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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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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 5-NITROACENAPHTHENE
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 5-nitroacenaphthene 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 5-nitroacenaphthene 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).

Histopathologic examinations were performed by Dr. R. W. Fleischman (3), Dr. D. W. Hayden (3), Dr. A. S. Krishna Murthy (3), Dr. D. S. Wyand (3), and Dr. Yoon (3) at the Mason Research Institute, the pathology narratives were written by Dr. D. S. Wyand (3), and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (4).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (5); the statistical analysis was performed by Mr. W. W. Belew (6,7), and Mr. R. M. Helfand (6), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (8).

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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 (1,9), Dr. R. A. Griesemer (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,10), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay of 5-nitroacenaphthene for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F1 mice. 5-Nitroacenaphthene was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. For male and female rats, the high and low dietary concentrations of 5-nitroacenaphthene were 0.24 and 0.12 percent, respectively. The high and low time-weighted average concentrations for mice were 0.12 and 0.06 percent, respectively, for males and 0.12 and 0.05 percent, respectively, for females. After a 78-week dosing period, observation of surviving rats continued for up to 22 weeks and observation of the mice continued for 18 weeks.

For the chronic rat bioassay, 49 male and 50 female rats were placed on test as high dose controls, and 50 rats of each sex served as low dose controls. For the mice, 50 males and 50 females were placed on test as controls.

Accelerated mortality was observed in all dosed groups except the low dose female mice. There was a positive association between mortality and dietary concentration of 5-nitroacenaphthene for both sexes of both species. Early deaths were most apparent among high dose male mice; half of the animals in this group were dead by week 20 and insufficient male mice survived to be at risk from late-developing tumors.

Among rats, the incidence of malignant tumors of the ear canal (incidences of ceruminous carcinomas and squamous-cell carcinomas were combined) was significant at each dose level in each sex. Among both dosed groups of female rats, the incidence of clitoral gland carcinoma and the incidence of mammary adenocarcinoma were each significant. A significant incidence of alveolar/bronchiolar carcinoma was observed in low dose rat groups of each sex.

Among female mice, the incidence of hepatocellular carcinoma was significant at each dose level. The combined incidence of granulosa-cell tumors, luteomas, and tubular-cell adenomas of the ovary was significant in the high dose female mouse group.

Under the conditions of this bioassay, 5-nitroacenaphthene was carcinogenic to Fischer 344 rats, causing increased incidences of malignant tumors of the ear canal and lung in both sexes, and of the clitoral gland and mammary gland in females. 5-Nitroacenaphthene was also carcinogenic to female but not male B6C3F1 mice, causing carcinomas of the liver and ovarian tumors.

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I. INTRODUCTION

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for 5-nitroacenaphthene (Figure 1) (NCI No. C01967), an aromatic nitro compound, is 1,2-dihydro-5-nitro-acenaphthylene.* It is also known as 5-nitroacenaphthylene.

5-Nitroacenaphthene has never had any known commercial application in the United States and is apparently produced in this country solely for research purposes (Urso, 1977). The compound is, however, used in Japan as a captive intermediate in the synthesis of naphthalimide dyes; 95 percent of these dyes find application as fluorescent whitening agents in laundry detergents while the remainder are used to dye paper (Urso, 1977).

Exposure to 5-nitroacenaphthene in the United States is presently restricted to experimentalists engaged in laboratory research.

5-Nitroacenaphthene exhibited significant mutagenic activity in two highly sensitive histidine-requiring tester strains of Salmonella typhimurium (The Ames Test using the frame-shift-mutagen sensitive strain TA 98 and the base-pair-substitution-mutagen sensitive strain TA 100) (Yahagi et al., 1975). Reversion to histidine independence occurred both in the presence and absence of microsomal activation, with the largest number of revertent colonies (more than 100 times the number expected as a result of spontaneous reversion) observed in strain TA 98 in the presence of rat liver microsomes.

*The CAS registry number is 602-87-9.

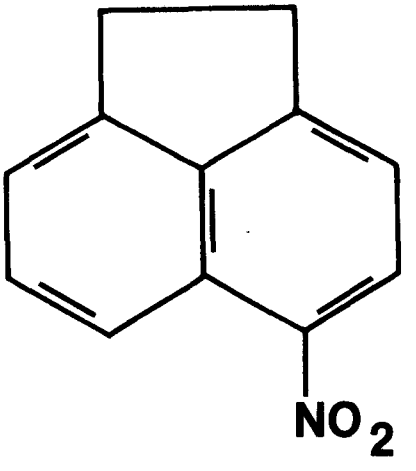


FIGURE 1
CHEMICAL STRUCTURE OF 5-NITROACENAPHTHENE

II. MATERIALS AND METHODS

A. Chemicals

One batch of 5-nitroacenaphthene was purchased from Carroll Products, Wood River Junction, Rhode Island by the NCI for Mason Research Institute, Worcester, Massachusetts. Spectroscopic analyses performed at Mason Research Institute confirmed the identity of the compound. The melting point of 98° to 100°C suggested a compound of fairly high purity due to its narrow range and proximity to the literature value of 103° to 104°C (Grasselli and Ritchey, 1975).

Throughout this report the term 5-nitroacenaphthene 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). 5-Nitroacenaphthene was administered to the dosed animals as a component of the diet.

Proper amounts of the chemical were removed from the stock bottle under a fume hood. The compound was ground to a powder and mixed with an aliquot of meal using a mortar and pestle. Once visual homogeneity was attained, the mixture was placed into a 6 kg capacity Patterson-Kelley twin-shell stainless steel V-blender 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 used for only one week.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. Fischer 344 rats and B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. High dose and all control rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Low dose rats were obtained from ARS/Sprague-Dawley, Madison, Wisconsin, as were high and low dose mice. Control mice were obtained from Charles River Breeding Laboratories, Inc. Dosed and control animals for both species were received in separate shipments.

Upon arrival a sample of animals was examined for parasites and other signs of disease. The remaining animals were quarantined by species for two 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 Corp., 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 13 months of study, high dose and high dose control rats were housed in galvanized- or stainless-steel wire-mesh cages

suspended above newspapers. Low dose rats and their controls were housed in galvanized- or stainless-steel wire-mesh cages during quarantine and for the first 7 months of study. Newspapers under cages were replaced daily and cages and racks washed weekly. For the remainder of the study, all rats were maintained in suspended polycarbonate cages equipped with disposable nonwoven fiber filter sheets. Clean bedding and cages were provided twice weekly. High dose rats and their controls were provided with corncob bedding (Bed-o-Cobs®, The Andersons Cob Division, Maumee, Ohio) while they were in polycarbonate cages. A second type of corncob bedding (SAN-I-CEL®, Paxton Processing Company, Paxton, Illinois) was supplied to low dose rats for 7 months and low dose control rats for 8 months after they were placed in polycarbonate cages. Bed-o-Cobs® was used for the remainder of the study. Stainless-steel cage racks were cleaned once every two weeks, and disposable filters were replaced at that time.

Mice were housed by sex in polycarbonate cages. During quarantine and periods of chemical administration, cages were fitted with perforated stainless steel lids. During the final observation period, stainless steel wire bar lids were used. Both types of lids were from Lab Products, Inc., Garfield, New Jersey. Nonwoven fiber filter bonnets were used over cage lids. Mice were housed ten per cage for the first 18 months of study and five per cage thereafter. Cages, lids, filters, and bedding were provided three times per week

when cage populations were ten and twice per week when cage populations were reduced to five. Ab-sorb-dri® hardwood chip bedding (Wilner Wood Products Company, Norway, Maine) was used through the first 7 months of study followed by SAN-I-CEL® for the next 12 months. After 19 months on study, Bed-o-Cobs® was utilized as bedding. Reusable filter bonnets and pipe racks were sanitized every two weeks throughout the study.

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

Wayne Lab-Blox® was supplied ad libitum throughout the entire test. Pelleted Wayne Lab-Blox® was supplied during the quarantine period and final observation period. During the chemical administration period, all dosed animals were fed Wayne Lab-Blox® meal containing the appropriate concentration of 5-nitroacenaphthene, while control animals had untreated meal available. Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc., Woburn, Massachusetts) containing stainless steel baffles were used to distribute powdered feed to high dose rats and their controls for the first 11 months of the study. These cups were used for all mice and for low dose rats and their controls during the entire study. During the last 7 months of chemical administration, high dose rats and their controls were fed from stainless steel gangstyle feed hoppers (Scientific Cages, Inc.,

Bryan, Texas). During the observation period following chemical administration, rats were fed pellets on the cage floor and mice were fed pellets from a wire bar hopper incorporated into the cage lid. Food hoppers were changed on the same schedule as were cages. Food was replenished daily in Alpine® feed cups.

Dosed rats were housed in a room with other rats receiving diets containing* 3-amino-4-ethoxyacetanilide (17026-81-2); 4-nitroanthranilic acid (619-17-0); 1-amino-2-methylanthraquinone (82-28-0); and 5-nitro-o-anisidine (99-59-2). Control rats were in a room with other rats receiving diets containing amitrole (61-82-5); 2-methyl-1-nitroanthraquinone (129-15-7); and 3-nitro-p-acetophenetide (1777-84-0).

Dosed and control mice were in a room with other mice receiving diets containing amitrole (61-82-5); N,N-dimethyl-p-nitrosoaniline (138-89-6); 2,5-toluenediamine sulfate (6369-59-1); 2,4-dinitrotoluene (121-14-2); 2-aminoanthraquinone (117-79-3); 3-amino-4-ethoxyacetanilide (17026-81-2); 3-amino-9-ethylcarbazole hydrochloride; 1-amino-2-methylanthraquinone (82-28-0); 2,4-diaminoanisole sulfate (615-05-4); APC (8003-03-0); 5-nitro-o-anisidine (99-59-2); 4-nitroanthranilic acid (619-17-0); 1-nitronaphthalene (86-57-7); and 3-nitro-p-acetophenetide (1777-84-0).

E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of 5-nitroacenaphthene for administration to dosed animals in the

*CAS registry numbers are given in parentheses.

chronic studies, subchronic toxicity studies were conducted with both rats and mice. Animals of each species were distributed among six groups, each consisting of five males and five females. 5-Nitroacenaphthene was incorporated into the basal laboratory diet and supplied ad libitum to five of the six rat groups and five of the six mouse groups in concentrations of 0.03, 0.06, 0.12, 0.24, and 0.48 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 a period of 4 weeks, followed by a 2-week observation period during which all animals were fed the basal diet.

The highest concentration causing no deaths, no compound-related gross abnormalities, and no mean group body weight depression in excess of 10 percent relative to controls was selected as the high concentration utilized for the rat and mouse chronic bioassays.

There were no rat deaths during the 6-week subchronic study. Mean body weight depression occurred in only one group of male rats, those receiving 0.48 percent, the highest concentration. At this concentration the mean body weight depression was in excess of 10 percent. Female rats experienced no mean group body weight depression. At 0.24 and 0.48 percent male rats were noted at gross necropsy to have enlarged submaxillary lymph nodes and white foci on external renal surfaces. Females exhibited no abnormalities. The high concentration selected for male and female rats in the chronic study was 0.24 percent.

There were three mouse deaths during the 6-week subchronic study, all in the male group receiving the highest concentration of 5-nitroacenaphthene. Mean group body weight depression was in excess of 10 percent at dosages of 0.48 percent for male mice, and 0.24 and 0.48 percent for female mice. The mice receiving 0.48 percent 5-nitroacenaphthene exhibited chronic convulsions, prostration, emaciation, and rapid respiration. Upon necropsy, discolored spleens and yellow kidneys and livers were noted. The high concentration selected for male and female mice in the chronic study was 0.12 percent.

F. Experimental Design

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

The dosed and control rats were all approximately 6 weeks old at the time they were placed on test. Low dose rats were started on test approximately 7 months prior to initiation of the high dose rats. The control rats for both dosed groups were approximately 1 month younger than the respective dosed groups and were, therefore, included in the bioassay approximately 1 month after their corresponding dosed group. The dietary concentrations of 5-nitroacenaphthene administered were 0.24 and 0.12 percent. Throughout this report those rats receiving the former concentration are referred to as the high dose groups, while those rats receiving the latter concentration

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
5-NITROACENAPHTHENE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	5-NITROACE-NAPHTHENE CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
LOW DOSE CONTROL	50	0	0	108
HIGH DOSE CONTROL	49	0	0	109
LOW DOSE	50	0.12 0	78	22
HIGH DOSE[b]	50	0.24	70	
FEMALE				
LOW DOSE CONTROL	50	0	0	108
HIGH DOSE CONTROL	50	0	0	110
LOW DOSE	50	0.12 0	78	22
HIGH DOSE	50	0.24 0	78	9

[a] Concentrations given in percentages of feed.

[b] This group was terminated in week 70, before the period of chemical administration was to be completed.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
5-NITROACENAPHTHENE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	5-NITROACE-NAPHTHENE CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION[b]
MALE					
CONTROL	50	0	0	96	0
LOW DOSE	50	0.06 0	78	18	0.06
HIGH DOSE	50	0.12 0	78	18	0.12
FEMALE					
CONTROL	50	0	0	97	0
LOW DOSE	50	0.06 0.03 0	51 27	18	0.05
HIGH DOSE	50	0.12 0	78	18	0.12

[a] Concentrations given in percentages of feed.

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

are referred to as the low dose groups. Except for the high dose male group, which was terminated in week 70, the dosed rats received feed containing 5-nitroacenaphthene for a period of 78 weeks, followed by an observation period of up to 22 weeks.

The dosed and control mice were all approximately 6 weeks old at the time they were placed on test. The dosed mice shared the same median date of birth and were, therefore, started on test at the same time. Control mice were approximately 3 weeks younger than the dosed mice and, as a result, were started on test approximately 3 weeks after the dosed groups. For both males and females, the initial dietary concentrations of 5-nitroacenaphthene administered were 0.12 and 0.06 percent. Throughout this report those mice initially receiving the former concentration are referred to as the high dose groups, while those initially receiving the latter concentration are referred to as the low dose groups. The concentration received by female low dose mice was reduced to 0.03 percent after 51 weeks because of excessive mortality. After the 78-week period of chemical administration, the dosed mouse groups were observed for up to 18 additional weeks.

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. From the first day, 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, or 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, brain, ear, testis, prostate, 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 for mice. 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 for mice. 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

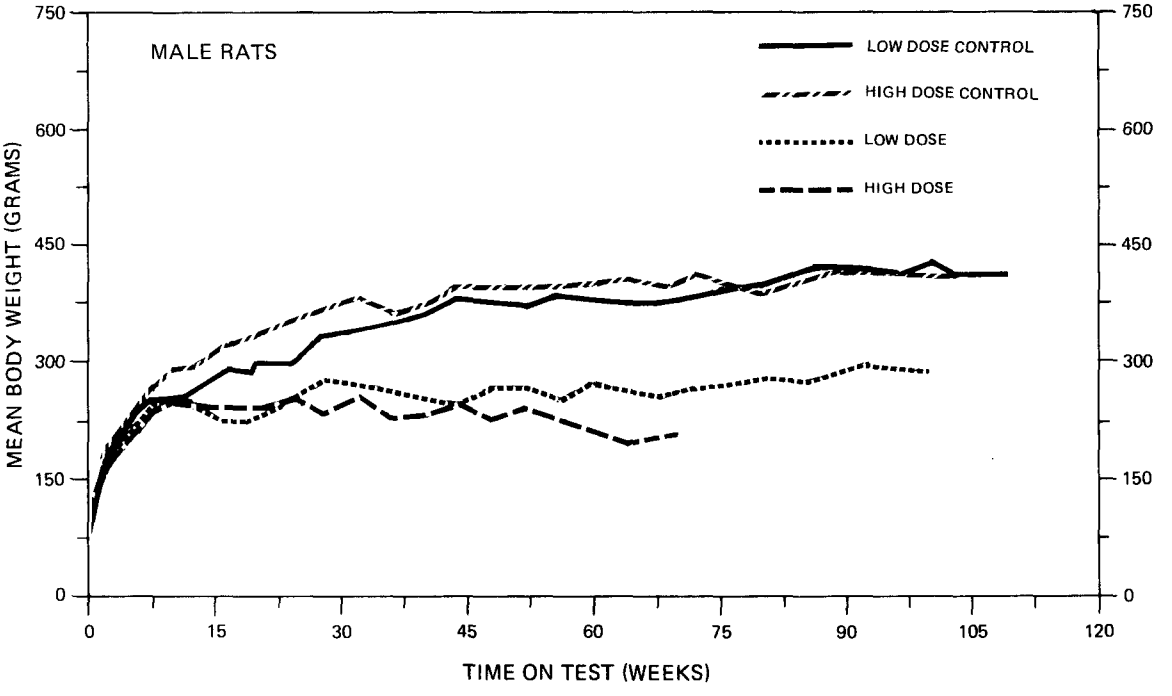
A. Body Weights and Clinical Observations

Severe compound-related mean group body weight depression was evident in male rats after week 20. Compound-related mean body weight depression, although not as extreme, was also apparent in the female rats (Figure 2). 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.

Subcutaneous and/or cutaneous masses were observed in 4 low dose control males, 4 low dose control females, 2 high dose control males, and 10 high dose control females. Four low dose males, 10 low dose females, and 4 high dose females had lesions or masses in the ear region. One high dose male developed a growth on the head and died shortly thereafter. One high dose female developed several subcutaneous vaginal masses and one low dose control female had a firm nodule on the nose. One case of exopthalmia and one of severe alopecia were observed among high dose control females.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 5-nitroacenaphthene-dosed groups are shown in Figure 3. For both male and female rats the Cox tests indicated that each dosed group had a significantly elevated mortality compared to its control group.



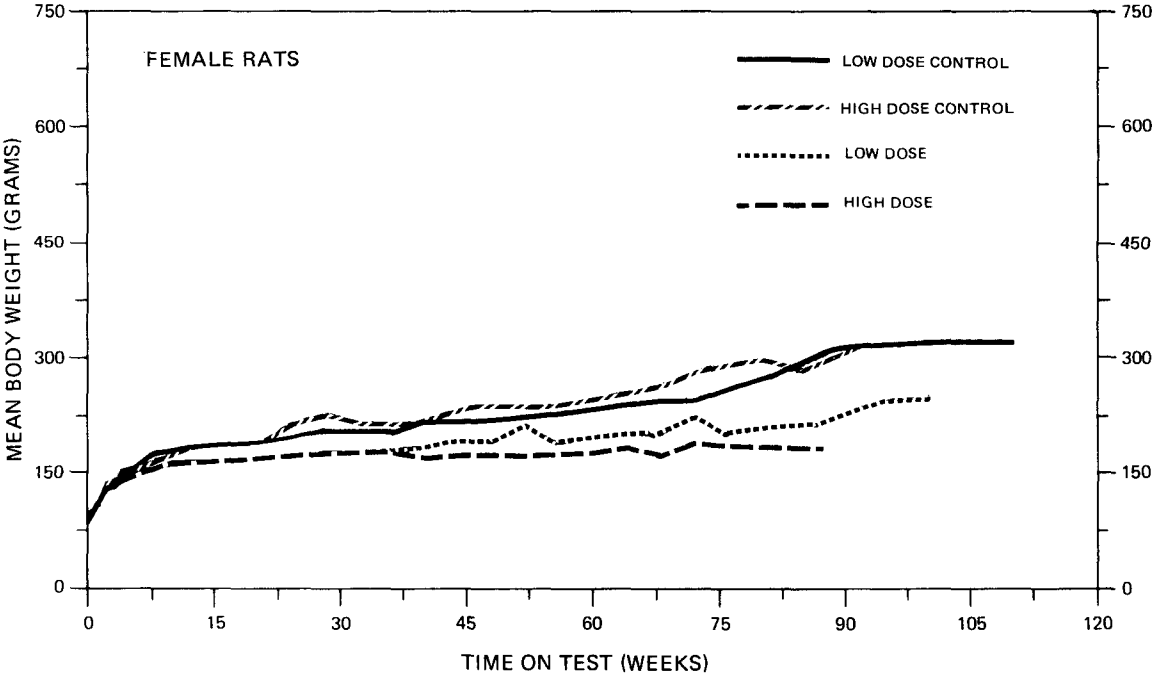
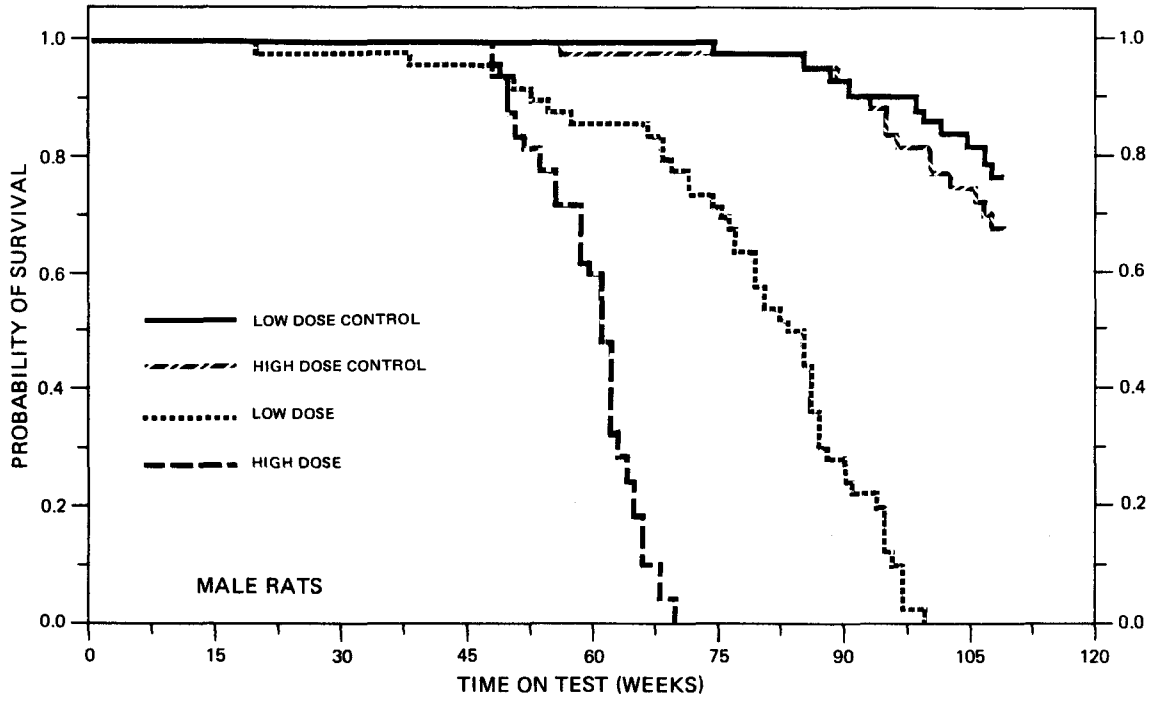


FIGURE 2
GROWTH CURVES FOR 5-NITROACENAPHTHENE CHRONIC STUDY RATS



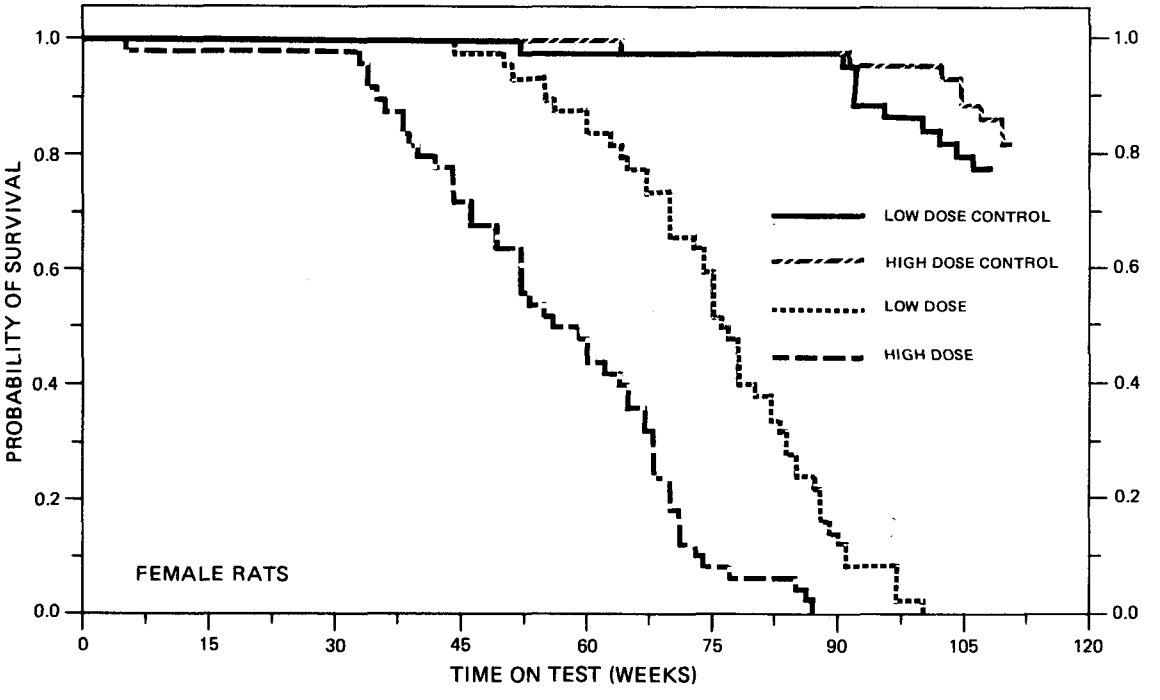


FIGURE 3
SURVIVAL COMPARISONS OF 5-NITROACENAPHTHENE CHRONIC STUDY RATS

For males five rats were sacrificed from the high dose control group in week 78 and five from the low dose control group in week 80. Survival was high in all groups through the first 45 weeks of the study, after which accelerated mortality was observed in the dosed groups. In the high dose group the median survival was 61 weeks, with the last animal dying in week 70. In the high dose group there were not adequate numbers of rats at risk long enough for meaningful statistical analyses of late-developing tumors. Survival was slightly better in the low dose group, with a median survival of 83 weeks. Thirty-six of 49 (73 percent) high dose control and 38/50 (76 percent) low dose control males survived on test at least 100 weeks.

For females five rats were sacrificed from the high dose control group in week 78 and five from the low dose control group in week 80. Survival was good in all groups through week 33, after which accelerated mortality was observed in the high dose group. In the high dose group the median survival was 56 weeks, with the last animal dying by week 88. In the high dose group there were not adequate numbers of rats at risk to permit meaningful statistical analyses of late-developing tumors. Survival was slightly better in the low dose group, with a median survival of 76 weeks, but no survivors after week 100. Forty-three of 50 (86 percent) high dose control and 39/50 (78 percent) low dose control females survived on test at least 100 weeks.

C. Pathology

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

The incidence of mammary gland adenocarcinoma was elevated in both dosed female groups (5/49 [10 percent] low dose and 5/48 [10 percent] high dose) when compared to controls (0/99); however, the incidence of mammary fibroadenoma was less in dosed groups than in controls. Morphologically the malignant mammary tumors were papillary adenocarcinomas which showed evidence of marked secretory activity (intracytoplasmic vacuoles and eosinophilic material in tumor spaces and glandular lumens). Cells grew in single layers of cuboidal epithelium or multiple layers of more pleomorphic cells which obliterated glandular patterns. Mitotic figures were numerous.

The incidence of clitoral gland carcinoma was elevated in dosed females (0/99 controls, 6/49 [12 percent] low dose and 5/48 [10 percent] high dose). This neoplasm was distinguished from squamous and sebaceous-type carcinomas by location and histologically, by the presence of prominent intracytoplasmic eosinophilic granules.

In low and high dose rats of both sexes, there was a marked increase in incidence of ceruminous or squamous-cell carcinoma arising in the region of the external ear canal (0/96 control males, 21/43 [49 percent] low dose males, 20/47 [43 percent] high dose males, 0/99

control females, 27/49 [55 percent] low dose females, 35/48 [73 percent] high dose females). These tumors probably arose from the auditory sebaceous glands (Zymbal's gland) in most instances. Ceruminous carcinoma usually contained some squamous epithelial cells although a few tumors containing only sebaceous cells were seen. The squamous-cell carcinomas were mostly composed of pleomorphic squamous epithelium with little keratinization.

Increased incidences of alveolar/bronchiolar adenomas or alveolar/bronchiolar carcinomas were observed in dosed rats (1/96 [1 percent] control males, 7/41 [17 percent] low dose males, 3/47 [6 percent] high dose males, 1/99 [1 percent] control females, 8/48 [17 percent] low dose females, 3/48 [6 percent] high dose females).

There was a variety of spontaneous inflammatory and degenerative lesions seen which commonly occur in aging Fischer 344 rats. These were not considered to be compound-related.

It is concluded from this pathologic examination that 5-nitroacenaphthene was carcinogenic to Fischer 344 rats, inducing carcinomas of the ear canal and lung in both sexes and carcinomas of the clitoral and mammary glands in females.

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

TABLE 3

TIME-ADJUSTED ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE RATS TREATED WITH 5-NITROACENAPHTHENE[a,e]

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Skin and Subcutaneous Tissue: Fibroma[b]	2/48(0.04)	3/48(0.06)	6/39(0.15)	1/40(0.03)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	3.692	0.400
Lower Limit	---	---	0.705	0.008
Upper Limit	---	---	35.610	4.739
Weeks to First Observed Tumor	99	95	77	62
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/48(0.00)	1/48(0.02)	5/37(0.14)	0/40(0.00)
P Values[c]	---	---	P = 0.013	N.S.
Relative Risk (Control)[d]	---	---	Infinite	0.000
Lower Limit	---	---	1.642	0.000
Upper Limit	---	---	Infinite	22.284
Weeks to First Observed Tumor	---	109	77	---
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	0/48(0.00)	1/48(0.02)	7/38(0.18)	3/45(0.07)
P Values[c]	---	---	P = 0.002	N.S.
Relative Risk (Control)[d]	---	---	Infinite	3.200
Lower Limit	---	---	2.462	0.268
Upper Limit	---	---	Infinite	164.119
Weeks to First Observed Tumor	---	109	75	49

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Kidney: Tubular-Cell Adenoma or Tubular-Cell Adenocarcinoma[b]	0/48(0.00)	0/48(0.00)	4/39(0.10)	0/39(0.00)
P Values[c]	---	---	P = 0.037	N.S.
Relative Risk (Control)[d]	---	---	Infinite	---
Lower Limit	---	---	1.144	---
Upper Limit	---	---	Infinite	---
Weeks to First Observed Tumor	---	---	90	---
Pituitary: Adenoma NOS[b]	1/41(0.02)	9/38(0.24)	0/28(0.00)	0/29(0.00)
P Values[c]	---	---	N.S.	P = 0.004(N)
Relative Risk (Control)[d]	---	---	0.000	0.000
Lower Limit	---	---	0.000	0.000
Upper Limit	---	---	26.911	0.487
Weeks to First Observed Tumor	108	85	---	---
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant[b]	10/47(0.21)	8/47(0.17)	2/34(0.06)	0/36(0.00)
P Values[c]	---	---	N.S.	P = 0.008(N)
Relative Risk (Control)[d]	---	---	0.276	0.000
Lower Limit	---	---	0.031	0.000
Upper Limit	---	---	1.186	0.565
Weeks to First Observed Tumor	99	107	76	---

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Preputial Gland: Carcinoma NOS[b]	2/48(0.04)	0/48(0.00)	3/39(0.08)	0/37(0.00)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	1.846	---
Lower Limit	---	---	0.222	---
Upper Limit	---	---	21.132	---
Weeks to First Observed Tumor	104	---	79	---
Testis: Interstitial-Cell Tumor[b]	45/47(0.96)	42/47(0.89)	19/33(0.58)	2/37(0.05)
P Values[c]	---	---	P < 0.001(N)	P < 0.001(N)
Relative Risk (Control)[d]	---	---	0.601	0.060
Lower Limit	---	---	0.526	0.010
Upper Limit	---	---	0.787	0.192
Weeks to First Observed Tumor	80	78	71	58
Ear Canal or Skin of Ear: Squamous-Cell Carcinoma[b]	1/48(0.02)	0/48(0.00)	7/39(0.18)	2/40(0.05)
P Values[c]	---	---	P = 0.014	N.S.
Relative Risk (Control)[d]	---	---	8.615	Infinite
Lower Limit	---	---	1.178	0.356
Upper Limit	---	---	376.472	Infinite
Weeks to First Observed Tumor	99	---	68	58

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Ear Canal: Ceruminous Carcinoma[b]	0/48(0.00)	0/48(0.00)	14/42(0.33)	18/47(0.38)
P Values[c]	---	---	P < 0.001	P < 0.001
Relative Risk (Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	5.006	5.895
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	38	47
Ear Canal or Skin of Ear: Squamous-Cell Carcinoma or Ceruminous Carcinoma[b]	1/48(0.02)	0/48(0.00)	21/42(0.50)	20/47(0.43)
P Values[c]	---	---	P < 0.001	P < 0.001
Relative Risk (Control)[d]	---	---	24.000	Infinite
Lower Limit	---	---	4.200	6.614
Upper Limit	---	---	946.120	Infinite
Weeks to First Observed Tumor	99	---	38	47
Body Cavities: Mesothelioma NOS or Mesothelioma, Malignant[b]	1/48(0.02)	2/48(0.04)	4/39(0.10)	2/40(0.05)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	4.923	1.200
Lower Limit	---	---	0.513	0.090
Upper Limit	---	---	235.669	15.868
Weeks to First Observed Tumor	108	106	79	66

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Papillary Adenoma or Follicular-Cell Adenoma[b]	0/39(0.00)	0/48(0.00)	4/33(0.12)	0/38(0.00)
P Values[c]	---	---	P = 0.040	N.S.
Relative Risk (Control)[d]	---	---	Infinite	---
Lower Limit	---	---	1.108	---
Upper Limit	---	---	Infinite	---
Weeks to First Observed Tumor	---	---	79	---
Thyroid: Papillary Adenocarcinoma or Follicular-Cell Carcinoma[b]	0/39(0.00)	0/48(0.00)	4/33(0.12)	1/38(0.03)
P Values[c]	---	---	P = 0.040	N.S.
Relative Risk (Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	1.108	0.068
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	75	68
Parathyroid: Adenoma NOS[b]	0/23(0.00)	1/28(0.04)	1/20(0.05)	2/19(0.11)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite	2.947
Lower Limit	---	---	0.063	0.164
Upper Limit	---	---	Infinite	163.981
Weeks to First Observed Tumor	---	109	91	62

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Pancreatic Islets: Islet-Cell Adenoma[b]	3/45(0.07)	0/46(0.00)	0/31(0.00)	1/35(0.03)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.000	Infinite
Lower Limit	---	---	0.000	0.071
Upper Limit	---	---	2.375	Infinite
Weeks to First Observed Tumor	85	---	---	62
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	6/48(0.13)	6/48(0.13)	2/39(0.05)	0/40(0.00)
P Values[c]	---	---	N.S.	P = 0.023(N)
Relative Risk (Control)[d]	---	---	0.410	0.000
Lower Limit	---	---	0.042	0.000
Upper Limit	---	---	2.138	0.745
Weeks to First Observed Tumor	98	93	96	---

[a]Treated groups received doses of 0.12 or 0.24 percent in feed.

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

[c]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. A negative designation (N) indicates a lower incidence in the treated group 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, except for sites where the first tumor of interest was observed earlier than 52 weeks, where the analyses were based upon all animals that survived until or past the date that the first tumor was observed.

TABLE 4

TIME-ADJUSTED ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE RATS TREATED WITH 5-NITROACENAPHTHENE[a,e]

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Skin: Squamous-Cell Carcinoma, Basal-Cell Carcinoma, or Sebaceous Adenocarcinoma[b]	0/49(0.00)	1/50(0.02)	0/46(0.00)	3/31(0.10)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	---	4.839
Lower Limit	---	---	---	0.408
Upper Limit	---	---	---	245.211
Weeks to First Observed Tumor	---	110	---	85
Subcutaneous Tissue: Fibroma[b]	0/49(0.00)	1/50(0.02)	3/46(0.07)	2/31(0.06)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite	3.226
Lower Limit	---	---	0.641	0.174
Upper Limit	---	---	Infinite	183.956
Weeks to First Observed Tumor	---	102	75	68
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/49(0.00)	0/50(0.00)	4/46(0.09)	1/31(0.03)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.989	0.086
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	65	87

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	0/49(0.00)	1/50(0.02)	8/46(0.17)	3/31(0.10)
P Values[c]	---	---	P = 0.002	N.S.
Relative Risk (Control)[d]	---	---	Infinite	4.839
Lower Limit	---	---	2.437	0.408
Upper Limit	---	---	Infinite	245.211
Weeks to First Observed Tumor	---	110	55	53
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	7/49(0.14)	5/50(0.10)	1/46(0.02)	0/30(0.00)
P Values[c]	---	---	P = 0.036(N)	N.S.
Relative Risk (Control)[d]	---	---	0.152	0.000
Lower Limit	---	---	0.003	0.000
Upper Limit	---	---	1.116	1.298
Weeks to First Observed Tumor	106	104	83	---
Pituitary: Adenoma NOS or Chromophobe Adenoma[b]	18/44(0.41)	17/40(0.43)	5/42(0.12)	0/28(0.00)
P Values[c]	---	---	P = 0.002(N)	P < 0.001(N)
Relative Risk (Control)[d]	---	---	0.291	0.000
Lower Limit	---	---	0.094	0.000
Upper Limit	---	---	0.727	0.257
Weeks to First Observed Tumor	90	78	60	---

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Adrenal: Pheochromocytoma[b]	2/49(0.04)	3/49(0.06)	1/45(0.02)	0/31(0.00)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.544	0.000
Lower Limit	---	---	0.009	0.000
Upper Limit	---	---	10.083	2.587
Weeks to First Observed Tumor	108	109	88	---
Thyroid: Follicular-Cell Carcinoma[b]	1/40(0.03)	1/45(0.02)	3/43(0.07)	2/27(0.07)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	2.791	3.333
Lower Limit	---	---	0.236	0.181
Upper Limit	---	---	142.985	188.994
Weeks to First Observed Tumor	106	109	77	60
Thyroid: Follicular-Cell Carcinoma or Follicular-Cell Adenoma[b]	1/40(0.02)	1/45(0.02)	4/44(0.09)	3/27(0.11)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	3.636	5.000
Lower Limit	---	---	0.381	0.424
Upper Limit	---	---	174.735	251.837
Weeks to First Observed Tumor	106	109	51	59

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	3/40(0.08)	2/45(0.04)	0/43(0.00)	0/27(0.00)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Control)[d]	---	---	0.000	0.000
Lower Limit	---	---	0.000	0.000
Upper Limit	---	---	1.538	5.519
Weeks to First Observed Tumor	108	110	---	---
Mammary Gland: Adenocarcinoma[b]	0/49(0.00)	0/50(0.00)	5/46(0.11)	5/31(0.16)
P Values[c]	---	---	P = 0.024	P = 0.007
Relative Risk (Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	1.345	2.044
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	60	65
Mammary Gland: Adenocarcinoma or Papillary Cystadenocarcinoma[b]	0/49(0.00)	0/50(0.00)	6/46(0.13)	5/31(0.16)
P Values[c]	---	---	P = 0.011	P = 0.007
Relative Risk (Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	1.706	2.044
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	60	65

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma[b]	16/49(0.33)	19/50(0.38)	12/46(0.26)	4/31(0.13)
P Values[c]	---	---	N.S.	P = 0.013(N)
Relative Risk (Control)[d]	---	---	0.799	0.340
Lower Limit	---	---	0.389	0.092
Upper Limit	---	---	1.593	0.902
Weeks to First Observed Tumor	80	107	78	74
Clitoral Gland: Carcinoma NOS[b]	0/49(0.00)	0/50(0.00)	6/46(0.13)	5/39(0.13)
P Values[c]	---	---	P = 0.011	P = 0.014
Relative Risk (Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	1.706	1.620
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	75	40
Uterus: Endometrial Stromal Polyp[b]	12/49(0.24)	10/50(0.20)	1/45(0.02)	1/26(0.04)
P Values[c]	---	---	P = 0.001(N)	N.S.
Relative Risk (Control)[d]	---	---	0.091	0.192
Lower Limit	---	---	0.002	0.005
Upper Limit	---	---	0.573	1.227
Weeks to First Observed Tumor	80	78	60	77

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
Ear Canal: Squamous-Cell Carcinoma[b]	0/49(0.00)	0/50(0.00)	3/49(0.06)	9/46(0.20)
P Values[c]	---	---	N.S.	P = 0.001
Relative Risk (Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.602	2.860
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	67	34
Ear Canal: Ceruminous Carcinoma[b]	0/49(0.00)	0/50(0.00)	25/49(0.51)	26/47(0.55)
P Values[c]	---	---	P < 0.001	P < 0.001
Relative Risk (Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	8.233	9.148
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	44	33
Ear Canal: Squamous-Cell Carcinoma, Ceruminous Carcinoma or Carcinosarcoma[b]	0/49(0.00)	0/50(0.00)	27/49(0.55)	35/47(0.74)
P Values[c]	---	---	P < 0.001	P < 0.001
Relative Risk (Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	8.947	12.674
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	44	33

TABLE 4 (CONCLUDED)

[a]Treated groups received doses of 0.12 or 0.24 percent in feed.

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

[c]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. A negative designation (N) indicates a lower incidence in the treated group 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, except for sites where the first tumor of interest was observed earlier than 52 weeks, where the analyses were based upon all animals that survived until or past the date that the first tumor was observed.

tumors were observed in at least one of the control or 5-nitroacenaphthene-dosed groups and where such tumors were observed in at least 5 percent of the group. Due to high early mortality these analyses have been based solely upon those rats surviving at least 52 weeks or, in the event that the tumor of interest was observed earlier, at least as long as the time at which the first tumor of interest was observed. Since all of the high dose males and all but 4/50 (8 percent) of the high dose females were dead after week 74, the statistical tests involving the high dose groups are not meaningful for late-developing tumors. Additionally, statistical results involving the low dose groups must be qualified since the low dose rats came from a different supplier.

In both male and female rats large numbers of ceruminous carcinomas and squamous-cell carcinomas of the ear canal or skin of the ear were observed. When the incidences were combined so that the numerator represented rats of that sex with either one of these tumors, then both for males and for females the Fisher exact tests comparing both the high dose and low dose groups to their respective controls were significant ($P < 0.001$). These tumors occurred in 3/334 (1 percent) of the male and 0/336 female Fischer 344 rats in the historical controls observed at Mason Research Institute for the NCI Carcinogenesis Testing Program. By comparison, these tumors were observed in 20/47 (43 percent) of the high dose and 21/42 (50 percent) of the low dose males surviving on test at least 38 weeks,

and in 35/47 (74 percent) of the high dose and 27/49 (55 percent) of the low dose females surviving on test at least 33 weeks. Based on these statistical results, the administration of 5-nitroacenaphthene was associated with an increased incidence of ceruminous carcinomas and squamous-cell carcinomas of the ear canal or skin of the ear in both male and female rats.

In female rats, the Fisher exact test for the incidence of clitoral gland carcinoma was significant when comparing both the high dose to the high dose control ($P = 0.014$) and the low dose to the low dose control ($P = 0.011$). Similarly, in female rats, the incidence of mammary adenocarcinomas was significant when comparing both the low dose ($P = 0.024$) and high dose ($P = 0.007$) to their respective controls. In historical control females, 0/336 and 4/336 (1 percent) of the untreated females had the clitoral or mammary tumor, respectively. Based on these statistical results, the administration of 5-nitroacenaphthene was associated with the increased incidence of clitoral gland carcinomas and mammary adenocarcinomas in female rats.

Significantly increased incidences of alveolar/bronchiolar neoplasms were noted with the Fisher exact tests for both low dose male ($P = 0.002$) and low dose female ($P = 0.002$) rats. In historical control data compiled by this laboratory for the NCI Carcinogenesis Testing Program 5/334 (1 percent) of the male and 4/336 (1 percent) of the female Fischer 344 rats had either an alveolar/bronchiolar

adenoma or an alveolar/bronchiolar carcinoma--compared to 7/38 (18 percent) low dose males and 8/46 (17 percent) low dose females in this study. The absence of significant results in the high dose groups probably reflected the poor survival in those high dose groups. The earliest observed tumor was in week 49 in the high dose males and week 53 in the high dose females, compared to week 109 in the high dose control males and week 110 in the high dose control females. Based on these statistical results, the administration of 5-nitroacenaphthene was associated with the development of alveolar/bronchiolar neoplasms in male and female Fischer 344 rats.

In male rats the incidence of kidney tubular-cell adenomas or tubular-cell adenocarcinomas was increased in the low dose group over that in the control. The Fisher exact test yielded a value of $P = 0.037$, a marginal result which was not significant under the Bonferroni criterion. Similarly, the incidences of thyroid adenomas and the incidences of thyroid carcinomas in males, plus the incidences of leukemias or malignant lymphomas in females were not significant under the Bonferroni criterion.

For interstitial-cell tumors of the testis, pituitary adenomas, and adrenal pheochromocytomas in males and for pituitary adenomas, mammary fibroadenomas, and endometrial stromal polyps in females, the possibility of a negative association between chemical administration and incidence was indicated. These results must be discounted, however, due to the poor survival of the dosed rats.

In summary, the statistical findings were that the administration of 5-nitroacenaphthene was associated with the increased incidence of carcinomas of the ear canal or skin of the ear and of alveolar/bronchiolar neoplasms in both male and female rats and with clitoral gland carcinomas and mammary gland adenocarcinomas in female rats.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

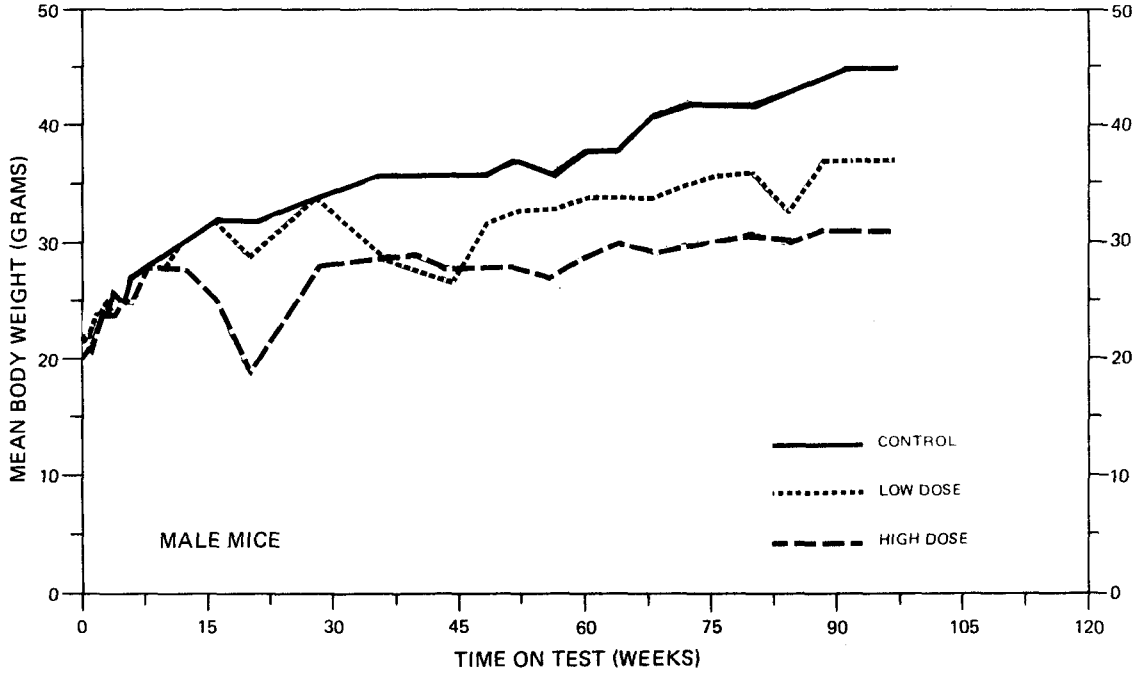
Compound-related mean body weight depression was apparent in male mice but not in females (Figure 4). Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variations.

No abnormal clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 5-nitroacenaphthene-dosed groups are shown in Figure 5. For male mice, the Tarone test for positive association between increased dosage and accelerated mortality was significant ($P < 0.001$). For female mice, the Cox test indicated a significantly ($P < 0.001$) greater mortality in the high dose than in the control group.

Five male mice were sacrificed from the control group in week 79. Survival was very poor in the male high dose group with a median survival of 20 weeks: 10 died in week 13, 26 in week 20, and 6 in week 42. Only 3 mice survived past week 42. These deaths were not related to tumor incidence since the one tumor found in a high dose male occurred in a mouse which survived until the end of the study. In the low dose group 14 deaths were observed in week 50. All 14 of these low dose males had a fatty metamorphosis of the liver; ten had



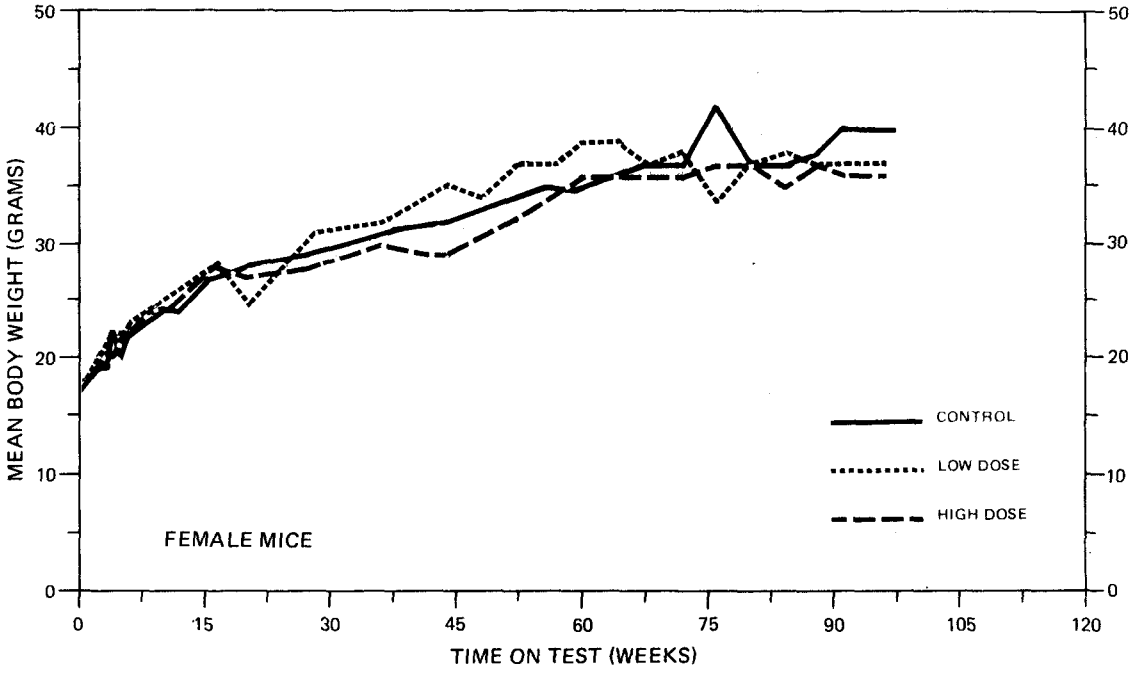
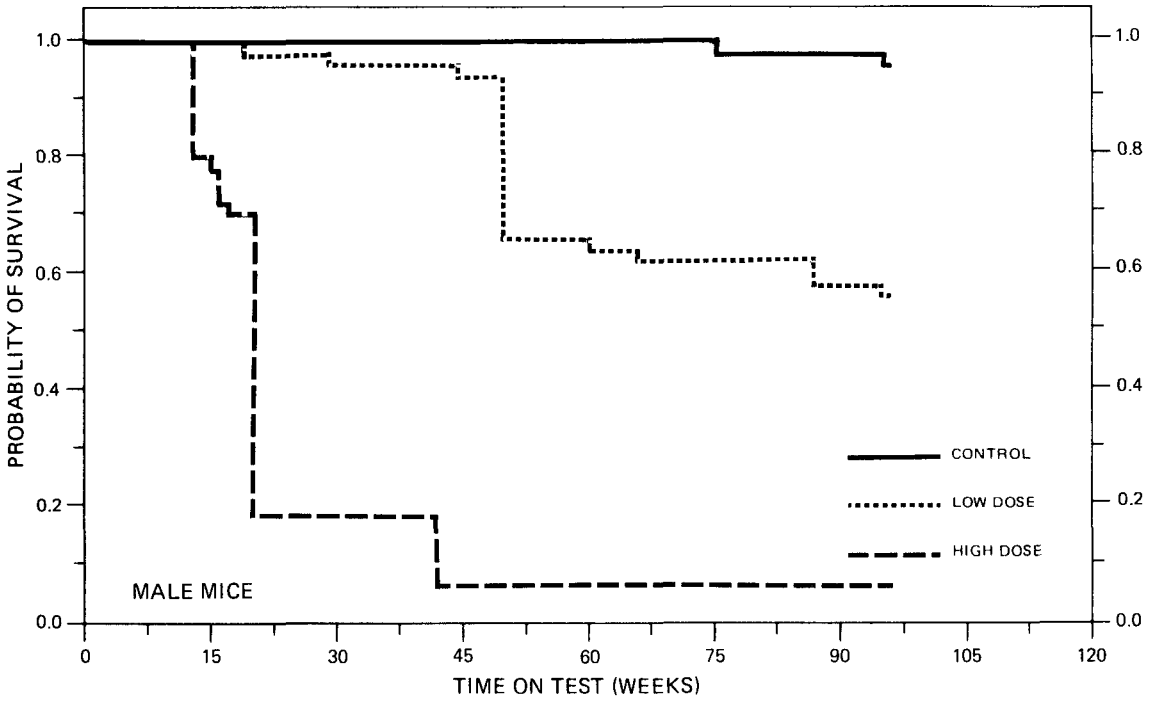


FIGURE 4
GROWTH CURVES FOR 5-NITROACENAPHTHENE CHRONIC STUDY MICE



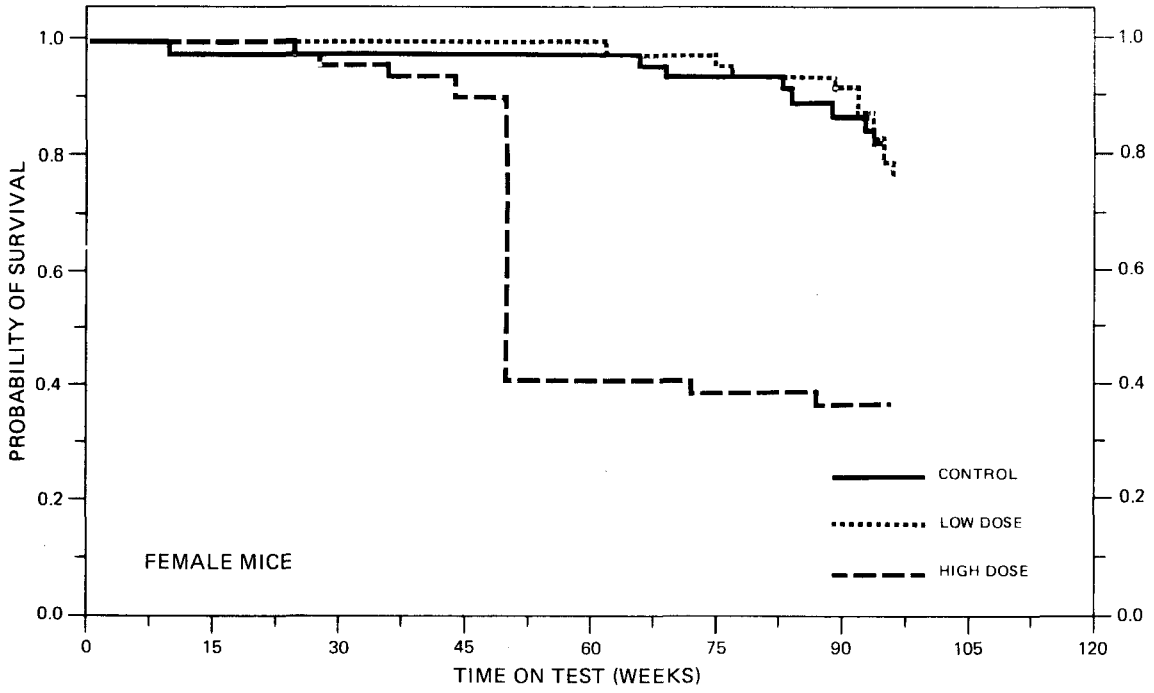


FIGURE 5
SURVIVAL COMPARISONS OF 5-NITROACENAPHTHENE CHRONIC STUDY MICE

calcification of the renal papilla. Twenty-eight low dose males survived on test until the termination of the study. In the control group survival was good, with 43/50 (86 percent) of the mice surviving on test until the termination of the study. Only in the low dose and control groups were adequate numbers of male mice at risk from late-developing tumors.

Among females five mice were sacrificed from the control group in week 79. The 24 deaths (48 percent) which occurred during week 50 in the high dose group were apparently due to toxicity, but only a few additional deaths were observed after that week. At the termination of the experiment 36 percent (18/50) of the high dose, 76 percent (38/50) of the low dose, and 72 percent (36/50) of the control mice were still alive on test. Adequate numbers of dosed and control females survived long enough to be at risk from late-developing tumors.

C. Pathology

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

The incidence of hepatocellular carcinoma was elevated in both low dose (23/47 [49 percent]) and high dose (18/45 [40 percent]) females compared with female controls (2/47 [4 percent]). There were no hepatocellular carcinomas in high dose males, although they occurred in both control and low dose animals. The lesions were typical of hepatocellular carcinoma in mice as described in the

literature (Reuber, 1975). The full range of lesions described by Reuber as hepatocellular carcinoma was seen (i.e., from well-differentiated to anaplastic). Four hepatocellular carcinomas in treated female mice and one in a control female mouse metastasized to the lung.

There was an increased incidence of ovarian neoplasms in dosed female mice. Granulosa-cell tumors were found in 2/41 (5 percent) low dose and 2/39 (5 percent) high dose mice. The incidence of tubular-cell adenomas appeared to be dose-related, occurring in 2/41 (5 percent) low dose and 4/39 (10 percent) high dose mice. One luteoma was observed in the high dose group. None of these neoplasms was observed in control female mice.

There were other tumors and many inflammatory and degenerative lesions reported which are commonly seen in aging mice. Most of these lesions were unrelated to the administration of the test compound, appearing with similar or lesser frequency in the dosed mice than in the controls. Fatty metamorphosis of the liver and calcification of renal papilla were, however, compound-related lesions in both sexes.

Based upon this pathologic examination, 5-nitroacenaphthene was carcinogenic in female B6C3F1 mice, inducing hepatocellular carcinomas and ovarian tumors.

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 5-nitroacenaphthene-dosed groups and where such tumors were observed in at least 5 percent of the group. The results for high dose male mice have been omitted since early mortality was so high that statistical analyses of late-developing tumors were meaningless for that group. Because of the high early mortality in both sexes, statistical analyses were based upon those mice surviving at least 52 weeks. All of the statistical results for mice must be qualified since the control mice were from a different supplier than the dosed mice.

For females the incidences of hepatocellular carcinoma were significant ($P < 0.001$) when comparing the dosed groups to the controls using the Cochran-Armitage test. These Cochran-Armitage results were supported by significant ($P < 0.001$) Fisher exact test results for the comparison of both high dose and low dose to control. In historical data compiled by this laboratory for the Carcinogenesis Testing Program, 12/250 (5 percent) of the untreated female B6C3F1 mice had one of these tumors, compared to the 2/47 (4 percent) observed in these control females. Based on these results the statistical conclusion is that the administration of 5-nitroacenaphthene was

TABLE 5

TIME-ADJUSTED ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH 5-NITROACENAPHTHENE[a,e]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	5/50(0.10)	0/31(0.00)
P Values[c]	---	N.S.
Relative Risk (Control)[d]	---	0.000
Lower Limit	---	0.000
Upper Limit	---	1.258
Weeks to First Observed Tumor	95	---
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	5/50(0.10)	4/45(0.09)
P Values[c]	---	N.S.
Relative Risk (Control)[d]	---	0.889
Lower Limit	---	0.187
Upper Limit	---	3.868
Weeks to First Observed Tumor	95	50
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	5/50(0.10)	3/33(0.09)
P Values[c]	---	N.S.
Relative Risk (Control)[d]	---	0.909
Lower Limit	---	0.149
Upper Limit	---	4.312
Weeks to First Observed Tumor	75	96

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE
Liver: Hepatocellular Carcinoma[b]	12/50(0.24)	7/31(0.23)
P Values[c]	---	N.S.
Relative Risk (Control)[d]	---	0.941
Lower Limit	---	0.348
Upper Limit	---	2.273
Weeks to First Observed Tumor	95	95

[a]Low dose group received a dose of 0.06 percent in feed.

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

[c]The probability level for the Fisher exact test for the comparison of the 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. A negative designation (N) indicates a lower incidence in the treated group 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, except for sites where the first tumor of interest was observed earlier than 52 weeks, where the analyses were based upon all animals that survived until or past the date that the first tumor was observed.

TABLE 6

TIME-ADJUSTED ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE MICE TREATED WITH 5-NITROACENAPHTHENE[a,e]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	2/46(0.04)	3/47(0.06)	2/19(0.11)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.468	2.421
Lower Limit	---	0.176	0.184
Upper Limit	---	16.917	30.577
Weeks to First Observed Tumor	96	92	96
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	7/47(0.15)	5/47(0.11)	4/46(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.714	0.584
Lower Limit	---	0.192	0.134
Upper Limit	---	2.423	2.132
Weeks to First Observed Tumor	83	92	25
Liver: Hepatocellular Carcinoma[b]	2/47(0.04)	23/47(0.49)	18/19(0.95)
P Values[c]	P < 0.001	P < 0.001	P < 0.001
Relative Risk (Control)[d]	---	11.500	22.263
Lower Limit	---	3.116	7.102
Upper Limit	---	94.161	44.718
Weeks to First Observed Tumor	94	92	87

TABLE 6 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma NOS[b]	5/43(0.12)	0/39(0.00)	0/18(0.00)
P Values[c]	P = 0.028(N)	P = 0.035(N)	N.S.
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	0.867	1.803
Weeks to First Observed Tumor	95	---	---
Ovary: Tubular-Cell Adenoma[b]	0/45(0.00)	2/41(0.05)	4/17(0.24)
P Values[c]	P = 0.001	N.S.	P = 0.004
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.326	2.497
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	96	96
Ovary: Granulosa-Cell Tumor, Luteoma, or Tubular-Cell Adenoma[b]	0/45(0.00)	4/41(0.10)	7/37(0.19)
P Values[c]	P = 0.003	P = 0.048	P = 0.003
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	1.022	2.376
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	96	36

TABLE 6 (CONCLUDED)

[a]Treated groups received time-weighted average doses of 0.05 or 0.12 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, except for sites where the first tumor of interest was observed earlier than 52 weeks, where the analyses were based upon all animals that survived until or past the date that the first tumor was observed.

associated with the incidence of hepatocellular carcinomas in female B6C3F1 mice.

For females the Cochran-Armitage test indicated a significant ($P = 0.003$) positive association between dose and the combined incidences of granulosa-cell tumors, luteomas, or tubular-cell adenomas of the ovary. The Fisher exact test comparing high dose to control was also significant ($P = 0.003$) under the Bonferroni criterion. The historical control data compiled by this laboratory indicated that 2/350 (0.57 percent) of the untreated female B6C3F1 mice had these tumors as compared to the absence of these tumors in the control females of this bioassay. Based upon these results the administration of 5-nitroacenaphthene was associated with the increased incidence of ovarian neoplasms.

In female mice, the Cochran-Armitage test indicated a significant negative association between dose and the incidence of pituitary adenomas NOS. The Fisher exact tests, however, were not significant when the Bonferroni criterion was applied.

In summary, the statistical results indicate that the administration of 5-nitroacenaphthene was associated with the increased incidence of hepatocellular carcinomas and ovarian neoplasms in female B6C3F1 mice under the conditions of this bioassay. No statistical inferences could be made with respect to male mice due to the elevated mortality among high dose males.

V. DISCUSSION

In spite of deficiencies in the conduct of the experiment (some controls improperly matched; the maximum tolerated doses exceeded), sufficient evidence was obtained to establish the carcinogenicity of 5-nitroacenaphthene.

The concentration of 5-nitroacenaphthene in the diet was associated with decreased survival in both species. Toxicity of 5-nitroacenaphthene at the dosages used in this experiment was indicated by accelerated mortality in all dosed groups and by depressed growth among dosed rats of both sexes and dosed male mice. Although mortality among dosed rats may have been related to tumor development, the very high rate of early deaths among male mice could not have been due to cancer since no tumors were found in high dose male mice that died before termination of the study. During week 50, 24/50 (48 percent) high dose female mice died, presumably due to toxicity of the test chemical. Survival in low dose and control mouse groups of both sexes was considered sufficient to provide an adequate number of mice at risk from late-developing tumors. Because of accelerated mortality in both species, statistical analyses were based on those animals surviving at least 52 weeks or until the first tumor at that site was observed in any group of that sex and species.

Dietary administration of 5-nitroacenaphthene was related to an increased incidence of several neoplasms in rats. The incidences of

malignant tumors of the ear canal (incidences of ceruminous carcinomas and squamous-cell carcinomas were combined so that the numerator represented rats with either one of these tumors) were statistically significant at each dose level in each sex. Tumors were found as early as week 33. Among female rats, the incidences of clitoral gland carcinoma and the incidences of mammary adenocarcinomas were each significant in both the high dose group and the low dose group. Significant incidences of alveolar/bronchiolar carcinomas were observed in low dose rat groups of each sex, but not in high dose groups. It is possible that high dose rats did not survive long enough to be at risk from these tumors. Hepatocellular carcinomas in four dosed female mice and one control female mouse metastasized to the lung.

Ovarian tumors of several histologic types were observed at increased incidences in dosed female mice. Although only tubular-cell adenomas were significant alone, when incidences of granulosa-cell tumors, luteomas, or tubular-cell adenomas were combined, there was a significant positive association between dosage level and tumor incidence. The number of high dose female mice having at least one of these tumors was significantly greater than the number of control mice with at least one of these ovarian tumors.

There was a significant positive association between dietary concentration of 5-nitroacenaphthene and the incidence of hepatocellular carcinomas in female mice. The incidences of these tumors were statistically significant for each dosed group.

In an earlier study conducted in Tokyo, Japan, 5-nitroacenaphthene was found to be carcinogenic in female Wistar rats following dietary administration of approximately 200 mg/day (1 percent of the diet) for a period of 4 months (Terasawa, 1974; Takemura et al., 1974). Malignancies were observed in all surviving animals and included rhabdomyosarcoma of the lower abdominal wall, sebaceous-cell carcinoma of the ear duct, intraductal carcinoma of the breast, and adenocarcinoma of the small intestine. In a similar experiment using female Syrian golden hamsters, cholangiomas were the only tumors observed in 54 percent of surviving hamsters fed a diet containing 1 percent 5-nitroacenaphthene for a period of 6 months (Terasawa, 1974; Takemura et al., 1974). No tumors were observed in male animals of either species. These indications of carcinogenicity in a different strain of rats and in a third rodent species provide supporting evidence for the carcinogenicity of this compound.

The numbers of high dose male B6C3F1 mice surviving long enough to be at risk from late-developing tumors did not constitute an adequate test for carcinogenicity; however, survival and dosage among low dose male mice was adequate. On this basis, no evidence was provided by this bioassay for the carcinogenicity of 5-nitroacenaphthene in male B6C3F1 mice.

Under the conditions of this bioassay, 5-nitroacenaphthene was carcinogenic to Fischer 344 rats, causing increased incidences of malignant tumors of the ear canal in both sexes, alveolar/bronchiolar

carcinoma in both sexes, clitoral gland carcinoma in females, and mammary adenocarcinomas in females. 5-Nitroacenaphthene was also carcinogenic to female B6C3F1 mice, causing hepatocellular carcinomas and ovarian tumors.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 5-NITROACENAPHTHENE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH 5-NITROACENAPHTHENE

	LOW DOSE CONTROL (UNTR) 01-0070	HIGH DOSE CONTROL (UNTR) 01-0118	LOW DOSE 01-0065	HIGH DOSE 01-0107
ANIMALS INITIALLY IN STUDY	50	@50	50	50
ANIMALS MISSING	1			
ANIMALS NECROPSIED	48	48	43	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	48	48	43	47
INTEGUMENTARY SYSTEM				
*SKIN	(48)	(48)	(43)	(47)
SQUAMOUS CELL PAPILLOMA	1 (2%)		1 (2%)	
SQUAMOUS CELL CARCINOMA	1 (2%)		1 (2%)	
BASAL-CELL TUMOR			1 (2%)	
BASAL-CELL CARCINOMA	1 (2%)			
SEBACEOUS ADENOCARCINOMA				1 (2%)
FIBROMA			4 (9%)	
*SUBCUT TISSUE	(48)	(48)	(43)	(47)
SARCOMA, NOS		1 (2%)		
FIBROMA	2 (4%)	3 (6%)	2 (5%)	1 (2%)
FIBROSARCOMA		1 (2%)		
LEIOMYOSARCOMA				1 (2%)
RESPIRATORY SYSTEM				
#LUNG	(48)	(48)	(41)	(47)
CARCINOMA, NOS, METASTATIC	1 (2%)			
SQUAMOUS CELL CARCINOMA			1 (2%)	
SQUAMOUS CELL CARCINOMA, METASTA			2 (5%)	1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA			2 (5%)	3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	5 (12%)	
CERUMINOUS CARCINOMA, METASTATIC				1 (2%)
PHEOCHROMOCYTOMA, METASTATIC		1 (2%)		
OSTEOSARCOMA, METASTATIC	1 (2%)			
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(48)	(48)	(43)	(47)
MALIGNANT LYMPHOMA, NOS		1 (2%)		
LEUKEMIA, NOS	1 (2%)	1 (2%)		
MYELOMONOCYTIC LEUKEMIA	5 (10%)	4 (8%)	2 (5%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS
@ 50 ANIMALS WERE INITIALLY IN STUDY BUT ONE WAS DELETED WHEN FOUND TO BE A FEMALE ANIMAL IN A MALE GROUP.

TABLE A1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0070	HIGH DOSE CONTROL (UNTR) 01-0118	LOW DOSE 01-0065	HIGH DOSE 01-0107
#SPLEEN	(48)	(48)	(38)	(47)
OSTEOSARCOMA, METASTATIC	1 (2%)			
#MEDIASTINAL L.NODE	(42)	(44)	(23)	(16)
ALVEOLAR/BRONCHIOLAR CA, METASTA			1 (4%)	
CIRCULATORY SYSTEM				
*PULMONARY ARTERY	(48)	(48)	(43)	(47)
SARCOMA, NOS			1 (2%)	
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(46)	(47)	(38)	(44)
ADENOCARCINOMA, NOS		1 (2%)		
SARCOMA, NOS		1 (2%)		
#SUBMAXILLARY GLAND	(46)	(47)	(38)	(44)
SARCOMA, NOS			1 (3%)	
#LIVER	(48)	(48)	(40)	(46)
NEOPLASTIC NODULE	1 (2%)			
HEPATOCELLULAR CARCINOMA	2 (4%)	1 (2%)	1 (3%)	
#ILEUM	(45)	(46)	(34)	(39)
SARCOMA, NOS		1 (2%)		
#COLON	(44)	(46)	(32)	(38)
ADENOCARCINOMA, NOS				1 (3%)
URINARY SYSTEM				
#KIDNEY	(48)	(48)	(43)	(46)
TUBULAR-CELL ADENOMA			3 (7%)	
TUBULAR-CELL ADENOCARCINOMA			1 (2%)	
SARCOMA, NOS				1 (2%)
#URINARY BLADDER	(46)	(43)	(37)	(45)
TRANSITIONAL-CELL PAPILLOMA	1 (2%)			
ENDOCRINE SYSTEM				
#PITUITARY	(41)	(38)	(31)	(33)
ADENOMA, NOS	1 (2%)	9 (24%)		

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

TABLE A1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0070	HIGH DOSE CONTROL (UNTR) 01-0118	LOW DOSE 01-0065	HIGH DOSE 01-0107
EPENDYMOMA			1 (3%)	
#ADRENAL	(47)	(47)	(37)	(42)
CORTICAL ADENOMA	1 (2%)			
PHEOCHROMOCYTOMA	10 (21%)	7 (15%)	2 (5%)	
PHEOCHROMOCYTOMA, MALIGNANT		1 (2%)		
GANGLIONEUROMA	1 (2%)			
#THYROID	(39)	(48)	(37)	(44)
PAPILLARY ADENOMA			2 (5%)	
PAPILLARY ADENOCARCINOMA			2 (5%)	
FOLLICULAR-CELL ADENOMA			2 (5%)	
FOLLICULAR-CELL CARCINOMA			2 (5%)	1 (2%)
C-CELL CARCINOMA		1 (2%)		
#PARATHYROID	(23)	(28)	(22)	(22)
ADENOMA, NOS		1 (4%)	1 (5%)	2 (9%)
#PANCREATIC ISLETS	(45)	(46)	(35)	(42)
ISLET-CELL ADENOMA	3 (7%)			1 (2%)
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(48)	(48)	(43)	(47)
PAPILLARY ADENOCARCINOMA	1 (2%)			
FIBROADENOMA	1 (2%)			
*PREPUTIAL GLAND	(48)	(48)	(43)	(47)
CARCINOMA,NOS	2 (4%)		3 (7%)	
#TESTIS	(47)	(47)	(37)	(44)
INTERSTITIAL-CELL TUMOR	45 (96%)	42 (89%)	19 (51%)	2 (5%)
NERVOUS SYSTEM				
#BRAIN	(47)	(48)	(37)	(44)
GLIOMA, NOS		1 (2%)		
SPECIAL SENSE ORGANS				
*EYE/LACRIMAL GLAND	(48)	(48)	(43)	(47)
ADENOMA, NOS			1 (2%)	
*EAR CANAL	(48)	(48)	(43)	(47)
SQUAMOUS CELL CARCINOMA			7 (16%)	2 (4%)
CERUMINOUS CARCINOMA			14 (33%)	18 (38%)

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

TABLE A1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0070	HIGH DOSE CONTROL (UNTR) 01-0118	LOW DOSE 01-0065	HIGH DOSE 01-0107
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*BODY CAVITIES	(48)	(48)	(43)	(47)
MESOTHELIOMA, NOS	1 (2%)			2 (4%)
MESOTHELIOMA, MALIGNANT		2 (4%)	4 (9%)	
ALL OTHER SYSTEMS				
DIAPHRAGM				
ADENOCARCINOMA, NOS			1	
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	50	50	50
NATURAL DEATH@	5	6	23	19
MORIBUND SACRIFICE	5	8	27	31
SCHEDULED SACRIFICE	5	5		
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	34	30		
ANIMAL MISSING	1			
ANIMAL DELETED/WRONG SEX		1		

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	LOW DOSE CONTROL (UNTR) 01-0070	HIGH DOSE CONTROL (UNTR) 01-0118	LOW DOSE 01-0065	HIGH DOSE 01-0107
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	45	44	39	30
TOTAL PRIMARY TUMORS	81	80	87	36
TOTAL ANIMALS WITH BENIGN TUMORS	45	43	25	7
TOTAL BENIGN TUMORS	66	62	40	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	17	31	25
TOTAL MALIGNANT TUMORS	13	18	47	25
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	1	3	2
TOTAL SECONDARY TUMORS	3	1	3	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	2			2
TOTAL UNCERTAIN TUMORS	2			2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH 5-NITROACENAPHTHENE

	LOW DOSE CONTROL (UNTR) 02-0070	HIGH DOSE CONTROL (UNTR) 02-0118	LOW DOSE 02-0065	HIGH DOSE 02-0107
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS NECROPSIED	49	50	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	50	48	48
INTEGUMENTARY SYSTEM				
*SKIN	(49)	(50)	(49)	(48)
SQUAMOUS CELL PAPILLOMA			1 (2%)	1 (2%)
SQUAMOUS CELL CARCINOMA				1 (2%)
BASAL-CELL CARCINOMA		1 (2%)		
SEBACEOUS ADENOMA			1 (2%)	
SEBACEOUS ADENOCARCINOMA				2 (4%)
*SUBCUT TISSUE	(49)	(50)	(49)	(48)
SEBACEOUS ADENOMA				1 (2%)
FIBROMA		1 (2%)	3 (6%)	2 (4%)
FIBROSARCOMA		1 (2%)		
RESPIRATORY SYSTEM				
#LUNG	(49)	(50)	(48)	(48)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	1 (2%)	1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	4 (8%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			4 (8%)	1 (2%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(49)	(50)	(49)	(48)
MALIGNANT LYMPHOMA, NOS	2 (4%)			
UNDIFFERENTIATED LEUKEMIA		1 (2%)		
MYELOMONOCYTIC LEUKEMIA	5 (10%)	3 (6%)		
#SPLEEN	(48)	(48)	(48)	(47)
UNDIFFERENTIATED LEUKEMIA		1 (2%)		
#LIVER	(49)	(50)	(48)	(47)
UNDIFFERENTIATED LEUKEMIA			1 (2%)	
CIRCULATORY SYSTEM				
NONE				

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

TABLE A2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 02-0070	HIGH DOSE CONTROL (UNTR) 02-0118	LOW DOSE 02-0065	HIGH DOSE 02-0107
DIGESTIVE SYSTEM				
*MOUTH	(49)	(50)	(49)	(48)
SQUAMOUS CELL PAPILLOMA			1 (2%)	
#PAROTID GLAND	(49)	(50)	(45)	(44)
ADENOMA, NOS			1 (2%)	
#LIVER	(49)	(50)	(48)	(47)
NEOPLASTIC NODULE	2 (4%)			
HEPATOCELLULAR CARCINOMA	1 (2%)			
#ILEUM	(49)	(48)	(48)	(47)
LEIOMYOSARCOMA		1 (2%)		
URINARY SYSTEM				
#KIDNEY	(49)	(50)	(48)	(48)
CARCINOMA,NOS	1 (2%)			
TUBULAR-CELL ADENOMA				1 (2%)
SARCOMA, NOS				1 (2%)
ENDOCRINE SYSTEM				
#PITUITARY	(44)	(40)	(44)	(43)
ADENOMA, NOS	18 (41%)	17 (43%)	4 (9%)	
CHROMOPHOBE ADENOMA			1 (2%)	
#ADRENAL	(49)	(49)	(47)	(48)
CORTICAL ADENOMA		1 (2%)		
CORTICAL CARCINOMA	1 (2%)			1 (2%)
PHEOCHROMOCYTOMA	2 (4%)	3 (6%)	1 (2%)	
#ADRENAL MEDULLA	(49)	(49)	(47)	(48)
GANGLIONEUROMA		1 (2%)		
#THYROID	(40)	(45)	(45)	(40)
FOLLICULAR-CELL ADENOMA			1 (2%)	1 (3%)
FOLLICULAR-CELL CARCINOMA	1 (3%)	1 (2%)	3 (7%)	2 (5%)
C-CELL ADENOMA	2 (5%)	1 (2%)		
C-CELL CARCINOMA	1 (3%)	1 (2%)		
#PANCREATIC ISLETS	(47)	(48)	(47)	(47)
ISLET-CELL ADENOMA	1 (2%)	2 (4%)		

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

TABLE A2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 02-0070	HIGH DOSE CONTROL (UNTR) 02-0118	LOW DOSE 02-0065	HIGH DOSE 02-0107
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(49)	(50)	(49)	(48)
ADENOMA, NOS	2 (4%)			
ADENOCARCINOMA, NOS			5 (10%)	5 (10%)
PAPILLARY CYSTADENOCARCINOMA,NOS			1 (2%)	
FIBROADENOMA	16 (33%)	19 (38%)	12 (24%)	4 (8%)
*CLITORAL GLAND	(49)	(50)	(49)	(48)
CARCINOMA,NOS			6 (12%)	5 (10%)
SQUAMOUS CELL PAPILLOMA		1 (2%)		
SQUAMOUS CELL CARCINOMA				1 (2%)
ADENOMA, NOS	1 (2%)	2 (4%)	1 (2%)	
#UTERUS	(49)	(50)	(47)	(42)
ADENOCARCINOMA, NOS		1 (2%)		
LEIOMYOSARCOMA	1 (2%)			
ENDOMETRIAL STROMAL POLYP	12 (24%)	10 (20%)	1 (2%)	1 (2%)
ENDOMETRIAL STROMAL SARCOMA		1 (2%)		
#UTERUS/ENDOMETRIUM	(49)	(50)	(47)	(42)
ADENOCARCINOMA, NOS	2 (4%)			
#OVARY	(47)	(49)	(45)	(45)
GRANULOSA-CELL TUMOR		1 (2%)		
NERVOUS SYSTEM				
*BRAIN	(49)	(50)	(48)	(47)
OLIGODENDROGLIOMA	1 (2%)			
SPECIAL SENSE ORGANS				
*EAR CANAL	(49)	(50)	(49)	(48)
SQUAMOUS CELL CARCINOMA			3 (6%)	9 (19%)
CERUMINOUS CARCINOMA			25 (51%)	26 (54%)
CARCINOSARCOMA			1 (2%)	
MUSCULOSKELETAL SYSTEM				
NONE				

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

TABLE A2 (CONCLUDED)

	LOW DOSE CONTROL (UNTR) 02-0070	HIGH DOSE CONTROL (UNTR) 02-0118	LOW DOSE 02-0065	HIGH DOSE 02-0107
BODY CAVITIES				
*PERITONEUM	(49)	(50)	(49)	(48)
MESOTHELIOMA, NOS	1 (2%)			
ALL OTHER SYSTEMS				
SITE UNKNOWN				
SQUAMOUS CELL CARCINOMA		1		
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	50	50	50
NATURAL DEATH@	3	5	9	11
MORIBUND SACRIFICE	7	3	41	39
SCHEDULED SACRIFICE	5	5		
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	35	37		
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	45	38	46	45
TOTAL PRIMARY TUMORS	73	73	81	67
TOTAL ANIMALS WITH BENIGN TUMORS	37	35	24	10
TOTAL BENIGN TUMORS	54	59	32	13
TOTAL ANIMALS WITH MALIGNANT TUMORS	13	12	39	44
TOTAL MALIGNANT TUMORS	16	13	49	54
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1	1
TOTAL SECONDARY TUMORS		1	1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	3	1		
TOTAL UNCERTAIN TUMORS	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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 5-NITROACENAPHTHENE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH 5-NITROACENAPHTHENE

	CONTROL (UNTR) 05-0070	LOW DOSE 05-0064	HIGH DOSE 05-0065
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	49	45
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	50	47	45
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(50)	(46)	(44)
UNDIFFERENTIATED CARCINOMA METAS		1 (2%)	
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA		4 (9%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	5 (10%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(45)
MALIGNANT LYMPHOMA, NOS	2 (4%)	2 (4%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	1 (2%)	
#SPLEEN	(50)	(42)	(41)
HEMANGIOMA		1 (2%)	
#LYMPH NODE	(45)	(31)	(30)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (4%)		
#PEYERS PATCH	(49)	(41)	(44)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(50)	(46)	(44)
HEPATOCELLULAR CARCINOMA	12 (24%)	7 (15%)	
HEMANGIOMA	1 (2%)		

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 05-0070	LOW DOSE 05-0064	HIGH DOSE 05-0065
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
*THYROID	(40)	(34)	(43)
ADENOCARCINOMA, NOS	1 (3%)		
*PANCREATIC ISLETS	(46)	(39)	(38)
ISLET-CELL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(45)
UNDIFFERENTIATED CARCINOMA		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(49)	(45)
ADENOMA, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(49)	(45)
NEUROFIBROSARCOMA	1 (2%)		

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

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 05-0070	LOW DOSE 05-0064	HIGH DOSE 05-0065
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	2	16	19
MORIBUND SACRIFICE		6	28
SCHEDULED SACRIFICE	5		
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	43	28	3
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	23	15	1
TOTAL PRIMARY TUMORS	27	16	1
TOTAL ANIMALS WITH BENIGN TUMORS	3	5	
TOTAL BENIGN TUMORS	3	5	
TOTAL ANIMALS WITH MALIGNANT TUMORS	22	10	1
TOTAL MALIGNANT TUMORS	24	11	1
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	
TOTAL SECONDARY TUMORS	1	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 5-NITROACENAPHTHENE

	CONTROL (UNTR) 06-0070	LOW DOSE 06-0064	HIGH DOSE 06-0065
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	1
ANIMALS NECROPSIED	48	47	46
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	47	47	46
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(48)	(47)	(46)
HEMANGIOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(46)	(47)	(46)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)	3 (6%)	1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (4%)	1 (2%)	
OSTEOSARCOMA, METASTATIC	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(48)	(47)	(46)
MALIGNANT LYMPHOMA, NOS	2 (4%)	3 (6%)	4 (9%)
LYMPHOCYTIC LEUKEMIA	1 (2%)		
ERYTHROCYTIC LEUKEMIA	1 (2%)		
#SPLEEN	(47)	(44)	(45)
HEMANGIOSARCOMA	1 (2%)		
MALIGNANT LYMPHOMA, NOS	1 (2%)		
#HILAR LYMPH NODE	(36)	(38)	(35)
HEPATOCELLULAR CARCINOMA, METAST			1 (3%)
#MESENTERIC L. NODE	(36)	(38)	(35)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (3%)	1 (3%)	
#PEYERS PATCH	(45)	(42)	(39)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
#THYMUS	(27)	(21)	(18)
MALIGNANT LYMPHOMA, NOS		1 (5%)	

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0070	LOW DOSE 06-0064	HIGH DOSE 06-0065
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(47)	(47)	(45)
HEPATOCELLULAR CARCINOMA	2 (4%)	23 (49%)	18 (40%)
#STOMACH	(45)	(44)	(43)
SQUAMOUS CELL CARCINOMA		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(43)	(39)	(39)
ADENOMA, NOS	5 (12%)		
#ADRENAL	(47)	(43)	(40)
ADENOCARCINOMA, NOS		1 (2%)	
CORTICAL ADENOMA	1 (2%)		
PHEOCHROMOCYTOMA			1 (3%)
REPRODUCTIVE SYSTEM			
#UTERUS	(43)	(39)	(42)
LEIOMYOMA	1 (2%)		
#UTERUS/ENDOMETRIUM	(43)	(39)	(42)
HEMANGIOMA			1 (2%)
#OVARY/OVIDUCT	(43)	(39)	(42)
PAPILLARY ADENOMA	1 (2%)		
#OVARY	(45)	(41)	(39)
LUTEOMA			1 (3%)
GRANULOSA-CELL TUMOR		2 (5%)	2 (5%)
TUBULAR ADENOMA		2 (5%)	4 (10%)
NERVOUS SYSTEM			
NONE			

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0070	LOW DOSE 06-0064	HIGH DOSE 06-0065
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(48)	(47)	(46)
ADENOMA, NOS	1 (2%)	1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM			
HEMANGIOSARCOMA	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	6	9	21
MORIBUND SACRIFICE	3	2	10
SCHEDULED SACRIFICE	5		
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	38	18
ANIMAL MISSING		1	1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 06-0070	LOW DOSE 06-0064	HIGH DOSE 06-0065
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	18	29	21
TOTAL PRIMARY TUMORS	22	39	33
TOTAL ANIMALS WITH BENIGN TUMORS	8	4	8
TOTAL BENIGN TUMORS	9	5	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	12	29	21
TOTAL MALIGNANT TUMORS	13	32	22
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	3	1
TOTAL SECONDARY TUMORS	2	3	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT		2	2
TOTAL UNCERTAIN TUMORS		2	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 5-NITROACENAPHTHENE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH 5-NITROACENAPHTHENE

	LOW DOSE CONTROL (UNTR) 01-0070	HIGH DOSE CONTROL (UNTR) 01-0118	LOW DOSE 01-0065	HIGH DOSE 01-0107
ANIMALS INITIALLY IN STUDY	50	@50	50	50
ANIMALS MISSING	1			
ANIMALS NECROPSIED	48	48	43	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	48	48	43	47
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(48)	(48)	(43)	(47)
INFLAMMATION, ACUTE				1 (2%)
ABSCESS, NOS				2 (4%)
NECROSIS, NOS				1 (2%)
METAPLASIA, OSSEOUS		1 (2%)		
RESPIRATORY SYSTEM				
#TRACHEA	(45)	(48)	(35)	(47)
INFLAMMATION, NOS		2 (4%)		
INFLAMMATION, ACUTE/CHRONIC	18 (40%)		3 (9%)	
INFLAMMATION, CHRONIC			3 (9%)	3 (6%)
#LUNG/BRONCHUS	(48)	(48)	(41)	(47)
BRONCHIECTASIS	3 (6%)	1 (2%)	1 (2%)	
INFLAMMATION, NOS		7 (15%)		
ABSCESS, NOS				1 (2%)
#LUNG/BRONCHIOLE	(48)	(48)	(41)	(47)
HYPERPLASIA, EPITHELIAL			1 (2%)	
#LUNG	(48)	(48)	(41)	(47)
CONGESTION, NOS	1 (2%)		7 (17%)	
EDEMA, NOS			2 (5%)	
HEMORRHAGE			2 (5%)	
INFLAMMATION, NOS			2 (5%)	
INFLAMMATION, FOCAL	2 (4%)			
INFLAMMATION, INTERSTITIAL		4 (8%)	1 (2%)	
INFLAMMATION, NECROTIZING		1 (2%)		
ABSCESS, NOS	1 (2%)			
PNEUMONIA, CHRONIC MURINE		1 (2%)		1 (2%)
INFLAMMATION, CHRONIC NECROTIZIN			1 (2%)	
GRANULOMA, NOS	1 (2%)			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS
@ 50 ANIMALS WERE INITIALLY IN STUDY BUT ONE WAS DELETED WHEN FOUND TO BE A FEMALE ANIMAL IN A MALE GROUP.

TABLE C1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0070	HIGH DOSE CONTROL (UNTR) 01-0118	LOW DOSE 01-0065	HIGH DOSE 01-0107
PERIVASCULITIS			1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)		
HYPERPLASIA, FOCAL	1 (2%)			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)			
#LUNG/ALVEOLI	(48)	(48)	(41)	(47)
MINERALIZATION			1 (2%)	
CALCIFICATION, NOS			1 (2%)	
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(48)	(47)	(39)	(43)
HYPERPLASIA, NOS			1 (3%)	
MYELOFIBROSIS	1 (2%)			
HYPERPLASIA, HEMATOPOIETIC	1 (2%)			
HYPERPLASIA, GRANULOCYTIC	1 (2%)			
HYPERPLASIA, MEGAKARYOCYTIC	1 (2%)			
#SPLEEN	(48)	(48)	(38)	(47)
FIBROSIS		1 (2%)		
NECROSIS, DIFFUSE				1 (2%)
HEMOSIDEROSIS		1 (2%)		10 (21%)
ATROPHY, NOS				7 (15%)
LYMPHOID DEPLETION			1 (3%)	
HYPERPLASIA, HEMATOPOIETIC		9 (19%)	1 (3%)	
HYPERPLASIA, ERYTHROID		10 (21%)	1 (3%)	
ERYTHROPOIESIS	1 (2%)			1 (2%)
#LYMPH NODE	(42)	(44)	(23)	(16)
HEMORRHAGE		1 (2%)		
ATROPHY, NOS			2 (9%)	
PLASMACYTOSIS		1 (2%)		
HYPERPLASIA, LYMPHOID		3 (7%)		
#MANDIBULAR L. NODE	(42)	(44)	(23)	(16)
DILATATION, NOS	1 (2%)			
HYPERPLASIA, NOS	1 (2%)			
HYPERPLASIA, PLASMA CELL			1 (4%)	
CIRCULATORY SYSTEM				
#HEART	(48)	(48)	(41)	(47)
MINERALIZATION			4 (10%)	
FIBROSIS, FOCAL	11 (23%)			

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

TABLE C1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0070	HIGH DOSE CONTROL (UNTR) 01-0118	LOW DOSE 01-0065	HIGH DOSE 01-0107
FIBROSIS, DIFFUSE	1 (2%)			
NECROSIS, NOS			4 (10%)	
#MYOCARDIUM	(48)	(48)	(41)	(47)
MINERALIZATION			1 (2%)	
INFLAMMATION, INTERSTITIAL	2 (4%)	23 (48%)		
INFLAMMATION, ACUTE/CHRONIC	3 (6%)		1 (2%)	
FIBROSIS		12 (25%)	1 (2%)	
FIBROSIS, FOCAL	2 (4%)		1 (2%)	
FIBROSIS, DIFFUSE			1 (2%)	
DEGENERATION, NOS	1 (2%)		5 (12%)	
NECROSIS, NOS			1 (2%)	
NECROSIS, FOCAL			1 (2%)	
#CARDIAC VALVE	(48)	(48)	(41)	(47)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)			
*BLOOD VESSEL	(48)	(48)	(43)	(47)
MINERALIZATION			1 (2%)	
MEDIAL CALCIFICATION			1 (2%)	
*ARTERY	(48)	(48)	(43)	(47)
MINERALIZATION			2 (5%)	
*AORTA	(48)	(48)	(43)	(47)
MEDIAL CALCIFICATION			1 (2%)	
*CORONARY ARTERY	(48)	(48)	(43)	(47)
PERIVASCULITIS	1 (2%)			
*PULMONARY ARTERY	(48)	(48)	(43)	(47)
MINERALIZATION	11 (23%)		5 (12%)	
*PANCREATIC ARTERY,	(48)	(48)	(43)	(47)
MINERALIZATION			1 (2%)	
ARTERIOSCLEROSIS, NOS			1 (2%)	
HYPERTROPHY, NOS			1 (2%)	
HYPERPLASIA, NOS			1 (2%)	
*RENAL ARTERY	(48)	(48)	(43)	(47)
INFLAMMATION PROLIFERATIVE			1 (2%)	
NECROSIS, NOS			1 (2%)	
*TESTICULAR ARTERY	(48)	(48)	(43)	(47)
DEGENERATION, EOSINOPHILIC			1 (2%)	

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

TABLE C1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0070	HIGH DOSE CONTROL (UNTR) 01-0118	LOW DOSE 01-0065	HIGH DOSE 01-0107
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(46)	(47)	(38)	(44)
ATYPIA, NOS				1 (2%)
HYPERPLASIA, PLASMA CELL			1 (3%)	
#LIVER	(48)	(48)	(40)	(46)
INFLAMMATION, NECROTIZING	1 (2%)			
FIBROSIS			1 (3%)	
FIBROSIS SEPTAL LIVER		2 (4%)		
NECROSIS, NOS			1 (3%)	1 (2%)
NECROSIS, FOCAL	8 (17%)	2 (4%)		
POSTMORTEM CHANGE			1 (3%)	
METAMORPHOSIS FATTY	4 (8%)		2 (5%)	
BASOPHILIC CYTO CHANGE			2 (5%)	
HYPERPLASIA, FOCAL	8 (17%)	15 (31%)	2 (5%)	
ANGIECTASIS	2 (4%)	1 (2%)		
ERYTHROPOIESIS	1 (2%)			
#LIVER/CENTRILOBULAR	(48)	(48)	(40)	(46)
DEGENERATION, EOSINOPHILIC	2 (4%)			
NECROSIS, NOS		1 (2%)		
#LIVER/HEPATOCYTES	(48)	(48)	(40)	(46)
NECROSIS, NOS			1 (3%)	
*BILE DUCT	(48)	(48)	(47)	(47)
INFLAMMATION, NOS		3 (6%)		
HYPERPLASIA, NOS	6 (13%)	43 (90%)		
#PANCREAS	(45)	(46)	(35)	(41)
THROMBOSIS, NOS			1 (3%)	
INFLAMMATION, NOS		17 (37%)		
INFLAMMATION, ACUTE/CHRONIC	6 (13%)			
INFLAMMATION, CHRONIC			1 (3%)	
PERIARTERITIS	1 (2%)		3 (9%)	
ATROPHY, FOCAL	1 (2%)			
#PANCREATIC ACINUS	(45)	(46)	(35)	(42)
ATROPHY, NOS			1 (3%)	
HYPERPLASIA, FOCAL		1 (2%)		
#ESOPHAGUS	(45)	(45)	(36)	(47)
MINERALIZATION			1 (3%)	
DYSPLASIA, NOS		1 (2%)		

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

TABLE C1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0070	HIGH DOSE CONTROL (UNTR) 01-0118	LOW DOSE 01-0065	HIGH DOSE 01-0107
#ESOPHAGEAL MUSCULARI	(45)	(45)	(36)	(47)
MINERALIZATION			1 (3%)	
#STOMACH	(48)	(48)	(35)	(41)
MINERALIZATION			4 (11%)	
EPIDERMAL INCLUSION CYST	1 (2%)			
INFLAMMATION, NOS		1 (2%)		
INFLAMMATION, ACUTE/CHRONIC			1 (3%)	
CALCIFICATION, NOS			1 (3%)	
HYPERPLASIA, BASAL CELL		1 (2%)		
HYPERKERATOSIS		2 (4%)		
ACANTHOSIS		2 (4%)		
#GASTRIC MUCOSA	(48)	(48)	(35)	(41)
CALCIFICATION, NOS			2 (6%)	1 (2%)
CALCIFICATION, FOCAL				1 (2%)
#PEYERS PATCH	(45)	(46)	(34)	(39)
HYPERPLASIA, NOS		12 (26%)		
HYPERPLASIA, RETICULUM CELL	1 (2%)			
#ILEUM	(45)	(46)	(34)	(39)
INFLAMMATION, NOS		2 (4%)		
HYPERPLASIA, LYMPHOID	1 (2%)			
#COLON	(44)	(46)	(32)	(38)
NEMATODIASIS	4 (9%)		6 (19%)	
PARASITISM		3 (7%)		
URINARY SYSTEM				
#KIDNEY	(48)	(48)	(43)	(46)
MINERALIZATION			3 (7%)	
HYDRONEPHROSIS			1 (2%)	
CYST, NOS			2 (5%)	
GLOMERULONEPHRITIS, NOS	3 (6%)	47 (98%)	2 (5%)	
INFLAMMATION, ACUTE/CHRONIC	1 (2%)			
GLOMERULONEPHRITIS, SUBACUTE			8 (19%)	
INFLAMMATION, CHRONIC				1 (2%)
FIBROSIS, DIFFUSE		6 (13%)		
PERIARTERITIS			1 (2%)	
NEPHROSIS, NOS	41 (85%)		31 (72%)	43 (93%)
#KIDNEY/CORTEX	(48)	(48)	(43)	(46)
CYST, NOS			2 (5%)	

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

TABLE C1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0070	HIGH DOSE CONTROL (UNTR) 01-0118	LOW DOSE 01-0065	HIGH DOSE 01-0107
*KIDNEY/PELVIS	(48)	(48)	(43)	(46)
MINERALIZATION	1 (2%)			
HYPERPLASIA, EPITHELIAL			1 (2%)	
*URINARY BLADDER	(46)	(43)	(37)	(45)
HYPERPLASIA, EPITHELIAL		1 (2%)	1 (3%)	
METAPLASIA, SQUAMOUS	1 (2%)			
ENDOCRINE SYSTEM				
*PITUITARY	(41)	(38)	(31)	(33)
HYPERPLASIA, NOS		1 (3%)		
HYPERPLASIA, FOCAL	3 (7%)	2 (5%)		
*ADRENAL	(47)	(47)	(37)	(42)
METAMORPHOSIS FATTY	1 (2%)			
HYPERPLASIA, NODULAR			2 (5%)	
ANGIECTASIS	3 (6%)			
*ADRENAL CORTEX	(47)	(47)	(37)	(42)
HYPERPLASIA, FOCAL	1 (2%)			
*ADRENAL MEDULLA	(47)	(47)	(37)	(42)
HYPERPLASIA, NODULAR		1 (2%)		
HYPERPLASIA, NOS			1 (3%)	
HYPERPLASIA, FOCAL		4 (9%)	2 (5%)	
*THYROID	(39)	(48)	(37)	(44)
FOLLICULAR CYST, NOS			1 (3%)	
HYPERPLASIA, C-CELL	1 (3%)	3 (6%)		
*PARATHYROID	(23)	(28)	(22)	(22)
CALCIFICATION, NOS			1 (5%)	
HYPERPLASIA, NOS		1 (4%)	11 (50%)	
*PANCREATIC ISLETS	(45)	(46)	(35)	(42)
HYPERPLASIA, NOS		1 (2%)	1 (3%)	
HYPERPLASIA, FOCAL	1 (2%)			
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(48)	(48)	(43)	(47)
GALACTOCELE	1 (2%)	2 (4%)		

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

TABLE C1 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 01-0070	HIGH DOSE CONTROL (UNTR) 01-0118	LOW DOSE 01-0065	HIGH DOSE 01-0107
HYPERPLASIA, NOS	3 (6%)	4 (8%)		
*PREPUTIAL GLAND	(48)	(48)	(43)	(47)
ABSCESS, NOS			1 (2%)	
*PROSTATE	(43)	(44)	(35)	(43)
INFLAMMATION, NOS		17 (39%)		
INFLAMMATION, FOCAL	1 (2%)			
INFLAMMATION, NECROTIZING			1 (3%)	
INFLAMMATION, ACUTE	6 (14%)		2 (6%)	
INFLAMMATION, ACUTE FOCAL	8 (19%)		2 (6%)	
INFLAMMATION, ACUTE/CHRONIC	2 (5%)			
*SEMINAL VESICLE	(48)	(48)	(43)	(47)
ATROPHY, NOS	2 (4%)			
*TESTIS	(47)	(47)	(37)	(44)
MINERALIZATION		1 (2%)		
PERIARTERITIS			1 (3%)	
DEGENERATION, NOS	39 (83%)		8 (22%)	
ATROPHY, NOS		6 (13%)		
HYPERPLASIA, INTERSTITIAL CELL	1 (2%)	3 (6%)	4 (11%)	
*TESTIS/TUBULE	(47)	(47)	(37)	(44)
DEGENERATION, NOS				5 (11%)
CALCIFICATION, FOCAL				2 (5%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EYE/CORNEA	(48)	(48)	(43)	(47)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	
*EYE/RETINA	(48)	(48)	(43)	(47)
DEGENERATION, NOS			1 (2%)	
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				

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

TABLE C1 (CONCLUDED)

	LOW DOSE CONTROL (UNTR) 01-0070	HIGH DOSE CONTROL (UNTR) 01-0118	LOW DOSE 01-0065	HIGH DOSE 01-0107
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(48)	(48)	(43)	(47)
POSTMORTEM CHANGE			2 (5%)	
ADIPOSE TISSUE				
INFLAMMATION, ACUTE/CHRONIC	2			
OMENTUM				
NECROSIS, FAT		2		
SPECIAL MORPHOLOGY SUMMARY				
ANIMAL MISSING/NO NECROPSY	1			
AUTO/NECROPSY/HISTO PERF	1			1
AUTOLYSIS/NO NECROPSY	1	†	7	3

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 5-NITROACENAPHTHENE

	LOW DOSE CONTROL (UNTR) 02-0070	HIGH DOSE CONTROL (UNTR) 02-0118	LOW DOSE 02-0065	HIGH DOSE 02-0107
ANIMALS INITIALLY IN STUDY	50	50	50	50
ANIMALS NECROPSIED	49	50	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	50	48	48
INTEGUMENTARY SYSTEM				
*SKIN	(49)	(50)	(49)	(48)
EPIDERMAL INCLUSION CYST	1 (2%)			
INFLAMMATION, NOS		1 (2%)		1 (2%)
NECROSIS, NOS				1 (2%)
*SUBCUT TISSUE	(49)	(50)	(49)	(48)
MINERALIZATION		1 (2%)		
ABSCESS, NOS		1 (2%)		
RESPIRATORY SYSTEM				
#TRACHEA	(49)	(49)	(48)	(47)
INFLAMMATION, ACUTE				1 (2%)
INFLAMMATION, ACUTE/CHRONIC	15 (31%)		4 (8%)	
INFLAMMATION, CHRONIC			8 (17%)	7 (15%)
#LUNG/BRONCHUS	(49)	(50)	(48)	(48)
BRONCHIECTASIS			2 (4%)	
INFLAMMATION, NOS		3 (6%)		
INFLAMMATION, ACUTE/CHRONIC	1 (2%)			
HYPERPLASIA, PAPILLARY			1 (2%)	
#LUNG	(49)	(50)	(48)	(48)
INFLAMMATION, FOCAL	2 (4%)			
INFLAMMATION, INTERSTITIAL	2 (4%)	6 (12%)	1 (2%)	
INFLAMMATION, SUPPURATIVE				1 (2%)
PNEUMONIA, CHRONIC MURINE			1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)		
HYPERPLASIA, FOCAL				1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)			
#LUNG/ALVEOLI	(49)	(50)	(48)	(48)
SEQUESTRATION			1 (2%)	

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

TABLE C2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 02-0070	HIGH DOSE CONTROL (UNTR) 02-0118	LOW DOSE 02-0065	HIGH DOSE 02-0107
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(46)	(46)	(47)	(43)
OSTEOSCLEROSIS	1 (2%)	1 (2%)		
HYPERPLASIA, NOS			1 (2%)	
#SPLEEN	(48)	(48)	(48)	(47)
HEMOSIDEROSIS		12 (25%)		2 (4%)
ATROPHY, NOS				4 (9%)
HYPERPLASIA, HEMATOPOIETIC	1 (2%)	25 (52%)		
HYPERPLASIA, ERYTHROID		19 (40%)		
HYPERPLASIA, RETICULUM CELL	1 (2%)			
ERYTHROPOIESIS			3 (6%)	
#SPLENIC CAPSULE	(48)	(48)	(48)	(47)
HEMORRHAGIC CYST		1 (2%)		
#LYMPH NODE	(42)	(47)	(42)	(34)
PLASMACYTOSIS		1 (2%)		
HYPERPLASIA, LYMPHOID		4 (9%)		
#MANDIBULAR L. NODE	(42)	(47)	(42)	(34)
HYPERPLASIA, PLASMA CELL			2 (5%)	
#MEDIASTINAL L.NODE	(42)	(47)	(42)	(34)
HYPERPLASIA, NOS			1 (2%)	
#PANCREATIC L.NODE	(42)	(47)	(42)	(34)
HYPERPLASIA, PLASMA CELL				1 (3%)
CIRCULATORY SYSTEM				
#HEART	(49)	(50)	(48)	(48)
FIBROSIS, FOCAL	1 (2%)			
FIBROSIS, DIFFUSE	1 (2%)			
#MYOCARDIUM	(49)	(50)	(48)	(48)
INFLAMMATION, NOS		1 (2%)		
INFLAMMATION, INTERSTITIAL	2 (4%)	23 (46%)		
INFLAMMATION, ACUTE FOCAL				1 (2%)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		1 (2%)	
FIBROSIS		15 (30%)		
FIBROSIS, FOCAL	2 (4%)			
CALCIFICATION, NOS			1 (2%)	

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

TABLE C2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 02-0070	HIGH DOSE CONTROL (UNTR) 02-0118	LOW DOSE 02-0065	HIGH DOSE 02-0107
CALCIFICATION, FOCAL				1 (2%)
#ENDOCARDIUM	(49)	(50)	(48)	(48)
INFLAMMATION, NOS		1 (2%)	1 (2%)	
#CARDIAC VALVE	(49)	(50)	(48)	(48)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)			
*AORTA	(49)	(50)	(49)	(48)
MEDIAL CALCIFICATION			1 (2%)	1 (2%)
*CORONARY ARTERY	(49)	(50)	(49)	(48)
INFLAMMATION, ACUTE/CHRONIC				1 (2%)
MEDIAL CALCIFICATION			1 (2%)	
*PULMONARY ARTERY	(49)	(50)	(49)	(48)
MINERALIZATION	9 (18%)			
*MESENTERIC ARTERY	(49)	(50)	(49)	(48)
MEDIAL CALCIFICATION			1 (2%)	1 (2%)
DIGESTIVE SYSTEM				
#LIVER	(49)	(50)	(48)	(47)
INFLAMMATION, ACUTE FOCAL				1 (2%)
DEGENERATION, EOSINOPHILIC	2 (4%)			
NECROSIS, FOCAL	3 (6%)	2 (4%)	2 (4%)	1 (2%)
METAMORPHOSIS FATTY	4 (8%)	6 (12%)	1 (2%)	
HYPERPLASIA, FOCAL	29 (59%)	38 (76%)		
ANGIECTASIS	1 (2%)			
HYPERPLASIA, ERYTHROID		1 (2%)		
HYPERPLASIA, RETICULUM CELL			1 (2%)	
HEMATOPOIESIS		2 (4%)		
#LIVER/CENTRILOBULAR	(49)	(50)	(48)	(47)
CONGESTION, CHRONIC PASSIVE			1 (2%)	
#LIVER/HEPATOCYTES	(49)	(50)	(48)	(47)
NECROSIS, NOS			1 (2%)	
*BILE DUCT	(49)	(50)	(49)	(48)
INFLAMMATION, NOS		1 (2%)		
HYPERPLASIA, NOS	5 (10%)	32 (64%)		
HYPERPLASIA, FOCAL	1 (2%)	1 (2%)		
#PANCREAS	(47)	(48)	(47)	(47)
THROMBOSIS, NOS			1 (2%)	

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

TABLE C2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 02-0070	HIGH DOSE CONTROL (UNTR) 02-0118	LOW DOSE 02-0065	HIGH DOSE 02-0107
INFLAMMATION, NOS		6 (13%)		
INFLAMMATION, ACUTE/CHRONIC	4 (9%)		1 (2%)	
ATROPHY, NOS	1 (2%)		1 (2%)	
#STOMACH	(49)	(48)	(48)	(46)
INFLAMMATION, NOS		1 (2%)		
ULCER, FOCAL	1 (2%)			
CALCIFICATION, NOS			1 (2%)	1 (2%)
ACANTHOSIS		2 (4%)		
#PEYERS PATCH	(49)	(48)	(48)	(47)
HYPERPLASIA, NOS		15 (31%)		
#COLON	(44)	(46)	(48)	(45)
NEMATODIASIS	2 (5%)		4 (8%)	1 (2%)
PARASITISM		2 (4%)		
URINARY SYSTEM				
#KIDNEY	(49)	(50)	(48)	(48)
MINERALIZATION	1 (2%)			
CYST, NOS			2 (4%)	
GLOMERULONEPHRITIS, NOS		43 (86%)	2 (4%)	
INFLAMMATION, SUPPURATIVE				1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)	1 (2%)
GLOMERULONEPHRITIS, SUBACUTE			15 (31%)	9 (19%)
FIBROSIS, DIFFUSE		1 (2%)		
NEPHROSIS, NOS	34 (69%)		29 (60%)	35 (73%)
#KIDNEY/TUBULE	(49)	(50)	(48)	(48)
CALCIFICATION, NOS				1 (2%)
#KIDNEY/PELVIS	(49)	(50)	(48)	(48)
HYPERPLASIA, FOCAL			1 (2%)	
ENDOCRINE SYSTEM				
#PITUITARY	(44)	(40)	(44)	(43)
PERIVASCULITIS		1 (3%)		
HYPERPLASIA, NOS	1 (2%)			
HYPERPLASIA, FOCAL	2 (5%)	3 (8%)		
#ADRENAL	(49)	(49)	(47)	(48)
METAMORPHOSIS FATTY	3 (6%)	1 (2%)		

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

TABLE C2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 02-0070	HIGH DOSE CONTROL (UNTR) 02-0078	LOW DOSE 02-0085	HIGH DOSE 02-0107
#ADRENAL CORTEX	(49)	(49)	(47)	(48)
CYST, NOS			1 (2%)	
METAMORPHOSIS FATTY	3 (6%)		1 (2%)	1 (2%)
HYPERPLASIA, FOCAL	1 (2%)			
ANGIECTASIS			1 (2%)	
#ADRENAL MEDULLA	(49)	(49)	(47)	(48)
HYPERPLASIA, NODULAR		3 (6%)		
HYPERPLASIA, NOS	1 (2%)			
HYPERPLASIA, FOCAL	1 (2%)	3 (6%)		
#THYROID	(40)	(45)	(45)	(40)
CYSTIC FOLLICLES		1 (2%)		
HYPERPLASIA, C-CELL		1 (2%)	1 (2%)	
#PARATHYROID	(23)	(29)	(23)	(23)
HYPERPLASIA, NOS			1 (4%)	
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(49)	(50)	(49)	(48)
GALACTOCELE	9 (18%)	16 (32%)		
CYSTIC DUCTS			1 (2%)	
INFLAMMATION, ACUTE	1 (2%)			
HYPERPLASIA, NOS	23 (47%)	8 (16%)	2 (4%)	
HYPERPLASIA, FOCAL	2 (4%)			
*CLITORAL GLAND	(49)	(50)	(49)	(48)
ABSCESS, NOS			1 (2%)	
*VAGINA	(50)	(50)	(49)	(48)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)			
#UTERUS	(49)	(50)	(47)	(42)
HYDROMETRA	6 (12%)		1 (2%)	1 (2%)
HEMATOMA, NOS			1 (2%)	
HYPERPLASIA, ADENOMATOUS		1 (2%)	1 (2%)	
#CERVIX UTERI	(49)	(50)	(47)	(42)
INFLAMMATION, ACUTE/CHRONIC	2 (4%)			
HYPERPLASIA, BASAL CELL	1 (2%)			
ACANTHOSIS	1 (2%)			
#UTERUS/ENDOMETRIUM	(49)	(50)	(47)	(42)
INFLAMMATION, NOS		22 (44%)		

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

TABLE C2 (CONTINUED)

	LOW DOSE CONTROL (UNTR) 02-0070	HIGH DOSE CONTROL (UNTR) 02-0118	LOW DOSE 02-0065	HIGH DOSE 02-0107
INFLAMMATION, SUPPURATIVE			1 (2%)	1 (2%)
INFLAMMATION, ACUTE	23 (47%)		2 (4%)	
HYPERPLASIA, NOS	5 (10%)	6 (12%)		
HYPERPLASIA, CYSTIC	5 (10%)		3 (6%)	
HYPERPLASIA, ADENOMATOUS		1 (2%)		
#OVARY/OVIDUCT	(49)	(50)	(47)	(42)
INFLAMMATION, NOS		10 (20%)		
INFLAMMATION, SUPPURATIVE		2 (4%)		
INFLAMMATION, ACUTE	1 (2%)			
#OVARY	(47)	(49)	(45)	(45)
CYST, NOS	2 (4%)	8 (16%)	1 (2%)	
NERVOUS SYSTEM				
#BRAIN/MENINGES	(49)	(50)	(48)	(47)
HEMORRHAGE				1 (2%)
SPECIAL SENSE ORGANS				
*EYE	(49)	(50)	(49)	(48)
INFLAMMATION, NOS				1 (2%)
SYNECHIA, NOS	1 (2%)			
CATARACT	1 (2%)	1 (2%)		
VASCULARIZATION			1 (2%)	
*EYE/CORNEA	(49)	(50)	(49)	(48)
INFLAMMATION, ACUTE			1 (2%)	
ULCER, ACUTE				1 (2%)
INFLAMMATION, ACUTE DIFFUSE			1 (2%)	
INFLAMMATION ACUTE AND CHRONIC				1 (2%)
INFLAMMATION, CHRONIC	1 (2%)			
*EYE/RETINA	(49)	(50)	(49)	(48)
DEGENERATION, NOS	1 (2%)			
ATROPHY, NOS		1 (2%)		
*EYE/CRYSTALLINE LENS	(49)	(50)	(49)	(48)
CALCIFICATION, FOCAL				1 (2%)
*HARDERIAN GLAND	(49)	(50)	(49)	(48)
HYPERPLASIA, NOS		1 (2%)		

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

TABLE C2 (CONCLUDED)

	LOW DOSE CONTROL (UNTR) 02-0070	HIGH DOSE CONTROL (UNTR) 02-0118	LOW DOSE 02-0065	HIGH DOSE 02-0107
MUSCULOSKELETAL SYSTEM				
*STERNUM	(49)	(50)	(49)	(48)
OSTEOPETROSIS	1 (2%)			
*MUSCLE OF NECK	(49)	(50)	(49)	(48)
HEMORRHAGE				1 (2%)
BODY CAVITIES				
*MEDIASTINUM	(49)	(50)	(49)	(48)
PERIARTERITIS	1 (2%)			
*PERICARDIUM	(49)	(50)	(49)	(48)
INFLAMMATION, ACUTE/CHRONIC				1 (2%)
ALL OTHER SYSTEMS				
ADIPOSE TISSUE				
INFLAMMATION, ACUTE/CHRONIC	3			
INFLAMMATION, CHRONIC	2			
OMENTUM				
MINERALIZATION	1			
NECROSIS, FAT		1		
SPECIAL MORPHOLOGY SUMMARY				
AUTO/NECROPSY/NO HISTO			1	
AUTOLYSIS/NO NECROPSY	1		1	2

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 5-NITROACENAPHTHENE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH 5-NITROACENAPHTHENE

	CONTROL (UNTR) 05-0070	LOW DOSE 05-0064	HIGH DOSE 05-0065
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	49	45
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	47	45
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(45)
ABSCESS, NOS	2 (4%)		
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
*SUBCUT TISSUE	(50)	(49)	(45)
NECROSIS, FAT	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(50)	(46)	(44)
INFLAMMATION, NOS	1 (2%)		
PERIVASCULITIS	1 (2%)		
#LUNG	(50)	(46)	(44)
CONGESTION, NOS			2 (5%)
HEMORRHAGE	2 (4%)		
PNEUMONIA, ASPIRATION			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	2 (4%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(48)	(44)	(43)
HYPERPLASIA, HEMATOPOIETIC			1 (2%)
HYPOPLASIA, HEMATOPOIETIC			11 (26%)
#SPLEEN	(50)	(42)	(41)
ATROPHY, NOS			10 (24%)
HYPERPLASIA, LYMPHOID	1 (2%)		
#SPLENIC FOLLICLES	(50)	(42)	(41)
HYPERPLASIA, NOS	2 (4%)		
#LYMPH NODE	(45)	(31)	(30)
HYPERPLASIA, NOS			1 (3%)

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0070	LOW DOSE 05-0064	HIGH DOSE 05-0065
HYPERPLASIA, LYMPHOID			1 (3%)
#CERVICAL LYMPH NODE	(45)	(31)	(30)
HYPERPLASIA, NOS			1 (3%)
#ABDOMINAL LYMPH NODE	(45)	(31)	(30)
HYPERPLASIA, NOS			1 (3%)
#MESENTERIC L. NODE	(45)	(31)	(30)
LYMPHOID DEPLETION			1 (3%)
HYPERPLASIA, NOS			2 (7%)
HYPERPLASIA, RETICULUM CELL	1 (2%)		
CIRCULATORY SYSTEM			
#HEART	(49)	(46)	(44)
INFLAMMATION, SUPPURATIVE			1 (2%)
#AORTIC VALVE	(49)	(46)	(44)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(49)	(44)	(42)
PERIVASCULITIS	1 (2%)		
#LIVER	(50)	(46)	(44)
CONGESTION, NOS			1 (2%)
CONGESTION, PASSIVE			1 (2%)
NECROSIS, FOCAL	1 (2%)		
METAMORPHOSIS FATTY	2 (4%)	15 (33%)	16 (36%)
HEPATOCYTOMEGALY	2 (4%)		
DEPLETION	1 (2%)		
HYPERPLASIA, NODULAR	2 (4%)		
HYPERPLASIA, FOCAL	1 (2%)		
HYPERPLASIA, DIFFUSE	1 (2%)		
#LIVER/CENTRILOBULAR	(50)	(46)	(44)
NECROSIS, NOS	1 (2%)		
#LIVER/KUPFFER CELL	(50)	(46)	(44)
HYPERPLASIA, NOS	1 (2%)		
#PANCREAS	(46)	(39)	(38)
INFLAMMATION, FOCAL	1 (2%)		

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0070	LOW DOSE 05-0064	HIGH DOSE 05-0065
INFLAMMATION, CHRONIC			1 (3%)
#ESOPHAGUS	(49)	(40)	(43)
HYPERKERATOSIS			4 (9%)
#STOMACH	(49)	(43)	(43)
ULCER, ACUTE		2 (5%)	
HYPERKERATOSIS			4 (9%)
#GASTRIC MUCOSA	(49)	(43)	(43)
INFLAMMATION, FOCAL	1 (2%)		
#PEYERS PATCH	(49)	(41)	(44)
HYPERPLASIA, NOS	1 (2%)		
#COLON	(46)	(39)	(43)
GRANULOMA, NOS	1 (2%)		
HYPOPLASIA, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(49)	(46)	(44)
EMBRYONAL DUCT CYST		1 (2%)	
HYDRONEPHROSIS			2 (5%)
CONGESTION, NOS			1 (2%)
INFLAMMATION, INTERSTITIAL	3 (6%)		
FIBROSIS			5 (11%)
FIBROSIS, FOCAL			1 (2%)
NEPHROPATHY			2 (5%)
METAMORPHOSIS FATTY			5 (11%)
CALCIFICATION, NOS			1 (2%)
#KIDNEY/CORTEX	(49)	(46)	(44)
SCAR			8 (18%)
DEGENERATION, NOS			1 (2%)
#KIDNEY/MEDULLA	(49)	(46)	(44)
DEGENERATION, NOS			1 (2%)
CALCIFICATION, NOS			1 (2%)
#RENAL PAPILLA	(49)	(46)	(44)
METAMORPHOSIS FATTY		1 (2%)	
CALCIFICATION, NOS		28 (61%)	4 (9%)
#KIDNEY/TUBULE	(49)	(46)	(44)
DILATATION, NOS			4 (9%)

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0070	LOW DOSE 05-0064	HIGH DOSE 05-0065
FIBROSIS			3 (7%)
DEGENERATION, NOS			21 (48%)
CALCIFICATION, NOS		2 (4%)	
#KIDNEY/PELVIS	(49)	(46)	(44)
DILATATION, NOS			1 (2%)
HYPERPLASIA, EPITHELIAL			2 (5%)
#URINARY BLADDER	(47)	(44)	(40)
INFLAMMATION, ACUTE		1 (2%)	
HYPERPLASIA, EPITHELIAL	1 (2%)		
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#TESTIS	(50)	(45)	(44)
ATROPHY, NOS			2 (5%)
ASPERMATOGENESIS			1 (2%)
#TESTIS/TUBULE	(50)	(45)	(44)
DEGENERATION, NOS		1 (2%)	
MULTINUCLEATE GIANT-CELL			4 (9%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(50)	(43)	(43)
HEMORRHAGE		1 (2%)	
#CEREBRUM	(50)	(43)	(43)
HEMORRHAGE		1 (2%)	
#CEREBELLUM	(50)	(43)	(43)
HEMORRHAGE		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 05-0070	LOW DOSE 05-0064	HIGH DOSE 05-0065
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	12	7	2
AUTO/NECROPSY/NO HISTO		2	
AUTOLYSIS/NO NECROPSY		1	5

* 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 5-NITROACENAPHTHENE

	CONTROL (UNTR) 06-0070	LOW DOSE 06-0064	HIGH DOSE 06-0065
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	1
ANIMALS NECROPSIED	48	47	46
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	47	47	46
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(46)	(47)	(46)
INFLAMMATION, NOS	1 (2%)		
#LUNG	(46)	(47)	(46)
INFLAMMATION, INTERSTITIAL	1 (2%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(46)	(46)	(44)
MYELOFIBROSIS	1 (2%)		
#SPLEEN	(47)	(44)	(45)
HYPERPLASIA, PLASMA CELL		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (2%)		
ERYTHROPOIESIS		1 (2%)	
#SPLENIC FOLLICLES	(47)	(44)	(45)
HYPERPLASIA, NOS	3 (6%)		
#LYMPH NODE	(36)	(38)	(35)
INFLAMMATION, NOS	1 (3%)		
HYPERPLASIA, NOS	1 (3%)		
HYPERPLASIA, PLASMA CELL	1 (3%)	1 (3%)	
#ABDOMINAL LYMPH NODE	(36)	(38)	(35)
PLASMACYTOSIS	1 (3%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(44)	(45)	(46)
FIBROSIS, FOCAL	1 (2%)		

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0070	LOW DOSE 06-0064	HIGH DOSE 06-0065
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(45)	(46)	(45)
PERIVASCULITIS	3 (7%)		
PERIVASCULAR CUFFING	1 (2%)		
#LIVER	(47)	(47)	(45)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
NECROSIS, FOCAL	2 (4%)		
METAMORPHOSIS FATTY		1 (2%)	24 (53%)
HYPERPLASIA, NODULAR		1 (2%)	
HYPERPLASTIC NODULE		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	
#BILE DUCT	(48)	(47)	(46)
INFLAMMATION, ACUTE/CHRONIC	4 (8%)		
#PANCREAS	(43)	(42)	(37)
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, INTERSTITIAL	1 (2%)		
PERIARTERITIS	1 (2%)	1 (2%)	
#PANCREATIC ACINUS	(43)	(42)	(37)
ATROPHY, NOS	1 (2%)		
#STOMACH	(45)	(44)	(43)
ULCER, FOCAL	1 (2%)		
#PEYERS PATCH	(45)	(42)	(39)
HYPERPLASIA, NOS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(45)	(46)	(45)
GLOMERULONEPHRITIS, NOS	3 (7%)		
GLOMERULONEPHRITIS, FOCAL	2 (4%)		
INFLAMMATION, INTERSTITIAL	1 (2%)	4 (9%)	
GLOMERULONEPHRITIS, MEMBRANOUS	2 (4%)		
PYELONGPHRITIS, ACUTE/CHRONIC	1 (2%)		
GLOMERULONEPHRITIS, CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC FOCAL			1 (2%)
PERIARTERITIS		1 (2%)	
#KIDNEY/CORTEX	(45)	(46)	(45)
DILATATION, NOS			1 (2%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0070	LOW DOSE 06-0064	HIGH DOSE 06-0065
INFLAMMATION, INTERSTITIAL		1 (2%)	
SCAR		4 (9%)	
#RENAL PAPILLA	(45)	(46)	(45)
CALCIFICATION, NOS			23 (51%)
#KIDNEY/TUBULE	(45)	(46)	(45)
CALCIFICATION, NOS		1 (2%)	6 (13%)
#URINARY BLADDER	(45)	(43)	(40)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		1 (3%)
PERIARTERITIS	1 (2%)		
HYPERPLASIA, EPITHELIAL			1 (3%)
ENDOCRINE SYSTEM			
#ADRENAL	(47)	(43)	(40)
HYPERPLASIA, NODULAR		1 (2%)	
#ADRENAL CORTEX	(47)	(43)	(40)
HYPERPLASIA, NODULAR		12 (28%)	13 (33%)
HYPERPLASIA, NOS		3 (7%)	
#THYROID	(41)	(43)	(40)
HYPERPLASIA, FOLLICULAR-CELL	1 (2%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(43)	(39)	(42)
HYDROMETRA	3 (7%)		
ABSCESS, NOS	2 (5%)		
#UTERUS/ENDOMETRIUM	(43)	(39)	(42)
INFLAMMATION, NOS	2 (5%)		
INFLAMMATION, SUPPURATIVE	2 (5%)		
INFLAMMATION, ACUTE	6 (14%)		
INFLAMMATION, ACUTE FOCAL	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC	3 (7%)		
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, CYSTIC	20 (47%)	21 (54%)	14 (33%)
METAPLASIA, SQUAMOUS	1 (2%)		
#OVARY/OVIDUCT	(43)	(39)	(42)
INFLAMMATION, SUPPURATIVE	4 (9%)		

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0070	LOW DOSE 06-0064	HIGH DOSE 06-0065
ABSCESS, NOS	1 (2%)		
HYPERPLASIA, ADENOMATOUS			2 (5%)
#OVARY	(45)	(41)	(39)
CYST, NOS		4 (10%)	4 (10%)
HEMORRHAGE		1 (2%)	
HEMORRHAGIC CYST			1 (3%)
INFLAMMATION, SUPPURATIVE	6 (13%)		
INFLAMMATION, CHRONIC	1 (2%)		
ABSCESS, CHRONIC	1 (2%)		
FIBROSIS		1 (2%)	
PERIARTERITIS	1 (2%)		
CALCIFICATION, NOS		1 (2%)	
HYPERPLASIA, CYSTIC		1 (2%)	
METAPLASIA, OSSEOUS			1 (3%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(46)	(45)	(43)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(48)	(47)	(46)
ABSCESS, NOS		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(48)	(47)	(46)
PERIVASCULITIS	1 (2%)		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		2	

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

TABLE D2 (CONCLUDED)

	CONTROL(UNTR) 06-0070	LOW DOSE 06-0064	HIGH DOSE 06-0065
ANIMAL MISSING/NO NECROPSY		1	1
AUTO/NECROPSY/HISTO PERF		2	
AUTO/NECROPSY/NO HISTO	1		
AUTOLYSIS/NO NECROPSY	2	2	3

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

Review of the Bioassay of 5-Nitroacenaphthene* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

June 29, 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 5-Nitroacenaphthene for carcinogenicity.

The reviewer agreed with the conclusion in the report that 5-Nitroacenaphthene was carcinogenic, under the conditions of test, in treated female mice and both sexes of rats. He said that poor survival among treated male mice precluded an analysis of this group. After a brief description of the experimental design, the reviewer commented on the various tumors induced by the test material. He opined that the study was adequate to support the conclusion on the carcinogenicity of 5-Nitroacenaphthene. The reviewer moved that the report on the bioassay of 5-Nitroacenaphthene be accepted as written. The motion was approved without objection.

Clearinghouse Members present:

Arnold L. Brown (Chairman), Mayo Clinic
Paul Nettesheim, National Institute of Environmental Health Sciences
Verne Ray, Pfizer Medical Research Laboratory
Verald K. Rowe, Dow Chemical U.S.A.
Michael B. Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center

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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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