National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 133
1979

BIOASSAY OF 3-NITRO-p-ACETOPHENETIDE FOR POSSIBLE CARCINOGENICITY

CAS No. 89-25-8

NCI-CG-TR-133

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
National Institutes of Health
Bethesda, Maryland 20205

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NIH Publication No. 79-1388

REPORT ON THE BIOASSAY OF 3-NITRO-p-ACETOPHENETIDE
FOR POSSIBLE CARCINOGENICITY

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

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

CONTRIBUTORS: This bioassay of 3-nitro-p-acetophenetide was conducted by Mason Research Institute, Worcester, Massachusetts, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. E. Smith (3) and Dr. A. Handler (3). Animal treatment and observation were supervised by Mr. G. Wade (3) and Ms. E. Zepp (3).

Histopathologic examinations were performed by Dr. D. S. Wyand (3) at the Mason Research Institute, and the diagnoses included in this report represent the interpretation of this pathologist. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (4).

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

This report was prepared at METREK, a Division of The MITRE Corporation (6) under the direction of the NCI. Those responsible for

this report at METREK are the project coordinator, Dr. L. W. Thomas (6), task leader Dr. M. R. Kornreich (6,9), senior biologist Ms. P. Walker (6), biochemist Mr. S. C. Drill (6), chemist Dr. N. Zimmerman (6), and the technical editor Ms. P. A. Miller (6). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1,9), Dr. R. A. Griesemer (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,10), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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

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

3. Mason Research Institute, 57 Union Street, Worcester, Massachusetts.

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

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

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

7. Now with the Solar Energy Research Institute, Cole Boulevard, Golden, Colorado.

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

9. Now with Clement Associates, Inc., 1010 Wisconsin Avenue, N.W., Washington, D.C.

10. Now with the Division of Comparative Medicine, Johns Hopkins University, School of Medicine, Traylor Building, Baltimore, Maryland.

SUMMARY

A bioassay for possible carcinogenicity of 3-nitro-p-acetophenetide was conducted using Fischer 344 rats and B6C3F1 mice. 3-Nitro-p-acetophenetide was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species, with the exception of low dose male mice, of which there were 49. Fifty animals of each sex and species were placed on test as controls. The high and low time-weighted average dietary concentrations of 3-nitro-p-acetophenetide were, respectively, 0.36 and 0.18 percent for rats and 1.46 and 0.73 percent for mice. The compound was administered in the diet for 78 weeks, followed by an observation period of up to 30 weeks for rats and 20 weeks for mice.

There were no significant positive associations between the concentrations of 3-nitro-p-acetophenetide administered and mortality in rats or mice of either sex. In addition, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

There was a statistically significant increased incidence of a combination of hepatocellular carcinomas and adenomas when high dose male mice were compared to controls. No other neoplasm in any other dosed group occurred in significant positive increased incidences when compared to controls.

Under the conditions of this bioassay, dietary administration of 3-nitro-p-acetophenetide was not carcinogenic in Fischer 344 rats of either sex or in female B6C3F1 mice. The compound, however, was considered carcinogenic in male B6C3F1 mice based on a significant increase in the combined incidence of hepatocellular carcinomas and hepatocellular adenomas in these animals.

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I. INTRODUCTION

3-Nitro-p-acetophenetide (Figure 1) (NCI No. C01978), a derivative of the analgesic phenacetin, was selected for bioassay by the National Cancer Institute because of the suspected renal pelvic carcinogenicity of the parent compound (Juusela, 1973; Weisburger, 1977; Odashima, 1978).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is N-(4-ethoxyphenyl)-3'-nitroacetamide.* It is also called 3'-nitro-p-acetophenetide (or 3'-nitro-p-acetophenetidin); and 4-acetamino-2-nitrophenetole.

3-Nitro-p-acetophenetide can be produced by the nitration of phenacetin, and this synthesis has been exploited for the detection of the latter (Oelschlaeger and Welsch, 1964).

Specific production data for 3-nitro-p-acetophenetide are not available; however, 1970 appears to have been the last year in which commercial production (in excess of 1000 pounds or $1000 in value annually) of the compound was reported in the United States (U.S. Tariff Commission, 1972; as cited in Urso, 1977).

The potential for exposure to 3-nitro-p-acetophenetide is apparently limited to researchers and workers engaged in the synthesis of this compound.

No information on the human toxicological properties of 3-nitro-p-acetophenetide is currently available; however, the para aminophenols

*The CAS registry number is 1777-84-0.

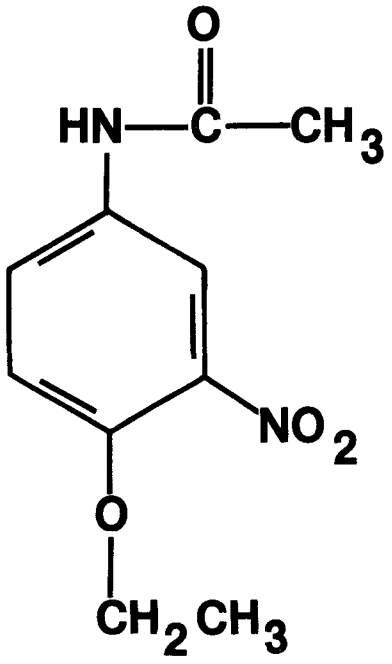


FIGURE 1
CHEMICAL STRUCTURE OF 3-NITRO-p-ACETOPHENETIDE

as a class have been implicated in the production of a variety of effects on the hematopoietic system of man (Barr and Penna, 1971).

II. MATERIALS AND METHODS

A. Chemicals

3-Nitro-p-acetophenetide was purchased from Carroll Products, Wood River Junction, Rhode Island. Chemical analysis was performed by Mason Research Institute, Worcester, Massachusetts. The experimentally determined range in the melting point was 116° to 118°C. Thin-layer chromatography utilizing ultraviolet light for detection showed only one spot. Ultraviolet analyses showed peaks at 245 and 353 nm. The compound was identified as 3-nitro-p-acetophenetide with a purity of 99 percent based on high pressure liquid chromatography analysis conducted three weeks after the chronic studies were terminated.

Throughout this report, the term 3-nitro-p-acetophenetide is used to represent this chemical.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® (Allied Mills, Inc., Chicago, Illinois). 3-Nitro-p-acetophenetide was administered to the dosed animals as a component of the diet.

The chemical was removed from its amber glass container, sifted, weighed out in proper amounts under an exhaust hood, and mixed in a mortar and pestle with an aliquot of the ground feed. Once visual homogeneity was attained, the mixture was placed into a 6 kg capacity Patterson-Kelley twin-shell V-blender along with the remainder of the meal. The blender was sealed and operated for 20 minutes. Prepared diets were placed in double plastic bags and stored in the dark at

4°C. The mixtures were prepared weekly and discarded 14 days after formulation.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. Fischer 344 rats and B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Rats and mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Dosed and control animals for each species were received in separate shipments. Upon arrival, a sample of animals was examined for parasites and other signs of disease. The remaining animals were quarantined by species for 2 weeks prior to initiation of test. Animals were assigned to groups and distributed among cages so that average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenance

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

Rats were housed five per cage by sex. During quarantine and for the first 7 months of study, rats were kept in galvanized- or stainless-steel wire-mesh cages (Fenco Cage Products, Boston, Massachusetts) suspended above newspapers. Newspapers under cages were

replaced daily and cages and racks washed weekly. For the duration of the study, all rats were held in suspended polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) equipped with disposable nonwoven fiber filter sheets. Clean corncob bedding and cages were provided twice weekly. Stainless steel cage racks (Fenco Cage Products) were cleaned once every 2 weeks, and disposable filters were replaced at that time.

Mice were housed by sex in polycarbonate shoe box type cages. During quarantine and periods of chemical administration, cages were fitted with perforated stainless steel lids. During the final observation period, stainless steel wire bar lids were used. Cages and both types of lids were from Lab Products, Inc. Nonwoven fiber filter bonnets were used over cage lids. Dosed mice were housed ten per cage for the first 17 months and five per cage thereafter. Control mice were changed from ten to five per cage after 14 months. Clean cages, lids, and bedding were provided three times per week when cage populations were ten and twice per week when cage populations were reduced to five. Hardwood chip bedding was used for the first 6 months of study and corncob bedding was used for the remainder of the study. Reusable filter bonnets and pipe racks were sanitized every 2 weeks throughout the study.

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

Pelleted Wayne Lab-Blox® was supplied to rats during the quarantine period and to both rats and mice during the final observation period. During the 78-week dosing period, all dosed animals were supplied with Wayne Lab-Blox® meal containing the appropriate concentration of 3-nitro-p-acetophenetide. Control animals had untreated meal available. Meal was dispensed from Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc., Woburn, Massachusetts) containing stainless steel baffles. Rats received pelleted feed on the cage floor. Mice were fed pellets from a hopper incorporated into the wire bar cage lids. Food hoppers were changed on the same schedule as were cages. Food was replenished daily in Alpine® feed cups.

All dosed and control rats were housed in a room with other rats receiving diets containing* amitrole (61-82-5) and 2-methyl-1-nitroanthraquinone (129-15-7).

Dosed mice were housed in a room with other mice receiving diets containing amitrole (61-82-5); N,N-dimethyl-p-nitrosoaniline (138-89-6); 2,5-toluenediamine sulfate (6369-59-1); 2,4-dinitrotoluene (121-14-2); 2-aminoanthraquinone (117-79-3); 3-amino-4-ethoxyacetanilide (17026-81-2); 3-amino-9-ethylcarbazole hydrochloride; APC (8003-03-0); 1-amino-2-methylanthraquinone (82-28-0); 5-nitro-o-anisidine (99-59-2); 4-nitroanthranilic acid (619-17-0); 1-nitronaphthalene (86-57-7); 5-nitroacenaphthene (602-87-9); and 2,4-diaminoanisole sulfate (615-05-4). Control mice were housed in a room with other mice receiving

*CAS registry numbers are given in parentheses.

diets containing 2-methyl-1-nitroanthraquinone (129-15-7); p-cresidine (120-71-8); 4-chloro-m-phenylenediamine (5131-60-2); acetylaminofluorene (53-96-3); and fenaminosulf (140-56-7).

E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of 3-nitro-p-acetophenetide for administration to dosed animals in the chronic study, subchronic toxicity studies were conducted with both rats and mice. Animals of each species were distributed among six groups, each consisting of five males and five females. 3-Nitro-p-acetophenetide was incorporated into the laboratory diet and supplied ad libitum to five of the six rat groups and five of the six mouse groups in concentrations of 0.05, 0.1, 0.2, 0.4, and 0.8 percent. The sixth group of each species served as a control group, receiving only the basal laboratory diet. The dosed dietary preparations were administered for 7 weeks, followed by a 1-week observation period during which animals were fed the basal diet. Survivors were sacrificed at the end of the test, and gross necropsies were performed.

The highest concentration causing no deaths, no compound-related gross abnormalities, and no mean body weight depression in excess of 10 percent relative to controls during the 8-week subchronic test was selected as the high concentration utilized for the chronic bioassays of both species.

All animals survived until the end of test. Mean body weight depression in female rats was 2.4, 6.0, and 15.0 percent at dietary

concentrations of 0.05, 0.1, and 0.2 percent, respectively. Mean body weight depression in male rats were 18.4, 8.3, and 19.4 percent at dietary concentrations of 0.05, 0.1, and 0.2 percent, respectively.

Enlarged submaxillary glands were noted in mice receiving concentrations of 0.8 percent. Mean body weight depression in female mice was 6.3 and 8.2 percent at dietary concentrations of 0.4 and 0.8 percent, respectively. Mean body weight depression in male mice was 2.6 percent at a dietary concentration of 0.4 percent and no mean body weight depression was observed at a dietary concentration of 0.8 percent.

The high concentrations chosen for the chronic study were 0.1 percent for rats and 0.4 percent for mice.

F. Experimental Design

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

The dosed and control rats were all approximately 6 weeks old at the time they were placed on test. The initial concentrations of 3-nitro-p-acetophenetide utilized were 0.1 and 0.05 percent. Throughout this report those rats initially receiving the former concentration are referred to as the high dose rat groups, while those initially receiving the latter concentration are referred to as the low dose rat groups. In week 10, concentrations were raised to 0.4 and 0.2

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
3-NITRO-p-ACETOPHENETIDE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	3-NITRO-p-ACETOPHENETIDE CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION[a]
MALE					
CONTROL	50	0	0	108	0
LOW DOSE	50	0.05	9		0.18
		0.2	69		
		0		28	
HIGH DOSE	50	0.1	9		0.36
		0.4	69		
		0		29	
FEMALE					
CONTROL	50	0	0	109	0
LOW DOSE	50	0.05	9		0.18
		0.2	69		
		0		29	
HIGH DOSE	50	0.1	9		0.36
		0.4	69		
		0		30	

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

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
3-NITRO-p-ACETOPHENETIDE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	3-NITRO-p-ACETOPHENETIDE CONCENTRATION (PERCENT)	OBSERVATION PERIOD		TIME-WEIGHTED AVERAGE CONCENTRATION[a]
			TREATED (WEEKS)	UNTREATED (WEEKS)	
MALE					
CONTROL	50	0	0	98	0
LOW DOSE	49	0.2 0.8 0	9 69	 20	0.73
HIGH DOSE	50	0.4 1.6 0	9 69	 20	1.46
FEMALE					
CONTROL	50	0	0	98	0
LOW DOSE	50	0.2 0.8 0	9 69	 20	0.73
HIGH DOSE	50	0.4 1.6 0	9 69	 20	1.46

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

percent for the rat groups because of insufficient mean body weight depression. These concentrations were not changed for the remainder of the 78-week dosing period. Five males and five females from the high dose and control groups were sacrificed and necropsied at the end of the compound administration phase according to existing protocol. The remaining rats were observed for an additional period of up to 30 weeks after compound administration ceased.

All mice were approximately 6 weeks old at the time they were placed on test. The initial concentrations of 3-nitro-p-acetophenetide utilized were 0.4 and 0.2 percent. Throughout this report those mice initially receiving the former concentration are referred to as the high dose groups, while those initially receiving the latter concentration are referred to as the low dose groups. In week 10, concentrations were raised to 1.6 and 0.8 percent for the high and low dose mouse groups, respectively, because of insufficient mean body weight depression. These concentrations were not changed for the remainder of the 78-week dosing period. Five low dose male mice were sacrificed and necropsied in week 14, and five males and five females from the high dose and control groups were sacrificed and necropsied at the end of the compound administration phase, according to existing protocol. All remaining mice were observed for an additional period of 20 weeks after compound administration ceased.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. Body weights were recorded twice weekly for the first

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

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

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

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

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

H. Data Recording and Statistical Analyses

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

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the

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

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control

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

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

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

found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

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

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

of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

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

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

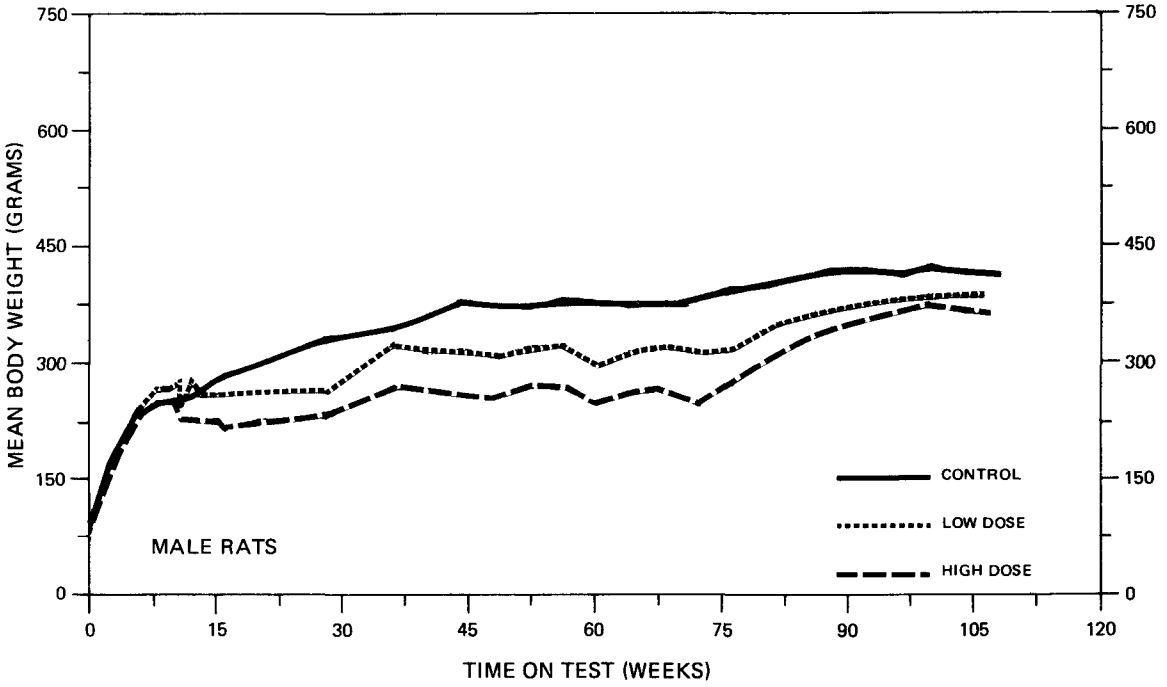
Mean body weight depression was apparent in both male and female dosed rats when compared to controls. Mean body weight depression was observed after 15 weeks in males and after 8 weeks in females (Figure 2).

Subcutaneous and/or cutaneous growths developed in one high dose male, one low dose male, one low dose female, four control males, and five control females. Exudate in the conjunctival sac of one high dose female and in the ear of one low dose female was observed. Alopecia was recorded in one low dose female. No other clinical abnormalities were observed.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 3-nitro-p-acetophenetide-dosed groups are shown in Figure 3. For both male and female rats the Tarone test for positive association between dosage and mortality was not significant.

For males five animals from the high dose and five from the control group were sacrificed in weeks 79 or 80. Sufficient numbers of male rats were at risk from late-developing tumors, with 80 percent (40/50) of the high dose, 84 percent (42/50) of the low dose, and 68 percent (34/50) of the control group surviving on test until the termination of the study.



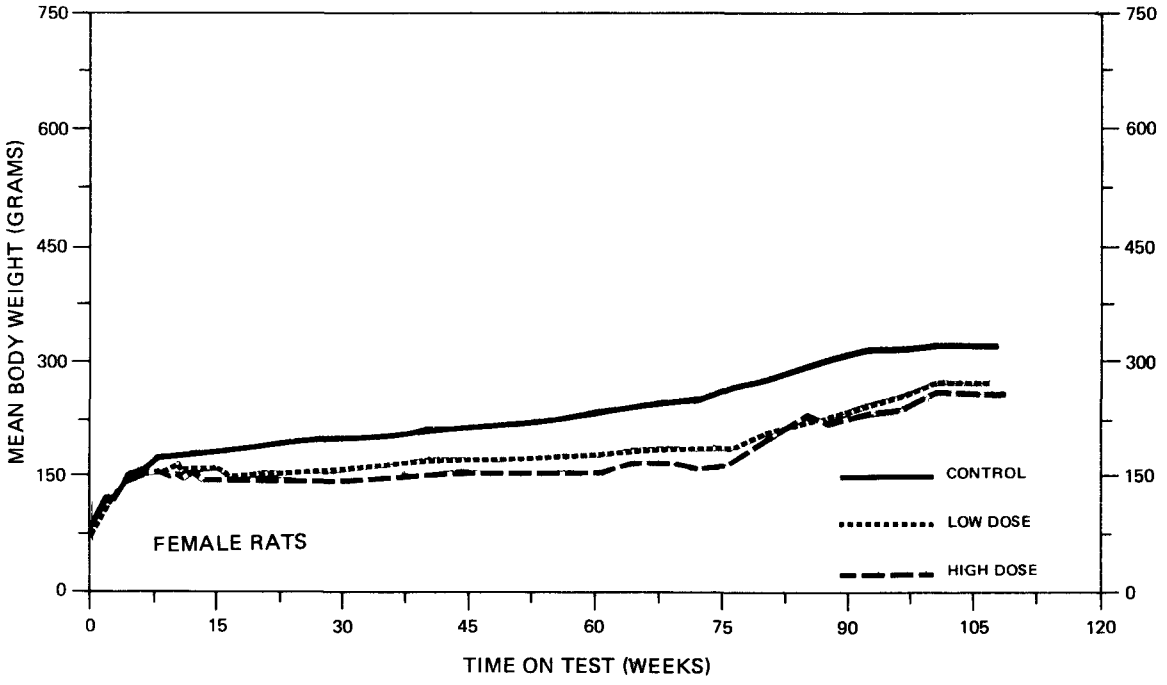
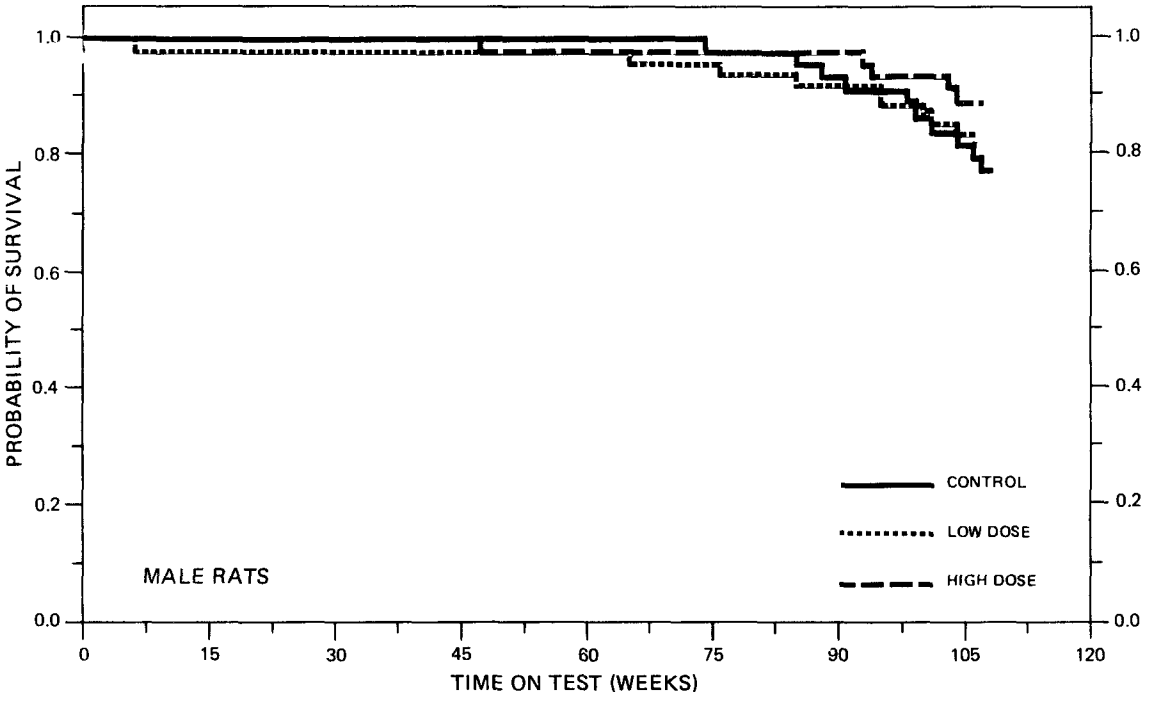


FIGURE 2
GROWTH CURVES FOR 3-NITRO-p-ACETOPHENETIDE CHRONIC STUDY RATS



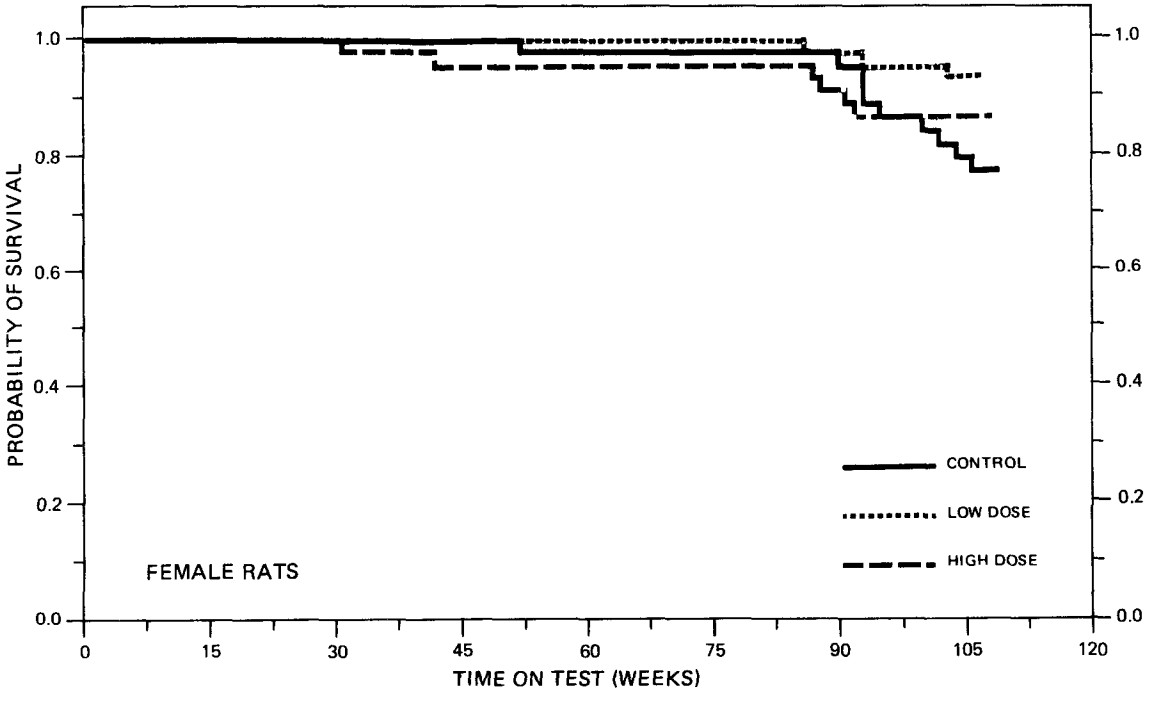


FIGURE 3
SURVIVAL COMPARISONS OF 3-NITRO-p-ACETOPHENETIDE CHRONIC STUDY RATS

For females five animals from the high dose and five from the control group were sacrificed in week 80. Sufficient numbers of female rats were at risk from late-developing tumors, with 78 percent (39/50) of the high dose, 94 percent (47/50) of the low dose, and 70 percent (35/50) of the control animals surviving on test until the end of the study.

C. Pathology

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

Tumors were found in comparable numbers in control and dosed animals and were not considered to be related to compound administration. There were instances in this study, as noted in the summary tables, where neoplastic lesions occurred only in dosed animals or with increased frequency when compared to the control group. However, the nature and incidence of these lesions are similar to those known to occur spontaneously in aged Fischer 344 rats.

A variety of inflammatory and degenerative lesions, which commonly occur in aging rats of this strain, was seen. None of these lesions was considered to be compound-induced.

Based upon the results of this pathologic examination, 3-nitro-p-acetophenetide is not carcinogenic in Fischer 344 rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 3-NITRO-p-ACETOPHENETIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma NOS[b]	1/41(0.02)	4/46(0.09)	0/44(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	3.565	0.000
Lower Limit	---	0.373	0.000
Upper Limit	---	171.546	17.344
Weeks to First Observed Tumor	108	105	---
Adrenal: Pheochromocytoma[b]	10/47(0.21)	1/49(0.02)	1/48(0.02)
P Values[c]	P = 0.001(N)	P = 0.003(N)	P = 0.003(N)
Departure from Linear Trend[e]	P = 0.045	---	---
Relative Risk (Control)[d]	---	0.096	0.098
Lower Limit	---	0.002	0.002
Upper Limit	---	0.633	0.646
Weeks to First Observed Tumor	99	105	107
Pancreatic Islets: Islet-Cell Adenoma[b]	3/45(0.07)	2/46(0.04)	1/45(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.652	0.333
Lower Limit	---	0.057	0.007
Upper Limit	---	5.426	3.964
Weeks to First Observed Tumor	85	106	107

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	6/48(0.13)	2/50(0.04)	1/50(0.02)
P Values[c]	P = 0.026(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.320	0.160
Lower Limit	---	0.033	0.004
Upper Limit	---	1.687	1.249
Weeks to First Observed Tumor	98	106	104
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	3/48(0.06)	0/50(0.00)	0/49(0.00)
P Values[c]	P = 0.035(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	1.596	1.628
Weeks to First Observed Tumor	99	---	---
Testis: Interstitial-Cell Tumor[b]	45/47(0.96)	47/50(0.94)	39/49(0.80)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.982	0.831
Lower Limit	---	0.911	0.765
Upper Limit	---	1.082	0.987
Weeks to First Observed Tumor	80	65	80

TABLE 3 (CONCLUDED)

[a]Treated groups received time-weighted average doses of 0.18 or 0.36 percent in feed.

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

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

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

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

TABLE 4
ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 3-NITRO-p-ACETOPHENETIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma NOS[b]	18/44(0.41)	3/45(0.07)	2/37(0.05)
P Values[c]	P < 0.001(N)	P < 0.001(N)	P < 0.001(N)
Departure from Linear Trend[e]	P = 0.020	---	---
Relative Risk (Control)[d]	---	0.163	0.132
Lower Limit	---	0.033	0.016
Upper Limit	---	0.506	0.499
Weeks to First Observed Tumor	90	106	108
Thyroid: C-Cell Adenoma[b]	2/40(0.05)	2/47(0.04)	0/45(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.851	0.000
Lower Limit	---	0.064	0.000
Upper Limit	---	11.301	2.995
Weeks to First Observed Tumor	108	107	---
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	3/40(0.08)	2/47(0.04)	3/45(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.567	0.889
Lower Limit	---	0.050	0.126
Upper Limit	---	4.717	6.296
Weeks to First Observed Tumor	108	107	107

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	7/49(0.14)	2/50(0.04)	1/49(0.02)
P Values[c]	P = 0.014(N)	N.S.	P = 0.030(N)
Relative Risk (Control)[d]	---	0.280	0.143
Lower Limit	---	0.030	0.003
Upper Limit	---	1.383	1.051
Weeks to First Observed Tumor	106	93	92
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	2/49(0.04)	0/50(0.00)	3/48(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	1.531
Lower Limit	---	0.000	0.183
Upper Limit	---	3.313	17.665
Weeks to First Observed Tumor	93	---	108
Mammary Gland: Fibroadenoma[b]	16/49(0.33)	0/50(0.00)	1/49(0.02)
P Values[c]	P < 0.001(N)	P < 0.001(N)	P < 0.001(N)
Departure from Linear Trend[e]	P = 0.002	---	---
Relative Risk (Control)[d]	---	0.000	0.063
Lower Limit	---	0.000	0.002
Upper Limit	---	0.194	0.375
Weeks to First Observed Tumor	80	---	108

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Uterus: Endometrial Stromal Polyp[b]	12/49(0.24)	0/47(0.00)	0/41(0.00)
P Values[c]	P < 0.001(N)	P < 0.001(N)	P < 0.001(N)
Departure from Linear Trend[e]	P = 0.015	---	---
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	0.284	0.324
Weeks to First Observed Tumor	80	---	---

[a]Treated groups received time-weighted average doses of 0.18 or 0.36 percent in feed.

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

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

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

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

every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 3-nitro-p-acetophenetide-dosed groups and where such tumors were observed in at least 5 percent of the group.

None of the statistical tests for any site in rats of either sex indicated a significant positive association between the administration of 3-nitro-p-acetophenetide and an increased tumor incidence. Thus, at the dose levels used in this experiment, there were no statistically significant results indicating that 3-nitro-p-acetophenetide was a carcinogen in Fischer 344 rats.

The Cochran-Armitage and Fisher exact tests indicated the possibility of a negative association between administration and the incidence of adrenal pheochromocytomas in the male, and the incidence of pituitary adenomas NOS, of mammary fibroadenomas, and of endometrial stromal polyps in the female rats.

The possibility of a negative association between dosage and incidence was also noted for liver tumors in males and for either leukemia or malignant lymphomas in both males and females. For these tumors, however, the Cochran-Armitage test and the Fisher exact tests were not significant under the Bonferroni criterion.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in

Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by 3-nitro-p-acetophenetide that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

Mean body weight depression relative to controls was apparent in both male and female dosed mice (Figure 4).

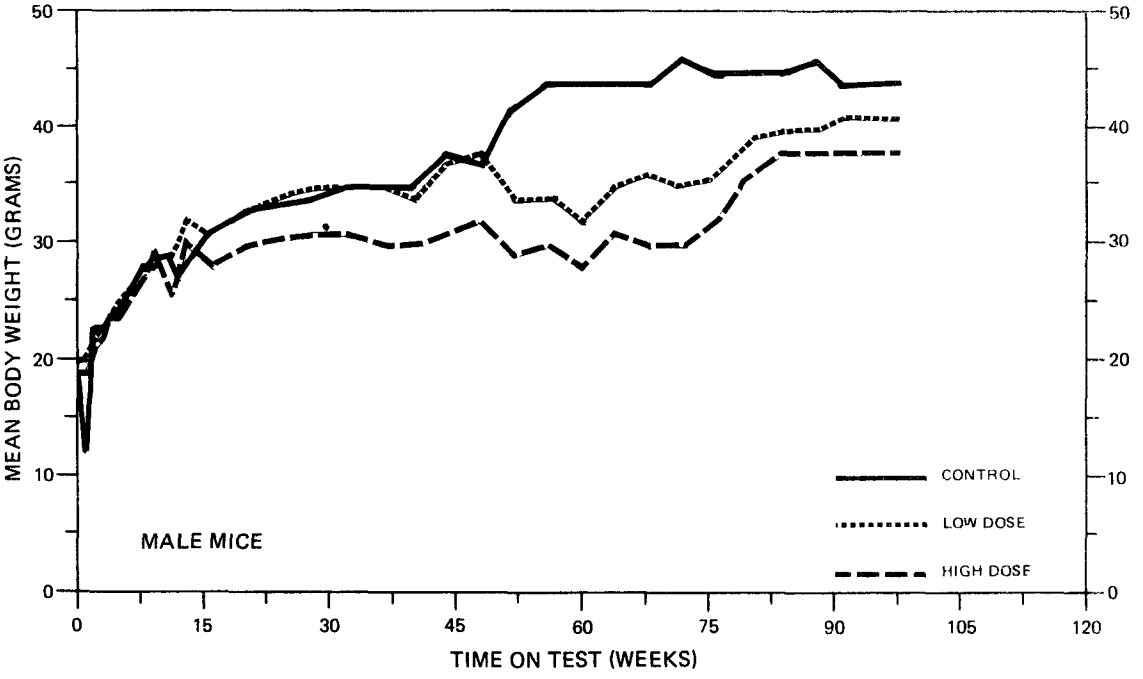
No clinical abnormalities were observed in mice of any group. Salivary glands were not enlarged.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 3-nitro-p-acetophenetide-dosed groups are shown in Figure 5. For both male and female mice the Tarone test for positive association between dosage and mortality was not significant.

For males five mice from the low dose group were sacrificed in week 14, five from the high dose group in week 80, and five from the control group in week 78. Sufficient numbers of male mice were at risk from late-developing tumors, with 74 percent (37/50) of the high dose, 82 percent (40/49) of the low dose, and 74 percent (37/50) of the control mice surviving on test until the termination of the study.

For females five mice from the high dose group were sacrificed in week 80 and five from the control group in week 78. Sufficient numbers of female mice were at risk from late-developing tumors, with 80 percent (40/50) of the high dose, 82 percent (41/50) of the low dose, and 70 percent (35/50) of the control mice surviving on test until the end of the study.



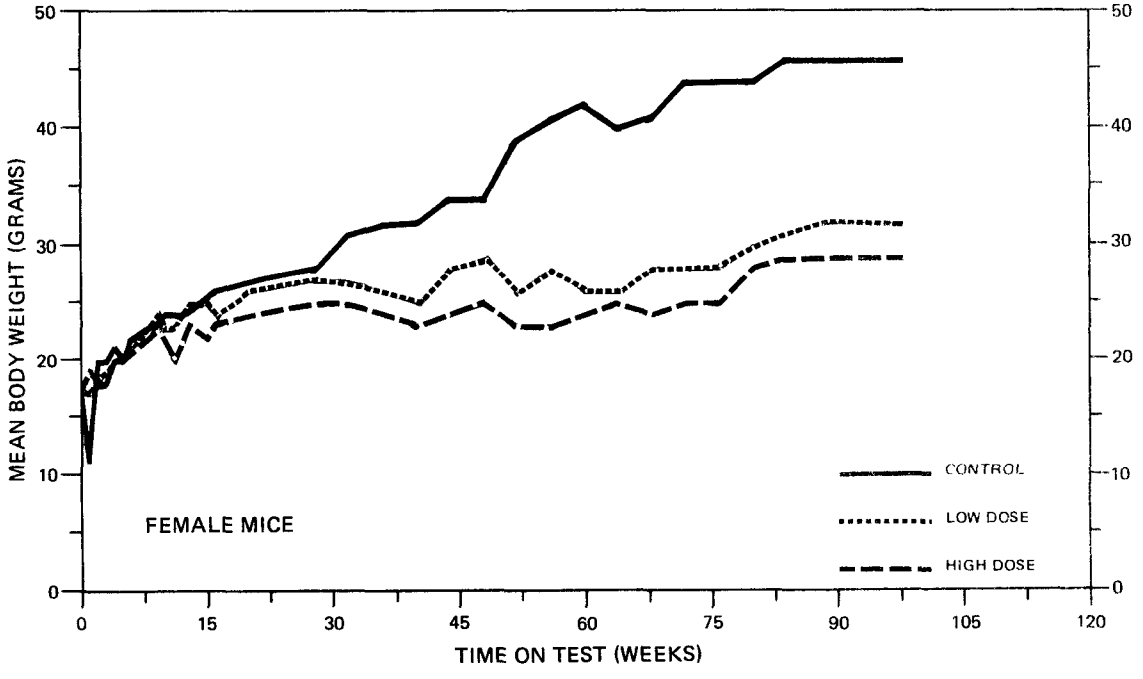


FIGURE 4
GROWTH CURVES FOR 3-NITRO-p-ACETOPHENETIDE CHRONIC STUDY MICE

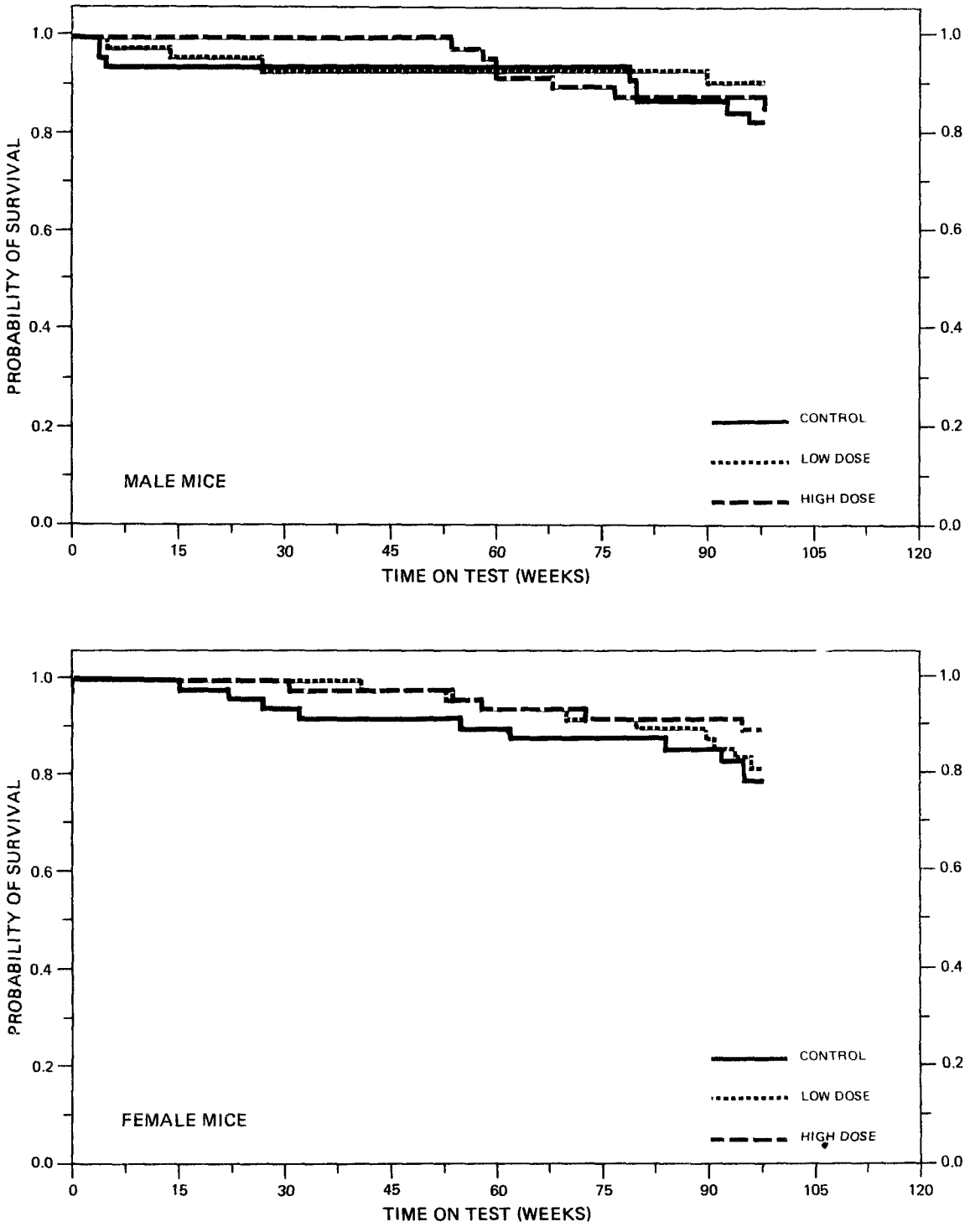


FIGURE 5
SURVIVAL COMPARISONS OF 3-NITRO-p-ACETOPHENETIDE CHRONIC STUDY MICE

C. Pathology

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

There was a dose-related increase in the incidence of hepatocellular carcinomas observed in the dosed male mice as shown below:

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
No. of Animals with Livers Examined Histopathologically	(45)	(41)	(43)	(45)	(47)	(49)
Hepatocellular Carcinoma	4	8	9	2	2	1
Hepatocellular Adenoma	6	5	14	2	1	0
Adenoma NOS	0	1	0	0	1	2

Hepatocellular carcinomas tended to lose their trabecular pattern and grew in solid sheets, papillary arrangements, or less commonly in adenomatous patterns with considerable variation in cytology. There was usually marked variation in cell size and cytoplasmic vacuolation and hyaline bodies were common findings. There were often bizarre hyperchromatic giant nuclei and multinucleate hepatocytes. Mitoses were usually numerous with occasional abnormal mitotic figures. Bile ducts were usually absent. Carcinomas often compressed the adjacent

normal parenchyma and invasion was difficult to demonstrate. Some tumors contained cystic and blood-filled spaces and areas of necrosis. Metastases were not detected in the dosed mice in this study.

Hepatocellular adenomas were roughly spherical, expanding unencapsulated tumors which compressed adjacent hepatic cords. They had a more trabecular pattern than carcinomas, being composed of closely packed cords of liver cells and sinusoids. Normal mitoses were present. Adenomas also had some cellular pleomorphism but to a lesser degree than carcinomas. There were areas of fatty change and cytoplasmic hyaline bodies. Bile ducts and triads were occasionally seen in adenomas. Generally, adenomas were smaller than carcinomas, with the majority occupying less than the width of a liver lobe.

The incidence of hepatic tumors was not significantly elevated in dosed female mice as compared with controls; thus the hepatic tumors observed in females were not considered to be compound-related. None of the other tumors noted in either sex were considered to be compound-related.

In a few mice the diagnosis of telangiectasis was made. This consisted of a focal area of dilated vascular spaces bounded usually by a single layer of hepatocytes and lined by somewhat enlarged endothelial cells.

A variety of other tumors was observed in both dosed and control mice. The incidence and distribution of these neoplasms were similar

to spontaneously occurring neoplasms in aged B6C3F1 mice and were not considered to be compound-related.

A variety of inflammatory and degenerative lesions which commonly occur in aging mice of this strain was seen. These nonneoplastic lesions were not considered to be compound-induced.

Based upon the results of this pathologic examination, there was an increased incidence in liver neoplasms in male B6C3F1 mice fed 3-nitro-p-acetophenetide.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 3-nitro-p-acetophenetide-dosed groups and where such tumors were observed in at least 5 percent of the group. Due to the sacrifice of five low dose males in week 14, the analyses in Table 5 include only those male mice surviving at least 15 weeks.

For male mice when incidences were combined so that the numerator represented males with either a hepatocellular carcinoma or a hepatocellular adenoma, the Cochran-Armitage test indicated a significant ($P = 0.002$) positive association between dose and incidence. The Fisher exact test comparing high dose to control was also significant ($P = 0.002$). In historical control data collected by this laboratory for the NCI Carcinogenesis Testing Program 51/350 (15 percent) of the

TABLE 5

TIME-ADJUSTED ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH 3-NITRO-p-ACETOPHENETIDE[a,f]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Malignant Lymphoma[b]	2/46(0.04)	4/41(0.10)	4/45(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.244	2.044
Lower Limit	---	0.340	0.310
Upper Limit	---	23.727	21.695
Weeks to First Observed Tumor	98	98	98
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	11/45(0.24)	2/41(0.05)	6/43(0.14)
P Values[c]	N.S.	P = 0.011(N)	N.S.
Departure from Linear Trend[e]	P = 0.032	---	---
Relative Risk (Control)[d]	---	0.200	0.571
Lower Limit	---	0.023	0.190
Upper Limit	---	0.843	1.525
Weeks to First Observed Tumor	78	98	98
Liver: Hepatocellular Carcinoma[b]	4/45(0.09)	8/41(0.20)	9/43(0.21)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.195	2.355
Lower Limit	---	0.639	0.715
Upper Limit	---	9.244	9.722
Weeks to First Observed Tumor	93	98	98

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	10/45(0.22)	13/41(0.32)	23/43(0.53)
P Values[c]	P = 0.002	N.S.	P = 0.002
Relative Risk (Control)[d]	---	1.427	2.407
Lower Limit	---	0.651	1.269
Upper Limit	---	3.212	4.829
Weeks to First Observed Tumor	93	98	80

[a]Treated groups received time-weighted average doses of 0.73 or 1.46 percent in feed.

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

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

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

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

[f]These analyses were based solely upon animals surviving at least 15 weeks.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 3-NITRO-p-ACETOPHENETIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	1/45(0.02)	4/47(0.09)	2/49(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	3.830	1.837
Lower Limit	---	0.399	0.099
Upper Limit	---	184.347	106.150
Weeks to First Observed Tumor	98	97	98
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	12/46(0.26)	7/47(0.15)	6/49(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.571	0.469
Lower Limit	---	0.209	0.158
Upper Limit	---	1.426	1.232
Weeks to First Observed Tumor	95	80	98
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	4/45(0.09)	3/47(0.06)	1/49(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.718	0.230
Lower Limit	---	0.111	0.005
Upper Limit	---	4.010	2.209
Weeks to First Observed Tumor	78	97	98

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Stomach: Squamous-Cell Papilloma[b]	3/42(0.07)	0/44(0.00)	0/47(0.00)
P Values[c]	P = 0.031(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	1.580	1.482
Weeks to First Observed Tumor	98	---	---
Pituitary: Adenoma NOS[b]	6/37(0.16)	1/41(0.02)	0/38(0.00)
P Values[c]	P = 0.004(N)	P = 0.040(N)	P = 0.012(N)
Relative Risk (Control)[d]	---	0.150	0.000
Lower Limit	---	0.003	0.000
Upper Limit	---	1.159	0.602
Weeks to First Observed Tumor	98	97	---

[a]Treated groups received time-weighted average doses of 0.73 or 1.46 percent in feed.

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

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

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

untreated male B6C3F1 mice had one of these tumors, compared to the 23/43 (53 percent) observed in the high dose group in this bioassay.

Based on these statistical results, the administration of 3-nitro-p-acethophenetide was associated with an elevated incidence of liver neoplasms in male mice under the conditions of this experiment.

Both the Cochran-Armitage test and the high dose Fisher exact comparison indicated a significant negative association between compound administration and the incidence of pituitary adenomas NOS in female mice. The low dose Fisher exact test was not significant under the Bonferroni criterion. For alveolar/bronchiolar neoplasms in the males and for squamous-cell papillomas of the stomach in females the possibility of a negative association was indicated; for both cases, however, the results of the Cochran-Armitage test did not agree with those of the Fisher exact test as to the significance of the results.

V. DISCUSSION

There were no significant positive associations between the concentrations of 3-nitro-p-acetophenetide administered and mortality in rats or mice of either sex. In addition, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

In rats of both sexes there were no significant positive associations between the administration of 3-nitro-p-acetophenetide and the incidence of any tumor.

In dosed male mice when compared to controls there were increased incidences of hepatocellular carcinomas (i.e., 4/45 [9 percent], 8/41 [20 percent], and 9/43 [21 percent] in the control, low dose, and high dose groups) and hepatocellular adenomas (i.e., 6/45 [13 percent], 5/41 [12 percent], and 14/43 [33 percent] in the control, low dose, and high dose groups). When the incidences of male mice having either of these neoplasms were combined, the resulting incidences (i.e., 10/45 [22 percent], 13/41 [32 percent], and 23/43 [53 percent] in the control, low dose, and high dose groups, respectively) indicated a significant positive association between concentration administered and tumor incidence. This finding was supported by a significant high dose to control Fisher exact comparison.

Under the conditions of this bioassay, dietary administration of 3-nitro-p-acetophenetide was not carcinogenic in Fischer 344 rats of either sex or in female B6C3F1 mice. The compound, however, was

considered carcinogenic in male B6C3F1 mice based on a significant increase in the combined incidence of hepatocellular carcinomas and hepatocellular adenomas in these animals.

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

June 29, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 3-Nitro-*p*-Acetophenetide for carcinogenicity.

The reviewer agreed with the conclusion in the report that the compound was not carcinogenic, under the conditions of test, in treated rats or female mice but did induce a statistically significant incidence of liver tumors in treated male mice. After a brief description of the experimental design, the reviewer noted several flaws that detracted from the study, e.g., analyses were not done to confirm the identification of the 3-Nitro-*p*-Acetophenetide or on its stability or concentration in the diet and the study was conducted in a room in which other compounds were under test. The reviewer opined that the flaws were sufficient to cast doubt on the validity of the bioassay. He also said that the "very slight response" in only one sex of one species was of considerable concern.

A discussion ensued as to whether the study flaws were sufficiently serious as to invalidate the conclusions drawn from the bioassay. A Program staff member noted that the

3-Nitro-*p*-Acetophenetide was procured from the manufacturer of the chemical. A Subgroup member said that the structure of the test material would indicate it to be unstable. It was agreed that further consideration of the report should be deferred until a sample of the reference 3-Nitro-*p*-Acetophenetide could be analyzed for identity and stability.

Clearinghouse Members present:

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

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

☆U S GOVERNMENT PRINTING OFFICE 1979—281 217 3221

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 3-NITRO-p-ACETOPHENETIDE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH 3-NITRO-p-ACETOPHENETIDE

	CONTROL (UNTR) 01-0070	LOW DOSE 01-0073	HIGH DOSE 01-0074
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	48	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	48	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(48)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
SQUAMOUS CELL CARCINOMA	1 (2%)		
BASAL-CELL CARCINOMA	1 (2%)		
KERATOACANTHOMA		1 (2%)	1 (2%)
*SUBCUT TISSUE	(48)	(50)	(50)
ADNEXAL ADENOMA			1 (2%)
FIBROMA	2 (4%)	1 (2%)	
NEUROFIBROMA		1 (2%)	
RESPIRATORY SYSTEM			
*LUNG	(48)	(50)	(49)
CARCINOMA, NOS, METASTATIC	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	
OSTEOSARCOMA, METASTATIC	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(48)	(50)	(50)
LEUKEMIA,NOS	1 (2%)		
MYELOMONOCYTIC LEUKEMIA	5 (10%)	2 (4%)	
*SKIN	(48)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
*SPLEEN	(48)	(50)	(49)
OSTEOSARCOMA, METASTATIC	1 (2%)		
CIRCULATORY SYSTEM			
NONE			

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0070	LOW DOSE 01-0073	HIGH DOSE 01-0074
DIGESTIVE SYSTEM			
#LIVER	(48)	(50)	(49)
NEOPLASTIC NODULE	1 (2%)		
HEPATOCELLULAR CARCINOMA	2 (4%)		
#STOMACH	(48)	(49)	(49)
SQUAMOUS CELL PAPILLOMA		1 (2%)	1 (2%)
#SMALL INTESTINE	(45)	(49)	(48)
CYSTADENOCAPCINOMA, NOS		1 (2%)	
LEIOMYOSARCOMA		1 (2%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(48)	(50)	(49)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	
HEMANGIOSARCOMA			1 (2%)
#KIDNEY/PELVIS	(48)	(50)	(49)
TRANSITIONAL-CELL CARCINOMA			1 (2%)
#URINARY BLADDER	(46)	(50)	(47)
TRANSITIONAL-CELL PAPILLOMA	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(41)	(46)	(44)
ADENOMA, NOS	1 (2%)	4 (9%)	
#ADRENAL	(47)	(49)	(48)
CORTICAL ADENOMA	1 (2%)		
PHEOCHROMOCYTOMA	10 (21%)	1 (2%)	1 (2%)
GANGLIONEUROMA	1 (2%)		
#THYROID	(39)	(45)	(47)
C-CELL ADENOMA		2 (4%)	2 (4%)
#PANCREATIC ISLETS	(45)	(46)	(45)
ISLET-CELL ADENOMA	3 (7%)	2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(48)	(50)	(50)
PAPILLARY ADENOCARCINOMA	1 (2%)		

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0070	LOW DOSE 01-0073	HIGH DOSE 01-0074
FIBROADENOMA	1 (2%)		
*PENIS	(48)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
*PREPUTIAL GLAND	(48)	(50)	(50)
CARCINOMA, NOS	2 (4%)		
#TESTIS	(47)	(50)	(49)
INTERSTITIAL-CELL TUMOR	45 (96%)	47 (94%)	39 (80%)
NERVOUS SYSTEM			
#CEREBRAL HEMISPHERE	(47)	(50)	(49)
OLIGODENDROGLIOMA			1 (2%)
#CEREBELLUM	(47)	(50)	(49)
ASTROCYTOMA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*BODY CAVITIES	(48)	(50)	(50)
MESOTHELIOMA, NOS	1 (2%)	1 (2%)	2 (4%)
*ABDOMINAL CAVITY	(48)	(50)	(50)
SARCOMA, NOS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			

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

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 01-0070	LOW DOSE 01-0073	HIGH DOSE 01-0074
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	5	3	3
MORIBUND SACRIFICE	5	5	2
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	34	42	40
ANIMAL MISSING	1		
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	45	49	42
TOTAL PRIMARY TUMORS	81	71	53
TOTAL ANIMALS WITH BENIGN TUMORS	45	49	41
TOTAL BENIGN TUMORS	66	62	46
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	8	5
TOTAL MALIGNANT TUMORS	13	8	5
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		
TOTAL SECONDARY TUMORS	3		
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	2	1	2
TOTAL UNCERTAIN TUMORS	2	1	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH 3-NITRO-p-ACETOPHENETIDE

	CONTROL (UNTR) 02-0070	LOW DOSE 02-0073	HIGH DOSE 02-0074
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	50	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(49)	(50)	(49)
TRICHOEPITHELIOMA			1 (2%)
FIBROMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(49)	(50)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(49)
MALIGNANT LYMPHOMA, NOS	2 (4%)		
LEUKEMIA,NOS		1 (2%)	1 (2%)
MYELOMONOCYTIC LEUKEMIA	5 (10%)	1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(48)
NEOPLASTIC NODULE	2 (4%)		1 (2%)
HEPATOCELLULAR CARCINOMA	1 (2%)		2 (4%)
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(48)
CARCINOMA,NOS	1 (2%)		

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0070	LOW DOSE 02-0073	HIGH DOSE 02-0074
TUBULAR-CELL ADENOCARCINOMA			1 (2%)
#KIDNEY/PELVIS	(49)	(50)	(48)
TRANSITIONAL-CELL CARCINOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(44)	(45)	(37)
ADENOMA, NOS	18 (41%)	3 (7%)	2 (5%)
#ADRENAL	(49)	(49)	(48)
CORTICAL CARCINOMA	1 (2%)		
PHEOCHROMOCYTOMA	2 (4%)		
#THYROID	(40)	(47)	(45)
FOLLICULAR-CELL CARCINOMA	1 (3%)		
C-CELL ADENOMA	2 (5%)	2 (4%)	
C-CELL CARCINOMA	1 (3%)		3 (7%)
#PANCREATIC ISLETS	(47)	(48)	(46)
ISLET-CELL ADENOMA	1 (2%)		
ISLET-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(50)	(49)
ADENOMA, NOS	2 (4%)		
ADENOCARCINOMA, NOS		2 (4%)	
FIBROADENOMA	16 (33%)		1 (2%)
*CLITORIS	(49)	(50)	(49)
ADENOMA, NOS			1 (2%)
*CLITORAL GLAND	(49)	(50)	(49)
ADENOMA, NOS	1 (2%)		
*VAGINA	(49)	(50)	(49)
LEIOMYOSARCOMA		1 (2%)	
#UTERUS	(49)	(47)	(41)
LEIOMYOSARCOMA	1 (2%)		
ENDOMETRIAL STROMAL POLYP	12 (24%)		
#UTERUS/ENDOMETRIUM	(49)	(47)	(41)
ADENOCARCINOMA, NOS	2 (4%)	1 (2%)	

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0070	LOW DOSE 02-0073	HIGH DOSE 02-0074
NERVOUS SYSTEM			
#BRAIN	(49)	(50)	(47)
OLIGODENDROGLIOMA	1 (2%)		
SPECIAL SENSE ORGANS			
*EYELID	(49)	(50)	(49)
SQUAMOUS CELL CARCINOMA		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(49)	(50)	(49)
MESOTHELIOMA, NOS	1 (2%)		
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	3	3	5
MORIBUND SACRIFICE	7		1
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	35	47	39
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 02-0070	LOW DOSE 02-0073	HIGH DOSE 02-0074
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	45	12	15
TOTAL PRIMARY TUMORS	73	12	17
TOTAL ANIMALS WITH BENIGN TUMORS	37	5	6
TOTAL BENIGN TUMORS	54	5	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	13	7	9
TOTAL MALIGNANT TUMORS	16	7	9
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	3		1
TOTAL UNCERTAIN TUMORS	3		1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 3-NITRO-p-ACETOPHENETIDE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH 3-NITRO-p-ACETOPHENETIDE

	CONTROL(UNTR) 05-0077	LOW DOSE 05-0075	HIGH DOSE 05-0076
ANIMALS INITIALLY IN STUDY	50	@50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	46	46	45
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	45	41	43
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(45)	(41)	(43)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	7 (16%)	2 (5%)	6 (14%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	4 (9%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(46)	(46)	(45)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#SPLEEN	(45)	(41)	(43)
HEMANGIOSARCOMA		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	2 (5%)	
#MANDIBULAR L. NODE	(35)	(36)	(35)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (3%)		
#MESENTERIC L. NODE	(35)	(36)	(35)
MALIGNANT LYMPHOMA, NOS		1 (3%)	
#RENAL LYMPH NODE	(35)	(36)	(35)
MALIGNANT LYMPHOMA, NOS		1 (3%)	
#PEYERS PATCH	(43)	(41)	(43)
MALIGNANT LYMPHOMA, NOS			2 (5%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE B1 (CONTINUED)

	CONTROL(UNTR) 05-0077	LOW DOSE 05-0075	HIGH DOSE 05-0076
DIGESTIVE SYSTEM			
#LIVER	(45)	(41)	(43)
ADENOMA, NOS		1 (2%)	
HEPATOCELLULAR ADENOMA	6 (13%)	5 (12%)	14 (33%)
HEPATOCELLULAR CARCINOMA	4 (9%)	8 (20%)	9 (21%)
HEMANGIOSARCOMA		1 (2%)	
#STOMACH	(42)	(41)	(43)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(46)	(46)	(45)
PAPILLARY ADENOMA			1 (2%)
*EAR CANAL	(46)	(46)	(45)
SQUAMOUS CELL CARCINOMA	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE B1 (CONCLUDED)

	CONTROL(UNTR) 05-0077	LOW DOSE 05-0075	HIGH DOSE 05-0076
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	7	4	7
MORIBUND SACRIFICE	1		
SCHEDULED SACRIFICE	5	5	5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	37	40	37
ANIMAL MISSING			1
ANIMAL DELETED(WRONG SEX)		1	
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	21	20	29
TOTAL PRIMARY TUMORS	25	22	34
TOTAL ANIMALS WITH BENIGN TUMORS	13	8	20
TOTAL BENIGN TUMORS	14	8	21
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	13	12
TOTAL MALIGNANT TUMORS	11	14	13
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		
TOTAL SECONDARY TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH 3-NITRO-p-ACETOPHENETIDE

	CONTROL(UNTR) 06-0077	LOW DOSE 06-0075	HIGH DOSE 06-0076
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	46	47	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	46	47	49
INTEGUMENTARY SYSTEM			
*SKIN	(46)	(47)	(49)
FIBROSARCOMA	2 (4%)		
*SUBCUT TISSUE	(46)	(47)	(49)
HEMANGIOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(45)	(47)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)	4 (9%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(46)	(47)	(49)
MALIGNANT LYMPHOMA, NOS	3 (7%)	5 (11%)	2 (4%)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (2%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	6 (13%)	1 (2%)	1 (2%)
LYMPHOCYTIC LEUKEMIA	1 (2%)		
#SPLEEN	(43)	(47)	(48)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#LIVER	(45)	(47)	(49)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#SMALL INTESTINE	(43)	(44)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#PEYERS PATCH	(43)	(44)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
#KIDNEY	(43)	(46)	(49)
MALIGNANT LYMPHOMA, NOS			1 (2%)

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

TABLE B2 (CONTINUED)

	CONTROL(UNTR) 06-0077	LOW DOSE 06-0075	HIGH DOSE 06-0076
#THYMUS	(27)	(32)	(36)
MALIGNANT LYMPHOMA, NOS			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(45)	(47)	(49)
ADENOMA, NOS		1 (2%)	2 (4%)
HEPATOCELLULAR ADENOMA	2 (4%)	1 (2%)	
HEPATOCELLULAR CARCINOMA	2 (4%)	2 (4%)	1 (2%)
#STOMACH	(42)	(44)	(47)
SQUAMOUS CELL PAPILLOMA	3 (7%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(37)	(41)	(38)
ADENOMA, NOS	6 (16%)	1 (2%)	
#ADRENAL	(43)	(45)	(47)
CORTICAL ADENOMA	1 (2%)		
#THYROID	(30)	(45)	(42)
ADENOCARCINOMA, NOS			1 (2%)
#PANCREATIC ISLETS	(41)	(44)	(48)
ISLET-CELL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(46)	(47)	(49)
ADENOCARCINOMA, NOS	1 (2%)	1 (2%)	

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

TABLE B2 (CONTINUED)

	CONTROL(UNTR) 06-0077	LOW DOSE 06-0075	HIGH DOSE 06-0076
#UTERUS	(43)	(47)	(42)
HEMANGIOMA			2 (5%)
#OVARY	(41)	(45)	(47)
LUTEOMA	1 (2%)		1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(46)	(47)	(49)
PAPILLARY CYSTADENOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	8	9	5
MORIBUND SACRIFICE	2		
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	35	41	40
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2 (CONCLUDED)

	CONTROL(UNTR) 06-0077	LOW DOSE 06-0075	HIGH DOSE 06-0076
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	22	17	15
TOTAL PRIMARY TUMORS	32	18	17
TOTAL ANIMALS WITH BENIGN TUMORS	13	7	8
TOTAL BENIGN TUMORS	15	7	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	16	10	7
TOTAL MALIGNANT TUMORS	17	11	8
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 3-NITRO-p-ACETOPHENETIDE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH 3-NITRO-p-ACETOPHENETIDE

	CONTROL (UNTR) 01-0070	LOW DOSE 01-0073	HIGH DOSE 01-0074
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	48	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	48	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(48)	(50)	(50)
DILATATION, NOS			1 (2%)
HEMATOMA, NOS			1 (2%)
ABSCESS, NOS		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#TRACHEA	(45)	(49)	(48)
INFLAMMATION, ACUTE/CHRONIC	18 (40%)		
#LUNG/BRONCHUS	(48)	(50)	(49)
BRONCHIECTASIS	3 (6%)		
#LUNG	(48)	(50)	(49)
CONGESTION, NOS	1 (2%)		
INFLAMMATION, FOCAL	2 (4%)		
ABSCESS, NOS	1 (2%)		
PNEUMONIA, CHRONIC MURINE		1 (2%)	
GRANULOMA, NOS	1 (2%)		
HYPERPLASIA, FOCAL	1 (2%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(48)	(49)	(47)
MYELOFIBROSIS	1 (2%)		
HYPERPLASIA, HEMATOPOIETIC	1 (2%)		
HYPERPLASIA, GRANULOCYTIC	1 (2%)		
HYPERPLASIA, MEGAKARYOCYTIC	1 (2%)		
#SPLEEN	(48)	(50)	(49)
CONGESTION, NOS		1 (2%)	

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0070	LOW DOSE 01-0073	HIGH DOSE 01-0074
ERYTHROPOIESIS	1 (2%)	1 (2%)	
#MANDIBULAR L. NODE	(42)	(40)	(46)
DILATATION, NOS	1 (2%)		
HYPERPLASIA, NOS	1 (2%)		
#MEDIASTINAL L.NODE	(42)	(40)	(46)
HYPERPLASIA, NOS		2 (5%)	
CIRCULATORY SYSTEM			
#HEART	(48)	(50)	(49)
FIBROSIS, FOCAL	11 (23%)		
FIBROSIS, DIFFUSE	1 (2%)		
CALCIFICATION, NOS			1 (2%)
#MYOCARDIUM	(48)	(50)	(49)
INFLAMMATION, INTERSTITIAL	2 (4%)		
INFLAMMATION, ACUTE/CHRONIC	3 (6%)		
FIBROSIS, FOCAL	2 (4%)	1 (2%)	
DEGENERATION, NOS	1 (2%)		1 (2%)
#CARDIAC VALVE	(48)	(50)	(49)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
*CORONARY ARTERY	(48)	(50)	(50)
PERIVASCULITIS	1 (2%)		
MEDIAL CALCIFICATION			1 (2%)
*PULMONARY ARTERY	(48)	(50)	(50)
MINERALIZATION	11 (23%)		
DIGESTIVE SYSTEM			
#LIVER	(48)	(50)	(49)
INFLAMMATION, NECROTIZING	1 (2%)		
PERIARTERITIS			1 (2%)
NECROSIS, FOCAL	8 (17%)		1 (2%)
METAMORPHOSIS FATTY	4 (8%)		
HYPERPLASIA, FOCAL	8 (17%)		
ANGIECTASIS	2 (4%)		
ERYTHROPOIESIS	1 (2%)		
#LIVER/CENTRILOBULAR	(48)	(50)	(49)
DEGENERATION, EOSINOPHILIC	2 (4%)		

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0070	LOW DOSE 01-0073	HIGH DOSE 01-0074
#LIVER/PERIPORTAL	(48)	(50)	(49)
FIBROSIS, FOCAL			1 (2%)
*BILE DUCT	(48)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
METAMORPHOSIS FATTY		1 (2%)	
HYPERPLASIA, NOS	6 (13%)	3 (6%)	4 (8%)
HYPERPLASIA, FOCAL			1 (2%)
HYPERPLASIA, DIFFUSE		1 (2%)	
#PANCREAS	(45)	(46)	(45)
INFLAMMATION, ACUTE/CHRONIC	6 (13%)		
PERIARTERITIS	1 (2%)		
ATROPHY, FOCAL	1 (2%)		
#STOMACH	(48)	(49)	(49)
EPIDERMAL INCLUSION CYST	1 (2%)		
#GASTRIC MUCOSA	(48)	(49)	(49)
EROSION			1 (2%)
#PEYERS PATCH	(45)	(49)	(48)
HYPERPLASIA, RETICULUM CELL	1 (2%)		
#ILEUM	(45)	(49)	(48)
HYPERPLASIA, LYMPHOID	1 (2%)		
#COLON	(44)	(42)	(45)
NEMATODIASIS	4 (9%)		
URINARY SYSTEM			
#KIDNEY	(48)	(50)	(49)
GLOMERULONEPHRITIS, NOS	3 (6%)		
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
SCAR			1 (2%)
NEPHROSIS, NOS	41 (85%)	45 (90%)	42 (86%)
#KIDNEY/PELVIS	(48)	(50)	(49)
MINERALIZATION	1 (2%)		
#URINARY BLADDER	(46)	(50)	(47)
POLYP			1 (2%)
METAPLASIA, SQUAMOUS	1 (2%)		

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0070	LOW DOSE 01-0073	HIGH DOSE 01-0074
ENDOCRINE SYSTEM			
#PITUITARY	(41)	(46)	(44)
CONGESTION, NOS		1 (2%)	
HYPERPLASIA, FOCAL	3 (7%)		
#ADRENAL	(47)	(49)	(48)
METAMORPHOSIS FATTY	1 (2%)		
ANGIECTASIS	3 (6%)		
#ADRENAL CORTEX	(47)	(49)	(48)
HYPERPLASIA, FOCAL	1 (2%)		
#THYROID	(39)	(45)	(47)
HYPERPLASIA, C-CELL	1 (3%)		
#PANCREATIC ISLETS	(45)	(46)	(45)
HYPERPLASIA, FOCAL	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(48)	(50)	(50)
GALACTOCELE	1 (2%)		1 (2%)
CYSTIC DUCTS			1 (2%)
HYPERPLASIA, NOS	3 (6%)		
#PROSTATE	(43)	(48)	(47)
INFLAMMATION, FOCAL	1 (2%)		
INFLAMMATION, SUPPURATIVE		3 (6%)	5 (11%)
INFLAMMATION, ACUTE	6 (14%)		
INFLAMMATION, ACUTE FOCAL	8 (19%)		
INFLAMMATION, ACUTE SUPPURATIVE		1 (2%)	1 (2%)
INFLAMMATION, ACUTE/CHRONIC	2 (5%)		
*SEMINAL VESICLE	(48)	(50)	(50)
ATROPHY, NOS	2 (4%)		
#TESTIS	(47)	(50)	(49)
DEGENERATION, NOS	39 (83%)		
ATROPHY, NOS		2 (4%)	4 (8%)
HYPERPLASIA, INTERSTITIAL CELL	1 (2%)		
#TESTIS/TUBULE	(47)	(50)	(49)
DEGENERATION, NOS			1 (2%)

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 01-0070	LOW DOSE 01-0073	HIGH DOSE 01-0074
CALCIFICATION, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(48)	(50)	(50)
ULCER, ACUTE			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, ACUTE/CHRONIC	2		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			4
ANIMAL MISSING/NO NECROPSY	1		
AUTO/NECROPSY/HISTO PERF	1	1	1
AUTO/NECROPSY/NO HISTO			1
AUTOLYSIS/NO NECROPSY	1		

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

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
TREATED WITH 3-NITRO-p-ACETOPHENETIDE

	CONTROL (UNTR) 02-0070	LOW DOSE 02-0073	HIGH DOSE 02-0074
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(49)
EPIDERMAL INCLUSION CYST	1 (2%)		
RESPIRATORY SYSTEM			
#TRACHEA	(49)	(48)	(47)
INFLAMMATION, ACUTE/CHRONIC	15 (31%)		
#LUNG/BRONCHUS	(49)	(50)	(49)
BRONCHIECTASIS			1 (2%)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
#LUNG	(49)	(50)	(49)
INFLAMMATION, FOCAL	2 (4%)		
INFLAMMATION, INTERSTITIAL	2 (4%)		
INFLAMMATION, SUPPURATIVE		1 (2%)	
GRANULOMA, NOS			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(46)	(50)	(49)
OSTEOSCLEROSIS	1 (2%)		
#SPLEEN	(48)	(50)	(48)
HYPERPLASIA, HEMATOPOIETIC	1 (2%)		
HYPERPLASIA, RETICULUM CELL	1 (2%)		
CIRCULATORY SYSTEM			
#HEART	(49)	(50)	(49)
FIBROSIS, FOCAL	1 (2%)		1 (2%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0070	LOW DOSE 02-0073	HIGH DOSE 02-0074
FIBROSIS, DIFFUSE	1 (2%)		
PERIARTERITIS			1 (2%)
NECROSIS, FOCAL			1 (2%)
CALCIFICATION, NOS			1 (2%)
#MYOCARDIUM	(49)	(50)	(49)
INFLAMMATION, INTERSTITIAL	2 (4%)		
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC		1 (2%)	
FIBROSIS, FOCAL	2 (4%)		
#CARDIAC VALVE	(49)	(50)	(49)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
*CORONARY ARTERY	(49)	(50)	(49)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
*PULMONARY ARTERY	(49)	(50)	(49)
MINERALIZATION	9 (18%)		
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(48)
DEGENERATION, EOSINOPHILIC	2 (4%)		
NECROSIS, FOCAL	3 (6%)		
METAMORPHOSIS FATTY	4 (8%)		
HYPERPLASIA, FOCAL	29 (59%)		
ANGIECTASIS	1 (2%)		
*BILE DUCT	(49)	(50)	(49)
HYPERPLASIA, NOS	5 (10%)	5 (10%)	1 (2%)
HYPERPLASIA, FOCAL	1 (2%)		1 (2%)
#PANCREAS	(47)	(48)	(46)
INFLAMMATION, INTERSTITIAL		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC	4 (9%)		
ATROPHY, NOS	1 (2%)		
#STOMACH	(49)	(50)	(46)
ULCER, FOCAL	1 (2%)		
#DUODENAL MUSCULARIS	(49)	(49)	(48)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
#COLON	(44)	(44)	(35)
NEMATODIASIS	2 (5%)		

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0070	LOW DOSE 02-0073	HIGH DOSE 02-0074
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(48)
EMBRYONAL DUCT CYST		1 (2%)	
MINERALIZATION	1 (2%)		
CYST, NOS		1 (2%)	
NEPHROSIS, NOS	34 (69%)	21 (42%)	15 (31%)
CALCINOSIS, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(44)	(45)	(37)
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, FOCAL	2 (5%)		
#ADRENAL	(49)	(49)	(48)
METAMORPHOSIS FATTY	3 (6%)		
#ADRENAL CORTEX	(49)	(49)	(48)
METAMORPHOSIS FATTY	3 (6%)		
HYPERPLASIA, FOCAL	1 (2%)		
#ADRENAL MEDULLA	(49)	(49)	(48)
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, FOCAL	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(50)	(49)
GALACTOCELE	9 (18%)		
INFLAMMATION, ACUTE	1 (2%)		
HYPERPLASIA, NOS	23 (47%)		
HYPERPLASIA, FOCAL	2 (4%)		
*VAGINA	(49)	(50)	(49)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
#UTERUS	(49)	(47)	(41)
DILATATION, NOS		1 (2%)	
HYDROMETRA	6 (12%)		
HEMORRHAGE		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	
#CERVIX UTERI	(49)	(47)	(41)
INFLAMMATION, ACUTE/CHRONIC	2 (4%)		

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0070	LOW DOSE 02-0073	HIGH DOSE 02-0074
HYPERPLASIA, BASAL CELL	1 (2%)		
ACANTHOSIS	1 (2%)		
#UTERUS/ENDOMETRIUM	(49)	(47)	(41)
INFLAMMATION, SUPPURATIVE		3 (6%)	3 (7%)
INFLAMMATION, ACUTE	23 (47%)		
GRANULATION, TISSUE		1 (2%)	
HYPERPLASIA, NOS	5 (10%)		
HYPERPLASIA, CYSTIC	5 (10%)		
#OVARY/OVIDUCT	(49)	(47)	(41)
INFLAMMATION, ACUTE	1 (2%)		
#OVARY	(47)	(50)	(46)
CYST, NOS	2 (4%)	1 (2%)	
ABSCESS, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(49)	(50)	(49)
SYNECHIA, NOS	1 (2%)		
CATARACT	1 (2%)		
*EYE/CORNEA	(49)	(50)	(49)
INFLAMMATION, CHRONIC	1 (2%)		
*EYE/RETINA	(49)	(50)	(49)
DEGENERATION, NOS	1 (2%)		
*EYE/CRYSTALLINE LENS	(49)	(50)	(49)
CALCIFICATION, NOS		1 (2%)	
*LENS CAPSULE	(49)	(50)	(49)
CALCIFICATION, NOS			1 (2%)
*EXTERNAL EAR	(49)	(50)	(49)
HEMATOMA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*STERNUM	(49)	(50)	(49)
OSTEOPETROSIS	1 (2%)		

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

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 02-0070	LOW DOSE 02-0073	HIGH DOSE 02-0074
BODY CAVITIES			
*MEDIASTINUM	(49)	(50)	(49)
PERIARTERITIS	1 (2%)		
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, ACUTE/CHRONIC	3		
INFLAMMATION, CHRONIC	2		
OMENTUM			
MINERALIZATION	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		12	22
AUTOLYSIS/NO NECROPSY	1		1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 3-NITRO-p-ACETOPHENETIDE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH 3-NITRO-p-ACETOPHENETIDE

	CONTROL(UNTR) 05-0077	LOW DOSE 05-0075	HIGH DOSE 05-0076
ANIMALS INITIALLY IN STUDY	50	@50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	46	46	45
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	45	41	43
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(46)	(46)	(45)
HYPERPLASIA, FOCAL			1 (2%)
HYPERKERATOSIS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(45)	(41)	(43)
ARTERIOSCLEROSIS, NOS	1 (2%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(45)	(41)	(43)
FIBROSIS	1 (2%)		1 (2%)
HEMOSIDEROSIS			1 (2%)
HYPERPLASIA, RETICULUM CELL	3 (7%)		
HYPERPLASIA, LYMPHOID			1 (2%)
HEMATOPOIESIS	1 (2%)	1 (2%)	
#MESENTERIC L. NODE	(35)	(36)	(35)
INFLAMMATION, ACUTE/CHRONIC		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(45)	(41)	(43)
CYST, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS
NOTE: ANIMAL #010 WAS DELETED FROM STUDY WHEN FOUND TO BE A FEMALE IN A MALE GROUP.

TABLE D1 (CONTINUED)

	CONTROL(UNTR) 05-0077	LOW DOSE 05-0075	HIGH DOSE 05-0076
INFLAMMATION, FOCAL	2 (4%)		
DEGENERATION, NOS	1 (2%)		
METAMORPHOSIS FATTY	2 (4%)		
ANGIECTASIS		1 (2%)	3 (7%)
#LIVER/CENTRILOBULAR	(45)	(41)	(43)
METAMORPHOSIS FATTY		1 (2%)*	
#LIVER/PERIPORTAL	(45)	(41)	(43)
INFLAMMATION, NOS	1 (2%)		
MEGALOCYTOSIS	1 (2%)		
*BILE DUCT	(46)	(46)	(45)
CYST, NOS		1 (2%)	
INFLAMMATION, NOS	1 (2%)		
HYPERPLASIA, FOCAL	1 (2%)		
#STOMACH	(42)	(41)	(43)
HYPERPLASIA, FOCAL	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(45)	(41)	(43)
CALCULUS, NOS	20 (44%)		
INFLAMMATION, INTERSTITIAL	5 (11%)		
INFLAMMATION, CHRONIC	1 (2%)		
PERIVASCULITIS	2 (4%)		
ARTERIOSCLEROSIS, NOS	1 (2%)		
NEPHROSIS, NOS	1 (2%)		
GLOMERULOSCLEROSIS, NOS		25 (61%)	22 (51%)
HYPERPLASIA, TUBULAR CELL	2 (4%)		
#KIDNEY/TUBULE	(45)	(41)	(43)
DEGENERATION, NOS	1 (2%)		
METAMORPHOSIS FATTY	9 (20%)		
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#TESTIS/TUBULE	(45)	(41)	(43)
DEGENERATION, NOS	2 (4%)		

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

TABLE D1 (CONCLUDED)

	CONTROL(UNTR) 05-0077	LOW DOSE 05-0075	HIGH DOSE 05-0076
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(46)	(46)	(45)
STEATITIS	1 (2%)		
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, FOCAL			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	8	10	6
ANIMAL MISSING/NO NECROPSY			1
NECROPSY PERF/NO HISTO PERFORMED		5	
AUTO/NECROPSY/NO HISTO	1		2
AUTOLYSIS/NO NECROPSY	4	3	4

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

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
TREATED WITH 3-NITRO-p-ACETOPHENETIDE

	CONTROL(UNTR) 06-0077	LOW DOSE 06-0075	HIGH DOSE 06-0076
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	46	47	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	46	47	49
INTEGUMENTARY SYSTEM			
*SKIN	(46)	(47)	(49)
FIBROSIS	1 (2%)		
FIBROSIS, FOCAL	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(45)	(47)	(49)
INFLAMMATION, INTERSTITIAL	2 (4%)		
PERIARTERITIS	1 (2%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(43)	(47)	(48)
HYPERPLASIA, RETICULUM CELL	2 (5%)		
HYPERPLASIA, LYMPHOID	4 (9%)		
HEMATOPOIESIS	1 (2%)	2 (4%)	
CIRCULATORY SYSTEM			
#HEART	(45)	(47)	(48)
PERIARTERITIS			1 (2%)
#MYOCARDIUM	(45)	(47)	(48)
CALCIFICATION, FOCAL	1 (2%)		
*PULMONARY ARTERY	(46)	(47)	(49)
HYPERPLASIA, NOS	1 (2%)		
*MESENTERIC ARTERY	(46)	(47)	(49)
HYPERTROPHY, NOS			1 (2%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0077	LOW DOSE 06-0075	HIGH DOSE 06-0076
DIGESTIVE SYSTEM			
#LIVER	(45)	(47)	(49)
INFLAMMATION, FOCAL	1 (2%)	1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		
CYTOPLASMIC CHANGE, NOS	1 (2%)		1 (2%)
BASOPHILIC CYTO CHANGE			1 (2%)
MEGALOCYTOSIS	1 (2%)		
HYPERTROPHY, NOS			1 (2%)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
ANGIECTASIS		7 (15%)	1 (2%)
#LIVER/PERIPORTAL	(45)	(47)	(49)
INFLAMMATION, NOS	1 (2%)		
#LIVER/KUPFFER CELL	(45)	(47)	(49)
HYPERPLASIA, NOS	1 (2%)		
*BILE DUCT	(46)	(47)	(49)
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
#STOMACH	(42)	(44)	(47)
PERIARTERITIS			2 (4%)
URINARY SYSTEM			
#KIDNEY	(43)	(46)	(49)
INFLAMMATION, INTERSTITIAL	3 (7%)		
PERIVASCULITIS	4 (9%)		
GLOMERULOSCLEROSIS, NOS		17 (37%)	25 (51%)
#KIDNEY/GLOMERULUS	(43)	(46)	(49)
AMYLOIDOSIS	1 (2%)		
SIDEROSIS		1 (2%)	
#KIDNEY/PELVIS	(43)	(46)	(49)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
#URINARY BLADDER	(41)	(47)	(46)
INFLAMMATION, ACUTE			1 (2%)
PERIARTERITIS			1 (2%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0077	LOW DOSE 06-0075	HIGH DOSE 06-0076
ENDOCRINE SYSTEM			
#THYROID	(30)	(45)	(42)
PERIARTERITIS			1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(43)	(47)	(42)
DILATATION, NOS		1 (2%)	
HYDROMETRA	4 (9%)	3 (6%)	1 (2%)
#UTERUS/ENDOMETRIUM	(43)	(47)	(42)
CYST, NOS	2 (5%)		
INFLAMMATION, SUPPURATIVE		7 (15%)	1 (2%)
INFLAMMATION, ACUTE		1 (2%)	1 (2%)
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, CYSTIC	35 (81%)	27 (57%)	6 (14%)
#OVARY	(41)	(45)	(47)
CYST, NOS	1 (2%)	4 (9%)	4 (9%)
HEMATOMA, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE		2 (4%)	
ABSCESS, NOS		2 (4%)	
INFLAMMATION, CHRONIC		4 (9%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(46)	(47)	(49)
ULCER, ACUTE			1 (2%)
MUSCULOSKELETAL SYSTEM			
*VERTEBRA	(46)	(47)	(49)
OSTEOSCLEROSIS	1 (2%)		
BODY CAVITIES			
*MESENTERY	(46)	(47)	(49)
PERIARTERITIS			2 (4%)

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

TABLE D2 (CONCLUDED)

	CONTROL(UNTR) 06-0077	LOW DOSE 06-0075	HIGH DOSE 06-0076
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	4	12
AUTO/NECROPSY/HISTO PERF	2	1	1
AUTOLYSIS/NO NECROPSY	4	3	1

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

NIH Publication No. 79-1388