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NITROFEN

FOR POSSIBLE CARCINOGENICITY

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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 NITROFEN
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 nitrofen 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 nitrofen was conducted by Litton Bionetics, Inc., Kensington, Maryland, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. N. P. Page (1,2), Dr. E. K. Weisburger (1) and Dr. J. H. Weisburger (1,3). The principal investigators for the contract were Dr. F. M. Garner (4) and Dr. B. M. Ulland (4,5). Mr. S. Johnson (4) was the coprincipal investigator for the contract. Animal treatment and observation were supervised by Mr. R. Cypher (4), Mr. D. S. Howard (4) and Mr. H. D. Thornett (4); Mr. H. Paulin (4) analyzed dosed feed mixtures. Ms. J. Blalock (4) was responsible for data collection and assembly. Chemical analysis was performed by Midwest Research Institute (6) and the analytical results were reviewed by Dr. N. Zimmerman (7).

Histopathologic examinations were performed at Litton Bionetics, Inc. (4). The slides for rats were reviewed at Experimental Pathology Laboratories, Inc. (8). The rat pathology narrative was written by Dr. J. F. Hardisty (8), the mouse pathology narrative was written by Dr. P. K. Hildebrandt (4), and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (9).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (10); the statistical analysis was performed by Mr. R. M. Helfand (7) and Dr. J. P. Dirkse, III (11), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (12).

This report was prepared at METREK, a Division of The MITRE Corporation (7) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (7), task leader Ms. P. Walker (7), senior biologist Mr. M. Morse (7), biochemist Mr. S. C. Drill (7), and technical editor Ms. P. A. Miller (7). The final report was reviewed by members of the participating organizations.

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

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SUMMARY

A bioassay for the possible carcinogenicity of nitrofen was conducted using Fischer 344 rats and B6C3F1 mice. Nitrofen was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of nitrofen were 6000 and 3000 ppm for both species. The compound was administered to rats and mice for 78 weeks, followed by a period of no compound administration of 26 weeks for rats and 13 weeks for mice.

There were no significant positive associations between the concentrations of nitrofen administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Dose-related mean body weight depression, relative to controls, was observed for males and females of both species, indicating that the concentrations of nitrofen administered to the animals in this bioassay may have approximated the maximum tolerated concentrations.

None of the statistical tests for any site in rats of either sex indicated a significant positive association between compound administration and tumor incidence. There was a significant positive association between the concentrations of nitrofen administered and the incidences of hepatocellular carcinomas in mice of both sexes.

In another bioassay of nitrofen for possible carcinogenicity, the compound was found to induce hepatocellular carcinomas in B6C3F1 mice of both sexes and hemangiosarcomas of the liver in male B6C3F1 mice. In addition, adenocarcinomas of the pancreas were induced in female Osborne-Mendel rats (U.S. Department of Health, Education, and Welfare, 1978).

Under the conditions of this bioassay, dietary administration of nitrofen was carcinogenic to B6C3F1 mice, causing hepatocellular carcinomas in both sexes. There was no evidence for carcinogenicity in Fischer 344 rats.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF TABLES (Concluded)

I. INTRODUCTION

Nitrofen (Figure 1) (NCI No. C00420), a substituted diphenyl ether, is one of several agricultural pesticides selected for bioassay by the National Cancer Institute because of a lack of carcinogenicity data.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 2,4-dichloro-1-(4-nitrophenoxy)-benzene.* It is also known as 2,4-dichlorophenyl-p-nitrophenyl ether, nitrophene, Tok E-25, and Nip.

Nitrofen is a selective contact herbicide used for pre- and post-emergence control of annual grasses and broadleaf weeds on a variety of food crops (Weed Science Society of America, 1974). Postemergence treatment is restricted to certain highly tolerant crops and involves spraying the crops with 4 to 6 pounds of active ingredient per acre in a water carrier. For preemergence treatment, the spray is applied at a similar rate directly to the soil (Weed Science Society of America, 1974).

Although specific production figures are not available, the listing of nitrofen in the 1975 Directory of Chemical Producers, U.S.A. (Stanford Research Institute, 1975) implies an annual commercial production in excess of 1000 pounds or $1000 in value.

Occupational exposure to nitrofen, primarily through inhalation and dermal contact, may occur among workers at pesticide production

*The CAS registry number is 1836-75-5.

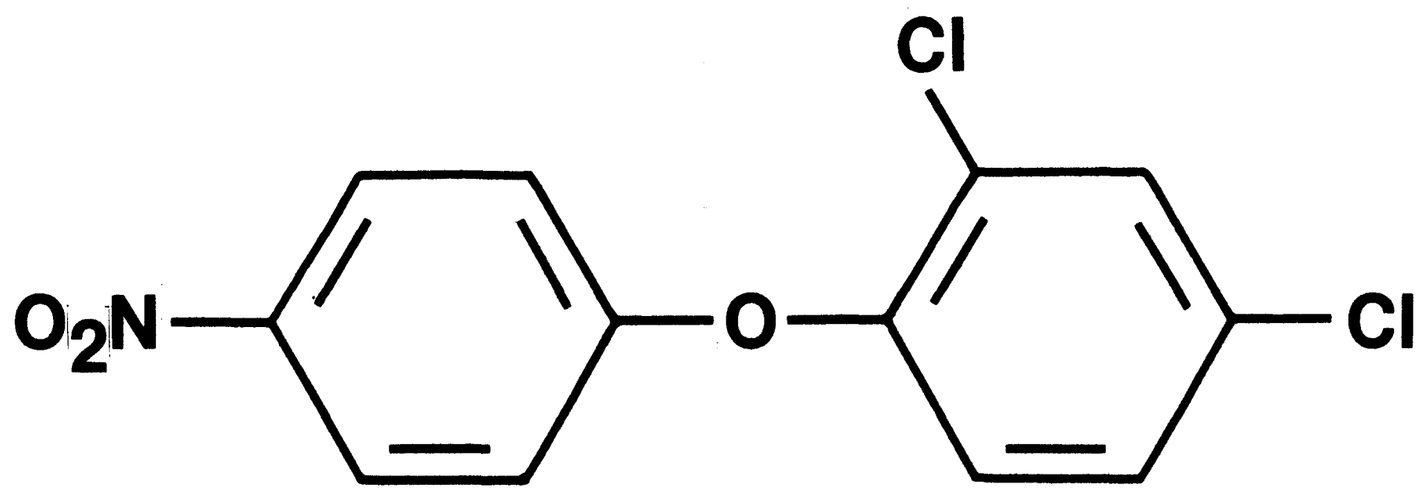

FIGURE 1
CHEMICAL STRUCTURE OF NITROFEN

facilities and among agricultural workers engaged in treatment of crops with the chemical. The route of potential exposure for the general population, however, is ingestion due to possible residual nitrofen on food crops.

Adverse effects noted in agricultural workers following excessive exposure to nitrofen over prolonged periods of time include reduction in hemoglobin and leukocyte counts, inhibition of serum cholinesterase and abnormalities in erythrocyte catalase and serum transaminase levels (Doroshenko, 1975). In addition, dermal contact with the concentrated emulsion (Tok E-25) may cause skin irritation (Weed Science Society of America, 1974).

II. MATERIALS AND METHODS

A. Chemicals

Technical-grade nitrofen was purchased from Rohm and Haas Chemical Company, Philadelphia, Pennsylvania. Chemical analysis was performed by Litton Bionetics, Inc., Kensington, Maryland. The experimentally determined melting point range was 53° to 62°C, as compared to that reported for the pure compound, 71° to 72°C. Ultraviolet/visible analysis revealed λ_{max} of 295 nm with a molar extinction coefficient of 9.1×10^3. Only one spot was revealed by thin-layer chromatography, performed utilizing two solvent systems and visualized with iodine vapor, silver nitrate, and 2-phenoxyethanol.

Throughout this report, the term nitrofen is used to represent this technical-grade material.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® meal (Allied Mills, Inc., Chicago, Illinois). Nitrofen was administered to the dosed animals as a component of the diet.

The chemical was removed from its container and a proper amount was blended with an aliquot of the feed using a mortar and pestle. Once visual homogeneity was attained, the mixture was placed in a 6 kg capacity Patterson-Kelley standard model twin-shell stainless

steel V-blender along with the remainder of the feed to be prepared. After 20 minutes of blending, the mixtures were placed in double plastic bags and stored in the dark at 4°C. The mixture was prepared once weekly.

Dosed feed preparations containing 600 and 300 ppm of nitrofen were analyzed spectrophotometrically. The mean result immediately after preparation was 95 percent of theoretical (ranging from 92 to 102 percent). After 9 days at ambient room temperature, the mean result was 89 percent of theoretical (ranging from 85 to 94 percent).

C. Animals

The two animal species, Fischer 344 rats and B6C3F1 mice, used in the carcinogenicity bioassay were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Rats were supplied by A. R. Schmidt, Madison, Wisconsin, and Laboratory Supply Company, Inc., Indianapolis, Indiana. Mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

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

D. Animal Maintenance

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

Rats were housed four per cage by sex and mice were housed five per cage by sex. Throughout the study dosed and control animals of both species were housed in polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) suspended from aluminum racks. Racks were fitted with a continuous piece of stainless steel mesh over which a sheet of filter paper was firmly secured. Filter paper was changed at 2-week intervals, when the racks were sanitized. Clean cages and bedding (Ab-sorb-dri® hardwood chip bedding [Wilner Wood Products Company, Norway, Maine]) were provided twice weekly.

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

All dosed and control rats were housed in a room with other rats receiving diets containing* 3-chloro-p-toluidine (95-74-9); 5-chloro-o-toluidine (95-74-4); 2-nitro-p-phenylenediamine (5307-14-2); amitrole (61-82-5); 1-phenyl-2-thiourea (103-85-5); dibutyltin diacetate (1067-33-0); copper acetate (4180-12-5); and zinc acetate (557-34-6).

All dosed and control mice were housed in a room with mice receiving diets containing amitrole (61-82-5); acetylaminofluorene (53-96-3); nitrilotriacetic acid (139-13-9); and p-nitrosodiphenylamine (156-10-5); and other mice intubated with styrene (100-42-5) and β-nitrostyrene (102-96-5).

E. Selection of Initial Concentrations

To establish the concentrations of nitrofen for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among six groups, each consisting of five males and five females. Nitrofen was incorporated into the basal laboratory diet and supplied ad libitum to five of the six rat groups in concentrations of 6800, 10,000, 14,670, 21,560 and 31,530 ppm and to five of the six mouse groups in concentrations of 1180, 2550, 5500, 13,900 and 25,520 ppm. The 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

*CAS registry numbers are given in parentheses.

animals were fed the basal laboratory diet. Individual body weights and food consumption data were recorded twice weekly throughout the study. Upon termination of the study all survivors were euthanized and necropsied.

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

RAT SUBCHRONIC STUDY RESULTS

ppm	Mean Body Weight Gain (%)*		Survival**		Observation of Arched Backs**	
	Males	Females	Males	Females	Males	Females
31,530	-58	-47	1/5	1/5	4/5	4/5
21,560	-52	-43	5/5	5/5	0/5	0/5
14,670	-36	-34	5/5	5/5	0/5	0/5
10,000	-17	-16	5/5	5/5	0/5	0/5
6,800	-13	-24	5/5	5/5	0/5	0/5
0	--	--	5/5	5/5	0/5	0/5

The high concentration selected for administration to dosed rats in the chronic bioassay was 6000 ppm.

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

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*- is indicative of mean body weight gain less than that of controls.

**Number of animals observed/number of animals originally in group.

MOUSE SUBCHRONIC STUDY RESULTS

ppm	Mean Body Weight Gain (%)[a] Males	Females	Survival[b] Males	Females	Observation of Abnormal Clinical Signs[b] Males	Females
25,520	+ 1	+ 3	2/5	3/5	5/5[c]	5/5[c,d]
13,900	- 4	- 2	5/5	5/5	5/5[c]	5/5[c]
5,500	- 4	+ 8	5/5	5/5	0/5	0/5
2,550	- 5	- 1	5/5	5/5	0/5	0/5
1,180	+ 5	+ 7	5/5	5/5	0/5	0/5
0	--	--	5/5	5/5	0/5	0/5

The high concentration selected for administration to dosed mice in the chronic bioassay was 6000 ppm.

F. Experimental Design

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

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed rats were supplied with diets containing 6000 and 3000 ppm nitrofen for 78 weeks followed by a 26-week observation period, when no test chemicals were used. Throughout this report those rats receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups.

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[a] + is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.

[b] Number of animals observed/number of animals originally in group.

[c] These mice had rough hair and arched backs.

[d] These mice also had mottled kidneys.

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
NITROFEN FEEDING EXPERIMENT

	INITIAL GROUP SIZE	NITROFEN CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	104
LOW DOSE	50	3000	78	
		0		26
HIGH DOSE	50	6000	78	
		0		26
FEMALE				
CONTROL	20	0	0	104
LOW DOSE	50	3000	78	
		0		26
HIGH DOSE	50	6000	78	
		0		26

[a]Concentrations given in parts per million.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
NITROFEN FEEDING EXPERIMENT

	INITIAL GROUP SIZE	NITROFEN CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	91
LOW DOSE	50	3000	78	
		0		13
HIGH DOSE	50	6000	78	
		0		13
FEMALE				
CONTROL	20	0	0	91
LOW DOSE	50	3000	78	
		0		13
HIGH DOSE	50	6000	78	
		0		13

[a]Concentrations given in parts per million.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed mice were supplied with diets containing 6000 and 3000 ppm nitrofen for 78 weeks followed by a 13-week observation period, when no test chemicals were used. Throughout this report those mice receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups.

G. Clinical and Histopathologic Examinations

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

All moribund animals, animals that developed large, palpable masses that jeopardized their health, or animals that survived until the end of the bioassay were euthanized using carbon dioxide inhalation. Necropsies were immediately performed on these animals and on all animals found dead during the bioassay. Gross and microscopic examinations were performed on all major tissues, organs, and gross lesions taken from killed animals and, whenever possible, from animals found dead.

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

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

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

H. Data Recording and Statistical Analyses

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

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

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

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of

tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

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

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

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

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

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

Dose-related mean body weight depression was apparent in male rats until week 75 and in female rats throughout the bioassay (Figure 2).

No other clinical signs were recorded.

B. Survival

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

There were adequate numbers of male rats at risk from late-developing tumors as 90 percent (45/50) of the high dose, 84 percent (42/50) of the low dose, and 85 percent (17/20) of the controls survived on test until the termination of the study.

There were adequate numbers of female rats at risk from late-developing tumors, as 76 percent (38/50) of the high dose, 84 percent (42/50) of the low dose, and 85 percent (17/20) of the controls survived on test until the termination of the study.

C. Pathology

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

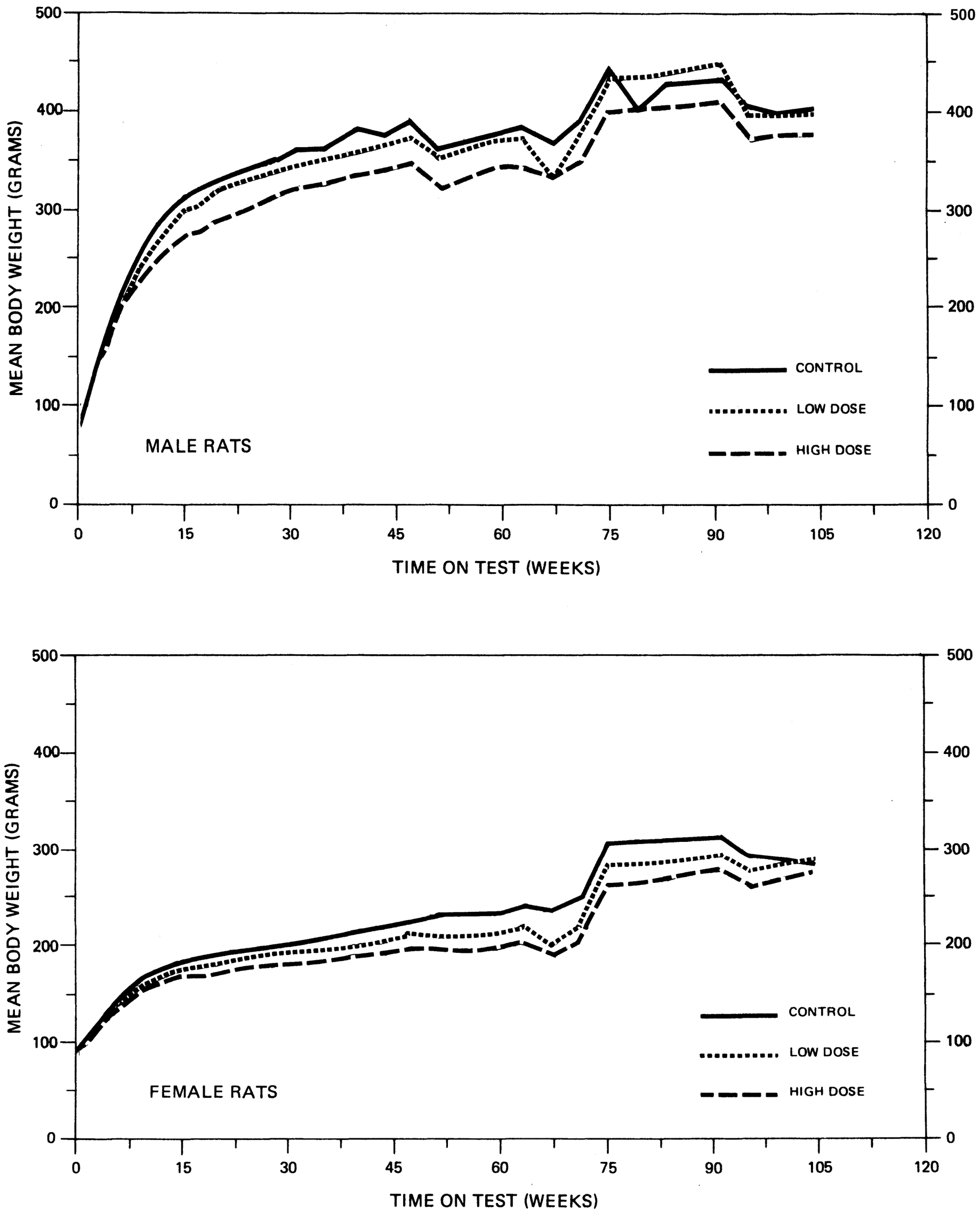

FIGURE 2
GROWTH CURVES FOR NITROFEN CHRONIC STUDY RATS

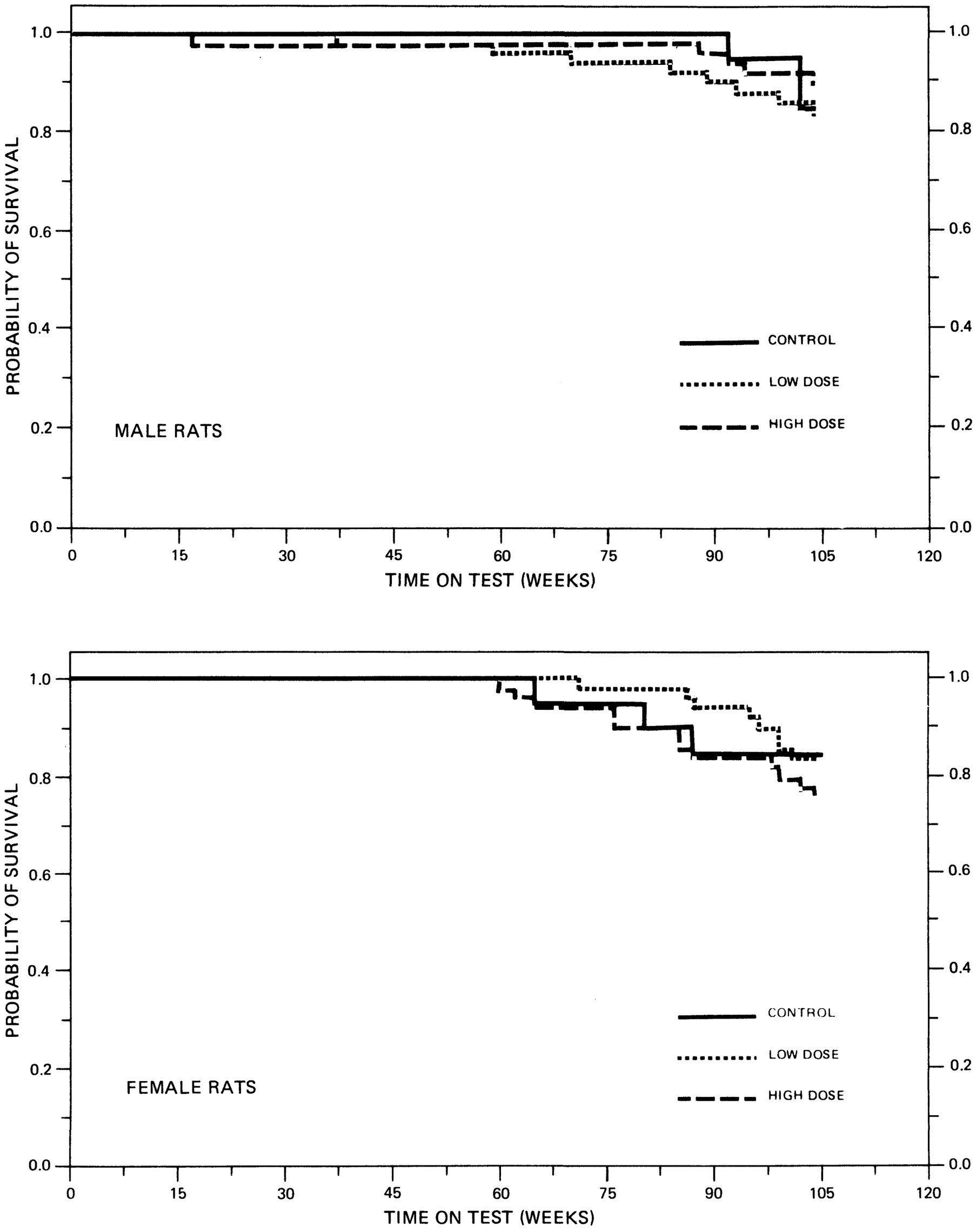

FIGURE 3
SURVIVAL COMPARISONS OF NITROFEN CHRONIC STUDY RATS

A variety of neoplasms was seen with approximately equal frequency in the control and dosed rats. A few types of neoplasms occurred only, or with increased frequency, in rats of dosed groups as compared with control groups. The nature and low incidence of these neoplasms are similar to those commonly observed in untreated aged male and/or female rats of this strain.

A variety of inflammatory, degenerative and proliferative lesions commonly seen in aged Fischer 344 rats were observed in dosed and control animals. None of these lesions appeared to be related to exposure to the compound.

Based on the results of this pathology examination, the administration of nitrofen was not carcinogenic to Fischer 344 male and female rats under the conditions of this bioassay.

D. Statistical Analyses of Results

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

None of the statistical tests at any site in rats of either sex indicated a significant positive association between the administration of nitrofen and an increased incidence of tumors.

TABLE 3

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

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	1/20(0.05)	3/50(0.06)	0/50(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.200	0.000
Lower Limit	---	0.106	0.000
Upper Limit	---	61.724	7.475
Weeks to First Observed Tumor	102	93	---
Pituitary: Chromophobe Adenoma[b]	1/18(0.06)	3/47(0.06)	2/45(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.149	0.800
Lower Limit	---	0.102	0.045
Upper Limit	---	59.033	46.162
Weeks to First Observed Tumor	104	104	104
Adrenal: Pheochromocytoma[b]	2/19(0.11)	3/49(0.06)	1/47(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.582	0.202
Lower Limit	---	0.074	0.004
Upper Limit	---	6.640	3.710
Weeks to First Observed Tumor	102	104	104

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Carcinoma[b]	2/19(0.11)	3/47(0.06)	2/48(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.606	0.396
Lower Limit	---	0.077	0.031
Upper Limit	---	6.913	5.211
Weeks to First Observed Tumor	104	104	104
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	3/19(0.16)	6/47(0.13)	6/48(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.809	0.792
Lower Limit	---	0.199	0.195
Upper Limit	---	4.644	4.552
Weeks to First Observed Tumor	104	104	104
Pancreatic Islets: Islet-Cell Adenoma[b]	2/20(0.10)	2/50(0.04)	0/48(0.00)
P Values[c]	P = 0.042(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.400	0.000
Lower Limit	---	0.032	0.000
Upper Limit	---	5.277	1.400
Weeks to First Observed Tumor	104	104	---

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Testis: Interstitial-Cell Tumor[b]	19/20(0.95)	48/49(0.98)	49/50(0.98)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.031	1.032
Lower Limit	---	0.961	0.962
Upper Limit	---	1.109	1.109
Weeks to First Observed Tumor	92	70	88

[a]Treated groups received doses of 3000 or 6000 ppm in feed.

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

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

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

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH NITROFEN[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	2/20(0.10)	2/50(0.04)	0/49(0.00)
P Values[c]	P = 0.041(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.400	0.000
Lower Limit	---	0.032	0.000
Upper Limit	---	5.277	1.372
Weeks to First Observed Tumor	104	87	---
Pituitary: Chromophobe Adenoma[b]	11/16(0.69)	8/48(0.17)	4/45(0.09)
P Values[c]	P < 0.001(N)	P < 0.001(N)	P < 0.001(N)
Departure from Linear Trend[e]	P = 0.008	---	---
Relative Risk (Control)[d]	---	0.242	0.129
Lower Limit	---	0.127	0.045
Upper Limit	---	0.542	0.362
Weeks to First Observed Tumor	80	99	76
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	2/17(0.12)	2/46(0.04)	5/42(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.370	1.012
Lower Limit	---	0.030	0.191
Upper Limit	---	4.848	10.027
Weeks to First Observed Tumor	104	104	104

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma[b]	4/20(0.20)	4/50(0.08)	0/49(0.00)
P Values[c]	P = 0.003(N)	N.S.	P = 0.006(N)
Relative Risk (Control)[d]	---	0.400	0.000
Lower Limit	---	0.085	0.000
Upper Limit	---	1.984	0.435
Weeks to First Observed Tumor	104	104	---
Mammary Gland: Fibroadenoma or Adenocarcinoma[b]	5/20(0.25)	4/50(0.08)	0/49(0.00)
P Values[c]	P = 0.001(N)	N.S.	P = 0.001(N)
Relative Risk (Control)[d]	---	0.320	0.000
Lower Limit	---	0.073	0.000
Upper Limit	---	1.358	0.319
Weeks to First Observed Tumor	104	104	---

[a]Treated groups received doses of 3000 or 6000 ppm in feed.

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

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

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

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

In female rats several significant negative associations were observed. The Cochran-Armitage test indicated significant negative associations between compound administration and the incidence of leukemia or malignant lymphomas, chromophobe adenomas of the pituitary, fibroadenomas, and the combination of fibroadenomas or adenocarcinomas NOS of the mammary gland. At these mammary gland sites the high dose to control Fisher exact tests were also significant. Both Fisher exact tests were significant for pituitary chromophobe adenomas, as was the test for departure from linear trend.

Thus, there was no evidence to indicate that nitrofen was a carcinogen in Fischer 344 rats under the conditions of this bioassay.

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

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

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

No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the control and nitrofen-dosed groups are shown in Figure 5. The Tarone test for association between dosage and mortality was not significant for either male or female mice.

There were adequate numbers of male mice at risk from late-developing tumors, as 80 percent (40/50) of the high dose, 96 percent (48/50) of the low dose and 95 percent (19/20) of the controls survived on test until termination of the study. Two high dose males were missing, one in week 7 and one in week 46.

There were adequate numbers of female mice at risk from late-developing tumors, as 96 percent (48/50) of the high dose, 86 percent (43/50) of the low dose and 60 percent (12/20) of the controls survived on test until the termination of the study. One low dose female was missing in week 61.

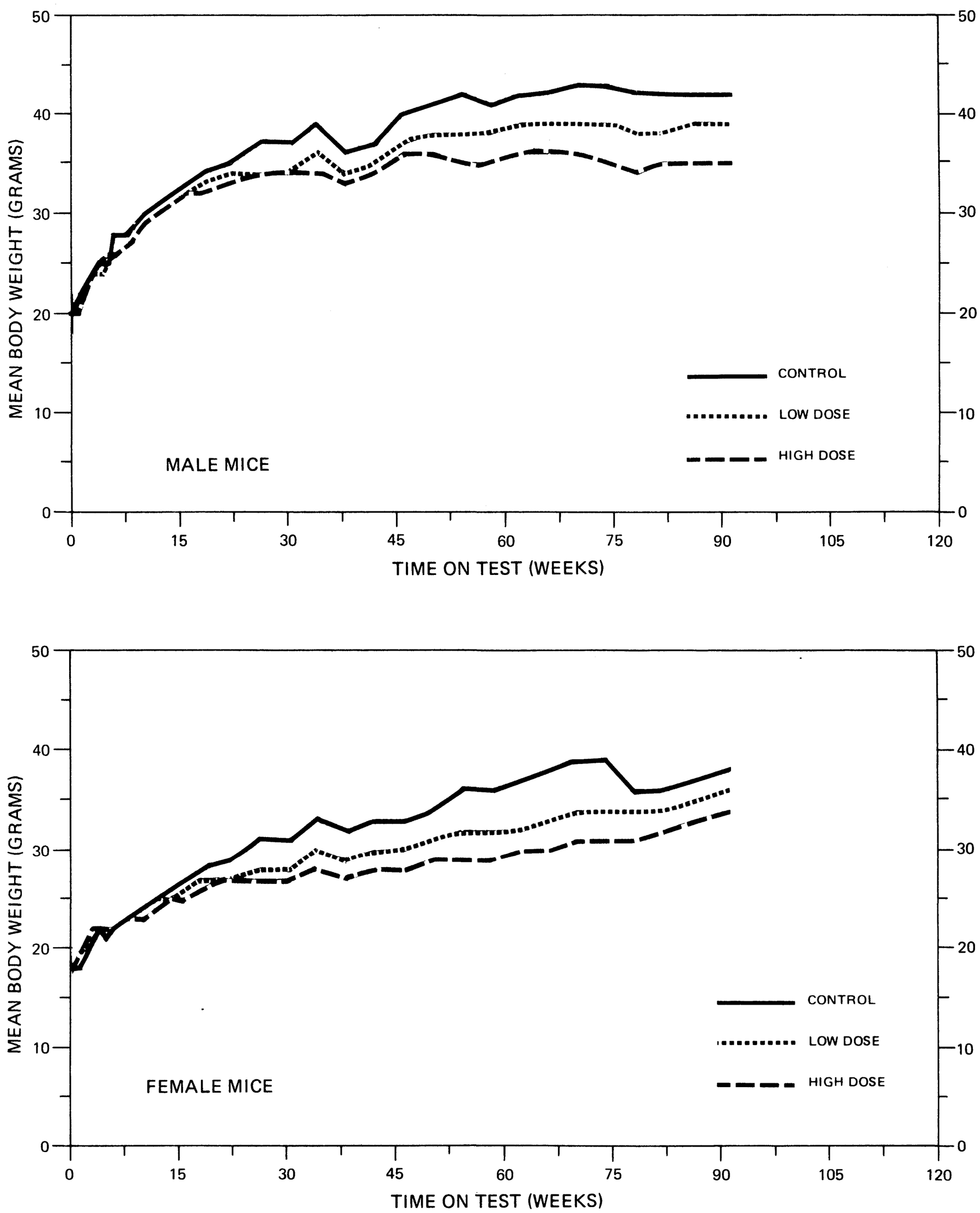

FIGURE 4
GROWTH CURVES FOR NITROFEN CHRONIC STUDY MICE

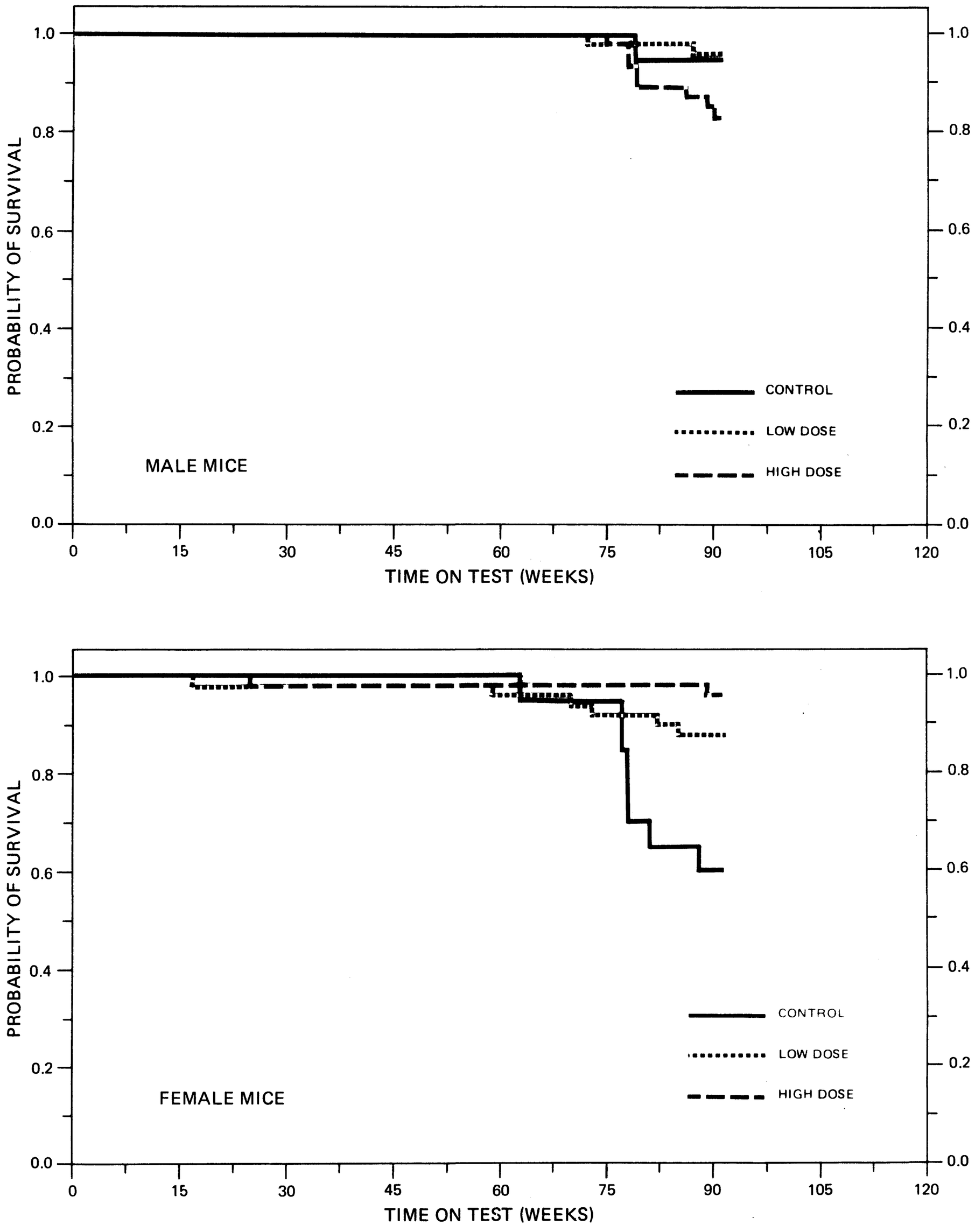

FIGURE 5
SURVIVAL COMPARISONS OF NITROFEN CHRONIC STUDY MICE

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).

There was a variety of neoplasms in control and dosed mice. Except for those of the liver, they seemed to be unrelated to chemical administration.

The incidences of liver neoplasms and hyperplasias are given below.

	MALES			FEMALES		
	CONTROL	LOW DOSE	HIGH DOSE	CONTROL	LOW DOSE	HIGH DOSE
NO. OF LIVERS EXAMINED	(20)	(49)	(48)	(18)	(48)	(50)
Hyperplasia, Focal	0	9(18%)	4(8%)	0	11(23%)	11(22%)
Hepatocellular Adenoma*	1(5%)	18(37%)	20(42%)	0	9(19%)	17(34%)
Hepatocellular Carcinoma	0	13(27%)	20(42%)	0	5(10%)	13(26%)
Bile Duct Carcinoma (Hepatoblastoma)	0	3(6%)	4(8%)	0	1(2%)	0

Livers diagnosed as hyperplasia contained solitary and occasionally multiple foci in which hepatocytes were larger, with enlarged nuclei. The cytoplasm was abundant and often vacuolated.

*These lesions were recorded only in the absence of malignant neoplasms.

Hepatocellular adenomas were expansile lesions with hepatocytes of uniform size and shape. The cells formed solid patterns. Hepatocellular carcinomas had cells of similar morphology to those in adenomas and also had areas of prominent trabecular formations where hepatocytes formed in cords several cells thick. Only one carcinoma metastasized to the lung.

The bile duct carcinomas (hepatoblastomas) consisted of small, elongated cells with scanty cytoplasm and very dark nuclei. These cells were arranged in sheets and clusters, and occasionally lined up around blood vessels.

In addition to the neoplastic lesions, a large number of degenerative, proliferative, and inflammatory changes were encountered in the dosed and control groups. There was no apparent association between the administration of nitrofen and the incidences of these nonneoplastic lesions.

The pathology examination provided evidence that nitrofen was carcinogenic in B6C3F1 mice, inducing liver tumors under the conditions of this bioassay.

D. Statistical Analyses of Results

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

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH NITROFEN[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	3/18(0.17)	0/49(0.00)	3/46(0.07)
P Values[c]	N.S.	P = 0.017(N)	N.S.
Departure from Linear Trend[e]	P = 0.010	---	---
Relative Risk (Control)[d]	---	0.000	0.391
Lower Limit	---	0.000	0.059
Upper Limit	---	0.604	2.723
Weeks to First Observed Tumor	91	---	91
Hematopoietic System: Malignant Lymphoma[b]	3/20(0.15)	0/49(0.00)	1/48(0.02)
P Values[c]	P = 0.043(N)	P = 0.022(N)	N.S.
Departure from Linear Trend[e]	P = 0.016	---	---
Relative Risk (Control)[d]	---	0.000	0.139
Lower Limit	---	0.000	0.003
Upper Limit	---	0.673	1.631
Weeks to First Observed Tumor	79	---	78
Liver: Bile Duct Carcinoma[b]	0/20(0.00)	3/49(0.06)	4/48(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.255	0.402
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	78

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	0/20(0.00)	13/49(0.27)	20/48(0.42)
P Values[c]	P = 0.001	P = 0.007	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	1.709	2.808
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	75
Liver: Hepatocellular Carcinoma or Heptocellular Adenoma[b]	1/20(0.05)	31/49(0.63)	40/48(0.83)
P Values[c]	P < 0.001	P = 0.001	P < 0.001
Departure from Linear Trend[e]	P = 0.037	---	---
Relative Risk (Control)[d]	---	12.653	16.667
Lower Limit	---	2.444	3.388
Upper Limit	---	487.770	592.272
Weeks to First Observed Tumor	91	91	75

[a]Treated groups received doses of 3000 or 6000 ppm in feed.

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

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

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

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

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH NITROFEN[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	0/20(0.00)	2/48(0.04)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.128	0.255
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	91
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	2/20(0.10)	3/49(0.06)	6/50(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.612	1.200
Lower Limit	---	0.078	0.243
Upper Limit	---	6.996	11.574
Weeks to First Observed Tumor	81	73	89
Liver: Hepatocellular Carcinoma[b]	0/18(0.00)	5/48(0.10)	13/50(0.26)
P Values[c]	P = 0.004	N.S.	P = 0.011
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.496	1.518
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	91

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	0/18(0.00)	14/48(0.29)	30/50(0.60)
P Values[c]	P < 0.001	P = 0.006	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	1.720	3.784
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	91
Pituitary: Chromophobe Adenoma[b]	0/10(0.00)	3/33(0.09)	0/35(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.203	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	91	---

[a]Treated groups received doses of 3000 or 6000 ppm in feed.

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

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

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

In male mice the Cochran-Armitage test indicated a significant ($P = 0.001$) positive association between dose and the incidence of hepatocellular carcinomas. This was supported by a significant ($P < 0.001$) positive Fisher exact high dose to control comparison and also by a significant ($P = 0.007$) positive Fisher exact low dose to control comparison. The Cochran-Armitage test further indicated a significant ($P < 0.001$) positive association between dose and the combined incidence of hepatocellular carcinomas or hepatocellular adenomas. This was again supported by Fisher exact tests, yielding a significant ($P < 0.001$) positive high dose to control comparison as well as a significant ($P = 0.001$) positive low dose to control comparison. The test for departure from linear trend was also significant for this site.

For female mice the Cochran-Armitage test indicated a significant ($P = 0.004$) positive association between dose and the incidence of hepatocellular carcinomas. This was supported by a significant ($P = 0.011$) positive Fisher exact test comparing the high dose group to the control group. The Cochran-Armitage test also indicated a significant ($P < 0.001$) positive association between dose and the combined incidence of hepatocellular carcinomas or hepatocellular adenomas. This result was supported by a significant ($P < 0.001$) positive Fisher exact high dose to control comparison and by a significant ($P = 0.006$) positive low dose to control comparison.

Based on these statistical results, there is sufficient evidence to indicate that nitrofen was carcinogenic to male and female B6C3F1 mice under the conditions of this bioassay.

In male mice the Cochran-Armitage test indicated a significant negative association between dose and the incidence of malignant lymphomas. The Fisher exact test comparing the low dose group to the control group was also significant, as was the test for departure from linear trend. The Fisher exact test comparing the low dose to the control groups indicated a significant negative association between dose and the combined incidence of alveolar/bronchiolar carcinomas or alveolar/bronchiolar adenomas and the test for departure from linear trend was also significant.

V. DISCUSSION

There were no significant positive associations between the concentrations of nitrofen administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Mean body weight depression, relative to controls, was observed for males and females of both species, indicating that the concentrations of nitrofen administered to the animals in this bioassay may have approximated the maximum tolerated concentrations.

None of the statistical tests for any site in rats of either sex indicated a significant positive association between compound administration and tumor incidence. There was a significant positive association between the concentrations of nitrofen administered and the incidences of hepatocellular carcinomas in mice of both sexes. The high dose to control Fisher exact comparisons were significant for both sexes and the low dose to control Fisher exact comparison for the male mice was also significant. When the incidences were combined so that the numerator represented mice having either hepatocellular carcinoma or hepatocellular adenoma, the Cochran-Armitage tests and the Fisher exact comparisons were all significant.

In another bioassay of nitrofen for possible carcinogenicity, the compound was found to induce hepatocellular carcinomas in B6C3F1 mice of both sexes and hemangiosarcomas of the liver in male B6C3F1 mice. In addition, adenocarcinomas of the pancreas were induced in

female Osborne-Mendel rats (U.S. Department of Health, Education, and Welfare, 1978).

Under the conditions of this bioassay, dietary administration of nitrofen was carcinogenic to B6C3F1 mice, causing hepatocellular carcinomas in both sexes. There was no evidence for carcinogenicity in Fischer 344 rats.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH NITROFEN

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH NITROFEN

	CONTROL (UNTR) 11-1145	LOW DOSE 11-1143	HIGH DOSE 11-1141
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
BASAL-CELL TUMOR			1 (2%)
FIBROSARCOMA	1 (5%)	1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(18)	(50)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (6%)	1 (2%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
LEUKEMIA,NOS	1 (5%)	2 (4%)	
#SPLEEN	(19)	(50)	(48)
FIBROSARCOMA		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#PANCREAS	(20)	(50)	(48)
MESOTHELIOMA, NOS		1 (2%)	
URINARY SYSTEM			
NONE			

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-11+5	LOW DOSE 11-11+3	HIGH DOSE 11-11+1
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(47)	(45)
CHROMOPHOBE ADENOMA	1 (6%)	3 (6%)	2 (4%)
#ADRENAL	(19)	(49)	(47)
PHEOCHROMOCYTOMA	2 (11%)	3 (6%)	1 (2%)
#THYROID	(19)	(47)	(48)
CARCINOMA,NOS			1 (2%)
FOLLICULAR-CELL CARCINOMA		1 (2%)	1 (2%)
C-CELL ADENOMA	1 (5%)	3 (6%)	4 (8%)
C-CELL CARCINOMA	2 (11%)	3 (6%)	2 (4%)
#PANCREATIC ISLETS	(20)	(50)	(48)
ISLET-CELL ADENOMA	2 (10%)	2 (4%)	
REPRODUCTIVE SYSTEM			
#TESTIS	(20)	(49)	(50)
INTERSTITIAL-CELL TUMOR	19 (95%)	48 (98%)	49 (98%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	1 (2%)
ALL OTHER SYSTEMS			
NONE			

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

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 11-11+5	LOW DOSE 11-11+3	HIGH DOSE 11-11+1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	7	4
MORIBUND SACRIFICE	2	1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	42	45
ANIMAL MISSING			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	49	49
TOTAL PRIMARY TUMORS	30	71	65
TOTAL ANIMALS WITH BENIGN TUMORS	20	48	49
TOTAL BENIGN TUMORS	26	60	59
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	7	5
TOTAL MALIGNANT TUMORS	4	9	5
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		2	1
TOTAL UNCERTAIN TUMORS		2	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

@ INCLUDES AUTOLYZED ANIMALS

* 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 NITROFEN

	CONTROL(UNTR) 11-1146	LOW DOSE 11-1144	HIGH DOSE 11-1142
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(49)
BASAL-CELL TUMOR		1 (2%)	2 (4%)
SARCOMA, NOS		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(49)
FIBROSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(18)	(49)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
FOLLICULAR-CELL CARCINOMA, METAS		1 (2%)	
FIBROSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
LEUKEMIA,NOS	1 (5%)	1 (2%)	
MONOCYTIC LEUKEMIA	1 (5%)		
CIRCULATORY SYSTEM			
#HEART	(18)	(50)	(47)
FIBROSARCOMA, METASTATIC		1 (2%)	
DIGESTIVE SYSTEM			
NONE			

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1146	LOW DOSE 11-1144	HIGH DOSE 11-1142
URINARY SYSTEM			
#URINARY BLADDER	(17)	(46)	(46)
TRANSITIONAL-CELL CARCINOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(48)	(45)
CHROMOPHOBE ADENOMA	11 (69%)	8 (17%)	4 (9%)
#ADRENAL	(20)	(49)	(48)
CORTICAL ADENOMA	1 (5%)	1 (2%)	
PHEOCHROMOCYTOMA	1 (5%)		1 (2%)
#THYROID	(17)	(46)	(42)
FOLLICULAR-CELL CARCINOMA		2 (4%)	1 (2%)
C-CELL ADENOMA	1 (6%)	2 (4%)	3 (7%)
C-CELL CARCINOMA	1 (6%)		2 (5%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
ADENOCARCINOMA, NOS	1 (5%)		
FIBROADENOMA	4 (20%)	4 (8%)	
*CLITORAL GLAND	(20)	(50)	(49)
ADENOMA, NOS		2 (4%)	
#UTERUS	(18)	(50)	(47)
ADENOCARCINOMA, NOS		1 (2%)	1 (2%)
PAPILLARY ADENOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP	1 (6%)	1 (2%)	1 (2%)
#CERVIX UTERI	(18)	(50)	(47)
SQUAMOUS CELL CARCINOMA		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1146	LOW DOSE 11-1144	HIGH DOSE 11-1142
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(49)
SARCOMA, NOS, UNC PRIM OR META			1 (2%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	5	9
MORIBUND SACRIFICE	1	3	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	42	38
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 11-1146	LOW DOSE 11-1144	HIGH DOSE 11-1142
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	15	21	15
TOTAL PRIMARY TUMORS	23	28	18
TOTAL ANIMALS WITH BENIGN TUMORS	14	16	9
TOTAL BENIGN TUMORS	19	20	12
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	8	5
TOTAL MALIGNANT TUMORS	4	8	5
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	
TOTAL SECONDARY TUMORS		3	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			1
TOTAL UNCERTAIN TUMORS			1

* 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 NITROFEN

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH NITROFEN

	CONTROL (UNTR) 22-2145	LOW DOSE 22-2143	HIGH DOSE 22-2141
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			2
ANIMALS NECROPSIED	20	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(49)	(48)
SEBACEOUS ADENOMA		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(18)	(49)	(46)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (17%)		2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(48)
MALIG.LYMPHOMA, UNDIFFER-TYPE	2 (10%)		1 (2%)
#SPLEEN	(20)	(46)	(44)
HEMANGIOSARCOMA			1 (2%)
#SMALL INTESTINE	(20)	(49)	(47)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(48)
BILE DUCT CARCINOMA		3 (6%)	4 (8%)

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 22-2145	LOW DOSE 22-2143	HIGH DOSE 22-2141
HEPATOCELLULAR ADENOMA	1 (5%)	18 (37%)	20 (42%)
HEPATOCELLULAR CARCINOMA		13 (27%)	20 (42%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#TESTIS	(20)	(48)	(45)
NEOPLASM, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*MUSCLE OF LEG	(20)	(49)	(48)
HEMANGIOMA	1 (5%)		
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(49)	(48)
FIBROSARCOMA			1 (2%)
*MESENTERY	(20)	(49)	(48)
HEMANGIOMA		1 (2%)	
ALL OTHER SYSTEMS			
NONE			

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

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 22-2145	LOW DOSE 22-2143	HIGH DOSE 22-2141
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	2	7
MORIBUND SACRIFICE			1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	19	48	40
ANIMAL MISSING			2
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	31	43
TOTAL PRIMARY TUMORS	8	36	52
TOTAL ANIMALS WITH BENIGN TUMORS	4	20	21
TOTAL BENIGN TUMORS	5	20	23
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	15	24
TOTAL MALIGNANT TUMORS	3	16	28
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH NITROFEN

	CONTROL (UNTR) 22-2146	LOW DOSE 22-2144	HIGH DOSE 22-2142
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHCLOGICALLY **	20	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(49)	(50)
PAPILLOMA, NOS		1 (2%)	1 (2%)
BASAL-CELL TUMOR		1 (2%)	
*SUBCUT TISSUE	(20)	(49)	(50)
FIBROSARCOMA	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	3 (6%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)	1 (2%)	1 (2%)
MALIG.LYMPHOMA, UNDIFFER-TYPE		2 (4%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		
*ABDOMINAL CAVITY	(20)	(49)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
*BLOOD	(20)	(49)	(50)
LEUKEMIA,NOS		1 (2%)	
#SPLEEN	(18)	(46)	(43)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			2 (5%)
#THYMUS		(2)	(5)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (20%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2146	LOW DOSE 22-2144	HIGH DOSE 22-2142
DIGESTIVE SYSTEM			
#LIVER	(18)	(48)	(50)
BILE DUCT CARCINOMA		1 (2%)	
HEPATOCELLULAR ADENOMA		9 (19%)	17 (34%)
HEPATOCELLULAR CARCINOMA		5 (10%)	13 (26%)
#DUODENUM	(20)	(48)	(47)
ADENOMA, NOS		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(33)	(35)
CHROMOPHOBE ADENOMA		3 (9%)	
#THYROID	(16)	(35)	(42)
FOLLICULAR-CELL ADENOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(50)
ADENOCARCINOMA, NOS			1 (2%)
#UTERUS	(20)	(48)	(49)
LEIOMYOMA		1 (2%)	
#OVARY	(13)	(27)	(36)
TERATOMA, NOS		1 (4%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2146	LOW DOSE 22-2144	HIGH DOSE 22-2142
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	5	5	2
MORIBUND SACRIFICE	3	1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	12	43	48
ANIMAL MISSING		1	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 22-2146.	LOW DOSE 22-2144	HIGH DOSE 22-2142
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	3	23	33
TOTAL PRIMARY TUMORS	3	30	41
TOTAL ANIMALS WITH BENIGN TUMORS		15	20
TOTAL BENIGN TUMORS		19	21
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	9	18
TOTAL MALIGNANT TUMORS	3	10	20
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH NITROFEN

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH NITROFEN

	CONTROL (UNTR) 11-1145	LOW DOSE 11-1143	HIGH DOSE 11-1141
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
CYST, NOS		1 (2%)	1 (2%)
METAPLASIA, SQUAMOUS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(18)	(50)	(48)
INFLAMMATION, SUPPURATIVE		1 (2%)	
PNEUMONIA, CHRONIC MURINE	12 (67%)	27 (54%)	22 (46%)
HEMATOPOIETIC SYSTEM			
*BLOOD	(20)	(50)	(50)
MYELOPROLIFERATIVE DISORDER			1 (2%)
#BONE MARROW	(19)	(44)	(46)
MYELOFIBROSIS			2 (4%)
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(48)
DEGENERATION, NOS	7 (35%)	18 (36%)	12 (25%)
#MYOCARDIUM	(20)	(50)	(48)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION ACUTE AND CHRONIC			1 (2%)
FIBROSIS		1 (2%)	
DEGENERATION, NOS		5 (10%)	
#ENDOCARDIUM	(20)	(50)	(48)
ENDOCARDIOSIS		1 (2%)	1 (2%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-11↓5	LOW DOSE 11-11↓3	HIGH DOSE 11-11↓1
DIGESTIVE SYSTEM			
#LIVER	(18)	(50)	(48)
INFLAMMATION, FOCAL		1 (2%)	
NECROSIS, FOCAL	1 (6%)	1 (2%)	1 (2%)
NECROSIS, DIFFUSE			1 (2%)
HYPERPLASIA, FOCAL			1 (2%)
ANGIECTASIS			1 (2%)
#LIVER/CENTRILOBULAR	(18)	(50)	(48)
NECROSIS, NOS	1 (6%)	1 (2%)	1 (2%)
#BILE DUCT	(18)	(50)	(48)
HYPERPLASIA, NOS		2 (4%)	
#PANCREAS	(20)	(50)	(48)
PERIARTERITIS		1 (2%)	
ATROPHY, NOS		1 (2%)	
ATROPHY, FOCAL	4 (20%)	7 (14%)	2 (4%)
ATROPHY, DIFFUSE		1 (2%)	
#PANCREATIC ACINUS	(20)	(50)	(48)
ATROPHY, NOS	3 (15%)		5 (10%)
#STOMACH	(19)	(50)	(48)
AMYLOIDOSIS		1 (2%)	
#SMALL INTESTINE	(19)	(50)	(48)
ULCER, NOS		1 (2%)	
#LARGE INTESTINE	(19)	(50)	(50)
NEMATODIASIS	7 (37%)	8 (16%)	21 (42%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(48)
INFLAMMATION, CHRONIC	16 (80%)	43 (86%)	44 (92%)
*URETER	(20)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
#URINARY BLADDER	(14)	(48)	(42)
INFLAMMATION, NOS		1 (2%)	

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-11+5	LOW DOSE 11-11+3	HIGH DOSE 11-11+1
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(47)	(45)
CYST, NOS			1 (2%)
#ADRENAL	(19)	(49)	(47)
HEMORRHAGIC CYST	1 (5%)		1 (2%)
#ADRENAL MEDULLA	(19)	(49)	(47)
HYPERPLASIA, FOCAL		1 (2%)	
#THYROID	(19)	(47)	(48)
ULTIMOBRANCHIAL CYST		1 (2%)	
HYPERPLASIA, C-CELL		4 (9%)	8 (17%)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	
#PARATHYROID	(12)	(30)	(21)
HYPERPLASIA, NOS			1 (5%)
#PANCREATIC ISLETS	(20)	(50)	(48)
HYPERPLASIA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
#PROSTATE	(19)	(48)	(44)
ABSCESS, NOS		1 (2%)	
#TESTIS	(20)	(49)	(50)
INFARCT, HEALED			1 (2%)
HYPERPLASIA, INTERSTITIAL CELL		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(20)	(50)	(50)
CATARACT			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 11-1145	LOW DOSE 11-1143	HIGH DOSE 11-1141
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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 NITROFEN

	CONTROL (UNTR) 11-1146	LOW DOSE 11-1144	HIGH DOSE 11-1142
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(49)
EPIDERMAL INCLUSION CYST		1 (2%)	
RESPIRATORY SYSTEM			
#TRACHEA	(16)	(49)	(44)
INFLAMMATION, NOS			2 (5%)
INFLAMMATION ACUTE AND CHRONIC			1 (2%)
#LUNG	(18)	(49)	(49)
PNEUMONIA, CHRONIC MURINE	6 (33%)	32 (65%)	32 (65%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(15)	(42)	(36)
MYELOFIBROSIS		1 (2%)	
#SPLEEN	(18)	(50)	(46)
HYPOPLASIA, LYMPHOID			1 (2%)
#MESENTERIC L. NODE	(16)	(45)	(39)
HYPERPLASIA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(18)	(50)	(47)
THROMBUS, ORGANIZED		1 (2%)	
PERIARTERITIS		1 (2%)	
DEGENERATION, NOS	2 (11%)	10 (20%)	8 (17%)
#MYOCARDIUM	(18)	(50)	(47)
MINERALIZATION			1 (2%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-11+6	LOW DOSE 11-11++	HIGH DOSE 11-11+2
DEGENERATION, NOS		1 (2%)	3 (6%)
#CARDIAC VALVE	(18)	(50)	(47)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
*CORONARY ARTERY	(20)	(50)	(49)
DEGENERATION, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(18)	(50)	(47)
INFLAMMATION, ACUTE NECROTIZING			1 (2%)
NECROSIS, NOS		1 (2%)	
METAMORPHOSIS FATTY	1 (6%)		
#LIVER/PERIPORTAL	(18)	(50)	(47)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
#BILE DUCT	(18)	(50)	(47)
HYPERPLASIA, NOS	1 (6%)	2 (4%)	1 (2%)
#PANCREAS	(18)	(50)	(47)
ATROPHY, NOS			2 (4%)
ATROPHY, FOCAL		3 (6%)	2 (4%)
#PANCREATIC ACINUS	(18)	(50)	(47)
ATROPHY, NOS	4 (22%)		3 (6%)
ATROPHY, FOCAL			1 (2%)
#STOMACH	(18)	(49)	(46)
INFLAMMATION, NOS		1 (2%)	
#LARGE INTESTINE	(18)	(49)	(47)
NEMATODIASIS	3 (17%)	14 (29%)	7 (15%)
#CECUM	(18)	(49)	(47)
ULCER, NOS	1 (6%)		
URINARY SYSTEM			
#KIDNEY	(19)	(50)	(49)
HYDRONEPHROSIS			1 (2%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1146	LOW DOSE 11-1144	HIGH DOSE 11-1142
INFLAMMATION, CHRONIC	12 (63%)	34 (68%)	38 (78%)
NEPHROPATHY, TOXIC		1 (2%)	
NEPHROSIS, CHOLEMIC	1 (5%)		
INFARCT, ACUTE		1 (2%)	
CALCINOSIS, NOS			2 (4%)
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(48)	(45)
CYST, NOS		2 (4%)	
CHOLESTEATOMA	1 (6%)		
#THYROID	(17)	(46)	(42)
CYSTIC FOLLICLES			1 (2%)
HYPERPLASIA, C-CELL		2 (4%)	
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	2 (5%)
#PARATHYROID	(9)	(28)	(23)
HYPERPLASIA, NOS			1 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
DILATATION/DUCTS		2 (4%)	
*MAMMARY DUCT	(20)	(50)	(49)
HYPERPLASIA, NOS		1 (2%)	
#UTERUS	(18)	(50)	(47)
CYST, NOS		4 (8%)	5 (11%)
INFLAMMATION, NOS	2 (11%)	5 (10%)	9 (19%)
INFLAMMATION, SUPPURATIVE			1 (2%)
ABSCESS, NOS		1 (2%)	
NECROSIS, FAT			1 (2%)
#CERVIX UTERI	(18)	(50)	(47)
CYST, NOS		13 (26%)	18 (38%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
ABSCESS, NOS		1 (2%)	4 (9%)
#UTERUS/ENDOMETRIUM	(18)	(50)	(47)
HYPERPLASIA, NOS	1 (6%)	1 (2%)	
#OVARY/OVIDUCT	(18)	(50)	(47)
FIBROSIS, FOCAL		1 (2%)	

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

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 11-11+6	LOW DOSE 11-11++	HIGH DOSE 11-1142
#OVARY	(18)	(50)	(47)
CYST, NOS	2 (11%)	2 (4%)	6 (13%)
FOLLICULAR CYST, NOS		3 (6%)	3 (6%)
NERVOUS SYSTEM			
#BRAIN	(17)	(50)	(48)
ABSCESS, NOS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1		
AUTOLYSIS/NO NECROPSY			1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH NITROFEN

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH NITROFEN

	CONTROL (UNTR) 22-2145	LOW DOSE 22-2143	HIGH DOSE 22-2141
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			2
ANIMALS NECROPSIED	20	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(18)	(49)	(46)
COLLAPSE	1 (6%)		
INFLAMMATION, INTERSTITIAL	1 (6%)	1 (2%)	
PNEUMONIA, ASPIRATION			1 (2%)
PNEUMONIA, CHRONIC MURINE	1 (6%)	2 (4%)	
INFLAMMATION, CHRONIC			1 (2%)
PERIVASCULAR CUFFING			1 (2%)
NECROSIS, FOCAL			1 (2%)
FOAM-CELL			1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	
HYPERPLASIA, ADENOMATOUS		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
#HEART	(20)	(49)	(47)
PERIVASCULITIS	2 (10%)		
*CORONARY ARTERY	(20)	(49)	(48)
INFLAMMATION, CHRONIC			1 (2%)
DEGENERATION, NOS	1 (5%)		
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(48)
THROMBOSIS, NOS		1 (2%)	2 (4%)

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2145	LOW DOSE 22-2143	HIGH DOSE 22-2141
CONGESTION, NOS			1 (2%)
HEMORRHAGE			1 (2%)
PERIVASCULAR CUFFING			1 (2%)
DEGENERATION, NOS		1 (2%)	1 (2%)
NECROSIS, NOS			3 (6%)
NECROSIS, FOCAL			1 (2%)
INFARCT, NOS			1 (2%)
HEPATOCYTOMEGALY			4 (8%)
HYPERPLASIA, NOS	1 (5%)		3 (6%)
HYPERPLASIA, FOCAL		9 (18%)	4 (8%)
#PANCREAS	(20)	(48)	(44)
INFLAMMATION, ACUTE	1 (5%)		
ATROPHY, NOS	1 (5%)	3 (6%)	
#STOMACH	(19)	(47)	(47)
INFLAMMATION, ACUTE		1 (2%)	
#GASTRIC MUCOSA	(19)	(47)	(47)
CALCIFICATION, FOCAL		1 (2%)	
#SMALL INTESTINE	(20)	(49)	(47)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
#LARGE INTESTINE	(20)	(49)	(45)
NEMATODIASIS	2 (10%)	15 (31%)	2 (4%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(46)
HYDRONEPHROSIS		1 (2%)	2 (4%)
INFLAMMATION, CHRONIC			1 (2%)
#SEROSA OF URINARY BL	(20)	(45)	(41)
PERIVASCULITIS		1 (2%)	
#NECK OF URINARY BLAD	(20)	(45)	(41)
PIGMENTATION, NOS			1 (2%)
ENDOCRINE SYSTEM			
NONE			

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

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 22-2145	LOW DOSE 22-2143	HIGH DOSE 22-2141
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(20)	(49)	(48)
PERIVASCULITIS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(49)	(48)
PERIVASCULITIS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(49)	(48)
HYPERPLASIA, LYMPHOID		1 (2%)	
ADIPOSE TISSUE			
NECROSIS, FOCAL		1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	6	9	
ANIMAL MISSING/NO NECROPSY			2
AUTOLYSIS/NO NECROPSY		1	

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

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH NITROFEN

	CONTROL (UNTR) 22-2146	LOW DOSE 22-2144	HIGH DOSE 22-2142
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(49)
CONGESTION, NOS		2 (4%)	
HEMORRHAGE		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC	1 (5%)		
GRANULOMA, NOS	1 (5%)		
PERIVASCULAR CUFFING	1 (5%)	1 (2%)	
HYPERPLASIA, LYMPHOID	1 (5%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(46)	(43)
HYPERPLASIA, LYMPHOID	1 (6%)		1 (2%)
#SPLENIC CAPSULE	(18)	(46)	(43)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
#LYMPH NODE	(12)	(36)	(29)
HYPERPLASIA, LYMPHOID			1 (3%)
#ABDOMINAL LYMPH NODE	(12)	(36)	(29)
INFLAMMATION, ACUTE/CHRONIC			1 (3%)
#MESENTERIC L. NODE	(12)	(36)	(29)
INFLAMMATION, ACUTE SUPPURATIVE			1 (3%)
INFLAMMATION, CHRONIC		1 (3%)	
INFLAMMATION, GRANULOMATOUS	1 (8%)		

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2146	LOW DOSE 22-2144	HIGH DOSE 22-2142
CIRCULATORY SYSTEM			
#MYOCARDIUM	(19)	(48)	(42)
INFLAMMATICN, ACUTE FOCAL			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(12)	(42)	(44)
PERIVASCULAR CUFFING			1 (2%)
#LIVER	(18)	(48)	(50)
MINERALIZATICN		1 (2%)	
HEMATOMA, CRGANIZED		1 (2%)	
INFLAMMATICN, MULTIFOCAL	1 (6%)		
INFLAMMATICN, ACUTE FOCAL		2 (4%)	
INFLAMMATICN, ACUTE/CHRONIC		1 (2%)	
INFLAMMATICN, CHRONIC FOCAL			1 (2%)
PERIVASCULAR CUFFING	2 (11%)		1 (2%)
DEGENERATICN, NOS			1 (2%)
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL		2 (4%)	
METAMORPHOSIS FATTY	1 (6%)		
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL		11 (23%)	11 (22%)
HYPERPLASIA, LYMPHOID	1 (6%)		
#HEPATIC CAPSULE	(18)	(48)	(50)
HEMATOMA, CRGANIZED	1 (6%)	1 (2%)	
#LIVER/PERIPORTAL	(18)	(48)	(50)
INFLAMMATICN, CHRONIC		1 (2%)	
#LIVER/HEPATOCYTES	(18)	(48)	(50)
INCLUSION, CYTOPLASMIC		1 (2%)	
#PANCREAS	(19)	(44)	(42)
ECTOPIA		1 (2%)	
DILATATICN/DUCTS		1 (2%)	
METAMORPHOSIS FATTY			1 (2%)
ATROPHY, NOS			1 (2%)
#SMALL INTESTINE	(20)	(48)	(47)
HYPERPLASIA, LYMPHOID		1 (2%)	

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2146	LOW DOSE 22-2144	HIGH DOSE 22-2142
#PEYERS PATCH	(20)	(48)	(47)
HYPERPLASIA, LYMPHOID	2 (10%)		
#LARGE INTESTINE	(20)	(44)	(47)
NEMATODIASIS		3 (7%)	
URINARY SYSTEM			
#KIDNEY	(19)	(48)	(44)
CONGESTION, NOS		1 (2%)	
INFLAMMATION, CHRONIC	3 (16%)	5 (10%)	
PERIVASCULAR CUFFING	2 (11%)	2 (4%)	5 (11%)
AMYLOIDOSIS	1 (5%)	2 (4%)	
#URINARY BLADDER	(20)	(43)	(43)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
PERIVASCULAR CUFFING			1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(16)	(35)	(42)
CYSTIC FOLLICLES		1 (3%)	
ATROPHY, NOS		1 (3%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(48)	(49)
DISTENTION			1 (2%)
HYDROMETRA	1 (5%)		
EPIDERMAL INCLUSION CYST		1 (2%)	
INFLAMMATION, NOS		2 (4%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, ACUTE		2 (4%)	1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE		1 (2%)	
INFLAMMATION, CHRONIC			1 (2%)
#UTERUS/ENDOMETRIUM	(20)	(48)	(49)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, ACUTE	1 (5%)	7 (15%)	3 (6%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)
HYPERPLASIA, CYSTIC	2 (10%)	1 (2%)	1 (2%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2146	LOW DOSE 22-2144	HIGH DOSE 22-2142
#OVARY/OVIDUCT	(20)	(48)	(49)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, ACUTE		1 (2%)	
#OVARY	(13)	(27)	(36)
FOLLICULAR CYST, NOS	1 (8%)	3 (11%)	
HEMORRHAGIC CYST	1 (8%)		
NERVOUS SYSTEM			
#BRAIN/MENINGES	(20)	(45)	(45)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE	1 (5%)		
#BRAIN	(20)	(45)	(45)
PERIVASCULAR CUFFING		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE	(20)	(49)	(50)
INFLAMMATION, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(49)	(50)
PARASITISM			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(49)	(50)
ABSCESS, NOS	1 (5%)		
*PERITONEUM	(20)	(49)	(50)
INFLAMMATION, NOS	1 (5%)		
*MESENTERY	(20)	(49)	(50)
HYPERPLASIA, LYMPHOID		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(49)	(50)
POSTMORTEM CHANGE	3 (15%)		

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

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 22-2146	LOW DOSE 22-2144	HIGH DOSE 22-2142
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	6	3
ANIMAL MISSING/NO NECROPSY		1	

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

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

October 25, 1978

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

The reviewer for the report on the bioassay of Nitrofen agreed with the conclusion in the report that Nitrofen was carcinogenic in both sexes of treated mice, inducing hepatocellular carcinomas. The compound was not carcinogenic in Fischer rats under the conditions of test. After briefly describing the experimental design, the reviewer noted a previous NCI bioassay of Nitrofen which demonstrated a significant number of pancreatic carcinomas in treated female Osborne-Mendel rats. He pointed out that the dose levels in this study were higher than in the previous one, thus suggesting that the difference in the response of the rats was due to species variation. He noted this as an interesting finding with respect to the reproducibility and significance of carcinogenicity studies. The reviewer suggested that the report discuss the difference between the results of the two Nitrofen studies. With that suggestion, there was no objection to the recommendation that the report be accepted as written.

Clearinghouse Members present:

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

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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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