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BIOASSAY OF 5-NITRO-o-TOLUIDINE FOR POSSIBLE CARCINOGENICITY

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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REPORT ON THE BIOASSAY OF 5-NITRO-o-TOLUIDINE
FOR POSSIBLE CARCINOGENICITY

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

FOREWORD: This report presents the results of the bioassay of 5-nitro-o-toluidine conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 5-nitro-o-toluidine was conducted by Mason Research Institute, Worcester, Massachusetts, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

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

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

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

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), senior biologist Ms. P. Walker (6), biochemist Dr. B. Fuller (6), chemist Dr. N. Zimmerman (6), and 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), Dr. R. A. Griesemer (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,8), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay of 5-nitro-o-toluidine for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F1 mice. 5-Nitro-o-toluidine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The time-weighted average high and low dietary concentrations of 5-nitro-o-toluidine were, respectively, 0.01 and 0.005 percent for rats, and 0.23 and 0.12 percent for mice. After a 78-week period of compound administration, observation of the rats continued for an additional 30 to 31 weeks and observation of the mice continued for up to an additional 20 weeks.

For each species, 50 animals of each sex were placed on test as controls and fed only the basal diet.

There were no significant positive associations between the concentration of 5-nitro-o-toluidine administered and mortality in rats or mice of either sex, and adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

No unusual tumors were observed in rats of either sex and no convincing statistical evidence was provided for a significant positive association between compound administration and the incidence of any neoplasm in rats.

Among mice there was a statistically significant positive association between administration of the chemical and the incidences of hepatocellular carcinomas in both males and females. The combined incidence of hemangiomas and hemangiosarcomas in male mice and the incidence of hemangiosarcomas in female mice were not statistically significant in relation to their respective control groups. However, they were considered to be associated with compound administration since they rarely occur in untreated B6C3F1 mice.

Under the conditions of this bioassay 5-nitro-o-toluidine was carcinogenic in B6C3F1 mice, causing hepatocellular carcinomas in both sexes, an increase in the combined incidence of hemangiomas and hemangiosarcomas in male mice, and an increased incidence of hemangiosarcomas in female mice. The compound was not carcinogenic in Fischer 344 rats.

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I. INTRODUCTION

5-Nitro-o-toluidine (NCI No. C01843), an intermediate in the synthesis of azo dyes, was selected for bioassay by the National Cancer Institute in an attempt to elucidate those chemicals which may be responsible for the increased incidence of bladder cancer observed among workers in the dye manufacturing industry (Wynder et al., 1963; Anthony and Thomas, 1970). Aromatic nitro and amino compounds are thought to contribute to the increased cancer risk in this industry (Wynder et al., 1963).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 2-methyl-5-nitro-benzeneamine.* Other frequently used chemical names include 4-nitro-2-aminotoluene and 6-methyl-3-nitroaniline. The dye industry commonly refers to this compound as Fast Scarlet Base G or Colour Index (C.I.) Azoic Diazo Component 12 (C.I. 37105).

According to the U.S. International Trade Commission (1977a), 1.25×10^5 pounds of Azoic Diazo Component 12, salt were produced by three companies in 1975. Two of these companies and one other company also produced additional quantities of the free base listed as either C.I. Azoic Diazo Component, base or as 5-nitro-o-toluidine. Although quantities produced were considered proprietary, there is reason to believe that production of the free base rivaled that of

*The CAS registry number is 99-55-8.

the salt, since in 1973, 3.53×10^5 pounds of 5-nitro-o-toluidine were produced as compared to 3.60×10^5 pounds of Azoic Diazo Component 12, salt (U.S. International Trade Commission, 1975; as cited in Urso, 1977). Imports of 5-nitro-o-toluidine through principle U.S. customs districts amounted to 4.63×10^3 pounds in 1975 (U.S. International Trade Commission, 1977b; as cited in Urso, 1977).

5-Nitro-o-toluidine finds application as an intermediate in the synthesis of Pigment Red 17 (C.I. 12 390) and Pigment Red 22 (C.I. 12 315). Used in conjunction with certain C.I. coupling components, 5-nitro-o-toluidine also serves as a precursor for a wide assortment of azo dyes of various red, yellow, orange, violet, and brown hues (Society of Dyers and Colourists, 1971a, Society of Dyers and Colourists, 1971b, and Schlopfer, 1973; as cited in Urso, 1977).

Since 5-nitro-o-toluidine and its salts appear to be used on a commercial scale exclusively by the dye and pigment industry, the potential for exposure to the compound may be greatest for workers at dye manufacturing facilities.

Methemoglobinemia is the major toxic effect observed following excessive absorption of 5-nitro-o-toluidine (Hamblin, 1967). Symptoms of exposure include bluish lips and/or fingernails, headache, nausea, and fatigue (Synalloy Corporation, 1977). In addition, upon dermal contact the compound may irritate the skin, resulting in dermatitis (Synalloy Corporation, 1977).

II. MATERIALS AND METHODS

A. Chemicals

5-Nitro-o-toluidine (Figure 1) was purchased from J. T. Baker Chemical Company and chemical analysis was performed by Mason Research Institute. The experimentally determined melting point of 104° to 106°C suggested a compound of high purity due to its narrow range and close proximity to the literature value (Weast, 1977) of 107° to 108°C. Ultraviolet analysis of the purchased chemical showed λ_{max} at 230.256 and 290. The literature (Sadtler Standard Spectra) listed a λ_{max} only at 227, thereby indicating the presence of impurities.

Throughout this report the term 5-nitro-o-toluidine is used to represent this compound.

B. Dietary Preparation

The basal laboratory diet for both treated and control animals consisted of Wayne Lab-Blox® (Allied Mills, Inc., Chicago, Illinois). 5-Nitro-o-toluidine was administered to the treated animals as a component of the diet. The chemical was mixed with the feed using a 6 kg capacity Patterson-Kelley twin-shell stainless-steel V-blender. After 20 minutes of blending, prepared diets were placed in double plastic bags and stored in the dark at 4°C. Mixtures were used for 1 week only.

C. Animals

Two animals species, rats and mice, were used in the carcinogenicity bioassay. Fischer 344 rats and B6C3F1 mice were obtained through

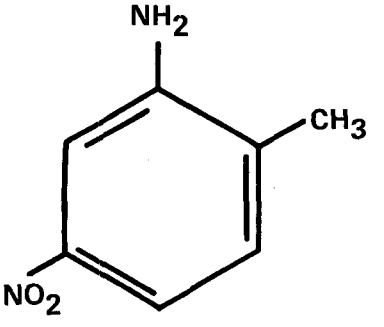


FIGURE 1
CHEMICAL STRUCTURE OF 5-NITRO-o-TOLUIDINE

contracts of the Division of Cancer Treatment, National Cancer Institute. Rats for use in the treated groups were supplied by ARS/Sprague-Dawley, Madison, Wisconsin. Rats to be used in the control groups were obtained from Laboratory Supply Company, Inc., Indianapolis, Indiana. All mice were supplied by Charles River Breeding Laboratories, Wilmington, Massachusetts. Upon arrival, a sample of animals was examined for parasites and other signs of disease. Some male rats from ARS/Sprague-Dawley were diagnosed as having pneumonia; only disease-free animals were used for the chronic bioassay. The remaining animals were quarantined for two weeks prior to initiation of test. Animals were assigned to groups and distributed among cages so that average body weight per cage was approximately equal for a given sex and species.

D. Animal Maintenance

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

Rats were housed five per cage by sex. During quarantine and for the first 11 months of the study, they were housed in stainless- and galvanized-steel wire-mesh cages suspended above newspapers. Newspapers were replaced daily and cages and racks washed weekly.

After the first 11 months and for the remainder of the study, rats were kept in suspended polycarbonate cages equipped with disposable nonwoven fiber filter sheets. Corncob bedding and clean cages were provided twice weekly. Stainless steel racks were cleaned once every two weeks and disposable filters were replaced at that time.

Mice were housed by sex in polycarbonate cages. During quarantine and periods of chemical administration, cages were fitted with perforated stainless-steel lids. During the observation period following chemical administration, stainless-steel wire bar lids were used. Both types of lids were from Lab Products, Inc., Garfield, New Jersey. Nonwoven fiber filter bonnets were used over cage lids. Animals were housed ten per cage for the first 16 months of study and five per cage thereafter. Cages, lids, filters, and bedding were provided three times per week when cage populations were ten and twice per week when cage populations were reduced to five. Ab-sorb-dri® hardwood chip bedding (Wilner Wood Products Company, Norway, Maine) was used through the first 7 months of study, followed by SAN-I-CEL® corncob bedding (Paxton Processing Company, Paxton, Illinois) for the next 12 months. After 19 months on study, animals were placed on a second corncob bedding (Bed-o-Cobs®, The Andersons Cob Division, Maumee, Ohio). Reusable filters and pipe racks were sanitized every 2 weeks throughout the study.

Water was available ad libitum for both species from 250 ml water bottles equipped with rubber stoppers and stainless steel sipper tubes.

Glass water bottles were used for the first 8 months of study; polycarbonate bottles were used for the remainder of the bioassay. Bottles were replaced twice weekly and, for rats only, water was supplied as needed between changes.

Wayne Lab-Blox® feed was used throughout the entire bioassay. Pelleted feed was supplied during the quarantine period and the final observation period. During chemical administration, all treated animals were supplied with meal containing the appropriate concentrations of 5-nitro-o-toluidine. Control animals were supplied with untreated meal. Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc., Woburn, Massachusetts) equipped with stainless steel baffles were used to dispense feed. Food was replenished daily and the food assembly was replaced weekly. During the untreated observation period, rats were fed pellets on the cage floor. During this same period, mice received pellets from a food hopper incorporated into the cage lid.

Treated and control rats were housed in a room with other rats receiving diets containing* hydrazobenzene (530-50-7); 2-aminoanthraquinone (117-79-3); 3-amino-9-ethylcarbazole hydrochloride; 4-nitroanthranilic acid (619-17-0); 6-nitrobenzimidazole (94-52-0); APC (8003-03-0); 1-nitronaphthalene (86-57-7); 2,4-diaminoanisole sulfate (615-05-4); and 2-methyl-1-nitroanthraquinone (129-15-7).

*CAS registry numbers are given in parentheses.

Treated and control mice were housed in a room with other mice receiving diets containing 2,5-toluenediamine sulfate (6369-59-1); hydrazobenzene (530-50-7); 3-amino-9-ethylcarbazole hydrochloride; 6-nitrobenzimidazole (94-52-0); 3-nitro-p-acetophenetide (1777-84-0); 5-nitro-o-anisidine (99-59-2); acetylaminofluorene (53-96-3); acetone (67-64-1); 1-amino-2-methylanthraquinone (82-28-0); 3-amino-4-ethoxy-acetanilide (17026-81-2); 1-nitronaphthalene (86-57-7); 2,4-diamino-anisole sulfate (615-05-4); APC (8003-03-0); 5-nitroacenaphthene (602-87-9); 3-amino-4-ethoxyacetanilide (17026-81-2); 2-aminoanthra-quinone (117-79-3); 2,4-dinitrotoluene (121-14-2); and N,N-dimethyl-p-nitrosoaniline (138-89-6).

E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of 5-nitro-o-toluidine for administration to treated animals in the chronic studies, subchronic toxicity studies were conducted with both rats and mice. In the initial phase of subchronic toxicity testing, 5-nitro-o-toluidine was incorporated into the basal laboratory diet and supplied to five of six rat groups (each consisting of five males and five females) at concentrations of 0.009, 0.019, 0.037, 0.07, and 0.15 percent. The sixth group served as the control, receiving only the basal laboratory diet. Each of these concentrations produced severe weight depression and deaths within the first 3 weeks of study. In the second phase of subchronic toxicity testing, animals of each species were distributed among five groups, each consisting of five

males and five females. 5-Nitro-o-toluidine was incorporated into the basal laboratory diet and supplied to four groups of each species in concentrations of 0.009, 0.019, 0.037, and 0.07 percent. The dosed dietary preparations were administered for 4 weeks, followed by a 2-week observation period during which animals were fed the basal diet. No deaths occurred at these concentrations. Based upon weight depression relative to untreated controls, the initial high and low concentrations selected for rats in the chronic bioassay were 0.009 and 0.0045 percent, respectively. The initial high and low concentrations selected for mice in the chronic bioassay were 0.01 and 0.005 percent, respectively.

F. Experimental Design

The experimental design parameters for the chronic bioassay (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.

At initiation of the study all the rats were approximately 6 weeks old. The initial dietary concentrations of 0.009 and 0.0045 percent were administered for the first 8 weeks. Throughout this report those rats initially receiving the former concentration are referred to as the high dose groups and those initially receiving the latter concentration are referred to as the low dose groups. High and low concentrations were increased in week 9 to 0.01 and 0.005 percent, respectively. Dosed feed was administered to treated rats for a

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
5-NITRO-o-TOLUIDINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	5-NITRO-o TOLUIDINE 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	0.0045 0.005 0	8 70	 30	0.005
HIGH DOSE	50	0.009 0.01 0	8 70	 30	0.01
FEMALE					
CONTROL	50	0	0	109	0
LOW DOSE	50	0.0045 0.005 0	8 70	 30	0.005
HIGH DOSE	50	0.009 0.01 0	8 70	 31	0.01

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

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
5-NITRO-o-TOLUIDINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	5-NITRO-o TOLUIDINE CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION[a]
MALE					
CONTROL	50	0	0	95	0
LOW DOSE	49	0.005 0.15 0	18 60	 19	0.12
HIGH DOSE	50	0.01 0.30 0	18 60	 19	0.23
FEMALE					
CONTROL	50	0	0	96	0
LOW DOSE	50	0.005 0.15 0	18 60	 19	0.12
HIGH DOSE	50	0.01 0.30 0	18 60	 20	0.23

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

78-week period, during which untreated meal was available to controls. The compound administration period was followed by an observation period of 30 to 31 weeks.

At initiation of the study all mice were approximately 6 weeks old. For the first 18 weeks dietary concentrations of 0.01 and 0.005 percent were administered. Throughout this report those mice initially receiving the former concentration are referred to as the high dose groups and those initially receiving the latter concentration are referred to as the low dose groups. Because of a lack of weight depression in the high and low dose mice, concentrations were increased in week 19 to 0.3 and 0.15 percent, respectively, for the remainder of the dosing period. After the 78-week period of compound administration, mice were observed for a period of up to 20 additional weeks. Control mice were fed the basal laboratory diet for 95 to 96 weeks.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. Body weights were recorded twice weekly for the first 12 weeks of the study and at monthly intervals thereafter. 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. From the first day, all animals were inspected twice daily for mortality. 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, brain, ear, testis, prostate, uterus, mammary gland, and ovary.

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

H. Data Recording and Statistical Analyses

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

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. 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

Slight mean body weight depression was observed for high dose male rats and for both high and low dose female rats when compared to their respective controls (Figure 2).

Palpable subcutaneous masses occurred in two high dose females, while one low dose female had a growth on its ear and a small crusted lesion was observed on one control male. No other clinical abnormalities were observed.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 5-nitro-o-toluidine-dosed groups are shown in Figure 3. For male rats the Tarone test did not indicate a significant positive association between dosage and mortality. The Cox tests did not indicate a significant difference in survival between either dosed group and the control group. For female rats the Tarone test did not show a significant association between dosage and mortality.

Five male rats were sacrificed from each group in week 78. Adequate numbers of males were at risk from late-developing tumors with 56 percent (28/50) of the high dose, 54 percent (27/50) of the low dose, and 66 percent (33/50) of the control rats alive on test until the termination of the study. Five of the seven low dose rats and three of the four high dose rats that died in weeks 2 through 4 had pneumonia.

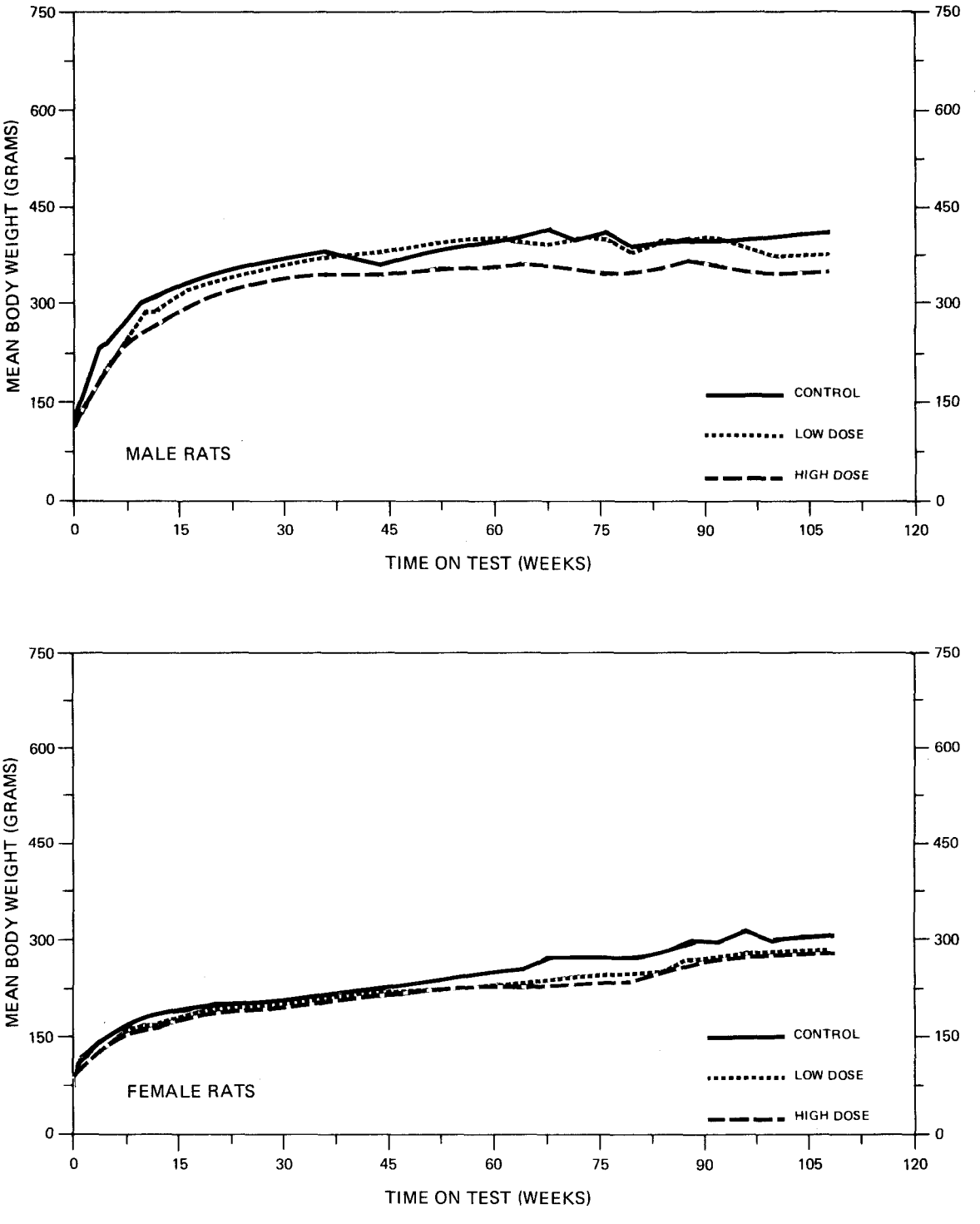


FIGURE 2
GROWTH CURVES FOR 5-NITRO-o-TOLUIDINE CHRONIC STUDY RATS

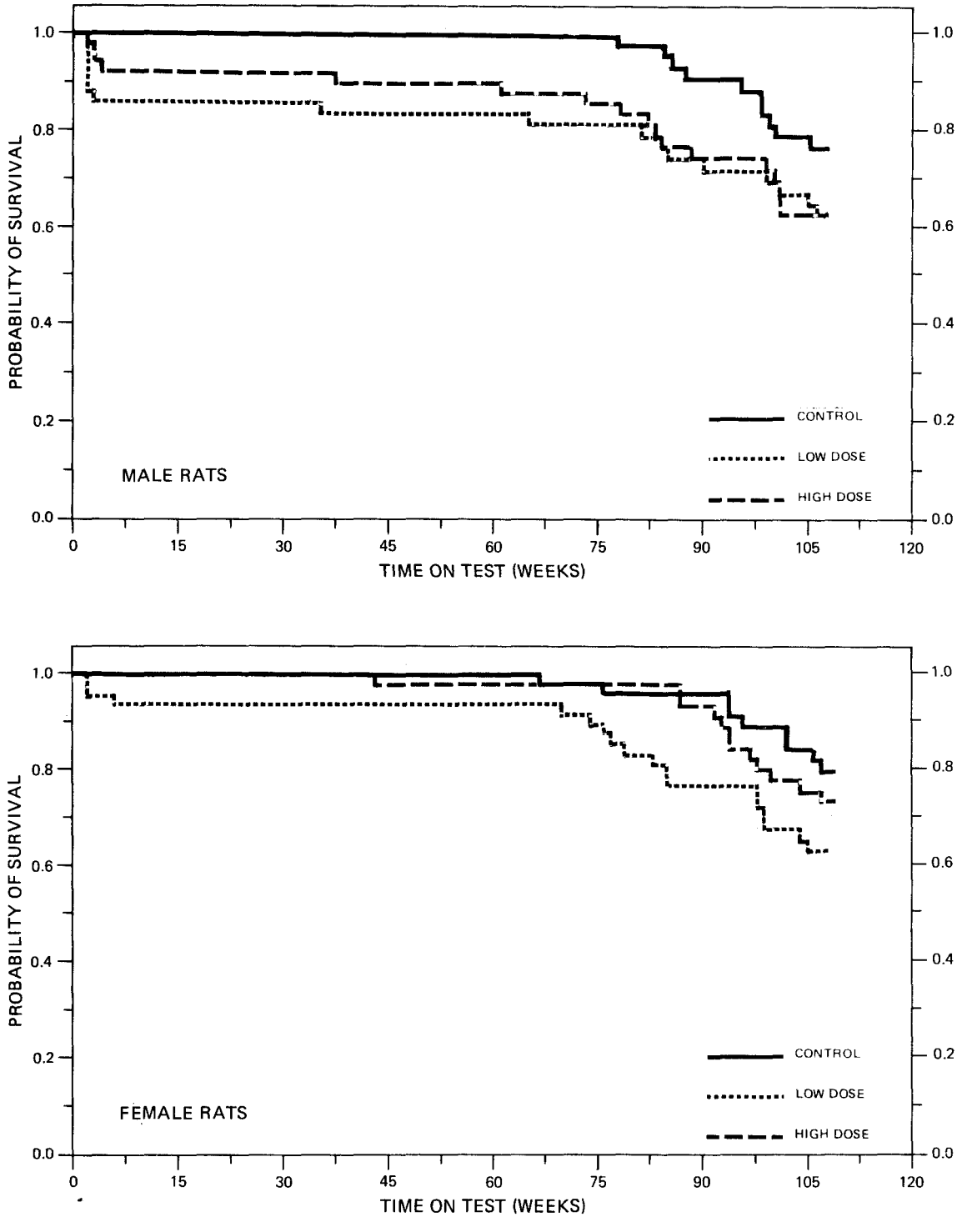


FIGURE 3
SURVIVAL COMPARISONS OF 5-NITRO-o-TOLUIDINE CHRONIC STUDY RATS

Five female rats were sacrificed from each group in week 78. With 66 percent (33/50) of the high dose, 56 percent (28/50) of the low dose, and 68 percent (34/50) of the control rats alive on test until the termination of the study, adequate numbers of females were at risk from late-developing tumors.

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).

Possible compound-related findings were limited to the occurrence of hepatocellular carcinomas in treated male rats. None of the 47 controls or 41 low dose animals developed the lesion; however, 3/46 (7 percent) of the high dose males had hepatocellular carcinomas. In contrast, 5/47 (11 percent) control males had neoplastic nodules in the liver as did 1/41 (2 percent) in the low dose and 1/46 (2 percent) in the high dose animals. The morphological features of the hepatocellular carcinomas and neoplastic nodules were similar to those described in the literature (Squire and Levitt, 1975). Neoplastic nodules were small and compressed the adjacent parenchyma in areas. Neoplastic cells were large and had acidophilic cytoplasm and hyperchromatic nuclei. A few mitotic figures were present. Hepatocellular carcinomas were composed of liver plates which were several cells in thickness. Pleomorphism in the size of neoplastic hepatocytes was noted. Cytoplasm of the cells was acidophilic or vacuolated and

nuclei were large with prominent nucleoli. Mitotic figures were not numerous.

Certain other neoplastic lesions occurred more frequently in the controls than in treated animals. However, their incidence is considered to be within the normal range of variation for Fischer 344 rats.

The variety of degenerative and inflammatory lesions was similar in control and treated rats and incidences of these lesions were within the normal range of variation for Fischer 344 rats. These nonneoplastic lesions were, therefore, not considered to be compound-related.

In this bioassay of 5-nitro-o-toluidine, the incidence of hepatocelluar carcinomas was higher in the high dose males than in the low dose or control groups. However, the numbers of animals with this lesion were too small to permit the determination that the effect was compound-related.

Under the conditions of this study, there was no conclusive evidence to indicate that the dietary administration of 5-nitro-o-toluidine was carcinogenic to 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 every type of tumor in either sex where at least two such tumors were observed in at least one of the control or 5-nitro-o-toluidine-dosed

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 5-NITRO-o-TOLUIDINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Skin and Subcutaneous Tissue: Fibroma[b]	7/47(0.15)	2/48(0.04)	1/48(0.02)
P Values[c]	P = 0.013(N)	N.S.	P = 0.027(N)
Relative Risk (Control)[d]	---	0.280	0.140
Lower Limit	---	0.030	0.003
Upper Limit	---	1.378	1.027
Weeks to First Observed Tumor	98	107	108
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	5/47(0.11)	6/48(0.13)	2/48(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.175	0.392
Lower Limit	---	0.321	0.039
Upper Limit	---	4.548	2.259
Weeks to First Observed Tumor	84	85	100
Liver: Hepatocellular Carcinoma[b]	0/47(0.00)	0/41(0.00)	3/46(0.07)
P Values[c]	P = 0.039	---	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.616
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	108

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	5/47(0.11)	1/41(0.02)	4/46(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.229	0.817
Lower Limit	---	0.005	0.172
Upper Limit	---	1.931	3.557
Weeks to First Observed Tumor	108	107	108
Pituitary: Adenoma[b]	8/47(0.17)	2/36(0.06)	8/43(0.19)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.326	1.093
Lower Limit	---	0.035	0.392
Upper Limit	---	1.507	3.040
Weeks to First Observed Tumor	108	78	82
Adrenal: Pheochromocytoma[b]	7/47(0.15)	1/41(0.02)	3/45(0.07)
P Values[c]	N.S.	P = 0.045(N)	N.S.
Relative Risk (Control)[d]	---	0.164	0.448
Lower Limit	---	0.004	0.079
Upper Limit	---	1.194	1.825
Weeks to First Observed Tumor	108	107	83

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Carcinoma[b]	2/46(0.04)	0/39(0.00)	2/41(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	1.122
Lower Limit	---	0.000	0.085
Upper Limit	---	3.960	14.845
Weeks to First Observed Tumor	108	---	101
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	4/46(0.09)	2/39(0.05)	3/41(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.590	0.842
Lower Limit	---	0.056	0.130
Upper Limit	---	3.867	4.671
Weeks to First Observed Tumor	108	107	101
Pancreatic Islets: Islet-Cell Adenoma or Islet-Cell Carcinoma[b]	4/45(0.09)	2/41(0.05)	5/44(0.11)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.549	1.278
Lower Limit	---	0.052	0.295
Upper Limit	---	3.606	6.041
Weeks to First Observed Tumor	85	107	88

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Testis: Interstitial-Cell Tumor[b]	44/47(0.94)	34/41(0.83)	38/46(0.83)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.886	0.882
Lower Limit	---	0.785	0.788
Upper Limit	---	1.054	1.048
Weeks to First Observed Tumor	78	78	78

[a]Treated groups received time-weighted average doses of 0.005 or 0.01 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.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 5-NITRO-o-TOLUIDINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	5/48(0.10)	5/48(0.10)	4/50(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.000	0.768
Lower Limit	---	0.246	0.162
Upper Limit	---	4.070	3.356
Weeks to First Observed Tumor	96	78	93
Pituitary: Adenoma NOS[b]	18/46(0.39)	14/40(0.35)	10/45(0.22)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.894	0.568
Lower Limit	---	0.476	0.266
Upper Limit	---	1.637	1.145
Weeks to First Observed Tumor	94	78	87
Adrenal: Cortical Adenoma or Cortical Carcinoma[b]	4/47(0.09)	1/44(0.02)	0/49(0.00)
P Values[c]	P = 0.025(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.267	0.000
Lower Limit	---	0.006	0.000
Upper Limit	---	2.561	1.034
Weeks to First Observed Tumor	96	108	---

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	3/46(0.07)	2/38(0.05)	1/47(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.807	0.326
Lower Limit	---	0.070	0.006
Upper Limit	---	6.662	3.887
Weeks to First Observed Tumor	109	108	98
Mammary Gland: Fibroadenoma[b]	14/48(0.29)	5/48(0.10)	12/50(0.24)
P Values[c]	N.S.	P = 0.019(N)	N.S.
Departure from Linear Trend[e]	P = 0.025	---	---
Relative Risk (Control)[d]	---	0.357	0.823
Lower Limit	---	0.109	0.389
Upper Limit	---	0.957	1.715
Weeks to First Observed Tumor	76	98	78
Uterus: Endometrial Stromal Polyp[b]	15/47(0.32)	13/44(0.30)	14/48(0.29)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.926	0.914
Lower Limit	---	0.460	0.463
Upper Limit	---	1.833	1.795
Weeks to First Observed Tumor	78	74	92

TABLE 4 (CONCLUDED)

[a]Treated groups received time-weighted average doses of 0.005 or 0.01 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 $P < 0.05$.

groups and where such tumors were observed in at least 5 percent of the group.

For male rats the Cochran-Armitage test indicated a significant (P = 0.039) positive association between compound administration and the incidence of hepatocellular carcinomas. The Fisher exact tests, however, were not significant.

Significant Cochran-Armitage test results in a negative direction were observed for fibroma of the skin or subcutaneous tissue in males (P = 0.013) and for the combined incidence of cortical adenomas or cortical carcinomas of the adrenal gland in females (P = 0.025), but Fisher exact tests were not significant under the Bonferroni criterion.

In females the Fisher exact test showed a significantly (P = 0.019) lower incidence of mammary fibroadenomas in the low dose than in the control. The Cochran-Armitage test and the high dose to control comparison, however, were not significant.

No statistical tests for other sites were significant under the Bonferroni criterion. Based upon these results, there was no convincing evidence that 5-nitro-o-toluidine was a carcinogen in male or female rats.

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 5-nitro-o-toluidine 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, as compared to controls, was observed in both sexes of treated mice. The effect was more pronounced among females than males (Figure 4).

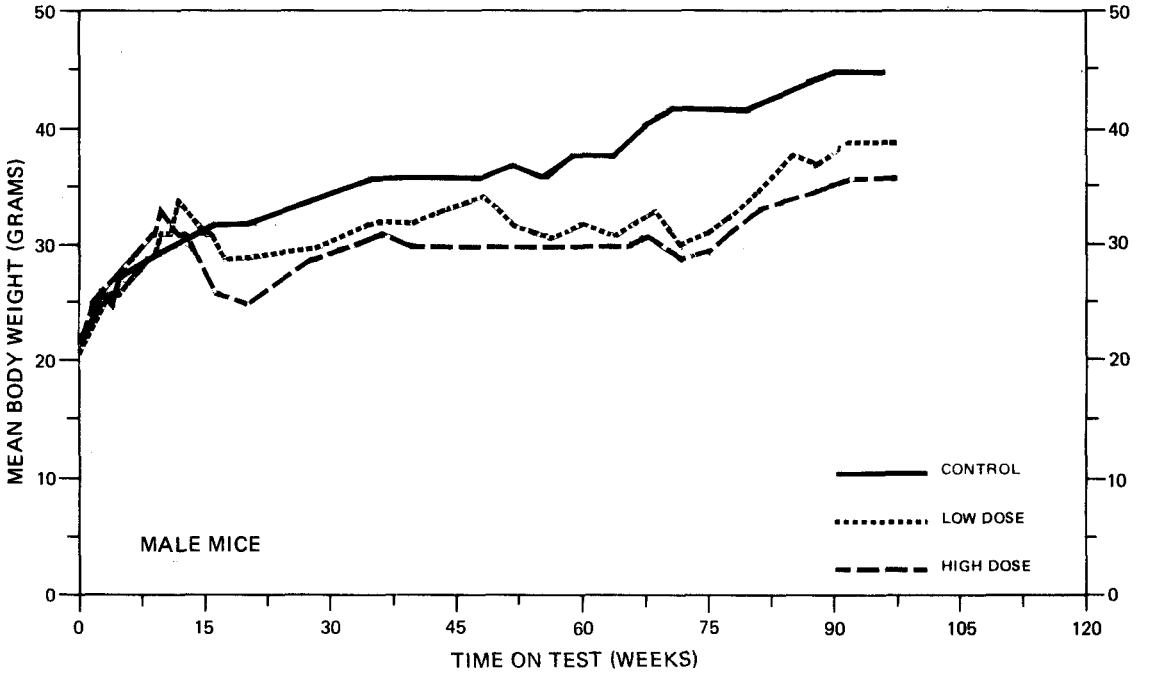
No differences in clinical abnormalities were observed between treated and untreated mice of either sex.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 5-nitro-o-toluidine-dosed groups are shown in Figure 5. For male mice the Tarone test did not indicate a positive association between dosage and mortality. For female mice the Tarone test did not show a significant association between dosage and mortality.

Five male mice were sacrificed from the high dose group in week 79 and five from the control group in week 78. Adequate numbers of males were at risk from late-developing tumors with 76 percent (38/50) of the high dose, 82 percent (41/50) of the low dose, and 86 percent (43/50) of the controls alive on test until the termination of the study.

Five female mice were sacrificed from the high dose group in week 79 and five from the control group in week 78. With 72 percent (36/50) of the high dose, 84 percent (42/50) of the low dose, and 72 percent (36/50) of the control mice alive on test until the



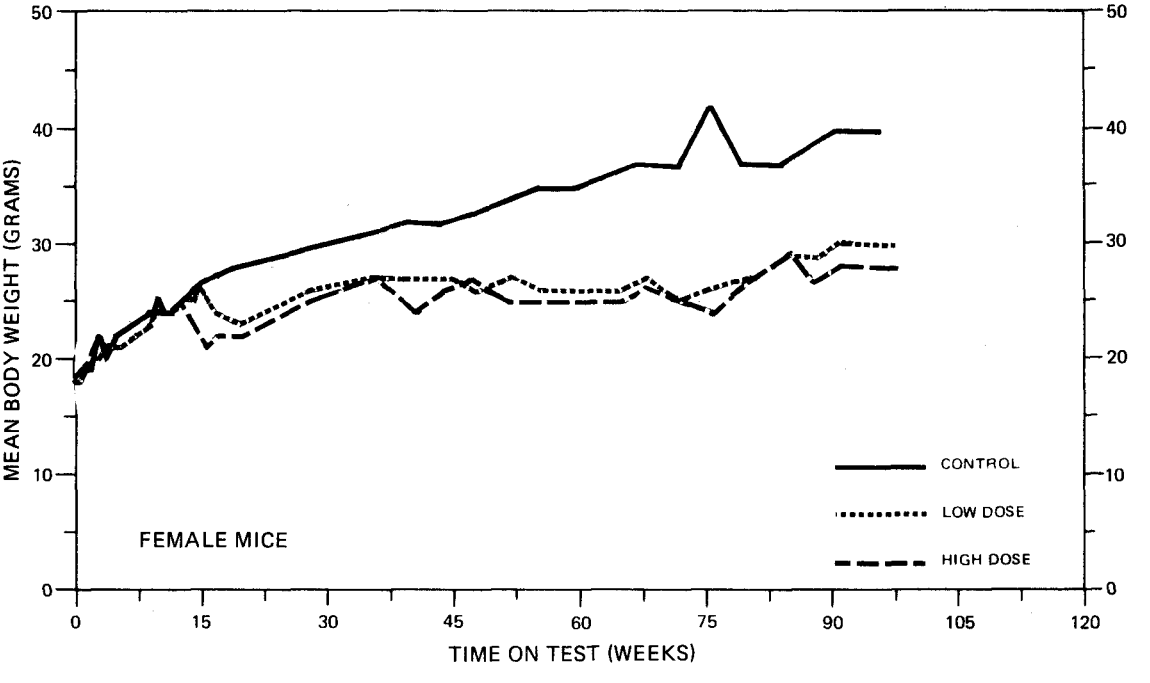
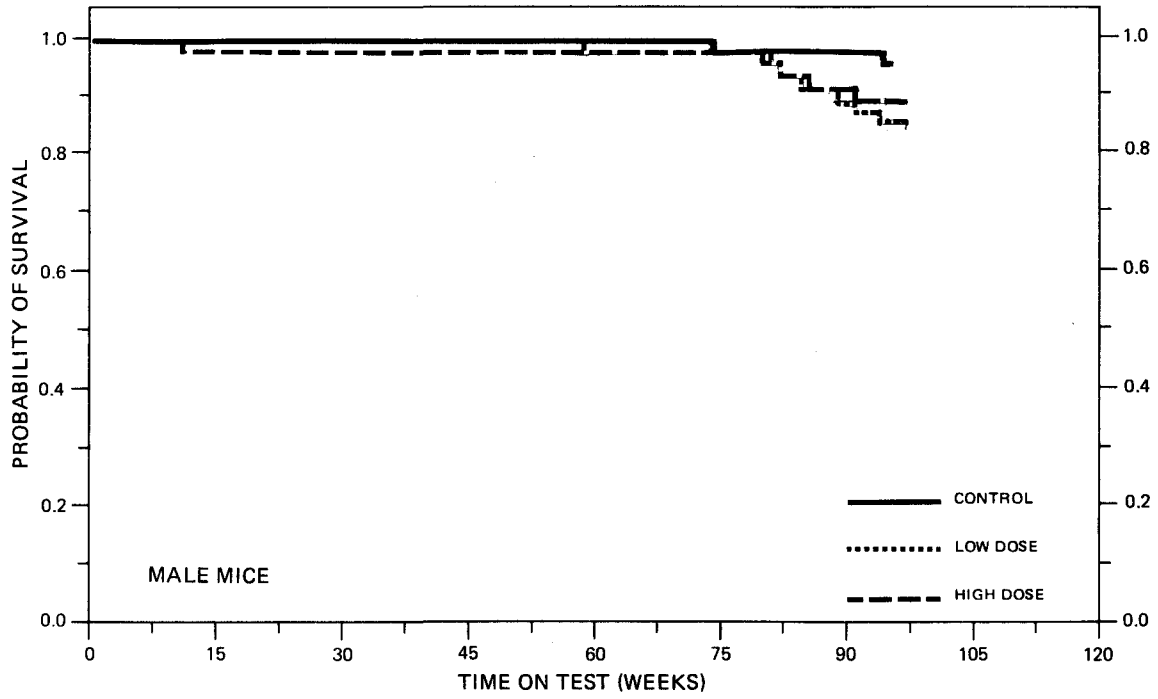


FIGURE 4
GROWTH CURVES FOR 5-NITRO-o-TOLUIDINE CHRONIC STUDY MICE



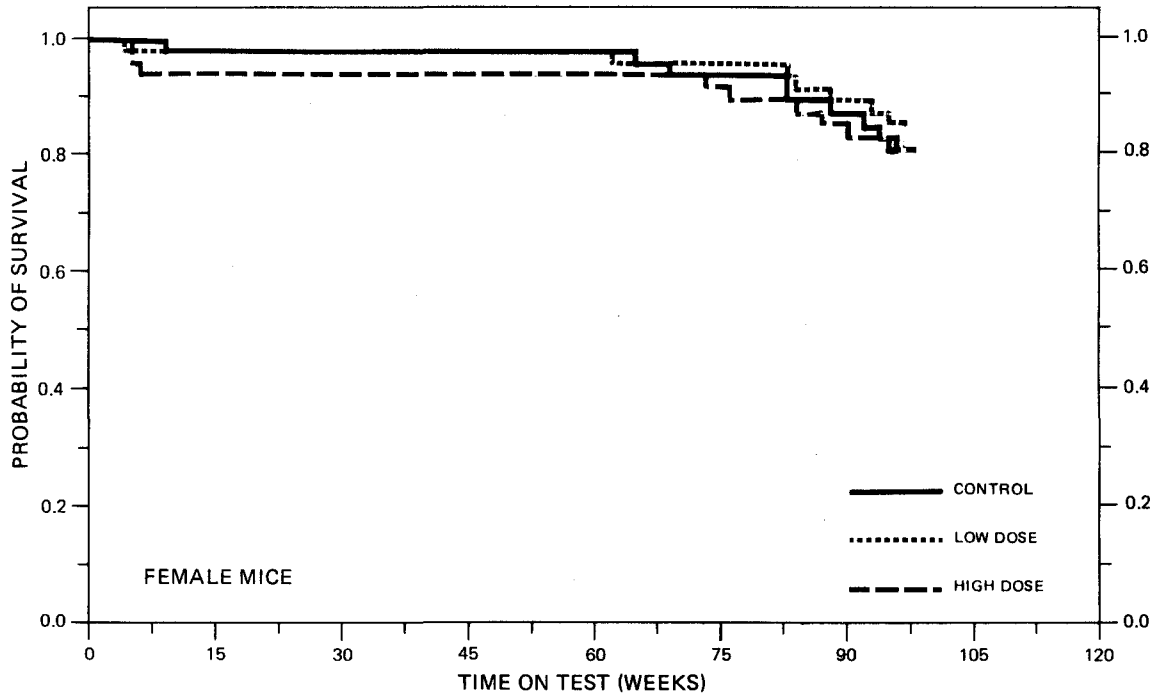


FIGURE 5
SURVIVAL COMPARISONS OF 5-NITRO-o-TOLUIDINE CHRONIC STUDY MICE

termination of the study, adequate numbers of females were at risk from late-developing tumors.

C. Pathology

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

Hepatocellular carcinoma was observed in 29/45 (64 percent) high dose male, 12/44 (27 percent) low dose male, and 12/50 (24 percent) control male mice. In females, the incidence was 20/45 (44 percent) high dose, 7/46 (15 percent) low dose, and 2/47 (4 percent) control mice. The first mouse that died with hepatocelluar carcinoma was a high dose male in week 79. This was in contrast to week 97 in high dose females, week 94 for control males, and week 94 for control females. The hepatocellular carcinomas were similar to those described by Reuber (1975). No hepatic tumor was judged to be benign, and hepatocellular carcinomas in two control animals had metastasized to the lungs. Hepatocellular carcinomas involved a part or an entire lobe of the liver. The lobular architecture was not preserved and sinusoids in areas were distended. Some pleomorphism in the size of neoplastic hepatocytes was evident. Cytoplasm was acidophilic; in some it was vacuolated, suggesting fatty infiltration. Nuclei were hyperchromatic. Mitotic figures were numerous.

The incidence of other types of tumors was sporadic in the treated groups of both sexes.

Low incidences of hemangiomas and hemangiosarcomas (all sites) were observed in dosed mice. The incidences of male mice having either hemangiomas or hemangiosarcomas were 1/50 (2 percent) controls, 0/47 low dose, and 4/48 (8 percent) high dose. Among female mice, no hemangiomas were observed, but the incidences of hemangiosarcomas (all sites) were 1/48 (2 percent) controls, 5/47 (11 percent) low dose and 3/47 (6 percent) high dose. These tumors are rarely seen in untreated B6C3F1 mice.

There were numerous inflammatory and degenerative lesions noted. None were judged to be related to the administration of 5-nitro-o-toluidine.

This histopathologic evaluation provides evidence for the carcinogenicity of 5-nitro-o-toluidine as hepatocellular carcinomas were induced in both male and female B6C3F1 mice. In addition, there may be an association between compound administration and the induction of hemangiomas and hemangiosarcomas.

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 tumor in either sex where at least two such tumors were observed in at least one of the control or 5-nitro-o-toluidine-dosed groups and where such tumors were observed in at least 5 percent of the group.

High incidences of hepatocellular carcinomas were observed in mice of both sexes. In both sexes the Cochran-Armitage tests

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH 5-NITRO-o-TOLUIDINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	5/50(0.10)	1/45(0.02)	0/46(0.00)
P Values[c]	P = 0.014(N)	N.S.	P = 0.035(N)
Relative Risk (Control)[d]	---	0.222	0.000
Lower Limit	---	0.005	0.000
Upper Limit	---	1.881	0.860
Weeks to First Observed Tumor	95	96	---
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	5/50(0.10)	3/45(0.07)	2/46(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.667	0.435
Lower Limit	---	0.109	0.043
Upper Limit	---	3.219	2.506
Weeks to First Observed Tumor	95	96	97
Hematopoietic System: Malignant Lymphoma[b]	5/50(0.10)	7/47(0.15)	0/48(0.00)
P Values[c]	N.S.	N.S.	P = 0.031(N)
Departure from Linear Trend[e]	P = 0.042	---	---
Relative Risk (Control)[d]	---	1.489	0.000
Lower Limit	---	0.438	0.000
Upper Limit	---	5.551	0.825
Weeks to First Observed Tumor	74	96	---

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	12/50(0.24)	12/44(0.27)	29/45(0.64)
P Values[c]	P < 0.001	N.S.	P < 0.001
Relative Risk (Control)[d]	---	1.136	2.685
Lower Limit	---	0.521	1.548
Upper Limit	---	2.463	4.775
Weeks to First Observed Tumor	94	96	79
Circulatory System: Hemangiosarcoma or Hemangioma[b]	1/50(0.02)	0/47(0.00)	4/48(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	4.167
Lower Limit	---	0.000	0.432
Upper Limit	---	19.825	200.683
Weeks to First Observed Tumor	95	---	85

[a]Treated groups received time-weighted average doses of 0.12 or 0.23 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.

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

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 5-NITRO-o-TOLUIDINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	2/46(0.04)	3/45(0.07)	0/45(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.533	0.000
Lower Limit	---	0.184	0.000
Upper Limit	---	17.640	3.446
Weeks to First Observed Tumor	96	88	---
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	7/48(0.15)	5/47(0.11)	5/47(0.11)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.729	0.729
Lower Limit	---	0.196	0.196
Upper Limit	---	2.475	2.475
Weeks to First Observed Tumor	83	97	79
Circulatory System: Hemangiosarcoma[b]	1/48(0.02)	5/47(0.11)	3/47(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	5.106	3.064
Lower Limit	---	0.602	0.257
Upper Limit	---	235.906	157.292
Weeks to First Observed Tumor	92	95	76

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	2/47(0.04)	7/46(0.15)	20/45(0.44)
P Values[c]	P < 0.001	N.S.	P < 0.001
Relative Risk (Control)[d]	---	3.576	10.440
Lower Limit	---	0.727	2.776
Upper Limit	---	33.800	86.450
Weeks to First Observed Tumor	94	97	97
Pituitary: Adenoma NOS[b]	5/43(0.12)	0/34(0.00)	0/39(0.00)
P Values[c]	P = 0.010(N)	P = 0.049(N)	P = 0.035(N)
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	0.989	0.867
Weeks to First Observed Tumor	95	---	---

[a]Treated groups received time-weighted average doses of 0.12 or 0.23 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.

indicated a significant ($P < 0.001$) positive association between compound administration and tumor incidence, supported by significant ($P < 0.001$) results for the Fisher exact test comparing the tumor incidence in the high dose treated group to that in the controls. Based on these results, the administration of 5-nitro-o-toluidine was associated with an elevated incidence of hepatocellular carcinomas in both male and female mice.

Hemangiosarcomas and hemangiomas were noted in both male and female mice, but Fisher exact tests and Cochran-Armitage tests were not significant. In historical data collected by this laboratory for the NCI Carcinogenesis Testing Program, 5/350 (1.4 percent) untreated male and 5/350 (1.4 percent) untreated female B6C3F1 mice had either hemangiomas or hemangiosarcomas at all body sites. When a binomial distribution with a probability of incidence of 5/350 was assumed, the probability of observing 4 or more mice with such tumors out of 48 males (as in the high dose males) was $P < 0.005$, a significant result. Similarly, the probability of observing 5 or more mice with such tumors out of 47 females (as in the low dose females) was $P < 0.001$, a significant result.

For females the Cochran-Armitage test indicated a significant ($P = 0.010$) negative association between dose and the incidence of pituitary adenomas NOS. The Fisher exact test results, however, were not significant under the Bonferroni criterion.

Similarly, a significant ($P = 0.014$) negative association was observed for the incidence of alveolar/bronchiolar carcinomas in male

mice. Again, the Fisher exact test results were not significant under the Bonferroni criterion. No other statistical tests for any site were significant under the Bonferroni criterion.

V. DISCUSSION

There were no significant positive associations between the concentration of 5-nitro-o-toluidine administered and mortality in rats or mice of either sex, and adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

No unusual tumors were observed in rats of either sex and no convincing statistical evidence was provided for a significant positive association between compound administration and the incidence of any neoplasm in rats.

In mice hepatocellular carcinomas were observed in 12/50 (24 percent), 12/44 (27 percent), and 29/45 (64 percent) of the control, low dose, and high dose males, respectively, and in 2/47 (4 percent), 7/46 (15 percent), and 20/45 (44 percent) of the control, low dose, and high dose females. There was a statistically significant positive association between administration of the chemical and tumor incidence in both males and females. The high dose to control Fisher exact comparisons supported the findings for both sexes.

The incidences of hemangiomas and hemangiosarcomas in dosed mice were increased relative to controls. Among male mice, either hemangiomas or hemangiosarcomas were found in 1/50 (2 percent) control, 0/47 low dose, and 4/48 (8 percent) high dose animals. Among female mice, no hemangiomas were observed, but the incidences of hemangiosarcomas were 1/48 (2 percent) in the control, 5/47 (11 percent) in the low dose, and 3/47 (6 percent) in the high dose group. Although

the incidences in dosed groups were not statistically significant when compared to the corresponding control groups, hemangiomas and hemangiosarcomas are rarely seen in untreated B6C3F1 mice. The incidences in the high dose male mouse group and low dose female mouse group, when considered in relation to historical data on untreated B6C3F1 mice at this laboratory (5/350 or 1.4 percent for each sex), are sufficiently high to be considered to be related to administration of the compound. When a binomial distribution and a spontaneous incidence rate corresponding to the appropriate historical control incidence were assumed, the incidences of hemangiomas and hemangiosarcomas observed in this bioassay were significant.

There were no other tumors in mice for which a statistically significant positive association was established between compound administration and incidence.

Under the conditions of this bioassay 5-nitro-o-toluidine was carcinogenic in B6C3F1 mice, causing hepatocellular carcinomas in both sexes, causing an increase in the combined incidence of hemangiomas and hemangiosarcomas in male mice, and causing an increased incidence of hemangiosarcomas in female mice. The compound was not carcinogenic in Fischer 344 rats.

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

April 26, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/ Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 5-Nitro-*o*-Toluidine for carcinogenicity.

The primary reviewer agreed with the conclusion given in the report, although his preference was to qualify 5-Nitro-*o*-Toluidine as an hepatocarcinogen. After a brief description of the experimental design, he said that the bioassay was valid within the experimental limitations of the study. He noted, however, that the mice were exposed at a concentration of about 100 times more than the rats. Since 5-Nitro-*o*-Toluidine is an azo dye intermediary, the primary reviewer suggested that the report indicate specifically that bladder tumors were not detected among the treated animals.

The secondary reviewer agreed that 5-Nitro-*o*-Toluidine was carcinogenic, based on the increased incidence of hepatocellular cinomas in treated mice. He opined that the bioassay was adequate on which to judge the carcinogenicity of 5-Nitro-*o*-Toluidine. The secondary reviewer

noted that the statement in the report, regarding Anthony and Thomas's work on bladder cancer in dye workers, may be questionable and should be reexamined. He concluded that 5-Nitro-*o*-Toluidine may be a potential human carcinogen.

A motion was approved unanimously that the report on the bioassay of 5-Nitro-*o*-Toluidine be accepted as written.

Members present were:

Michael Shimkin (Acting Chairman), University of California at San Diego
Joseph Highland, Environmental Defense Fund
George Roush, Jr., Monsanto Company
Louise Strong, University of Texas Health Sciences Center
John Weisburger, American Health Foundation
(David Clayson, Eppley Institute for Cancer Research, submitted a written review)

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

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 5-NITRO-o-TOLUIDINE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH 5-NITRO-o-TOLUIDINE

	CONTROL(UNTR) 01-0055	LOW DOSE 01-0053	HIGH DOSE 01-0054
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	2	1	
ANIMALS NECROPSIED	47	48	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	47	46	47
INTEGUMENTARY SYSTEM			
*SKIN	(47)	(48)	(48)
SQUAMOUS CELL PAPILLOMA	1 (2%)	1 (2%)	1 (2%)
BASAL-CELL CARCINOMA		1 (2%)	
SEBACEOUS ADENOCARCINOMA			1 (2%)
FIBROMA		1 (2%)	
LIPOMA		1 (2%)	
LEIOMYOSARCOMA	1 (2%)		
*SUBCUT TISSUE	(47)	(48)	(48)
BASAL-CELL CARCINOMA	1 (2%)		
FIBROMA	7 (15%)	1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(47)	(46)	(47)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)		
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	1 (2%)
CORTICAL CARCINOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(47)	(48)	(48)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
LEUKEMIA,NOS	1 (2%)		
UNDIFFERENTIATED LEUKEMIA	1 (2%)		
MYELOMONOCYTIC LEUKEMIA	1 (2%)	5 (10%)	1 (2%)
#BONE MARROW	(46)	(41)	(45)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#SPLEEN	(47)	(41)	(46)
MUCINOUS ADENOCARCINOMA, METASTA	1 (2%)		

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0055	LOW DOSE 01-0053	HIGH DOSE 01-0054
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)		
#MEDIASTINAL L.NODE	(42)	(36)	(44)
MUCINOUS ADENOCARCINOMA, METASTA	1 (2%)		
#PEYERS PATCH	(47)	(40)	(45)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*ORAL CAVITY	(47)	(48)	(48)
FIBROSARCOMA	1 (2%)		
#LIVER	(47)	(41)	(46)
NEOPLASTIC NODULE	5 (11%)	1 (2%)	1 (2%)
HEPATOCELLULAR CARCINOMA			3 (7%)
#PANCREAS	(45)	(41)	(44)
MUCINOUS ADENOCARCINOMA, METASTA	1 (2%)		
#STOMACH	(47)	(40)	(46)
MUCINOUS ADENOCARCINOMA, METASTA	1 (2%)		
#ILEUM	(47)	(40)	(45)
LEIOMYOSARCOMA			1 (2%)
#COLON	(46)	(40)	(43)
MUCINOUS ADENOCARCINOMA	1 (2%)		1 (2%)
URINARY SYSTEM			
#KIDNEY	(47)	(41)	(46)
LIPOMA	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(47)	(36)	(43)
CARCINOMA,NOS		1 (3%)	1 (2%)

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0055	LOW DOSE 01-0053	HIGH DOSE 01-0054
ADENOMA, NOS	8 (17%)	2 (6%)	8 (19%)
#ADRENAL	(47)	(41)	(45)
CORTICAL CARCINOMA			1 (2%)
PHEOCHROMOCYTOMA	7 (15%)	1 (2%)	3 (7%)
#THYROID	(46)	(39)	(41)
ADENOCARCINOMA, NOS			1 (2%)
FOLLICULAR-CELL ADENOMA			1 (2%)
C-CELL ADENOMA	2 (4%)	2 (5%)	1 (2%)
C-CELL CARCINOMA	2 (4%)		2 (5%)
#PARATHYROID	(24)	(24)	(23)
ADENOMA, NOS	1 (4%)		
#PANCREATIC ISLETS	(45)	(41)	(44)
ISLET-CELL ADENOMA	3 (7%)	2 (5%)	5 (11%)
ISLET-CELL CARCINOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(47)	(48)	(48)
ADENOMA, NOS		1 (2%)	
FIBROADENOMA		1 (2%)	1 (2%)
*PREPUTIAL GLAND	(47)	(48)	(48)
SQUAMOUS CELL CARCINOMA		1 (2%)	
ADENOMA, NOS	1 (2%)		
*SEMINAL VESICLE	(47)	(48)	(48)
MUCINOUS ADENOCARCINOMA, METASTA	1 (2%)		
#TESTIS	(47)	(41)	(46)
INTERSTITIAL-CELL TUMOR	44 (94%)	34 (83%)	38 (83%)
NERVOUS SYSTEM			
#CEREBELLUM	(45)	(40)	(45)
GLIOMA, NOS			1 (2%)
SPECIAL SENSE ORGANS			
*EAR CANAL	(47)	(48)	(48)
SQUAMOUS CELL CARCINOMA	1 (2%)		

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

TABLE A1 (CONTINUED)

	CONTROL(UNTR) 01-0055	LOW DOSE 01-0053	HIGH DOSE 01-0054
MUSCULOSKELETAL SYSTEM			
*STERNUM	(47)	(48)	(48)
MUCINOUS ADENOCARCINOMA, METASTA	1 (2%)		
BODY CAVITIES			
*BODY CAVITIES	(47)	(48)	(48)
MESOTHELIOMA, NOS		1 (2%)	1 (2%)
MESOTHELIOMA, MALIGNANT	2 (4%)		
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	2	9	11
MORIBUND SACRIFICE	8	8	6
SCHEDULED SACRIFICE	5	5	5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	33	27	28
ANIMAL MISSING	2	1	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 01-0055	LOW DOSE 01-0053	HIGH DOSE 01-0054
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	47	37	42
TOTAL PRIMARY TUMORS	96	59	76
TOTAL ANIMALS WITH BENIGN TUMORS	47	34	40
TOTAL BENIGN TUMORS	76	47	59
TOTAL ANIMALS WITH MALIGNANT TUMORS	11	10	15
TOTAL MALIGNANT TUMORS	15	10	15
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		1
TOTAL SECONDARY TUMORS	6		1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	5	2	2
TOTAL UNCERTAIN TUMORS	5	2	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH 5-NITRO-o-TOLUIDINE

	CONTROL(UNTR) 02-0055	LOW DOSE 02-0053	HIGH DOSE 02-0054
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	2		
ANIMALS NECROPSIED	48	48	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	47	47	50
INTEGUMENTARY SYSTEM			
*SKIN	(48)	(48)	(50)
SQUAMOUS CELL PAPILLOMA		1 (2%)	
BASAL-CELL CARCINOMA			1 (2%)
FIBROMA		1 (2%)	
FIBROSARCOMA		1 (2%)	2 (4%)
*SUBCUT TISSUE	(48)	(48)	(50)
FIBROMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(47)	(47)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	2 (4%)
FIBROSARCOMA		1 (2%)	
FIBROSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(48)	(48)	(50)
MALIGNANT LYMPHOMA, NOS	2 (4%)	1 (2%)	
MYELOMONOCYTIC LEUKEMIA	3 (6%)	4 (8%)	3 (6%)
#SPLEEN	(47)	(44)	(49)
MYELOMONOCYTIC LEUKEMIA			1 (2%)
#THYMUS	(39)	(25)	(37)
THYMOMA	1 (3%)		
CIRCULATORY SYSTEM			
#HEART	(47)	(44)	(50)
FIBROSARCOMA, METASTATIC			1 (2%)

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

TABLE A2 (CONTINUED)

	CONTROL(UNTR) 02-0055	LOW DOSE 02-0053	HIGH DOSE 02-0054
DIGESTIVE SYSTEM			
#LIVER	(47)	(43)	(50)
NEOPLASTIC NODULE		1 (2%)	
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)
FIBROSARCOMA, METASTATIC			1 (2%)
URINARY SYSTEM			
#KIDNEY	(47)	(41)	(48)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(40)	(45)
CARCINOMA,NOS	2 (4%)		1 (2%)
ADENOMA, NOS	18 (39%)	14 (35%)	10 (22%)
#ADRENAL	(47)	(44)	(49)
CORTICAL ADENOMA	3 (6%)		
CORTICAL CARCINOMA	1 (2%)	1 (2%)	
PHEOCHROMOCYTOMA	1 (2%)		1 (2%)
LIPOMA		1 (2%)	
GANGLIONEUROMA			1 (2%)
#THYROID	(46)	(38)	(47)
C-CELL ADENOMA		1 (3%)	1 (2%)
C-CELL CARCINOMA	3 (7%)	1 (3%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(48)	(48)	(50)
ADENOMA, NOS		1 (2%)	
ADENOCARCINOMA, NOS	1 (2%)		3 (6%)
PAPILLARY CYSTADENOMA, NOS	1 (2%)		
FIBROADENOMA	14 (29%)	5 (10%)	12 (24%)
*VAGINA	(48)	(48)	(50)
SARCOMA, NOS	1 (2%)		
#UTERUS	(47)	(44)	(48)
ADENOCARCINOMA, NOS	2 (4%)		

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0055	LOW DOSE 02-0053	HIGH DOSE 02-0054
LEIOMYOSARCOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP	15 (32%)	13 (30%)	14 (29%)
#UTERUS/ENDOMETRIUM	(47)	(44)	(48)
CARCINOMA,NOS			2 (4%)
#OVARY	(46)	(44)	(48)
GRANULOSA-CELL CARCINOMA	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(47)	(44)	(48)
ASTROCYTOMA	1 (2%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*MUSCLE OF NECK	(48)	(48)	(50)
FIBROSARCOMA, METASTATIC			1 (2%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	3	11	4
MORIBUND SACRIFICE	6	6	8
SCHEDULED SACRIFICE	5	5	5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	34	28	33
ANIMAL MISSING	2		

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 02-0055	LOW DOSE 02-0053	HIGH DOSE 02-0054
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	42	33	35
TOTAL PRIMARY TUMORS	70	51	57
TOTAL ANIMALS WITH BENIGN TUMORS	38	30	28
TOTAL BENIGN TUMORS	53	39	41
TOTAL ANIMALS WITH MALIGNANT TUMORS	14	11	13
TOTAL MALIGNANT TUMORS	17	11	16
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		1	4
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 5-NITRO-o-TOLUIDINE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH 5-NITRO-o-TOLUIDINE

	CONTROL (UNTR) 05-0070	LOW DOSE 05-0053	HIGH DOSE 05-0054
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	1
ANIMALS NECROPSIED	50	47	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	50	46	48
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(47)	(48)
SARCOMA, NOS		1 (2%)	
HEMANGIOSARCOMA			3 (6%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(45)	(46)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	5 (10%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(47)	(48)
MALIGNANT LYMPHOMA, NOS	2 (4%)	4 (9%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
#LYMPH NODE	(45)	(34)	(36)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (4%)		
#MESENTERIC L. NODE	(45)	(34)	(36)
MALIGNANT LYMPHOMA, NOS		2 (6%)	
#PEYERS PATCH	(49)	(43)	(44)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 05-0070	LOW DOSE 05-0053	HIGH DOSE 05-0054
DIGESTIVE SYSTEM			
#LIVER	(50)	(44)	(45)
HEPATOCELLULAR CARCINOMA	12 (24%)	12 (27%)	29 (64%)
HEMANGIOMA	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(49)	(45)	(45)
TUBULAR-CELL ADENOCARCINOMA		1 (2%)	
#KIDNEY/CORTEX	(49)	(45)	(45)
TUBULAR-CELL ADENOMA			1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(40)	(38)	(40)
ADENOCARCINOMA, NOS	1 (3%)		
#PANCREATIC ISLETS	(46)	(44)	(41)
ISLET-CELL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
#TESTIS	(50)	(45)	(45)
HEMANGIOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(47)	(48)
ADENOMA, NOS	1 (2%)	1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 05-0070	LOW DOSE 05-0053	HIGH DOSE 05-0054
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(47)	(48)
NEUROFIBROSARCOMA	1 (2%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	2	6	5
MORIBUND SACRIFICE		1	1
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	43	41	38
ANIMAL MISSING		1	1
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	23	20	31
TOTAL PRIMARY TUMORS	27	25	36
TOTAL ANIMALS WITH BENIGN TUMORS	3	3	4
TOTAL BENIGN TUMORS	3	3	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	22	19	30
TOTAL MALIGNANT TUMORS	24	22	32
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 5-NITRO-o-TOLUIDINE

	CONTROL(UNTR) 06-0070	LOW DOSE 06-0053	HIGH DOSE 06-0054
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	48	47	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	47	47	47
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(48)	(47)	(47)
HEMANGIOSARCOMA		2 (4%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(46)	(45)	(45)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA		3 (7%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (4%)		
OSTEOSARCOMA, METASTATIC	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(48)	(47)	(47)
MALIGNANT LYMPHOMA, NOS	2 (4%)	4 (9%)	4 (9%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	1 (2%)
LYMPHOCYTIC LEUKEMIA	1 (2%)		
ERYTHROCYTIC LEUKEMIA	1 (2%)		
#SPLEEN	(47)	(45)	(45)
HEMANGIOSARCOMA	1 (2%)		
MALIGNANT LYMPHOMA, NOS	1 (2%)		
#MESENTERIC L. NODE	(36)	(31)	(42)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (3%)		
#PEYERS PATCH	(45)	(46)	(42)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
CIRCULATORY SYSTEM			
#HEART/VENTRICLE	(44)	(46)	(45)
HEMANGIOSARCOMA			1 (2%)

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

TABLE B2 (CONTINUED)

	CONTROL(UNTR) 06-0070	LOW DOSE 06-0053	HIGH DOSE 06-0054
DIGESTIVE SYSTEM			
#LIVER	(47)	(46)	(45)
HEPATOCELLULAR CARCINOMA	2 (4%)	7 (15%)	20 (44%)
URINARY SYSTEM			
#KIDNEY	(45)	(46)	(45)
HEMANGIOSARCOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(43)	(34)	(39)
ADENOMA, NOS	5 (12%)		
#ADRENAL	(47)	(44)	(42)
CORTICAL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(48)	(47)	(47)
ADENOCARCINOMA, NOS		1 (2%)	
#UTERUS	(43)	(44)	(41)
LEIOMYOMA	1 (2%)		
#OVARY/OVIDUCT	(43)	(44)	(41)
PAPILLARY ADENOMA	1 (2%)		
#OVARY	(45)	(45)	(41)
TUBULAR ADENOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(48)	(47)	(47)
ADENOMA, NOS	1 (2%)		

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0070	LOW DOSE 06-0053	HIGH DOSE 06-0054
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(48)	(47)	(47)
HEMANGIOSARCOMA		2 (4%)	1 (2%)
ALL OTHER SYSTEMS			
OMENTUM			
HEMANGIOSARCOMA	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	6	5	8
MORIBUND SACRIFICE	3	2	1
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	42	36
ANIMAL MISSING		1	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 06-0070	LOW DOSE 06-0053	HIGH DOSE 06-0054
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	18	19	25
TOTAL PRIMARY TUMORS	22	21	29
TOTAL ANIMALS WITH BENIGN TUMORS	8	3	1
TOTAL BENIGN TUMORS	9	3	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	12	17	24
TOTAL MALIGNANT TUMORS	13	18	28
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		
TOTAL SECONDARY TUMORS	2		
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 5-NITRO-o-TOLUIDINE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH 5-NITRO-o-TOLUIDINE

	CONTROL(UNTR) 01-0055	LOW DOSE 01-0053	HIGH DOSE 01-0054
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	2	1	
ANIMALS NECROPSIED	47	48	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	47	46	47
INTEGUMENTARY SYSTEM			
*SKIN	(47)	(48)	(48)
EPIDERMAL INCLUSION CYST	1 (2%)		
INFLAMMATION, NECROTIZING	1 (2%)		
INFLAMMATION, ACUTE FOCAL	1 (2%)		
FIBROSIS, FOCAL		1 (2%)	
HYPERPLASIA, PSEUDOEPITHELIOMATO		1 (2%)	
HYPERPLASIA, BASAL CELL		1 (2%)	
ACANTHOSIS		1 (2%)	
RESPIRATORY SYSTEM			
#TRACHEA	(46)	(44)	(46)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC		19 (43%)	22 (48%)
INFLAMMATION, CHRONIC		1 (2%)	
METAPLASIA, SQUAMOUS		1 (2%)	
#LUNG/BRONCHUS	(47)	(46)	(47)
BRONCHIECTASIS		4 (9%)	3 (6%)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
ABSCESS, NOS	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC			5 (11%)
METAPLASIA, SQUAMOUS		1 (2%)	
#LUNG/BRONCHIOLE	(47)	(46)	(47)
HYPERPLASIA, LYMPHOID		5 (11%)	
#LUNG	(47)	(46)	(47)
INFLAMMATION, FOCAL		3 (7%)	2 (4%)
INFLAMMATION, INTERSTITIAL		2 (4%)	
ABSCESS, NOS		1 (2%)	1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)

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

TABLE C1 (CONTINUED)

	CONTROL(UNTR) 01-0055	LOW DOSE 01-0053	HIGH DOSE 01-0054
PNEUMONIA, CHRONIC MURINE		4 (9%)	3 (6%)
INFLAMMATION, CHRONIC		1 (2%)	1 (2%)
HYPERPLASIA, ADENOMATOUS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(46)	(41)	(45)
MYELOFIBROSIS		1 (2%)	
#SPLEEN	(47)	(41)	(46)
FIBROSIS, FOCAL		1 (2%)	
INFARCT HEMORRHAGIC	1 (2%)		
ATROPHY, NOS		1 (2%)	1 (2%)
HYPERPLASIA, LYMPHOID			1 (2%)
HEMATOPOIESIS			1 (2%)
#TRACHEAL LYMPH NODE	(42)	(36)	(44)
CONGESTION, NOS		1 (3%)	1 (2%)
#PANCREATIC L.NODE	(42)	(36)	(44)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
#ILEOCOLIC LYMPH NODE	(42)	(36)	(44)
LYMPHADENOPATHY	1 (2%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(47)	(41)	(46)
INFLAMMATION, INTERSTITIAL		1 (2%)	5 (11%)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	3 (7%)
FIBROSIS		15 (37%)	2 (4%)
FIBROSIS, FOCAL		5 (12%)	8 (17%)
FIBROSIS, DIFFUSE		1 (2%)	6 (13%)
DEGENERATION, NOS	10 (21%)		
*AORTIC TUNICA MEDIA	(47)	(48)	(48)
MINERALIZATION		1 (2%)	
*CORONARY ARTERY	(47)	(48)	(48)
MINERALIZATION			1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
*PULMONARY ARTERY	(47)	(48)	(48)
MINERALIZATION	1 (2%)		

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0055	LOW DOSE 01-0053	HIGH DOSE 01-0054
*MEDIASTINAL ARTERY	(47)	(48)	(48)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(47)	(41)	(46)
CONGESTION, PASSIVE			1 (2%)
CHOLANGIOFIBROSIS		1 (2%)	
DEGENERATION, HYALINE	1 (2%)		
DEGENERATION, EOSINOPHILIC		5 (12%)	3 (7%)
NECROSIS, FOCAL	1 (2%)	2 (5%)	1 (2%)
NECROSIS, HEMORRHAGIC			1 (2%)
METAMORPHOSIS FATTY	3 (6%)	4 (10%)	7 (15%)
BASOPHILIC CYTO CHANGE		16 (39%)	20 (43%)
FOCAL CELLULAR CHANGE	12 (26%)		
HYPERPLASIA, NODULAR			1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	1 (2%)
#LIVER/CENTRILOBULAR	(47)	(41)	(46)
METAMORPHOSIS FATTY		5 (12%)	5 (11%)
*BILE DUCT	(47)	(48)	(48)
FIBROSIS		1 (2%)	
HYPERPLASIA, NOS	4 (9%)	1 (2%)	1 (2%)
HYPERPLASIA, FOCAL			3 (6%)
#PANCREAS	(45)	(41)	(44)
INFLAMMATION, FOCAL		2 (5%)	4 (9%)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)	3 (7%)	4 (9%)
PERIARTERITIS			1 (2%)
#PANCREATIC ACINUS	(45)	(41)	(44)
ATROPHY, NOS	3 (7%)		
ATROPHY, FOCAL	1 (2%)		
HYPERPLASIA, NOS	1 (2%)		
#STOMACH	(47)	(40)	(46)
PERIARTERITIS			1 (2%)
#GASTRIC MUCOSA	(47)	(40)	(46)
NECROSIS, FOCAL		1 (3%)	
#COLON	(46)	(40)	(43)
NEMATODIASIS			1 (2%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0055	LOW DOSE 01-0053	HIGH DOSE 01-0054
PARASITISM		3 (8%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(47)	(41)	(46)
CYST, NOS	1 (2%)		
GLOMERULONEPHRITIS, NOS		33 (80%)	35 (76%)
GLOMERULONEPHRITIS, FOCAL			1 (2%)
GLOMERULONEPHRITIS, SUBACUTE		1 (2%)	
INFLAMMATION, CHRONIC	39 (83%)		
#KIDNEY/TUBULE	(47)	(41)	(46)
HEMOSIDEROSIS		2 (5%)	
#URINARY BLADDER	(47)	(40)	(43)
HYPERPLASIA, EPITHELIAL	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(47)	(36)	(43)
HEMORRHAGE			2 (5%)
HYPERPLASIA, FOCAL	3 (6%)	1 (3%)	5 (12%)
#ADRENAL	(47)	(41)	(45)
METAMORPHOSIS FATTY			2 (4%)
HYPERPLASIA, FOCAL	1 (2%)		
ANGIECTASIS		3 (7%)	
#ADRENAL CORTEX	(47)	(41)	(45)
METAMORPHOSIS FATTY		1 (2%)	1 (2%)
HYPERPLASIA, FOCAL			2 (4%)
#ADRENAL MEDULLA	(47)	(41)	(45)
CYST, NOS			1 (2%)
HEMORRHAGIC CYST			1 (2%)
HYPERPLASIA, NODULAR	3 (6%)		
HYPERPLASIA, FOCAL		1 (2%)	
#THYROID	(46)	(39)	(41)
HYPERPLASIA, PAPILLARY			1 (2%)
HYPERPLASIA, CYSTIC			1 (2%)
HYPERPLASIA, C-CELL	4 (9%)	1 (3%)	3 (7%)
METAPLASIA, SQUAMOUS			1 (2%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0055	LOW DOSE 01-0053	HIGH DOSE 01-0054
#PARATHYROID	(24)	(24)	(23)
HYPERPLASIA, NOS	1 (4%)		
#PANCREATIC ISLETS	(45)	(41)	(44)
HYPERPLASIA, FOCAL		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(47)	(48)	(48)
GALACTOCELE	1 (2%)		
HYPERPLASIA, NOS	1 (2%)		2 (4%)
HYPERPLASIA, FOCAL			1 (2%)
*PREPUTIAL GLAND	(47)	(48)	(48)
INFLAMMATION, ACUTE	1 (2%)		
#PROSTATE	(46)	(41)	(44)
INFLAMMATION, ACUTE	7 (15%)	7 (17%)	5 (11%)
INFLAMMATION, ACUTE FOCAL	3 (7%)	3 (7%)	10 (23%)
ABSCESS, NOS			1 (2%)
INFLAMMATION, ACUTE/CHRONIC	2 (4%)	3 (7%)	2 (5%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
ATROPHY, NOS			1 (2%)
*SEMINAL VESICLE	(47)	(48)	(48)
ATROPHY, NOS			1 (2%)
#TESTIS	(47)	(41)	(46)
DEGENERATION, NOS	1 (2%)	31 (76%)	31 (67%)
HYPERPLASIA, INTERSTITIAL CELL		1 (2%)	
#TESTIS/TUBULE	(47)	(41)	(46)
DEGENERATION, NOS	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(45)	(40)	(45)
CONGESTION, NOS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 01-0055	LOW DOSE 01-0053	HIGH DOSE 01-0054
MUSCULOSKELETAL SYSTEM			
*STERNUM	(47)	(48)	(48)
OSTEOSCLEROSIS			1 (2%)
BODY CAVITIES			
*MESENTERY	(47)	(48)	(48)
STEATITIS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(47)	(48)	(48)
POSTMORTEM CHANGE			3 (6%)
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC		1	
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY	2	1	
AUTO/NECROPSY/NO HISTO		2	1
AUTOLYSIS/NO NECROPSY	1	1	2

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

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
TREATED WITH 5-NITRO-o-TOLUIDINE

	CONTROL (UNTR) 02-0055	LOW DOSE 02-0053	HIGH DOSE 02-0054
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	2		
ANIMALS NECROPSIED	48	48	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	47	47	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(47)	(45)	(48)
INFLAMMATION, ACUTE/CHRONIC		20 (44%)	19 (40%)
INFLAMMATION, CHRONIC		1 (2%)	
METAPLASIA, SQUAMOUS		1 (2%)	
#LUNG/BRONCHUS	(47)	(47)	(50)
BRONCHIECTASIS		5 (11%)	2 (4%)
POLYP, INFLAMMATORY		1 (2%)	
#LUNG/BRONCHIOLE	(47)	(47)	(50)
HYPERPLASIA, PAPILLARY			1 (2%)
#LUNG	(47)	(47)	(50)
CONGESTION, NOS			2 (4%)
EDEMA, NOS		2 (4%)	1 (2%)
INFLAMMATION, FOCAL		1 (2%)	3 (6%)
INFLAMMATION, INTERSTITIAL		1 (2%)	1 (2%)
ABSCESS, NOS		3 (6%)	
PNEUMONIA, CHRONIC MURINE		2 (4%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(47)	(44)	(49)
CONGESTION, NOS		1 (2%)	
HEMATOPOIESIS	1 (2%)		1 (2%)
#SUBMANDIBULAR L.NODE	(40)	(41)	(46)
HYPERPLASIA, NOS			2 (4%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0055	LOW DOSE 02-0053	HIGH DOSE 02-0054
#THYMUS	(39)	(25)	(37)
CYST, NOS			1 (3%)
CIRCULATORY SYSTEM			
#HEART	(47)	(44)	(50)
PERIARTERITIS			1 (2%)
#MYOCARDIUM	(47)	(44)	(50)
INFLAMMATION, INTERSTITIAL		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC			6 (12%)
FIBROSIS		2 (5%)	6 (12%)
FIBROSIS, FOCAL		3 (7%)	2 (4%)
FIBROSIS, DIFFUSE		3 (7%)	
DEGENERATION, NOS	7 (15%)		
#ENDOCARDIUM	(47)	(44)	(50)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
#CARDIAC VALVE	(47)	(44)	(50)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
*AORTA	(48)	(48)	(50)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
*CORONARY ARTERY	(48)	(48)	(50)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
*PULMONARY ARTERY	(48)	(48)	(50)
MINERALIZATION		1 (2%)	3 (6%)
DIGESTIVE SYSTEM			
#PAROTID GLAND	(46)	(43)	(48)
HYPERPLASIA, FOCAL			1 (2%)
#SUBMAXILLARY GLAND	(46)	(43)	(48)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
#LIVER	(47)	(43)	(50)
DEGENERATION, NOS	1 (2%)		

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0055	LOW DOSE 02-0053	HIGH DOSE 02-0054
NECROSIS, FOCAL		2 (5%)	
NECROSIS, DIFFUSE		1 (2%)	
NECROSIS, HEMORRHAGIC		1 (2%)	
METAMORPHOSIS FATTY	2 (4%)	8 (19%)	7 (14%)
BASOPHILIC CYTO CHANGE		30 (70%)	43 (86%)
FOCAL CELLULAR CHANGE	25 (53%)		
ANGIECTASIS			1 (2%)
#LIVER/CENTRILOBULAR	(47)	(43)	(50)
METAMORPHOSIS FATTY		1 (2%)	
*BILE DUCT	(48)	(48)	(50)
HYPERPLASIA, NOS	2 (4%)		3 (6%)
#PANCREAS	(46)	(44)	(47)
INFLAMMATION, FOCAL			5 (11%)
INFLAMMATION, ACUTE/CHRONIC		6 (14%)	3 (6%)
ATROPHY, FOCAL		2 (5%)	
HYPERPLASIA, NOS		1 (2%)	
#PANCREATIC ACINUS	(46)	(44)	(47)
ATROPHY, NOS	8 (17%)		
#STOMACH	(46)	(44)	(46)
ULCER, FOCAL		1 (2%)	
#COLON	(45)	(41)	(45)
NEMATODIASIS		6 (15%)	7 (16%)
HYPERPLASIA, LYMPHOID			2 (4%)
URINARY SYSTEM			
#KIDNEY	(47)	(41)	(48)
GLOMERULONEPHRITIS, NOS		15 (37%)	25 (52%)
GLOMERULONEPHRITIS, FOCAL		3 (7%)	2 (4%)
INFLAMMATION, CHRONIC	29 (62%)		
POSTMORTEM CHANGE	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(40)	(45)
CYST, NOS			1 (2%)
HYPERPLASIA, FOCAL	1 (2%)	2 (5%)	

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0055	LOW DOSE 02-0053	HIGH DOSE 02-0054
#ADRENAL	(47)	(44)	(49)
HEMORRHAGIC CYST		1 (2%)	
#ADRENAL CORTEX	(47)	(44)	(49)
CYST, NOS	1 (2%)		
DEGENERATION, NOS	3 (6%)		
METAMORPHOSIS FATTY	1 (2%)	5 (11%)	4 (8%)
HYPERPLASIA, NODULAR	2 (4%)		
HYPERPLASIA, FOCAL	3 (6%)	1 (2%)	4 (8%)
ANGIECTASIS		5 (11%)	
#ADRENAL MEDULLA	(47)	(44)	(49)
THROMBOSIS, NOS	1 (2%)		
HYPERPLASIA, FOCAL	1 (2%)		
#THYROID	(46)	(38)	(47)
INFLAMMATION, ACUTE/CHRONIC		1 (3%)	
HYPERPLASIA, C-CELL	4 (9%)	1 (3%)	1 (2%)
#PANCREATIC ISLETS	(46)	(44)	(47)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(48)	(48)	(50)
DILATATION/DUCTS	3 (6%)		
GALACTOCELE	7 (15%)	10 (21%)	20 (40%)
INFLAMMATION, ACUTE			1 (2%)
HYPERPLASIA, NOS	4 (8%)	18 (38%)	22 (44%)
HYPERPLASIA, FOCAL		1 (2%)	
*MAMMARY DUCT	(48)	(48)	(50)
FIBROSIS	2 (4%)		
#UTERUS	(47)	(44)	(48)
HYDROMETRA	2 (4%)		
#UTERUS/ENDOMETRIUM	(47)	(44)	(48)
INFLAMMATION, VESICULAR			1 (2%)
INFLAMMATION, ACUTE	9 (19%)	24 (55%)	24 (50%)
INFLAMMATION, ACUTE VESICULAR			1 (2%)
INFLAMMATION, CHRONIC	1 (2%)		

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0055	LOW DOSE 02-0053	HIGH DOSE 02-0054
HYPERPLASIA, NOS		2 (5%)	5 (10%)
HYPERPLASIA, CYSTIC	3 (6%)	6 (14%)	6 (13%)
HYPERPLASIA, STROMAL	1 (2%)		
#OVARY/OVIDUCT	(47)	(44)	(48)
INFLAMMATION, ACUTE	3 (6%)	1 (2%)	3 (6%)
INFLAMMATION ACTIVE CHRONIC	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
INFLAMMATION, CHRONIC	1 (2%)		
#OVARY/PAROVARIAN	(47)	(44)	(48)
ABSCESS, NOS			1 (2%)
#OVARY	(46)	(44)	(48)
CYST, NOS			1 (2%)
ABSCESS, NOS		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		
#OVARY/FOLLICLE	(46)	(44)	(48)
HYPERPLASIA, NOS	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(47)	(44)	(48)
NECROSIS, HEMORRHAGIC		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE/CRYSTALLINE LENS	(48)	(48)	(50)
CATARACT			1 (2%)
MUSCULOSKELETAL SYSTEM			
*BONE	(48)	(48)	(50)
OSTEOSCLEROSIS	1 (2%)		
BODY CAVITIES			
*PLEURA	(48)	(48)	(50)
INFLAMMATION, CHRONIC	1 (2%)		
*EPICARDIUM	(48)	(48)	(50)
INFLAMMATION, CHRONIC	1 (2%)		

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

TABLE C2 (CONCLUDED)

	CONTROL(UNTR) 02-0055	LOW DOSE 02-0053	HIGH DOSE 02-0054
*MESENTERY	(48)	(48)	(50)
STEATITIS			1 (2%)
NECROSIS, FAT			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(48)	(48)	(50)
POSTMORTEM CHANGE		3 (6%)	3 (6%)
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY	2		
AUTO/NECROPSY/NO HISTO	1	1	
AUTOLYSIS/NO NECROPSY		2	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 5-NITRO-o-TOLUIDINE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH 5-NITRO-o-TOLUIDINE

	CONTROL (UNTR) 05-0070	LOW DOSE 05-0053	HIGH DOSE 05-0054
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	1
ANIMALS NECROPSIED	50	47	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	50	46	48
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(47)	(48)
ABSCESS, NOS	2 (4%)		
*SUBCUT TISSUE	(50)	(47)	(48)
ABSCESS, NOS			1 (2%)
NECROSIS, FAT	1 (2%)		
NECROSIS, HEMORRHAGIC		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(50)	(45)	(46)
INFLAMMATION, NOS	1 (2%)		
PERIVASCULITIS	1 (2%)		
#LUNG	(50)	(45)	(46)
HEMORRHAGE	2 (4%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM	2 (4%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(43)	(45)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, LYMPHOID	1 (2%)		
HEMATOPOIESIS			2 (4%)
ERYTHROPOIESIS		3 (7%)	1 (2%)
#SPLENIC FOLLICLES	(50)	(43)	(45)
HYPERPLASIA, NOS	2 (4%)		
#LYMPH NODE	(45)	(34)	(36)
HYPERPLASIA, NOS			1 (3%)

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0070	LOW DOSE 05-0053	HIGH DOSE 05-0054
#MESENTERIC L. NODE	(45)	(34)	(36)
HYPERPLASIA, RETICULUM CELL	1 (2%)		
CIRCULATORY SYSTEM			
#AORTIC VALVE	(49)	(45)	(45)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(49)	(42)	(45)
PERIVASCULITIS	1 (2%)		
#LIVER	(50)	(44)	(45)
HEPATITIS, TOXIC		1 (2%)	
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL	1 (2%)		
METAMORPHOSIS FATTY	2 (4%)	1 (2%)	
HEPATOCYTOMEGALY	2 (4%)		
MEGALOCYTOSIS		1 (2%)	
DEPLETION	1 (2%)		
HYPERPLASIA, NODULAR	2 (4%)		
HYPERPLASTIC NODULE			1 (2%)
HYPERPLASIA, FOCAL	1 (2%)		
HYPERPLASIA, DIFFUSE	1 (2%)		
HEMATOPOIESIS			2 (4%)
#LIVER/CENTRILOBULAR	(50)	(44)	(45)
NECROSIS, NOS	1 (2%)		
#LIVER/KUPFFER CELL	(50)	(44)	(45)
HYPERPLASIA, NOS	1 (2%)		
*BILE DUCT	(50)	(47)	(48)
CYST, NOS		1 (2%)	
HYPERPLASIA, NOS			1 (2%)
#PANCREAS	(46)	(44)	(41)
INFLAMMATION, FOCAL	1 (2%)		
INFLAMMATION, INTERSTITIAL		1 (2%)	1 (2%)
#GASTRIC MUCOSA	(49)	(42)	(44)
INFLAMMATION, FOCAL	1 (2%)		

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

TABLE D1 (CONTINUED)

	CONTROL(UNTR) 05-0070	LOW DOSE 05-0053	HIGH DOSE 05-0054
#PEYERS PATCH	(49)	(43)	(44)
HYPERPLASIA, NOS	1 (2%)	1 (2%)	1 (2%)
#COLON	(46)	(39)	(43)
GRANULOMA, NOS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(49)	(45)	(45)
HYDRONEPHROSIS			1 (2%)
GLOMERULONEPHRITIS, NOS			1 (2%)
INFLAMMATION, INTERSTITIAL	3 (6%)		
#URINARY BLADDER	(47)	(41)	(42)
HYPERPLASIA, EPITHELIAL	1 (2%)		
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(47)	(48)
ABSCESS, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(47)	(48)
HEMATOMA, NOS		1 (2%)	

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

TABLE D1 (CONCLUDED)

	CONTROL(UNTR) 05-0070	LOW DOSE 05-0053	HIGH DOSE 05-0054
*MESENTERY	(50)	(47)	(48)
CONGESTION, NOS		1 (2%)	
HEMORRHAGE		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	12	19	10
ANIMAL MISSING/NO NECROPSY		1	1
AUTO/NECROPSY/HISTO PERF		1	2
AUTO/NECROPSY/NO HISTO		1	
AUTOLYSIS/NO NECROPSY		1	1

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

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
TREATED WITH 5-NITRO-o-TOLUIDINE

	CONTROL (UNTR) 06-0070	LOW DOSE 06-0053	HIGH DOSE 06-0054
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	48	47	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	47	47	47
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(46)	(45)	(45)
INFLAMMATION, NOS	1 (2%)		
#LUNG	(46)	(45)	(45)
INFLAMMATION, INTERSTITIAL	1 (2%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(46)	(45)	(44)
MYELOFIBROSIS	1 (2%)		
#SPLEEN	(47)	(45)	(45)
ANGIECTASIS			1 (2%)
HYPERPLASIA, LYMPHOID	1 (2%)		
HEMATOPOIESIS		1 (2%)	1 (2%)
ERYTHROPOIESIS		2 (4%)	2 (4%)
#SPLENIC FOLLICLES	(47)	(45)	(45)
HYPERPLASIA, NOS	3 (6%)		
#LYMPH NODE	(36)	(31)	(42)
INFLAMMATION, NOS	1 (3%)		
HYPERPLASIA, NOS	1 (3%)		
HYPERPLASIA, PLASMA CELL	1 (3%)		
#ABDOMINAL LYMPH NODE	(36)	(31)	(42)
PLASMACYTOSIS	1 (3%)		

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0070	LOW DOSE 06-0053	HIGH DOSE 06-0054
CIRCULATORY SYSTEM			
#HEART/ATRIUM	(44)	(46)	(45)
THROMBOSIS, NOS		1 (2%)	
#MYOCARDIUM	(44)	(46)	(45)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
FIBROSIS, FOCAL	1 (2%)		
#ENDOCARDIUM	(44)	(46)	(45)
INFLAMMATION, CHRONIC		1 (2%)	
#AORTIC VALVE	(44)	(46)	(45)
INFLAMMATION, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(45)	(45)	(42)
PERIVASCULITIS	3 (7%)		
PERIVASCULAR CUFFING	1 (2%)		
#LIVER	(47)	(46)	(45)
MULTIPLE CYSTS		1 (2%)	
INFLAMMATION, ACUTE FOCAL	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
NECROSIS, FOCAL	2 (4%)		
NECROSIS, COAGULATIVE			1 (2%)
METAMORPHOSIS FATTY		1 (2%)	2 (4%)
HYPERPLASTIC NODULE		1 (2%)	2 (4%)
HYPERPLASIA, FOCAL		1 (2%)	1 (2%)
#LIVER/CAUDATE LOBE	(47)	(46)	(45)
HEMORRHAGE			1 (2%)
*BILE DUCT	(48)	(47)	(47)
INFLAMMATION, ACUTE/CHRONIC	4 (8%)		
#PANCREAS	(43)	(44)	(41)
HEMORRHAGIC CYST			1 (2%)
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, INTERSTITIAL	1 (2%)		
PERIARTERITIS	1 (2%)		
#PANCREATIC ACINUS	(43)	(44)	(41)
ATROPHY, NOS	1 (2%)		

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0070	LOW DOSE 06-0053	HIGH DOSE 06-0054
#STOMACH	(45)	(45)	(42)
ULCER, FOCAL	1 (2%)		
#PEYERS PATCH	(45)	(46)	(42)
HYPERPLASIA, NOS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(45)	(46)	(45)
HYDRONEPHROSIS			1 (2%)
GLOMERULONEPHRITIS, NOS	3 (7%)		
GLOMERULONEPHRITIS, FOCAL	2 (4%)		
INFLAMMATION, INTERSTITIAL	1 (2%)		
GLOMERULONEPHRITIS, MEMBRANOUS	2 (4%)		
PYELONGPHRITIS, ACUTE/CHRONIC	1 (2%)		
GLOMERULONEPHRITIS, CHRONIC	1 (2%)		
#URINARY BLADDER	(45)	(45)	(43)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
PERIARTERITIS	1 (2%)		
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(47)	(44)	(42)
HEMORRHAGE		1 (2%)	
#THYROID	(41)	(32)	(29)
HYPERPLASIA, FOLLICULAR-CELL	1 (2%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(43)	(44)	(41)
DILATATION, NOS		2 (5%)	
HYDROMETRA	3 (7%)	1 (2%)	
INFLAMMATION, ACUTE		1 (2%)	
ABSCESS, NOS	2 (5%)		
#UTERUS/ENDOMETRIUM	(43)	(44)	(41)
INFLAMMATION, NOS	2 (5%)		
INFLAMMATION, SUPPURATIVE	2 (5%)	1 (2%)	1 (2%)
INFLAMMATION, ACUTE	6 (14%)		

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0070	LOW DOSE 06-0053	HIGH DOSE 06-0054
INFLAMMATION, ACUTE FOCAL	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC	3 (7%)		
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, CYSTIC	20 (47%)	18 (41%)	15 (37%)
METAPLASIA, SQUAMOUS	1 (2%)		
#OVARY/OVIDUCT	(43)	(44)	(41)
INFLAMMATION, SUPPURATIVE	4 (9%)		
ABSCESS, NOS	1 (2%)		
#OVARY	(45)	(45)	(41)
CYST, NOS		1 (2%)	4 (10%)
FOLLICULAR CYST, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE	6 (13%)		
INFLAMMATION, CHRONIC	1 (2%)		
ABSCESS, CHRONIC	1 (2%)		
PERIARTERITIS	1 (2%)		
NERVOUS SYSTEM			
#BRAIN/MENINGES	(46)	(46)	(44)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(48)	(47)	(47)
HEMATOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(48)	(47)	(47)
PERIVASCULITIS	1 (2%)		

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

TABLE D2 (CONCLUDED)

	CONTROL(UNTR) 06-0070	LOW DOSE 06-0053	HIGH DOSE 06-0054
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		10	7
ANIMAL MISSING/NO NECROPSY		1	
AUTO/NECROPSY/HISTO PERF		1	2
AUTO/NECROPSY/NO HISTO	1		
AUTOLYSIS/NO NECROPSY	2	2	3

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

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