84	77

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma NOS, Chromophobe Adenoma, or Basophil Adenoma[b]	2/22(0.09)	15/44(0.34)	7/47(0.15)
P Values[c]	N.S.	P = 0.025	N.S.
Departure from Linear Trend[e]	P = 0.007	---	---
Relative Risk (Control)[d]	---	3.750	1.638
Lower Limit	---	1.000	0.351
Upper Limit	---	31.675	15.363
Weeks to First Observed Tumor[f]	99	61	107
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant[b]	2/24(0.08)	9/48(0.19)	7/49(0.14)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.250	1.714
Lower Limit	---	0.522	0.364
Upper Limit	---	20.345	16.124
Weeks to First Observed Tumor[f]	110	62	107
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	2/21(0.10)	3/47(0.06)	6/49(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.670	1.286
Lower Limit	---	0.084	0.259
Upper Limit	---	7.654	12.403
Weeks to First Observed Tumor[f]	97	107	103

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pancreatic Islets: Islet-Cell Adenoma or Islet-Cell Carcinoma[b]	1/25(0.04)	1/47(0.02)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.532	1.531
Lower Limit	---	0.007	0.132
Upper Limit	---	40.896	78.672
Weeks to First Observed Tumor[f]	99	107	94
Testis: Interstitial-Cell Tumor[b]	21/25(0.84)	46/48(0.96)	40/49(0.82)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.036	---	---
Relative Risk (Control)[d]	---	1.141	0.972
Lower Limit	---	0.958	0.801
Upper Limit	---	1.289	1.277
Weeks to First Observed Tumor[f]	97	84	102

[a]Treated groups received doses of 0.05 or 0.1 percent in feed.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

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

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

[f]Controls were terminally censored in week 110; low dose in week 107; and high dose in weeks 107 and 108.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	3/24(0.13)	4/50(0.08)	0/50(0.00)
P Values[c]	P = 0.019(N)	N.S.	P = 0.031(N)
Relative Risk (Control)[d]	---	0.640	0.000
Lower Limit	---	0.120	0.000
Upper Limit	---	4.113	0.793
Weeks to First Observed Tumor[f]	95	80	---
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	0/24(0.00)	8/50(0.16)	1/50(0.02)
P Values[c]	N.S.	P = 0.036	N.S.
Departure from Linear Trend[e]	P = 0.003	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	1.130	0.026
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor[f]	---	101	108
Pituitary: Carcinoma NOS or Chromophobe Carcinoma[b]	0/21(0.00)	3/47(0.06)	0/42(0.00)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.047	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.279	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor[f]	---	107	---

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Carcinoma NOS, Chromophobe Carcinoma, Adenoma NOS, Chromophobe Adenoma, or Acidophil Adenoma[b]	6/21(0.29)	19/47(0.40)	6/42(0.14)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.032	---	---
Relative Risk (Control)[d]	---	1.415	0.500
Lower Limit	---	0.659	0.157
Upper Limit	---	3.774	1.671
Weeks to First Observed Tumor[f]	92	101	108
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	1/21(0.05)	2/46(0.04)	8/50(0.16)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.913	3.360
Lower Limit	---	0.051	0.504
Upper Limit	---	52.715	145.685
Weeks to First Observed Tumor[f]	109	107	108
Mammary Gland: Fibroadenoma[b]	4/24(0.17)	5/50(0.10)	1/50(0.02)
P Values[c]	P = 0.021(N)	N.S.	P = 0.036(N)
Relative Risk (Control)[d]	---	0.600	0.120
Lower Limit	---	0.145	0.003
Upper Limit	---	2.807	1.140
Weeks to First Observed Tumor[f]	109	107	108

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Uterus: Endometrial Stromal Polyp[b]	2/24(0.08)	5/50(0.10)	9/48(0.19)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.200	2.250
Lower Limit	---	0.217	0.522
Upper Limit	---	12.045	20.345
Weeks to First Observed Tumor[f]	103	107	108
Body Cavities: Mesothelioma NOS[b]	0/24(0.00)	1/50(0.02)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.026	0.297
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor[f]	---	107	108

[a]Treated groups received doses of 0.05 or 0.1 percent in feed.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

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

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

[f]Controls were terminally censored in week 110; low dose in week 107; and high dose in weeks 107 and 108.

ethylenediamine dihydrochloride-dosed groups and where such tumors were observed in at least 5 percent of the group.

For male rats, the Fisher exact test comparing low dose to control for the incidence of pituitary adenomas NOS, chromophobe adenomas or basophil adenomas was significant (P = 0.025). However, the Fisher exact test comparing high dose to control and the Cochran-Armitage test for association between dosage and incidence were not significant.

The Fisher exact test comparing the incidence of hepatocellular carcinomas or neoplastic nodules in low dose female rats to the control had a probability level of P = 0.036; however, this probability is above the 0.025 level required by the Bonferroni criterion.

For both male and female rats there were no other sites for which the statistical tests showed a significant positive association between dosage and an elevated incidence of tumors. Thus, at the dose levels used in this bioassay there was no convincing statistical evidence that N-(1-naphthyl)ethylenediamine dihydrochloride was a carcinogen in male or female Fischer 344 rats.

The Cochran-Armitage test indicated a significant negative association between dosage and the incidence of leukemia or malignant lymphomas and between dosage and the incidence of mammary gland fibroadenomas in female rats. In both instances, however, the Fisher exact tests were not significant under the Bonferroni criterion.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by N-(1-naphthyl)ethylenediamine dihydrochloride that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

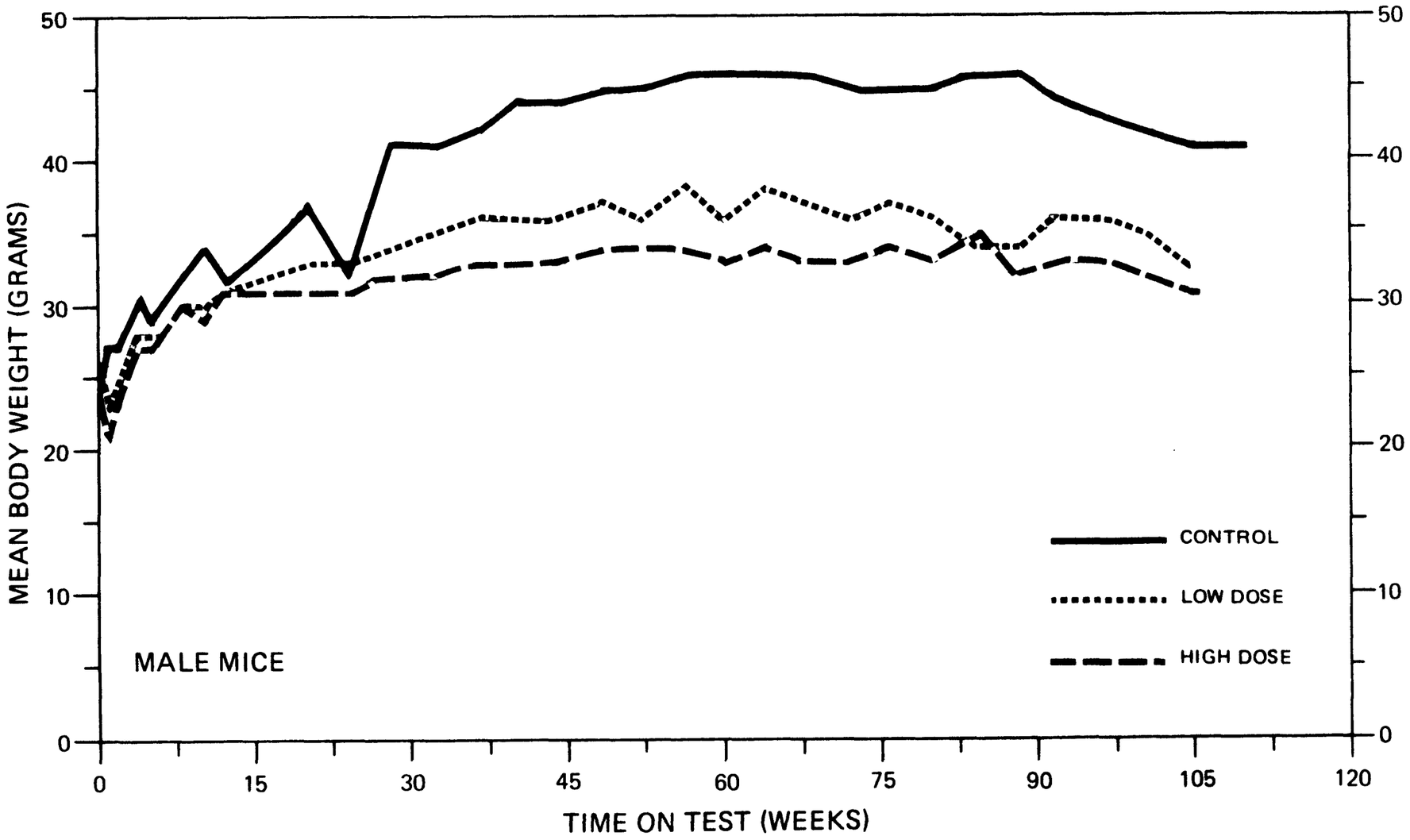
A distinct and consistent dose-related mean body weight depression was apparent in male mice throughout the bioassay. Dosed female mice evidenced mean body weight depression relative to the control group (Figure 4). Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variations.

Although isolated clinical observations were recorded, there were none that were consistently related to administration of the compound.

B. Survival

The estimated probabilities of survival for male and female mice in the control and N-(1-naphthyl)ethylenediamine dihydrochloride-dosed groups are shown in Figure 5.

For male mice the Tarone test did not show a significant positive association between dosage and mortality and the Cox tests were also not significant. For female mice, the Tarone test did indicate a significant ($P < 0.001$) positive association between dosage and mortality and the Cox test comparing high dose to control was significant ($P < 0.001$). The Tarone test for departure from linear trend was also significant ($P < 0.001$) due to the high early mortality of the high dose group, and the increased mortality of the control group relative to the low dose group starting with week 70.



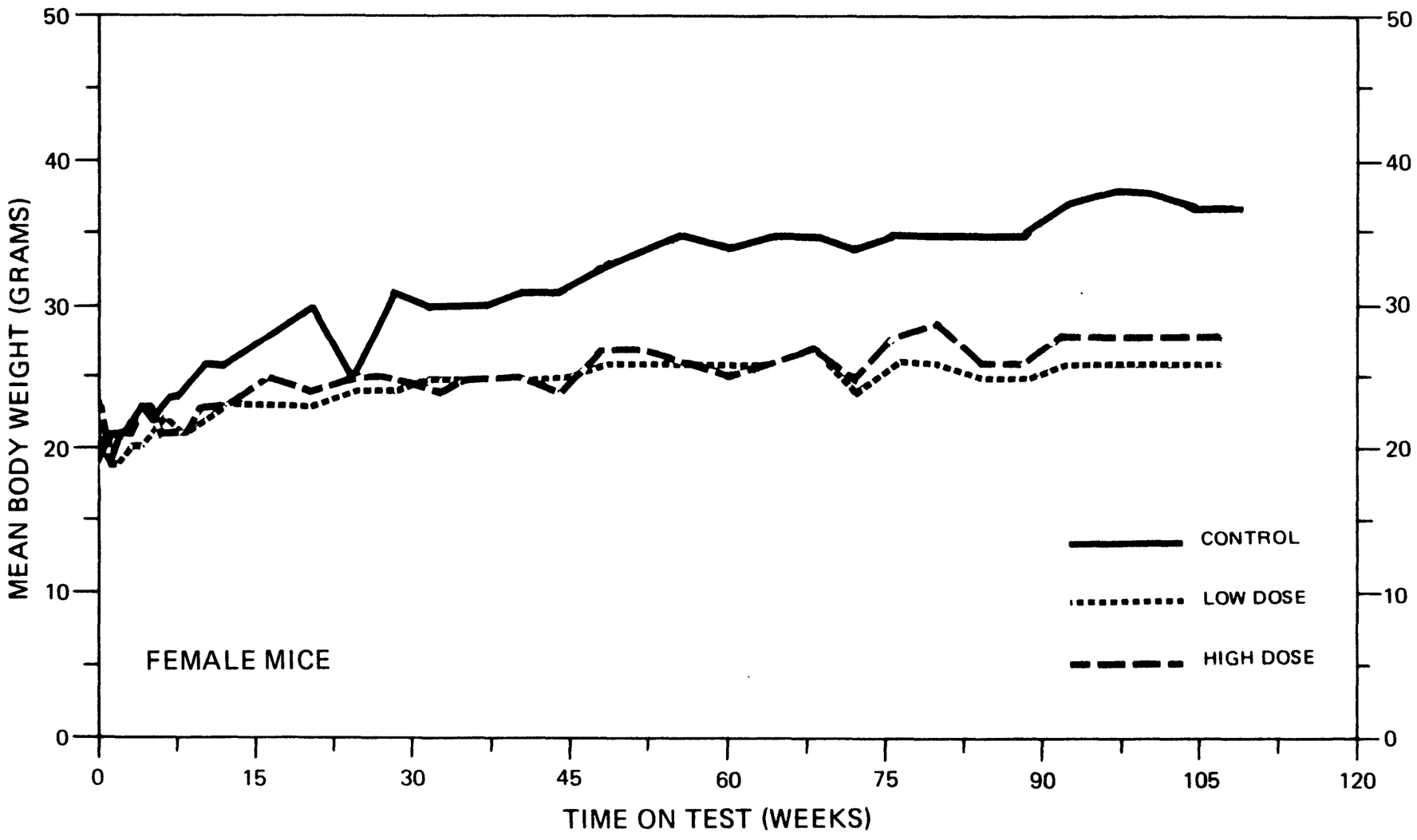


FIGURE 4
GROWTH CURVES FOR
N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE
CHRONIC STUDY MICE

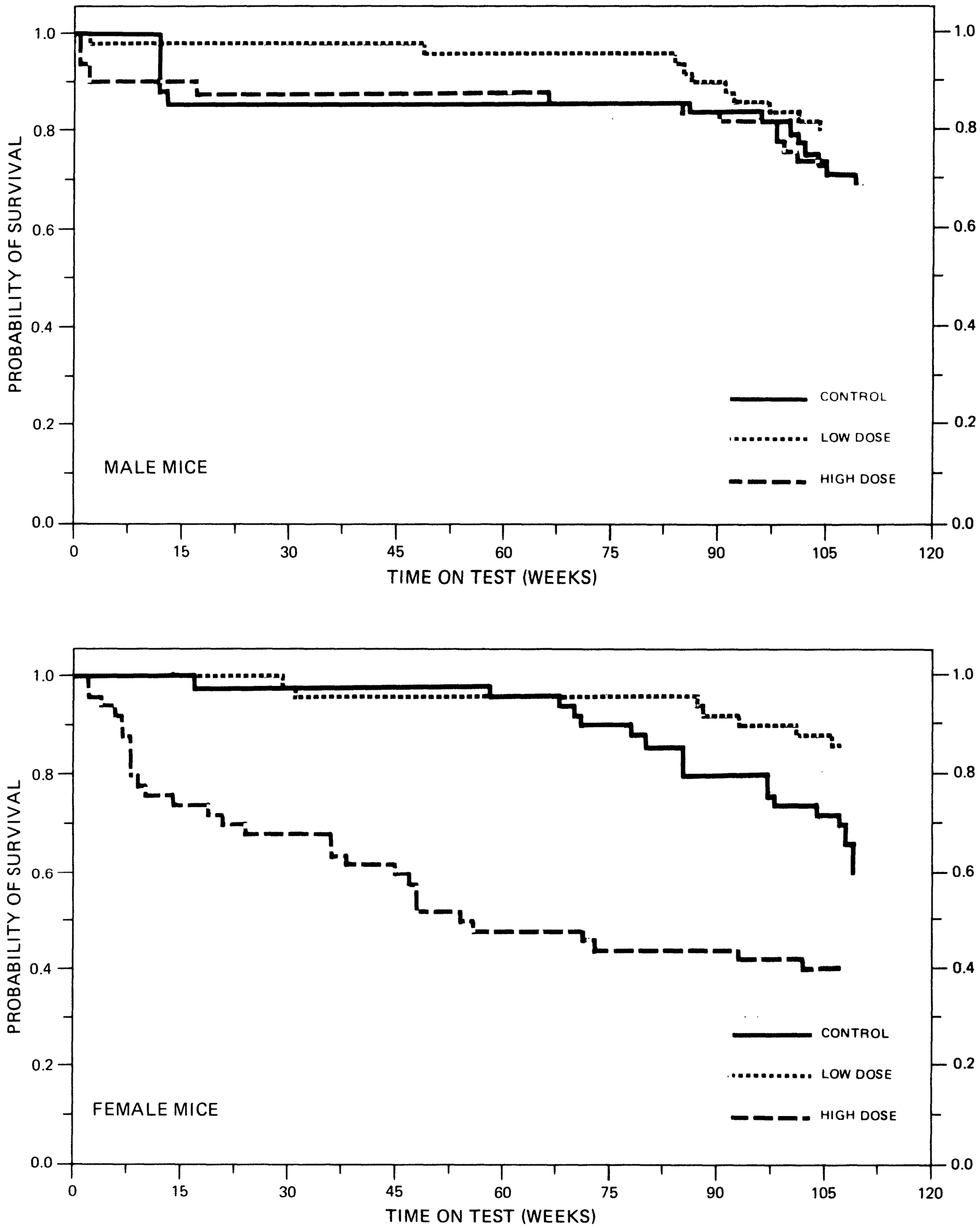


FIGURE 5
SURVIVAL COMPARISONS OF
N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE
CHRONIC STUDY MICE

Adequate numbers of male mice were at risk from late-developing tumors as 74 percent (37/50) of the high dose, 82 percent (41/50) of the low dose, and 72 percent (36/50) of the control group survived on test for at least 104 weeks.

For female mice 40 percent (20/50) of the high dose, 88 percent (44/50) of the low dose, and 72 percent (36/50) of the control group survived on test for at least 106 weeks. In the high dose female group, 24/50 (48 percent) died before week 52. No cause of death for these mice was recorded.

C. Pathology

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

A variety of neoplasms occurred with approximately equal frequency in the dosed and control mice. Occasionally, as shown in the summary tables, neoplasms occurred only in the dosed mice or with an increased frequency when compared with the control animals. The nature and incidence of these neoplasms was similar to spontaneously occurring neoplasms in B6C3F1 mice.

The dosed mice had a variety of nonneoplastic lesions. The incidence and severity of the lesions was approximately equal in the dosed and control groups. The lesions were not observed in the high dose female mice that died early in the bioassay. Hyperplasia of the renal pelvic epithelium was not found in mice of either sex.

Based on the results of this pathologic examination, the administration of N-(1-naphthyl)ethylenediamine dihydrochloride was not carcinogenic to B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or N-(1-naphthyl) ethylenediamine dihydrochloride-dosed groups and where such tumors were observed in at least 5 percent of the group. Due to the early mortality of high dose female and high dose and control male mice, the analyses for mice have been based upon those males and females surviving at least 52 weeks or, in the event that the tumor of interest appeared earlier, at least as long as the time at which the first tumor of interest was observed.

None of the statistical tests for any site in mice of either sex indicated a significant positive association between the administration of N-(1-naphthyl)ethylenediamine dihydrochloride and an increased tumor incidence. Thus, at the dose levels used in this bioassay there was no evidence that N-(1-naphthyl)ethylenediamine dihydrochloride was a carcinogen in B6C3F1 mice.

For female mice the Cochran-Armitage test indicated a significant negative association between dosage and the incidence of pituitary adenomas NOS and between dosage and the incidence of adrenal

TABLE 5

TIME-ADJUSTED ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE[a,e]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	2/39(0.05)	2/48(0.04)	2/43(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.812	0.907
Lower Limit	---	0.062	0.069
Upper Limit	---	10.794	12.006
Weeks to First Observed Tumor[f]	110	101	104
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	4/39(0.10)	7/48(0.15)	3/43(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.422	0.680
Lower Limit	---	0.393	0.106
Upper Limit	---	6.199	3.773
Weeks to First Observed Tumor[f]	109	85	104
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	13/39(0.33)	8/48(0.17)	4/44(0.09)
P Values[c]	P = 0.004(N)	N.S.	P = 0.007(N)
Relative Risk (Control)[d]	---	0.500	0.273
Lower Limit	---	0.202	0.071
Upper Limit	---	1.165	0.799
Weeks to First Observed Tumor[f]	101	86	85

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	12/39(0.31)	5/48(0.10)	9/43(0.21)
P Values[c]	N.S.	P = 0.017(N)	N.S.
Relative Risk (Control)[d]	---	0.339	0.680
Lower Limit	---	0.103	0.286
Upper Limit	---	0.937	1.562
Weeks to First Observed Tumor[f]	86	104	99

[a]Treated groups received doses of 0.05 or 0.1 percent in feed.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when P < 0.05; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when P < 0.05; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

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

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

[f]Controls were terminally censored in weeks 109 and 110; low dose in week 104; and high dose in weeks 104 and 105.

TABLE 6

TIME-ADJUSTED ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE MICE TREATED WITH N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE[a,e]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	13/49(0.27)	9/48(0.19)	4/25(0.16)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.707	0.603
Lower Limit	---	0.295	0.156
Upper Limit	---	1.612	1.701
Weeks to First Observed Tumor[f]	58	87	106
Pituitary: Adenoma NOS[b]	3/34(0.09)	0/38(0.00)	0/20(0.00)
P Values[c]	P = 0.047(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	1.472	2.713
Weeks to First Observed Tumor[f]	110	---	---
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant[b]	4/46(0.09)	0/47(0.00)	0/24(0.00)
P Values[c]	P = 0.025(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	1.053	2.008
Weeks to First Observed Tumor[f]	68	---	---

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Carcinoma or Follicular-Cell Adenoma[b]	4/44(0.09)	0/39(0.00)	1/23(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	0.478
Lower Limit	---	0.000	0.010
Upper Limit	---	1.207	4.417
Weeks to First Observed Tumor[f]	80	---	106

[a]The low dose treated group received a dose of 0.2 percent in feed. The high dose treated group received a time-weighted average dose of 0.3 percent in feed.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

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

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

[f]Controls were terminally censored in week 109; low dose in week 107; and high dose in weeks 106 and 107.

pheochromocytomas or malignant pheochromocytomas. The Fisher exact tests, however, were not significant for either site.

A significant negative Fisher exact test was indicated for the comparison between the incidence of hepatocellular carcinomas in the low dose male mice to that in the control group. However, the high dose Fisher exact test and the Cochran-Armitage test for linear trend were not significant. Both the Cochran-Armitage test and the Fisher exact test comparing high dose to control had significant negative results for the incidence of leukemias or malignant lymphomas in male mice. It should be noted, however, that in historical control data collected by this laboratory for the NCI Carcinogenesis Testing Program, only 22/275 (8 percent) of the untreated B6C3F1 male mice had leukemias or malignant lymphomas as compared with 13/39 (33 percent) of the controls in this bioassay.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by N-(1-naphthyl)ethylenediamine dihydrochloride that could not be established under the conditions of this test.

V. DISCUSSION

There were no significant positive associations between the concentrations of N-(1-naphthyl)ethylenediamine dihydrochloride administered and mortality in rats of either sex or in male mice. There was a significant positive association between concentration and mortality in female mice. In all groups, except for high dose females, adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors. Mean body weight depression, in relation to controls, was apparent for both sexes of rats and mice, indicating that higher concentrations of the test chemical would not have been tolerated by these animals.

In rats or mice of either sex, there were no statistically significant positive associations between the concentration of N-(1-naphthyl)ethylenediamine dihydrochloride and tumor incidence. The only tumors occurring in statistically significant higher incidences in a dosed group, when compared to controls, were pituitary adenomas in low dose male rats. This finding was not supported in the high dose male rats. The incidence of hepatocellular neoplasms was higher in low dose female rats than in control females; however, this difference was not statistically significant.

Under the conditions of this bioassay, dietary administration of N-(1-naphthyl)ethylenediamine dihydrochloride was not carcinogenic in Fischer 344 rats or B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH N-(1-NAPHTHYL) ETHYLENEDIAMINE DIHYDROCHLORIDE

TABLE D2 (CONCLUDED)

	CONTROL(UNTR) 06-0330	LOW DOSE 06-0305	HIGH DOSE 06-0310
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
STEATITIS	1		
NECROSIS, FAT	1		
LESSER OMENTUM			
INFLAMMATION, CHRONIC			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	15	10
AUTO/NECROPSY/HISTO PERF	3		
AUTO/NECROPSY/NO HISTO			4
AUTOLYSIS/NO NECROPSY	1	2	15

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

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH
N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL(UNTR) 01-0330	LOW DOSE 01-0295	HIGH DOSE 01-0300
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	48	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	25	48	49
INTEGUMENTARY SYSTEM			
*SKIN	(25)	(48)	(50)
SQUAMOUS CELL PAPILLOMA	2 (8%)	1 (2%)	
BASAL-CELL CARCINOMA		1 (2%)	1 (2%)
*SUBCUT TISSUE	(25)	(48)	(50)
FIBROMA	1 (4%)		
LIPOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(25)	(48)	(49)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
PHEOCHROMOCYTOMA, METASTATIC	1 (4%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(25)	(48)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
UNDIFFERENTIATED LEUKEMIA		9 (19%)	2 (4%)
MYELOMONOCYTIC LEUKEMIA	1 (4%)		
LYMPHOCYTIC LEUKEMIA		1 (2%)	
GRANULOCYTIC LEUKEMIA			1 (2%)
#MANDIBULAR L. NODE	(24)	(47)	(49)
C-CELL CARCINOMA, METASTATIC	1 (4%)		
CIRCULATORY SYSTEM			
NONE			

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

TABLE A1 (CONTINUED)

	CONTROL(UNTR) 01-0330	LOW DOSE 01-0295	HIGH DOSE 01-0300
DIGESTIVE SYSTEM			
#LIVER	(25)	(48)	(49)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
NEOPLASTIC NODULE	1 (4%)	2 (4%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(25)	(48)	(49)
TUBULAR-CELL ADENOMA		1 (2%)	
HAMARTOMA+			1 (2%)
#URINARY BLADDER	(25)	(48)	(49)
TRANSITIONAL-CELL PAPILLOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(22)	(44)	(47)
ADENOMA, NOS	2 (9%)		
CHROMOPHOBE ADENOMA		11 (25%)	3 (6%)
CHROMOPHOBE CARCINOMA			1 (2%)
BASOPHIL ADENOMA		4 (9%)	4 (9%)
PHEOCHROMOCYTOMA, METASTATIC			1 (2%)
#ADRENAL	(24)	(48)	(49)
CORTICAL ADENOMA		1 (2%)	
PHEOCHROMOCYTOMA	1 (4%)	8 (17%)	6 (12%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (4%)	1 (2%)	1 (2%)
GANGLIONEUROBLASTOMA		1 (2%)	
#THYROID	(21)	(47)	(49)
FOLLICULAR-CELL ADENOMA	1 (5%)		
C-CELL ADENOMA		2 (4%)	4 (8%)
C-CELL CARCINOMA	2 (10%)	1 (2%)	2 (4%)
PAPILLARY CYSTADENOMA, NOS		1 (2%)	
#PARATHYROID	(13)	(22)	(28)
ADENOMA, NOS			1 (4%)
#PANCREATIC ISLETS	(25)	(47)	(49)
ISLET-CELL ADENOMA	1 (4%)	1 (2%)	1 (2%)
ISLET-CELL CARCINOMA			2 (4%)

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

\+ THIS IS CONSIDERED TO BE A BENIGN FORM OF THE MIXED TUMOR OF THE KIDNEY AND CONSISTS OF PROLIFERATIVE LIPOCYTES, TUBULAR STRUCTURES, FIBROBLASTS, AND VASCULAR SPACES IN VARYING PROPORTIONS.

TABLE A1 (CONTINUED)

	CONTROL(UNTR) 01-0330	LOW DOSE 01-0295	HIGH DOSE 01-0300
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(25)	(48)	(50)
ADENOMA, NCS		1 (2%)	
*TESTIS	(25)	(48)	(49)
INTERSTITIAL-CELL TUMOR	21 (84%)	46 (96%)	40 (82%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EAR CANAL	(25)	(48)	(50)
SQUAMOUS CELL CARCINOMA	1 (4%)		
*ZYMBAL'S GLAND	(25)	(48)	(50)
SEBACECUS ADENOCCARCINOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*BODY CAVITIES	(25)	(48)	(50)
MESOTHELIOMA, NOS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			

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

TABLE A1 (CONCLUDED)

	CONTROL(UNTR) 01-0330	LOW DOSE 01-0295	HIGH DOSE 01-0300
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	25	50	50
NATURAL DEATH@	5	4	2
MORIBUND SACRIFICE	3	3	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	43	44
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	21	48	47
TOTAL PRIMARY TUMORS	35	97	76
TOTAL ANIMALS WITH BENIGN TUMORS	21	47	43
TOTAL BENIGN TUMORS	29	80	62
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	13	11
TOTAL MALIGNANT TUMORS	5	14	13
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	1	2
TOTAL SECONDARY TUMORS	2	1	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	3	1
TOTAL UNCERTAIN TUMORS	1	3	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH
N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL(UNTR) 02-0330	LOW DOSE 02-0295	HIGH DOSE 02-0300
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	24	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	24	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(24)	(50)	(50)
TRICHOEPITHELICMA		1 (2%)	
*SUBCUT TISSUE	(24)	(50)	(50)
FIBRCSARCCMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(24)	(50)	(50)
UNDIFFERENTIATED CARCINOMA METAS			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA			2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE CRGANS	(24)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (4%)		
UNDIFFERENTIATED LEUKEMIA		4 (8%)	
MYELOMONOCYTIC LEUKEMIA	2 (8%)		
#MESENTERIC L. NODE	(19)	(48)	(50)
UNDIFFEPENTIATED CARCINOMA METAS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(24)	(50)	(50)
ANITSCHKOW-CELI SARCOMA			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(24)	(50)	(50)
NEOPLASTIC NODULE	6 (12%)	1 (2%)	

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0330	LOW DOSE 02-0295	HIGH DOSE 02-0300
HEPATCCELLULAR CARCINOMA		2 (4%)	
HEMANGIOMA		1 (2%)	
URINARY SYSTEM			
#URINARY BLADDER	(24)	(50)	(49)
TRANSITIONAL-CELL PAPILLOMA	1 (4%)	1 (2%)	
TRANSITIONAL-CELL CARCINOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(21)	(47)	(42)
CARCINOMA, NOS		2 (4%)	
ADENOMA, NOS	6 (29%)		
CHROMOPHOBE ADENOMA		14 (30%)	6 (14%)
CHROMOPHOBE CARCINOMA		1 (2%)	
ACIDOPHIL ADENOMA		2 (4%)	
#ADRENAL	(24)	(50)	(49)
CORTICAL ADENOMA		3 (6%)	1 (2%)
PHEOCHROMOCYTOMA	1 (4%)	2 (4%)	1 (2%)
PHEOCHROMOCYTOMA, MALIGNANT			1 (2%)
LIPOMA		2 (4%)	1 (2%)
ANGIOLIPOMA			1 (2%)
OSTEOSARCOMA		1 (2%)	
#THYROID	(21)	(46)	(50)
FOLLICULAR-CELL CARCINOMA	1 (5%)		1 (2%)
C-CELL ADENOMA			8 (16%)
C-CELL CARCINOMA	1 (5%)	2 (4%)	
CYSTADENOCARCINOMA, NOS		1 (2%)	
#PANCREATIC ISLETS	(22)	(50)	(50)
ISLET-CELL CARCINOMA	1 (5%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(24)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
PAPILLARY ADENOCARCINOMA		1 (2%)	
FIBROADENOMA	4 (17%)	5 (10%)	1 (2%)
*CLITORAL GLAND	(24)	(50)	(50)
CARCINOMA, NOS	1 (4%)	1 (2%)	

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

TABLE A2 (CONTINUED)

	CONTROL(UNTR) 02-0330	LOW DOSE 02-0295	HIGH DOSE 02-0300
#UTERUS	(24)	(50)	(48)
ADENOCARCINOMA, NOS	1 (4%)		
ENDOMETRIAL STROMAL POLYP	2 (8%)	5 (10%)	9 (19%)
ENDOMETRIAL STROMAL SARCOMA	1 (4%)	2 (4%)	
#OVARY	(24)	(50)	(50)
UNDIFFERENTIATED CARCINOMA			1 (2%)
GRANULOSA-CELL TUMOR			1 (2%)
SERTOLI-CELL TUMOR		2 (4%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EAR CANAL	(24)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (4%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*BODY CAVITIES	(24)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	3 (6%)
*ABDOMINAL CAVITY	(24)	(50)	(50)
LEIOMYOSARCOMA	1 (4%)		
ALL OTHER SYSTEMS			
NONE			

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

TABLE A2 (CONCLUDED)

	CONTROL(UNTR) 02-0330	LOW DOSE 02-0295	HIGH DOSE 02-0300
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	25	50	50
NATURAL DEATH@	4	4	1
MORIBUND SACRIFICE	5	3	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	43	47
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	17	36	30
TOTAL PRIMARY TUMORS	25	64	40
TOTAL ANIMALS WITH BENIGN TUMORS	10	26	23
TOTAL BENIGN TUMORS	14	38	30
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	17	5
TOTAL MALIGNANT TUMORS	11	19	5
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			2
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT		7	5
TOTAL UNCERTAIN TUMORS		7	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH N-(1-NAPHTHYL) ETHYLENEDIAMINE DIHYDROCHLORIDE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL (UNTR) 05-0330	LOW DOSE 05-0295	HIGH DOSE 05-0300
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	2		
ANIMALS NECROPSIED	39	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	39	49	47
INTEGUMENTARY SYSTEM			
*SKIN	(39)	(50)	(49)
SARCOMA, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(39)	(49)	(45)
HEPATOCELLULAR CARCINOMA, METAST	2 (5%)		1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (5%)	5 (10%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (5%)	2 (4%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(39)	(50)	(49)
MALIGNANT LYMPHOMA, NOS	11 (28%)	4 (8%)	4 (8%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#SPLEEN	(38)	(48)	(45)
MALIGNANT LYMPHOMA, NOS	1 (3%)		
#MEDIASTINAL L.NODE	(36)	(43)	(45)
ADENOCARCINOMA, NOS		1 (2%)	
#MESENTERIC L. NODE	(36)	(43)	(45)
MALIGNANT LYMPHOMA, NOS		2 (5%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (3%)	1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(39)	(49)	(45)
ADENOCARCINOMA, NOS		1 (2%)	

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

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL(UNTR) 05-0330	LOW DOSE 05-0295	HIGH DOSE 05-0300
DIGESTIVE SYSTEM			
#LIVER	(39)	(49)	(45)
HEPATOCELLULAR CARCINOMA	12 (31%)	5 (10%)	9 (20%)
#STOMACH	(37)	(49)	(44)
SQUAMOUS CELL PAPILLOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY	(39)	(49)	(46)
ADENOCARCINOMA, NOS		1 (2%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B1 (CONCLUDED)

	CONTROL(UNTR) 05-0330	LOW DOSE 05-0295	HIGH DOSE 05-0300
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	13	7	11
MORIBUND SACRIFICE	2	3	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	33	40	37
ANIMAL MISSING	2		
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	24	19	16
TOTAL PRIMARY TUMORS	29	24	17
TOTAL ANIMALS WITH BENIGN TUMORS	2	5	2
TOTAL BENIGN TUMORS	2	5	2
TOTAL ANIMALS WITH MALIGNANT TUMORS	22	14	14
TOTAL MALIGNANT TUMORS	27	19	15
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		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 B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH
N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL(UNTR) 06-0330	LOW DOSE 06-0305	HIGH DOSE 06-0310
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	48	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	48	31
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(49)	(48)	(35)
FIBROSARCOMA		2 (4%)	
HEMANGIOSARCOMA			1 (3%)
RESPIRATORY SYSTEM			
#LUNG	(49)	(48)	(31)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	1 (3%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(48)	(35)
MALIGNANT LYMPHOMA, NOS	10 (20%)	6 (13%)	2 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
MALIGNANT LYMPHOMA, MIXED TYPE			1 (3%)
#SPLEEN	(45)	(48)	(30)
HEMANGIOSARCOMA	1 (2%)		
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#PANCREATIC L.NODE	(44)	(45)	(27)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
#MESENTERIC L. NODE	(44)	(45)	(27)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	1 (4%)
#LIVER	(46)	(48)	(30)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
CIRCULATORY SYSTEM			
#ENDOCARDIUM	(49)	(47)	(31)
HEMANGIOSARCOMA, METASTATIC			1 (3%)

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0330	LOW DOSE 06-0305	HIGH DOSE 06-0310
DIGESTIVE SYSTEM			
#LIVER	(46)	(48)	(30)
HEPATOCELLULAR CARCINOMA	1 (2%)	1 (2%)	1 (3%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(34)	(38)	(24)
ADENOMA, NOS	3 (9%)		
#ADRENAL	(46)	(47)	(30)
PHEOCHROMOCYTOMA	3 (7%)		
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)		
#THYROID	(44)	(39)	(28)
FOLLICULAR-CELL ADENOMA	2 (5%)		
FOLLICULAR-CELL CARCINOMA	2 (5%)		1 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(48)	(35)
ADENOCARCINOMA, NOS		1 (2%)	1 (3%)
#UTERUS	(44)	(47)	(25)
ENDOMETRIAL STROMAL POLYP	1 (2%)		
#OVARY	(44)	(47)	(27)
TUBULAR ADENOMA	1 (2%)	1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(49)	(48)	(35)
PAPILLARY ADENOMA		1 (2%)	

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

TABLE B2 (CONTINUED)

	CONTROL(UNTR) 06-0330	LOW DOSE 06-0305	HIGH DOSE 06-0310
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	17	6	26
MORIBUND SACRIFICE	3	1	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	30	43	20
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2 (CONCLUDED)

	CONTROL(UNTR) 06-0330	LOW DOSE 06-0305	HIGH DOSE 06-0310
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	21	15	9
TOTAL PRIMARY TUMORS	28	17	9
TOTAL ANIMALS WITH BENIGN TUMORS	9	4	1
TOTAL BENIGN TUMORS	10	4	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	16	12	8
TOTAL MALIGNANT TUMORS	18	13	8
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH N-(1-NAPHTHYL) ETHYLENEDIAMINE DIHYDROCHLORIDE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH
N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL(UNTR) 01-0330	LOW DOSE 01-0295	HIGH DOSE 01-0300
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	48	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	25	48	49
INTEGUMENTARY SYSTEM			
*SKIN	(25)	(48)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
SCAB	1 (4%)		
*SUBCUT TISSUE	(25)	(48)	(50)
ABSCESS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(25)	(48)	(49)
BRONCHIECTASIS		2 (4%)	5 (10%)
#LUNG	(25)	(48)	(49)
BRONCHOPNEUMONIA, NOS			1 (2%)
BRONCHOPNEUMONIA NECROTIZING	1 (4%)		
ABSCESS, NOS		1 (2%)	1 (2%)
PNEUMONIA, CHRONIC MURINE		5 (10%)	5 (10%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(23)	(45)	(49)
HYPERPLASIA, HEMATOPOIETIC		2 (4%)	1 (2%)
#SPLEEN	(25)	(48)	(49)
CONGESTION, NOS	1 (4%)		
HEMOSIDEROSIS		1 (2%)	
#LYMPH NODE	(24)	(47)	(49)
HYPERPLASIA, NOS		1 (2%)	
#MANDIBULAR L. NODE	(24)	(47)	(49)
HYPERPLASIA, PLASMA CELL	2 (8%)		

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

TABLE C1 (CONTINUED)

	CONTROL(UNTR) 01-0330	LOW DOSE 01-0295	HIGH DOSE 01-0300
#MESENTERIC L. NODE	(24)	(47)	(49)
HEMOSIDEROSIS			1 (2%)
#RENAL LYMPH NODE	(24)	(47)	(49)
HEMOSIDEROSIS			5 (10%)
HISTIOCYTOSIS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(25)	(48)	(49)
THROMBUS, MURAL			1 (2%)
#MYOCARDIUM	(25)	(48)	(49)
INFLAMMATION, CHRONIC	1 (4%)		
DEGENERATION, NOS		8 (17%)	14 (29%)
DIGESTIVE SYSTEM			
#LIVER	(25)	(48)	(49)
INFLAMMATION, ACUTE FOCAL			1 (2%)
DEGENERATION, NOS	1 (4%)		
METAMORPHOSIS FATTY	1 (4%)	1 (2%)	
BASOPHILIC CYTO CHANGE	1 (4%)	4 (8%)	3 (6%)
CLEAR-CELL CHANGE		1 (2%)	1 (2%)
HYPERPLASIA, FOCAL	1 (4%)		
ANGIECTASIS	1 (4%)		
#BILE DUCT	(25)	(48)	(49)
FIBROSIS		1 (2%)	
#PANCREAS	(25)	(47)	(49)
ATROPHY, FOCAL		2 (4%)	
#STOMACH	(24)	(48)	(49)
HYPERPLASIA, BASAL CELL		5 (10%)	8 (16%)
URINARY SYSTEM			
#KIDNEY	(25)	(48)	(49)
GLOMERULONEPHRITIS, NOS		1 (2%)	
PYELONEPHRITIS, CHRONIC		1 (2%)	3 (6%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0330	LOW DOSE 01-0295	HIGH DOSE 01-0300
NEPHROSIS, NOS	21 (84%)	25 (52%)	25 (51%)
NECROSIS, MEDULLARY			1 (2%)
#KIDNEY/CORTEX	(25)	(48)	(49)
MULTILOCULAR CYST	1 (4%)		
#KIDNEY/PELVIS	(25)	(48)	(49)
HYPERPLASIA, EPITHELIAL		1 (2%)	7 (14%)
#URINARY BLADDER	(25)	(48)	(49)
INFLAMMATION, ACUTE			1 (2%)
HYPERPLASIA, EPITHELIAL			1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(24)	(48)	(49)
HYPERPLASIA, FOCAL	1 (4%)		
#ADRENAL MEDULLA	(24)	(48)	(49)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL	4 (17%)		
#THYROID	(21)	(47)	(49)
COLLOID CYST		1 (2%)	
#PARATHYROID	(13)	(22)	(28)
HYPERPLASIA, NOS		3 (14%)	
#PANCREATIC ISLETS	(25)	(47)	(49)
HYPERPLASIA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(25)	(48)	(50)
GALACTOCELE		1 (2%)	
*MAMMARY DUCT	(25)	(48)	(50)
HEMORRHAGE	1 (4%)		
#PROSTATE	(25)	(42)	(48)
INFLAMMATION, SUPPURATIVE	2 (8%)		
#TESTIS	(25)	(48)	(49)
ATROPHY, NOS	5 (20%)		

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

TABLE C1 (CONCLUDED)

	CONTROL(UNTR) 01-0330	LOW DOSE 01-0295	HIGH DOSE 01-0300
HYPERPLASIA, INTERSTITIAL CELL		1 (2%)	1 (2%)
NERVOUS SYSTEM			
ACNE			
SPECIAL SENSE ORGANS			
*EYE	(25)	(48)	(50)
HEMORRHAGE		1 (2%)	
SYNECHIA, POSTERIOR		1 (2%)	
*EYE/CRYSTALLINE LENS	(25)	(48)	(50)
CALCIFICATION, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(25)	(48)	(50)
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4		
AUTO/NECROPSY/NO HISTO			1
AUTOLYSIS/NO NECROPSY		2	

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

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH
N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL(UNTR) 02-0330	LOW DOSE 02-0295	HIGH DOSE 02-0300
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	24	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	24	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(24)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(24)	(50)	(50)
PNEUMONIA, CHRONIC MURINE		3 (6%)	10 (20%)
REACTION, FOREIGN BODY		1 (2%)	
HYPERPLASIA, ADENOMATOUS		2 (4%)	
METAPLASIA, NOS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(23)	(50)	(50)
INFARCT, HEALED		1 (2%)	
ERYTHROPOIESIS	1 (4%)		
#MANDIBULAR L. NODE	(19)	(48)	(50)
HYPERPLASIA, PLASMA CELL	2 (11%)		
#MESENTERIC L. NODE	(19)	(48)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
#RENAL LYMPH NODE	(19)	(48)	(50)
HEMOSIDEROSIS			2 (4%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(24)	(50)	(50)
DEGENERATION, NOS		5 (10%)	5 (10%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0330	LOW DOSE 02-0295	HIGH DOSE 02-0300
CALCIFICATION, FOCAL	1 (4%)		
*AORTA	(24)	(50)	(50)
MEDIAL CALCIFICATION	1 (4%)		
*CORONARY ARTERY	(24)	(50)	(50)
MEDIAL CALCIFICATION	1 (4%)		
DIGESTIVE SYSTEM			
#LIVER	(24)	(50)	(50)
METAMORPHOSIS FATTY		1 (2%)	
BASOPHILIC CYTC CHANGE	10 (42%)	11 (22%)	3 (6%)
ANGIECTASIS	1 (4%)		
HYPERPLASIA, BASOPHILIC	1 (4%)		
#PANCREAS	(22)	(50)	(50)
INFLAMMATICN, CHRONIC FOCAL		1 (2%)	
#STOMACH	(24)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
ULCER, NOS		1 (2%)	
HYPERPLASIA, BASAL CELL		5 (10%)	8 (16%)
URINARY SYSTEM			
#KIDNEY	(24)	(50)	(50)
HYDRONEPHROSIS			2 (4%)
PYELONEPHRITIS, FOCAL		1 (2%)	
PYELONEPHRITIS, CHRONIC		3 (6%)	
NEPHROSIS, NOS	8 (33%)	1 (2%)	
CALCIFICATION, FOCAL			1 (2%)
#KIDNEY/TUBULE	(24)	(50)	(50)
CALCIFICATION, NOS	1 (4%)		
ENDOCRINE SYSTEM			
#PITUITARY	(21)	(47)	(42)
HEMOSIDEROSIS	1 (5%)		
#ADRENAL CORTEX	(24)	(50)	(49)
HYPERPLASIA, NOS		1 (2%)	1 (2%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0330	LOW DOSE 02-0295	HIGH DOSE 02-0300
#THYROID	(21)	(46)	(50)
HYPERPLASIA, C-CELL	1 (5%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(24)	(50)	(50)
GALACTOCELE		2 (4%)	
#UTERUS	(24)	(50)	(48)
HYDROMETRA			2 (4%)
#UTERUS/ENDOMETRIUM	(24)	(50)	(48)
INFLAMMATION, SUPPURATIVE	3 (13%)		
METAPLASIA, SQUAMOUS	1 (4%)		
#OVARY/OVIDUCT	(24)	(50)	(48)
INFLAMMATION, SUPPURATIVE	5 (21%)		
#OVARY	(24)	(50)	(50)
CYST, NOS	2 (8%)		
INFLAMMATION, SUPPURATIVE	1 (4%)		
ABSCESS, NOS		2 (4%)	1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(23)	(48)	(50)
HYDROCEPHALUS, NOS		1 (2%)	1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE C2 (CONCLUDED)

	CONTROL(UNTR) 02-0330	LOW DOSE 02-0295	HIGH DOSE 02-0300
ALL OTHER SYSTEMS			
CRANICBUCCAL POUCH			
CYST, NOS			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	3	8
AUTO/NECROPSY/HISTO PERF		2	1
AUTOLYSIS/NO NECROPSY	1		

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH N-(1-NAPHTHYL) ETHYLENEDIAMINE DIHYDROCHLORIDE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH
N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL(UNTR) 05-0330	LOW DOSE 05-0295	HIGH DOSE 05-0300
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	2		
ANIMALS NECROPSIED	39	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	39	49	47
INTEGUMENTARY SYSTEM			
*SKIN	(39)	(50)	(49)
EPIDERMAL INCLUSION CYST	1 (3%)		
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, CHRONIC	1 (3%)		
FIBROSIS	1 (3%)		
*SUBCUT TISSUE	(39)	(50)	(49)
INFLAMMATION, ACUTE			1 (2%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(38)	(48)	(45)
HYPERPLASIA, LYMPHOID	1 (3%)		
HEMATOPOIESIS	1 (3%)	3 (6%)	1 (2%)
ERYTHROPOIESIS	3 (8%)		
#MANDIBULAR L. NODE	(36)	(43)	(45)
HYPERPLASIA, PLASMA CELL	1 (3%)		
#LUMBAR LYMPH NODE	(36)	(43)	(45)
HYPERPLASIA, LYMPHOID			1 (2%)
#MESENTERIC L. NODE	(36)	(43)	(45)
CONGESTION, NOS			6 (13%)
HYPERPLASIA, NOS	4 (11%)	7 (16%)	5 (11%)
HYPERPLASIA, LYMPHOID	4 (11%)	1 (2%)	12 (27%)
HEMATOPOIESIS		1 (2%)	

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

TABLE D1 (CONTINUED)

	CONTROL(UNTR) 05-0330	LOW DOSE 05-0295	HIGH DOSE 05-0300
#THYMUS	(13)	(28)	(27)
DEGENERATION, HYALINE			1 (4%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(39)	(49)	(45)
INFLAMMATION, ACUTE FOCAL			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(39)	(49)	(45)
NECROSIS, NOS			1 (2%)
METAMORPHOSIS FATTY		2 (4%)	
HEPATOCYTOMEGALY			1 (2%)
HYPERPLASIA, FOCAL	3 (8%)		
ANGIECTASIS			1 (2%)
#BILE DUCT	(39)	(49)	(45)
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION, CHRONIC DIFFUSE		6 (12%)	2 (4%)
#PANCREAS	(36)	(47)	(43)
DILATATION/DUCTS			1 (2%)
CYSTIC DUCTS			1 (2%)
THROMBOSIS, NOS			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION, CHRONIC FOCAL			2 (5%)
#PANCREATIC ACINUS	(36)	(47)	(43)
ATROPHY, NOS			1 (2%)
#STOMACH	(37)	(49)	(44)
ECTOPIA		1 (2%)	
INFLAMMATION, ACUTE		1 (2%)	1 (2%)
EROSION		1 (2%)	
#JEJUNUM	(37)	(48)	(43)
AMYLOIDOSIS	1 (3%)		
#ILEUM	(37)	(48)	(43)
AMYLOIDOSIS	2 (5%)		

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

TABLE D1 (CONTINUED)

	CONTROL(UNTR) 05-0330	LOW DOSE 05-0295	HIGH DOSE 05-0300
URINARY SYSTEM			
#KIDNEY	(39)	(49)	(46)
POLYCYSTIC KIDNEY			1 (2%)
PYELONEPHRITIS, NOS		1 (2%)	
GLOMERULONEPHRITIS, CHRONIC			1 (2%)
GLOMERULOSCLEROSIS, NOS	3 (8%)		
#KIDNEY/CORTEX	(39)	(49)	(46)
SCAR	1 (3%)		
#KIDNEY/GLOMERULUS	(39)	(49)	(46)
AMYLOIDOSIS	2 (5%)		
#URINARY BLADDER	(37)	(49)	(45)
INFLAMMATION, ACUTE		1 (2%)	
ENDOCRINE SYSTEM			
#ADRENAL	(36)	(49)	(45)
AMYLOIDOSIS	2 (6%)		
#THYROID	(38)	(42)	(41)
CYSTIC FOLLICLES	1 (3%)		
AMYLOIDOSIS	1 (3%)		
HYPERPLASIA, FOLLICULAR-CELL	2 (5%)		
#PARATHYROID	(28)	(22)	(22)
HYPERPLASIA, NOS	1 (4%)		
#PANCREATIC ISLETS	(36)	(47)	(43)
HYPERPLASIA, NOS	1 (3%)		
REPRODUCTIVE SYSTEM			
#PROSTATE	(37)	(48)	(44)
INFLAMMATION, ACUTE		1 (2%)	
#TESTIS	(38)	(49)	(44)
ATROPHY, NOS			1 (2%)
#TESTIS/TUBULE	(38)	(49)	(44)
DEGENERATION, NOS		1 (2%)	

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

TABLE D1 (CONCLUDED)

	CONTROL(UNTR) 05-0330	LOW DOSE 05-0295	HIGH DOSE 05-0300
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(39)	(50)	(49)
AMYLOIDOSIS	1 (3%)		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	19	13
ANIMAL MISSING/NO NECROPSY	2		
AUTO/NECROPSY/HISTO PERF			1
AUTO/NECROPSY/NO HISTO		1	2
AUTOLYSIS/NO NECROPSY	9		1

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

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH
N-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE

	CONTROL(UNTR) 06-0330	LOW DOSE 06-0305	HIGH DOSE 06-0310
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	48	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	48	31
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(48)	(35)
INFLAMMATION, ACUTE	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(49)	(48)	(31)
LEUKOCYTOSIS, NOS			1 (3%)
#LUNG/ALVEOLI	(49)	(48)	(31)
HEMORRHAGE			1 (3%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(45)	(48)	(30)
SIDEROSIS			1 (3%)
HYPERPLASIA, LYMPHOID	1 (2%)	2 (4%)	1 (3%)
HEMATOPOIESIS		1 (2%)	
ERYTHROPOIESIS	4 (9%)	1 (2%)	2 (7%)
#LYMPH NODE OF THORAX	(44)	(45)	(27)
HYPERPLASIA, NOS	1 (2%)		
#PANCREATIC L.NODE	(44)	(45)	(27)
HEMATOPOIESIS	1 (2%)		
#LUMBAR LYMPH NODE	(44)	(45)	(27)
HYPERPLASIA, NOS	1 (2%)		
#MESENTERIC L. NODE	(44)	(45)	(27)
LYMPHANGIECTASIS		1 (2%)	
INFLAMMATION, ACUTE			1 (4%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0330	LOW DOSE 06-0305	HIGH DOSE 06-0310
HYPERPLASIA, NCS	1 (2%)		4 (15%)
HYPERPLASIA, LYMPHOID	1 (2%)	3 (7%)	2 (7%)
HEMATOPOIESIS	1 (2%)		
#RENAL LYMPH NODE	(44)	(45)	(27)
HEMATOPOIESIS	1 (2%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(46)	(48)	(30)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
NECROSIS, FOCAL			1 (3%)
HYPERPLASTIC NODULE	1 (2%)		
ANGIECTASIS		1 (2%)	
HEMATOPOIESIS	1 (2%)		1 (3%)
#BILE DUCT	(46)	(48)	(30)
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC DIFFUSE		2 (4%)	1 (3%)
#PANCREAS	(38)	(47)	(29)
INFLAMMATION, CHRONIC			1 (3%)
#ILEUM	(42)	(48)	(27)
AMYLOIDOSIS		1 (2%)	1 (4%)
URINARY SYSTEM			
#KIDNEY	(46)	(48)	(30)
INFLAMMATION, CHRONIC			1 (3%)
GLOMERULOSCLEROSIS, NOS	2 (4%)	1 (2%)	
#KIDNEY/GLOMERULUS	(46)	(48)	(30)
INFLAMMATION, CHRONIC		1 (2%)	
#URINARY BLADDER	(43)	(48)	(29)
INFLAMMATION, CHRONIC			1 (3%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0330	LOW DOSE 06-0305	HIGH DOSE 06-0310
ENDOCRINE SYSTEM			
#THYROID	(44)	(39)	(28)
INFLAMMATION, ACUTE		1 (3%)	
INFLAMMATION, ACUTE FOCAL	1 (2%)		
INFLAMMATION, CHRONIC FOCAL		1 (3%)	
AMYLOIDOSIS		1 (3%)	
HYPERPLASIA, CYSTIC			1 (4%)
HYPERPLASIA, C-CELL			1 (4%)
HYPERPLASIA, FOLLICULAR-CELL	2 (5%)		
#PARATHYROID	(24)	(22)	(15)
AMYLOIDOSIS		1 (5%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(44)	(47)	(25)
HYDROMETRA		8 (17%)	2 (8%)
INFLAMMATION, SUPPURATIVE			1 (4%)
#UTERUS/ENDOMETRIUM	(44)	(47)	(25)
INFLAMMATION, SUPPURATIVE		1 (2%)	
HYPERPLASIA, CYSTIC	30 (68%)	2 (4%)	7 (28%)
#OVARY	(44)	(47)	(27)
CYST, NOS	7 (16%)		3 (11%)
HEMORRHAGIC CYST	4 (9%)		2 (7%)
ABSCESS, NOS	1 (2%)		
INFLAMMATION, CHRONIC	2 (5%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(49)	(48)	(35)
ABSCESS, NOS	1 (2%)		

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

Review of the Bioassay of N-1(1-Naphthyl)ethylenediamine Hydrochloride*
for Carcinogenicity by the
Data Evaluation/Risk Assessment Subgroup of the
Clearinghouse on Environmental Carcinogens

August 31, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of N-1(1-Naphthyl)ethylenediamine Hydrochloride for carcinogenicity.

The primary reviewer agreed with the conclusion in the report that N-1(1-Naphthyl)ethylenediamine Hydrochloride was not carcinogenic in rats or mice, under the conditions of test. He noted that the survival was adequate in all groups except high dose female mice. He thought that there would have been some benefit to have analyzed the compound for free alpha- and beta- naphthylamine. The primary reviewer pointed out the nonstatistically significant increases in the incidence of urinary tract lesions, including neoplasms, among treated rats. He suggested that the lesions should not be totally ignored since the compound is a naphthylamine. Because of the nature of the compound, he said that hamsters, dogs, or monkeys, would be better experimental models for testing the carcinogenicity of N-1(1-Napththyl)ethylenediamine Hydrochloride. As a result of the relative insensitivity of the test species to naphthylamine carcinogenicity, the primary reviewer said that an assessment of human risk would be difficult to derive based on the results of this study.

The secondary reviewer agreed with the conclusion in the report that N-1(1-Naphthyl)ethylenediamine Hydrochloride was not carcinogenic, under the conditions of test. He noted, however, an increased incidence of hepatocellular carcinomas in treated female rats. Although the tumors were not statistically significant, he recommended that they be noted in the summary or discussion sections in the report. He also recommended that the early mortality among high dose female mice be noted. Despite its deficiencies, the secondary reviewer concluded that the study was still valid. He said that the data do not suggest that N-1(1-Naphthyl)ethylenediamine Hydrochloride would present any significant hazard to humans.

A recommendation that the report be accepted with the addition of a statement to the text regarding the increased, but statistically insignificant, incidence of hepatocellular tumors in treated rats was accepted without objection.

Members present were:

Arnold Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center
(Verald Rowe, Dow Chemical USA, submitted a written review)

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

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