National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 52
1978

BIOASSAY OF 3-NITROPROPIONIC ACID FOR POSSIBLE CARCINOGENICITY

CAS No. 504-88-1

NCI-CG-TR-52

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

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

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DHEW Publication No. (NIH) 78-1302

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FOREWORD: This report presents the results of the bioassay of 3-nitropropionic acid conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected environmental chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: The bioassay of 3-nitropropionic acid was conducted by The Dow Chemical Company, Indianapolis, Indiana, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design and doses were determined by Dr. E. K. Weisburger[1]. Dr. C. G. Gerbig[2] supervised the preparation of the gavage solutions and was responsible for animal care. Histopathologic examinations were performed by Dr. J. L. Emerson[2,3], the principal investigator, and the diagnoses included in this report represent his interpretations. Drs. Emerson and Gerbig prepared the data for the methodology section of this report.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[4]. The statistical analyses were performed by Dr. J. R. Joiner[5], using methods selected for the bioassay program by Dr. J. J. Gart[6]. Chemicals used in this bioassay were analyzed under the direction of Dr. E. Murrill[7], and the analytical results were reviewed by Dr. S. S. Olin[5].

This report was prepared at Tracor Jitco[5] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg, Director of the Bioassay Program; Dr. L. A. Campbell, Deputy Director for Science; Drs. J. F. Robens and C. H. Williams, toxicologists; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Waitz, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of NCI[6]: Dr. John J. Gart, Mr. Jun-mo Nam, Dr. Hugh M. Pettigrew, and Dr. Robert E. Tarone.

The following other scientists at NCI were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings:

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SUMMARY

A bioassay of 3-nitropropionic acid (95% pure) for possible carcinogenicity was conducted by administering the test chemical by gavage to Fischer 344 rats and B6C3F1 mice.

Groups of 50 rats and 50 mice of each sex were administered 3-nitropropionic acid at one of the following doses by gavage 5 days per week. For male rats, the doses were 0.425 or 0.85 mg/animal/day; for females, they were 0.6 or 1.2 mg/animal/day. For both sexes of mice, the doses were 0.375 or 0.75 mg/animal/day. The rats were administered the chemical for 110 weeks and the mice for 104 weeks. The controls consisted of 50 untreated rats and 50 untreated mice of each sex. All surviving rats were killed at 111 weeks and all surviving mice at 104 or 105 weeks.

Mean body weights and mortality of the dosed animals were not markedly affected by 3-nitropropionic acid under the conditions of this bioassay, indicating that the maximum tolerated dose may not have been reached. The various clinical signs observed were common to both dosed and control groups.

In rats, the combination of neoplastic nodule of the liver and hepatocellular carcinoma occurred in the males with a significant dose-related trend ($P = 0.010$) and with a higher incidence ($P = 0.012$) in the high-dose group of animals than in the controls (controls 0/49, low-dose 3/50, high-dose 6/49). All but one of these tumors were neoplastic nodules. In the females, only two neoplastic nodules occurred, one in each of the dosed groups. Biliary hyperplasia occurred at a higher incidence in the dosed males than in the corresponding controls (controls 19/50, low-dose 32/50, high-dose 36/50), but the incidence of this lesion in the dosed females was not increased as compared with controls. There was also a dose-related trend ($P = 0.033$) in the incidence of pancreatic islet-cell adenoma in the male rats (controls 4/49, low-dose 6/50, high-dose 11/50); however, direct comparisons of incidences in the dosed and control groups were not statistically significant. The historical incidence of

pancreatic islet-cell adenoma among 100 control Fischer 344 rats at the laboratory was 7/100 (7%). In addition, focal myocardial fibrosis was observed at a higher incidence in dosed rats than among controls (males: controls 1/4, low-dose 17/49, high-dose 24/48; females: controls 2/48, low-dose 9/46, high-dose 9/50).

In mice, each type of neoplasm found in the dosed and control mice has been encountered previously as a spontaneous lesion. No specific tumor was found to occur at a statistically significantly higher incidence among dosed mice than among the respective control groups.

It is concluded that under the conditions of this bioassay, there was an elevated incidence of hepatocellular neoplasms, primarily benign, and of islet-cell adenomas of the pancreas in male Fischer 344 rats receiving 3-nitropropionic acid as compared with controls; however, there was no conclusive evidence that 3-nitropropionic acid was carcinogenic in these animals. The chemical was not carcinogenic in female rats or in male or female B6C3F1 mice.

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I. INTRODUCTION

3-Nitropropionic acid (CAS 504-88-1; NCI C03076), also called β-nitropropionic acid or hiptagenic acid, has been isolated from plants, including a tropical forage plant (Cooke, 1955; Finnegan and Mueller, 1965; Morris et al., 1954), and from nuts that are eaten in the New Zealand area as a food staple (Bell, 1974; Carter, 1951). It has been isolated from Streptomyces found in soil (Anzai and Suzuki, 1960) and as a metabolite of certain fungal species of Aspergillus (Bush et al., 1951; Iwasaki and Kosikowski, 1973) and Penicillium (Hylin and Matsumoto, 1960; Raistrick and Stossl, 1958). These species of fungi are commonly present in several oriental fermented foodstuffs, both domestically and commercially produced, in which 3-nitropropionic acid has been identified (Kinosita et al., 1968). Other fungal strains that are frequent contaminants in many kinds of food have been found to produce mycotoxins that have exhibited carcinogenic activity in experimental animals (Butler et al., 1969; IARC, 1972; Wogan and Newberne, 1967).

3-Nitropropionic acid was selected for testing for carcinogenic activity because it was known to demonstrate varying degrees of toxicity in man and animals (Hutton et al., 1958; Morris et al., 1954; Bell, 1974), and because its use in food preparations and

its identification as a contaminant in foods suggested there was a possibility of long-term human exposure.

II. MATERIALS AND METHODS

A. Chemical

3-Nitropropionic acid, synthesized from β-propiolactone, was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin, in a single batch (Lot No. 111627) for the chronic study.

Analysis at Midwest Research Institute confirmed the identity of the chemical. Infrared and nuclear magnetic resonance (nmr) spectra were as expected for 3-nitropropionic acid, with the exception that the nmr spectra revealed a 5% impurity which was identified as a dimeric ester of 3-hydroxypropionic acid. Elemental analyses for carbon and hydrogen agreed with the theoretical values for $C_3H_5NO_4$, the molecular formula for 3-nitropropionic acid, but the results for nitrogen were slightly low. Titration with sodium hydroxide gave 100.7 $\pm$ 0.3% of the theoretical value. High-pressure liquid chromatography showed a single peak (uv detector, 254 nm), whereas thin-layer chromatography indicated two trace impurities. Water content by Karl Fischer analysis was 0.35 $\pm$ 0.01%. In summary, the analyses indicated that the batch used for the chronic study was approximately 95% pure, with a single major organic impurity, apparently a dimeric ester of 3-hydroxypropionic acid, comprising most of the remainder.

The chemical was stored at 4°C in the original glass container.

B. Dosage Preparation

3-Nitropropionic acid was administered in feed during the subchronic study. Polarographic and chromatographic analyses of extracts of samples of the test diets suggested partial decomposition of the chemical. To maintain adequate doses during the chronic study, the chemical was administered by gavage in an aqueous solution. A 1-mg/ml solution of 3-nitropropionic acid in distilled water was prepared once per day and used within 1-1/2 hours after preparation. This solution was stable for 3 hours at ambient temperature, as verified by both high-pressure liquid chromatographic and polarographic analyses.

C. Animals

Rats and mice of each sex, obtained through contracts of the Division of Cancer Treatment, National Cancer Institute, were used in these bioassays. The rats were of the Fischer 344 strain obtained from A. R. Schmidt/Sprague-Dawley, Madison, Wisconsin, and the mice were B6C3F1 hybrids obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. The rats and mice were approximately 28 days of age when received. On arrival at the laboratory, all animals were quarantined (rats for 7 days, mice for 14 days) and then assigned to control or dosed

groups. All animals were individually identified: rats were earmarked and mice were toe-clipped.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature was maintained at 22-25°C, and the relative humidity was maintained at 45-55%. The room air was changed 15 times per hour. Illumination was provided by fluorescent lighting 14 hours per day. Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) and chlorinated well water that was deionized were available ad libitum.

Initially, rats in the chronic study were housed individually in suspended cages made of stainless-steel wire mesh (Ford Fence Co., Indianapolis, Ind.). At week 45, all rats were housed three per cage in suspended polycarbonate cages (Maryland Plastics, Federalsburg, Md.) lined with autoclaved Absorb-Dri® bedding (Lab Products, Inc., Garfield, N. J.) and equipped with filters and an automatic watering system. The cages were changed, washed, and sanitized at 82°C twice per week. The feeders were changed, washed, and sterilized once per week, and the filters were changed every 2 weeks.

Mice were housed five per cage in filtered, prebedded cages made of disposable polypropylene (Lab Products, Inc., Garfield, N.J.).

The cages were changed twice per week and the used cages were incinerated. Feeders, water bottles, and cage lids were also changed twice per week, and cage filters were changed once per week. Feeders and sipper tubes were washed and sterilized prior to use. Water bottles and cage lids were sanitized at 82°C.

Rats and mice were housed in separate rooms. The animal racks were rotated once per week and the cages were kept in fixed positions on the racks. The rats administered 3-nitropropionic acid were housed in the same room as rats fed 2-amino-5-nitrothiazole (CAS 121-66-4) and the positive control, N-9H-fluoren-2-ylacetamide (CAS 53-96-3), in the diet. The mice administered 3-nitropropionic acid were housed in the same room as mice fed 2-amino-5-nitrothiazole, N,N′-dicyclohexylthiourea (CAS 1212-29-9), proflavine hydrochloride (CAS 952-23-8), 1,3-dichloro-5,5-dimethylhydantoin (CAS 118-52-5), and N-9H-fluoren-2-ylacetamide in the diet. The control animals were housed in the same room with respective dosed animals.

E. Subchronic Studies

Subchronic feeding studies were conducted with rats and mice to estimate the maximum tolerated doses of 3-nitropropionic acid, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high doses") were determined for

administration in the chronic studies. In the subchronic studies, 3-nitropropionic acid was added to the animal feed in concentrations ranging from 100 to 900 ppm for rats and from 150 to 800 ppm for mice. Five males and five females of each species were tested at the different doses, and equal numbers of males and females were used as untreated controls. All animals were fed the chemical for 6 weeks, then observed for 2 weeks. All animals were necropsied and gross lesions were examined histologically.

In male rats, mean body weight gain was 77% of that of the controls at 100 ppm, 59% at 150 ppm, and 57% at 250 ppm. All males at 500 and 900 ppm died. In females, mean body weight gain was 97% of the controls at 100 ppm, 87% at 150 ppm, 71% at 250 ppm, and 62% at 500 ppm. Two females died at 250 ppm, four at 500 ppm, and five at 900 ppm. On histologic examination, testicular atrophy with spermatogenic arrest was found in male rats and malacia in the midbrain in both sexes of rats given doses of 150 ppm and above. For male rats, the low and high doses for the chronic studies were set at 25 and 50 ppm; for females, they were set at 50 and 100 ppm.

In male mice, mean body weight gain of groups receiving 150 or 600 ppm was not affected. An early weight depression was observed at 800 ppm; however, these animals recovered, and their

final weights were comparable to those of control mice. Mean body weights in female mice were not markedly affected at any dose tested. One male died at 600 ppm, and one male died at 800 ppm. Hydronephrosis was found in nine mice, but the incidence was not dose related. For both male and female mice, the low and high doses for the chronic studies were set at 75 and 150 ppm.

F. Designs of Chronic Studies

The designs of the chronic studies are shown in tables 1 and 2.

Because the test chemical was unstable in feed, the method of administration used for the chronic study was gavage. Doses were converted from parts per million to milligrams per animal per day (mg/animal/day) based on an estimated food consumption of 17 g/day for male rats, 12 g/day for female rats, and 5 g/day for mice (both sexes). The doses in mg/animal/day that are stated in the tables were used throughout the study; thus, as the weights of the animals increased, the amounts per unit of body weight decreased. Since water was used as the vehicle, no control groups administered a vehicle by gavage were included. The control animals were those started with another chemical on test at the same time in the diet; thus, they received control diet only.

Table 1. Design of Chronic Studies of 3-Nitropropionic Acid in Rats

Sex and Test Group	Initial No. of Animals[a]	3-Nitropropionic Acid Dose (mg/animal/day)[b]	Time on Study[c]	
			Dosed (weeks)	Observed (weeks)
Male				
Control	50	0[d]		111
Low-Dose	50	0.425	110	1
High-Dose	50	0.85	110	1
Female				
Control	50	0[d]		111
Low-Dose	50	0.6	110	1
High-Dose	50	1.2	110	1

[a]Rats were approximately 50 days of age when placed on study.

[b]Animals were administered the chemical by gavage 5 days per week.

[c]All animals were started on study on the same day.

[d]The control groups were not administered the chemical.

Table 2. Design of Chronic Studies of 3-Nitropropionic Acid in Mice

Sex and Test Group	Initial No. of Animals[a]	3-Nitropropionic Acid Dose (mg/animal/day)[b]	Time on Study[c]	
			Dosed (weeks)	Observed (weeks)
Male				
Control	50	0[d]		104
Low-Dose	50	0.375	104	1
High-Dose	50	0.75	104	1
Female				
Control	50	0[d]		104
Low-Dose	50	0.375	104	1
High-Dose	50	0.75	104	1

[a]Mice were approximately 53 days of age when placed on study.

[b]Animals were administered the chemical by gavage 5 days per week.

[c]All animals were started on study on the same day.

[d]The control groups were not administered the chemical.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity and were weighed every 14 days during the first 3 months and every 28 days thereafter. Clinical observations were recorded at weekly intervals. Animals that were moribund at the time of daily examination were killed and necropsied; however, some moribund animals were isolated from their cage-mates for a few days prior to being killed.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, colon, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, testis or ovary, prostate or uterus, brain, and eyes. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

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

H. Data Recording and Statistical Analyses

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

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

Probabilities of survival were estimated by the product-limit

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

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a

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

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

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the

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

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

The approximate 95 percent confidence interval for the relative risk of each dosed group compared with its control was calculated

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

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result ($P < 0.025$ 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. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of both dosed groups of each sex were not appreciably lower than those of the controls (figure 1). Throughout the study, the dosed rats were generally comparable to the controls in appearance and behavior. Early during the second year of the study, approximately 75% of the rats, including the controls, developed acute swelling of the submaxillary salivary glands. The clinical appearance was consistent with that of sialodacryoadenitis. Both control and dosed animals developed this condition, which lasted for approximately 14 days. The animals developed partial anorexia and rough coats, and in some cases the animals lost weight. Unilateral and occasionally bilateral cataracts appeared in both control and dosed rats at the end of the first year and continued through the second year.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered 3-nitropropionic acid by gavage at the doses of this bioassay, together with those of the controls, are shown in figure 2.

The result of the Tarone test for positive dose-related trend in

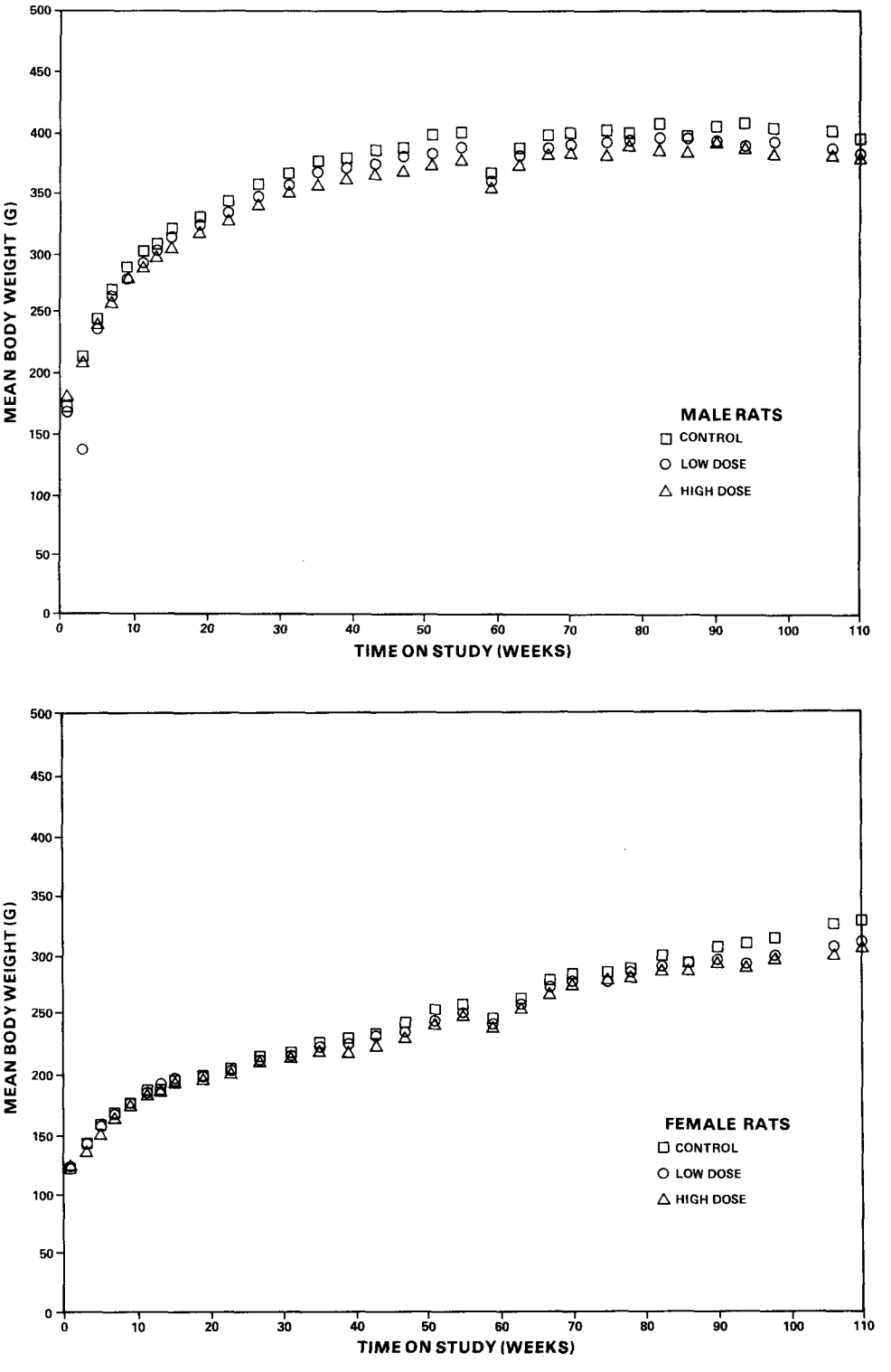


Figure 1. Growth Curves for Rats Administered 3-Nitropropionic Acid by Gavage

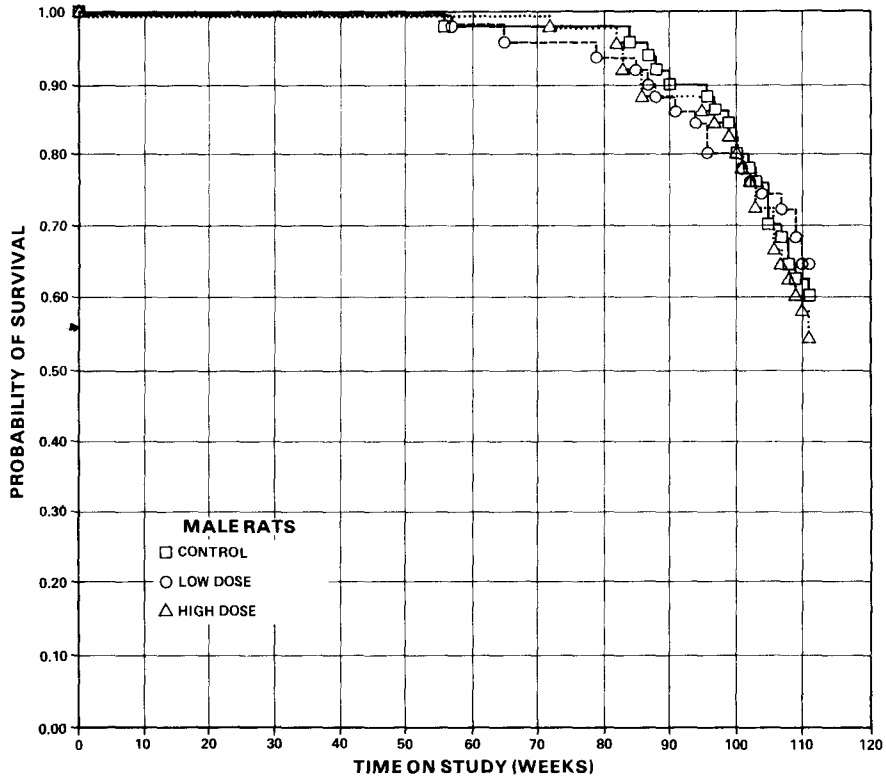
mortality is not significant at the 0.05 level in either sex. In male rats, 30/50 (60%) of the controls, 33/50 (66%) of the low-dose group, and 30/50 (60%) of the high-dose group lived to the last week of the study. In females, 33/50 (66%) of the controls, 26/50 (52%) of the low-dose group, and 32/50 (64%) of the high-dose group survived to the last week of the study. A sufficient number of rats of each sex was at risk for the development of late-appearing tumors.

C. Pathology (Rats)

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

A variety of neoplasms occurred in both the control and dosed groups. Each type of neoplasm represented in the tables has been encountered previously as a spontaneous lesion in rats.

In male rats, only one hepatocellular carcinoma was observed; this tumor was present in a high-dose animal. The incidence of neoplastic nodules, as described by Squire and Levitt (1975), was as follows in males: controls 0/49 (0%), low-dose 3/50 (6%), high-dose 5/49 (10%). In female rats, neoplastic nodules were observed in 1/50 (2%) of each dosed group, but in none of the controls.



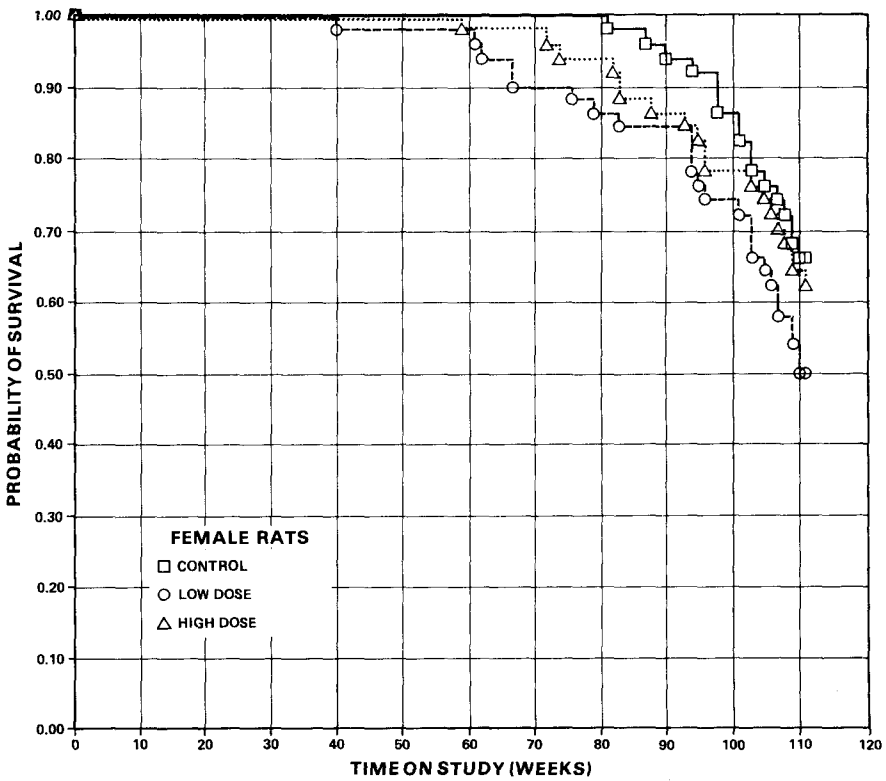


Figure 2. Survival Curves for Rats Administered 3-Nitropropionic Acid by Gavage

The incidence of pancreatic islet-cell adenoma was dose related in males (controls 4/49 [8%], low-dose 6/50 [12%], high-dose 11/50 [22%]). This trend was not evident in females.

Nonneoplastic lesions consisted of degenerative, proliferative, and inflammatory changes that are commonly observed in aging rats (Davey and Moloney, 1970; Sass et al., 1975). These conditions occurred in a random fashion and did not appear to be related to administration of the chemical.

Focal myocardial fibrosis occurred in 1/48 (2%) control males, 17/49 (35%) low-dose males, 24/48 (50%) high-dose males; 2/48 (4%) control females, 9/46 (20%) low-dose females, and 9/50 (18%) high-dose females.

Biliary hyperplasia occurred in 19/50 (38%) control males, 32/50 (64%) low-dose males, and 36/50 (72%) high-dose males; 15/50 (30%) control females, 17/50 (34%) low-dose females, and 18/50 (36%) high-dose females.

In the judgment of the pathologist, 3-nitropropionic acid was not carcinogenic in Fischer 344 rats when administered under the conditions of this study, although chemical administration may be associated with a slightly increased incidence of benign tumors of the pancreatic islets and of the liver in males.

D. Statistical Analyses of Results (Rats)

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

In male rats, the results of the Cochran-Armitage test for positive dose-related trend in combined tumor incidences of those animals with either neoplastic nodules or hepatocellular carcinoma of the liver are significant ($P = 0.010$) and the results of the Fisher exact test show that the incidence in the high-dose group is significantly higher ($P = 0.012$) than that in the controls. At this laboratory, none out of a total of 100 control male rats receiving only the control diet used in this study were observed to have neoplastic nodules or hepatocellular carcinomas. The statistical analysis suggests that the incidence of this combination of tumors in male rats is dose associated. The results of statistical tests on the incidence of these tumors in females are not significant.

In the analyses of the incidence of islet-cell adenoma of the pancreatic islets in male rats, the result of the Cochran-Armitage test is significant ($P = 0.033$). The Fisher exact test

shows a probability level of 0.049 when the incidence in the high-dose group is compared with that in the controls, but this level is above that of 0.025, which is required by the multiple comparison criterion. The laboratory historical controls have an incidence of 7/100 (7%) of islet-cell adenoma. No significant incidence of islet-cell adenoma is obtained for the females, and no islet-cell carcinoma was observed in either sex. No other tumors appeared in significant incidences in the dosed groups when compared with the control groups.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of both low- and high-dose males and females were lower than those of the controls during the greater part of the bioassay (figure 3). Fluctuations in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to wide variation. Throughout the study, the dosed animals were generally comparable to the controls in appearance and behavior. Focal alopecia, focal dermatitis, and small palpable nodules in the perineal area were observed in increasing numbers of male mice after 7 months on study. These lesions were associated with fighting.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered 3-nitropropionic acid by gavage at the doses of this bioassay, together with those of the controls, are shown in figure 4.

In each sex, the result of the Tarone test for positive dose-related trend in mortality is not significant at the 0.05 level. In male mice, 38/50 (76%) of the controls, 36/50 (72%) of the low-dose group, and 38/50 (76%) of the high-dose group lived to

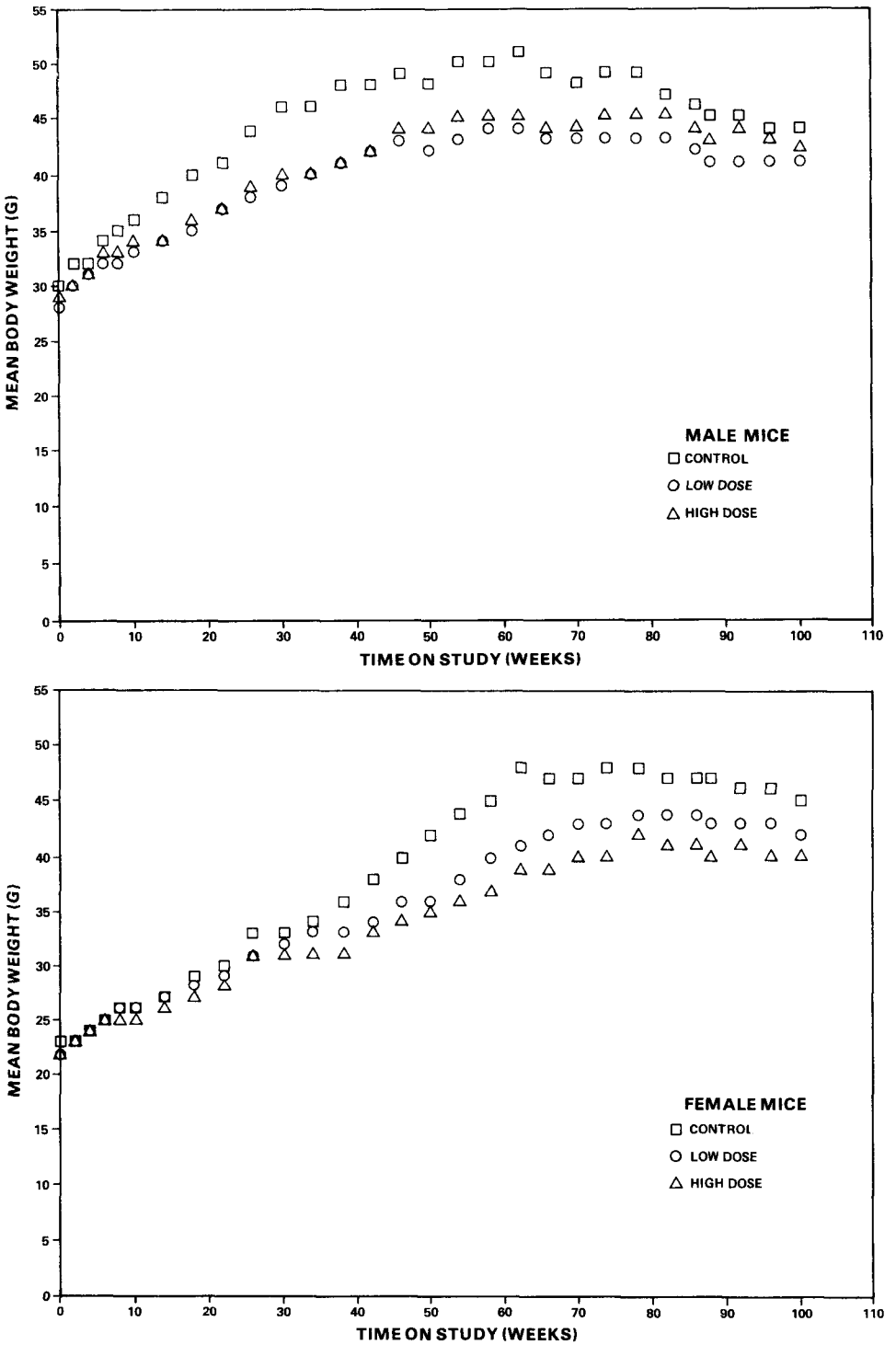


Figure 3. Growth Curves for Mice Administered 3-Nitropropionic Acid by Gavage

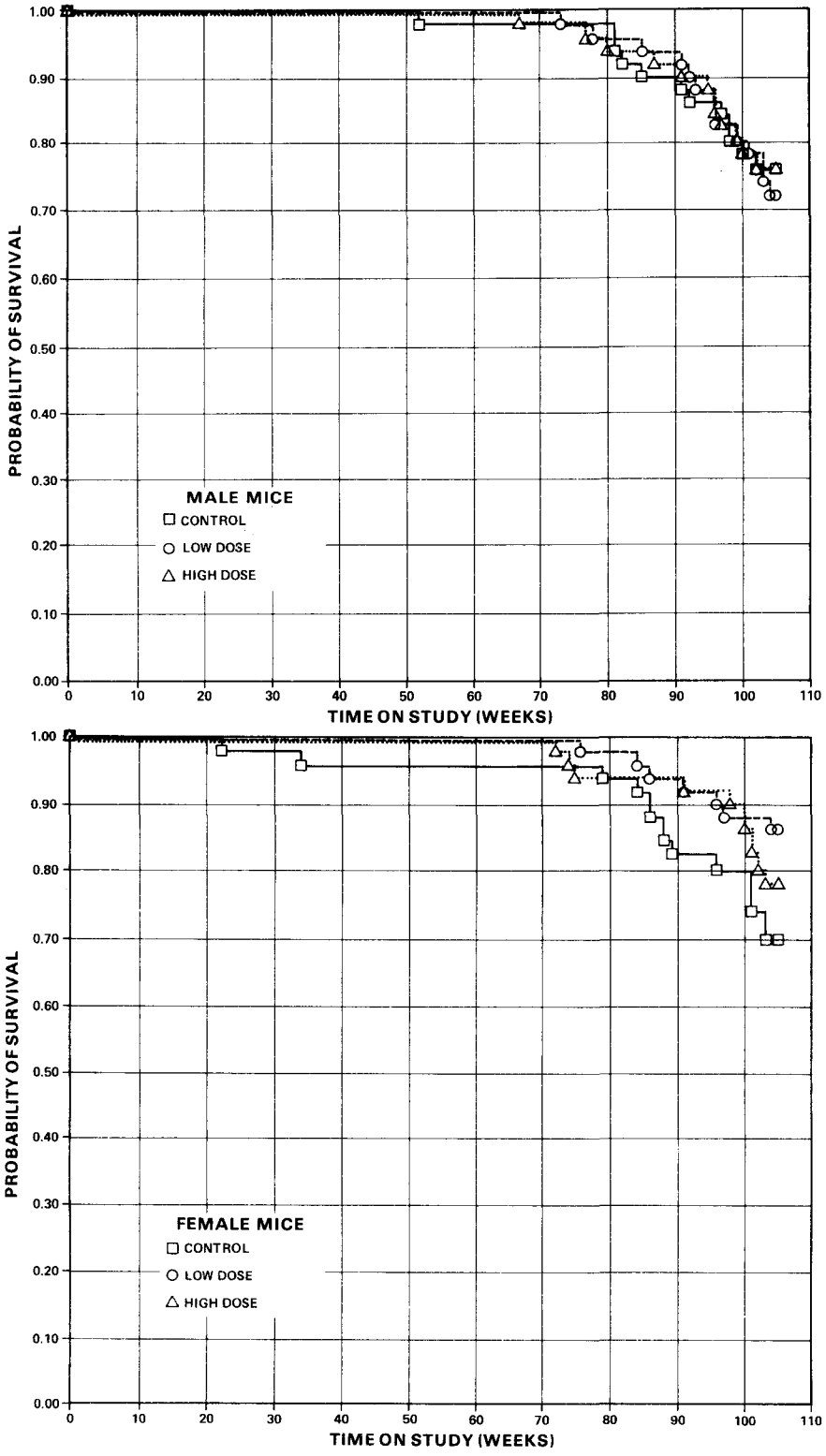


Figure 4. Survival Curves for Mice Administered 3-Nitropropionic Acid by Gavage

the end of the study. In females, 35/50 (70%) of the controls, 43/50 (86%) of the low-dose group, and 39/50 (78%) of the high-dose group survived to termination of the study. A sufficient number of mice of each sex was at risk for the development of late-appearing tumors.

C. Pathology (Mice)

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

A variety of neoplasms occurred in both the control and dosed groups. Each of the types of neoplasms represented in the tables has been encountered previously as a spontaneous lesion in the mouse.

The incidences of hepatocellular carcinomas, hepatocellular adenomas, and hyperplastic lesions (nodular hyperplasia and hyperplastic nodule) of the liver in mice are summarized below:

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Number of animals with tissues examined microscopically	49	50	49	49	50	50
Hepatocellular carcinoma	16 (33%)	8 (16%)	12 (24%)	1 (2%)	1 (2%)	2 (4%)
Hepatocellular adenoma	4 (8%)	2 (4%)	4 (8%)	1 (2%)	0 (0%)	2 (4%)
Hyperplastic lesions	1 (2%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

The incidences of proliferative hepatocellular lesions were greater in males than in females; however, there was no indication that these lesions were related to administration of the test chemical.

Other lesions that occurred among control and dosed groups were considered to be spontaneous.

Several chronic inflammatory, degenerative, and proliferative conditions were observed in all groups. These conditions were considered to be of common occurrence, spontaneous, and not related to administration of the test chemical.

In the judgment of the pathologist, 3-nitropropionic acid was not carcinogenic in B6C3F1 mice when administered under the conditions of this study.

D. Statistical Analyses of Results (Mice)

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

There is no specific incidence of tumors in either sex of mice for which the results of the Cochran-Armitage test or of the Fisher exact test are significant at the 0.05 level in the positive direction. In two instances the control groups had a significantly higher incidence than dosed groups. The incidence of hepatocellular adenoma or carcinoma in male mice is lower ($P = 0.021$) in the low-dose group than in the control group. In female mice, the occurrence of the combination of tumors in the hematopoietic system is lower ($P = 0.015$) in the high-dose group than in the control group. These results in the negative direction cannot be explained by differential mortality, since survivals of these groups within each sex are comparable.

In each of the 95% confidence intervals, shown in the tables, the value of one or less than one is included; this indicates the

absence of significant positive results. It should also be noted that each of the intervals (except that for the incidence of hepatocellular adenoma and carcinoma in the low-dose group of male mice and that for the incidence of hematopoietic tumors in the high-dose group of female mice) has an upper limit greater than one, indicating the theoretical possiblity of the induction of tumors by 3-nitropropionic acid, which could not be detected under the conditions of this test.

V. DISCUSSION

Mean body weights and mortality of the dosed rats were not markedly affected by 3-nitropropionic acid under the conditions of the bioassay. Mean body weights of dosed mice were slightly lower than those of controls throughout the greater part of the bioassay. The various clinical signs observed were common to both dosed and control groups.

In rats, the combination of neoplastic nodule of the liver and hepatocellular carcinoma occurred in the males with a significant dose-related trend ($P = 0.010$) and with a higher incidence ($P = 0.012$) in the high-dose group of animals than in the controls (controls 0/49, low-dose 3/50, high-dose 6/49). All but one of these tumors were neoplastic nodules. In the females, only two neoplastic nodules occurred, one in each of the dosed groups. Biliary hyperplasia occurred at a higher incidence in the dosed males than in the corresponding controls (controls 19/50, low-dose 32/50, high-dose 36/50), but the incidence of this lesion in the dosed females was not increased as compared with controls. There was also a dose-related trend ($P = 0.033$) in the incidence of pancreatic islet-cell adenoma in the male rats (controls 4/49, low-dose 6/50, high-dose 11/50); however, direct comparisons of incidences in the dosed and control groups were not statistically significant. The historical incidence of

pancreatic islet-cell adenomas among 100 control Fischer 344 rats at the laboratory was 7/100 (7%). In addition, focal myocardial fibrosis was observed at a higher incidence in dosed rats than among controls (males: controls 1/4, low-dose 17/49, high-dose 24/48; females: controls 2/48, low-dose 9/46, high-dose 9/50).

In mice, each type of neoplasm found in the dosed and control mice has been encountered previously as a spontaneous lesion. No specific tumor was found to occur at a statistically significantly higher incidence among dosed mice than among the respective control groups.

The minimum acute lethal dose of 3-nitropropionic acid has been reported to be 100 mg/kg for rats (Bell, 1974). Rabbits treated with a total of 5.5 g over a period of 34 days showed no toxic effects (Hutton et al., 1958). There have been no previous long-term toxicity studies of this chemical. The compound first attracted attention when Morris et al. (1954) found that it was present in a potential pasture legume (Indigofera endecaphylla) grown in tropical countries. This legume was severely toxic to grazing animals and the toxic principle was thought to be 3-nitropropionic acid. Hutton et al. (1958), however, fed the leaves of the legume and also pure 3-nitropropionic acid to rabbits and found the leaves caused severe liver damage, while the pure acid had no effect on the liver. 3-Nitropropionic acid

is one of the metabolites of fungi such as Aspergillus flavus, which is a widespread contaminant of foodstuffs.

It is concluded that under the conditions of this bioassay, there was an elevated incidence of hepatocellular neoplasms, primarily benign, and of islet-cell adenomas of the pancreas in male Fischer 344 rats receiving 3-nitropropionic acid as compared with controls; however, there was no conclusive evidence that 3-nitropropionic acid was carcinogenic in these animals. The chemical was not carcinogenic in female rats or in male or female B6C3F1 mice.

VI. BIBLIOGRAPHY

Anzai, K. and Suzuki, S., A new antibiotic bovinocidin, identified as β-nitropropionic acid. J. Antibiotics 13(2):133-136, 1960.

Armitage, P., Statistical Methods in Medical Research, John Wiley & Sons, Inc., New York, 1971, pp. 362-365.

Bell, M. E., Toxicology of karaka kernel, karakin, and β-nitropropionic acid. New Zealand J. Sci. 17:327-334, 1974.

Berenblum, I., ed., Carcinogenicity Testing, A Report of the Panel on Carcinogenicity of the Cancer Research Commission of the UICC, Vol. 2, International Union Against Cancer, Geneva, 1969.

Bush, M. T., Touster, O., and Brockman, J. E., The production of β-nitropropionic acid by a strain of Aspergillus flavis. J. Biol. Chem. 188(2):685-693, 1951.

Butler, W. H., Greenblatt, M., and Lijinsky, W., Carcinogenesis in rats by aflatoxins B_1, G_1 and B_2. Cancer Res. 29:2206-2211, 1969.

Carter, C. L., The constitition of karakin. J. Sci. Food Agric. 2:54-55, 1951.

Cooke, A. R., The toxic constituent of Indigofera endecaphylla. Arch. Biochem. Biophys. 55:114-120, 1955.

Cox, D. R., Regression models and life tables. J. R. Statist. Soc. B 34(2):187-220, 1972.

Cox, D. R., Analysis of Binary Data, Methuen & Co., Ltd., London, 1970, pp. 48-52.

Davey, F. R. and Moloney, W. C., Postmortem observations on Fischer rats with leukemia and other disorders. Lab. Investigations 23(3):327-334, 1970.

Finnegan, R. A. and Mueller, W. H., Chemical examination of a toxic extract of Indigofera endecaphylla. The endecaphyllins. J. Pharmaceut. Sci. 54:1136-1144, 1965.

Gart, J. J., The comparison of proportions: a review of significance tests, confidence limits and adjustments for stratification. Rev. Int. Statist. Inst. 39:148-169, 1971.

Hutton, E. M., Windrum, G. M. and Kratzing, C. C., Studies on the toxicity of Indigofera endecaphylla, I. Toxicity for rabbits. J. Nutrition 64:321-333, 1958.

Hylin, J. W. and Matsumoto, H., The biosynthesis of β-nitropropionic acid by Penicilluim atrovenetum. Arch. Biophys. 93:542-545, 1960.

International Agency for Research on Cancer, IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man, Vol. 1, World Health Organization, Geneva, 1972, pp. 145-156, 1972.

Iwasaki, T. and Kosikowski, F. V., Production of β-nitropropionic acid in foods. J. Food Science 38:1162-1165, 1973.

Kaplan, E. L. and Meier, P., Nonparametric estimation from incomplete observations. J. Amer. Statist. Assoc. 53:457-481, 1958.

Kinosita, R., Ishiko, T., Sugiyama, S., Seto, T., Igarasi, S., and Goetz, I. E., Mycotoxins in fermented food, Cancer Res. 28:2296-2307, 1968.

Linhart, M. S., Cooper, J., Martin, R. L., Page, N., and Peters, J., Carcinogenesis bioassay data system. Comp. and Biomed. Res. 7:230-248, 1974.

Miller, R. G., Jr., Simultaneous Statistical Inference, McGraw-Hill Book Co., New York, 1966, pp. 6-10.

Morris, M. P., Pagan, C., and Warmke, H. E., Hiptagenic acid, a toxic component of Indigofera endecaphylla. Science 119:322-323, 1954.

Raistrick, H. and Stossl, A., Studies in the biochemistry of micro-organisms. Metabolites of Pencillium atrovenetum G. Smith: β-nitropropionic acid, a major metabolite. Biochem. Journal 68:647-653, 1958.

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F., and Kaufman, D. G., Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo(a) pyrene and ferric oxide. Cancer Res. 32:1073-1081, 1972.

Sass, B., Rabstein, L. S., Madison, R., Nims, R. M., Peters, R. L., and Kelloff, G. J., Incidence of spontaneous neoplasms in F344 rats throughout the natural life-span. J. NCI 54(6):1449-1453, 1975.

Squire, R. A. and Levitt, M. H., Report of a workshop on classification of specific hepatocellular lesions in rats. Cancer Res. 35:3214-3223, 1975.

Tarone, R. E., Tests for trend in life table anlaysis. Biometrika 62(3):679-682, 1975.

Wogan, G. N. and Newberne, P. M., Dose-response characteristics of aflatoxin B_1 carcinogenesis in the rat. Cancer Res. 27:2370-2376, 1967.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED 3-NITROPROPIONIC ACID BY GAVAGE

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED 3-NITROPROPIONIC ACID BY GAVAGE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	1 (2%)	1 (2%)	
BASAL-CELL CARCINOMA		2 (4%)	1 (2%)
TRICHOEPITHELIOMA	1 (2%)		1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
BASAL-CELL CARCINOMA		1 (2%)	
FIBROMA	1 (2%)	1 (2%)	
FIBROSARCOMA	1 (2%)		
LIPOMA	1 (2%)		1 (2%)
MESENCHYMOMA, BENIGN		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(48)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (6%)	1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	3 (6%)
CORTICAL CARCINOMA, METASTATIC			1 (2%)
C-CELL CARCINOMA, METASTATIC	1 (2%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (2%)	1 (2%)	7 (14%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	4 (8%)	4 (8%)	4 (8%)
UNDIFFERENTIATED LEUKEMIA	2 (4%)		1 (2%)
LYMPHOCYTIC LEUKEMIA	4 (8%)		2 (4%)
GRANULOCYTIC LEUKEMIA	2 (4%)	1 (2%)	2 (4%)
GRANULOCYTIC SARCOMA		1 (2%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#SPLEEN	(49)	(49)	(49)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (2%)		1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
UNDIFFERENTIATED LEUKEMIA	1 (2%)		
#MEDIASTINAL L.NODE	(41)	(43)	(43)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(48)	(49)	(48)
HEMANGIOMA		1 (2%)	
ANITSCHKOW-CELL SARCOMA	1 (2%)		
DIGESTIVE SYSTEM			
*PALATE	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		
#LIVER	(49)	(50)	(49)
NEOPLASTIC NODULE		3 (6%)	5 (10%)
HEPATOCELLULAR CARCINOMA			1 (2%)
URINARY SYSTEM			
#URINARY BLADDER	(47)	(42)	(45)
TRANSITIONAL-CELL PAPILLOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(48)	(49)
CARCINOMA,NOS		1 (2%)	
CHROMOPHOBE ADENOMA	3 (7%)	5 (10%)	4 (8%)
#ADRENAL	(49)	(50)	(50)
CORTICAL CARCINOMA	1 (2%)		1 (2%)
PHEOCHROMOCYTOMA	4 (8%)	5 (10%)	5 (10%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)		
#THYROID	(46)	(49)	(47)
FOLLICULAR-CELL CARCINOMA	1 (2%)		4 (9%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
C-CELL ADENOMA	3 (7%)	8 (16%)	4 (9%)
C-CELL CARCINOMA	1 (2%)	2 (4%)	
#PANCREATIC ISLETS	(49)	(50)	(50)
ISLET-CELL ADENOMA	4 (8%)	6 (12%)	11 (22%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
FIBROADENOMA	1 (2%)		
*PREPUTIAL GLAND	(50)	(50)	(50)
CARCINOMA,NOS	1 (2%)		
ADENOMA, NOS	2 (4%)	1 (2%)	4 (8%)
#TESTIS	(50)	(49)	(49)
INTERSTITIAL-CELL TUMOR	48 (96%)	44 (90%)	48 (98%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
#CEREBRUM	(50)	(50)	(50)
ASTROCYTOMA			1 (2%)
#BRAIN	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
#MIDBRAIN	(50)	(50)	(50)
ASTROCYTOMA	1 (2%)		
#CEREBELLUM	(50)	(50)	(50)
ASTROCYTOMA			1 (2%)
SPECIAL SENSE ORGANS			
*EAR CANAL	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(50)	(50)	(50)
LIPOMA		1 (2%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*PERITONEUM	(50)	(50)	(50)
MESOTHELIOMA, NOS	1 (2%)	3 (6%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT	1 (2%)		
MESOTHELIOMA, MALIGNANT	1 (2%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	15	9	12
MORIBUND SACRIFICE	5	9	11
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	30	32	27
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	49	49	49
TOTAL PRIMARY TUMORS	99	99	112
TOTAL ANIMALS WITH BENIGN TUMORS	48	48	48
TOTAL BENIGN TUMORS	72	76	78
TOTAL ANIMALS WITH MALIGNANT TUMORS	23	14	24
TOTAL MALIGNANT TUMORS	26	17	29
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	3	1
TOTAL SECONDARY TUMORS	1	5	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	6	5
TOTAL UNCERTAIN TUMORS	1	6	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED 3-NITROPROPIONIC ACID BY GAVAGE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
PAPILLOMA, NOS			1 (2%)
SEBACEOUS ADENOMA	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(49)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
C-CELL CARCINOMA, METASTATIC			1 (2%)
LIPOSARCOMA, METASTATIC	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE	4 (8%)	1 (2%)	4 (8%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)	2 (4%)	2 (4%)
LEUKEMIA,NOS			1 (2%)
LYMPHOCYTIC LEUKEMIA	1 (2%)		
GRANULOCYTIC LEUKEMIA	2 (4%)		1 (2%)
MONOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(50)	(50)	(49)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	1 (2%)
GRANULOCYTIC LEUKEMIA		1 (2%)	
#LYMPH NODE	(44)	(41)	(45)
C-CELL CARCINOMA, METASTATIC	2 (5%)		

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MANDIBULAR L. NODE	(44)	(41)	(45)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*TONGUE	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
#LIVER	(49)	(50)	(50)
NEOPLASTIC NODULE		1 (2%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(50)
MIXED TUMOR, MALIGNANT		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(46)	(47)
CHROMOPHOBE ADENOMA	19 (42%)	15 (33%)	20 (43%)
#ADRENAL	(49)	(50)	(49)
PHEOCHROMOCYTOMA	3 (6%)	1 (2%)	1 (2%)
#THYROID	(50)	(44)	(44)
FOLLICULAR-CELL ADENOMA			1 (2%)
FOLLICULAR-CELL CARCINOMA	1 (2%)		
C-CELL ADENOMA	3 (6%)	3 (7%)	3 (7%)
C-CELL CARCINOMA	2 (4%)	1 (2%)	2 (5%)
#PANCREATIC ISLETS	(49)	(49)	(47)
ISLET-CELL ADENOMA	1 (2%)		1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOMA, NOS			1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ADENOCARCINOMA, NOS	1 (2%)		3 (6%)
FIBROADENOMA	12 (24%)	14 (28%)	13 (26%)
*PREPUTIAL GLAND	(50)	(50)	(50)
CARCINOMA,NOS	1 (2%)		
ADENOMA, NOS	2 (4%)	2 (4%)	
#UTERUS	(50)	(48)	(49)
LEIOMYOMA	1 (2%)		
ENDOMETRIAL STROMAL POLYP	2 (4%)	4 (8%)	5 (10%)
#CERVIX UTERI	(50)	(48)	(49)
FIBROSARCOMA			1 (2%)
#OVARY	(50)	(47)	(48)
SERTOLI-CELL TUMOR	1 (2%)		
NERVOUS SYSTEM			
#CEREBRUM	(49)	(50)	(50)
OLIGODENDROGLIOMA		1 (2%)	
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
*MANDIBLE	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
BODY CAVITIES			
*MESENTERY	(50)	(50)	(50)
FIBROSARCOMA			1 (2%)
ALL OTHER SYSTEMS			
LUMBOSACRAL REGION			
LIPOSARCOMA	1		

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	4	15	9
MORIBUND SACRIFICE	13	10	11
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	33	25	30
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	40	30	39
TOTAL PRIMARY TUMORS	59	50	66
TOTAL ANIMALS WITH BENIGN TUMORS	35	27	29
TOTAL BENIGN TUMORS	45	39	46
TOTAL ANIMALS WITH MALIGNANT TUMORS	11	10	19
TOTAL MALIGNANT TUMORS	14	10	19
TOTAL ANIMALS WITH SECONDARY TUMORS#	3	1	2
TOTAL SECONDARY TUMORS	3	1	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	1
TOTAL UNCERTAIN TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED 3-NITROPROPIONIC ACID BY GAVAGE

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED 3-NITROPROPIONIC ACID BY GAVAGE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(49)	(50)	(50)
FIBROMA		1 (2%)	
FIBROSARCOMA	2 (4%)	2 (4%)	
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(49)	(48)	(50)
HEPATOCELLULAR CARCINOMA, METAST	3 (6%)	3 (6%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	10 (20%)	5 (10%)	8 (16%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	4 (8%)	3 (6%)	3 (6%)
CORTICAL CARCINOMA, METASTATIC	1 (2%)		
FIBROSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	4 (8%)	5 (10%)	4 (8%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (4%)	1 (2%)
LYMPHOCYTIC LEUKEMIA		1 (2%)	
GRANULOCYTIC LEUKEMIA		2 (4%)	1 (2%)
MONOCYTIC LEUKEMIA		1 (2%)	
GRANULOCYTIC SARCOMA	1 (2%)		
*SUBCUT TISSUE	(49)	(50)	(50)
MAST-CELL TUMOR			1 (2%)
#SPLEEN	(46)	(50)	(46)
HEMANGIOMA		1 (2%)	2 (4%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEMANGIOSARCOMA	4 (9%)		2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		1 (2%)
#MESENTERIC L. NODE	(40)	(31)	(30)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (3%)		1 (3%)
#LIVER	(49)	(50)	(49)
GRANULOCYTIC LEUKEMIA	1 (2%)		
#PEYERS PATCH	(47)	(49)	(49)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	3 (6%)
#THYMUS	(35)	(38)	(41)
MALIGNANT LYMPHOMA, NOS		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(49)
HEPATOCELLULAR ADENOMA	4 (8%)	2 (4%)	4 (8%)
HEPATOCELLULAR CARCINOMA	16 (33%)	8 (16%)	12 (24%)
CORTICAL CARCINOMA, METASTATIC	1 (2%)		
HEMANGIOMA	1 (2%)		
HEMANGIOSARCOMA	2 (4%)	1 (2%)	4 (8%)
ANGIOSARCOMA			1 (2%)
#STOMACH	(48)	(50)	(46)
ADENOMATOUS POLYP, NOS			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(46)	(49)	(50)
CORTICAL CARCINOMA	1 (2%)		

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#THYROID	(43)	(44)	(48)
FOLLICULAR-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
#TESTIS	(47)	(49)	(50)
INTERSTITIAL-CELL TUMOR	1 (2%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(49)	(50)	(50)
PAPILLARY ADENOMA	1 (2%)		
PAPILLARY CYSTADENOMA, NOS		1 (2%)	2 (4%)
MUSCULOSKELETAL SYSTEM			
*SKULL	(49)	(50)	(50)
OSTEOMA			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(49)	(50)	(50)
CORTICAL CARCINOMA, METASTATIC	1 (2%)		
ALL OTHER SYSTEMS			
THORAX			
FIBROSARCOMA, METASTATIC		1	
DIAPHRAGM			
FIBROSARCOMA, METASTATIC		1	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	10	11	12
MORIBUND SACRIFICE	2	3	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	38	36	38
ANIMAL MISSING			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	39	28	38
TOTAL PRIMARY TUMORS	54	39	55
TOTAL ANIMALS WITH BENIGN TUMORS	15	9	19
TOTAL BENIGN TUMORS	17	11	19
TOTAL ANIMALS WITH MALIGNANT TUMORS	31	24	27
TOTAL MALIGNANT TUMORS	37	28	35
TOTAL ANIMALS WITH SECONDARY TUMORS#	4	4	
TOTAL SECONDARY TUMORS	6	6	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

@ INCLUDES AUTOLYZED ANIMALS

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED 3-NITROPROPIONIC ACID BY GAVAGE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
FIBROSARCOMA		1 (2%)	2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(47)	(49)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (4%)	4 (8%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		2 (4%)	2 (4%)
FIBROSARCOMA, METASTATIC			1 (2%)
OSTEOSARCOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	11 (22%)	10 (20%)	7 (14%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	6 (12%)	2 (4%)	
UNDIFFERENTIATED LEUKEMIA		2 (4%)	
LYMPHOCYTIC LEUKEMIA	2 (4%)		
GRANULOCYTIC LEUKEMIA	1 (2%)	2 (4%)	
GRANULOCYTIC SARCOMA		1 (2%)	
#BONE MARROW	(46)	(48)	(50)
GRANULOCYTIC SARCOMA			1 (2%)
#SPLEEN	(47)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)	3 (6%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#LYMPH NODE	(38)	(36)	(33)
GRANULOCYTIC SARCOMA			1 (3%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE	(38)	(36)	(33)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (3%)		1 (3%)
#PEYERS PATCH	(48)	(48)	(49)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		3 (6%)	1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(50)
HEPATOCELLULAR ADENOMA	1 (2%)		2 (4%)
HEPATOCELLULAR CARCINOMA	1 (2%)	1 (2%)	2 (4%)
HEMANGIOSARCOMA	1 (2%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(43)	(48)	(42)
CHROMOPHOBE ADENOMA	2 (5%)	4 (8%)	1 (2%)
#ADRENAL	(48)	(50)	(49)
CORTICAL CARCINOMA			1 (2%)
PHEOCHROMOCYTOMA			1 (2%)
#THYROID	(40)	(47)	(45)
FOLLICULAR-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	1 (2%)
FIBROADENOMA		1 (2%)	
*VAGINA	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#UTERUS	(47)	(49)	(48)
LEIOMYOSARCOMA	2 (4%)	1 (2%)	
#OVARY	(39)	(47)	(47)
PAPILLARY CYSTADENOMA, NOS			1 (2%)
GRANULOSA-CELL TUMOR	1 (3%)		1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(47)	(50)	(50)
OSTEOSARCOMA, METASTATIC			1 (2%)
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(50)
PAPILLARY CYSTADENOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(50)	(50)	(50)
FIBROSARCOMA		1 (2%)	
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
DIAPHRAGM			
OSTEOSARCOMA, METASTATIC			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	14	7	11
MORIBUND SACRIFICE	1		
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	35	43	39
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	26	32	26
TOTAL PRIMARY TUMORS	31	39	33
TOTAL ANIMALS WITH BENIGN TUMORS	3	9	8
TOTAL BENIGN TUMORS	5	9	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	23	25	19
TOTAL MALIGNANT TUMORS	25	30	24
TOTAL ANIMALS WITH SECONDARY TUMORS#			2
TOTAL SECONDARY TUMORS			3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1		1
TOTAL UNCERTAIN TUMORS	1		1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS ADMINISTERED 3-NITROPROPIONIC ACID BY GAVAGE

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED 3-NITROPROPIONIC ACID BY GAVAGE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
CYST, NOS	1 (2%)		
EPIDERMAL INCLUSION CYST			1 (2%)
HYPERKERATOSIS	2 (4%)		1 (2%)
ACANTHOSIS			1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
RESPIRATORY SYSTEM			
*NASAL CAVITY	(50)	(50)	(50)
INFLAMMATION, CHRONIC	1 (2%)		
#TRACHEA	(49)	(50)	(47)
INFLAMMATION, NOS	17 (35%)	22 (44%)	14 (30%)
INFLAMMATION, CHRONIC	1 (2%)	3 (6%)	2 (4%)
HYPERPLASIA, LYMPHOID	3 (6%)	2 (4%)	1 (2%)
#LUNG/BRONCHUS	(50)	(49)	(48)
BRONCHIECTASIS	4 (8%)	2 (4%)	2 (4%)
INFLAMMATION, SUPPURATIVE			1 (2%)
HYPERPLASIA, FOCAL		2 (4%)	
HYPERPLASIA, LYMPHOID	8 (16%)	19 (39%)	24 (50%)
#LUNG	(50)	(49)	(48)
ATELECTASIS	1 (2%)		
CONGESTION, NOS	2 (4%)	1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)
BRONCHOPNEUMONIA ACUTE SUPPURATI	1 (2%)	2 (4%)	
PNEUMONIA, CHRONIC MURINE	12 (24%)	16 (33%)	10 (21%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, GRANULOMATOUS		1 (2%)	
GRANULOMA, NOS		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
FIBROSIS	1 (2%)		
NECROSIS, FOCAL	1 (2%)		1 (2%)
PIGMENTATION, NOS	1 (2%)		
HEMOSIDEROSIS	1 (2%)		
ALVEOLAR MACROPHAGES	5 (10%)	2 (4%)	2 (4%)
#LUNG/ALVEOLI	(50)	(49)	(48)
CONGESTION, NOS	1 (2%)		1 (2%)
EDEMA, NOS	1 (2%)		1 (2%)
HEMORRHAGE	1 (2%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(49)	(48)	(50)
HYPERPLASIA, NOS	4 (8%)	1 (2%)	
HYPERPLASIA, HEMATOPOIETIC	4 (8%)	8 (17%)	16 (32%)
HYPERPLASIA, ERYTHROID	1 (2%)	1 (2%)	1 (2%)
HYPERPLASIA, GRANULOCYTIC			1 (2%)
HYPOPLASIA, ERYTHROID		1 (2%)	
#SPLEEN	(49)	(49)	(49)
CONGESTION, NOS	1 (2%)		2 (4%)
FIBROSIS	1 (2%)		
HEMOSIDEROSIS	23 (47%)	36 (73%)	31 (63%)
ATROPHY, NOS	1 (2%)		
LEUKEMOID REACTION	1 (2%)	1 (2%)	
HYPERPLASIA, RETICULUM CELL		1 (2%)	
HEMATOPOIESIS	25 (51%)	39 (80%)	34 (69%)
ERYTHROPOIESIS		1 (2%)	1 (2%)
GRANULOPOIESIS	1 (2%)		
#LYMPH NODE	(41)	(43)	(43)
HEMOSIDEROSIS	1 (2%)		
#SUBMANDIBULAR L.NODE	(41)	(43)	(43)
LYMPHANGIECTASIS			1 (2%)
#MANDIBULAR L. NODE	(41)	(43)	(43)
LYMPHANGIECTASIS		2 (5%)	1 (2%)
#BRONCHIAL LYMPH NODE	(41)	(43)	(43)
LYMPHANGIECTASIS			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE	(41)	(43)	(43)
HEMOSIDEROSIS		1 (2%)	
#THYMUS	(37)	(28)	(29)
LYMPHANGIECTASIS			1 (3%)
HEMOSIDEROSIS			1 (3%)
CIRCULATORY SYSTEM			
#HEART	(48)	(49)	(48)
FIBROSIS, DIFFUSE			1 (2%)
#MYOCARDIUM	(48)	(49)	(48)
INFLAMMATION, FOCAL	2 (4%)		
INFLAMMATION, INTERSTITIAL		6 (12%)	5 (10%)
ABSCESS, NOS	1 (2%)		
FIBROSIS	4 (8%)		
FIBROSIS, FOCAL	1 (2%)	17 (35%)	24 (50%)
SCAR			1 (2%)
DEGENERATION, NOS	6 (13%)		1 (2%)
CALCIFICATION, DYSTROPHIC		1 (2%)	
#ENDOCARDIUM	(48)	(49)	(48)
INFLAMMATION, FOCAL	2 (4%)		
*PULMONARY ARTERY	(50)	(50)	(50)
MEDIAL CALCIFICATION		1 (2%)	
CALCIFICATION, FOCAL		1 (2%)	2 (4%)
#HEPATIC SINUSOID	(49)	(50)	(49)
CONGESTION, NOS		1 (2%)	4 (8%)
DIGESTIVE SYSTEM			
*MOUTH	(50)	(50)	(50)
ABSCESS, NOS			1 (2%)
#SALIVARY GLAND	(49)	(49)	(46)
EDEMA, NOS			1 (2%)
#LIVER	(49)	(50)	(49)
CONGESTION, NOS	1 (2%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEMORRHAGE			1 (2%)
NECROSIS, NOS	1 (2%)		
NECROSIS, COAGULATIVE			1 (2%)
METAMORPHOSIS FATTY	1 (2%)	2 (4%)	5 (10%)
FOCAL CELLULAR CHANGE		1 (2%)	3 (6%)
PHAGOCYTIC CELL	1 (2%)		
ANGIECTASIS			1 (2%)
HYPERPLASIA, RETICULUM CELL		3 (6%)	4 (8%)
HEMATOPOIESIS	4 (8%)		
#LIVER/CENTRILOBULAR	(49)	(50)	(49)
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL			1 (2%)
NECROSIS, COAGULATIVE		1 (2%)	
METAMORPHOSIS FATTY	2 (4%)	3 (6%)	1 (2%)
PIGMENTATION, NOS	1 (2%)		
*BILE DUCT	(50)	(50)	(50)
INFLAMMATION, FOCAL			1 (2%)
HYPERPLASIA, NOS	1 (2%)	2 (4%)	
HYPERPLASIA, FOCAL	18 (36%)	30 (60%)	34 (68%)
HYPERPLASIA, DIFFUSE			2 (4%)
#PANCREAS	(49)	(50)	(50)
EDEMA, NOS	1 (2%)		1 (2%)
PERIARTERITIS	1 (2%)	1 (2%)	
HEMOSIDEROSIS		1 (2%)	
#PANCREATIC DUCT	(49)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, EPITHELIAL			1 (2%)
HYPERPLASIA, FOCAL	2 (4%)	8 (16%)	6 (12%)
#ESOPHAGUS	(46)	(44)	(45)
PERFORATION, INFLAMMATORY			1 (2%)
#STOMACH	(49)	(50)	(49)
ULCER, NOS	1 (2%)		
ULCER, FOCAL	1 (2%)		
EROSION	1 (2%)		
#PEYERS PATCH	(49)	(50)	(45)
HYPERPLASIA, LYMPHOID	5 (10%)	3 (6%)	6 (13%)
#ILEUM	(49)	(50)	(45)
MUCOCELE	1 (2%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#COLON	(32)	(30)	(39)
NEMATODIASIS	3 (9%)	2 (7%)	6 (15%)
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(49)
CAST, NOS	1 (2%)		1 (2%)
CONGESTION, NOS	1 (2%)		1 (2%)
INFLAMMATION, INTERSTITIAL	1 (2%)	1 (2%)	
ABSCESS, NOS	1 (2%)		
INFLAMMATION, CHRONIC	8 (16%)	9 (18%)	10 (20%)
INFLAMMATION, CHRONIC FOCAL	26 (52%)	29 (59%)	25 (51%)
INFLAMMATION, CHRONIC DIFFUSE	1 (2%)	1 (2%)	
SCLEROSIS		1 (2%)	
NEPHROSIS, NOS		1 (2%)	
GLOMERULOSCLEROSIS, NOS	1 (2%)		
CALCIFICATION, NOS		1 (2%)	
#KIDNEY/CAPSULE	(50)	(49)	(49)
CYST, NOS		1 (2%)	
#KIDNEY/CORTEX	(50)	(49)	(49)
CAST, NOS		1 (2%)	
CYST, NOS			1 (2%)
PIGMENTATION, NOS			6 (12%)
#KIDNEY/TUBULE	(50)	(49)	(49)
CAST, NOS	1 (2%)		1 (2%)
PIGMENTATION, NOS	3 (6%)		
#CONVOLUTED TUBULES	(50)	(49)	(49)
DEGENERATION, HYALINE			1 (2%)
PIGMENTATION, NOS		3 (6%)	1 (2%)
#KIDNEY/PELVIS	(50)	(49)	(49)
INFLAMMATION, SUPPURATIVE			1 (2%)
#URINARY BLADDER	(47)	(42)	(45)
HEMORRHAGE			1 (2%)
INFLAMMATION, HEMORRHAGIC			1 (2%)
#U. BLADDER/SUBMUCOSA	(47)	(42)	(45)
HEMORRHAGE	1 (2%)	1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(48)	(49)
CYST, NOS	1 (2%)		
HEMORRHAGE	1 (2%)		1 (2%)
HEMOSIDEROSIS			1 (2%)
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, FOCAL			1 (2%)
ANGIECTASIS	2 (4%)	4 (8%)	3 (6%)
#ADRENAL	(49)	(50)	(50)
ANGIECTASIS	1 (2%)	1 (2%)	4 (8%)
#ADRENAL CORTEX	(49)	(50)	(50)
HYPERPLASIA, NODULAR	1 (2%)	1 (2%)	
#ADRENAL MEDULLA	(49)	(50)	(50)
HYPERPLASIA, NODULAR	2 (4%)		
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL	1 (2%)	5 (10%)	9 (18%)
#THYROID	(46)	(49)	(47)
CYSTIC FOLLICLES		4 (8%)	2 (4%)
PIGMENTATION, NOS			1 (2%)
HYPERPLASIA, C-CELL	23 (50%)	29 (59%)	26 (55%)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	2 (4%)
#THYROID FOLLICLE	(46)	(49)	(47)
PIGMENTATION, NOS		3 (6%)	2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
GALACTOCELE			1 (2%)
*PREPUTIAL GLAND	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	2 (4%)	1 (2%)	5 (10%)
INFLAMMATION, CHRONIC	2 (4%)		
NECROSIS, NOS			1 (2%)
#PROSTATE	(44)	(44)	(47)
INFLAMMATION, FOCAL			4 (9%)
INFLAMMATION, SUPPURATIVE	2 (5%)	4 (9%)	4 (9%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, NOS		1 (2%)	
#TESTIS	(50)	(49)	(49)
CALCIFICATION, FOCAL		1 (2%)	
ATROPHY, NOS	32 (64%)	39 (80%)	38 (78%)
ATROPHY, FOCAL	7 (14%)	1 (2%)	2 (4%)
ASPERMATOGENESIS	4 (8%)	1 (2%)	3 (6%)
HYPERTROPHY, NOS			1 (2%)
HYPERPLASIA, INTERSTITIAL CELL	1 (2%)	1 (2%)	6 (12%)
#TESTIS/TUBULE	(50)	(49)	(49)
CALCIFICATION, NOS	1 (2%)		
CALCIFICATION, FOCAL		1 (2%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(50)	(50)	(50)
THROMBOSIS, NOS	1 (2%)		
#BRAIN STEM	(50)	(50)	(50)
HEMORRHAGE			1 (2%)
NECROSIS, NOS	1 (2%)		
MALACIA			1 (2%)
#MIDBRAIN	(50)	(50)	(50)
NECROSIS, NOS	1 (2%)		
MALACIA	1 (2%)		
*SPINAL CORD	(50)	(50)	(50)
HEMORRHAGE			1 (2%)
DEGENERATION, NOS			1 (2%)
MALACIA		1 (2%)	1 (2%)
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	
INFLAMMATION, NOS		1 (2%)	
PERIVASCULITIS		1 (2%)	
DEGENERATION, NOS	1 (2%)		
CATARACT	13 (26%)	16 (32%)	12 (24%)
HEMOSIDEROSIS			1 (2%)
ANEMIA, NOS			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*EYE/CORNEA	(50)	(50)	(50)
ULCER, NOS		1 (2%)	
INFLAMMATION, INTERSTITIAL	1 (2%)	2 (4%)	
*LENS CAPSULE	(50)	(50)	(50)
CALCIFICATION, NOS	1 (2%)		
*MIDDLE EAR	(50)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(50)	(50)	(50)
ATROPHY, NOS			1 (2%)
*MUSCLE HIP/THIGH	(50)	(50)	(50)
ATROPHY, NOS			1 (2%)
BODY CAVITIES			
*MEDIASTINUM	(50)	(50)	(50)
THROMBOSIS, NOS			1 (2%)
*ABDOMINAL CAVITY	(50)	(50)	(50)
NECROSIS, FAT	1 (2%)		
*PLEURA	(50)	(50)	(50)
HYDROTHORAX	1 (2%)		
*MESENTERY	(50)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
FIBROSIS		1 (2%)	1 (2%)
PERIARTERITIS		1 (2%)	
NECROSIS, FAT	2 (4%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
CONGESTION, NOS			2 (4%)
JAUNDICE, NOS			1 (2%)
DIAPHRAGM			
HERNIA, NOS			1

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ADIPOSE TISSUE			
INFLAMMATION, NOS		1	
INFLAMMATION, FOCAL	1		
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED 3-NITROPROPIONIC ACID BY GAVAGE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
NECROSIS, FOCAL	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
ABSCESS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#TRACHEA	(49)	(48)	(49)
INFLAMMATION, NOS	17 (35%)	10 (21%)	11 (22%)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, CHRONIC			3 (6%)
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	2 (4%)
#LUNG/BRONCHUS	(50)	(49)	(49)
BRONCHIECTASIS	2 (4%)		1 (2%)
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, SUPPURATIVE		2 (4%)	
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	
HYPERPLASIA, LYMPHOID	27 (54%)	27 (55%)	31 (63%)
#LUNG	(50)	(49)	(49)
CONGESTION, NOS		1 (2%)	1 (2%)
EDEMA, NOS		1 (2%)	
BRONCHOPNEUMONIA, NOS	1 (2%)		
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, INTERSTITIAL		1 (2%)	
PNEUMONIA, ASPIRATION			1 (2%)
BRONCHOPNEUMONIA SUPPURATIVE		1 (2%)	
BRONCHOPNEUMONIA, ACUTE		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, ACUTE SUPPURATIVE		1 (2%)	1 (2%)
BRONCHOPNEUMONIA ACUTE SUPPURATI		1 (2%)	1 (2%)
PNEUMONIA, CHRONIC MURINE	5 (10%)	4 (8%)	4 (8%)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	1 (2%)
PERIVASCULAR CUFFING	2 (4%)		
HEMOSIDEROSIS		1 (2%)	1 (2%)
ALVEOLAR MACROPHAGES	2 (4%)	5 (10%)	
HYPERPLASIA, LYMPHOID	1 (2%)		
#LUNG/ALVEOLI	(50)	(49)	(49)
CONGESTION, NOS	1 (2%)	3 (6%)	3 (6%)
EDEMA, NOS		3 (6%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(50)	(47)	(50)
HEMOSIDEROSIS			1 (2%)
HYPOPLASIA, NOS		1 (2%)	
HYPERPLASIA, NOS	1 (2%)	1 (2%)	
HYPERPLASIA, HEMATOPOIETIC	3 (6%)	2 (4%)	6 (12%)
HYPERPLASIA, GRANULOCYTIC	2 (4%)		1 (2%)
ERYTHROPOIESIS		2 (4%)	
GRANULOPOIESIS		1 (2%)	
#SPLEEN	(50)	(50)	(49)
ECTOPIA		1 (2%)	
CONGESTION, NOS	1 (2%)	1 (2%)	
HEMOSIDEROSIS	34 (68%)	44 (88%)	40 (82%)
LEUKEMOID REACTION	1 (2%)		
HYPERPLASIA, RETICULUM CELL	1 (2%)		
HEMATOPOIESIS	40 (80%)	37 (74%)	36 (73%)
ERYTHROPOIESIS		3 (6%)	2 (4%)
GRANULOPOIESIS		2 (4%)	2 (4%)
#SPLENIC CAPSULE	(50)	(50)	(49)
INFLAMMATION, FOCAL		1 (2%)	
#LYMPH NODE	(44)	(41)	(45)
LYMPHANGIECTASIS			1 (2%)
HEMOSIDEROSIS	1 (2%)		
#MANDIBULAR L. NODE	(44)	(41)	(45)
PIGMENTATION, NOS		1 (2%)	
#CERVICAL LYMPH NODE	(44)	(41)	(45)
CONGESTION, NOS	1 (2%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEMOSIDEROSIS	1 (2%)		
#THYMUS	(39)	(30)	(31)
LYMPHANGIECTASIS		1 (3%)	
CONGESTION, NOS			1 (3%)
HEMOSIDEROSIS	1 (3%)		
CIRCULATORY SYSTEM			
#HEART/ATRIUM	(48)	(46)	(50)
THROMBOSIS, NOS		1 (2%)	
#MYOCARDIUM	(48)	(46)	(50)
INFLAMMATION, FOCAL			2 (4%)
INFLAMMATION, INTERSTITIAL		7 (15%)	2 (4%)
FIBROSIS	1 (2%)		
FIBROSIS, FOCAL	2 (4%)	9 (20%)	9 (18%)
SCAR		1 (2%)	
#ENDOCARDIUM	(48)	(46)	(50)
INFLAMMATION, FOCAL		1 (2%)	
*PULMONARY ARTERY	(50)	(50)	(50)
CALCIFICATION, NOS			1 (2%)
CALCIFICATION, FOCAL			1 (2%)
#HEPATIC SINUSOID	(49)	(50)	(50)
CONGESTION, NOS		4 (8%)	1 (2%)
HYPERPLASIA, GRANULOCYTIC		1 (2%)	
DIGESTIVE SYSTEM			
*TONGUE	(50)	(50)	(50)
HYPERKERATOSIS		1 (2%)	
ACANTHOSIS		1 (2%)	
#LIVER	(49)	(50)	(50)
HERNIA, NOS		1 (2%)	1 (2%)
NECROSIS, FOCAL		1 (2%)	1 (2%)
METAMORPHOSIS FATTY	9 (18%)	11 (22%)	3 (6%)
PIGMENTATION, NOS			1 (2%)
FOCAL CELLULAR CHANGE		1 (2%)	
ANGIECTASIS	3 (6%)	1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, RETICULUM CELL		4 (8%)	1 (2%)
HEMATOPOIESIS	1 (2%)	2 (4%)	
ERYTHROPOIESIS	1 (2%)		
#LIVER/CENTRILOBULAR	(49)	(50)	(50)
NECROSIS, FOCAL	1 (2%)		
METAMORPHOSIS FATTY	2 (4%)	2 (4%)	2 (4%)
#LIVER/PERIPORTAL	(49)	(50)	(50)
METAMORPHOSIS FATTY	1 (2%)		1 (2%)
*BILE DUCT	(50)	(50)	(50)
INFLAMMATION, FOCAL		2 (4%)	
HYPERPLASIA, NOS			2 (4%)
HYPERPLASIA, FOCAL	15 (30%)	17 (34%)	16 (32%)
#PANCREAS	(49)	(49)	(47)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		
PERIARTERITIS			1 (2%)
#PANCREATIC DUCT	(49)	(49)	(47)
HYPERPLASIA, FOCAL	5 (10%)	6 (12%)	9 (19%)
#PANCREATIC ACINUS	(49)	(49)	(47)
NODULE		1 (2%)	
#ESOPHAGUS	(49)	(48)	(42)
ABSCESS, NOS		1 (2%)	
#STOMACH	(50)	(50)	(49)
ULCER, NOS	1 (2%)		
ULCER, FOCAL		1 (2%)	
#CARDIAC STOMACH	(50)	(50)	(49)
ULCER, NOS	1 (2%)		
EROSION		1 (2%)	
#PEYERS PATCH	(49)	(45)	(50)
HYPERPLASIA, LYMPHOID	4 (8%)	2 (4%)	4 (8%)
#COLON	(35)	(39)	(39)
NEMATODIASIS	5 (14%)	4 (10%)	5 (13%)
HYPERPLASIA, RETICULUM CELL		1 (3%)	
HYPERPLASIA, LYMPHOID		1 (3%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(50)
CAST, NOS		1 (2%)	
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, INTERSTITIAL	1 (2%)	1 (2%)	
INFLAMMATION, CHRONIC	2 (4%)	2 (4%)	2 (4%)
INFLAMMATION, CHRONIC FOCAL	12 (24%)	7 (14%)	13 (26%)
INFLAMMATION, CHRONIC DIFFUSE		1 (2%)	
SCAR		1 (2%)	
NEPHROSIS, NOS	1 (2%)		
INFARCT, ACUTE			1 (2%)
PIGMENTATION, NOS	2 (4%)	2 (4%)	1 (2%)
HYPERPLASIA, TUBULAR CELL		1 (2%)	
#KIDNEY/CORTEX	(49)	(50)	(50)
SCAR		1 (2%)	
INFARCT, NOS			1 (2%)
PIGMENTATION, NOS	17 (35%)	26 (52%)	20 (40%)
HYPERPLASIA, LYMPHOID	1 (2%)		
#KIDNEY/TUBULE	(49)	(50)	(50)
CAST, NOS		1 (2%)	
PIGMENTATION, NOS	2 (4%)	2 (4%)	1 (2%)
ATROPHY, NOS		1 (2%)	
#CONVOLUTED TUBULES	(49)	(50)	(50)
CAST, NOS	1 (2%)		
HYALINE MEMBRANE	1 (2%)		
PIGMENTATION, NOS			1 (2%)
#KIDNEY/PELVIS	(49)	(50)	(50)
CALCIFICATION, FOCAL	1 (2%)	1 (2%)	
#URINARY BLADDER	(35)	(42)	(41)
CALCULUS, NOS	1 (3%)		
INFLAMMATION, CHRONIC	1 (3%)		
HYPERPLASIA, EPITHELIAL	1 (3%)		
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(46)	(47)
CYST, NOS	1 (2%)		3 (6%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEMORRHAGE	2 (4%)	1 (2%)	2 (4%)
HEMORRHAGIC CYST	2 (4%)	3 (7%)	
HEMOSIDEROSIS	1 (2%)	3 (7%)	3 (6%)
HYPERPLASIA, NOS	3 (7%)	1 (2%)	
HYPERPLASIA, FOCAL	1 (2%)	1 (2%)	
ANGIECTASIS	3 (7%)	14 (30%)	20 (43%)
#ADRENAL	(49)	(50)	(49)
CYST, NOS			1 (2%)
DEGENERATION, NOS	1 (2%)		
HEMOSIDEROSIS		1 (2%)	
ANGIECTASIS	3 (6%)	6 (12%)	7 (14%)
#ADRENAL CORTEX	(49)	(50)	(49)
HEMORRHAGE	1 (2%)		
#ADRENAL MEDULLA	(49)	(50)	(49)
HYPERPLASIA, FOCAL		1 (2%)	2 (4%)
#THYROID	(50)	(44)	(44)
CYSTIC FOLLICLES	1 (2%)	1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	1 (2%)
HYPERPLASIA, C-CELL	39 (78%)	24 (55%)	21 (48%)
#THYROID FOLLICLE	(50)	(44)	(44)
PIGMENTATION, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
GALACTOCELE	5 (10%)	4 (8%)	10 (20%)
NECROSIS, FOCAL			1 (2%)
METAPLASIA, SQUAMOUS			1 (2%)
ADENOSIS	1 (2%)	1 (2%)	
*PREPUTIAL GLAND	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	7 (14%)	2 (4%)	1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)
NECROSIS, NOS			1 (2%)
HYPERPLASIA, NOS	1 (2%)		2 (4%)
HYPERPLASIA, CYSTIC		1 (2%)	
*VAGINA	(50)	(50)	(50)
HEMATOMA, NOS		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#UTERUS	(50)	(48)	(49)
HEMORRHAGE		1 (2%)	
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	
PYOMETRA		1 (2%)	
NECROSIS, NOS		1 (2%)	
#CERVIX UTERI	(50)	(48)	(49)
HYPERPLASIA, NOS		1 (2%)	
#UTERUS/ENDOMETRIUM	(50)	(48)	(49)
CYST, NOS	1 (2%)		
HEMORRHAGE	1 (2%)		
INFLAMMATION, FOCAL		1 (2%)	
ULCER, FOCAL	1 (2%)		
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		
INFLAMMATION, SUPPURATIVE	8 (16%)	8 (17%)	14 (29%)
INFLAMMATION, VESICULAR		1 (2%)	
INFLAMMATION, CHRONIC SUPPURATIV		1 (2%)	
NECROSIS, NOS			2 (4%)
HYPERPLASIA, CYSTIC	2 (4%)	1 (2%)	2 (4%)
#OVARY/OVIDUCT	(50)	(48)	(49)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE	5 (10%)	13 (27%)	12 (24%)
#OVARY	(50)	(47)	(48)
CYST, NOS	9 (18%)	8 (17%)	10 (21%)
FOLLICULAR CYST, NOS			1 (2%)
INFLAMMATION, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(49)	(50)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#CEREBRUM	(49)	(50)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)	
ABSCESS, NOS			1 (2%)
#BRAIN	(49)	(50)	(50)
COMPRESSION		2 (4%)	
INFLAMMATION, NOS		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MALACIA	1 (2%)		
#MIDBRAIN	(49)	(50)	(50)
COMPRESSION			2 (4%)
GLIOSIS		1 (2%)	
*SPINAL CORD	(50)	(50)	(50)
HEMORRHAGE	1 (2%)		
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
HEMORRHAGE			1 (2%)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, INTERSTITIAL			1 (2%)
PUS			1 (2%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
CATARACT	11 (22%)	15 (30%)	16 (32%)
*EYE/CORNEA	(50)	(50)	(50)
ULCER, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	
*EAR	(50)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
*EAR CANAL	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(50)	(50)	(50)
ATROPHY, NOS	1 (2%)		
BODY CAVITIES			
*MESENTERY	(50)	(50)	(50)
FIBROSIS		2 (4%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
CONGESTION, NOS		2 (4%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
JAUNDICE, NOS		1 (2%)	
THORAX			
HEMORRHAGE			1
DIAPHRAGM			
HERNIA, NOS	1	1	2
ADIPOSE TISSUE			
INFLAMMATION, NOS		2	

SPECIAL MORPHOLOGY SUMMARY

NONE

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN MICE ADMINISTERED 3-NITROPROPIONIC ACID

BY GAVAGE

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED 3-NITROPROPIONIC ACID BY GAVAGE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(50)
CYST, NOS	1 (2%)		
ULCER, NOS		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
*SUBCUT TISSUE	(49)	(50)	(50)
ABSCESS, NOS		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(49)	(48)	(50)
METAPLASIA, SQUAMOUS	1 (2%)		
HYPERPLASIA, LYMPHOID	11 (22%)	1 (2%)	2 (4%)
#LUNG	(49)	(48)	(50)
CONGESTION, NOS	1 (2%)		
HEMORRHAGE	1 (2%)		
PNEUMONIA, ASPIRATION			1 (2%)
PERIVASCULITIS		1 (2%)	
ALVEOLAR MACROPHAGES			1 (2%)
HYPERPLASIA, ADENOMATOUS	1 (2%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM			1 (2%)
#LUNG/ALVEOLI	(49)	(48)	(50)
CONGESTION, NOS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(46)	(49)	(50)
HYPERPLASIA, NOS		3 (6%)	1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, HEMATOPOIETIC	2 (4%)	2 (4%)	
HYPERPLASIA, GRANULOCYTIC	2 (4%)		1 (2%)
#SPLEEN	(46)	(50)	(46)
HEMORRHAGE	1 (2%)		
ANGIECTASIS	1 (2%)		
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
HYPERPLASIA, RETICULUM CELL		2 (4%)	1 (2%)
HYPERPLASIA, LYMPHOID	2 (4%)	5 (10%)	
HEMATOPOIESIS	24 (52%)	23 (46%)	17 (37%)
ERYTHROPOIESIS	2 (4%)	1 (2%)	2 (4%)
GRANULOPOIESIS	1 (2%)		
#LYMPH NODE	(40)	(31)	(30)
HEMATOPOIESIS	1 (3%)		
#MESENTERIC L. NODE	(40)	(31)	(30)
THROMBOSIS, NOS	1 (3%)		
CONGESTION, NOS	3 (8%)		
HEMOSIDEROSIS			1 (3%)
ERYTHROPHAGOCYTOSIS			2 (7%)
HYPERPLASIA, HEMATOPOIETIC		1 (3%)	
HYPERPLASIA, LYMPHOID		1 (3%)	
#THYMUS	(35)	(38)	(41)
HYPERPLASIA, RETICULUM CELL		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*TONGUE	(49)	(50)	(50)
HYPERPLASIA, EPITHELIAL			1 (2%)
HYPERKERATOSIS			1 (2%)
ACANTHOSIS			1 (2%)
#LIVER	(49)	(50)	(49)
HEMORRHAGE	1 (2%)		1 (2%)
FIBROSIS, FOCAL	1 (2%)		
DEGENERATION, NOS			1 (2%)
NECROSIS, FOCAL		1 (2%)	7 (14%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
METAMORPHOSIS FATTY	4 (8%)	1 (2%)	3 (6%)
CLEAR-CELL CHANGE		1 (2%)	
HYPERPLASIA, NODULAR		1 (2%)	
HYPERPLASTIC NODULE	1 (2%)		
ANGIECTASIS	1 (2%)		1 (2%)
HYPERPLASIA, HEMATOPOIETIC	1 (2%)		
HYPERPLASIA, RETICULUM CELL		3 (6%)	2 (4%)
HYPERPLASIA, LYMPHOID	1 (2%)		
HEMATOPOIESIS	1 (2%)	1 (2%)	1 (2%)
ERYTHROPOIESIS		1 (2%)	
#HEPATIC CAPSULE	(49)	(50)	(49)
HEMATOMA, NOS	1 (2%)		
FIBROSIS, FOCAL		1 (2%)	
#LIVER/CENTRILOBULAR	(49)	(50)	(49)
NECROSIS, NOS			1 (2%)
METAMORPHOSIS FATTY	1 (2%)		
#LIVER/PERIPORTAL	(49)	(50)	(49)
HYPERPLASIA, LYMPHOID	1 (2%)		
#LIVER/HEPATOCYTES	(49)	(50)	(49)
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL		2 (4%)	
*BILE DUCT	(49)	(50)	(50)
CYST, NOS		1 (2%)	
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, FOCAL		1 (2%)	1 (2%)
INFLAMMATION, SUPPURATIVE	1 (2%)		
FOCAL CELLULAR CHANGE			1 (2%)
HYPERPLASIA, NOS	4 (8%)		
HYPERPLASIA, FOCAL		1 (2%)	2 (4%)
#PANCREAS	(48)	(50)	(47)
CYSTIC DUCTS	1 (2%)		
FIBROSIS	1 (2%)		
NECROSIS, NOS	1 (2%)		
#PANCREATIC DUCT	(48)	(50)	(47)
CYST, NOS		3 (6%)	
HYPERPLASIA, FOCAL	1 (2%)		
#PEYERS PATCH	(47)	(49)	(49)
INFLAMMATION, SUPPURATIVE			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, LYMPHOID	2 (4%)	10 (20%)	5 (10%)
#COLON	(22)	(44)	(35)
NEMATODIASIS	4 (18%)	6 (14%)	1 (3%)
URINARY SYSTEM			
#KIDNEY	(48)	(49)	(50)
HYDRONEPHROSIS		1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR	2 (4%)		
INFLAMMATION, INTERSTITIAL	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
FIBROSIS	1 (2%)		
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	1 (2%)
#KIDNEY/CORTEX	(48)	(49)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
INFARCT, NOS	1 (2%)		
#U.BLADDER/SUBMUCOSA	(47)	(46)	(49)
HYPERPLASIA, LYMPHOID			1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL/CAPSULE	(46)	(49)	(50)
HYPERPLASIA, FOCAL	28 (61%)	41 (84%)	38 (76%)
#ADRENAL CORTEX	(46)	(49)	(50)
HYPERPLASIA, NOS	2 (4%)		
#THYROID	(43)	(44)	(48)
CYSTIC FOLLICLES	1 (2%)		1 (2%)
HYPERPLASIA, FOLLICULAR-CELL	2 (5%)	2 (5%)	2 (4%)
#PANCREATIC ISLETS	(48)	(50)	(47)
HYPERPLASIA, NOS	2 (4%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(49)	(50)	(50)
CYST, NOS		3 (6%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, SUPPURATIVE	2 (4%)	2 (4%)	1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION, CHRONIC SUPPURATIV			1 (2%)
HYPERKERATOSIS			1 (2%)
#TESTIS	(47)	(49)	(50)
GRANULOMA, SPERMATIC			1 (2%)
ATROPHY, NOS			2 (4%)
ATROPHY, FOCAL		4 (8%)	1 (2%)
ASPERMATOGENESIS	1 (2%)		
*EPIDIDYMIS	(49)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
FIBROSIS		1 (2%)	
FIBROSIS, FOCAL			1 (2%)
NECROSIS, FAT		1 (2%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(48)	(49)	(49)
INFLAMMATION, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE	(49)	(50)	(50)
CATARACT		1 (2%)	
*EYE/CORNEA	(49)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
*LENS CAPSULE	(49)	(50)	(50)
DEGENERATION, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(49)	(50)	(50)
HEMORRHAGE			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, NOS			1 (2%)
NECROSIS, FOCAL	1 (2%)		
*ABDOMINAL VISCERA	(49)	(50)	(50)
ADHESION, NOS			1 (2%)
*PLEURA	(49)	(50)	(50)
HYDROTHORAX		1 (2%)	
HEMOTHORAX		2 (4%)	
INFLAMMATION, FOCAL			1 (2%)
*MESENTERY	(49)	(50)	(50)
FIBROSIS		4 (8%)	1 (2%)
FIBROSIS, FOCAL			1 (2%)
NECROSIS, FAT	2 (4%)	3 (6%)	3 (6%)
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, NOS	2	1	
FIBROSIS	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	1
AUTO/NECROPSY/HISTO PERF			1
AUTOLYSIS/NO NECROPSY	1		

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED 3-NITROPROPIONIC ACID BY GAVAGE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
ALOPECIA		1 (2%)	
HYPERKERATOSIS	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(47)	(49)	(49)
HYPERPLASIA, LYMPHOID	18 (38%)	3 (6%)	3 (6%)
#LUNG	(47)	(49)	(49)
CONGESTION, NOS		1 (2%)	2 (4%)
INFLAMMATION, FOCAL	1 (2%)		
INFLAMMATION, SUPPURATIVE			1 (2%)
ALVEOLAR MACROPHAGES			1 (2%)
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(46)	(48)	(50)
HYPERPLASIA, HEMATOPOIETIC	3 (7%)	1 (2%)	
HYPERPLASIA, GRANULOCYTIC	1 (2%)		1 (2%)
GRANULOPOIESIS			1 (2%)
#SPLEEN	(47)	(50)	(50)
RUPTURE			1 (2%)
THROMBOSIS, NOS			1 (2%)
LEUKEMOID REACTION	1 (2%)		2 (4%)
HYPERPLASIA, LYMPHOID	6 (13%)	8 (16%)	11 (22%)
HEMATOPOIESIS	19 (40%)	22 (44%)	22 (44%)
ERYTHROPOIESIS			1 (2%)
MYELOPOIESIS	1 (2%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LYMPH NODE	(38)	(36)	(33)
EDEMA, NOS			1 (3%)
HYPERPLASIA, LYMPHOID	1 (3%)		
#MANDIBULAR L. NODE	(38)	(36)	(33)
HEMORRHAGE			1 (3%)
#MESENTERIC L. NODE	(38)	(36)	(33)
INFLAMMATION, GRANULOMATOUS	1 (3%)		
HYPERPLASIA, LYMPHOID			1 (3%)
#THYMUS	(38)	(42)	(35)
HYPERPLASIA, LYMPHOID	1 (3%)	1 (2%)	
CIRCULATORY SYSTEM			
#HEART/ATRIUM	(49)	(50)	(49)
THROMBOSIS, NOS			1 (2%)
#CARDIAC VALVE	(49)	(50)	(49)
PIGMENTATION, NOS		1 (2%)	1 (2%)
*UTERINE ARTERY	(50)	(50)	(50)
THROMBOSIS, NOS	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(50)
HEMORRHAGIC CYST		1 (2%)	
NECROSIS, FOCAL	1 (2%)		
NECROSIS, ISCHEMIC			1 (2%)
METAMORPHOSIS FATTY	2 (4%)	2 (4%)	
ANGIECTASIS		1 (2%)	
LEUKEMOID REACTION			1 (2%)
HYPERPLASIA, RETICULUM CELL	1 (2%)		2 (4%)
HYPERPLASIA, LYMPHOID	2 (4%)		1 (2%)
HEMATOPOIESIS	3 (6%)		1 (2%)
#LIVER/HEPATOCYTES	(49)	(50)	(50)
DEGENERATION, NOS		1 (2%)	
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL	1 (2%)	1 (2%)	3 (6%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC DUCT	(44)	(49)	(50)
CYST, NOS		1 (2%)	
#PEYERS PATCH	(48)	(48)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	3 (6%)
#DUODENUM	(48)	(48)	(49)
HYPERPLASIA, LYMPHOID			1 (2%)
#COLON	(36)	(38)	(35)
NEMATODIASIS		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(50)
CONGESTION, NOS		1 (2%)	
GLOMERULONEPHRITIS, NOS	1 (2%)		
PYELONEPHRITIS, NOS			1 (2%)
PYELONEPHRITIS DIFFUSE			1 (2%)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
GLOMERULOSCLEROSIS, NOS			1 (2%)
HYPERPLASIA, LYMPHOID	10 (20%)		4 (8%)
#KIDNEY/CORTEX	(49)	(50)	(50)
SCAR	1 (2%)		
DEGENERATION, HYALINE	1 (2%)		
#KIDNEY/TUBULE	(49)	(50)	(50)
DEGENERATION, HYALINE		1 (2%)	1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(43)	(48)	(42)
HEMORRHAGIC CYST			1 (2%)
HYPERPLASIA, NOS		1 (2%)	2 (5%)
ANGIECTASIS		2 (4%)	2 (5%)
#ADRENAL/CAPSULE	(48)	(50)	(49)
HYPERPLASIA, FOCAL	43 (90%)	47 (94%)	42 (86%)
#ADRENAL CORTEX	(48)	(50)	(49)
HYPERPLASIA, FOCAL			1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#THYROID	(40)	(47)	(45)
CYSTIC FOLLICLES	1 (3%)		1 (2%)
INFLAMMATION, FOCAL			1 (2%)
HYPERPLASIA, FOLLICULAR-CELL	6 (15%)	13 (28%)	5 (11%)
REPRODUCTIVE SYSTEM			
#UTERUS	(47)	(49)	(48)
HYDROMETRA		1 (2%)	
HEMORRHAGE		1 (2%)	
METAPLASIA, SQUAMOUS		2 (4%)	1 (2%)
#UTERUS/ENDOMETRIUM	(47)	(49)	(48)
HEMATOMA, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE	3 (6%)	1 (2%)	1 (2%)
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, CYSTIC	19 (40%)	37 (76%)	30 (63%)
#OVARY/OVIDUCT	(47)	(49)	(48)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)	1 (2%)	
INFLAMMATION, SUPPURATIVE	3 (6%)		
NECROSIS, NOS	1 (2%)		
#OVARY	(39)	(47)	(47)
CYST, NOS	4 (10%)	10 (21%)	12 (26%)
FOLLICULAR CYST, NOS		4 (9%)	4 (9%)
HEMORRHAGIC CYST	1 (3%)		1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
INFLAMMATION, SUPPURATIVE	1 (3%)		1 (2%)
INFLAMMATION, CHRONIC	1 (3%)		
CALCIFICATION, FOCAL			1 (2%)
HYPERPLASIA, LYMPHOID			1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(47)	(50)	(50)
PERIVASCULAR CUFFING		1 (2%)	
#CEREBRUM	(47)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
PHTHISIS BULBI		1 (2%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*HARDERIAN GLAND	(50)	(50)	(50)
ABSCESS, NOS		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(50)	(50)	(50)
CYST, NOS	1 (2%)		
HEMORRHAGE			1 (2%)
*PLEURA	(50)	(50)	(50)
HYDROTHORAX	1 (2%)		
HEMOTHORAX			1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/HISTO PERF	1		

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED 3-NITROPROPIONIC ACID BY GAVAGE

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 3-Nitropropionic Acid by Gavage[a]

Topography: Morphology	Control	Low Dose	High Dose
All Sites: Skin Tumors[b]	2/50 (4)	6/50 (12)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		3.000	1.000
Lower Limit		0.569	0.075
Upper Limit		29.254	13.326
Weeks to First Observed Tumor	111	91	111
Hematopoietic System: Malignant Lymphoma, Undifferentiated Leukemia, or Lymphocytic Leukemia[b]	11/50 (22)	6/50 (12)	14/50 (28)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.545	1.273
Lower Limit		0.179	0.597
Upper Limit		1.477	2.785
Weeks to First Observed Tumor	96	96	83

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
All Neoplasms[b]	13/50 (26)	7/50 (14)	16/50 (32)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.043		
Relative Risk (Control)[f]		0.539	1.231
Lower Limit		0.199	0.624
Upper Limit		1.323	2.474
Weeks to First Observed Tumor	90	96	83
Liver: Neoplastic Nodule[b]	0/49 (0)	3/50 (6)	5/49 (10)
P Values[c,d]	P = 0.022	N.S.	P = 0.028
Relative Risk (Control)[f]		Infinite	Infinite
Lower Limit		0.590	1.262
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	111	109

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Neoplastic Nodule or Hepatocellular Carcinoma[b]	0/49 (0)	3/50 (6)	6/49 (12)
P Values[c,d]	P = 0.010	N.S.	P = 0.012
Relative Risk (Control)[f]		Infinite	Infinite
Lower Limit		0.590	1.601
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	111	109
Pituitary: Chromophobe Adenoma[b]	3/46 (7)	5/48 (10)	4/49 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		1.597	1.252
Lower Limit		0.331	0.224
Upper Limit		9.773	8.137
Weeks to First Observed Tumor	111	101	95

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Adrenal: Pheochromocytoma[b]	4/49 (8)	5/50 (10)	5/50 (10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		1.225	1.225
Lower Limit		0.280	0.280
Upper Limit		5.833	5.833
Weeks to First Observed Tumor	88	79	106
Thyroid: Follicular-cell Carcinoma[b]	1/46 (2)	0/49 (0)	4/47 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.000	3.915
Lower Limit		0.000	0.407
Upper Limit		17.510	188.454
Weeks to First Observed Tumor	111	--	99

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma[b]	1/46 (2)	2/49 (4)	0/47 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		1.878	0.000
Lower Limit		0.101	0.000
Upper Limit		108.485	18.240
Weeks to First Observed Tumor	111	111	--
Thyroid: C-cell Adenoma or Carcinoma[b]	4/46 (9)	10/49 (20)	4/47 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.044		
Relative Risk (Control)[f]		2.347	0.979
Lower Limit		0.735	0.193
Upper Limit		9.593	4.955
Weeks to First Observed Tumor	111	107	103

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Pancreatic Islets: Islet-cell Adenoma[b]	4/49 (8)	6/50 (12)	11/50 (22)
P Values[c,d]	P = 0.033	N.S.	P = 0.049
Relative Risk (Control)[f]		1.470	2.695
Lower Limit		0.372	0.865
Upper Limit		6.681	10.868
Weeks to First Observed Tumor	88	87	83
Preputial Gland: Adenoma, NOS (not otherwise specified)[b]	2/50 (4)	1/50 (2)	4/50 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.500	2.000
Lower Limit		0.009	0.301
Upper Limit		9.290	21.316
Weeks to First Observed Tumor	111	111	106

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor[b]	48/50 (96)	44/49 (90)	48/49 (98)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.935	1.020
Lower Limit		0.871	0.954
Upper Limit		1.058	1.061
Weeks to First Observed Tumor	84	65	82
Peritoneum: Mesothelioma, NOS[b]	1/50 (2)	3/50 (6)	0/50 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		3.000	0.000
Lower Limit		0.287	0.000
Upper Limit		74.701	18.658
Weeks to First Observed Tumor	111	79	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/50 (0)	1/49 (2)	3/48 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		Infinite	Infinite
Lower Limit		0.055	0.627
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	111	86
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	3/50 (6)	2/49 (4)	3/48 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.680	1.042
Lower Limit		0.059	0.146
Upper Limit		5.680	7.419
Weeks to First Observed Tumor	102	111	86

[a]Treated groups received doses of 0.425 or 0.85 mg/animal/day.

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

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in each treated group is the probability level for the Fisher exact test for the comparison of that treated group with the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 3-Nitropropionic Acid by Gavage[a]

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma, Lymphocytic Leukemia, or Leukemia, NOS[b]	5/50 (10)	5/50 (10)	8/50 (16)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		1.000	1.600
Lower Limit		0.245	0.497
Upper Limit		4.082	5.808
Weeks to First Observed Tumor	98	106	83
Hematopoietic System: All Neoplasms[b]	7/50 (14)	6/50 (12)	10/50 (20)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.857	1.429
Lower Limit		0.268	0.535
Upper Limit		2.684	4.072
Weeks to First Observed Tumor	98	105	83

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	19/45 (42)	15/46 (33)	20/47 (43)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.772	1.008
Lower Limit		0.423	0.596
Upper Limit		1.393	1.711
Weeks to First Observed Tumor	90	96	95
Adrenal: Pheochromocytoma[b]	3/49 (6)	1/50 (2)	1/49 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.327	0.333
Lower Limit		0.013	0.013
Upper Limit		3.417	3.486
Weeks to First Observed Tumor	107	103	107

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma[b]	2/50 (4)	1/44 (2)	2/44 (5)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.568	1.136
Lower Limit		0.010	0.085
Upper Limit		10.516	15.083
Weeks to First Observed Tumor	111	111	59
Thyroid: C-cell Adenoma or Carcinoma[b]	5/50 (10)	4/44 (9)	5/44 (11)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.909	1.136
Lower Limit		0.191	0.279
Upper Limit		3.952	4.608
Weeks to First Observed Tumor	111	111	59

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	12/50 (24)	14/50 (28)	13/50 (26)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		1.167	1.083
Lower Limit		0.560	0.509
Upper Limit		2.475	2.334
Weeks to First Observed Tumor	94	67	88
Uterus: Endometrial Stromal Polyp[b]	2/50 (4)	4/48 (8)	5/49 (10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		2.083	2.551
Lower Limit		0.314	0.441
Upper Limit		22.174	25.786
Weeks to First Observed Tumor	111	96	111

[a]Treated groups received doses of 0.6 or 1.2 mg/animal/day.

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

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in each treated group is the probability level for the Fisher exact test for the comparison of that treated group with the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED 3-NITROPROPIONIC ACID BY GAVAGE

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 3-Nitropropionic Acid by Gavage[a]

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	4/49 (8)	3/48 (6)	3/50 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.766	0.735
Lower Limit		0.118	0.113
Upper Limit		4.285	4.120
Weeks to First Observed Tumor	104	96	105
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	14/49 (29)	8/48 (17)	10/50 (20)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.583	0.700
Lower Limit		0.234	0.309
Upper Limit		1.345	1.525
Weeks to First Observed Tumor	85	96	105

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma or Lymphocytic Leukemia[b]	6/49 (12)	10/50 (20)	11/50 (22)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		1.633	1.797
Lower Limit		0.585	0.664
Upper Limit		5.059	5.463
Weeks to First Observed Tumor	91	73	91
Hematopoietic System: All Neoplasms[b]	8/49 (16)	12/50 (24)	12/50 (24)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		1.470	1.470
Lower Limit		0.608	0.608
Upper Limit		3.783	3.783
Weeks to First Observed Tumor	91	73	91

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	16/49 (33)	8/50 (16)	12/49 (24)
P Values[c,d]	N.S.	P = 0.044 (N)	N.S.
Relative Risk (Control)[f]		0.490	0.750
Lower Limit		0.201	0.364
Upper Limit		1.094	1.504
Weeks to First Observed Tumor	97	92	87
Liver: Hepatocellular Adenoma or Carcinoma[b]	20/49 (41)	10/50 (20)	16/49 (33)
P Values[c,d]	N.S.	P = 0.021 (N)	N.S.
Departure from Linear Trend[e]	P = 0.037		
Relative Risk (Control)[f]		0.490	0.800
Lower Limit		0.231	0.446
Upper Limit		0.975	1.419
Weeks to First Observed Tumor	97	92	87

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
All Sites: Hemangiosarcoma[b]	5/49 (10)	1/50 (2)	6/50 (12)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.196	1.176
Lower Limit		0.004	0.320
Upper Limit		1.665	4.565
Weeks to First Observed Tumor	85	96	99
All Sites: Hemangioma or Hemangiosarcoma[b]	6/49 (12)	2/50 (4)	8/50 (16)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.327	1.307
Lower Limit		0.033	0.430
Upper Limit		1.723	4.243
Weeks to First Observed Tumor	85	96	80

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

[a]Treated groups received doses of 0.375 or 0.75 mg/animal/day.

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

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in each treated group is the probability level for the Fisher exact test for the comparison of that treated group with the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 3-Nitropropionic Acid by Gavage[a]

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/47 (0)	2/49 (4)	2/49 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		Infinite	Infinite
Lower Limit		0.284	0.284
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	105
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	2/47 (4)	6/49 (12)	3/49 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		2.878	1.439
Lower Limit		0.547	0.173
Upper Limit		28.023	16.603
Weeks to First Observed tumor	104	105	105

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma, Undifferentiated Leukemia, or Lymphocytic Leukemia[b]	20/50 (40)	18/50 (36)	10/50 (20)
P Values[c,d]	P = 0.021 (N)	N.S.	P = 0.024 (N)
Relative Risk (Control)[f]		0.900	0.500
Lower Limit		0.516	0.235
Upper Limit		1.561	0.996
Weeks to First Observed Tumor	79	84	100
Hematopoietic System: All Neoplasms[b]	21/50 (42)	21/50 (42)	10/50 (20)
P Values[c,d]	P = 0.014 (N)	N.S.	P = 0.015 (N)
Relative Risk (Control)[f]		1.000	0.476
Lower Limit		0.603	0.226
Upper Limit		1.659	0.939
Weeks to First Observed Tumor	79	84	100

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	1/49 (2)	1/50 (2)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		0.980	1.960
Lower Limit		0.013	0.106
Upper Limit		75.404	113.310
Weeks to First Observed Tumor	104	--	105
All Sites: Hemangiosarcoma[b]	1/50 (2)	1/50 (2)	3/50 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		1.000	3.000
Lower Limit		0.013	0.251
Upper Limit		76.970	154.270
Weeks to First Observed Tumor	104	105	101

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

Topography: Morphology	Control	Low Dose	High Dose
All Sites: Sarcoma of All Kinds[b]	2/50 (4)	4/50 (8)	6/50 (12)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		2.000	3.000
Lower Limit		0.301	0.569
Upper Limit		21.316	29.254
Weeks to First Observed Tumor	104	96	98
Pituitary: Chromophobe Adenoma[b]	2/43 (5)	4/48 (8)	1/42 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[f]		1.792	0.512
Lower Limit		0.272	0.009
Upper Limit		19.046	9.452
Weeks to First Observed Tumor	104	105	105

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 3-Nitropropionic Acid by Gavage[a]

(continued)

[a]Treated groups received doses of 0.375 or 0.75 mg/animal/day.

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

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in each treated group is the probability level for the Fisher exact test for the comparison of that treated group with the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the control group.

Review of the Bioassay of 3-Nitroproprionic Acid* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

January 18, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976 under the authority of the National Cancer Act of 1971 (P.L. 92-218). The purpose of the Clearinghouse is to advise on the National Cancer Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in organic chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of NCI bioassay reports on chemicals studied for carcinogenicity. In this context, below is the edited excerpt from the minutes of the Subgroup's meeting at which 3-Nitroproprionic Acid was reviewed.

The primary reviewer briefly described the experimental design under which 3-Nitroproprionic Acid was tested. He noted that there was no marked effect on weight gain or mortality in the treated animals. He agreed with the conclusion in the report that 3-Nitroproprionic Acid was not carcinogenic in either sex of mice or female rats, however, he pointed out a dose-related trend in the incidence of hepatic neoplasms and pancreatic islet-cell adenomas. Based on the neoplasms in the treated male rats, the primary reviewer questioned the conclusion that the evidence was insufficient to state that 3-Nitroproprionic Acid was not carcinogenic.

A Program staff member pointed out that there was also a significant increase in the incidence of hepatocellular carcinomas in previous studies where a chemical induced neoplastic nodules and was classified as a carcinogen. In this study only a single hepatocellular carcinoma was found in the treated male rats. Despite the lack of evidence for

the carcinogenicity of 3-Nitroproprionic Acid, he continued that the benign liver tumors were clearly treatment-related. He pointed out, however, that the biological effect was restricted to one species, one sex, and one organ site.

A Subgroup member argued that hyperplastic nodules and carcinomas should be combined for the purposes of analysis, since the former may represent a premalignant lesion. He added that the ratio of hyperplastic nodules to hepatocellular carcinomas is a function of the strength of the carcinogen and the time to tumor detection. Since the ratio of hyperplastic nodules to liver carcinomas is higher in the case of 3-Nitroproprionic Acid than for the other organochlorine carcinogens, he concluded that it was not as powerful a carcinogen as the others. Further discussion ensued as to the appropriateness of combining benign and malignant tumors for the purposes of statistical analysis.

The secondary reviewer opined that the evidence was inconclusive as to the carcinogenicity of 3-Nitroproprionic Acid in the treated male rats. He pointed out that the chemical was tested at the same time and in the same room with a number of other compounds (some of which were carcinogenic) and, as a result, cross-contamination may have occurred.

It was moved that the conclusion in the report be accepted with an addition noting that the hyperplastic nodules, which occurred in a statistically significant incidence, are generally thought to be premalignant. The motion was seconded and, in further discussion, a Subgroup member objected to combining neoplastic nodules and hepatoceullular carcinomas for the purposes of obtaining a statistically significant result. He opined that this could set a bad precedent for combining benign and malignant tumors. Voting in favor of the motion were Dr. Wolfe, Dr. Highland, Dr. Strong, Dr. Brown, and Mr. Samuels. Those opposed to the motion were Mr. Garfinkel, Dr. Kensler, and Dr. Rowe.

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

*U.S. GOVERNMENT PRINTING OFFICE: 1978 260-899/3043 1-3

DHEW Publication No. (NIH) 78-1302