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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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A SOLUTION OF β-NITROSTYRENE AND STYRENE

FOR POSSIBLE CARCINOGENICITY

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 A SOLUTION OF β-NITROSTYRENE AND STYRENE 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 a solution of β-nitrostyrene and styrene 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 a solution of β-nitrostyrene and styrene was conducted by Litton Bionetics, Inc., Kensington, Maryland, 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. N. P. Page (1,2), Dr. E. K. Weisburger (1) and Dr. J. H. Weisburger (1,3). The principal investigators for the contract were Dr. F. M. Garner (4) and Dr. B. M. Ulland (4,5). Mr. S. Johnson (4) was the coprincipal investigator for the contract. Animal treatment and observation were supervised by Mr. R. Cypher (4), Mr. D. S. Howard (4) and Mr. H. D. Thornett (4); Mr. H. Paulin (4) analyzed dosed feed mixtures. Ms. J. Blalock (4) was responsible for data collection and assembly.

Histopathologic examinations were performed by Dr. A. DePaoli (4), Dr. E. Gorgacz (4), and Dr. C. Montgomery (4) at Litton Bionetics, Inc. (4), and reviewed by Dr. J. F. Hardisty (6); the pathology narratives were written by Dr. J. F. Hardisty (6), and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (7).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (8); the statistical analysis was performed by Mr. R. M. Helfand (9) and Dr. J. P. Dirkse, III (10), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (11).

This report was prepared at METREK, a Division of The MITRE Corporation (9) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (9), task leader Ms. P. Walker (9), senior biologist Mr. M. Morse (9), biochemist Mr. S. C. Drill (9), chemist Dr. N. Zimmerman (9), and technical editor Ms. P. A. Miller (9). 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 (1,12), Dr. R. A. Griesemer (1), Dr. T. E. Hamm (1), Dr. W. V. Hartwell (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,13), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay of a solution of 30 percent β-nitrostyrene and 70 percent styrene for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F1 mice. The solution of the two test materials in corn oil was administered by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. The high and low dosages utilized in the study were, respectively, 300 and 150 mg/kg for male rats; 150 and 75 mg/kg for female rats; and 175 and 87.5 mg/kg for mice of both sexes. These dosages are expressed in terms of the β-nitrostyrene contained in the styrene solution. Twenty animals of each species and sex were placed on test as controls, and were gavaged with corn oil on the same schedule as dosed animals.

A 79-week period of chemical administration was followed by an additional observation period of 29 weeks for rats, and a 78-week period of chemical administration was followed by an additional 14-week observation period for mice.

There was no significant difference between the survival of rats dosed with the test solution and that of their controls, and there was no significant association between dosage and mortality among female mice. There was a significant positive association between dosage and mortality among male mice; however, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. There was distinct mean body weight depression when high dose female mice or male rats were compared to their controls, indicating that the dosages administered to these animals may have approximated the maximum tolerated dosage. Since no distinct mean body weight depression, no significantly accelerated mortality, and no other toxic effects were associated with the administration of β-nitrostyrene and styrene to female rats or male mice, it is possible that these animals may have been able to tolerate a higher dosage.

There were no significant positive associations between administration of the solution and increased tumor incidence in rats of either sex.

When those male mice having either alveolar/bronchiolar carcinoma or alveolar/bronchiolar adenoma were combined and the resulting tumor incidences for each group were statistically analyzed, the low dose to control Fisher exact comparison was significant. The Cochran-Armitage test and the high dose to control comparison, however, were not. No other tests for tumors of any site in either male or female mice were significant.

Under the conditions of this bioassay, there was no convincing evidence for the carcinogenicity of a solution of β-nitrostyrene and styrene in Fischer 344 rats or in B6C3F1 mice.

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I. INTRODUCTION

β-Nitrostyrene (Figure 1) (NCI No. CO2211), an intermediate in polymerization reactions, was selected for bioassay by the National Cancer Institute because of a lack of adequate carcinogenicity data. The compound is usually supplied as a 30 percent solution in styrene* (Figure 1; NCI No. CO2200)(Gosselin et al., 1976) and this commercial product was selected as the material to be tested.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is (2-nitroethenyl)benzene.** It is also called ω-nitrostyrene and BNS.

β-Nitrostyrene is used as a chain stopper in styrene type polymerization reactions for the production of polystyrene plastics, synthetic rubber, and resins (Hawley, 1971). β-Nitrostyrene also possesses antibacterial, antifungal, and insecticidal activities and has been suggested for use as a repellent for bats and other rodents (Gosselin et al., 1976); however, this compound does not appear to be currently registered for pesticide use with the U.S. Environmental Protection Agency (Neylen, 1977).

Specific production data for β-nitrostyrene are not available; however, this compound is produced in commercial quantities (in excess of 1000 pounds or $1000 in value annually) by one U.S. company (Stanford Research Institute, 1977).

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*The CAS registry number for styrene is 100-42-5.

**The CAS registry number is 102-96-5.

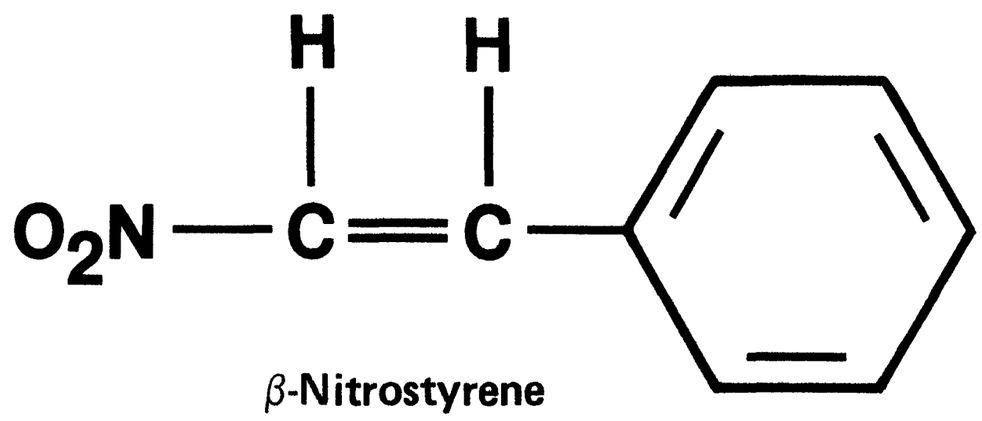

FIGURE 1
CHEMICAL STRUCTURES OF β-NITROSTYRENE AND STYRENE

The potential for exposure to β-nitrostyrene is greatest for workers in facilities which produce this compound or which utilize the compound in polymer manufacturing processes. Simultaneous exposure to styrene is likely to occur in cases of occupational exposure to β-nitrostyrene.

β-Nitrostyrene is a primary irritant to the skin and eyes (Gosselin et al., 1976).

II. MATERIALS AND METHODS

A. Chemicals

β-Nitrostyrene solution (30 percent β-nitrostyrene and 70 percent styrene) was purchased from Upjohn Laboratories, North Haven, Connecticut. The manufacturer's analysis indicated that the β-nitrostyrene content of the solution was 30 ± 0.5 percent and that 10 to 15 ppm t-butylcatechol was present as an inhibitor. The t-butylcatechol was removed from the solution by treatment with anhydrous calcium sulfate prior to testing in animals; chemical analysis of β-nitrostyrene was performed prior to removal of the inhibitor.*

Chemical analysis was performed at Litton Bionetics, Inc., Kensington, Maryland, on two batches. Nuclear magnetic resonance analysis indicated that the β-nitrostyrene concentration was 29.4 percent in the first batch and 30.6 percent in the second batch. The infrared spectra for each batch included the absorption bands characteristic of β-nitrostyrene as well as those characteristic of styrene. The presence of styrene interfered with the ultraviolet and visible spectra analyses of β-nitrostyrene.

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*When stored at -30°C, as it was for this bioassay, styrene is stable for one to three years (according to the Monsanto Company, the supplier of the styrene used in the Upjohn Laboratories' β-nitrostyrene solution). Removal of the inhibitor from the solution should have no effect on the β-nitrostyrene and the presence of β-nitrostyrene, a free radical scavenger, should confer added stability to the styrene (according to Upjohn Laboratories).

Stability studies of the first batch using the techniques of infrared analysis and methanol solubility (styrene polymer insoluble in methanol) indicated that solvent polymerization occurred after one year. The same analyses performed on the second batch revealed no evidence of compound degradation or solvent polymerization. Only the second batch of the compound was used for the chronic bioassay.

Throughout this report, the term β-nitrostyrene is used to refer to this material in styrene.

B. Dosage Preparation

Fresh solutions of β-nitrostyrene in shelf-grade A&P corn oil (Great Atlantic and Pacific Tea Company, Baltimore, Maryland) were prepared on each day that intubation was performed. Excess portions of the mixtures were disposed of rather than stored. The concentrations of β-nitrostyrene in corn oil ranged from 1.38 to 5.50 percent for rats, and from 1.61 to 3.22 percent for mice.

C. Animals

The two animal species, Fischer 344 rats and B6C3F1 mice, used in the carcinogenicity bioassay, were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Rats were supplied by A. R. Schmidt, Madison, Wisconsin, and Harlan Industries, Inc., Cumberland, Indiana. Mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

Rats and mice were approximately 4 weeks old when received. Upon receipt, animals were examined and obviously ill or runted

animals were killed. The remaining animals were quarantined for 2 weeks prior to initiation of test. Animals which did not manifest clinical signs of disease were placed on test at this time. Animals were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenance

All animals were housed by species in temperature- and humidity-controlled rooms. The temperature range was 20° to 26°C and the relative humidity was maintained between 45 and 55 percent. Incoming air was filtered through HEPA filters (Flanders Filters, McLean, Virginia) at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided 8 hours per day (9:00 a.m. to 5:00 p.m.).

All rats were housed four per cage by sex and all mice were housed five per cage by sex. Throughout the study dosed and control animals of both species were housed in polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) suspended from aluminum racks. Racks were fitted with a continuous piece of stainless steel mesh over which a sheet of filter paper was firmly secured. Filter paper was changed at 2-week intervals, when the racks were sanitized. Clean cages and bedding were provided twice weekly. Ab-sorb-dri® hardwood chip bedding (Wilner Wood Products Company, Norway, Maine) was used in polycarbonate cages for the entire study.

Acidulated water (pH 2.5) was supplied to animals in water bottles filled by an automated metering device, which was checked daily for diluting accuracy. Water bottles were changed and washed twice weekly and sipper tubes were washed at weekly intervals. All animals were supplied with Wayne Lab-Blox® meal (Allied Mills, Inc., Chicago, Illinois) in hanging stainless steel hoppers, which were refilled three times per week and sanitized weekly. Food and water were available ad libitum for both species.

All dosed and control rats were housed in a room with other rats receiving diets containing* triphenyltin hydroxide (76-87-9); diaminozide (1596-84-5); and carbromal (77-65-6).

All dosed and control mice were housed in a room with other mice receiving diets containing nitrofen (1836-75-5); p-nitrosodiphenylamine (156-10-5); acetylaminofluorene (53-96-3); nitrilotriacetic acid (139-13-9); amitrole (61-82-5); NTA trisodium salt (5064-31-3); and other mice intubated with styrene (100-42-5).

E. Gastric Intubation

Intubation was performed for three days per week on a mg/kg body weight basis, utilizing the most recently observed group mean body weight as a guide for determining the dose. All animals were weighed and dosages adjusted once monthly, based on group mean body weight.

*CAS registry numbers are given in parentheses.

Thus, although the ratio of dose to weight remained constant, the total dosage administered fluctuated with an increase or decrease in group mean body weight. Animals of each sex within a dosed group received the same dosage.

F. Selection of Initial Dose Levels

To establish the maximum tolerated dosages of β-nitrostyrene for administration to dosed animals in the chronic study, subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among several groups, each consisting of five males and five females. β-Nitrostyrene mixed with corn oil was introduced by gavage to seven of eight rat groups at dosages of 100, 147, 215, 316, 464, 681, and 1000 mg/kg and to five of six mouse groups at dosages of 100, 147, 215, 316, and 464 mg/kg. The remaining group of each species served as a control group, receiving only corn oil by gavage. Intubation was performed three times per week for 4 weeks, followed by a 2-week observation period to detect any delayed toxicity for rats; and 7 weeks, followed by a 1-week observation period, for mice. Individual body weights were recorded weekly. At the end of the observation period, all survivors were sacrificed and necropsied.

The following table indicates the mean body weight gain, relative to controls, survival, and the incidence of spotted livers observed in each of the rat groups at the end of the subchronic test.

RAT SUBCHRONIC STUDY RESULTS

mg/kg	Mean Body Weight Gain (%)[a] Males	Females	Survival Males	Females	Observation of Spotted Livers Males	Females
1000	--	--	0/5	0/5	0/5	0/5
681	--	-17	0/5	3/5	0/5	0/5
464	-21	+26	5/5	5/5	0/5	0/5
316	- 5	+34	5/5	4/5	1/5	0/5
215	-22	+34	5/5	5/5	0/5	0/5
147	- 7	+28	5/5	4/5	0/5	0/5
100	-18	+26	5/5	5/5	0/5	0/5
0	--	--	5/5	5/5	0/5	0/5

The high dosages selected for administration to dosed rats in the chronic bioassay were 300 and 150 mg/kg for males and females, respectively.

The following table indicates the mean body weight gain, relative to controls, survival and the incidence of clinical signs observed in each of the mouse groups at the end of the subchronic test.

MOUSE SUBCHRONIC STUDY RESULTS

mg/kg	Mean Body Weight Gain (%)[a] Males	Females	Survival Males	Females	Observation of Clinical Signs Males	Females
464	--	-2	0/5	1/5	0/5	0/5
316	-8	-5	4/5	2/5	1/5[b]	1/5[b]
215	+8	-6	4/5	5/5	0/5	1/5[c]
147	+3	+4	5/5	5/5	0/5	1/5[c]
100	-6	-2	5/5	5/5	0/5	0/5
0	--	--	5/5	5/5	0/5	0/5

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[a] + is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.

[b] Pale and necrotic areas observed on the liver.

[c] Pale livers observed.

The high dosage selected for administration to both male and female mice in the chronic bioassay was 175 mg/kg.

G. Experimental Design

The experimental design parameters for the chronic bioassay (species, sex, group size, dosages administered, and duration of treated and untreated observation periods) are summarized in Tables 1 and 2.

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test simultaneously. The animals were received from 2 suppliers and were combined for distribution among groups. The dosages administered to male rats were 300 and 150 mg/kg. Throughout this report those male rats receiving the former dosage are referred to as the high dose group and those receiving the latter dosage are referred to as the low dose group. The dosages administered to female rats were 150 and 75 mg/kg. Throughout this report those female rats receiving the former dosage are referred to as the high dose group and those receiving the latter dosage are referred to as the low dose group. All dosed rats were administered β-nitrostyrene at the dosages indicated for 79 weeks, followed by a 29-week observation period. The dosages are expressed in terms of the β-nitrostyrene contained in the styrene solution.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test simultaneously. The dosages administered were 175 and 87.5 mg/kg. Throughout this report those

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
β-NITROSTYRENE GAVAGE EXPERIMENT

INITIAL GROUP SIZE	β-NITROSTYRENE DOSAGE[a]	OBSERVATION PERIOD	
		TREATED (WEEKS)	UNTREATED (WEEKS)
MALE			
CONTROL	20	0	108
LOW DOSE	50	150 0	79 29
HIGH DOSE	50	300 0	79 29
FEMALE			
CONTROL	20	0	108
LOW DOSE	50	75 0	79 29
HIGH DOSE	50	150 0	79 29

[a]Dosages, administered by gavage 3 days/week, are given in mg/kg body weight and are based on β-nitrostyrene contained in the styrene solution.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
β-NITROSTYRENE GAVAGE EXPERIMENT

INITIAL GROUP SIZE	β-NITROSTYRENE DOSAGE[a]	OBSERVATION PERIOD	
		TREATED (WEEKS)	UNTREATED (WEEKS)
MALE			
CONTROL	20	0	92
LOW DOSE	50	87.5 0	78 14
HIGH DOSE	50	175 0	78 14
FEMALE			
CONTROL	20	0	92
LOW DOSE	50	87.5 0	78 14
HIGH DOSE	50	175 0	78 14

[a]Dosages, administered by gavage 3 days/week, are given in mg/kg body weight and are based on β-nitrostyrene contained in the styrene solution.

mice receiving the former dosage are referred to as the high dose groups and those receiving the latter dosage are referred to as the low dose groups. All dosed mice were administered β-nitrostyrene at the dosages indicated for 78 weeks, followed by a 14-week observation period. The dosages are expressed in terms of the β-nitrostyrene contained in the styrene solution.

Controls were gavaged with corn oil with the same frequency and in the same volumes administered to the high dose groups.

H. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment and body weights were recorded once a week for the first 6 weeks, every 2 weeks for the next 12 weeks, and at monthly intervals for the remainder of the bioassay. All animals were inspected twice daily for mortality. Food consumption data were collected at monthly intervals from 20 percent of the animals in each group.

All moribund animals or animals that developed large, palpable masses that jeopardized their health were sacrificed. A necropsy was performed on each animal regardless of whether it died, was sacrificed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized using carbon dioxide, and were immediately necropsied. Gross and microscopic examinations were performed on all major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in a 10 percent neutral buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, 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 recorded in each group at the time that the test was initiated.

I. 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

A distinct and consistent mean body weight depression was associated with dosage administration in male rats. There was no significant mean body weight depression apparent in dosed females (Figure 2).

No other clinical signs were recorded.

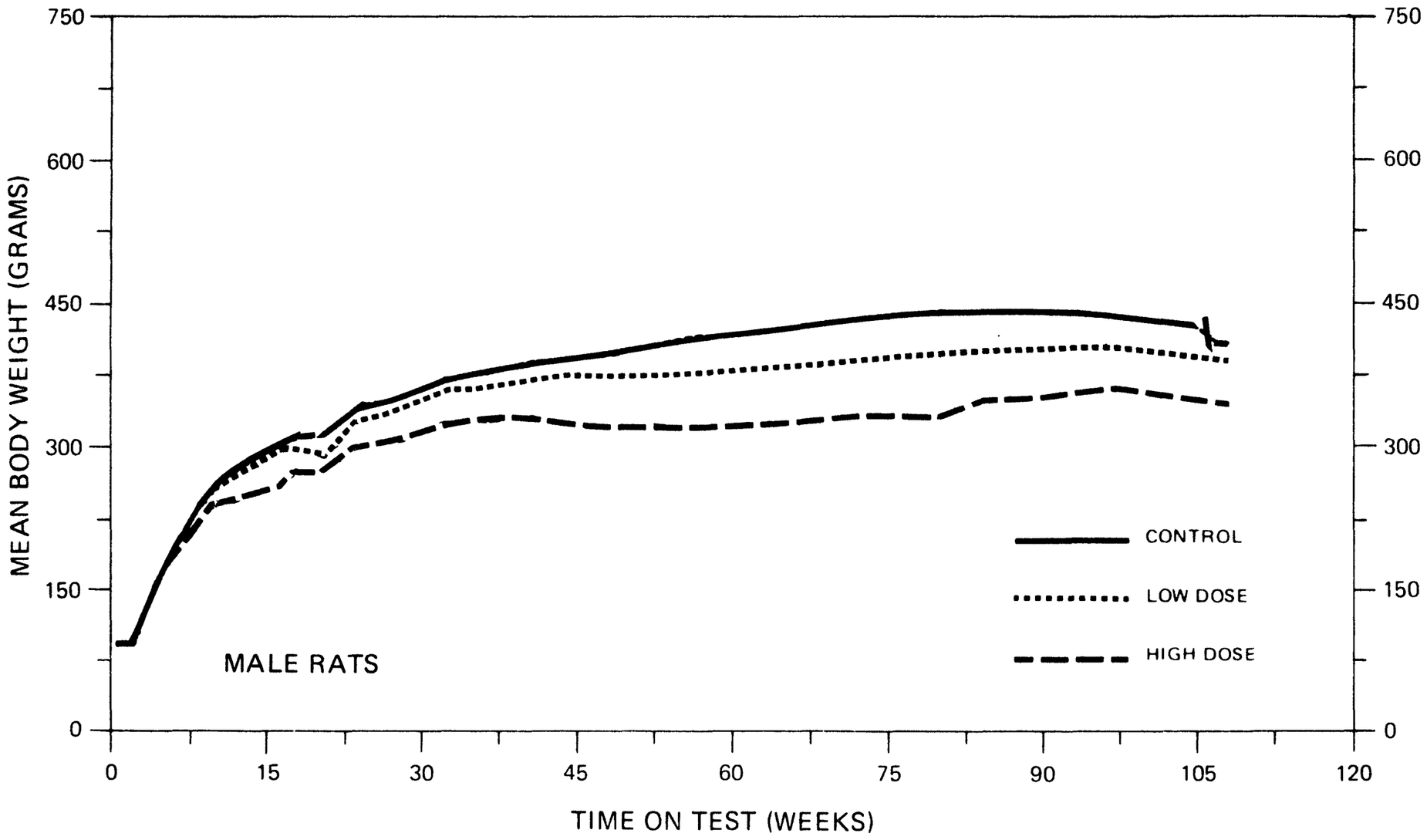
B. Survival

The estimated probabilities of survival for male and female rats in the control and β-nitrostyrene-dosed groups are shown in Figure 3. For both male and female rats there were no significant differences between the survival of the dosed groups and that of the control groups.

For the males adequate numbers of rats were at risk from late-developing tumors, as 31/50 (62 percent) of the high dose, 34/50 (68 percent) of the low dose, and 16/20 (80 percent) of the controls survived on test until the end of the study. For females survival was also adequate, as 31/50 (62 percent) of the high dose, 33/50 (66 percent) of the low dose, and 12/20 (60 percent) of the controls survived on test until the end of the study.

C. Pathology

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



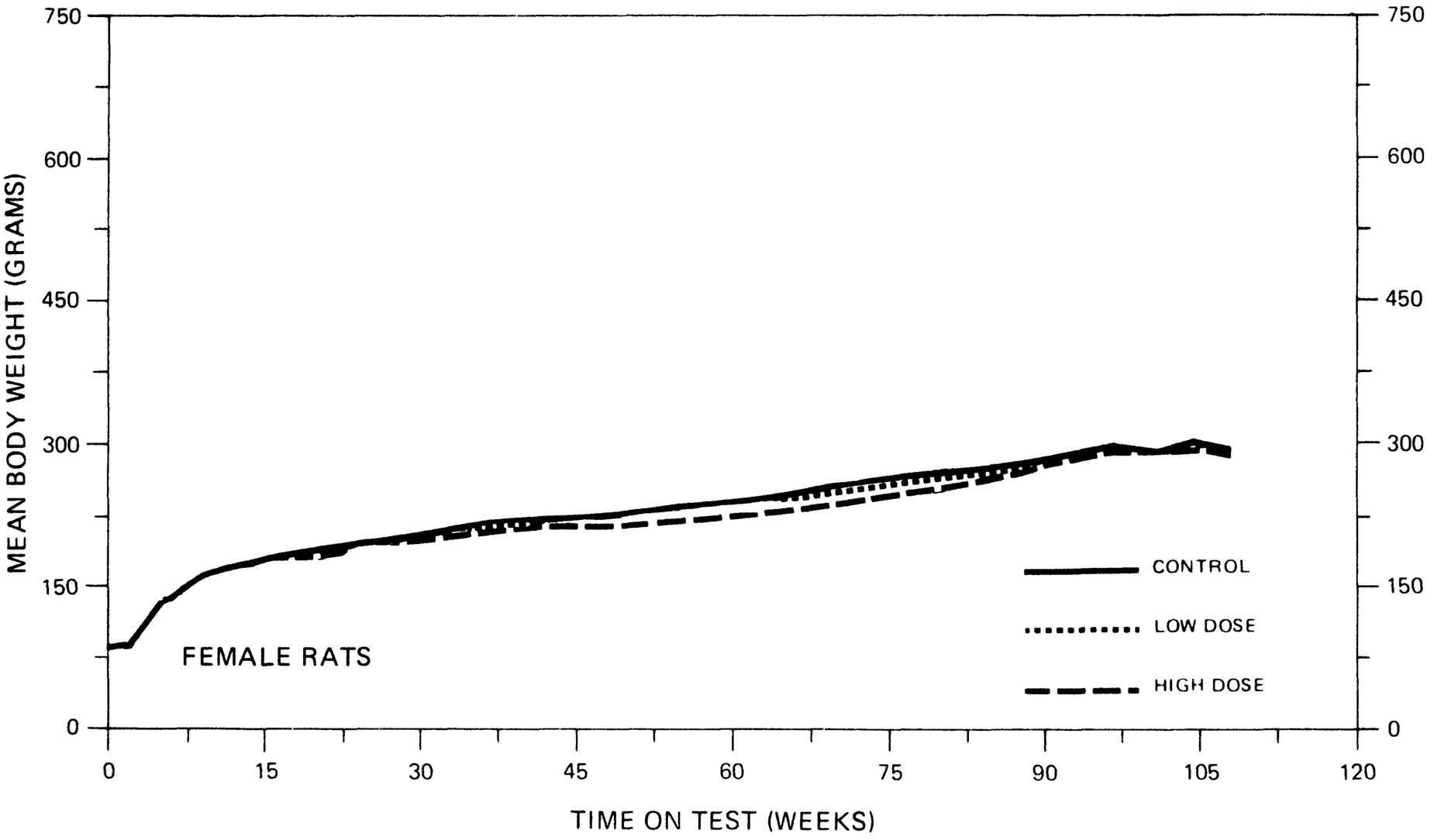


FIGURE 2
GROWTH CURVES FOR β-NITROSTYRENE CHRONIC STUDY RATS

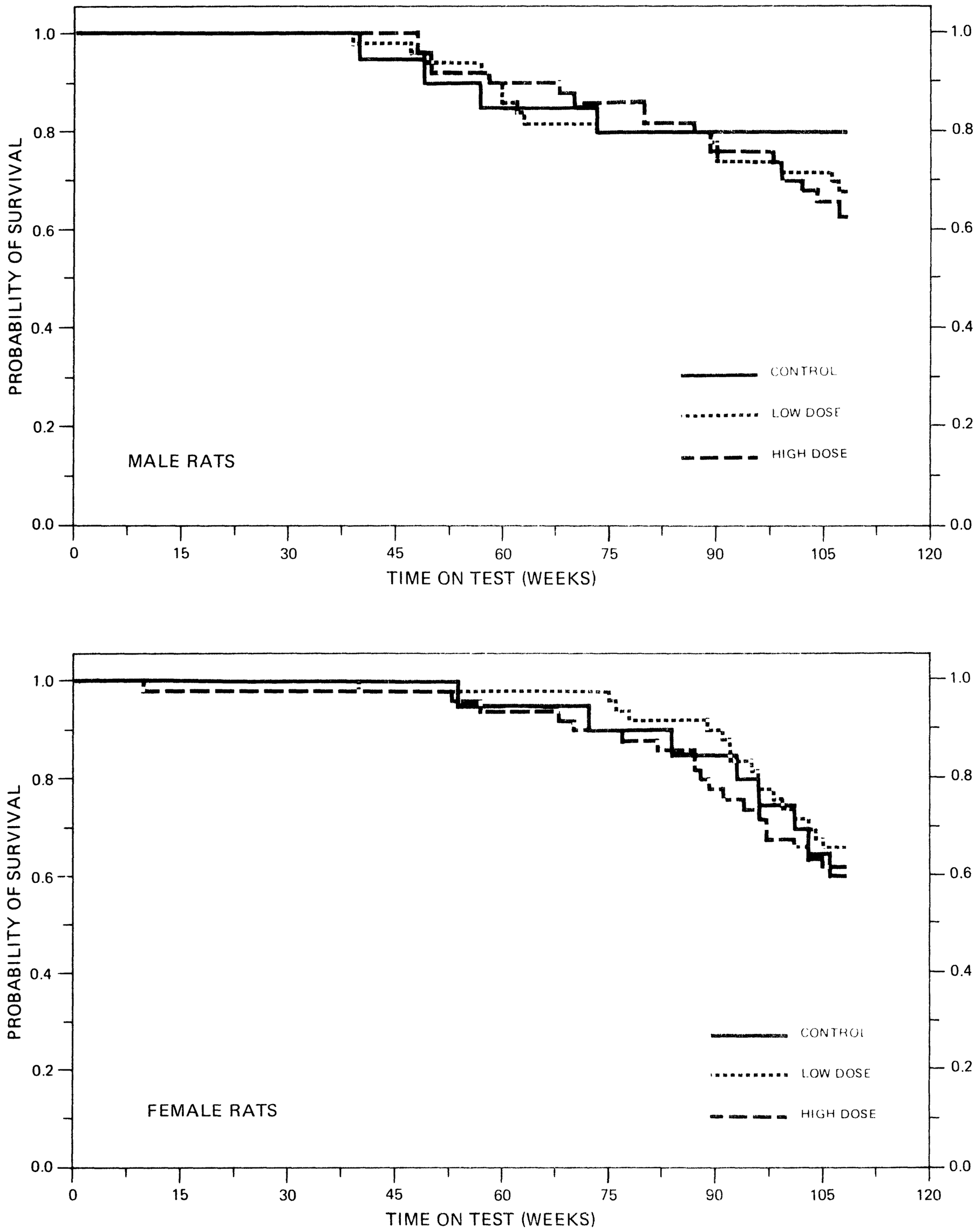


FIGURE 3
SURVIVAL COMPARISONS OF β-NITROSTYRENE CHRONIC STUDY RATS

A variety of neoplastic lesions was seen with approximately equal frequency in the control and dosed rats. The most frequently observed neoplasm in the male rats was interstitial-cell tumors of the testis. A high incidence of this neoplasm is characteristic of aged male Fischer 344 rats. Chromophobe adenomas of the pituitary and fibroadenomas of the mammary gland were the most frequently observed neoplasms in the female rats. Some types of neoplasms occurred only, or with increased frequency, in rats of dosed groups as compared with control groups. The nature and incidence of these neoplasms are similar to that seen spontaneously in aged Fischer 344 rats.

A variety of inflammatory, degenerative and proliferative lesions commonly seen in aged Fischer 344 rats were seen with approximately equal frequency in dosed and control animals of each sex.

Based on the results of this pathology examination, β-nitrostyrene was not carcinogenic to Fischer 344 rats under the conditions of this study.

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

TABLE 3

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

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	4/17(0.24)	4/42(0.10)	1/44(0.02)
P Values[c]	P = 0.010(N)	N.S.	P = 0.019(N)
Relative Risk (Control)[d]	---	0.405	0.097
Lower Limit	---	0.088	0.002
Upper Limit	---	1.976	0.902
Weeks to First Observed Tumor	108	99	107
Adrenal: Pheochromocytoma[b]	1/19(0.05)	3/48(0.06)	1/46(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.187	0.413
Lower Limit	---	0.105	0.006
Upper Limit	---	61.031	31.749
Weeks to First Observed Tumor	73	108	108
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	0/18(0.00)	1/47(0.02)	3/41(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.021	0.277
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	108	108

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Pancreatic Islets: Islet-Cell Adenoma[b]	2/18(0.11)	1/42(0.02)	0/42(0.00)
P Values[c]	P = 0.039(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.214	0.000
Lower Limit	---	0.004	0.000
Upper Limit	---	3.916	1.434
Weeks to First Observed Tumor	108	108	---
Testis: Interstitial-Cell Tumor[b]	15/19(0.79)	38/47(0.81)	39/46(0.85)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.024	1.074
Lower Limit	---	0.805	0.847
Upper Limit	---	1.453	1.481
Weeks to First Observed Tumor	107	62	80

[a]Treated groups received doses of 150 or 300 mg/kg by gavage of β-nitrostyrene in a styrene solution.

[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 β-NITROSTYRENE[a]

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	3/20(0.15)	4/50(0.08)	2/50(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.533	0.267
Lower Limit	---	0.102	0.024
Upper Limit	---	3.410	2.190
Weeks to First Observed Tumor	93	78	68
Pituitary: Chromophobe Adenoma[b]	5/18(0.28)	15/49(0.31)	18/44(0.41)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.102	1.473
Lower Limit	---	0.466	0.650
Upper Limit	---	3.434	4.418
Weeks to First Observed Tumor	96	92	88
Mammary Gland: Fibroadenoma[b]	2/20(0.10)	5/50(0.10)	7/50(0.14)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.000	1.400
Lower Limit	---	0.184	0.303
Upper Limit	---	10.007	13.138
Weeks to First Observed Tumor	84	108	88

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Uterus: Adenocarcinoma NOS[b]	1/20(0.05)	3/48(0.06)	0/45(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.250	0.000
Lower Limit	---	0.110	0.000
Upper Limit	---	64.251	8.288
Weeks to First Observed Tumor	108	92	---
Uterus: Endometrial Stromal Polyp[b]	1/20(0.05)	9/48(0.19)	8/45(0.18)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	3.750	3.556
Lower Limit	---	0.585	0.537
Upper Limit	---	160.325	153.667
Weeks to First Observed Tumor	108	91	87

[a]Treated groups received doses of 75 or 150 mg/kg by gavage of β-nitrostyrene in a styrene solution.

[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.

None of the statistical tests for any site in rats of either sex indicated a significant positive association between the administration of β-nitrostyrene and an increased tumor incidence. Thus, at the dose levels used in this bioassay there was no statistical evidence that β-nitrostyrene was a carcinogen in Fischer 344 rats.

For male rats the possibility of a negative association between dose and incidence was noted for pituitary chromophobe adenomas. The Cochran-Armitage test also showed a significant negative association between dose and the incidence of islet-cell adenomas of the pancreatic islets, but the Fisher exact tests were not significant.

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 β-nitrostyrene that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

There was no consistent mean body weight depression associated with administration of the compound in male mice or low dose female mice. High dose females evidenced a distinct mean body weight depression when compared with the control group (Figure 4).

No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female rats in the control and β-nitrostyrene-dosed groups are shown in Figure 5. For male mice the Tarone test indicated a significant (P = 0.007) association between dosage and mortality. For female mice no significant results for either Tarone or Cox tests were observed.

For the males adequate numbers of mice were at risk from late-developing tumors, as 33/50 (66 percent) of the high dose, 43/50 (86 percent) of the low dose, and 18/20 (90 percent) of the controls survived on test until the end of the study. Of the 14 high dose males that died in week 36, all had hemorrhage or a hemorrhagic necrosis of the liver.

For the females survival was also adequate, as 38/50 (76 percent) of the high dose, 47/50 (94 percent) of the low dose, and 17/20 (85 percent) of the controls survived on test until the end of the study.

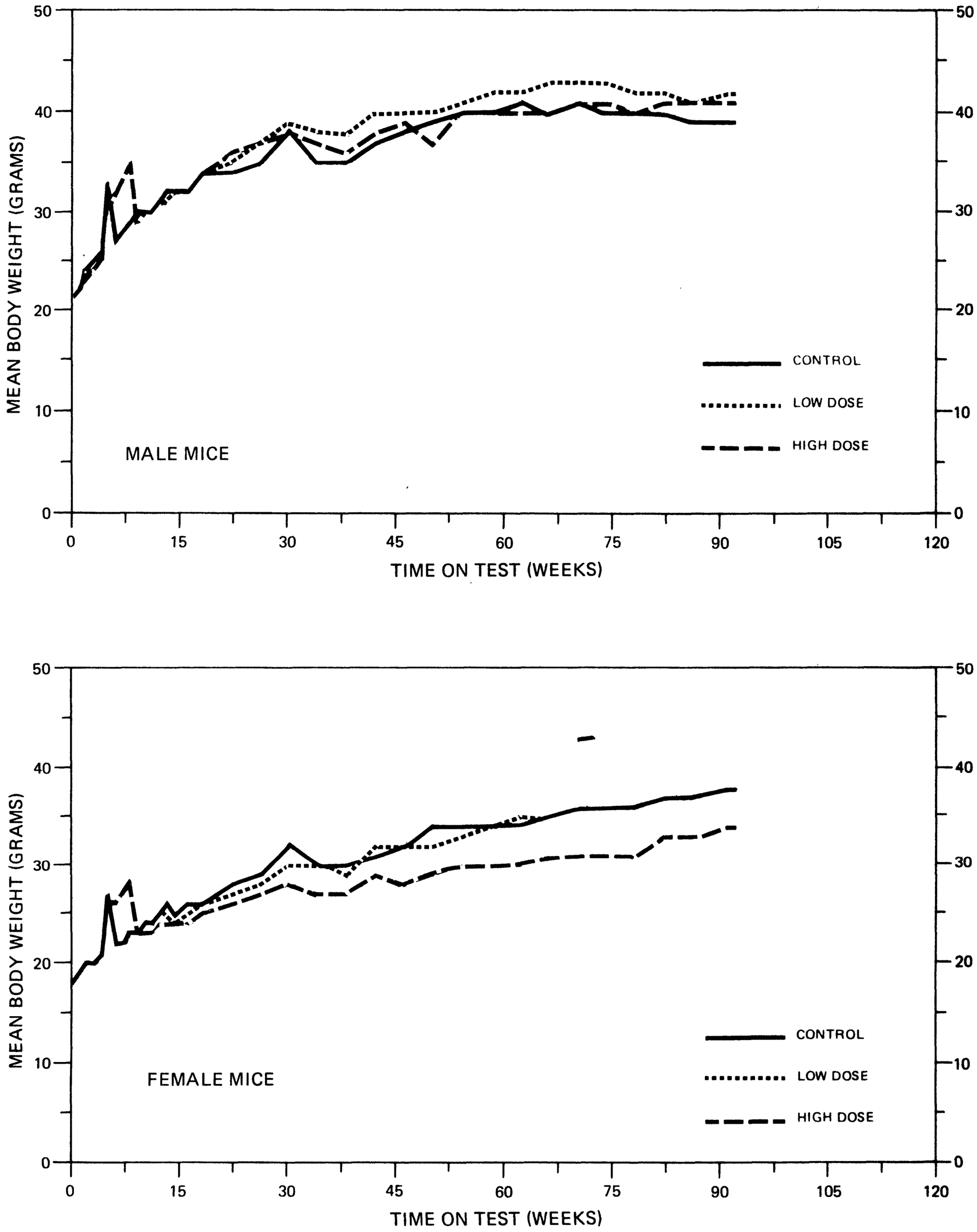


FIGURE 4
GROWTH CURVES FOR β-NITROSTYRENE CHRONIC STUDY MICE

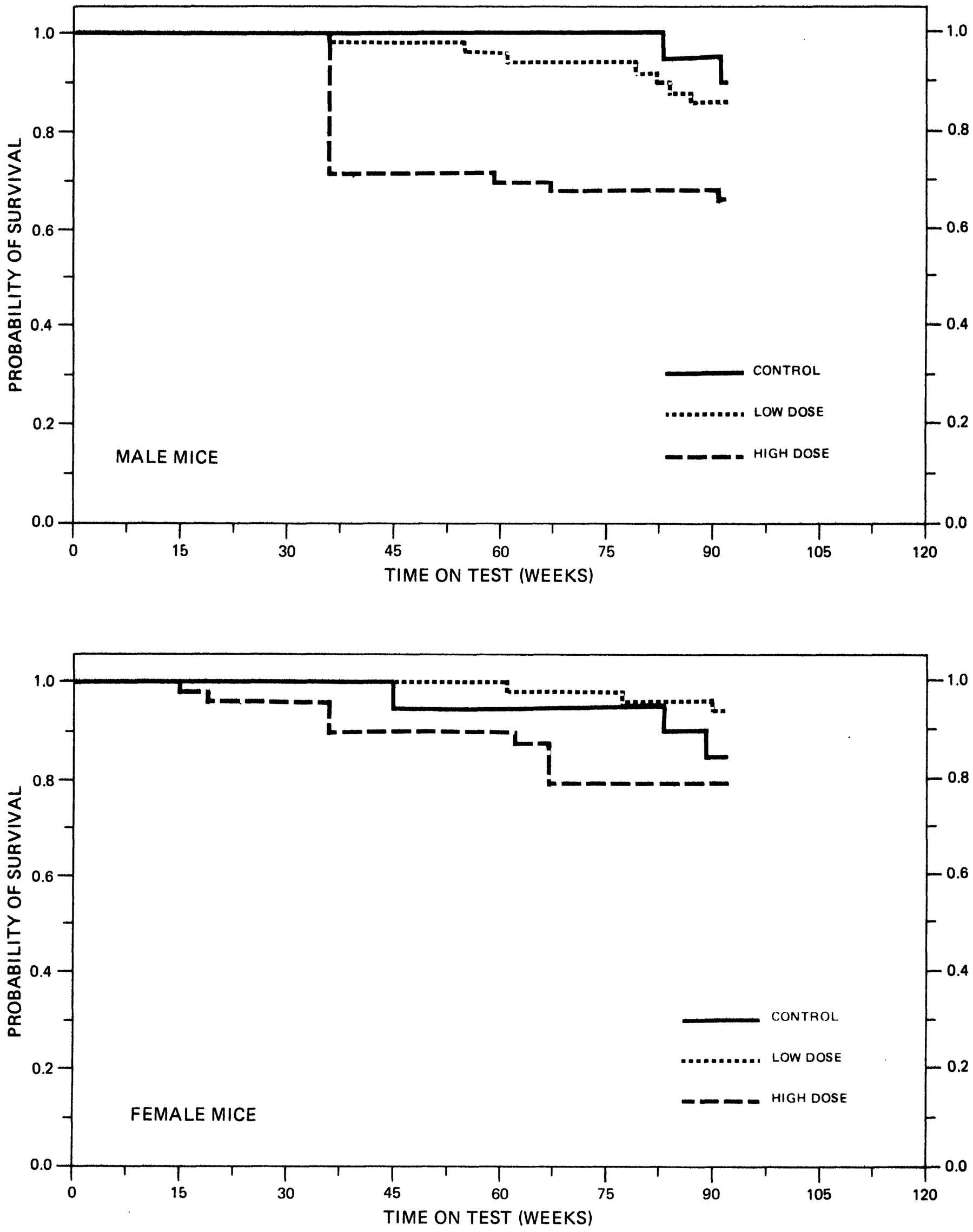


FIGURE 5
SURVIVAL COMPARISONS OF β-NITROSTYRENE CHRONIC STUDY MICE

C. Pathology

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

A variety of neoplastic lesions was present in the dosed and control groups. There were instances in this study where neoplastic lesions occurred only, or with increased frequency, in mice of dosed groups as compared with control groups. All observed neoplasms were of types and incidences known to occur spontaneously in B6C3F1 mice.

There was an increased incidence of hemorrhage and necrosis in the livers of high dose male mice when compared with low dose or control mice (16/50 [32 percent] high dose, 3/50 [6 percent] low dose, 1/20 [5 percent] controls).

A variety of other nonneoplastic lesions commonly seen in B6C3F1 mice occurred with approximately equal frequency in dosed and control mice.

Based on the results of this pathology examination, β-nitrostyrene was not carcinogenic in B6C3F1 mice.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or β-nitrostyrene dosed groups and where such tumors were observed in at least 5 percent of the group. Because of the number of early deaths observed,

TABLE 5

TIME-ADJUSTED ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH β-NITROSTYRENE[a,f]

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/20(0.00)	3/49(0.06)	1/36(0.03)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.255	0.031
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	61	92
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	0/20(0.00)	11/49(0.22)	2/36(0.06)
P Values[c]	N.S.	P = 0.016	N.S.
Departure from Linear Trend[e]	P = 0.003	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	1.413	0.171
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	61	92
Hematopoietic System: Malignant Lymphoma[b]	2/20(0.10)	3/49(0.06)	1/36(0.03)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.612	0.278
Lower Limit	---	0.078	0.005
Upper Limit	---	6.996	5.057
Weeks to First Observed Tumor	91	82	91

TABLE 5 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	2/20(0.10)	1/49(0.02)	7/36(0.19)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.030	---	---
Relative Risk (Control)[d]	---	0.204	1.944
Lower Limit	---	0.004	0.423
Upper Limit	---	3.754	17.964
Weeks to First Observed Tumor	92	92	91
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	6/20(0.30)	6/49(0.12)	8/36(0.22)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.408	0.741
Lower Limit	---	0.129	0.271
Upper Limit	---	1.372	2.267
Weeks to First Observed Tumor	92	92	91
Thyroid: Follicular-Cell Adenoma or Follicular-Cell Carcinoma[b]	0/18(0.00)	0/42(0.00)	2/26(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.214
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	92

TABLE 5 (CONCLUDED)

[a]Treated groups received doses of 87.5 or 175 mg/kg by gavage of β-nitrostyrene in a styrene solution.

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

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

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

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

[f]These analyses were based solely upon animals surviving at least 52 weeks, except for sites where the first tumor of interest was observed earlier than 52 weeks in any group of this sex and species, where the analyses were based upon all animals that survived until or past the date that the first tumor was observed.

TABLE 6

TIME-ADJUSTED ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE MICE TREATED WITH β-NITROSTYRENE[a,e]

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Malignant Lymphoma[b]	1/19(0.05)	5/50(0.10)	3/43(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.900	1.326
Lower Limit	---	0.238	0.117
Upper Limit	---	87.985	67.933
Weeks to First Observed Tumor	83	92	92
Pituitary: Chromophobe Adenoma[b]	0/15(0.00)	0/35(0.00)	2/28(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.168
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	92
Thyroid: Follicular-Cell Adenoma or Follicular-Cell Carcinoma[b]	0/17(0.00)	0/40(0.00)	2/34(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.155
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	92

TABLE 6 (CONCLUDED)

[a]Treated groups received doses of 87.5 or 175 mg/kg by gavage of β-nitrostyrene in a styrene solution.

[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]These analyses were based solely upon animals surviving at least 52 weeks, except for sites where the first tumor of interest was observed earlier than 52 weeks in any group of this sex and species, where the analyses were based upon all animals that survived until or past the date that the first tumor was observed.

time-adjusted analyses were performed. In these analyses only those mice surviving at least 52 weeks were considered.

For male mice the Fisher exact test indicated a significantly ($P = 0.016$) higher combined incidence of alveolar/bronchiolar carcinomas or alveolar/bronchiolar adenomas in the low dose group than in the control group. The high dose Fisher exact test and the Cochran-Armitage test, however, were not significant.

No other tests for any site in either male or female mice were significant. Thus, based upon these results there was no conclusive statistical evidence that β-nitrostyrene was a carcinogen in B6C3F1 mice under the conditions of this bioassay.

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 5 and 6, 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 mice by β-nitrostyrene that could not be established under the conditions of this test.

V. DISCUSSION

There was no significant difference between the survival of rats dosed with the solution of β-nitrostyrene and styrene and that of their controls, and there was no significant association between dosage and mortality among female mice. There was a significant positive association between dosage and mortality among male mice; however, adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. There was distinct mean body weight depression when high dose female mice or male rats were compared to their controls, indicating that the dosages administered to these animals may have approximated the maximum tolerated dosage. Although 16/50 (32 percent) high dose male mice had hemorrhagic necrosis of the liver, 14 of these 16 died as a group during week 36; it was considered that the death of these animals was due to a handling accident and that the effect was not associated with compound administration. Since no distinct mean body weight depression, no significant accelerated mortality, and no other toxic effects were associated with the administration of β-nitrostyrene to female rats or male mice, it is possible that these animals may have been able to tolerate a higher dosage.

There were no significant positive associations between administration of the solution and increased tumor incidence in rats of either sex.

When those male mice having either alveolar/bronchiolar carcinoma or alveolar/bronchiolar adenoma were combined and the resulting tumor incidences for each group were statistically analyzed, the low dose to control Fisher exact comparison was significant. The Cochran-Armitage test and the high dose to control comparison, however, were not. No other tests for tumors of any site in either male or female mice were significant.

Under the conditions of this bioassay, there was no convincing evidence for the carcinogenicity of a solution of β-nitrostyrene and styrene in Fischer 344 rats or in B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH β-NITROSTYRENE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH β-NITROSTYRENE

	CONTROL (VEH) 11-1085	LOW DOSE 11-1083	HIGH DOSE 11-1081
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	46
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
PAPILLOMA, NOS	1 (5%)	1 (2%)	
TRICHOEPITHELIOMA			2 (4%)
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA	1 (5%)		
LIPOMA		1 (2%)	
OSTEOSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(45)
NEOPLASM, NOS, METASTATIC		1 (2%)	
CARCINOMA, NOS, METASTATIC		1 (2%)	
INTERSTITIAL-CELL TUMOR, METASTA	1 (5%)		
SARCOMA, NOS		2 (4%)	
CARCINOSARCOMA		1 (2%)	
OSTEOSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
LEUKEMIA,NOS		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(16)	(49)	(45)
SARCOMA, NOS		1 (2%)	

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

TABLE A1 (CONTINUED)

	CONTROL (VEH) 11-1085	LOW DOSE 11-1083	HIGH DOSE 11-1081
#LIVER	(18)	(49)	(46)
CARCINOMA, NOS, METASTATIC		1 (2%)	
HEPATOCELLULAR ADENOMA			1 (2%)
HEPATOCELLULAR CARCINOMA		1 (2%)	
#PANCREAS	(18)	(42)	(42)
CARCINOMA, NOS, METASTATIC		1 (2%)	
#STOMACH	(19)	(48)	(45)
CARCINOMA, NOS, METASTATIC		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(19)	(48)	(46)
CARCINOMA,NOS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(42)	(44)
CHROMOPHOBE ADENOMA	4 (24%)	4 (10%)	1 (2%)
#ADRENAL	(19)	(48)	(46)
CORTICAL ADENOMA			1 (2%)
CORTICAL CARCINOMA		1 (2%)	
PHEOCHROMOCYTOMA	1 (5%)	3 (6%)	1 (2%)
#THYROID	(18)	(47)	(41)
ADENOMA, NOS	1 (6%)		
C-CELL ADENOMA		1 (2%)	1 (2%)
C-CELL CARCINOMA			2 (5%)
#PANCREATIC ISLETS	(18)	(42)	(42)
ISLET-CELL ADENOMA	2 (11%)	1 (2%)	
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(20)	(50)	(50)
CARCINOMA,NOS		1 (2%)	
ADENOMA, NOS		1 (2%)	
#PROSTATE	(16)	(44)	(40)
CARCINOMA, NOS, METASTATIC		1 (2%)	

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

TABLE A1 (CONTINUED)

	CONTROL (VEH) 11-1085	LOW DOSE 11-1083	HIGH DOSE 11-1081
#TESTIS	(19)	(47)	(46)
INTERSTITIAL-CELL TUMOR	15 (79%)	38 (81%)	39 (85%)
INTERSTITIAL-CELL TUMOR, MALIGNA	1 (5%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
SARCOMA, NOS			1 (2%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	12	16
MORIBUND SACRIFICE	1	4	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	34	31
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	CONTROL (VEH) 11-1085	LOW DOSE 11-1083	HIGH DOSE 11-1081
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	17	41	42
TOTAL PRIMARY TUMORS	26	60	53
TOTAL ANIMALS WITH BENIGN TUMORS	16	40	42
TOTAL BENIGN TUMORS	25	50	46
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	8	6
TOTAL MALIGNANT TUMORS	1	9	6
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	2	1
TOTAL SECONDARY TUMORS	1	6	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	1
TOTAL UNCERTAIN TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH β-NITROSTYRENE

	CONTROL (VEH) 11-1086	LOW DOSE 11-1084	HIGH DOSE 11-1082
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SARCOMA, NOS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(47)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		2 (4%)	
LEUKEMIA,NOS	2 (10%)	1 (2%)	2 (4%)
UNDIFFERENTIATED LEUKEMIA	1 (5%)		
#SPLEEN	(20)	(47)	(45)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
MAST-CELL TUMOR		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(46)
HEPATOCELLULAR ADENOMA		1 (2%)	
URINARY SYSTEM			
NONE			

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

TABLE A2 (CONTINUED)

	CONTROL (VEH) 11-1086	LOW DOSE 11-1084	HIGH DOSE 11-1082
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(49)	(44)
CHROMOPHOBE ADENOMA	5 (28%)	15 (31%)	18 (41%)
#ADRENAL	(19)	(47)	(47)
CORTICAL ADENOMA			1 (2%)
PHEOCHROMOCYTOMA	1 (5%)		
#THYROID	(19)	(46)	(41)
FOLLICULAR-CELL ADENOMA			1 (2%)
C-CELL ADENOMA		2 (4%)	2 (5%)
#PANCREATIC ISLETS	(19)	(44)	(46)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
FIBROADENOMA	2 (10%)	5 (10%)	7 (14%)
*PREPUTIAL GLAND	(20)	(50)	(50)
SEBACEOUS ADENOMA			1 (2%)
#UTERUS	(20)	(48)	(45)
ADENOCARCINOMA, NOS	1 (5%)	3 (6%)	
LEIOMYOMA		1 (2%)	1 (2%)
LEIOMYOSARCOMA		1 (2%)	
ENDOMETRIAL STROMAL POLYP	1 (5%)	9 (19%)	8 (18%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(20)	(50)	(50)
SQUAMOUS CELL PAPILLOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A2 (CONCLUDED)

	CONTROL (VEH) 11-1086	LOW DOSE 11-1084	HIGH DOSE 11-1082
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
NEOPLASM, NOS, MALIGNANT			1 (2%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	11	9
MORIBUND SACRIFICE	7	6	10
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	12	33	31
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	34	30
TOTAL PRIMARY TUMORS	13	43	45
TOTAL ANIMALS WITH BENIGN TUMORS	9	28	27
TOTAL BENIGN TUMORS	9	34	41
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	8	4
TOTAL MALIGNANT TUMORS	4	8	4
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
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 β-NITROSTYRENE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH β-NITROSTYRENE

	CONTROL (VEH) 22-2085	LOW DOSE 22-2083	HIGH DOSE 22-2081
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (5%)		
ALVEOLAR/BRONCHIOLAR ADENOMA		8 (16%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		3 (6%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	2 (10%)	1 (2%)	
#MESENTERIC L. NODE	(17)	(21)	(33)
MALIGNANT LYMPHOMA, NOS		1 (5%)	1 (3%)
#PEYERS PATCH	(19)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
HEPATOCELLULAR ADENOMA	4 (20%)	5 (10%)	1 (2%)
HEPATOCELLULAR CARCINOMA	2 (10%)	1 (2%)	7 (14%)

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

	CONTROL (VEH) 22-2085	LOW DOSE 22-2083	HIGH DOSE 22-2081
HEMANGIOMA		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(18)	(43)	(35)
FOLLICULAR-CELL ADENOMA			1 (3%)
FOLLICULAR-CELL CARCINOMA			1 (3%)
REPRODUCTIVE SYSTEM			
#TESTIS	(20)	(46)	(47)
SEMINOMA/DYSGERMINOMA		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B1 (CONCLUDED)

	CONTROL (VEH) 22-2085	LOW DOSE 22-2083	HIGH DOSE 22-2081
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATUPAL DEATH@	2	6	16
MORIBUND SACRIFICE		1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	43	33
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	19	12
TOTAL PRIMARY TUMORS	8	22	13
TOTAL ANIMALS WITH BENIGN TUMORS	4	13	3
TOTAL BENIGN TUMORS	4	14	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	7	9
TOTAL MALIGNANT TUMORS	4	8	10
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 β-NITROSTYRENE

	CONTROL (VEH) 22-2086	LOW DOSE 22-2084	HIGH DOSE 22-2082
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			2
ANIMALS NECROPSIED	20	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	47
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(48)
CARCINOSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(46)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(48)
MALIGNANT LYMPHOMA, NOS	1 (5%)	4 (8%)	2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#SPLEEN	(19)	(43)	(44)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(47)	(47)
HEPATOCELLULAR ADENOMA	1 (5%)	1 (2%)	
URINARY SYSTEM			
NONE			

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

TABLE B2 (CONTINUED)

	CONTROL (VEH) 22-2086	LOW DOSE 22-2084	HIGH DOSE 22-2082
ENDOCRINE SYSTEM			
#PITUITARY	(15)	(35)	(28)
CHROMOPHOBE ADENOMA			2 (7%)
#THYROID	(17)	(40)	(36)
FOLLICULAR-CELL ADENOMA			1 (3%)
FOLLICULAR-CELL CARCINOMA			1 (3%)
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(48)	(46)
LEIOMYOMA		1 (2%)	1 (2%)
ENDOMETRIAL STROMAL POLYP		1 (2%)	
HEMANGIOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B2 (CONCLUDED)

	CONTROL (VEH) 22-2086	LOW DOSE 22-2084	HIGH DOSE 22-2082
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	2	9
MORIBUND SACRIFICE	1	1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	47	38
ANIMAL MISSING			2
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	10	10
TOTAL PRIMARY TUMORS	2	10	10
TOTAL ANIMALS WITH BENIGN TUMORS	1	4	5
TOTAL BENIGN TUMORS	1	4	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	6	5
TOTAL MALIGNANT TUMORS	1	6	5
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

@ INCLUDES AUTOLYZED ANIMALS

* 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 β-NITROSTYRENE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH β-NITROSTYRENE

	CONTROL (VEH) 11-1085	LOW DOSE 11-1083	HIGH DOSE 11-1081
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	46
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
CUTANEOUS HORN			1 (2%)
RESPIRATORY SYSTEM			
#TRACHEA	(19)	(48)	(45)
INFLAMMATION, NOS	1 (5%)		
HYPERPLASIA, NOS			1 (2%)
#LUNG	(19)	(49)	(45)
EMPHYSEMA, NOS			1 (2%)
CONGESTION, NOS			1 (2%)
EDEMA, NOS			1 (2%)
HEMORRHAGE	1 (5%)	1 (2%)	2 (4%)
BRONCHOPNEUMONIA, NOS	1 (5%)	1 (2%)	1 (2%)
PNEUMONIA, CHRONIC MURINE	5 (26%)	28 (57%)	10 (22%)
HYPERPLASIA, ADENOMATOUS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(17)	(46)	(41)
HYPERPLASIA, NOS		1 (2%)	
#SPLEEN	(19)	(47)	(44)
FIBROSIS, FOCAL		1 (2%)	
HEMOSIDEROSIS			1 (2%)
#LYMPH NODE	(16)	(44)	(40)
CYST, NOS	1 (6%)	2 (5%)	

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

TABLE C1 (CONTINUED)

	CONTROL (VEH) 11-1085	LOW DOSE 11-1083	HIGH DOSE 11-1081
CIRCULATORY SYSTEM			
#MYOCARDIUM	(19)	(49)	(45)
INFLAMMATION, FOCAL		1 (2%)	
FIBROSIS	3 (16%)	5 (10%)	4 (9%)
FIBROSIS, FOCAL			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(18)	(49)	(46)
CONGESTION, NOS		1 (2%)	
CIRRHOSIS, BILIARY			1 (2%)
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY	1 (6%)	1 (2%)	1 (2%)
HYPERPLASIA, NOS	1 (6%)	1 (2%)	
LEUKOCYTOSIS, NOS		1 (2%)	
#BILE DUCT	(18)	(49)	(46)
DILATATION, NOS		1 (2%)	
#PANCREAS	(18)	(42)	(42)
ATROPHY, NOS		1 (2%)	
#PANCREATIC ACINUS	(18)	(42)	(42)
ATROPHY, NOS		1 (2%)	
#STOMACH	(19)	(48)	(45)
ULCER, NOS	1 (5%)		
#SMALL INTESTINE	(19)	(48)	(38)
NEMATODIASIS		1 (2%)	
#LARGE INTESTINE	(14)	(48)	(39)
NEMATODIASIS	5 (36%)	6 (13%)	3 (8%)
#COLON	(14)	(48)	(39)
NEMATODIASIS	1 (7%)		
HYPERPLASIA, LYMPHOID		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(19)	(48)	(46)
HEMORRHAGE		1 (2%)	

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

TABLE C1 (CONTINUED)

	CONTROL (VEH) 11-1085	LOW DOSE 11-1083	HIGH DOSE 11-1081
INFLAMMATION, CHRONIC	10 (53%)	18 (38%)	12 (26%)
FIBROSIS, FOCAL		1 (2%)	
AMYLOIDOSIS			1 (2%)
#URINARY BLADDER	(18)	(47)	(32)
CALCULUS, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(42)	(44)
CYST, NOS		2 (5%)	
HEMOSIDEROSIS		1 (2%)	
#ADRENAL CORTEX	(19)	(48)	(46)
METAMORPHOSIS FATTY	1 (5%)		
#THYROID	(18)	(47)	(41)
HYPERPLASIA, C-CELL		1 (2%)	
#PANCREATIC ISLETS	(18)	(42)	(42)
HYPERPLASIA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
DILATATION/DUCTS	1 (5%)		
INFLAMMATION, CHRONIC			1 (2%)
LACTATION		1 (2%)	
*PREPUTIAL GLAND	(20)	(50)	(50)
CYSTIC DUCTS			1 (2%)
#TESTIS	(19)	(47)	(46)
ATROPHY, NOS	2 (11%)	1 (2%)	3 (7%)
NERVOUS SYSTEM			
#BRAIN	(19)	(49)	(46)
CONGESTION, NOS			1 (2%)
GLIOSIS	1 (5%)		
#MEDULLA OBLONGATA	(19)	(49)	(46)
PERIVASCULAR CUFFING			1 (2%)

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

TABLE C1 (CONCLUDED)

	CONTROL (VEH) 11-1085	LOW DOSE 11-1083	HIGH DOSE 11-1081
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(50)	(50)
HYPERPLASIA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*INGUINAL REGION	(20)	(50)	(50)
NECROSIS, FAT		2 (4%)	1 (2%)
*MESENTERY	(20)	(50)	(50)
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
CONGESTION, NOS		1 (2%)	
LEUKEMOID REACTION	1 (5%)		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	2	
AUTO/NECROPSY/NO HISTO			4

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 β-NITROSTYRENE

	CONTROL (VEH) 11-1086	LOW DOSE 11-1084	HIGH DOSE 11-1082
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*NASAL CAVITY	(20)	(50)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
#LUNG	(20)	(49)	(47)
BRONCHOPNEUMONIA, NOS		2 (4%)	1 (2%)
BRONCHOPNEUMONIA, ACUTE			1 (2%)
PNEUMONIA, CHRONIC MURINE	6 (30%)	17 (35%)	6 (13%)
ARTERIOSCLEROSIS, NOS		1 (2%)	
HYPERPLASIA, ADENOMATOUS		2 (4%)	2 (4%)
LEUKOCYTOSIS, NOS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(49)	(47)
HYPERPLASIA, NOS			2 (4%)
#SPLEEN	(20)	(47)	(45)
HEMOSIDEROSIS		2 (4%)	1 (2%)
HEMATOPOIESIS			2 (4%)
CIRCULATORY SYSTEM			
#HEART	(19)	(49)	(47)
PERIARTERITIS		1 (2%)	
#MYOCARDIUM	(19)	(49)	(47)
INFLAMMATION, NOS		1 (2%)	

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

TABLE C2 (CONTINUED)

	CONTROL (VEH) 11-1086	LOW DOSE 11-1084	HIGH DOSE 11-1082
INFLAMMATION, FOCAL		1 (2%)	
FIBROSIS	2 (11%)	1 (2%)	4 (9%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(46)
HEPATITIS, TOXIC		3 (6%)	2 (4%)
NECROSIS, NOS			1 (2%)
NECROSIS, COAGULATIVE		1 (2%)	
METAMORPHOSIS FATTY	2 (10%)	1 (2%)	3 (7%)
BASOPHILIC CYTO CHANGE		1 (2%)	1 (2%)
FOCAL CELLULAR CHANGE	2 (10%)	1 (2%)	
MEGALOCYTOSIS	1 (5%)		
HYPERPLASIA, NOS	4 (20%)	3 (6%)	7 (15%)
#PANCREAS	(19)	(44)	(46)
FIBROSIS, FOCAL			1 (2%)
ATROPHY, NOS		2 (5%)	
#PANCREATIC ACINUS	(19)	(44)	(46)
ATROPHY, NOS		1 (2%)	
#SMALL INTESTINE	(19)	(47)	(47)
NEMATODIASIS			1 (2%)
#LARGE INTESTINE	(11)	(47)	(47)
NEMATODIASIS	1 (9%)	9 (19%)	4 (9%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(47)
HYDRONEPHROSIS		1 (2%)	
INFLAMMATION, CHRONIC	2 (10%)	6 (12%)	4 (9%)
SCAR			1 (2%)
INFARCT HEMORRHAGIC		1 (2%)	
CALCINOSIS, NOS			1 (2%)
PIGMENTATION, NOS	1 (5%)		
HEMOSIDEROSIS		1 (2%)	1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(49)	(44)
CYST, NOS			1 (2%)

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

TABLE C2 (CONTINUED)

	CONTROL (VEH) 11-1086	LOW DOSE 11-1084	HIGH DOSE 11-1082
HEMORRHAGIC CYST	1 (6%)		
HYPERPLASIA, FOCAL			1 (2%)
#ADRENAL MEDULLA	(19)	(47)	(47)
CYST, NOS			1 (2%)
#THYROID	(19)	(46)	(41)
ULTIMOBRANCHIAL CYST	1 (5%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
CYSTIC DUCTS		1 (2%)	
#UTERUS	(20)	(48)	(45)
CYST, NOS	2 (10%)	1 (2%)	4 (9%)
INFLAMMATION, NOS		1 (2%)	1 (2%)
PYOMETRA	1 (5%)	3 (6%)	1 (2%)
HYPERPLASIA, NODULAR			1 (2%)
POLYP, INFLAMMATORY	1 (5%)	3 (6%)	4 (9%)
ADENOMYOSIS	1 (5%)		
#UTERUS/ENDOMETRIUM	(20)	(48)	(45)
CYST, NOS		1 (2%)	3 (7%)
INFLAMMATION, NOS		1 (2%)	6 (13%)
INFLAMMATION, SUPPURATIVE	1 (5%)	1 (2%)	2 (4%)
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION PROLIFERATIVE			1 (2%)
HYPERPLASIA, NOS	1 (5%)	1 (2%)	4 (9%)
HYPERPLASIA, CYSTIC			1 (2%)
#OVARY/OVIDUCT	(20)	(48)	(45)
INFLAMMATION, NOS		5 (10%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, ACUTE	2 (10%)		
#OVARY	(20)	(47)	(46)
CYST, NOS	1 (5%)	1 (2%)	4 (9%)
FOLLICULAR CYST, NOS		1 (2%)	
PAROVARIAN CYST	2 (10%)	5 (11%)	
INFLAMMATION, NOS		1 (2%)	
ATROPHY, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(46)
HYDROCEPHALUS, NOS		1 (2%)	1 (2%)

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

TABLE C2 (CONCLUDED)

	CONTROL (VEH) 11-1086	LOW DOSE 11-1084	HIGH DOSE 11-1082
HEMOPRHAGE			1 (2%)
RETICULOCYTOSIS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
NECROSIS, FAT		1 (2%)	
*INGUINAL REGION	(20)	(50)	(50)
NECROSIS, FAT		2 (4%)	1 (2%)
*PERICARDIUM	(20)	(50)	(50)
CYST, NOS			1 (2%)
ALL CTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKOCYTOSIS, NOS		1 (2%)	
LEUKFMOID REACTION			1 (2%)
ADIPOSE TISSUE			
HEMOPRHAGE			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		2	1
AUTO/NECROPSY/NO HISTO		1	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 β-NITROSTYRENE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH β-NITROSTYRENE

	CONTROL(VEH) 22-2085	LOW DOSE 22-2083	HIGH DOSE 22-2081
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
CYST, NOS		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
ABSCESS, NOS	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
ATELECTASIS			1 (2%)
HEMORRHAGE			4 (8%)
INFLAMMATION, INTERSTITIAL	1 (5%)	1 (2%)	1 (2%)
PNEUMONIA, CHRONIC MURINE	3 (15%)		3 (6%)
HYPERPLASIA, ADENOMATOUS			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(45)	(45)
HYPERPLASIA, LYMPHOID		1 (2%)	
#LYMPH NODE	(17)	(21)	(33)
INFLAMMATION, NOS	1 (6%)		
#MESENTERIC L. NODE	(17)	(21)	(33)
EPIDERMAL INCLUSION CYST			1 (3%)
HYPERPLASIA, RETICULUM CELL		1 (5%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(50)	(49)
INFLAMMATION, FOCAL			1 (2%)

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

TABLE D1 (CONTINUED)

	CONTROL (VEH) 22-2085	LOW DOSE 22-2083	HIGH DOSE 22-2081
#ENDOCARDIUM	(20)	(50)	(49)
CALCIFICATION, FOCAL		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
HEMORRHAGE			9 (18%)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
NECROSIS, NOS	1 (5%)	1 (2%)	
NECROSIS, FOCAL		2 (4%)	1 (2%)
NECROSIS, HEMORRHAGIC			3 (6%)
NECROSIS, CENTRAL			1 (2%)
NECROSIS, PERIPHERAL			1 (2%)
INFARCT, NOS		1 (2%)	
BASOPHILIC CYTO CHANGE	1 (5%)		
HEPATOCYTOMEGALY		1 (2%)	
#LIVER/CENTRILOBULAR	(20)	(50)	(50)
HEMORRHAGE			3 (6%)
#LIVER/PERIPORTAL	(20)	(50)	(50)
HEMORRHAGE			2 (4%)
#BILE DUCT	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)		
#PANCREAS	(20)	(45)	(50)
INFLAMMATION, NOS	1 (5%)		
PERIARTERITIS		1 (2%)	
#STOMACH	(20)	(47)	(48)
INFLAMMATION, ACUTE			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(49)
HYDRONEPHROSIS		2 (4%)	1 (2%)
PYELONEPHRITIS, NOS	1 (5%)		
INFLAMMATION, INTERSTITIAL			2 (4%)
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, LYMPHOID	1 (5%)		
#KIDNEY/CORTEX	(20)	(50)	(49)
FIBROSIS, FOCAL		1 (2%)	

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

TABLE D1 (CONTINUED)

	CONTROL (VEH) 22-2085	LOW DOSE 22-2083	HIGH DOSE 22-2081
#URINARY BLADDER	(20)	(42)	(44)
INFLAMMATION, NOS	1 (5%)		
HYPERPLASIA, LYMPHOID		1 (2%)	
ENDOCRINE SYSTEM			
#THYROID	(18)	(43)	(35)
FOLLICULAR CYST, NOS	1 (6%)		
INFLAMMATION, GRANULOMATOUS		1 (2%)	
#PANCREATIC ISLETS	(20)	(45)	(50)
HYPERPLASIA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
#PROSTATE	(20)	(44)	(22)
HYPERPLASIA, CYSTIC		2 (5%)	1 (5%)
#TESTIS	(20)	(46)	(47)
HYPERPLASIA, INTERSTITIAL CELL		1 (2%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(20)	(50)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
#CEREBRUM	(20)	(50)	(50)
CORPORA AMYLACEA		1 (2%)	
#BRAIN	(20)	(50)	(50)
CORPORA AMYLACEA	7 (35%)	8 (16%)	8 (16%)
#CEREBELLUM	(20)	(50)	(50)
CALCIFICATION, DYSTROPHIC		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*STERNUM	(20)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	

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

TABLE D1 (CONCLUDED)

	CONTROL (VEH) 22-2085	LOW DOSE 22-2083	HIGH DOSE 22-2081
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
AMYLOIDOSIS		1 (2%)	
ADIPOSE TISSUE			
INFLAMMATION, GRANULOMATOUS		1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	12	14

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 β-NITROSTYRENE

	CONTROL (VEH) 22-2086	LOW DOSE 22-2084	HIGH DOSE 22-2082
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			2
ANIMALS NECROPSIED	20	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	47
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(19)	(49)	(46)
CONGESTION, NOS			1 (2%)
HYPEREMIA			1 (2%)
HEMORRHAGE			1 (2%)
PNEUMONIA, CHRONIC MURINE	3 (16%)	6 (12%)	2 (4%)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
PERIARTERITIS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(42)	(37)
MYELOFIBROSIS		2 (5%)	1 (3%)
MYELOSCLEROSIS		1 (2%)	1 (3%)
HYPERPLASIA, HEMATOPOIETIC			1 (3%)
#SPLEEN	(19)	(43)	(44)
HEMOSIDEROSIS	4 (21%)	4 (9%)	
HYPERPLASIA, RETICULUM CELL			1 (2%)
HYPERPLASIA, LYMPHOID	2 (11%)	3 (7%)	5 (11%)
HEMATOPOIESIS			2 (5%)
#LYMPH NODE	(18)	(32)	(31)
INFLAMMATION, SUPPURATIVE	1 (6%)		
#MESENTERIC L. NODE	(18)	(32)	(31)
INFLAMMATION, GRANULOMATOUS			1 (3%)
HYPERPLASIA, LYMPHOID	1 (6%)		

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

TABLE D2 (CONTINUED)

	CONTROL (VEH) 22-2086	LOW DOSE 22-2084	HIGH DOSE 22-2082
CIRCULATORY SYSTEM			
#HEART	(20)	(44)	(47)
PERIARTERITIS		1 (2%)	
#MYOCARDIUM	(20)	(44)	(47)
DEGENERATION, NOS		1 (2%)	
*PULMONARY ARTERY	(20)	(50)	(48)
HYPERTROPHY, NOS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(47)	(47)
HAMARTOMA		1 (2%)	
HEMORRHAGE			3 (6%)
INFLAMMATION, NECROTIZING	1 (5%)		
NECROSIS, FOCAL		1 (2%)	
METAMORPHOSIS FATTY			1 (2%)
LIPOIDOSIS		1 (2%)	
BASOPHILIC CYTO CHANGE			1 (2%)
HEPATOCYTOMEGALY	1 (5%)		
HYPERPLASIA, LYMPHOID			1 (2%)
HEMATOPOIESIS			1 (2%)
#PANCREAS	(19)	(45)	(43)
INFLAMMATION, GRANULOMATOUS	1 (5%)		
#PEYERS PATCH	(20)	(49)	(47)
INFLAMMATION, GRANULOMATOUS			1 (2%)
#ILEUM	(20)	(49)	(47)
INFLAMMATION, GRANULOMATOUS			1 (2%)
HYPERPLASIA, LYMPHOID			1 (2%)
#LARGE INTESTINE	(20)	(12)	(47)
NEMATODIASIS			1 (2%)
#COLON	(20)	(12)	(47)
HYPERPLASIA, LYMPHOID			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(47)	(47)
INFLAMMATION, CHRONIC	1 (5%)	1 (2%)	

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

	CONTROL (VEH) 22-2086	LOW DOSE 22-2084	HIGH DOSE 22-2082
AMYLOID, NOS	1 (5%)		
#URINARY BLADDER	(17)	(39)	(46)
HYPERPLASIA, LYMPHOID	1 (6%)		
ENDOCRINE SYSTEM			
#THYROID	(17)	(40)	(36)
GOITER COLLOID			1 (3%)
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(48)	(46)
HYDROMETRA			1 (2%)
CYST, NOS		2 (4%)	
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, SUPPURATIVE	1 (5%)		1 (2%)
PYOMETRA		3 (6%)	
#CERVIX UTERI	(20)	(48)	(46)
POLYP			1 (2%)
#UTERUS/ENDOMETRIUM	(20)	(48)	(46)
CYST, NOS	6 (30%)	6 (13%)	4 (9%)
INFLAMMATION, SUPPURATIVE			2 (4%)
HYPERPLASIA, NOS		2 (4%)	1 (2%)
HYPERPLASIA, CYSTIC	1 (5%)	6 (13%)	7 (15%)
#UTERUS/MYOMETRIUM	(20)	(48)	(46)
INFLAMMATION, NOS			1 (2%)
#OVARY	(14)	(23)	(41)
CYST, NOS		2 (9%)	
PAROVARIAN CYST	2 (14%)		1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE	1 (7%)		
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(47)
CORPORA AMYLACEA	1 (5%)	7 (14%)	9 (19%)
SPECIAL SENSE ORGANS			
NONE			

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

TABLE D2 (CONCLUDED)

	CONTROL (VEH) 22-2086	LOW DOSE 22-2084	HIGH DOSE 22-2082
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(50)	(48)
INFLAMMATION, SUPPURATIVE	1 (5%)		
BODY CAVITIES			
*MESENTERY	(20)	(50)	(48)
NECROSIS, FAT	1 (5%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	13	12
ANIMAL MISSING/NO NECROPSY			2
AUTO/NECROPSY/NO HISTO			1

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

Review of the Bioassay of a Solution of β-Nitrostyrene and Styrene*
for Carcinogenicity by the
Data Evaluation/Risk Assessment Subgroup of the
Clearinghouse on Environmental Carcinogens

August 31, 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 a solution of β-Nitrostyrene and Styrene for carcinogenicity.

The primary reviewer agreed with the conclusion in the report that, under the conditions of test, a 30% solution of β-Nitrostyrene and Styrene was not carcinogenic in rats or mice. He considered the study well conducted and the survival good in all groups except high dose male mice. The weight gain data suggested that maximum tolerated doses may not have been achieved for treated female rats and male mice. The primary reviewer said that the shortcomings did not interfer with the adequacy of the study. He indicated that the study should not be interpreted as a bioassay of β-Nitrostyrene or of Styrene, since it was the mixture that was tested.

The secondary reviewer agreed with the primary reviewer's critique of the study.

A motion was approved unanimously that the report on the bioassay of a solution of β-Nitrostyrene and Styrene be accepted as written.

Members present were:

Arnold L. Brown (Chairman), University of Wisconsin School of Medicine
Joseph Highland, Environmental Defense Fund
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center

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

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