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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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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 2-METHYL-1-NITROANTHRAQUINONE
FOR POSSIBLE CARCINOGENICITY

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

CONTRIBUTORS: This report presents the results of the bioassay of 2-methyl-1-nitroanthraquinone conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This bioassay 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 Bioassay Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. E. Smith (3) and Dr. A. Handler (3). Animal treatment and observation were supervised by Mr. G. Wade (3) and Ms. E. Zepp (3). Chemical analysis was performed by Midwest Research Institute (4) and the analytical results were reviewed by Dr. N. Zimmerman (5).

Histopathologic examinations were performed by Dr. A. S. Krishna Murthy (3) at the Mason Research Institute, and the diagnoses included in this report represent the interpretation of this pathologist. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (6).

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

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SUMMARY

A bioassay of 2-methyl-1-nitroanthraquinone for possible carcinogenicity was conducted using Fischer 344 rats. 2-Methyl-1-nitroanthraquinone was administered in the feed at either of two concentrations to groups of 50 male and 50 female animals. The high and low dietary concentrations used were 0.12 and 0.06 percent, respectively, for the male and female rats. After a 78-week treatment period, observation of the rats continued for an additional 31 weeks. Fifty rats of each sex were placed on test as controls. No 2-methyl-1-nitroanthraquinone was added to their diet.

Survival in both the male and female rats was adequate for a meaningful statistical analysis of late-developing tumors; however, there was a significant positive association between increased dosage and elevated mortality in female rats.

Hepatocellular carcinomas and neoplastic nodules of the liver occurred in both the male and female treated rats. A statistically significant association between increased dosage and an elevated incidence of hepatocellular carcinomas was indicated by the Cochran-Armitage test for the males (1/48, 5/48, and 9/49 in control, low dose, and high dose, respectively); however, the Fisher exact tests supported these results only for the high dose males. The incidence of neoplastic nodules was statistically significant in the male rats (0/48, 2/48, and 6/49 in control, low dose, and high dose, respectively), as indicated by the Cochran-Armitage test and supported by the Fisher exact test for the high dose group. When those rats having either hepatocellular carcinomas or neoplastic nodules of the liver were combined and evaluated simultaneously, the Cochran-Armitage tests indicated statistically significant associations between increased dosages and elevated tumor incidences in both the males and females. This was supported by the Fisher exact tests for males but not for females. The incidences of one tumor type, subcutaneous fibroma, were found to be statistically significant in both male and female rats. No other tumors occurred in treated animals in statistically significant incidences when compared to controls.

Squamous-cell papillomas and squamous-cell carcinomas of the forestomach were observed only in high dose rats. Although the incidences of these gastric tumors were not statistically significant, historical data indicate that these tumors are rare in Fischer 344 rats. The occurrence of these tumors in high dose rats, together with the frequent occurrence of nonneoplastic proliferative lesions of the forestomach in treated rats, indicates that the occurrence of

these tumors was related to administration of 2-methyl-1-nitroanthraquinone. An increased incidence of bladder tumors (papillomas, transitional-cell papillomas, and sarcomas) was observed among female rats.

Under the conditions of this bioassay, the results indicate that orally administered 2-methyl-1-nitroanthraquinone is carcinogenic in male Fischer 344 rats, producing hepatocellular carcinomas. Increased incidences of subcutaneous fibromas in both male and female Fischer 344 rats were also associated with the administration of the compound. Tumors of the forestomach and bladder in these animals may also have been related to the administration of the test chemical.

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I. INTRODUCTION

2-Methyl-1-nitroanthraquinone (NCI No. C01923), an intermediate in the synthesis of anthraquinone 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 compounds are one of several classes of chemicals 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-1-nitro-9,10-anthracenedione.* It is also frequently listed as 1-nitro-2-methyl-anthraquinone.

In the past, 2-methyl-1-nitroanthraquinone served as an intermediate in the manufacture of certain wool dyes of the alizarin series (Cofrancesco, 1963) and of anthraquinone vat dyes such as C.I. (Color Index) Vat Red 39 (Society of Dyers and Colourists, 1971). However, the compound is no longer used by the dye industry in the United States and has not been produced commercially in this country since 1970 (Urso, 1977).

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*The CAS registry number is 129-15-7.

Although anthraquinone vat dyes on the whole are considered to possess relatively low toxicity (Hawley, 1971), specific information is not available on the toxicity of the 2-methyl-1-nitroanthraquinone precursor. The risk of exposure to this compound is at present negligible; however, workers at dye manufacturing facilities may have experienced significant contact with the chemical in the past.

II. MATERIALS AND METHODS

A. Chemicals

2-Methyl-1-nitroanthraquinone was purchased from Carroll Products, Wood River Junction, Rhode Island. Analysis was performed by Midwest Research Institute. The experimentally determined melting point range (265° to 268°C) suggested a compound of high purity and was close to the value reported in the literature (270° to 271°C).

Throughout this report the term 2-methyl-1-nitroanthraquinone is used to represent this material.

B. Dietary Preparation

The basal laboratory diet for both treated and control animals was Wayne Lab-Blox® (Allied Mills, Inc.). 2-Methyl-1-nitroanthraquinone was administered to the treated animals as a component of their diet. The amount of 2-methyl-1-nitroanthraquinone needed for preparation of the treated feed was mixed with an aliquot of ground Wayne Lab-Blox®. Once visual homogeneity was obtained, the mixture was placed into a 6 kg capacity Patterson-Kelley twin-shell stainless steel V-blender along with the remainder of the meal and blended for 20 minutes. Prepared diets were placed in labeled plastic bags and stored in the dark at 4°C. All transfer and mixing of the chemical was carried out under a fume hood. The prepared mixture was used for only 1 week.

C. Animals

Fischer 344 rats were used in the 2-methyl-1-nitroanthraquinone bioassay. They were supplied by Charles River Breeding Laboratories,

Inc., Wilmington, Massachusetts, in accordance with contracts with the Division of Cancer Treatment, National Cancer Institute. Upon arrival, a sample of the animals was examined both internally and externally for parasites and other signs of disease. Rats to be used were quarantined for 2 weeks prior to initiation of test. Animals were assigned to groups and distributed among cages so that average body weight per cage was approximately equal for a given sex.

D. Animal Maintenance

All animals were housed in rooms having a temperature range of 23° to 34°C and a range in relative humidity of 10 to 85 percent. Incoming air was filtered through Tri-Dek® 15/40 denier Dacron® filters providing six changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

The animals were housed five per cage by sex. During quarantine and for the first 13 months of study, they were kept in stainless- and galvanized-steel wire-mesh cages suspended above newspapers. Newspapers were replaced daily, and cages and racks washed weekly. After the first 13 months and for the remainder of the study, the animals were kept in suspended polycarbonate cages equipped with disposable nonwoven fiber filter sheets. Corncob bedding and clean cages were provided twice weekly. Stainless steel cage racks (Fenco Cage Products) were cleaned once every 2 weeks, and disposable filters were replaced at that time.

Water was available from 250 ml water bottles equipped with rubber stoppers and stainless steel sipper tubes. Glass water bottles were used for the first 2 months of the study. Polycarbonate bottles were used thereafter. Bottles were replaced twice weekly and water was supplied as needed between changes. Food and water were available ad libitum.

Pelleted Wayne Lab-Blox® was supplied during the quarantine and final observation periods. During treatment, all treated animals received treated Wayne Lab-Blox® meal; control animals had untreated meal available. Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc.) equipped with stainless steel baffles were used to dispense food during the first 11 months of study. Thereafter, the rats were fed from stainless steel gangstyle hoppers. The food assemblies were replaced weekly. Food was replenished daily in Alpine® feed cups, twice weekly in gangstyle hoppers. During the observation period following treatment, rats were fed pellets on the cage floor.

The 2-methyl-1-nitroanthraquinone-treated rats were housed in rooms with other rats receiving diets treated with* amitrole (61-82-5); 3-nitro-p-acetophenetide (1777-84-0); tris (2,3-dibromopropyl) phosphate (126-72-7); and o-anisidine hydrochloride (134-29-0). Control rats for this study were in the same room as the treated animals for the first part of the study but later the treated animals were moved to a separate room. Control rats were exposed only to other

*CAS registry numbers are given in parentheses.

rats receiving diets treated with amitrole (61-82-5) and 3-nitro-p-acetophenetide (1777-84-0).

E. Selection of Initial Concentrations

An 8-week subchronic toxicity test was performed to determine the high dose to be used in the chronic study. During the subchronic study, Fischer 344 rats received 2-methyl-1-nitroanthraquinone mixed in the diet for 6 weeks, followed by 2 weeks of observation during which they received untreated feed. Ten groups, each consisting of five male and five female rats, received feed containing the following concentrations of the chemical: 0.005, 0.01, 0.02, 0.04, 0.05, 0.08, 0.15, 0.5, 1.5, and 5.0 percent. Ten male and ten female rats served as controls and received untreated feed. Individual body weights were recorded at weekly intervals throughout the study. Food consumption was monitored by cage for the entire test period. All survivors were sacrificed at the end of the test and gross necropsies were performed on all animals.

A dosage inducing no mortality and resulting in a depression in mean group body weight of approximately 20 percent relative to controls was to be selected as the initial high dose. At a concentration of 0.15 percent, the depression of mean body weight in male and female rats was 14 and 3 percent, respectively; and one female rat died. At concentrations of 0.15 percent and above, the animals were observed at necropsy to have reddened thyroids and parathyroids, and granularity

of the spleen. The high dietary concentration selected for the chronic study was 0.12 percent.

F. Experimental Design

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

The low dose, high dose and control rats were all approximately 6 weeks old at the time they were placed on test. Treated rats were started on test approximately 6 weeks earlier than control rats. The high and low concentrations of 2-methyl-1-nitroanthraquinone in diets were 0.12 and 0.06 percent, respectively. Treated rats were supplied with dosed feed for a total of 78 weeks followed by a 31-week observation period. At the end of the treatment phase, five males and five females from the high dose and control groups were sacrificed and necropsied in accordance with the pathology protocol then in effect. At the end of the observation period, surviving animals were sacrificed and necropsied.

G. Clinical and Histopathologic Examinations

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

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
2-METHYL-1-NITROANTHRAQUINONE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2-METHYL-1-NITRO-ANTHRAQUINONE CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	50	0	0	109
LOW DOSE	50	0.06	78	
		0		31
HIGH DOSE	50	0.12	78	
		0		31
FEMALE				
CONTROL	50	0	0	110
LOW DOSE	50	0.06	78	
		0		31
HIGH DOSE	50	0.12	78	
		0		31

the bioassay and for three consecutive days each month thereafter. The presence of tissue masses and lesions was determined by monthly observation and palpation of each animal.

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

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

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

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to

preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were placed on experiment in each group.

H. Data Recording and Statistical Analyses

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

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

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

equality and used Tarone's (1975) extensions of Cox's methods for testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison

be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

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

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week

during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

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

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would

be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

A barely discernible depression in mean body weight was noted among the male and female treated rat groups when compared to their controls (Figure 1).

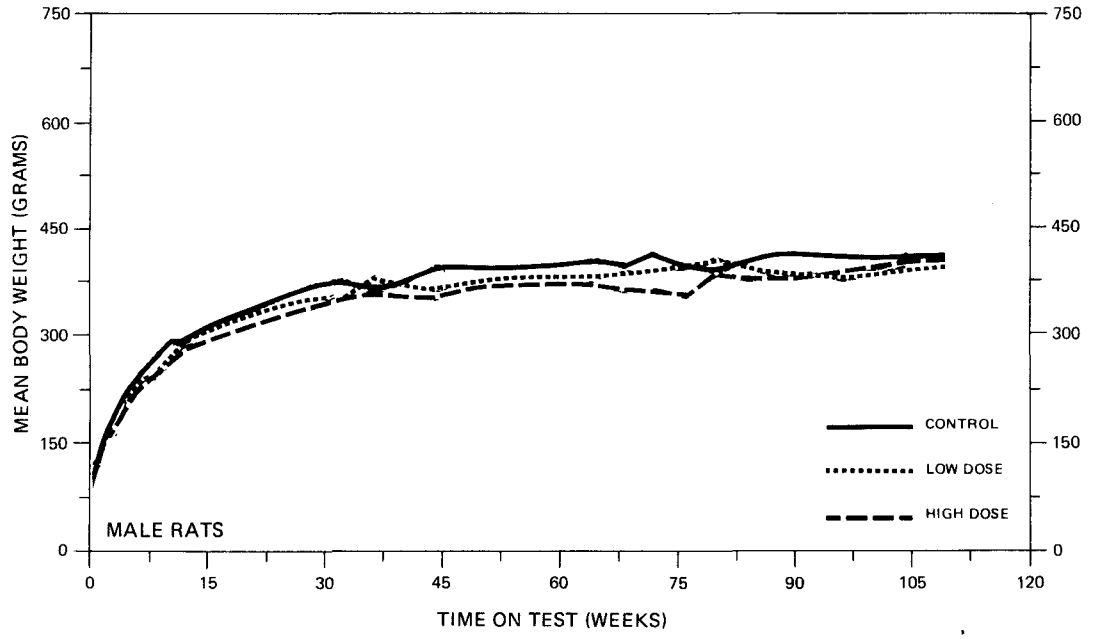
Firm subcutaneous masses were the clinical sign most often observed in rats. They were palpated in ten female controls, two male controls, six high dose males, three high dose females, and one low dose male. One of the ten female controls having palpable, firm subcutaneous masses also exhibited severe alopecia, and two other female controls were observed to have pale discoloration of the eyes.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 2-methyl-1-nitroanthraquinone-treated groups are shown in Figure 2.

In male rats the Tarone test did not show a significant association between increased dosage and elevated mortality. The actual survival was adequate for statistical analysis of tumor incidence, despite the five high dose and five control rats sacrificed in week 78. Fifty-four percent of the high dose, 70 percent of the low dose, and 60 percent of the control group male rats survived until the end of the study.

In female rats the Tarone test showed a significant ($P = 0.043$) positive association between dosage and mortality. The actual



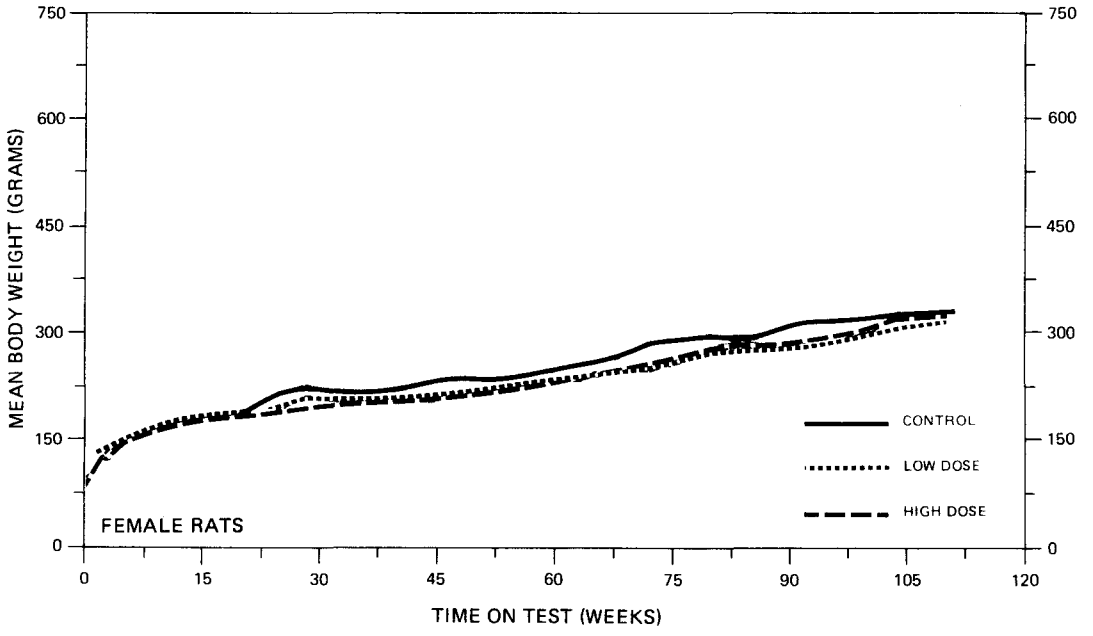
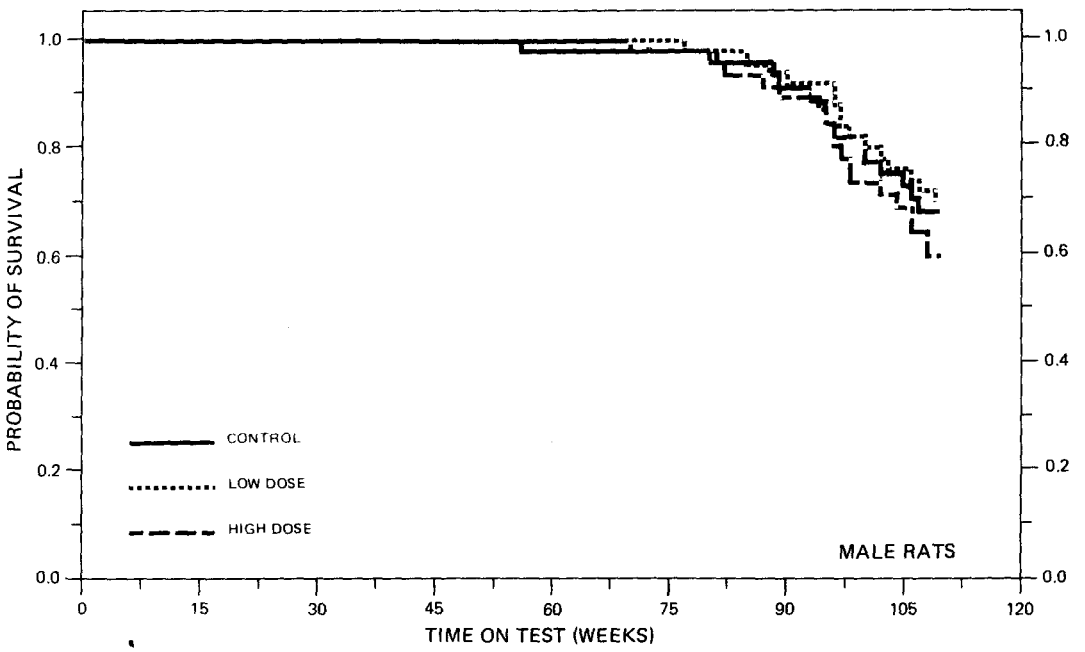


FIGURE 1
GROWTH CURVES FOR 2-METHYL-1-NITROANTHRAQUINONE CHRONIC STUDY RATS



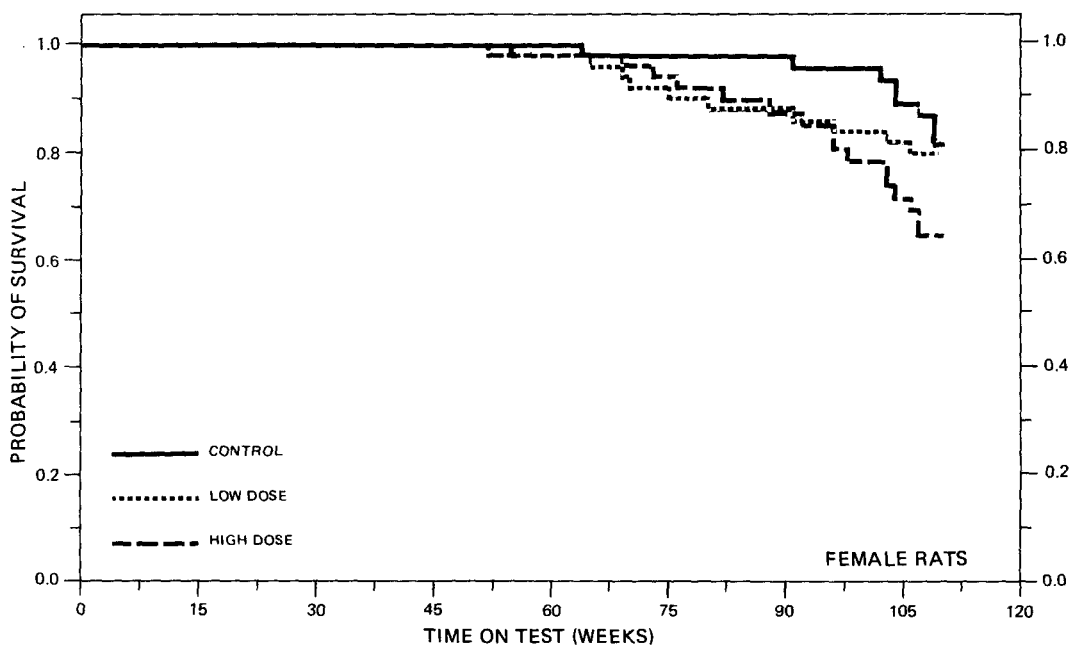


FIGURE 2
SURVIVAL COMPARISONS OF 2-METHYL-1-NITROANTHRAQUINONE CHRONIC STUDY RATS

survival, however, was adequate for statistical analysis of tumor incidence despite the sacrifice of five high dose and five control females in week 78. Fifty-eight percent of the high dose, 80 percent of the low dose and 74 percent of the control group female rats survived until the end of the study.

Thus, in both sexes, survival was adequate for a meaningful statistical analysis of late-developing tumors.

C. Pathology

Histopathologic findings on neoplasms in rats are tabulated in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are tabulated in Appendix B (Tables B1 and B2).

There was an increased incidence of subcutaneous, gastric, and hepatic tumors in both male and female rats treated with this chemical.

Subcutaneous fibroma was found in four control rats (3/48 males and 1/50 females). Incidences of fibromas in treated rats were 10/49 low dose and 34/49 high dose males and 13/49 high dose females. Some fibromas in these rats were single, others were multiple, and all were well circumscribed. Fibroblasts were arranged in a whorled pattern and mature collagen appeared in various portions of the tumor. Only a few mitotic figures were observed.

Tumors of the forestomach (i.e., squamous-cell papillomas and carcinomas) occurred only in the male and female high dose rats: squamous-cell papillomas were observed in 2/47 high dose males and

2/48 high dose females and squamous-cell carcinomas were observed in 1/47 high dose males and 3/48 high dose females. Nonneoplastic stomach lesions were observed less frequently in the control rats than among the treated animals. Focal hyperplasia was observed only in treated rats. Basal cell hyperplasia occurred in 1/48, 9/47, and 17/47 of the control, low dose, and high dose males, respectively; and in 0/48, 4/48, and 16/48 of the control, low dose, and high dose females, respectively. Hyperkeratosis was found in 2/48, 4/47, and 5/47 of the control, low dose, and high dose males, respectively, and in 0/48, 5/48, and 8/48 of the corresponding female groups. Acanthosis was observed in 2/48, 13/47, and 8/47 of the control, low dose, and high dose males, respectively, and in 2/48, 8/48, and 16/48 of the control, low dose, and high dose females.

One control rat had a hepatocellular carcinoma. In contrast, there were neoplastic nodules in ten treated male and female rats (2/48 low dose and 6/49 high dose males and 1/50 low dose and 1/49 high dose females). Hepatocellular carcinomas were observed in 5/48 low dose male rats and in 12 rats fed the higher concentration of the chemical (9/49 high dose males, 3/49 high dose females). In two males, hepatocellular carcinoma had metastasized to the lung. Morphology of these lesions was similar to that described by Squire and Levitt (1975).

Neoplasms of the urinary bladder were detected only in treated female rats. Included were papillomas (1/43 and 1/44 in the low and

high dose groups, respectively), transitional-cell papillomas (2/43 and 1/44 in the low and high dose groups, respectively), and sarcomas (2/44 in the high dose group).

Subcutaneous hemangiosarcomas were observed only in 3/49 high dose male rats.

Other neoplasms that occurred in this bioassay were observed at similar frequencies in control and treated rats.

The following provided the basis for the histopathologic conclusion of carcinogenicity of 2-methyl-1-nitroanthraquinone in Fischer 344 rats:

1. There was an increase in the incidence of neoplastic nodules of the liver and hepatocellular carcinomas in treated rats.
2. There was an increase in the incidence of subcutaneous fibromas in treated rats.
3. Gastric tumors and subcutaneous hemangiosarcomas, though very few in number, occurred only in high dose rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 2 and 3. The analysis for every type of tumor that was observed in more than 5 percent of any of the 2-methyl-1-nitroanthraquinone-treated groups of either sex is included.

Hepatocellular carcinomas and neoplastic nodules of the liver were observed in both male and female rats. The Cochran-Armitage test indicated a significant positive association between dosage and

TABLE 2

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 2-METHYL-1-NITROANTHRAQUINONE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	1/48(0.02)	5/48(0.10)	9/49(0.18)
P Values[c]	P = 0.007	N.S.	P = 0.008
Relative Risk (Control)[d]	---	5.000	8.816
Lower Limit	---	0.590	1.298
Upper Limit	---	231.144	376.984
Weeks to First Observed Tumor	109	103	106
Liver: Neoplastic Nodule[b]	0/48(0.00)	2/48(0.04)	6/49(0.12)
P Values[c]	P = 0.008	N.S.	P = 0.014
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.296	1.569
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	109	109
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	1/48(0.02)	7/48(0.15)	15/49(0.31)
P Values[c]	P < 0.001	P = 0.030	P < 0.001
Relative Risk (Control)[d]	---	7.000	14.690
Lower Limit	---	0.953	2.421
Upper Limit	---	307.993	600.781
Weeks to First Observed Tumor	109	103	106

TABLE 2 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	3/48(0.06)	10/49(0.20)	34/49(0.69)
P Values[c]	P < 0.001	P = 0.039	P < 0.001
Departure from Linear Trend[e]	P = 0.028	---	---
Relative Risk (Control)[d]	---	3.265	11.100
Lower Limit	---	0.906	3.945
Upper Limit	---	17.460	49.676
Weeks to First Observed Tumor	97	77	70
Adrenal: Pheochromocytoma[b]	7/47(0.15)	4/47(0.09)	10/48(0.21)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.571	1.399
Lower Limit	---	0.131	0.527
Upper Limit	---	2.089	3.969
Weeks to First Observed Tumor	107	109	78
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	1/48(0.02)	3/46(0.07)	3/44(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	3.130	3.273
Lower Limit	---	0.263	0.275
Upper Limit	---	160.659	167.729
Weeks to First Observed Tumor	109	90	87

TABLE 2 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma[b]	0/48(0.00)	2/49(0.04)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.290	0.590
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	109	109
Testis: Interstitial-Cell Tumor[b]	42/47(0.89)	46/48(0.96)	47/49(0.96)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.072	1.073
Lower Limit	---	0.941	0.943
Upper Limit	---	1.161	1.161
Weeks to First Observed Tumor	78	77	70
Hematopoietic System: Leukemia[b]	5/48(0.10)	6/49(0.12)	0/49(0.00)
P Values[c]	P = 0.040(N)	N.S.	P = 0.027(N)
Relative Risk (Control)[d]	---	1.176	0.000
Lower Limit	---	0.321	0.000
Upper Limit	---	4.557	0.766
Weeks to First Observed Tumor	95	97	---

TABLE 2 (Concluded)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Circulatory System: Hemangiosarcoma[b]	0/48(0.00)	1/49(0.02)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.053	0.590
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	109	106
Pituitary: Adenoma[b]	9/38(0.24)	15/43(0.35)	8/42(0.19)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.473	0.804
Lower Limit	---	0.690	0.302
Upper Limit	---	3.361	2.109
Weeks to First Observed Tumor	85	97	102

[a]Treated groups received time-weighted average doses of 0.06 or 0.12 percent in feed.

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

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

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

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

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 2-METHYL-1-NITROANTHRAQUINONE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	0/50(0.00)	0/50(0.00)	3/49(0.06)
P Values[c]	P = 0.036	---	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.614
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	96
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	0/50(0.00)	1/50(0.02)	4/49(0.08)
P Values[c]	P = 0.025	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.054	0.946
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	109	96
Subcutaneous Tissue: Fibroma[b]	1/50(0.02)	0/50(0.00)	13/49(0.27)
P Values[c]	P < 0.001	N.S.	P < 0.001
Departure from Linear Trend[e]	P = 0.005	---	---
Relative Risk (Control)[d]	---	0.000	13.260
Lower Limit	---	0.000	2.126
Upper Limit	---	18.660	548.387
Weeks to First Observed Tumor	102	---	52

TABLE 3 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia[b]	5/50(0.10)	3/50(0.06)	1/49(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.600	0.204
Lower Limit	---	0.098	0.004
Upper Limit	---	2.910	1.733
Weeks to First Observed Tumor	104	70	104
Stomach: Squamous-Cell Carcinoma[b]	0/48(0.00)	0/48(0.00)	3/48(0.06)
P Values[c]	P = 0.038	---	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.602
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	109
Urinary Bladder: Sarcoma; Papilloma NOS; or Transitional-Cell Papilloma[b]	0/46(0.00)	3/43(0.07)	4/44(0.09)
P Values[c]	P = 0.045	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.645	0.972
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	109	76

TABLE 3 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma[b]	17/40(0.43)	26/41(0.63)	25/41(0.61)
P Values[c]	N.S.	P = 0.048	N.S.
Relative Risk (Control)[d]	---	1.492	1.435
Lower Limit	---	0.941	0.897
Upper Limit	---	2.366	2.302
Weeks to First Observed Tumor	78	65	73
Mammary Gland: Fibroadenoma[b]	19/50(0.38)	23/50(0.46)	23/49(0.47)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.210	1.235
Lower Limit	---	0.730	0.746
Upper Limit	---	2.021	2.056
Weeks to First Observed Tumor	107	80	88
Uterus: Adenocarcinoma[b]	1/50(0.02)	1/49(0.02)	3/48(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.020	3.125
Lower Limit	---	0.013	0.267
Upper Limit	---	78.488	160.536
Weeks to First Observed Tumor	109	109	109

TABLE 3 (Concluded)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Uterus: Endometrial Stromal Polyp[b]	10/50(0.20)	11/49(0.22)	8/48(0.17)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.122	0.833
Lower Limit	---	0.477	0.312
Upper Limit	---	2.674	2.140
Weeks to First Observed Tumor	78	70	103

[a]Treated groups received time weighted average doses of 0.06 or 0.12 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 $P < 0.05$.

the incidence of hepatocellular carcinomas for both males ($P = 0.007$) and females ($P = 0.036$). The Fisher exact tests, however, only confirmed this result for the high dose males ($P = 0.008$). For neoplastic nodules the Cochran-Armitage test was only significant for male rats ($P = 0.008$), a result which was confirmed ($P = 0.014$) by the Fisher exact test comparing high dose to control.

When the incidences of hepatocellular carcinoma or neoplastic nodule were combined so that the numerator of the incidence rate indicated the number of animals with either or both lesions, the Cochran-Armitage test was significant for both males ($P < 0.001$) and females ($P = 0.025$). For males, the Fisher exact test comparing high dose to control ($P < 0.001$) was significant; the comparison of low dose to control was $P = 0.03$, a marginal result which was not significant under the Bonferroni criterion. For females the significant Cochran-Armitage result was not supported by the Fisher exact tests.

Based upon these results, the statistical conclusion is that the administration of 2-methyl-1-nitroanthraquinone to male Fischer 344 rats at the dose levels in this study was associated with the increased incidence of hepatocellular carcinoma and neoplastic nodules of the liver.

Fibromas of the subcutaneous tissue were also observed in both male and female rats. The Cochran-Armitage test showed a significant ($P < 0.001$) association of dosage to tumor rate for both sexes. The

departure from linear trend was significant for both males ($P = 0.028$) and females ($P = 0.005$) because of the high tumor rates observed in the high dose groups. In both sexes, the Fisher exact tests showed the incidence of subcutaneous fibromas in the high dose group to be significantly ($P < 0.001$) higher than the incidence in the control group. In the males, the comparison of low dose to control had a probability level of $P = 0.039$, a marginal result which was not significant under the Bonferroni criterion. For the high dose groups of both sexes, the lower limit of the 95 percent confidence interval on the relative risk was greater than the value one.

The statistical conclusion derived from these results is that the administration of 2-methyl-1-nitroanthraquinone to male and female rats at the dose levels of this study was associated with an increased incidence of fibromas of the subcutaneous tissue.

The Cochran-Armitage test showed a significant positive association betwen dose and incidence for squamous-cell carcinomas of the stomach in the females ($P = 0.038$). The Fisher exact tests, however, did not confirm this result. Likewise, in the female rats the Cochran-Armitage test was significant ($P = 0.045$) for tumors of the urinary bladder, but the Fisher exact tests did not confirm these results.

For several tumors, the incidence rate in the control group was significantly ($P < 0.05$) higher than that observed in Fischer 344 rat historical controls at Mason Research Institute included in the NCI

Bioassay Program. Pituitary adenomas were observed in 9/38 (24 percent) of the male control group, as compared to 64/534 (12 percent) of the male historical controls. Fibroadenomas of the mammary gland were observed in 19/50 (38 percent) of the female controls, compared to 112/589 (19 percent) of the female historical controls.

IV. DISCUSSION

Under the conditions of this bioassay, dietary administration of 2-methyl-1-nitroanthraquinone to Fischer 344 rats was associated with a dose-related increased incidence of hepatocellular carcinomas and neoplastic nodules of the liver in males and subcutaneous fibromas in both sexes. There was a significant association between increased dosage and elevated mortality in females but not in males. However, survival was still adequate for meaningful statistical analyses of late-developing tumors in both sexes.

Hepatocellular carcinomas were observed in 1/48 (2 percent), 5/48 (10 percent), and 9/49 (18 percent) of the control, low dose, and high dose male rats, respectively, and in 3/49 (6 percent) of the high dose female rats. Using the Cochran-Armitage test, a significant positive dose-related association was indicated for the incidence of these tumors in males. The incidence in the high dose male group was significantly higher than that in the male control group. Neoplastic nodules of the liver occurred in treated males and females but not in controls (i.e., 2/48 or 4 percent low dose males, 6/49 or 12 percent high dose males, 1/50 or 2 percent low dose females, and 1/49 or 2 percent high dose females). These incidences were significant in males, as indicated by both the Cochran-Armitage test and the Fisher exact test for the high dose group. When those rats having either hepatocellular carcinomas or neoplastic nodules of the liver were combined and evaluated simultaneously, the Cochran-Armitage tests for

both males and females indicated significant associations between increased dosage and elevated tumor incidences; the Fisher exact test confirmed this result for high dose males but not for low dose males or for low dose or high dose females.

Gastric tumors of the forestomach occurred only in high dose rats (i.e., squamous-cell papillomas in 2/47 [4 percent] high dose males and 2/48 [4 percent] high dose females, and squamous-cell carcinomas in 1/47 [2 percent] high dose males and 3/48 [6 percent] high dose females). Statistical analysis using the Cochran-Armitage test indicated a significant positive association between dosage and incidence of squamous-cell carcinomas in females. The significance of this tumor incidence was not confirmed, however, by the Fisher exact test. Although the incidences of these neoplasms in this bioassay were not found to be statistically significant, these tumors occur only infrequently in Fischer 344 rats. The historical incidences, collated from all untreated rats of this strain used in the NCI Bioassay Program at Mason Research Institute, of squamous-cell papillomas of the stomach (1/534 and 1/589 for males and females, respectively) and stomach squamous-cell carcinomas (2/534 and 1/589 for males and females, respectively) indicate the rarity of these tumors. In addition, it is felt that the frequency with which nonneoplastic stomach lesions (i.e., hyperplasia, hyperkeratosis, and acanthosis) were observed in treated animals provided supplementary

evidence for concluding that occurrence of these tumors and nonneoplastic lesions was treatment-related.

Various tumors were observed in the urinary bladder of treated female rats (i.e., papillomas, transitional-cell papillomas, and sarcomas). Statistical analysis, using the Cochran-Armitage test, indicated a significant association between dosage and incidence of these tumors but the Fisher exact tests did not support the association.

Common neoplasms observed in 2-methyl-1-nitroanthraquinone-treated and control rats included testicular interstitial-cell adenomas in males, endometrial stromal polyps and mammary fibroadenomas in females, and subcutaneous fibromas in both sexes. The Cochran-Armitage tests indicated a significant positive association between dosage and incidence of subcutaneous fibromas in both sexes (i.e., 3/48 or 6 percent, 10/49 or 20 percent, and 34/49 or 69 percent in control, low dose, and high dose males, respectively, and 1/50 or 2 percent and 13/49 or 27 percent in control and high dose females, respectively). For both sexes the Fisher exact test comparing high dose to control confirmed this association. Statistical evaluation of the occurrence of the other tumors did not indicate any significant associations between treatment and tumor incidence.

A bioassay conducted at the same laboratory during the same time period as this Fischer 344 rat bioassay indicated that 2-methyl-1-nitroanthraquinone was a carcinogen in male and female B6C3F1 mice.

Subcutaneous hemangiosarcomas developed in 97 percent of the male and female mice treated with the compound (Murthy et al., 1977). (This article has been fully reproduced in Appendix C.) In the bioassay of Fischer 344 rats, subcutaneous hemangiosarcomas were observed only in 3/49 (6 percent) of the high dose group.

Under the conditions of this bioassay, the results indicate that 2-methyl-1-nitroanthraquinone is carcinogenic in male Fischer 344 rats, producing hepatocellular carcinomas. Increased incidences of subcutaneous fibromas in both male and female rats were also associated with administration of the compound. Tumors of the forestomach and bladder in these animals may also have been related to administration of the test chemical.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 2-METHYL-1-NITROANTHRAQUINONE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED
WITH 2-METHYL-1-NITROANTHRAQUINONE

	CONTROL (UNTR) 01-0118	LOW DOSE 01-0116	HIGH DOSE 01-0117
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	48	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	48	48	49
INTEGUMENTARY SYSTEM			
*SKIN	(48)	(49)	(49)
SQUAMOUS CELL PAPILLOMA		1 (2%)	1 (2%)
BASAL-CELL CARCINOMA		1 (2%)	1 (2%)
*SUBCUT TISSUE	(48)	(49)	(49)
SARCOMA, NOS	1 (2%)		1 (2%)
FIBROMA	3 (6%)	10 (20%)	34 (69%)
FIBROSARCOMA	1 (2%)	1 (2%)	1 (2%)
LEIOMYOSARCOMA			1 (2%)
HEMANGIOSARCOMA			3 (6%)
RESPIRATORY SYSTEM			
#LUNG	(48)	(48)	(49)
NEOPLASM, NOS			1 (2%)
HEPATOCELLULAR CARCINOMA, METAST			2 (4%)
ALVEOLAR/BRONCHIOLAR ADENOMA			2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)	1 (2%)	
PHEOCHROMOCYTOMA, METASTATIC	1 (2%)		
HEMATOPOIETIC SYSTEM			
#BRAIN	(48)	(48)	(48)
LEUKEMIA,NOS		1 (2%)	
*MULTIPLE ORGANS	(48)	(49)	(49)
MALIGNANT LYMPHOMA, NOS	1 (2%)	1 (2%)	
LEUKEMIA,NOS	1 (2%)		
MYELOMONOCYTIC LEUKEMIA	4 (8%)	2 (4%)	
LYMPHOCYTIC LEUKEMIA		3 (6%)	
#SPLEEN	(48)	(48)	(49)
HEMANGIOSARCOMA		1 (2%)	

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0118	LOW DOSE 01-0116	HIGH DOSE 01-0117
#MESENTERIC L. NODE	(44)	(35)	(22)
MALIGNANT LYMPHOMA, NOS		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(47)	(41)	(46)
ADENOCARCINOMA, NOS	1 (2%)		
SARCOMA, NOS	1 (2%)		
#LIVER	(48)	(48)	(49)
NEOPLASTIC NODULE		2 (4%)	6 (12%)
HEPATOCELLULAR CARCINOMA	1 (2%)	5 (10%)	9 (18%)
#STOMACH	(48)	(47)	(47)
SQUAMOUS CELL PAPILLOMA			2 (4%)
SQUAMOUS CELL CARCINOMA			1 (2%)
#SMALL INTESTINE	(46)	(45)	(48)
ADENOMATOUS POLYP, NOS			1 (2%)
#ILEUM	(46)	(45)	(48)
SARCOMA, NOS	1 (2%)		
#COLON	(46)	(34)	(38)
ADENOCARCINOMA, NOS			1 (3%)
URINARY SYSTEM			
#KIDNEY	(48)	(48)	(49)
TUBULAR-CELL ADENOMA			1 (2%)
SARCOMA, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(38)	(43)	(42)
ADENOMA, NOS	9 (24%)	14 (33%)	8 (19%)
CHROMOPHOBE ADENOMA		1 (2%)	

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

TABLE A1 (CONTINUED)

	CONTROL(UNTR) 01-0118	LOW DOSE 01-0116	HIGH DOSE 01-0117
#ADRENAL	(47)	(47)	(48)
PHEOCHROMOCYTOMA	7 (15%)	4 (9%)	10 (21%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)		
#ADRENAL MEDULLA	(47)	(47)	(48)
GANGLIONEUROMA			1 (2%)
#THYROID	(48)	(46)	(44)
FOLLICULAR-CELL ADENOMA		1 (2%)	
C-CELL ADENOMA		3 (7%)	2 (5%)
C-CELL CARCINOMA	1 (2%)		1 (2%)
#PARATHYROID	(28)	(23)	(22)
ADENOMA, NOS	1 (4%)	1 (4%)	
#PANCREATIC ISLETS	(46)	(44)	(48)
ISLET-CELL ADENOMA		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(48)	(49)	(49)
ADENOMA, NOS			1 (2%)
FIBROADENOMA		2 (4%)	3 (6%)
*PREPUTIAL GLAND	(48)	(49)	(49)
SQUAMOUS CELL CARCINOMA		1 (2%)	
ADENOMA, NOS			2 (4%)
#TESTIS	(47)	(48)	(49)
INTERSTITIAL-CELL TUMOR	42 (89%)	46 (96%)	47 (96%)
NERVOUS SYSTEM			
#BRAIN	(48)	(48)	(48)
GLIOMA, NOS	1 (2%)		1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A1 (CONCLUDED)

	CONTROL(UNTR) 01-0118	LOW DOSE 01-0116	HIGH DOSE 01-0117
BODY CAVITIES			
*BODY CAVITIES	(48)	(49)	(49)
MESOTHELIOMA, NOS		1 (2%)	2 (4%)
MESOTHELIOMA, MALIGNANT	2 (4%)		2 (4%)
ALL OTHER SYSTEMS			
SITE UNKNOWN			
UNDIFFERENTIATED CARCINOMA		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	6	4	4
MORIBUND SACRIFICE	8	11	14
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	30	34	27
ANIMAL MISSING		1	
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	44	48	49
TOTAL PRIMARY TUMORS	80	106	148
TOTAL ANIMALS WITH BENIGN TUMORS	43	47	48
TOTAL BENIGN TUMORS	62	84	116
TOTAL ANIMALS WITH MALIGNANT TUMORS	17	18	18
TOTAL MALIGNANT TUMORS	18	19	23
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		2
TOTAL SECONDARY TUMORS	1		2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		3	8
TOTAL UNCERTAIN TUMORS		3	9
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 2-METHYL-1-NITROANTHRAQUINONE

	CONTROL (UNTR) 02-0118	LOW DOSE 02-0116	HIGH DOSE 02-0117
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(49)
BASAL-CELL CARCINOMA	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(49)
SARCOMA, NOS			1 (2%)
FIBROMA	1 (2%)		13 (27%)
FIBROSARCOMA	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(49)
SQUAMOUS CELL CARCINOMA, METASTA	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(49)
UNDIFFERENTIATED LEUKEMIA	1 (2%)	1 (2%)	
MYELOMONOCYTIC LEUKEMIA	3 (6%)	2 (4%)	
LYMPHOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(48)	(50)	(49)
UNDIFFERENTIATED LEUKEMIA	1 (2%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(49)
NEOPLASTIC NODULE		1 (2%)	1 (2%)

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0118	LOW DOSE 02-0116	HIGH DOSE 02-0117
HEPATOCELLULAR CARCINOMA			3 (6%)
#STOMACH	(48)	(48)	(48)
SQUAMOUS CELL PAPILLOMA			2 (4%)
SQUAMOUS CELL CARCINOMA			3 (6%)
#SMALL INTESTINE	(48)	(47)	(49)
LEIOMYOSARCOMA			1 (2%)
#ILEUM	(48)	(47)	(49)
LEIOMYOSARCOMA	1 (2%)		
URINARY SYSTEM			
#URINARY BLADDER	(46)	(43)	(44)
PAPILLOMA, NOS		1 (2%)	1 (2%)
TRANSITIONAL-CELL PAPILLOMA		2 (5%)	1 (2%)
SARCOMA, NOS			2 (5%)
ENDOCRINE SYSTEM			
#PITUITARY	(40)	(41)	(41)
ADENOMA, NOS	17 (43%)	26 (63%)	25 (61%)
#ADRENAL	(49)	(47)	(45)
CORTICAL ADENOMA	1 (2%)	1 (2%)	2 (4%)
PHEOCHROMOCYTOMA	3 (6%)	1 (2%)	1 (2%)
#ADRENAL MEDULLA	(49)	(47)	(45)
GANGLIONEUROMA	1 (2%)		
#THYROID	(44)	(45)	(47)
FOLLICULAR-CELL CARCINOMA	1 (2%)	1 (2%)	
C-CELL ADENOMA	1 (2%)	1 (2%)	2 (4%)
C-CELL CARCINOMA	1 (2%)	1 (2%)	
#PANCREATIC ISLETS	(48)	(47)	(44)
ISLET-CELL ADENOMA	2 (4%)	1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(49)
ADENOCARCINOMA, NOS			1 (2%)

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

TABLE A2 (CONTINUED)

	CONTROL(UNTR) 02-0118	LOW DOSE 02-0116	HIGH DOSE 02-0117
FIBROADENOMA	19 (38%)	23 (46%)	23 (47%)
*CLITORAL GLAND	(50)	(50)	(49)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
ADENOMA, NOS	2 (4%)		
PAPILLARY ADENOMA			1 (2%)
#UTERUS	(50)	(49)	(48)
CARCINOMA,NOS		1 (2%)	
ADENOCARCINOMA, NOS	1 (2%)	1 (2%)	3 (6%)
LEIOMYOSARCOMA		2 (4%)	
ENDOMETRIAL STROMAL POLYP	10 (20%)	11 (22%)	8 (17%)
ENDOMETRIAL STROMAL SARCOMA	1 (2%)		
#OVARY	(49)	(47)	(48)
GRANULOSA-CELL TUMOR	1 (2%)	1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(49)
OLIGODENDROGLIOMA		1 (2%)	
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(50)	(50)	(49)
SQUAMOUS CELL CARCINOMA		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
SITE UNKNOWN			
SQUAMOUS CELL CARCINOMA	1		

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

TABLE A2 (CONCLUDED)

	CONTROL(UNTR) 02-0118	LOW DOSE 02-0116	HIGH DOSE 02-0117
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	5	4	3
MORIBUND SACRIFICE	3	6	13
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	37	40	29
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	38	43	44
TOTAL PRIMARY TUMORS	73	80	96
TOTAL ANIMALS WITH BENIGN TUMORS	35	41	43
TOTAL BENIGN TUMORS	59	67	80
TOTAL ANIMALS WITH MALIGNANT TUMORS	12	11	15
TOTAL MALIGNANT TUMORS	13	11	15
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		
TOTAL SECONDARY TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	2	1
TOTAL UNCERTAIN TUMORS	1	2	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 NONNEOPLASTIC LESIONS IN RATS TREATED WITH 2-METHYL-1-NITROANTHRAQUINONE

TABLE B1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH 2-METHYL-1-NITROANTHRAQUINONE

	CONTROL (UNTR) 01-0118	LOW DOSE 01-0116	HIGH DOSE 01-0117
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	48	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	48	48	49
INTEGUMENTARY SYSTEM			
*SKIN	(48)	(49)	(49)
INFLAMMATION, NOS			2 (4%)
ULCER, NOS		1 (2%)	1 (2%)
KELOID		2 (4%)	
FIBROMATOSIS		1 (2%)	
*SUBCUT TISSUE	(48)	(49)	(49)
FIBROSIS		1 (2%)	
NECROSIS, NOS		1 (2%)	
METAPLASIA, OSSEOUS	1 (2%)		
RESPIRATORY SYSTEM			
#TRACHEA	(48)	(37)	(46)
INFLAMMATION, NOS	2 (4%)		
#LUNG/BRONCHUS	(48)	(48)	(49)
BRONCHIECTASIS	1 (2%)	1 (2%)	1 (2%)
INFLAMMATION, NOS	7 (15%)	3 (6%)	4 (8%)
INFLAMMATION, FOCAL		3 (6%)	
INFLAMMATION, SUPPURATIVE			1 (2%)
HYPERPLASIA, PAPILLARY		1 (2%)	
#LUNG	(48)	(48)	(49)
INFLAMMATION, INTERSTITIAL	4 (8%)	14 (29%)	8 (16%)
INFLAMMATION, NECROTIZING	1 (2%)	1 (2%)	1 (2%)
ABSCESS, NOS		1 (2%)	
PNEUMONIA, CHRONIC MURINE	1 (2%)	1 (2%)	1 (2%)
HYPERPLASIA, EPITHELIAL	1 (2%)	5 (10%)	2 (4%)
HYPERPLASIA, ADENOMATOUS			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(48)	(48)	(49)
INFLAMMATION, NOS		1 (2%)	1 (2%)

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 01-0118	LOW DOSE 01-0116	HIGH DOSE 01-0117
FIBROSIS	1 (2%)	1 (2%)	
DEGENERATION, NOS		1 (2%)	
HEMOSIDEROSIS	1 (2%)	3 (6%)	2 (4%)
HYPERPLASIA, HEMATOPOIETIC	9 (19%)	7 (15%)	10 (20%)
HYPERPLASIA, ERYTHROID	10 (21%)	15 (31%)	15 (31%)
HEMATOPOIESIS			1 (2%)
#LYMPH NODE	(44)	(35)	(22)
HEMORRHAGE	1 (2%)		
HYPERPLASIA, NOS		5 (14%)	2 (9%)
RETICULOCYTOSIS		1 (3%)	2 (9%)
PLASMACYTOSIS	1 (2%)	1 (3%)	2 (9%)
HYPERPLASIA, LYMPHOID	3 (7%)	2 (6%)	4 (18%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(48)	(48)	(48)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, INTERSTITIAL	23 (48%)	22 (46%)	15 (31%)
FIBROSIS	12 (25%)	22 (46%)	22 (46%)
*AORTA	(48)	(49)	(49)
INFLAMMATION, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(47)	(41)	(46)
HYPERTROPHY, FOCAL			1 (2%)
HYPERPLASIA, FOCAL			1 (2%)
#LIVER	(48)	(48)	(49)
FIBROSIS SEPTAL LIVER	2 (4%)		
NECROSIS, FOCAL	2 (4%)	3 (6%)	
NECROSIS, COAGULATIVE		2 (4%)	
METAMORPHOSIS FATTY		3 (6%)	2 (4%)
CYTOPLASMIC VACUOLIZATION		1 (2%)	
HYPERPLASIA, FOCAL	15 (31%)	14 (29%)	10 (20%)
HYPERPLASIA, DIFFUSE		1 (2%)	
ANGIECTASIS	1 (2%)	2 (4%)	1 (2%)
HEMATOPOIESIS			1 (2%)
#LIVER/CENTRILOBULAR	(48)	(48)	(49)
NECROSIS, NOS	1 (2%)		
*BILE DUCT	(48)	(49)	(49)
INFLAMMATION, NOS	3 (6%)		1 (2%)

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 01-0118	LOW DOSE 01-0116	HIGH DOSE 01-0117
HYPERPLASIA, NOS	43 (90%)	27 (55%)	11 (22%)
#PANCREAS	(46)	(44)	(48)
INFLAMMATION, NOS	17 (37%)	14 (32%)	18 (38%)
PERIVASCULITIS		1 (2%)	
#PANCREATIC DUCT	(46)	(44)	(48)
HYPERPLASIA, NOS		2 (5%)	
#PANCREATIC ACINUS	(46)	(44)	(48)
HYPERTROPHY, FOCAL		2 (5%)	1 (2%)
HYPERPLASIA, FOCAL	1 (2%)		
#ESOPHAGUS	(45)	(26)	(44)
DYSPLASIA, NOS	1 (2%)		
#STOMACH	(48)	(47)	(47)
INFLAMMATION, NOS	1 (2%)	1 (2%)	5 (11%)
INFLAMMATION, FOCAL		1 (2%)	
DEGENERATION, NOS			2 (4%)
HYPERPLASIA, FOCAL		2 (4%)	2 (4%)
HYPERPLASIA, BASAL CELL	1 (2%)	9 (19%)	17 (36%)
HYPERKERATOSIS	2 (4%)	4 (9%)	5 (11%)
ACANTHOSIS	2 (4%)	13 (28%)	8 (17%)
#PEYERS PATCH	(46)	(45)	(48)
HYPERPLASIA, NOS	12 (26%)	17 (38%)	9 (19%)
#ILEUM	(46)	(45)	(48)
INFLAMMATION, NOS	2 (4%)		
#COLON	(46)	(34)	(38)
PARASITISM	3 (7%)		2 (5%)
URINARY SYSTEM			
#KIDNEY	(48)	(48)	(49)
MINERALIZATION			1 (2%)
GLOMERULONEPHRITIS, NOS	46 (96%)	46 (96%)	46 (94%)
FIBROSIS		1 (2%)	
FIBROSIS, FOCAL			1 (2%)
FIBROSIS, DIFFUSE	6 (13%)	13 (27%)	5 (10%)
HYPERPLASIA, EPITHELIAL			1 (2%)
#KIDNEY/TUBULE	(48)	(48)	(49)
NECROSIS, NOS		1 (2%)	

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 01-0118	LOW DOSE 01-0116	HIGH DOSE 01-0117
HYPERPLASIA, TUBULAR CELL			1 (2%)
#URINARY BLADDER	(43)	(44)	(47)
HYPERPLASIA, EPITHELIAL	1 (2%)	2 (5%)	2 (4%)
ENDOCRINE SYSTEM			
#PITUITARY	(38)	(43)	(42)
HYPERPLASIA, NOS	1 (3%)		1 (2%)
HYPERPLASIA, FOCAL	2 (5%)	2 (5%)	4 (10%)
HYPERPLASIA, LYMPHOID		1 (2%)	
#ADRENAL	(47)	(47)	(48)
LIPOIDOSIS		1 (2%)	
#ADRENAL MEDULLA	(47)	(47)	(48)
HYPERPLASIA, NODULAR	1 (2%)	3 (6%)	1 (2%)
HYPERPLASIA, FOCAL	4 (9%)	1 (2%)	
#THYROID	(48)	(46)	(44)
LYMPHOCYTIC INFILTRATE			1 (2%)
HYPERPLASIA, C-CELL	3 (6%)	1 (2%)	
#PARATHYROID	(28)	(23)	(22)
HYPERPLASIA, NOS	1 (4%)		
#PANCREATIC ISLETS	(46)	(44)	(48)
HYPERPLASIA, NOS	1 (2%)	2 (5%)	3 (6%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(48)	(49)	(49)
GALACTOCELE	2 (4%)	2 (4%)	
HYPERPLASIA, NOS	4 (8%)	10 (20%)	8 (16%)
#PROSTATE	(44)	(44)	(44)
INFLAMMATION, NOS	17 (39%)	21 (48%)	14 (32%)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
#TESTIS	(47)	(48)	(49)
MINERALIZATION	1 (2%)		2 (4%)
FIBROSIS			2 (4%)
ATROPHY, NOS	6 (13%)	2 (4%)	5 (10%)

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

TABLE B1 (CONCLUDED)

	CONTROL(UNTR) 01-0118	LOW DOSE 01-0116	HIGH DOSE 01-0117
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, INTERSTITIAL CELL	3 (6%)	2 (4%)	1 (2%)
#TESTIS/TUBULE	(47)	(48)	(49)
DEGENERATION, NOS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(48)	(48)	(48)
NECROSIS, CORTICAL		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, NOS		2	
NECROSIS, FAT	2		
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY		1	
NECROPSY PERF/NO HISTO PERFORMED		1	
AUTOLYSIS/NO NECROPSY	1		1

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

TABLE B2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH 2-METHYL-1-NITROANTHRAQUINONE

	CONTROL (UNTR) 02-0118	LOW DOSE 02-0116	HIGH DOSE 02-0117
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	50	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(49)
INFLAMMATION, NOS	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(49)
MINERALIZATION	1 (2%)		
ABSCESS, NOS	1 (2%)		
FIBROMATOSIS		1 (2%)	
RESPIRATORY SYSTEM			
*NASAL CAVITY	(50)	(50)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)	
METAPLASIA, SQUAMOUS		1 (2%)	
#TRACHEA	(49)	(48)	(48)
INFLAMMATION, NOS			1 (2%)
#LUNG/BRONCHUS	(50)	(50)	(49)
INFLAMMATION, NOS	3 (6%)	1 (2%)	4 (8%)
INFLAMMATION, FOCAL			1 (2%)
#LUNG	(50)	(50)	(49)
INFLAMMATION, INTERSTITIAL	6 (12%)	10 (20%)	15 (31%)
HYPERPLASIA, EPITHELIAL	1 (2%)	2 (4%)	4 (8%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(46)	(39)	(38)
OSTEOSCLEROSIS	1 (2%)		
MEGAKARYOCYTOSIS		1 (3%)	
#SPLEEN	(48)	(50)	(49)
HEMOSIDEROSIS	12 (25%)	19 (38%)	15 (31%)

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 02-0118	LOW DOSE 02-0116	HIGH DOSE 02-0117
HYPERPLASIA, HEMATOPOIETIC	25 (52%)	23 (46%)	28 (57%)
HYPERPLASIA, ERYTHROID	19 (40%)	30 (60%)	29 (59%)
HYPERPLASIA, LYMPHOID			1 (2%)
HEMATOPOIESIS		1 (2%)	
#SPLENIC CAPSULE	(48)	(50)	(49)
HEMORRHAGIC CYST	1 (2%)		
#LYMPH NODE	(47)	(23)	(31)
HYPERPLASIA, NOS		1 (4%)	3 (10%)
LYMPHOCYTOSIS			2 (6%)
PLASMACYTOSIS	1 (2%)		1 (3%)
HYPERPLASIA, LYMPHOID	4 (9%)		2 (6%)
CIRCULATORY SYSTEM			
#HEART	(50)	(50)	(48)
PERIVASCULITIS		1 (2%)	
#MYOCARDIUM	(50)	(50)	(48)
INFLAMMATION, NOS	1 (2%)	1 (2%)	
INFLAMMATION, INTERSTITIAL	23 (46%)	24 (48%)	16 (33%)
FIBROSIS	15 (30%)	9 (18%)	10 (21%)
#ENDOCARDIUM	(50)	(50)	(48)
INFLAMMATION, NOS	1 (2%)	1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(49)
NECROSIS, FOCAL	2 (4%)	1 (2%)	1 (2%)
METAMORPHOSIS FATTY	6 (12%)	1 (2%)	3 (6%)
CYTOPLASMIC VACUOLIZATION			1 (2%)
HYPERPLASTIC NODULE			1 (2%)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
HYPERPLASIA, FOCAL	38 (76%)	33 (66%)	24 (49%)
HYPERPLASIA, ERYTHROID	1 (2%)		
HEMATOPOIESIS	2 (4%)		
*BILE DUCT	(50)	(50)	(49)
INFLAMMATION, NOS	1 (2%)	1 (2%)	
METAMORPHOSIS FATTY		1 (2%)	
HYPERPLASIA, NOS	32 (64%)	32 (64%)	16 (33%)
HYPERPLASIA, FOCAL	1 (2%)		

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 02-0118	LOW DOSE 02-0116	HIGH DOSE 02-0117
#PANCREAS	(48)	(47)	(44)
INFLAMMATION, NOS	6 (13%)	15 (32%)	13 (30%)
PERIARTERITIS		1 (2%)	
#PANCREATIC ACINUS	(48)	(47)	(44)
HYPERTROPHY, FOCAL		1 (2%)	1 (2%)
#STOMACH	(48)	(48)	(48)
INFLAMMATION, NOS	1 (2%)	4 (8%)	
ULCER, NOS			1 (2%)
INFLAMMATION, FOCAL			2 (4%)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
HYPERPLASIA, FOCAL		2 (4%)	2 (4%)
HYPERPLASIA, BASAL CELL		4 (8%)	16 (33%)
HYPERKERATOSIS		5 (10%)	8 (17%)
ACANTHOSIS	2 (4%)	8 (17%)	16 (33%)
#SMALL INTESTINE	(48)	(47)	(49)
HYPERPLASIA, NOS		1 (2%)	
#PEYERS PATCH	(48)	(47)	(49)
HYPERPLASIA, NOS	15 (31%)	12 (26%)	17 (35%)
#COLON	(46)	(36)	(36)
PARASITISM	2 (4%)		2 (6%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(49)
GLOMERULONEPHRITIS, NOS	43 (86%)	41 (82%)	35 (71%)
FIBROSIS, DIFFUSE	1 (2%)		1 (2%)
#URINARY BLADDER	(46)	(43)	(44)
HYPERPLASIA, EPITHELIAL			2 (5%)
ENDOCRINE SYSTEM			
#PITUITARY	(40)	(41)	(41)
PERIVASCULITIS	1 (3%)		
HYPERPLASIA, FOCAL	3 (8%)	1 (2%)	
#ADRENAL	(49)	(47)	(45)
METAMORPHOSIS FATTY	1 (2%)		

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 02-0118	LOW DOSE 02-0116	HIGH DOSE 02-0117
#ADRENAL CORTEX	(49)	(47)	(45)
NODULE		1 (2%)	
HYPERPLASIA, NODULAR			1 (2%)
HYPERPLASIA, FOCAL			1 (2%)
#ADRENAL MEDULLA	(49)	(47)	(45)
HYPERPLASIA, NODULAR	3 (6%)		
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL	3 (6%)	1 (2%)	
#THYROID	(44)	(45)	(47)
CYSTIC FOLLICLES	1 (2%)		
HYPERPLASIA, C-CELL	1 (2%)	4 (9%)	2 (4%)
#PANCREATIC ISLETS	(48)	(47)	(44)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(49)
GALACTOCELE	16 (32%)	23 (46%)	19 (39%)
HYPERPLASIA, NOS	8 (16%)	10 (20%)	12 (24%)
*CLITORIS	(50)	(50)	(49)
HYPERPLASIA, NOS		1 (2%)	
#UTERUS	(50)	(49)	(48)
HYDROMETRA		3 (6%)	
HYPERPLASIA, ADENOMATOUS	1 (2%)		1 (2%)
#UTERUS/ENDOMETRIUM	(50)	(49)	(48)
INFLAMMATION, NOS	22 (44%)	21 (43%)	15 (31%)
HYPERPLASIA, NOS	6 (12%)	9 (18%)	2 (4%)
HYPERPLASIA, ADENOMATOUS	1 (2%)		
#OVARY/OVIDUCT	(50)	(49)	(48)
INFLAMMATION, NOS	10 (20%)	12 (24%)	7 (15%)
INFLAMMATION, SUPPURATIVE	2 (4%)		
INFLAMMATION, NECROTIZING			1 (2%)
#OVARY	(49)	(47)	(48)
CYST, NOS	8 (16%)	13 (28%)	4 (8%)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE		1 (2%)	

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 02-0118	LOW DOSE 02-0116	HIGH DOSE 02-0117
DEGENERATION, CYSTIC			2 (4%)
HYPERPLASIA, NOS		2 (4%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, NECROTIZING			1 (2%)
CATARACT	1 (2%)		1 (2%)
*EYE/RETINA	(50)	(50)	(49)
ATROPHY, NOS	1 (2%)		
*HARDERIAN GLAND	(50)	(50)	(49)
HYPERPLASIA, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*SKULL	(50)	(50)	(49)
OSTEOSCLEROSIS		1 (2%)	
*STERNUM	(50)	(50)	(49)
OSTEOSCLEROSIS		1 (2%)	
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, FAT	1	1	
SPECIAL MORPHOLOGY SUMMARY			
AUTOLYSIS/NO NECROPSY			1

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

APPENDIX C

DEVELOPMENT OF HEMANGIOSARCOMAS IN B6C3F1 MICE FED 2-METHYL-1-NITROANTHRAQUINONE

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DEVELOPMENT OF HEMANGIOSARCOMAS IN B6C3F$_1$ MICE FED 2-METHYL-1-NITROANTHRAQUINONE

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Subcutaneous hemangiosarcomas developed in 97% of 172 B6C3F$_1$ mice of both sexes fed either 0.03% or 0.06% 2-methyl-1-nitroanthraquinone in the diet. There was no significant relationship to dose or sex. In addition, similar vascular tumors occurred in the mesentery of 14 mice. 2-Methyl-1-nitroanthraquinone is carcinogenic in B6C3F$_1$ mice when given in food. One of 97 control mice had a splenic hemangiosarcoma.

Vascular tumors, either spontaneous or induced, are comparatively rare in rodents. A spontaneous hemangioendothelioma in the epididymis of a BALB/c mouse (Stewart *et al.*, 1959), hemangiomas of the liver, spleen, uterus, and subcutis in CD-1-HaM mice (Homburger *et al.*, 1975; Percy and Jonas, 1971), and subcutaneous hemangiosarcomas in two Oregon strain rats (MacKenzie and Garner, 1973), have been described. Vascular tumors have developed following treatment with dimethylnitrosamine (mice) (Kuwahara *et al.*, 1972; Otsuka and Kuwahara, 1971), dimethylhydrazine (mice and hamsters) (Toth, 1972 and 1973), 5-acetamido-3(5-nitro-2-furyl)-6H-1,2,4 oxadiazine (rats) (Ertürk *et al.*, 1969), vinyl chloride (rats and mice) (Maltoni and Lefemine, 1975), 4,4′-methylene-bis-(2-chloroaniline) (mice) (Russfield *et al.*, 1975), β-phenylethylhydrazine sulfate (mice) (Toth, 1976), and following irradiation (Talerman, 1972).

We describe herein the incidence and histopathology of subcutaneous hemangiosarcomas in B6C3F$_1$ fed two concentrations of 2-Methyl-1-nitroanthraquinone, a vat dye.

MATERIAL AND METHODS

2-Methyl-1-nitroanthraquinone (2-Me-1-NA) was procured from Carroll Chemicals, Wood River Junction, R. I. B6C3F$_1$ (hybrid of C57BL/6J female × C3H/He male) mice of both sexes, 42 days old, were used in this study.

The mice were randomly distributed into three groups: group 1: control (stock diet) (50 M; 50F); group 2: 0.03% 2-Me-1-NA (50 M; 50 F); Group 3: 0.06% 2-Me-1-NA (50 M; 50 F).

The animals were housed in groups of ten in polycarbonate cages, and maintained on a 12 h light—dark schedule at 21-24° C. Wayne Laboratory meal (Allied Mills Inc., Chicago, Ill.) and water were available to all animals.

(The two concentrations of 2-Me-1-NA were chosen on the basis of subacute studies carried out in this laboratory. Five mice of each sex were fed 2-Me-1-NA mixed with the stock diet at one of five concentrations (0.05, 0.15, 0.5, 1.5 and 5.0%) for 7 weeks followed by 1 week on stock diet. Five male and five female mice fed only the stock diet served as controls. The dose which produced a depression of approximately 10% in weight gain in comparison with control mice was chosen as the maximum tolerated dose (MTD). The planned length of

TABLE I

INCIDENCE OF HEMANGIOSARCOMAS IN B6C3F$_1$ MICE FED EITHER OF TWO CONCENTRATIONS OF 2-METHYL-1-NITROANTHRAQUINONE

Group	Days in study	Mice with hemangiosarcoma
Control		
Male (49) [1]	345 (5)	0
	547 (5)	0
	671 (39)	1 (2%)
Female (48)	345 (5)	0
	547 (5)	0
	671 (38)	
2-Methyl-1-nitroanthraquinone		
0.03%		
Male (43)	288 (249-326) [2]	42 (98%)
Female (38)	297 (221-338)	35 (92%)
0.06%		
Male (47)	258 (216-324)	46 (98%)
Female (44)	272 (221-338)	44 (100%)

[1] Numbers in parenthesis indicate the effective number of animals. — [2] Mean and range.

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TABLE II

INCIDENCE OF NEOPLASMS IN CONTROL B6C3F$_1$ MICE

Group	Tumor type	Male (49)	Female (48)
Pituitary	Adenoma	0	3
Lung	Adenoma	6	2
	Carcinoma	4	1
Liver	Hepatocellular carcinoma	6	1
Spleen and lymph node	Lymphoreticular neoplasm	4	2
Spleen	Hemangiosarcoma	1	0
Adrenal medulla	Pheochromocytoma	1	0
Oviduct	Adenoma	—	1
Hardeian gland	Adenoma	1	1

FIGURE 1

Hemangiosarcoma in the subcutaneous tisse of a B6C3F$_1$ mouse. Vascular spaces are lined by transformed endothelial cells. H. and E., ×340.

chronic study was feeding the MTD (0.06%) and 1/2 MTD (0.03%) of 2-Me-1-NA mixed with the diet for 548 days followed by 120 days of stock diet).

Five control mice of each sex were killed at 345 and 547 days respectively, and the survivors at 671 days after commencement of the study. The treated animals had to be killed much earlier as they were moribund.

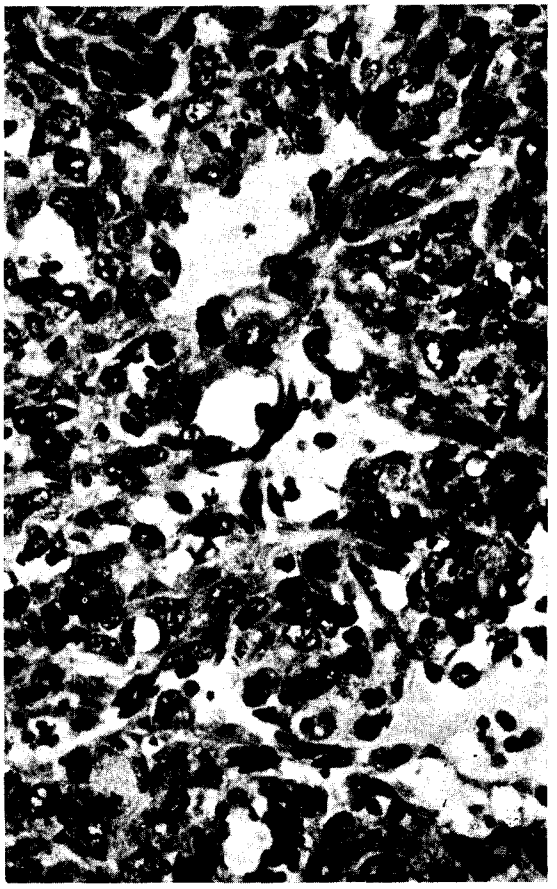
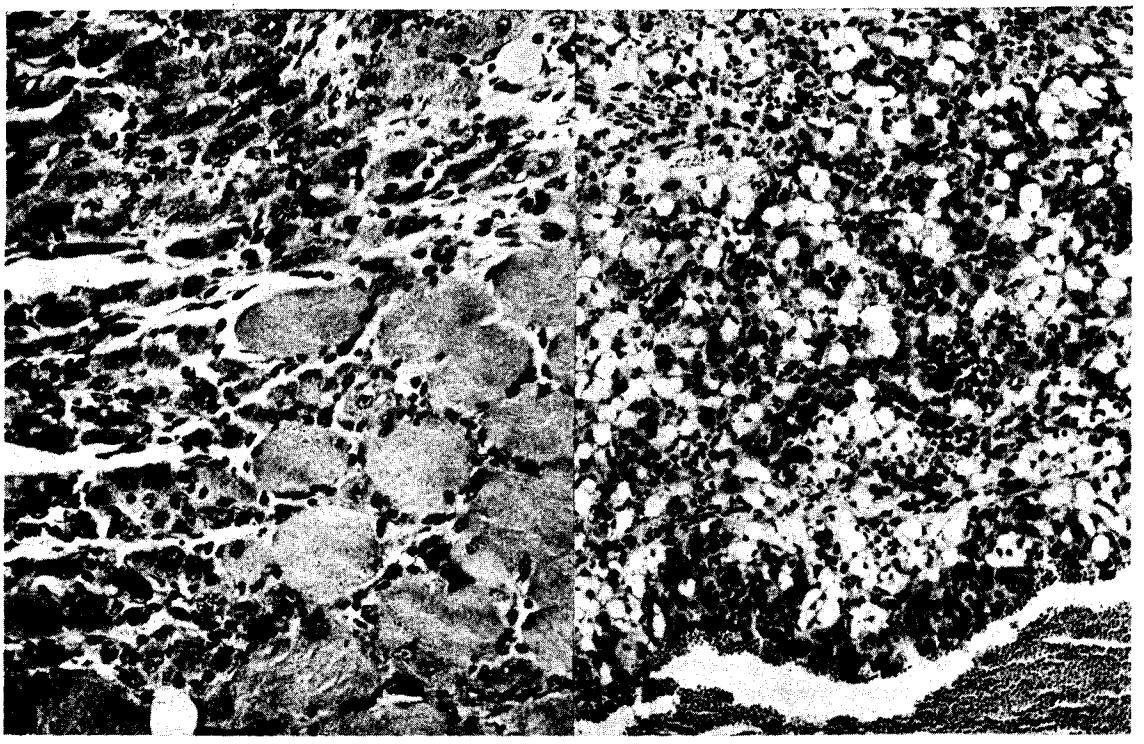


FIGURE 2

Cells lining both the vascular spaces and in the interstitium are large. Nuclei are pleomorphic, and there are mitotic figures in this field. H. and E., ×540.

At necropsy, brain, spinal cord, pituitary, thyroid, thymus, adrenal, lung, heart, esophagus, stomach, intestine, liver, pancreas, bone and bone marrow, spleen, lymph nodes, kidney, urinary bladder, gonads, accessory sex organs, skin, and salivary and mammary glands were dissected, fixed in 10% buffered formalin, and processed for histologic examination. Selected sections from neoplastic lesions were stained with MacManus-PAS, Masson trichrome, Movat pentachrome, and Gomori reti-



FIGURES 3 and 4

Infiltration of neoplastic cells into the adjacent skeletal muscle (Fig. 3) and the adipose tissue (Fig. 4). H. and E., ×140.

culum procedures. Data from 97 control (49 M; 48 F) and 172 treated (90 M; 82 F) mice were analyzed and are presented here.

RESULTS

Differences in body weight between control and treated mice were not significant. Control mice ate more food than those fed either concentration of 2-Me-1-NA.

The number of mice in the three groups, days in study, and number of treated animals with vascular tumors are summarized in Table 1. In the control group, 77 of 80 mice survived until the planned limit of chronic study (671 days). Nineteen of 44 male and 18 of 38 female mice in Group 2, and 13 of 47 male and 8 of 44 female mice in Group 3 died, and the others had to be killed as they were moribund. None of the mice in either of the treated groups survived longer than 338 days, although the projected study period was 671 days.

Angiosarcomas developed in 88 of 90 male and in 79 of 82 female mice. There appeared to be no significant difference in occurrence of tumors between animals fed either of two concentrations of the chemical. Other neoplasms besides vascular tumors in these mice were adenomas of the lung in two, and lymphoreticular neoplasms in 14. In control mice

FIGURE 5

Hemangiosarcoma metastasis in the lung. H. and E., ×140.

there were a few age-related neoplasms, of which one was a hemangiosarcoma of the spleen (Table II).

Tumors ranging in size from 0.2 to 3 cm occurred on the back or in the axilla in treated mice. Microscopically, these consisted of anatomosing blood-filled spaces with large cavernous vascular cysts or capillaries lined by transformed endothelial cells, which were either polygonal or fusiform (Fig. 1). Cytoplasm of the cells did not contain neutral fat, and was PAS-negative. Myofibrils could not be demonstrated in tumor cells. Nuclei were hyperchromatic and pleomorphic, often bizarre. Mitotic figures, both normal and abnormal, were numerous (Fig. 2). There were a few multinucleate giant cells. Some of the more solid tumors had the cytologic characteristics of a fibrosarcoma, except that there were vascular clefts containing a few erythrocytes and leukocytes.

The tumor infiltrated both adjacent adipose tissue (Fig. 3) and skeletal muscle (Fig. 4). Strands of tumor cells, with clumps of chromatin and macronuclei, trapped muscle fibers, many of which were atrophic. Extensive necrosis, hemorrhage and thrombi (in the vessels) occurred in the tumor. Invariably, clusters of polymorphonuclear leukocytes, lymphocytes and intact and fragmented erythrocytes were in vascular channels, and in interstitial spaces of the tumor.

Mesenteric hemangiosarcomas, similar to subcutaneous tumors, occurred in six male and eight female mice. The subcutaneous tumor metastasized to the lung in three males and in one female (Fig. 5). Hemangiosarcoma involving either the liver, spleen, or uterus was lacking in any of the animals.

DISCUSSION

The salient features of this study were that, (1) subcutaneous hemangiosarcomas developed in 97% of $B6C3F_1$ mice of both sexes fed either of two concentrations of 2-Me-1-NA, and (2) such tumors did not develop in the liver, spleen, or uterus.

The present data from this preliminary study are insufficient to explain the concentration of tumors in subcutaneous tissue and mesentery, and the absence of similar lesions in other organs. Either the chemical has a predilection for the subcutaneous tissue surrounded by neutral or brown fat, or its yet unknown metabolite, if any, acts at this site. A correlation between chemical structure and organ specificity seems unlikely, as a variety of chemicals can induce these tumors.

The environmental impact created by vinyl chloride as a causative factor for hemangiosarcomas of the liver in several men working in a rubber factory has elicited much interest in this type of tumor (Block, 1974; Creech and Johnson, 1974; Falk *et al.*, 1974). The hemangiosarcomas induced by 2-Me-1-NA vary considerably in morphology and have both cutaneous and generalized forms. Further studies on the metabolism of this chemical, and on the biology of these tumors, would be needed to develop a suitable model for human vascular tumors.

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DÉVELOPPEMENT D'HÉMANGIOSARCOMES CHEZ LES SOURIS QUI ABSORBENT DE LA 2-MÉTHYL-1-NITROANTHRAQUINONE

Des hémangiosarcomes sous-cutanés sont apparus chez 97% des 172 souris $B6C3F_1$ des deux sexes qui ont absorbé 0.03 ou 0.06% de 2-méthyl-1-nitroanthraquinone en même temps que leur nourriture. Aucune relation significative avec la dose ou le sexe n'a été mise en évidence. De plus, des tumeurs vasculaires analogues se sont développées dans le mésentère de 14 souris. La 2-méthyl-1-nitroanthraquinone est donc cancérogène chez les souris $B6C3F_1$ lorsqu'elle est mélangée à leur nourriture. L'une des 97 souris-témoins a développé un hémangiosarcome splénique.

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