National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 26
1978

BIOASSAY OF NITROFEN FOR POSSIBLE CARCINOGENICITY

CAS No. 1836-75-5

NCI-CG-TR-26

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

BIOASSAY OF

NITROFEN

FOR POSSIBLE CARCINOGENICITY

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

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

DHEW Publication No. (NIH) 78-826

REPORT ON THE BIOASSAY OF NITROFEN
FOR POSSIBLE CARCINOGENICITY

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

CONTRIBUTORS: This report presents the results of the bioassay of nitrofen conducted for the Carcinogen Bioassay and Program Resources Branch, Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This bioassay was conducted by Hazleton Laboratories America, Inc., Vienna, Virginia, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Bioassay 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. M. B. Powers (3), Dr. R. W. Voelker (3), Dr. W. A. Olson (3,4) and Dr. W. M. Weatherholtz (3). Chemical analysis was performed by Dr. C. L. Guyton (3,5); the technical supervisor of animal treatment and observation was Ms. K. J. Petrovics (3).

Histopathology was performed by Dr. R. H. Habermann (3) and reviewed by Dr. R. W. Voelker (3) at the Hazleton Laboratories America, Inc., and the diagnoses included in this report represent the interpretation of these pathologists. Pathologists from NCI (1) and Tracor Jitco (6) have reviewed selected slides and concur with the overall histopathologic evaluation of the study.

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (7); the statistical analysis was performed by Dr. J. R. Joiner (6) and Mr. W. W. Belew (8), using methods selected for the Bioassay Program by Dr. J. J. Gart (9).

This report was prepared at METREK, a Division of The MITRE Corporation (8) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (8), the task leader, Dr. M. R. Kornreich (8), and the senior biologist, Ms. P. Walker (8). The final report was reviewed by members of the participating organizations.

The following 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. C.

Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1), Dr. R. A. Griesemer (1), Dr. R. A. Squire (1), and Dr. J. M. Ward (1).

---

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

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

3. Hazleton Laboratories America, Inc., 9200 Leesburg Turnpike, Vienna, Virginia.

4. Now with the Center for Regulatory Services, 2347 Paddock Lane, Reston, Virginia.

5. Now with Rhodia, Inc., 23 Belmont Drive, Somerset, New Jersey.

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

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

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

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

SUMMARY

A bioassay of technical-grade nitrofen for possible carcinogenicity was conducted using Osborne-Mendel 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. The time-weighted average high and low dietary concentrations of nitrofen were 3656 and 2300 ppm for male rats, 2600 and 1300 ppm for female rats, and 4696 and 2348 ppm for both male and female mice, respectively. After a 78-week treatment period, observation of the low dose and control male and all female rats continued for an additional 32 weeks; observation of the high dose male rats continued for an additional 4 weeks. All mice were observed for an additional 12 weeks after the 78-week treatment period.

For each species, 20 animals of each sex were placed on test as controls. No nitrofen was added to their diet.

The incidence of carcinomas of the pancreas had a statistically significant positive association with concentration of nitrofen in the diet of female rats. The incidence of this tumor in high dose female rats was significant when compared to controls. Poor survival related to chemical toxicity precluded the evaluation of the carcinogenicity of nitrofen in male rats.

In mice of both sexes, the incidence of hepatocellular carcinoma at both high and low dose levels was highly significant when compared to the controls. The incidence of hemangiosarcoma of the liver had a statistically significant relationship with nitrofen concentration in the diet for mice of both sexes, and the incidence in high dose male mice was significant when compared to controls.

The results of this study indicate that orally administered technical-grade nitrofen is a liver carcinogen in B6C3F1 mice of both sexes. Nitrofen is also carcinogenic to female Osborne-Mendel rats.

TABLE OF CONTENTS

		Page
I.	INTRODUCTION	1
II.	MATERIALS AND METHODS	3
	A. Chemicals	3
	B. Dietary Preparation	3
	C. Animals	4
	D. Animal Maintenance	4
	E. Selection of Initial Concentrations	6
	F. Experimental Design	7
	G. Clinical and Histopathologic Examinations	10
	H. Data Recording and Statistical Analyses	12
III.	CHRONIC TESTING RESULTS: RATS	17
	A. Body Weights and Clinical Observations	17
	B. Survival	19
	C. Pathology	21
	D. Statistical Analyses of Results	23
IV.	CHRONIC TESTING RESULTS: MICE	35
	A. Body Weights and Clinical Observations	35
	B. Survival	35
	C. Pathology	38
	D. Statistical Analyses of Results	39
V.	DISCUSSION	46
VI.	BIBLIOGRAPHY	48
APPENDIX A	SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH NITROFEN	A-1
APPENDIX B	SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH NITROFEN	B-1
APPENDIX C	SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH NITROFEN	C-1
APPENDIX D	SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH NITROFEN	D-1

LIST OF ILLUSTRATIONS

Figure Number		Page
1	GROWTH CURVES FOR NITROFEN CHRONIC STUDY RATS	18
2	SURVIVAL COMPARISONS OF NITROFEN CHRONIC STUDY RATS	20
3	GROWTH CURVES FOR NITROFEN CHRONIC STUDY MICE	36
4	SURVIVAL COMPARISONS OF NITROFEN CHRONIC STUDY MICE	37

LIST OF TABLES

Table Number		Page
1	DESIGN SUMMARY FOR OSBORNE-MENDEL RATS--NITROFEN FEEDING EXPERIMENT	8
2	DESIGN SUMMARY FOR B6C3F1 MICE--NITROFEN FEEDING EXPERIMENT	9
3	ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE RATS TREATED WITH NITROFEN	24
4	ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE RATS TREATED WITH NITROFEN	27
5	ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH NITROFEN	40
6	ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE MICE TREATED WITH NITROFEN	43
A1	SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH NITROFEN	A-3

LIST OF TABLES (Concluded)

Table Number		Page
A2	SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH NITROFEN	A-7
B1	SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH NITROFEN	B-3
B2	SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH NITROFEN	B-6
C1	SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH NITROFEN	C-3
C2	SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH NITROFEN	C-8
D1	SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH NITROFEN	D-3
D2	SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH NITROFEN	D-6

I. INTRODUCTION

Nitrofen (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 adequate chronic toxicity 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 unavailable, 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.

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

Nitrofen, 2,4-dichloro-1-(4-nitrophenoxy) benzene, was purchased from Rohm and Haas Chemical Company by Hazleton Laboratories America, Inc., Vienna, Virginia, where the chemical analysis was performed. The manufacturer's analysis indicated a purity of approximately 87 percent. Gas-liquid chromatography (GLC), utilizing the internal standard assay, suggested a purity of greater than 80 percent. The observed melting point (58° to 68°C) suggested the presence of significant impurities, because of its wide range and variance from that reported as an FDA standard (71° to 72°C). GLC total area analysis indicated the presence of at least five impurities.

The material was analyzed by GLC total area analysis after having been stored for one year. Five impurities were again detected and, although the change in area suggested a different distribution of these substances, no significant change in purity of the compound over the 12-month period was indicated. The nature of the impurities, as suggested by the manufacturer, include xylene, dichlorophenol, p-chloronitrobenzene, and chloronitrodiphenyl ethers.

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

B. Dietary Preparation

The basal laboratory diet for both control and dosed animals consisted of 2 percent Duke's® corn oil (S. F. Sauer Company)

by weight added to Wayne Lab-Blox® meal (Allied Mills, Inc.). Fresh mixtures of nitrofen in corn oil were prepared each week and stored in the dark. The mixtures of nitrofen in corn oil were incorporated into the appropriate amount of the basal laboratory diet in a twin-shell blender fitted with an accelerator bar.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. The Osborne-Mendel rat was selected on the basis of a comparative study of the tumorigenic responsiveness to carbon tetrachloride of five different strains of rats (Reuber and Glover, 1970). The B6C3F1 mouse was selected because it has been used by the NCI for carcinogenesis bioassays and has proved satisfactory in this capacity.

Rats and mice of both sexes were obtained through contracts with the Division of Cancer Treatment, National Cancer Institute. The Osborne-Mendel rats were procured from the Battelle Memorial Institute, Columbus, Ohio, and the B6C3F1 mice were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Upon receipt, animals were quarantined for at least 10 days, observed for visible signs of disease or parasites, and assigned to the various treatment and control groups.

D. Animal Maintenance

All animals were housed by species in temperature- and humidity-controlled rooms. The temperature range was 20° to 24°C and the relative humidity was maintained between 45 and 55 percent. The air

conditioning system in the laboratory provided filtered air at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle. The rats were individually housed in suspended galvanized-steel wire-mesh cages with perforated floors. Mice were housed by sex in groups of 10 in solid-bottom polypropylene cages equipped with filter tops. Sanitized cages with fresh bedding (Sanichips®, Shurfire) were provided once each week for mice. Rats received sanitized cages with no bedding with the same frequency. Food hoppers were changed and heat-sterilized once a week for the first 10 weeks and once a month thereafter. Fresh heat-sterilized glass water bottles were provided three times a week. Food and water were available ad libitum.

The nitrofen-treated and control rats were housed in the same room with rats treated with* trifluralin (1582-09-8), dioxathion (78-34-2), dicofol (115-32-2), endosulfan (115-29-7), and mexacarbate (315-18-4). All mice used in the nitrofen study, including controls, were housed in the same room with mice treated with trifluralin (1582-09-8), dioxathion (78-34-2), sulfallate (95-06-7), p,p'-DDT (50-29-3), methoxychlor (72-43-5), p,p'-DDE (72-55-9), p,p'-TDE (72-54-8), dicofol (115-32-2), pentachloronitrobenzene (82-68-8), clonitralid (1420-04-8), endosulfan (115-29-7), chlorobenzilate (510-15-6), mexacarbate (315-18-4), amitrole (61-82-5), acetylaminofluorene (53-96-3), and safrole (94-59-7).

*CAS registry numbers are given in parentheses.

E. Selection of Initial Concentrations

In order to establish the maximum tolerated doses of nitrofen for administration to treated 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 premixed with a small amount of corn oil. The mixture was then incorporated into the basal laboratory diet and fed *ad libitum* to five of the six rat groups at concentrations of 1000, 1780, 3160, 5620, and 10,000 ppm and to five of the six mouse groups at concentrations of 1780, 3160, 5620, 10,000 and 17,800 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 6 weeks, followed by a 2-week observation period during which all animals were fed the basal diet of corn oil and laboratory chow.

A dosage inducing no mortality and resulting in a retardation in body weight gain (a retardation of approximately 20 percent) was to be selected as the initial high dose. When weight gain criteria were not applicable, mortality data alone were utilized.

In the male and female rats, no deaths were observed at any concentration. In males, body weight gain retardation, expressed as a percentage of the weight gain of the controls, was 10 and 25 percent at concentrations of 3160 and 5620 ppm, respectively. In females, body weight gain retardation was 17 and 26 percent at

concentrations of 1780 and 3160 ppm, respectively. The initial high doses selected for the rat chronic bioassay were 4600 ppm for males and 2600 ppm for females.

In mice, retardation in body weight gain, although not clearly dose-related, was observed at concentrations of 3160 ppm and above. At the 3160 ppm concentration, body weight gain reduction was 12 percent for male mice and 8 percent for female mice. At 5620 ppm, body weight gain reduction was 37 percent for male mice and 40 percent for female mice. One male died at 5620 ppm. Mortality increased with concentration in both sexes. The initial high dose selected for the chronic study was 3550 ppm for both male and female mice.

F. Experimental Design

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

At the initiation of the study the high dose, low dose, and control rats were all approximately 7 weeks old. The high and low concentrations of nitrofen initially utilized for male rats were 4600 and 2300 ppm, respectively. For female rats the initial high and low concentrations were 2600 and 1300 ppm, respectively. During week 46 the concentration administered to the high dose male rats was decreased to 2300 ppm as intolerance to the higher dosage was observed. All high and low dose rats were treated for 78 weeks. The low dose

TABLE 1

DESIGN SUMMARY FOR OSBORNE-MENDEL RATS
NITROFEN FEEDING EXPERIMENT

	INITIAL GROUP SIZE	NITROFEN CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION[b]
MALE					
CONTROL	20	0		110	0
LOW DOSE	50	2300	78		2300
		0		32	
HIGH DOSE[c]	50	4600	45		3627
		2300	33		
		0		5	
FEMALE					
CONTROL	20	0		110	0
LOW DOSE	50	1300	78		1300
		0		32	
HIGH DOSE	50	2600	78		2600
		0		32	

[a]Concentrations in parts per million.

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

[c]These animals were terminated in week 83.

TABLE 2

DESIGN SUMMARY FOR B6F3F1 MICE
NITROFEN FEEDING EXPERIMENT

	INITIAL GROUP SIZE	NITROFEN CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION[b]
MALE					
CONTROL	20	0		90	0
LOW DOSE	50	1775	6		2348
		2000	15		
		2500	57		
		0		12	
HIGH DOSE	50	3550	6		4696
		4000	15		
		5000	57		
		0		12	
FEMALE					
CONTROL	20	0		90	0
LOW DOSE	50	1775	6		2348
		2000	15		
		2500	57		
		0		12	
HIGH DOSE	50	3550	6		4696
		4000	15		
		5000	57		
		0		12	

[a]Concentrations in parts per million.

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

and control males were observed for an additional 32 weeks during which they were maintained on the basal laboratory diet and corn oil mixture. The high dose males were observed for an additional 4 weeks after treatment, during which they were maintained on the basal laboratory diet and corn oil mixture. All surviving high dose male rats were sacrificed during week 83 of the study. High and low dose female rats were treated for 78 weeks and then received the basal diet and corn oil for an additional 32-week observation period.

At the initiation of the study all mice were approximately weeks old. The high and low doses initially administered to the male and female mice were 3550 and 1775 ppm, respectively. During week 7 the high and low dosages administered to the male and female mice were increased to 4000 and 2000 ppm, respectively, as the animals had apparently tolerated the previous dosages. Dosages were increased again during week 22, to 5000 ppm for the high dose male and female mice, and to 2500 ppm for the low dose male and female mice. The treated mice were maintained on these nitrofen concentrations for 57 weeks, followed by a 12-week observation period during which the animals received the basal diet and corn oil. Control mice received the basal diet and corn oil for the entire study.

Both rat and mouse control groups were maintained and observed in the same manner as the treated animals.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. From the first day, all animals were inspected daily

for mortality. Body weights, food consumption, and data concerning appearance, behavior, signs of toxic effects, and incidence, size, and location of tissue masses were recorded at weekly intervals for the first 10 weeks and at monthly intervals thereafter. The presence of tissue masses was determined by 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 exsanguination under sodium pentobarbital anesthesia, 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.

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) and bile duct, pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, pancreatic islets, testis, prostate, brain, muscle, uterus, mammary gland, and ovary.

Tissues for which slides were prepared 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.

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) for testing two groups for equality and used Tarone's (1975) extensions of Cox's methods for 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. 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

As indicated in Figure 1, a dose-related depression of body weight gain was observed in males and females throughout the 78-week treatment period. Growth curves for rats surviving beyond the treatment period tend to converge.

All animals exhibited generally normal appearance and behavior during the first 10 weeks of the study with the exception of intermittent observations of hunched appearance, abdominal urine stains, and labored respiration in a few treated rats. Beginning in week 14, a hunched appearance was observed in a gradually increasing number of treated rats and by week 78, at cessation of treatment, 75 percent of the low dose and 95 percent of the high dose rats appeared hunched. Urine stains and a slight decrease in body weight gain were also evident, particularly in the high dose groups. A bloody-appearing vaginal discharge was intermittently observed in one to five females in each of the treatment groups during the second year of the study and was consistently noted in these animals during the the last 3 months.

Respiratory signs, characterized by labored respiration, wheezing, and/or nasal discharge were observed at a low to moderate incidence in all groups during the second year of the study. The incidence increased as the animals aged. At termination of the study in week 110, most of the surviving treated and control rats

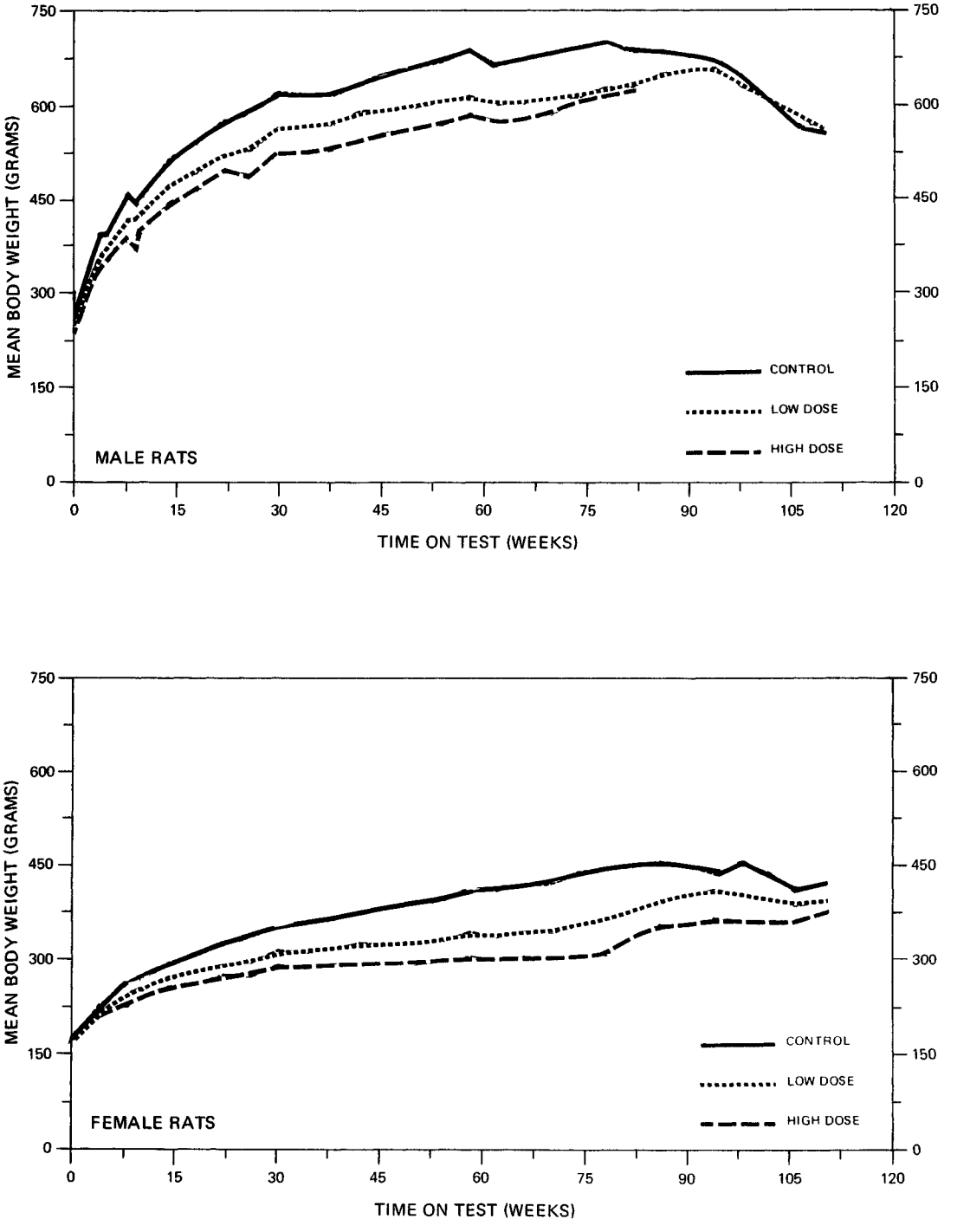


FIGURE 1
GROWTH CURVES FOR NITROFEN CHRONIC STUDY RATS

appeared hunched and were showing respiratory signs. Other signs often associated with aging that were noted at comparable rates in all groups included sores on the body and/or extremities, localized alopecia, reddened or squinted eyes, rough or stained fur, bloating, and palpable nodules or tissue masses. Isolated, apparently spontaneous symptoms observed in one or two rats included incoordination, ataxia, pale appearance, and hyperactivity.

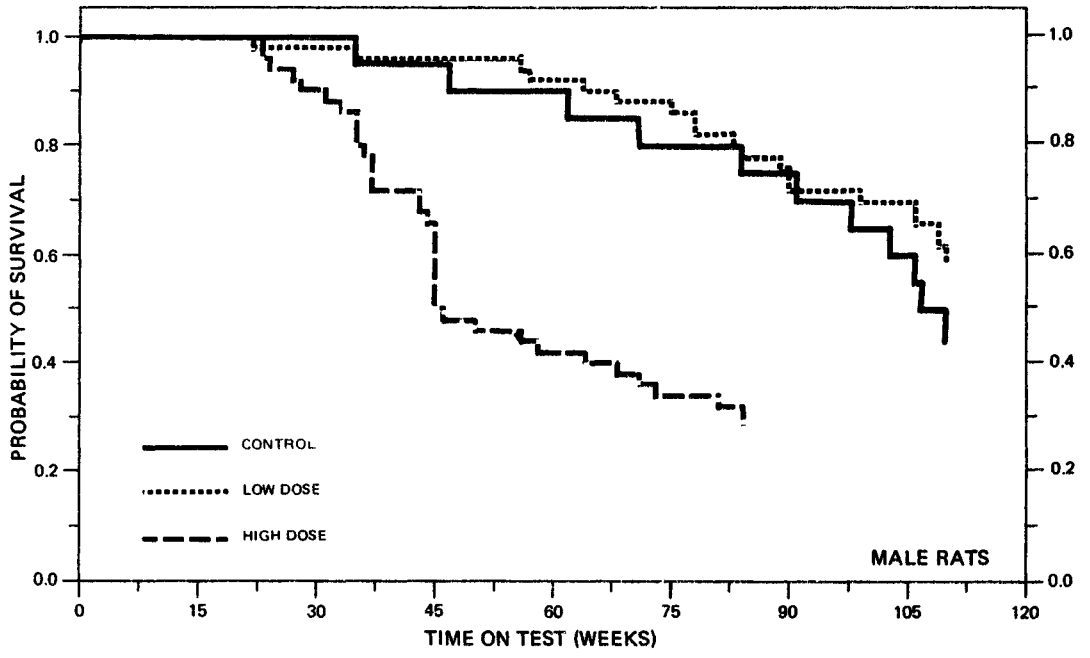
B. Survival

The estimated probabilities of survival for male and female rats in the control and nitrofen-treated groups are shown in Figure 2.

For male rats the Tarone test for positive association between increased dosage and accelerated mortality was significant ($P < 0.001$). The departure from linear trend was also significant ($P < 0.001$), principally because of the accelerated mortality in the high dose group. Fifty percent of the high dose males were dead by week 45; the 15 males still surviving by week 83 were then sacrificed.

Sixty percent of the low dose and 45 percent of the control group survived until the end of the study. As such, there were adequate numbers of low dose and control males, but inadequate numbers of high dose males, at risk to perform a meaningful statistical analysis of the incidence of late-developing tumors.

For female rats the Tarone test indicated a significant ($P = 0.032$) positive association between increased dosage and accelerated



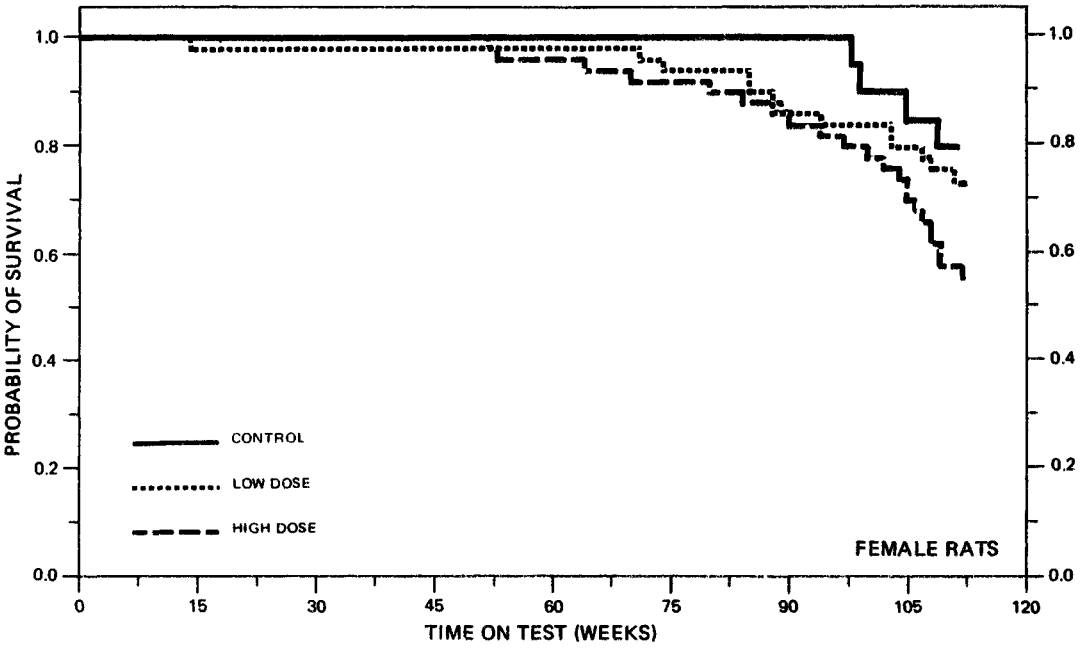


FIGURE 2
SURVIVAL COMPARISONS OF NITROFEN CHRONIC STUDY RATS

mortality. Survival was adequate in all groups as 56 percent of the high dose, 74 percent of the low dose, and 80 percent of the control female rats survived until the end of the study.

C. Pathology

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

Long-term dietary intake of nitrofen was associated with an increased incidence of carcinoma of the pancreas in female rats. This unusual neoplasm occurred in 2/50 (4 percent) low dose and 7/50 (14 percent) high dose females. These were highly invasive neoplasms characterized microscopically by the proliferation of anaplastic epithelial cells forming glands, often sequestered in fibrous tissue, and ducts and papillary structures that were lined by one or more cell layers. Marked desmoplasia, ischemic necrosis, inflammation, and hemorrhage were often associated with these tumors. Most appeared to be ductal carcinomas, but in some areas, were so highly anaplastic and poorly differentiated that little pattern was observed. Poorly formed acini occasionally were recognized, consisting of polygonal, highly basophilic cells with abundant cytoplasm and hyperchromatic nuclei, but zymogen granules were not observed. Peritoneal spread and invasion of abdominal viscera were common, and all metastasized to the lung.

An equivocal increased incidence of neoplasms affecting the reproductive system of female rats was observed including the following: in the vagina, a squamous-cell carcinoma in 1/50 (2 percent) high dose females; in the uterus, a squamous-cell carcinoma in 1/50 (2 percent) low dose females; and uterine adenocarcinomas in 1/50 (2 percent) low dose and 1/49 (2 percent) high dose females. In the ovary, a granulosa-cell carcinoma appeared in 1/20 (5 percent) control and 1/50 (2 percent) low dose females, cystadenocarcinoma in 1/50 (2 percent) low dose females, and granulosa-cell tumors in 4/49 (8 percent) high dose females. Although these types of neoplasms occurred in small numbers, the vaginal and uterine carcinomas represent unusual forms of neoplasia in this strain. There were 3/50 (6 percent) histiocytic malignant lymphomas of multiple organs and 1/49 (2 percent) lymphocytic malignant lymphoma of the uterus in high dose females. A variety of other neoplasms were seen in all groups but appeared unrelated to treatment.

In male rats, a life-shortening effect related to intake of nitrofen was observed, particularly in high dose males. The high dose male group was terminated in week 83 because only 15 animals remained in the study. The principal toxic effect of nitrofen in the high dose male group was massive hemorrhage involving the genitalia and pelvic cavity. Massive centrilobular necrosis, a sequela of hypoxia due to acute hemorrhage, was frequently recognized in the livers of high dose animals. In low dose males, a high incidence

of chronic pneumonia, probably exacerbated by stress, was observed. Although a carcinogenic effect was not demonstrated in male rats, the possible masking effects of toxicity with early mortality should not be dismissed.

Increased incidences of malignant tumors of the pancreas and of the reproductive system provided evidence of carcinogenicity in female rats. The absence of histopathologic evidence of carcinogenicity in male rats could be the result of abbreviated life spans due to compound-related toxicity.

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 for every type of tumor that was observed in more than 5 percent of any of the nitrofen-dosed groups of either sex is included.

Two control groups were used for statistical analyses: the control group originally assigned to nitrofen in the experimental design (designated in this section as the "matched" control group) and a pooled control group which combined the controls from the studies of nitrofen, chlorobenzilate, endosulfan, and mexacarbate. Each chlorobenzilate control group had 50 rats, each of the other groups had 20. The control rats used for the pool were of the same strain, were housed in the same room, were tested concurrently for over a year, and were diagnosed by the same pathologists.

TABLE 3

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

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma or Fibrosarcoma[b]	7/109(0.06)	3/20(0.15)	3/50(0.06)	0/50(0.00)
P Values[c]	N.S.	P = 0.012(N)	N.S.	P = 0.021**(N)
Relative Risk (Pooled Control)[d]	---	---	0.934	0.000
Lower Limit	---	---	0.161	0.000
Upper Limit	---	---	3.880	1.128
Relative Risk (Matched Control)[d]	---	---	0.400	0.000
Lower Limit	---	---	0.060	0.000
Upper Limit	---	---	2.801	0.659
Weeks to First Observed Tumor	---	107	89	---
Pituitary: Chromophobe Adenoma[b]	12/100(0.12)	2/20(0.10)	9/46(0.20)	1/44(0.02)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend	P = 0.025	P = 0.031	---	---
Relative Risk (Pooled Control)[d]	---	---	1.630	0.189
Lower Limit	---	---	0.647	0.004
Upper Limit	---	---	3.868	1.212
Relative Risk (Matched Control)[d]	---	---	1.957	0.227
Lower Limit	---	---	0.462	0.004
Upper Limit	---	---	17.603	4.167
Weeks to First Observed Tumor	---	71	99	84
Thyroid: Follicular-Cell Adenoma[b]	6/108(0.06)	1/20(0.05)	7/50(0.14)	0/47(0.00)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend	P = 0.006	P = 0.019	---	---
Relative Risk (Pooled Control)[d]	---	---	2.520	0.000
Lower Limit	---	---	0.760	0.000
Upper Limit	---	---	8.549	1.440
Relative Risk (Matched Control)[d]	---	---	2.800	0.000
Lower Limit	---	---	0.402	0.000
Upper Limit	---	---	123.408	7.942
Weeks to First Observed Tumor	---	103	90	---

TABLE 3
(CONTINUED)

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Adenoma or Carcinoma[b]	9/108(0.08)	2/20(0.10)	8/50(0.16)	0/47(0.00)
P Values[c]	N.S.	N.S.	N.S.	N.S. P = 0.035*(N)
Departure from Linear Trend	P = 0.010	P = 0.042	---	---
Relative Risk (Pooled Control)[d]	---	---	1.920	0.000
Lower Limit	---	---	0.681	0.000
Upper Limit	---	---	5.226	0.877
Relative Risk (Matched Control)[d]	---	---	1.600	0.000
Lower Limit	---	---	0.364	0.000
Upper Limit	---	---	14.699	1.429
Weeks to First Observed Tumor	---	103	90	---
Thyroid: C-Cell Adenoma[b]	0/108(0.00)	0/20(0.00)	1/50(0.02)	2/47(0.04)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.114	0.671
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.022	0.130
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	112	58
Mammary Gland: Adenocarcinoma, NOS[b]	3/109(0.03)	1/20(0.05)	2/50(0.04)	0/50(0.00)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	1.453	0.000
Lower Limit	---	---	0.124	0.000
Upper Limit	---	---	12.215	3.635
Relative Risk (Matched Control)[d]	---	---	0.800	0.000
Lower Limit	---	---	0.045	0.000
Upper Limit	---	---	46.273	7.475
Weeks to First Observed Tumor	---	111	83	---

TABLE 3
(CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma[b]	1/109(0.01)	0/20(0.00)	2/50(0.04)	0/50(0.00)
P Values[c]	N.S.	N.S.	N.S.	
Relative Risk (Pooled Control)[d]	---	---	4.360	0.000
Lower Limit	---	---	0.231	0.000
Upper Limit	---	---	252.025	40.652
Relative Risk (Matched Control)[d]	---	---	Infinite	---
Lower Limit	---	---	0.123	---
Upper Limit	---	---	Infinite	---
Weeks to First Observed Tumor	---	---	111	---

[a]Dosed groups received time-weighted average doses of 2300 and 3627 ppm in feed.

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

[c]Beneath the incidence of each of the controls is the probability level for the Cochran-Armitage test for dose-related trend in proportions when it is below 0.05, otherwise N.S. - not significant. Departure from linear trend is noted when it is below 0.05 for any comparison. Beneath the dose group incidence is the probability level for the Fisher exact (conditional) test for the comparison of that dose group with the pooled control group (*) and the matched control group (**) when either is below 0.05, otherwise N.S. - not significant.
(N) Less incidence in the dose group(s) than in a control group results in a negative indication.

[d]Relative Risk of the treated group versus the control group is shown along with the lower and upper limit of the 95% confidence interval for that relative risk.

TABLE 4

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

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	6/110(0.05)	2/20(0.10)	0/50(0.00)	0/50(0.00)
P Values[c]	P = 0.029(N)	P = 0.024(N)	N.S.	N.S.
Departure from Linear Trend	---	P = 0.042	---	---
Relative Risk (Pooled Control)[d]	---	---	0.000	0.000
Lower Limit	---	---	0.000	0.000
Upper Limit	---	---	1.381	1.381
Relative Risk (Matched Control)[d]	---	---	0.000	0.000
Lower Limit	---	---	0.000	0.000
Upper Limit	---	---	1.345	1.345
Weeks to First Observed Tumor	---	111	---	---
Hematopoietic System: Lymphoma[b]	1/110(0.01)	0/20(0.00)	0/50(0.00)	4/50(0.08)
P Values[c]	P = 0.016	P = 0.039	N.S.	P = 0.033*
Relative Risk (Pooled Control)[d]	---	---	0.000	8.800
Lower Limit	---	---	0.000	0.098
Upper Limit	---	---	41.028	424.104
Relative Risk (Matched Control)[d]	---	---	---	Infinite
Lower Limit	---	---	---	0.386
Upper Limit	---	---	---	Infinite
Weeks to First Observed Tumor	---	---	---	88
Pituitary: Chromophobe Adenoma[b]	37/107(0.35)	3/18(0.17)	15/49(0.31)	18/48(0.38)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	0.885	1.085
Lower Limit	---	---	0.496	0.645
Upper Limit	---	---	1.469	1.717
Relative Risk (Matched Control)[d]	---	---	1.837	2.250
Lower Limit	---	---	0.618	0.783
Upper Limit	---	---	9.084	10.870
Weeks to First Observed Tumor	---	98	103	105

TABLE 4
(CONTINUED)

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Adenoma[b]	4/108(0.04)	1/19(0.05)	2/49(0.04)	4/50(0.08)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	1.102	2.160
Lower Limit	---	---	0.103	0.418
Upper Limit	---	---	7.363	11.071
Relative Risk (Matched Control)[d]	---	---	0.776	1.520
Lower Limit	---	---	0.044	0.168
Upper Limit	---	---	44.838	73.309
Weeks to First Observed Tumor	---	111	103	112
Thyroid: Follicular-Cell Adenoma or Carcinoma[b]	5/108(0.05)	2/19(0.11)	3/49(0.06)	4/50(0.08)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	1.322	1.728
Lower Limit	---	---	0.211	0.357
Upper Limit	---	---	6.470	7.632
Relative Risk (Matched Control)[d]	---	---	0.582	0.760
Lower Limit	---	---	0.074	0.122
Upper Limit	---	---	6.640	8.007
Weeks to First Observed Tumor	---	111	103	112
Thyroid: C-Cell Adenoma or Carcinoma[b]	6/108(0.06)	0/19(0.00)	3/49(0.06)	2/50(0.04)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	1.102	0.720
Lower Limit	---	---	0.184	0.073
Upper Limit	---	---	4.897	3.836
Relative Risk (Matched Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.243	0.117
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	112	112

TABLE 4
(CONTINUED)

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Pancreas: Carcinoma, NOS[b]	0/110(0.00)	0/20(0.00)	2/50(0.04)	7/50(0.14)
P Values[c]	P < 0.001	P = 0.021	N.S.	P < 0.001*
Relative Risk (Pooled Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.640	4.206
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.123	0.809
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	94	70
Mammary Gland: Adenocarcinoma, NOS[b]	3/110(0.03)	1/20(0.05)	3/50(0.06)	4/50(0.08)
P Values[c]	P = 0.094	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	2.200	2.933
Lower Limit	---	---	0.303	0.509
Upper Limit	---	---	15.792	19.228
Relative Risk (Matched Control)[d]	---	---	1.200	1.600
Lower Limit	---	---	0.105	0.175
Upper Limit	---	---	61.724	77.169
Weeks to First Observed Tumor	---	98	14	94
Mammary Gland: Fibroadenoma[b]	32/110(0.29)	7/20(0.35)	10/50(0.20)	12/50(0.24)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	0.688	0.825
Lower Limit	---	---	0.324	0.419
Upper Limit	---	---	1.300	1.483
Relative Risk (Matched Control)[d]	---	---	0.571	0.686
Lower Limit	---	---	0.240	0.305
Upper Limit	---	---	1.558	1.806
Weeks to First Observed Tumor	---	111	89	112

TABLE 4
(CONTINUED)

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroma or Fibroadenoma[b]	32/110(0.29)	7/20(0.35)	11/50(0.22)	12/50(0.24)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	0.756	0.825
Lower Limit	---	---	0.371	0.427
Upper Limit	---	---	1.392	1.493
Relative Risk (Matched Control)[d]	---	---	0.629	0.686
Lower Limit	---	---	0.272	0.305
Upper Limit	---	---	1.683	1.806
Weeks to First Observed Tumor	---	111	89	112
Uterus: Endometrial Stromal Polyp[b]	8/109(0.07)	2/20(0.10)	3/50(0.06)	1/49(0.02)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	0.834	0.284
Lower Limit	---	---	0.147	0.006
Upper Limit	---	---	3.222	1.977
Relative Risk (Matched Control)[d]	---	---	0.612	0.208
Lower Limit	---	---	0.077	0.004
Upper Limit	---	---	6.860	3.754
Weeks to First Observed Tumor	---	111	107	112
Ovary: Granulosa-Cell Tumor[b]	1/109(0.01)	0/20(0.00)	0/50(0.00)	4/49(0.08)
P Values[c]	P = 0.015	P = 0.038	N.S.	P = 0.032*
Relative Risk (Pooled Control)[d]	---	---	0.000	8.898
Lower Limit	---	---	0.000	0.905
Upper Limit	---	---	40.652	428.730
Relative Risk (Matched Control)[d]	---	---	---	Infinite
Lower Limit	---	---	---	0.394
Upper Limit	---	---	---	Infinite
Weeks to First Observed Tumor	---	---	---	112

TABLE 4
(CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Ovary: Granulosa-Cell Tumor or Carcinoma[b]	2/109(0.02)	1/20(0.05)	1/50(0.02)	4/49(0.08)
P Values[c]	P = 0.050	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	1.090	4.449
Lower Limit	---	---	0.019	0.658
Upper Limit	---	---	20.319	47.561
Relative Risk (Matched Control)[d]	---	---	0.400	1.633
Lower Limit	---	---	0.005	0.179
Upper Limit	---	---	30.802	78.704
Weeks to First Observed Tumor	---	99	112	112

[a]Dosed groups received time-weighted average doses of 1300 and 2600 ppm in feed.

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

[c]Beneath the incidence of each of the controls is the probability level for the Cochran-Armitage test for dose-related trend in proportions when it is below 0.05, otherwise N.S. - not significant. Departure from linear trend is noted when it is below 0.05 for any comparison. Beneath the dose group incidence is the probability level for the Fisher exact (conditional) test for the comparison of that dose group with the pooled control group (*) and the matched control group (**) when either is below 0.05, otherwise N.S. - not significant.
(N) Less incidence in the dose group(s) than in a control group results in a negative indication.

[d]Relative Risk of the treated group versus the control group is shown along with the lower and upper limit of the 95% confidence intercal for that relative risk.

The incidence of carcinomas of the pancreas was high in female rats. The Cochran-Armitage test indicated a significant positive association between increased dosage and elevated tumor incidence when comparing either the pooled ($P < 0.001$) or the matched ($P = 0.021$) control. The Fisher exact test confirmed these results with a statistically significant comparison ($P < 0.001$) of the high dose group to the pooled control. The lower limit of the 95 percent confidence interval on the relative risk of the high dose to the pooled control was greater than the value one.

In female rats the Cochran-Armitage test showed a significant positive association between increased dosage and an elevated incidence of lymphomas whether compared to the matched control ($P = 0.039$) or to the pooled control ($P = 0.016$). The Fisher exact test did not confirm this increased incidence of lymphomas as the probability level ($P = 0.033$) of the comparison of the high dose to the pooled control was not significant under the Bonferroni criterion.

The incidence of granulosa-cell tumors of the ovary was also noted in female rats. The Cochran-Armitage test was significant in comparisons involving both the pooled ($P = 0.015$) and the matched ($P = 0.038$) controls. The Fisher exact tests did not support these findings, however, when the Bonferroni criterion was applied. When the incidences of ovarian carcinomas were combined with the incidences of ovarian granulosa-cell tumors, again the Fisher exact tests

did not indicate that the relationship between treatment and incidence was statistically significant.

Based upon these results, the statistical conclusion is that in female Osborne-Mendel rats the incidences of carcinomas of the pancreas were associated with the administration of nitrofen at the dose levels used in this experiment.

In male rats, for C-cell adenomas of the thyroid, the Fisher exact test comparing the high dose to the pooled control was significant ($P = 0.031$). The Cochran-Armitage test and the Fisher exact test comparing low dose to control did not confirm this finding. Because of the poor survival in the high dose male rats, an additional analysis was conducted based upon males that survived at least 52 weeks; however, no statistically significant results were found. Because of the lack of supporting results and because only two such tumors were found in the high dose group, there was insufficient statistical evidence to conclude that the tumor incidence was associated with the administration of nitrofen.

To provide additional insight, 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 a

significantly increased rate of tumor incidence induced 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

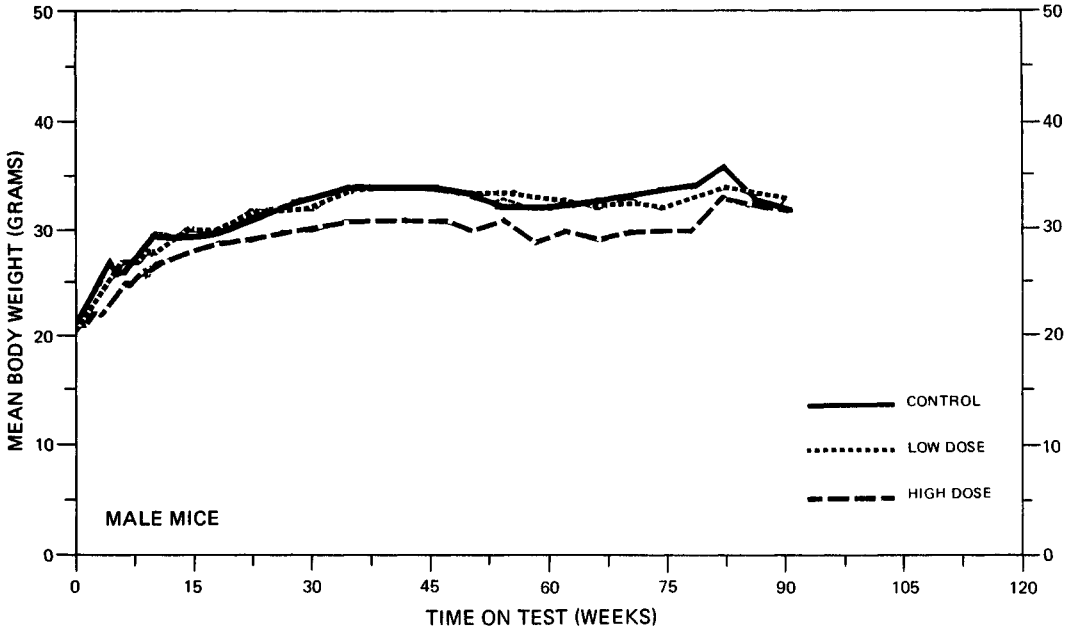
As indicated in Figure 3, weight gain of high dose male mice was depressed relative to low dose male mice. Mean body weights of male controls were close to those of low dose males but occasionally fell as low as those of high dose males. A dose-related depression in body weight gain was evident for female mice.

During the first year of the bioassay, patterns of appearance and behavior were generally comparable for treated and control mice except that body sores and alopecia (usually associated with fighting) were observed as early as week 2 in the treated males. Other clinical signs often observed in group-housed laboratory mice were noted at a comparable rate in control and treated groups. These signs included a hunched appearance, penile, vulvar, or anal irritation, squinted or reddened eyes, palpable nodules, and rough or stained fur.

Beginning in week 54 and until termination of the study, bloating or pronounced abdominal distension was displayed by an increasing number of treated mice, particularly in the females. Necropsy of these animals revealed liver tumors that were subsequently diagnosed as hepatocellular carcinomas.

B. Survival

The estimated probabilities of survival for male and female mice in the control and nitrofen-treated groups are shown in Figure 4.



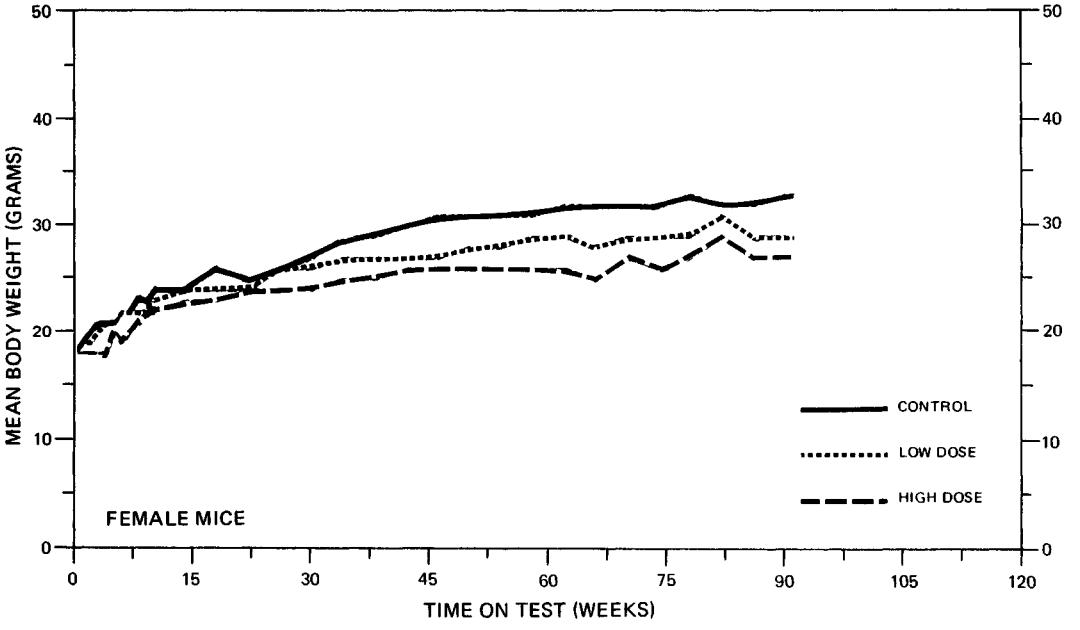
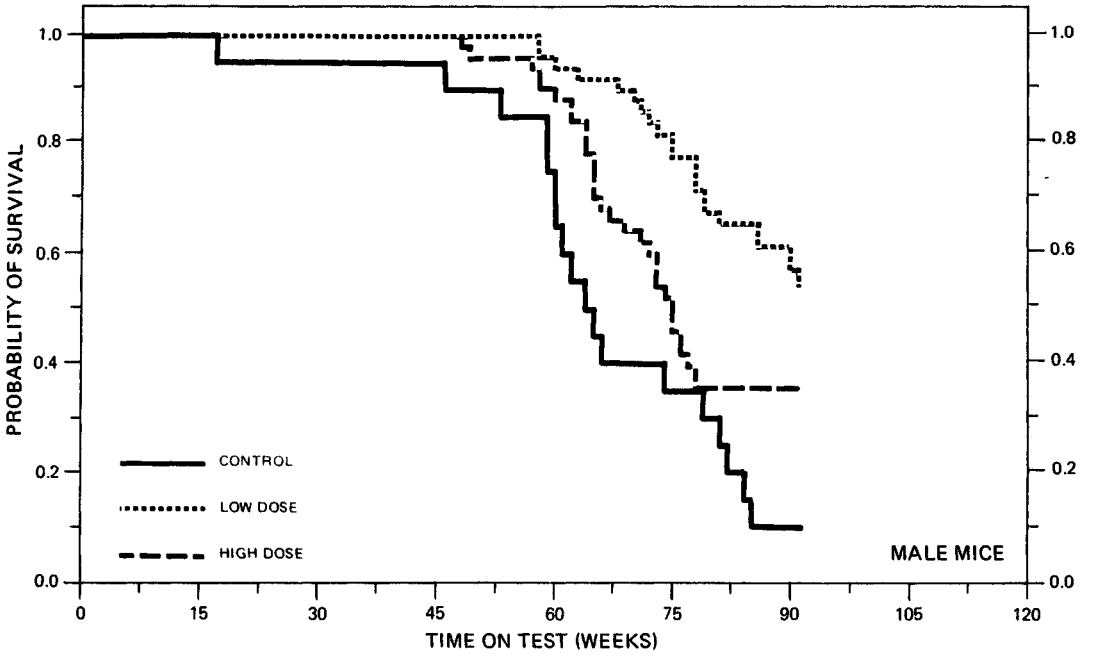


FIGURE 3
GROWTH CURVES FOR NITROFEN CHRONIC STUDY MICE



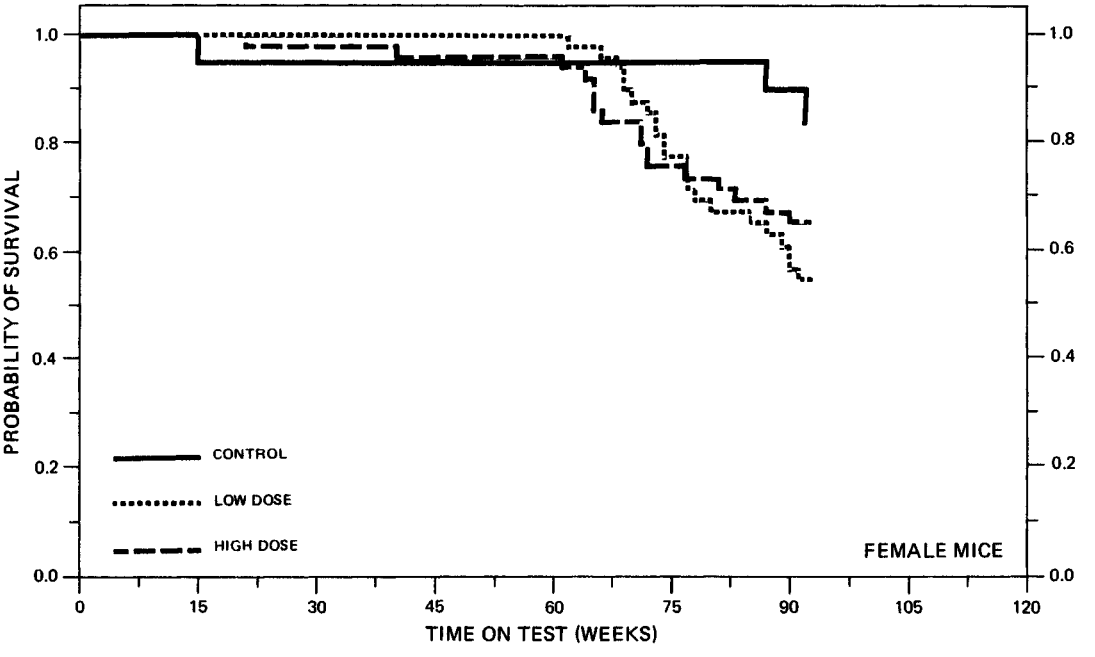


FIGURE 4
SURVIVAL COMPARISONS OF NITROFEN CHRONIC STUDY MICE

For male mice the Tarone test did not indicate a significant positive association between dosage and mortality. The survival of the control group was unexpectedly poor, as only 10 percent survived until the end of the test compared to 34 percent of the high dose and 54 percent of the low dose male mice. Sixty percent of the control mice were dead by week 67, leaving only eight animals at risk from late-developing tumors. No common cause for these early deaths could be detected, but 11 of the 12 had amyloidosis at multiple sites and chronic inflammation of the kidney.

For female mice, again, the Tarone test did not indicate a significant positive association between dosage and mortality. Survival was adequate in all groups as 62 percent of the high dose, 54 percent of the low dose, and 85 percent of the control female mice survived until the end of the study.

C. Pathology

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

Long-term dietary intake of nitrofen was associated with a high incidence of hepatocellular carcinoma in both sexes and at all dose levels. This liver tumor occurred in 4/20 (20 percent) control males, 36/49 (73 percent) low dose males, 46/48 (96 percent) high dose males, 36/41 (88 percent) low dose females, and 43/44 (98 percent) high dose females. These were primarily confined to the liver, but a few

metastasized. The hepatocellular carcinomas were characterized microscopically by proliferating eosinophilic or basophilic swollen hepatocytes forming liver plates usually one or more cells in thickness and by compressed adjacent parenchyma. Although most tumors were relatively well-differentiated, occasional anaplastic lesions were composed of intensely basophilic staining hepatocytes forming pseudo-acini and thick blunted liver plates.

Hemangiosarcoma of the liver occurred in 1/49 (2 percent) low dose males, 2/48 (4 percent) high dose males, and 4/44 (9 percent) high dose females. Also considered spontaneous were subcutaneous fibrosarcomas which were observed in 8/44 (18 percent) low dose males but not in high dose males, matched controls or treated females.

In the urinary bladder, 2/40 (5 percent) high dose males had transitional-cell carcinomas, while 1/41 (2 percent) high dose females had a transitional-cell papilloma. These are normally very infrequent tumors in the urinary bladder of this strain. However, the low incidence of these lesions does not provide adequate evidence for a carcinogenic effect.

The increased incidence of hepatocellular carcinomas in all groups of mice receiving nitrofen in the diet provides histopathologic evidence of carcinogenicity in B6C3F1 mice of both sexes.

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 for every type

TABLE 5

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

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	0/74(0.00)	0/20(0.00)	2/44(0.05)	0/46(0.00)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend	P = 0.020	---	---	---
Relative Risk (Pooled Control)[d]	---	---	Infinite	---
Lower Limit	---	---	0.494	---
Upper Limit	---	---	Infinite	---
Relative Risk (Matched Control)[d]	---	---	Infinite	---
Lower Limit	---	---	0.140	---
Upper Limit	---	---	Infinite	---
Weeks to First Observed Tumor	---	---	92	---
Subcutaneous Tissue: Fibrosarcoma[b]	3/74(0.04)	0/20(0.00)	8/44(0.18)	0/46(0.00)
P Values[c]	N.S.	N.S.	P = 0.040** P = 0.014*	N.S.
Departure from Linear Trend	P < 0.001	P < 0.001	---	---
Relative Risk (Pooled Control)[d]	---	---	4.485	0.000
Lower Limit	---	---	1.142	0.000
Upper Limit	---	---	24.852	2.673
Relative Risk (Matched Control)[d]	---	---	Infinite	---
Lower Limit	---	---	1.086	---
Upper Limit	---	---	Infinite	---
Weeks to First Observed Tumor	---	---	75	---
Subcutaneous Tissue: Fibroma or Fibrosarcoma[b]	3/74(0.04)	0/20(0.00)	10/44(0.23)	0/46(0.00)
P Values[c]	N.S.	N.S.	P = 0.016** P = 0.003*	N.S.
Departure from Linear Trend	P < 0.001	P < 0.001	---	---
Relative Risk (Pooled Control)[d]	---	---	5.606	0.000
Lower Limit	---	---	1.536	0.000
Upper Limit	---	---	29.925	2.673
Relative Risk (Matched Control)[d]	---	---	Infinite	---
Lower Limit	---	---	1.412	---
Upper Limit	---	---	Infinite	---
Weeks to First Observed Tumor	---	---	75	---

TABLE 5
(CONTINUED)

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Lymphoma[b]	1/74(0.01)	0/20(0.00)	1/44(0.02)	0/46(0.00)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	1.682	0.000
Lower Limit	---	---	0.022	0.000
Upper Limit	---	---	129.041	29.957
Relative Risk (Matched Control)[d]	---	---	Infinite	---
Lower Limit	---	---	0.025	---
Upper Limit	---	---	Infinite	---
Weeks to First Observed Tumor	---	---	58	---
Liver: Hepatocellular Carcinoma[b]	9/74(0.12)	4/20(0.20)	36/49(0.73)	46/48(0.96)
P Values[c]	P < 0.001	P < 0.001	P < 0.001** P < 0.001*	P < 0.001** P < 0.001*
Departure from Linear Trend	P = 0.016	---	---	---
Relative Risk (Pooled Control)[d]	---	---	6.041	7.880
Lower Limit	---	---	3.285	4.927
Upper Limit	---	---	11.623	10.091
Relative Risk (Matched Control)[d]	---	---	3.673	4.792
Lower Limit	---	---	1.616	2.360
Upper Limit	---	---	11.735	8.369
Weeks to First Observed Tumor	---	62	58	57
Urinary Bladder: Transitional-Cell Carcinoma[b]	0/69(0.00)	0/20(0.00)	0/47(0.00)	2/40(0.05)
P Values[c]	P = 0.051	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	---	Infinite
Lower Limit	---	---	---	0.510
Upper Limit	---	---	---	Infinite
Relative Risk (Matched Control)[d]	---	---	---	Infinite
Lower Limit	---	---	---	0.153
Upper Limit	---	---	---	Infinite
Weeks to First Observed Tumor	---	---	---	92

TABLE 5
(CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
All Sites: Hemangiosarcoma[b]	0/74(0.00)	0/20(0.00)	1/44(0.02)	4/48(0.08)
P Values[c]	P = 0.010	P = 0.074	N.S.	P = 0.022*
Relative Risk (Pooled Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.090	1.417
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	0.023	0.402
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	92	92

[a]Dosed groups received time-weighted average doses of 2348 and 4696 ppm in feed.

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

[c]Beneath the incidence of each of the controls is the probability level for the Cochran-Armitage test for dose-related trend in proportions when it is below 0.05, otherwise N.S. - not significant. Departure from linear trend is noted when it is below 0.05 for any comparison. Beneath the dose group incidence is the probability level for the Fisher exact (conditional) test for the comparison of that dose group with the pooled control group (*) and the matched control group (**) when either is below 0.05, otherwise N.S. - not significant.
(N) Less incidence in the dose group(s) than in a control group results in a negative indication.

[d]Relative Risk of the treated group versus the control group is shown along with the lower and upper limit of the 95% confidence interval for that relative risk.

TABLE 6

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

TOPOGRAPHY:MORPHOLOGY	POOLED CONTROL	MATCHED CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Lymphoma[b]	11/80(0.14)	2/20(0.10)	3/40(0.08)	2/45(0.05)
P Values[c]	P = 0.056(N)	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	0.545	0.323
Lower Limit	---	---	0.102	0.036
Upper Limit	---	---	1.913	1.390
Relative Risk (Matched Control)[d]	---	---	0.750	0.444
Lower Limit	---	---	0.095	0.035
Upper Limit	---	---	8.508	5.844
Weeks to First Observed Tumor	---	92	91	81
Liver: Hepatocellular Carcinoma[b]	0/80(0.00)	0/19(0.00)	36/41(0.88)	43/44(0.98)
P Values[c]	P < 0.001	P < 0.001	P < 0.001** P < 0.001*	P < 0.001** P < 0.001*
Departure from Linear Trend	P < 0.001	P < 0.001	---	---
Relative Risk (Pooled Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	24.549	30.137
Upper Limit	---	---	Infinite	Infinite
Relative Risk (Matched Control)[d]	---	---	Infinite	Infinite
Lower Limit	---	---	6.116	7.434
Upper Limit	---	---	Infinite	Infinite
Weeks to First Observed Tumor	---	---	62	64
All Sites: Hemangiosarcoma[b]	2/80(0.03)	1/18(0.06)	0/41(0.00)	5/44(0.11)
P Values[c]	P = 0.032	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[d]	---	---	0.000	4.545
Lower Limit	---	---	0.000	0.777
Upper Limit	---	---	6.570	45.880
Relative Risk (Matched Control)[d]	---	---	0.000	2.045
Lower Limit	---	---	0.000	0.258
Upper Limit	---	---	8.171	94.395
Weeks to First Observed Tumor	---	87	---	87

[a]Dosed groups received time-weighted average doses of 2348 and 4696 ppm in feed.

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

[c]Beneath the incidence of each of the controls is the probability level for the Cochran-Armitage test for dose-related trend in proportions when it is below 0.05, otherwise N.S. - not significant. Departure from linear trend is noted when it is below 0.05 for any comparison. Beneath the dose group incidence is the probability level for the Fisher exact (conditional) test for the comparison of that dose group with the pooled control group (*) and the matched control group (**) when either is below 0.05, otherwise N.S. - not significant.
(N) Less incidence in the dose group(s) than in a control group results in a negative indication.

[d]Relative Risk of the treated group versus the control group is shown along with the lower and upper limit of the 95% confidence interval for that relative risk.

of tumor that was observed in more than 5 percent of any of the nitrofen-dosed groups of either sex is included.

Two control groups were used for statistical analyses: the control group originally assigned to the nitrofen bioassay in the experimental design (designated in this section as the "matched" control group) and a pooled control group that combined the controls from the studies of nitrofen, chlorobenzilate, endosulfan, and mexacarbate. Each of the control groups had 20 mice. The control mice used for the pool were of the same strain, were housed in the same room, were tested concurrently for at least one year, and were diagnosed by the same pathologists.

A high incidence of hepatocellular carcinoma was observed in dosed mice. For both sexes the Cochran-Armitage test indicated a highly significant ($P < 0.001$) positive association between dosage and tumor incidence when compared with either the matched controls or the pooled controls. The departure from linear trend was significant because of the extremely high incidence in both high and low dose groups. For both sexes, both the high dose and low dose groups had a significantly ($P < 0.001$) higher incidence of hepatocellular carcinomas than either of the controls. In all cases the lower limit of the confidence interval on the relative risk was greater than the value one. Finally, in the historical controls on B6C3F1 mice (compiled to date for this specific laboratory by the NCI Bioassay Program) hepatocellular carcinomas were found in 13/180 (7 percent) of

the males and 4/180 (2 percent) of the females, substantially lower rates than observed in the dosed mice.

Based upon these results the statistical conclusion is that nitrofen had a carcinogenic effect on the livers of B6C3F1 mice at the dose levels of this experiment.

Hemangiosarcomas were also noted in both male and female mice. The Cochran-Armitage test showed a significant positive association between increased dose and elevated tumor incidence for both males (P = 0.010) and females (P = 0.032) when compared to the pooled controls. The Fisher exact test showed a significantly (P = 0.022) higher incidence in the high dose than in the pooled control for males, but for females the results were not significant.

Based on these results the statistical conclusion is that the incidence of hemangiosarcomas in male B6C3F1 mice was associated with the administration of nitrofen at the dose levels of this experiment.

In low dose male mice the Fisher exact tests showed a significantly higher incidence of either fibromas or fibrosarcomas of the subcutaneous tissue than in the pooled control (P = 0.014); the comparison of matched control to low dose was not significant under the Bonferroni criterion. It was questionable, however, whether this tumor was dose-related since none of these tumors were observed in the high dose group.

V. DISCUSSION

Under the conditions of this study the administration of nitrofen was associated with a highly significant incidence of hepatocellular carcinomas and an elevated incidence of hemangiosarcoma of the liver in mice of both sexes. In female rats, nitrofen was associated with an increased incidence of carcinomas of the pancreas, and various tumors of the reproductive system. Because of an accelerated early mortality, the number of male rats at risk from late-developing tumors was inadequate for meaningful statistical analyses.

In mice of both sexes, the incidence of hepatocellular carcinoma showed a highly significant positive association with concentration of nitrofen in the diet. The incidences of hepatocellular carcinomas for each dosed group were highly significant when compared to either control group. A statistically significant positive association between dosage and incidence of hemangiosarcomas of the liver was observed in mice of both sexes. The incidence in the high dose male mice, but not female or low dose male mice, was significantly higher than controls.

The incidence of carcinomas of the pancreas had a statistically significant positive association with concentration of nitrofen in diets of female rats. The high dose group had a statistically significant incidence of carcinoma of the pancreas when compared to the pooled controls. The fact that this is an unusual neoplasm in the

Osborne-Mendel rat is further indication that occurrence of this tumor is related to administration of nitrofen.

The results of this study indicate that nitrofen is a liver carcinogen, causing hepatocellular carcinomas in B6C3F1 mice of both sexes and hemangiosarcoma of the liver in male mice. In addition, the compound is carcinogenic to female Osborne-Mendel rats, causing an increased incidence of pancreatic carcinomas. Survival of the high dose male rats was not adequate for meaningful statistical analysis of tumor incidence.

VI. BIBLIOGRAPHY

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

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

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

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

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

Doroshenko, G.V., "Hygenic Characteristics of Working Conditions and Health Status of Persons Handling a Complex of Pesticides in Gardening." Gigiena Truda I Professional-nye Zabolevaniya 2:12-14, 1975.

Environmental Protection Agency, Compendium of Registered Pesticides, Vol. I supplement. June 30, 1975.

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

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

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

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

Reuber, M.D., and E.L. Glover, "Cirrhosis and Carcinoma of the Liver in Male Rats Given Subcutaneous Carbon Tetrachloride." Journal of the National Cancer Institute 44:419-423, 1970.

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

Stanford Research Institute, 1975 Directory of Chemical Producers, U.S.A. Menlo Park California, 1975.

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

Weed Science Society of America, "Nitrofen." Herbicide Handbook. Champaign, Illinois, 1974.

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 (VEH) 01-M060	LOW DOSE 01-M061	HIGH DOSE 01-M062
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
PAPILLOMA, NOS		2 (4%)	
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA	1 (5%)	2 (4%)	
FIBROSARCOMA	2 (10%)	1 (2%)	
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
LIPOMA		2 (4%)	1 (2%)
RESPIRATORY SYSTEM			
*LUNG	(20)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
CORTICAL CARCINOMA, METASTATIC		1 (2%)	
FIBROUS HISTIOCYTOMA, METASTATIC		1 (2%)	
MIXED TUMOR, METASTATIC	1 (5%)		
HEMATOPOIETIC SYSTEM			
*SPLEEN	(20)	(50)	(50)
FIBROSARCOMA, METASTATIC		1 (2%)	
FIBROUS HISTIOCYTOMA, METASTATIC		1 (2%)	
HEMANGIOMA	1 (5%)		
HEMANGIOSARCOMA	2 (10%)	2 (4%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*LIVER	(20)	(50)	(50)
FIBROUS HISTIOCYTOMA, METASTATIC		1 (2%)	

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

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	CONTROL (VEH) 01-M060	LOW DOSE 01-M061	HIGH DOSE 01-M062
#PANCREAS	(18)	(50)	(48)
FIBROSARCOMA, METASTATIC		1 (2%)	
#COLON	(20)	(50)	(49)
ADENOMATOUS POLYP, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
CORTICAL CARCINOMA, METASTATIC		1 (2%)	
MIXED TUMOR, MALIGNANT	1 (5%)	2 (4%)	
#URINARY BLADDER	(20)	(49)	(48)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	
FIBROUS HISTIOCYTOMA, METASTATIC		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(46)	(44)
CHROMOPHOBE ADENOMA	2 (10%)	9 (20%)	1 (2%)
#ADRENAL	(20)	(50)	(49)
CORTICAL CARCINOMA		1 (2%)	
MIXED TUMOR, METASTATIC	1 (5%)		
#THYROID	(20)	(50)	(47)
FOLLICULAR-CELL ADENOMA	1 (5%)	7 (14%)	
FOLLICULAR-CELL CARCINOMA	1 (5%)	1 (2%)	
C-CELL ADENOMA		1 (2%)	2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOCARCINOMA, NOS	1 (5%)	2 (4%)	
FIBROADENOMA		2 (4%)	
#TESTIS	(20)	(50)	(49)
INTERSTITIAL-CELL TUMOR		2 (4%)	1 (2%)
NERVOUS SYSTEM			
NONE			

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

TABLE A1 (CONTINUED)

	CONTROL (VEH) 01-M060	LOW DOSE 01-M061	HIGH DOSE 01-M062
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(20)	(50)	(50)
FIBROSARCOMA, METASTATIC		1 (2%)	
*ABDOMINAL CAVITY	(20)	(50)	(50)
FIBROSARCOMA		1 (2%)	
*MESENTERY	(20)	(50)	(50)
FIBROSARCOMA, METASTATIC		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	10	19	35
MORIBUND SACRIFICE	1	1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	9	30	15
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	CONTROL (VEH) 01-M060	LOW DOSE 01-M061	HIGH DOSE 01-M062
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	10	30	4
TOTAL PRIMARY TUMORS	12	40	5
TOTAL ANIMALS WITH BENIGN TUMORS	5	23	4
TOTAL BENIGN TUMORS	5	28	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	11	
TOTAL MALIGNANT TUMORS	7	12	
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	4	
TOTAL SECONDARY TUMORS	2	11	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH NITROFEN

	CONTROL (VEH) 01-F060	LOW DOSE 01-F063	HIGH DOSE 01-F064
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%)
KERATOACANTHOMA			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA	2 (10%)		
RESPIRATORY SYSTEM			
*LUNG	(20)	(50)	(50)
CARCINOMA, NOS, METASTATIC		2 (4%)	7 (14%)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
**MULTIPLE ORGANS	(20)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			3 (6%)
*SPLEEN	(20)	(50)	(50)
CARCINOMA, NOS, METASTATIC		1 (2%)	4 (8%)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
GRANULOSA-CELL CARCINOMA, METAST	1 (5%)		
HEMANGIOSARCOMA			1 (2%)
*UTERUS	(20)	(50)	(49)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE A2 (CONTINUED)

	CONTROL (VEH) 01-F060	LOW DOSE 01-F063	HIGH DOSE 01-F064
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
CARCINOMA, NOS, METASTATIC		2 (4%)	3 (6%)
#PANCREAS	(20)	(50)	(50)
CARCINOMA,NOS		2 (4%)	7 (14%)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
#ESOPHAGUS	(20)	(50)	(39)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
#STOMACH	(20)	(49)	(50)
CARCINOMA, NOS, METASTATIC		2 (4%)	7 (14%)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
#SMALL INTESTINE	(19)	(50)	(49)
CARCINOMA, NOS, METASTATIC			5 (10%)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
#LARGE INTESTINE	(20)	(49)	(49)
CARCINOMA, NOS, METASTATIC			3 (6%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
CARCINOMA, NOS, METASTATIC		1 (2%)	2 (4%)
TUBULAR-CELL ADENOCARCINOMA			1 (2%)
MIXED TUMOR, MALIGNANT	1 (5%)		1 (2%)
HAMARTOMA			1 (2%)
#URINARY BLADDER	(18)	(48)	(49)
CARCINOMA, NOS, METASTATIC		1 (2%)	2 (4%)
ENDOMETRIAL STROMAL SARCOMA, MET		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(49)	(48)
CHROMOPHOBE ADENOMA	3 (17%)	15 (31%)	18 (38%)
#ADRENAL	(20)	(50)	(49)
CARCINOMA, NOS, METASTATIC			2 (4%)
PHEOCHROMOCYTOMA			1 (2%)

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

TABLE A2 (CONTINUED)

	CONTROL (VEH) 01-F060	LOW DOSE 01-F063	HIGH DOSE 01-F064
*THYROID	(19)	(49)	(50)
FOLLICULAR-CELL ADENOMA	1 (5%)	2 (4%)	4 (8%)
FOLLICULAR-CELL CARCINOMA	1 (5%)	1 (2%)	
C-CELL ADENOMA		2 (4%)	1 (2%)
C-CELL CARCINOMA		1 (2%)	1 (2%)
*PANCREATIC ISLETS	(20)	(50)	(50)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOCARCINOMA, NOS	1 (5%)	3 (6%)	4 (8%)
FIBROMA		1 (2%)	
FIBROADENOMA	7 (35%)	10 (20%)	12 (24%)
*VAGINA	(20)	(50)	(50)
CARCINOMA, NOS, METASTATIC			1 (2%)
SQUAMOUS CELL CARCINOMA			1 (2%)
*UTERUS	(20)	(50)	(49)
CARCINOMA, NOS, METASTATIC			2 (4%)
SQUAMOUS CELL CARCINOMA		1 (2%)	
ADENOCARCINOMA, NOS		1 (2%)	1 (2%)
ENDOMETRIAL STROMAL POLYP	2 (10%)	3 (6%)	1 (2%)
ENDOMETRIAL STROMAL SARCOMA		1 (2%)	
*OVARY	(20)	(50)	(49)
CARCINOMA, NOS, METASTATIC		1 (2%)	2 (4%)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
CYSTADENOCARCINOMA, NOS		1 (2%)	
GRANULOSA-CELL TUMOR			4 (8%)
GRANULOSA-CELL CARCINOMA	1 (5%)	1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(20)	(50)	(50)
SQUAMOUS CELL PAPILLOMA		1 (2%)	

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

TABLE A2 (CONTINUED)

	CONTROL (VEH) 01-F060	LOW DOSE 01-F063	HIGH DOSE 01-F064
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)
*MESENTERY	(20)	(50)	(50)
CARCINOMA, NOS, METASTATIC		1 (2%)	2 (4%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	12	22
MORIBUND SACRIFICE		1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	37	28
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL (VEH) 01-P060	LOW DOSE 01-P063	HIGH DOSE 01-P064
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	31	42
TOTAL PRIMARY TUMORS	19	47	66
TOTAL ANIMALS WITH BENIGN TUMORS	13	26	25
TOTAL BENIGN TUMORS	15	35	39
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	10	20
TOTAL MALIGNANT TUMORS	4	12	23
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	5	8
TOTAL SECONDARY TUMORS	1	19	44
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			4
TOTAL UNCERTAIN TUMORS			4
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 NITROFEN

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

	CONTROL (VEH) 02-M067	LOW DOSE 02-M068	HIGH DOSE 02-M069
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	20	44	46
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	44	46
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(44)	(46)
LYMPHANGIOMA	1 (5%)		
*SUBCUT TISSUE	(20)	(44)	(46)
FIBROMA		2 (5%)	
FIBROSARCOMA		8 (18%)	
HEMANGIOPERICYTOMA, NOS		1 (2%)	
NEUROFIBROMA		1 (2%)	
RESPIRATORY SYSTEM			
*LUNG	(20)	(48)	(46)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(44)	(46)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
*SPLEEN	(20)	(49)	(47)
HEMANGIOSARCOMA			2 (4%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*LIVER	(20)	(49)	(48)
HEPATOCELLULAR CARCINOMA	4 (20%)	36 (73%)	46 (96%)

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

TABLE B1 (CONTINUED)

	CONTROL (VEH) 02-M067	LOW DOSE 02-M068	HIGH DOSE 02-M069
HEMANGIOSARCOMA		1 (2%)	2 (4%)
*BILE DUCT	(20)	(44)	(46)
BILE DUCT CARCINOMA		1 (2%)	
URINARY SYSTEM			
*URINARY BLADDER	(20)	(47)	(40)
TRANSITIONAL-CELL CARCINOMA			2 (5%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*PROSTATE	(18)	(42)	(28)
PAPILLOMA, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B1 (CONCLUDED)

	CONTROL (VEH) 02-M067	LOW DOSE 02-M068	HIGH DOSE 02-M069
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	18	22	32
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	2	27	17
ANIMAL MISSING			1
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	38	46
TOTAL PRIMARY TUMORS	6	53	53
TOTAL ANIMALS WITH BENIGN TUMORS	2	5	1
TOTAL BENIGN TUMORS	2	5	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	38	46
TOTAL MALIGNANT TUMORS	4	47	52
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
THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH NITROFEN

	CONTROL (VEH) 02-F067	LOW DOSE 02-F070	HIGH DOSE 02-F071
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	2
ANIMALS NECROPSIED	20	39	43
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	39	43
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(38)	(45)
HEPATOCELLULAR CARCINOMA, METAST			2 (4%)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (3%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(39)	(43)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)	2 (5%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		
#SPLEEN	(18)	(39)	(43)
HEMANGIOSARCOMA	1 (6%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	
#MESENTERIC L. NODE	(20)	(40)	(45)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(41)	(44)
HEPATOCELLULAR CARCINOMA		36 (88%)	43 (98%)
HEMANGIOSARCOMA			4 (9%)

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

TABLE B2 (CONTINUED)

	CONTROL (VEH) 02-F067	LOW DOSE 02-F070	HIGH DOSE 02-F071
URINARY SYSTEM			
#URINARY BLADDER	(19)	(35)	(41)
TRANSITIONAL-CELL PAPILLOMA			1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(20)	(35)	(39)
FOLLICULAR-CELL CARCINOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
#OVARY	(19)	(37)	(40)
CYSTADENOMA, NOS		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(39)	(43)
HEMANGIOSARCOMA			1 (2%)
ALL OTHER SYSTEMS			
NONE			

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

TABLE B2 (CONCLUDED)

	CONTROL (VEH) 02-P067	LOW DOSE 02-P070	HIGH DOSE 02-P071
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	22	17
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	17	26	31
ANIMAL MISSING		1	2
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	3	36	43
TOTAL PRIMARY TUMORS	3	42	51
TOTAL ANIMALS WITH BENIGN TUMORS		2	1
TOTAL BENIGN TUMORS		2	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	36	43
TOTAL MALIGNANT TUMORS	3	40	50
TOTAL ANIMALS WITH SECONDARY TUMORS#			2
TOTAL SECONDARY TUMORS			2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

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

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

	CONTROL (VEH) 01-M060	LOW DOSE 01-M061	HIGH DOSE 01-M062
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
METAPLASIA, OSSEOUS		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
INFLAMMATION, GRANULOMATOUS			1 (2%)
NECROSIS, FAT		1 (2%)	
RESPIRATORY SYSTEM			
#TRACHEA	(20)	(50)	(48)
INFLAMMATION, NOS			2 (4%)
*LUNG	(20)	(50)	(50)
THROMBOSIS, NOS		1 (2%)	
EDEMA, NOS			1 (2%)
HEMORRHAGE		2 (4%)	8 (16%)
INFLAMMATION, NOS			1 (2%)
PNEUMONIA, CHRONIC MURINE	13 (65%)	35 (70%)	17 (34%)
FIBROSIS, FOCAL		1 (2%)	
CALCIUM DEPOSIT		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(50)	(48)
METAMORPHOSIS FATTY		2 (4%)	
#SPLEEN	(20)	(50)	(50)
THROMBUS, ORGANIZED		1 (2%)	
HEMATOPOIESIS		2 (4%)	
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(49)
THROMBUS, ORGANIZED		2 (4%)	

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

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	CONTROL (VEH) 01-M060	LOW DOSE 01-M061	HIGH DOSE 01-M062
MEDIAL CALCIFICATION		1 (2%)	
CALCIUM DEPOSIT	2 (10%)	1 (2%)	1 (2%)
#MYOCARDIUM	(20)	(50)	(49)
INFLAMMATION, NOS		2 (4%)	
FIBROSIS	1 (5%)	2 (4%)	1 (2%)
DEGENERATION, NOS		1 (2%)	1 (2%)
*AORTA	(20)	(50)	(50)
MEDIAL CALCIFICATION	3 (15%)	6 (12%)	
*CORONARY ARTERY	(20)	(50)	(50)
MEDIAL CALCIFICATION			1 (2%)
*MESENTERIC ARTERY	(20)	(50)	(50)
MEDIAL CALCIFICATION	1 (5%)	3 (6%)	
CALCIUM DEPOSIT		1 (2%)	
DIGESTIVE SYSTEM			
*LIVER	(20)	(50)	(50)
THROMBUS, ORGANIZED		2 (4%)	
INFLAMMATION, FOCAL	1 (5%)		
NECROSIS, NOS			1 (2%)
METAMORPHOSIS FATTY	3 (15%)	9 (18%)	1 (2%)
FOCAL CELLULAR CHANGE			1 (2%)
ANGIECTASIS		7 (14%)	1 (2%)
*LIVER/CENTRILOBULAR	(20)	(50)	(50)
NECROSIS, NOS			21 (42%)
*BILE DUCT	(20)	(50)	(50)
DILATATION, NOS		1 (2%)	
HYPERPLASIA, NOS	7 (35%)	9 (18%)	3 (6%)
*PANCREAS	(18)	(50)	(48)
INFLAMMATION, NOS	1 (6%)	1 (2%)	
PERIARTERITIS	1 (6%)	1 (2%)	
ATROPHY, NOS		2 (4%)	
ATROPHY, FOCAL		1 (2%)	
*STOMACH	(20)	(50)	(50)
ULCER, FOCAL		1 (2%)	1 (2%)
CALCIUM DEPOSIT	3 (15%)	4 (8%)	
HYPERKERATOSIS		1 (2%)	

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

TABLE C1 (CONTINUED)

	CONTROL (VEH) 01-M060	LOW DOSE 01-M061	HIGH DOSE 01-M062
#COLON	(20)	(50)	(49)
NEMATODIASIS	1 (5%)		1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
HYDRONEPHROSIS		1 (2%)	1 (2%)
PYELONEPHRITIS, NOS		5 (10%)	2 (4%)
INFLAMMATION, CHRONIC	13 (65%)	37 (74%)	28 (56%)
CALCIUM DEPOSIT	2 (10%)	4 (8%)	1 (2%)
CALCIFICATION, NOS			1 (2%)
#URINARY BLADDER	(20)	(49)	(48)
CALCULUS, NOS		1 (2%)	1 (2%)
HEMORRHAGE			2 (4%)
INFLAMMATION, HEMORRHAGIC			1 (2%)
INFLAMMATION, CHRONIC		2 (4%)	1 (2%)
HYPERPLASIA, EPITHELIAL		1 (2%)	
POLYP, INFLAMMATORY		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(46)	(44)
ANGIECTASIS		3 (7%)	
#ADRENAL	(20)	(50)	(49)
HEMORRHAGE			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
ANGIECTASIS		2 (4%)	
#ADRENAL CORTEX	(20)	(50)	(49)
DEGENERATION, NOS		3 (6%)	
#THYROID	(20)	(50)	(47)
FOLLICULAR CYST, NOS		10 (20%)	3 (6%)
HYPERPLASIA, C-CELL		1 (2%)	
#PARATHYROID	(3)	(3)	
HYPERPLASIA, NOS	3 (100%)	3 (100%)	
REPRODUCTIVE SYSTEM			
#PROSTATE	(18)	(44)	(34)
HEMORRHAGE			4 (12%)

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

TABLE C1 (CONTINUED)

	CONTROL (VEH) 01-M060	LOW DOSE 01-M061	HIGH DOSE 01-M062
INFLAMMATION, NOS	1 (6%)	10 (23%)	3 (9%)
INFLAMMATION, CHRONIC		2 (5%)	4 (12%)
*SEMINAL VESICLE	(20)	(50)	(50)
HEMORRHAGE			5 (10%)
INFLAMMATION, NOS		1 (2%)	4 (8%)
*TESTIS	(20)	(50)	(49)
HEMORRHAGE			5 (10%)
INFLAMMATION, NOS			1 (2%)
PERIARTERITIS		1 (2%)	
DEGENERATION, NOS			5 (10%)
ATROPHY, NOS	5 (25%)	23 (46%)	5 (10%)
*EPIDIDYMIS	(20)	(50)	(50)
HEMORRHAGE			5 (10%)
INFLAMMATION, NOS			2 (4%)
INFLAMMATION, CHRONIC			2 (4%)
FIBROSIS			1 (2%)
NECROSIS, FAT			3 (6%)
*SCROTUM	(20)	(50)	(50)
HEMORRHAGE			4 (8%)
NERVOUS SYSTEM			
*BRAIN	(20)	(50)	(49)
HYDROCEPHALUS, NOS			1 (2%)
HEMORRHAGE			4 (8%)
INFLAMMATION, NOS		1 (2%)	
ABSCESS, NOS		1 (2%)	1 (2%)
CALCIUM DEPOSIT			1 (2%)
*PINEAL BODY	(20)	(50)	(50)
FIBROSIS		1 (2%)	
CALCIFICATION, NOS		1 (2%)	
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 (VEH) 01-M060	LOW DOSE 01-M061	HIGH DOSE 01-M062
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)		
*PERICARDIUM	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)	2 (4%)	
*MESENTERY	(20)	(50)	(50)
PERIARTERITIS	1 (5%)	3 (6%)	
ALL OTHER SYSTEMS			
PERINEUM			
HEMORRHAGE			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	1

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

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH NITROFEN

	CONTROL (VEH) 01-F060	LOW DOSE 01-F063	HIGH DOSE 01-F064
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
HYPERKERATOSIS	1 (5%)		
ACANTHOSIS	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(50)
HEMATOMA, NOS	1 (5%)		
RESPIRATORY SYSTEM			
*LUNG	(20)	(50)	(50)
PNEUMONIA, CHRONIC MURINE	12 (60%)	36 (72%)	34 (68%)
HEMATOPOIETIC SYSTEM			
*SPLEEN	(20)	(50)	(50)
THROMBOSIS, NOS		1 (2%)	
HEMATOPOIESIS		5 (10%)	6 (12%)
*THYMUS	(17)	(39)	(38)
CYST, NOS		1 (3%)	
INFLAMMATION, NOS	1 (6%)		
CIRCULATORY SYSTEM			
*MYOCARDIUM	(20)	(50)	(50)
FIBROSIS		1 (2%)	3 (6%)
DEGENERATION, NOS		1 (2%)	3 (6%)
*ENDOCARDIUM	(20)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
*AORTA	(20)	(50)	(50)
MEDIAL CALCIFICATION			2 (4%)

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

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL (VEH) 01-F060	LOW DOSE 01-F063	HIGH DOSE 01-F064
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(42)	(42)
CYST, NOS			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
#LIVER	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)		1 (2%)
INFLAMMATION, FOCAL			1 (2%)
ABSCESS, NOS	1 (5%)		1 (2%)
FIBROSIS, FOCAL			1 (2%)
PELIOSIS HEPATIS			2 (4%)
METAMORPHOSIS FATTY		1 (2%)	
FOCAL CELLULAR CHANGE		1 (2%)	1 (2%)
HYPERPLASIA, NOS	1 (5%)		
HYPERPLASIA, CYSTIC			1 (2%)
ANGIECTASIS		1 (2%)	1 (2%)
#LIVER/CENTRILOBULAR	(20)	(50)	(50)
NECROSIS, NOS		2 (4%)	2 (4%)
#BILE DUCT	(20)	(50)	(50)
DILATATION, NOS			1 (2%)
HYPERPLASIA, NOS	3 (15%)	9 (18%)	5 (10%)
HYPERPLASIA, CYSTIC			3 (6%)
#STOMACH	(20)	(49)	(50)
ULCER, FOCAL	2 (10%)	3 (6%)	3 (6%)
#COLON	(20)	(49)	(49)
NEMATODIASIS	1 (5%)	2 (4%)	
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
HYDRONEPHROSIS		1 (2%)	1 (2%)
PYELONEPHRITIS, NOS	2 (10%)		2 (4%)
INFLAMMATION, CHRONIC	4 (20%)	27 (54%)	24 (48%)
CALCIUM DEPOSIT			1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL	(20)	(50)	(49)
ANGIECTASIS	2 (10%)	3 (6%)	4 (8%)

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

TABLE C2 (CONTINUED)

	CONTROL (VEH) 01-F060	LOW DOSE 01-F063	HIGH DOSE 01-F064
#ADRENAL CORTEX	(20)	(50)	(49)
DEGENERATION, NOS	2 (10%)	3 (6%)	4 (8%)
#THYROID	(19)	(49)	(50)
FOLLICULAR CYST, NOS		3 (6%)	3 (6%)
HYPERPLASIA, C-CELL		1 (2%)	2 (4%)
REPRODUCTIVE SYSTEM			
*VAGINA	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
POLYP, INFLAMMATORY		1 (2%)	
#UTERUS	(20)	(50)	(49)
INTUSSUSCEPTION			1 (2%)
HYDROMETRA	1 (5%)	8 (16%)	6 (12%)
HEMORRHAGE			1 (2%)
INFLAMMATION, NOS		1 (2%)	1 (2%)
#UTERUS/ENDOMETRIUM	(20)	(50)	(49)
HYPERPLASIA, CYSTIC		6 (12%)	1 (2%)
#OVARY	(20)	(50)	(49)
CYST, NOS	1 (5%)	4 (8%)	6 (12%)
INFLAMMATION, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)		
SPECIAL SENSE ORGANS			
*EYE	(20)	(50)	(50)
PANNUS	1 (5%)		
*EYE/IRIS	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)		
*EYE/LACRIMAL GLAND	(20)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE C2 (CONCLUDED)

	CONTROL (VEH) 01-F060	LOW DOSE 01-F063	HIGH DOSE 01-F064
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
*PERICARDIUM	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)	1 (2%)	
*MESENTERY	(20)	(50)	(50)
PERIARTERITIS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	1	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 (VEH) 02-M067	LOW DOSE 02-M068	HIGH DOSE 02-M069
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	** 20	44	46
ANIMALS EXAMINED HISTOPATHOLOGICALLY	** 20	44	46
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(44)	(46)
EPIDERMAL INCLUSION CYST	2 (10%)	1 (2%)	
INFLAMMATION, NOS		1 (2%)	1 (2%)
ACANTHOSIS	1 (5%)		1 (2%)
*SUBCUT TISSUE	(20)	(44)	(46)
ABSCESS, NOS	2 (10%)	1 (2%)	
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*SPLEEN	(20)	(49)	(47)
AMYLOIDOSIS	15 (75%)	4 (8%)	
HEMATOPOIESIS		3 (6%)	2 (4%)
*LYMPH NODE	(20)	(50)	(46)
INFLAMMATION, NOS		1 (2%)	
*MESENTERIC L. NODE	(20)	(50)	(46)
LYMPHANGIECTASIS	1 (5%)		
INFLAMMATION, NOS	1 (5%)	1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
*HEART	(20)	(48)	(46)
THROMBUS, ORGANIZED	2 (10%)		
*MYOCARDIUM	(20)	(48)	(46)
INFLAMMATION, NOS	2 (10%)		

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

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL (VEH) 02-M067	LOW DOSE 02-M068	HIGH DOSE 02-M069
#ENDOCARDIUM	(20)	(48)	(46)
INFLAMMATION, NOS	2 (10%)		
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(48)
THROMBUS, ORGANIZED		6 (12%)	2 (4%)
AMYLOIDOSIS	13 (65%)		
HYPERPLASIA, NODULAR		2 (4%)	
#PANCREAS	(19)	(46)	(43)
THROMBUS, ORGANIZED		1 (2%)	
INFLAMMATION, NOS		1 (2%)	
PERIARTERITIS		1 (2%)	1 (2%)
CALCIUM DEPOSIT		1 (2%)	
#STOMACH	(20)	(48)	(46)
HYPERKERATOSIS		1 (2%)	
ACANTHOSIS		1 (2%)	
#COLON	(20)	(49)	(44)
PARASITISM		3 (6%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(46)
HYDRONEPHROSIS			3 (7%)
PYELONEPHRITIS, NOS		1 (2%)	
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, CHRONIC	16 (80%)	3 (6%)	
AMYLOIDOSIS	15 (75%)	1 (2%)	
#URINARY BLADDER	(20)	(47)	(40)
INFLAMMATION, NOS		1 (2%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*PENIS	(20)	(44)	(46)
INFLAMMATION, NOS		2 (5%)	

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

TABLE D1 (CONCLUDED)

	CONTROL (VEH) 02-M067	LOW DOSE 02-M068	HIGH DOSE 02-M069
*PREPUTIAL GLAND	(20)	(44)	(46)
INFLAMMATION, NOS		1 (2%)	
*TESTIS	(18)	(49)	(45)
CALCIUM DEPOSIT		1 (2%)	
ATROPHY, NOS		1 (2%)	1 (2%)
*EPIDIDYMIS	(20)	(44)	(46)
GRANULOMA, SPERMATIC		1 (2%)	
ATROPHY, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(44)	(46)
PERIARTERITIS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	4	
ANIMAL MISSING/NO NECROPSY			1
AUTO/NECROPSY/HISTO PERF	1		
AUTOLYSIS/NO NECROPSY		6	3

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 (VEH) 02-F067	LOW DOSE 02-F070	HIGH DOSE 02-F071
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	2
ANIMALS NECROPSIED **	20	39	43
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	39	43
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(38)	(45)
PNEUMONIA, CHRONIC MURINE			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(39)	(43)
HEMATOPOIESIS		1 (3%)	
#LUMBAR LYMPH NODE	(20)	(40)	(45)
HYPERPLASIA, NOS	1 (5%)		
#MESENTERIC L. NODE	(20)	(40)	(45)
LYMPHANGIECTASIS		1 (3%)	3 (7%)
INFLAMMATION, NOS		2 (5%)	
ANGIECTASIS	1 (5%)		
#RENAL LYMPH NODE	(20)	(40)	(45)
HYPERPLASIA, NOS	1 (5%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(41)	(44)
CYST, NOS		1 (2%)	

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

TABLE D2 (CONTINUED)

	CONTROL (VEH) 02-F067	LOW DOSE 02-F070	HIGH DOSE 02-F071
THROMBUS, ORGANIZED		3 (7%)	2 (5%)
INFLAMMATION, NOS		2 (5%)	
CALCIUM DEPOSIT			4 (9%)
#PANCREAS	(20)	(37)	(38)
DILATATION/DUCTS		1 (3%)	
CYST, NOS	1 (5%)		2 (5%)
INFLAMMATION, NOS			1 (3%)
ATROPHY, NOS	1 (5%)	2 (5%)	
#PANCREATIC DUCT	(20)	(37)	(38)
DILATATION, NOS			1 (3%)
HYPERPLASIA, NOS		1 (3%)	
#STOMACH	(20)	(39)	(42)
HYPERKERATOSIS			3 (7%)
ACANTHOSIS			3 (7%)
URINARY SYSTEM			
#KIDNEY	(20)	(40)	(44)
HYDRONEPHROSIS		1 (3%)	2 (5%)
INFLAMMATION, CHRONIC		1 (3%)	
PIGMENTATION, NOS			1 (2%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#UTERUS	(19)	(37)	(40)
HYDROMETRA	2 (11%)	4 (11%)	7 (18%)
THROMBOSIS, NOS			1 (3%)
INFLAMMATION, NOS		5 (14%)	
#UTERUS/ENDOMETRIUM	(19)	(37)	(40)
INFLAMMATION, NOS	7 (37%)		
HYPERPLASIA, CYSTIC	4 (21%)	3 (8%)	1 (3%)
#OVARY/OVIDUCT	(19)	(37)	(40)
INFLAMMATION, NOS	1 (5%)	2 (5%)	
#OVARY	(19)	(37)	(40)
CYST, NOS	8 (42%)	10 (27%)	4 (10%)

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

TABLE D2 (CONCLUDED)

	CONTROL (VEH) 02-P067	LOW DOSE 02-P070	HIGH DOSE 02-P071
INFLAMMATION, NOS	7 (37%)	13 (35%)	
CALCIUM DEPOSIT			1 (3%)
ANGIECTASIS			1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(39)	(43)
INFLAMMATION, NOS	1 (5%)	1 (3%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	1	
ANIMAL MISSING/NO NECROPSY		1	2
AUTO/NECROPSY/HISTO PERF	1		
AUTOLYSIS/NO NECROPSY		10	5

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

☆U S GOVERNMENT PRINTING OFFICE 1978—260-899 3202

DHEW Publication No. (NIH) 78-826