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Carcinogenesis Technical Report Series

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

United States
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FOREWORD: This report presents the results of the bioassay of azobenzene 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. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. A positive results demonstrates that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic in animals requires a wider analysis.

CONTRIBUTORS: This bioassay of azobenzene was conducted at the NCI Frederick Cancer Research Center (FCRC) (1), Frederick, Maryland, for NCI (2) by Litton Bionetics, Inc.

The manager of the bioassay at FCRC was Dr. B. Ulland, the toxicologist was Dr. E. Gordon, and Drs. R. Cardy and D. Creasia compiled the data. Ms. S. Toms was responsible for management of data, Mr. D. Cameron for management of histopathology, Mr. L. Callahan for management of the computer branch, and Mr. R. Cypher for management of the facilities. Mr. A. Butler performed the computer services. Histopathologic evaluations for rats and mice were performed by Dr. B. Ulland (1). The diagnoses included in this report represent his interpretations.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (3). Statistical analyses were performed by Dr. J. R. Joiner (4) and Ms. P. L. Yong (4), using methods selected for the bioassay program by Dr. J. J. Gart (5). The chemicals used in this bioassay were analyzed at FCRC by Dr. W. Zielinsky (1). The chemical narrative and analyses were reviewed and approved by Dr. W. Lijinsky (1).

This report was prepared at Tracor Jitco (4) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. C. R. Angel, Acting Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. J. F. Robens, toxicologist; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Owen, Ms. M. S. King, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The following scientists at NCI were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. Morton H. Levitt, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. A. R. Patel, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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(1) Frederick Cancer Research Center, P.O. Box B, Frederick, Maryland.

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

(3) EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

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

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

SUMMARY

A bioassay of azobenzene for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F1 mice.

Groups of 50 rats of each sex were administered azobenzene at one of two doses, either 200 or 400 ppm, for 105 or 106 weeks. Matched controls consisted of 20 untreated rats of each sex. All surviving rats were killed at the end of administration of the test chemical.

Groups of 50 male mice were administered azobenzene at one of two doses, either 200 or 400 ppm, for 105 weeks. Groups of 50 female mice were administered the test chemical at one of two doses, initially 400 or 800 ppm, for 38 weeks. Because of excessively lowered body weights in the dosed groups of the females, doses for the females were then reduced to 100 and 400 ppm, respectively, and administration at the lowered doses was continued for 67 or 68 weeks. The time-weighted average doses for the female mice were either 208 or 545 ppm. Matched controls consisted of 20 untreated mice of each sex. All surviving mice were killed at the end of administration of the test chemical.

Mean body weights of dosed rats and mice of each sex were lower than those of corresponding controls, and were generally dose related throughout the bioassay. Mortality was dose related in the male rats and the female mice, but was not significantly affected in either the female rats or the male mice. Survival was 70% or greater at week 90 on study in all dosed and control groups of each species and sex; thus, sufficient numbers of animals were at risk in all groups for the development of late-appearing tumors.

In rats, a large number of sarcomas, including fibrosarcomas, hemangiosarcomas, and osteosarcomas in both males and females and malignant hemangiopericytomas in females, occurred in the spleen and other abdominal organs at incidences that were dose related in each sex (P less than 0.001) and that in direct comparisons were significantly higher (P less than 0.001) in the high-dose groups of each sex than in the corresponding control groups (males: controls 0/20, low-dose 6/49, high-dose 31/49; females: controls 0/20, low-dose 5/50, high-dose 21/50).

In mice, no tumors occurred in either males or females at

incidences that were significantly higher in the dosed groups than in the corresponding control groups.

It is concluded that under the conditions of this bioassay, azobenzene was carcinogenic (sarcomagenic) for F344 rats, inducing various types of sarcomas in the spleen and other abdominal organs of both males and females. The test chemical was not carcinogenic for B6C3F1 mice of either sex.

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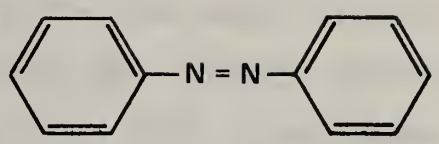
I. INTRODUCTION

Azobenzene (CAS 103-33-3; NCI C02926) occurs as a by-product during the manufacture of benzidine (Noller, 1965; Lurie, 1964). Benzidine is a widely used intermediate for the azo dyes and other organic chemicals and is a carcinogen (Department of Labor, 1974). Azobenzene itself has no known uses as a dyestuff and is produced only in small quantities for research purposes (International Agency for Research on Cancer, 1975).



Azobenzene

Since 1950, there has been documented evidence of an increased risk of bladder cancer in persons employed in the dye industries (International Agency for Research on Cancer, 1975). Although it has not been possible to identify the causative dyes or intermediates by these epidemiological studies, some compounds have been shown to be carcinogenic in animal studies. Azobenzene has been regarded in the literature as a noncarcinogen (Daoust and Calamai, 1971; Eldredge and Luck, 1952), as a result of a

long-term study by Spitz et al. (1950) in which Sherman rats were given subcutaneous injections of the compound for life. More recently, azobenzene was tested by Innes et al. (1969) in a large-scale screen of industrial compounds for carcinogenic activity. Since the results of this preliminary bioassay in mice did not clearly associate the incidence of any tumor with administration of the test chemical, azobenzene was selected for further testing in the Carcinogenesis Testing Program.

II. MATERIALS AND METHODS

A. Chemical

Azobenzene (diphenyldiimide; azobenzide) was obtained from Eastman Chemical Company as a hard, dark-orange, crystalline material. Its purity was determined at Frederick Cancer Research Center using gas-liquid chromatography (GLC) to be 99.5%, with up to six minor contaminants and a melting point of 66°C (literature: 68°C). Mass spectral analysis gave a molecular ion at m/e 182 and a base peak at m/e 77. The infrared spectrum was consistent with its structure, and was identical to that of a standard.

B. Dietary Preparation

Test diets containing azobenzene were prepared in 6-kg batches at the appropriate doses. A known weight of the chemical was first mixed with an equal weight of autoclaved Wayne® Sterilizable Lab Meal with 4% fat (Allied Mills, Inc., Chicago, Ill.), using a mortar and pestle. The mixing was continued with second and third additions of feed, and final mixing was performed with the

remaining quantity of feed for a minimum of 15 minutes in a Patterson-Kelly twin-shell blender with an intensifier bar.

Detailed GLC analyses of aliquots of azobenzene-feed mixtures taken from various locations in the blender showed that the mixture was homogeneous.

C. Animals

Male and female F344 (Fischer) rats and B6C3F1 mice were obtained as 4-week-old weanlings, all within 3 days of the same age, from the NCI Frederick Cancer Research Center animal farm (Frederick, Md.). The animals were housed within the test facility for 2 weeks and were then assigned four rats to a cage and five mice to a cage on a weight basis for each cage of animals of a given species and sex. For use in the chronic study, the male rats were required to weigh 90 to 105 g, averaging at least 100 g; the female rats, 80 to 95 g, averaging at least 90 g; the male mice, 18 to 22 g, averaging at least 19.5 g; and the female mice, 17 to 21 g, averaging at least 18.5 g. Individual animals were identified by ear punch.

D. Animal Maintenance

The animals were housed in polycarbonate cages (Lab Products, Inc., Garfield, N.J.), 19 x 10-1/2 x 8 inches for the rats and 11-1/2 x 7-1/2 x 5 inches for the mice, which were suspended from aluminum racks (Scientific Cages, Inc., Bryan, Tex.) and were covered by nonwoven polyester-fiber 12-mil-thick filter paper (Hoeltge, Inc., Cincinnati, Ohio). The bedding used was Absorb-dri® hardwood chips (Northeastern Products, Inc., Warrenburg, N. Y.). The feed supplied was presterilized Wayne® Sterilizable Lab Meal, provided ad libitum in suspended stainless steel hoppers and replenished at least three times per week. Water, acidified to pH 2.5, was supplied ad libitum from glass bottles. Sipper tubes (Lab Products, Inc.) were suspended through the tops of the cages.

The contaminated bedding was disposed of through an enclosed vacuum line that led to a holding tank from which the bedding was fed periodically into an incinerator. The cages were sanitized twice per week and the feed hoppers twice per month at 82 to 88°C in a tunnel-type cagewasher (Industrial Washing Corp., Mataway, N. J.), using the detergents, Clout® (Pharmacal Research Laboratories, Greenwich, Conn.) or Oxford D'Chlor (Oxford Chemicals, Atlanta, Ga.).

The glass bottles and sipper tubes were sanitized at 82 to 88°C in a tunnel-type bottle washer (Consolidated Equipment Supply Co., Mercersburg, Pa.) three times per week, using a Calgen Commercial Division detergent (St. Louis, Mo.). The racks for the cages were sanitized at or above 82°C in a rack washer (Consolidated Equipment Supply Co.) once per month, using Calgen Commercial Division detergent, and the filter paper was changed at the same time.

The air in the animal rooms was maintained at 22 to 24°C and 45 to 55% relative humidity. Fresh air was passed through a filter of 65% efficiency and a bag filter of 95% efficiency at the intake and through a "Z"-type roughing filter of 30% efficiency and a bag system of 90 to 95% efficiency at the exhaust (American Air Filters, Louisville, Ky.; Mine Safety Appliances, Pittsburgh, Pa.); the air was not recirculated. Room air was changed 15 times per hour. The air pressure was maintained negative to a clean hallway and positive to a return hallway. Fluorescent lighting was provided automatically on a 12-hour-per-day cycle.

Rats administered azobenzene and their corresponding controls were housed in the same room as rats on feeding studies of the following chemicals:

(CAS 72-56-0) p,p'-ethyl-DDD
(CAS 120-62-7) piperonyl sulfoxide

Mice administered azobenzene and their corresponding controls were housed in the same room as mice on feeding studies of the following chemicals:

(CAS 128-66-5)	C. I. vat yellow 4
(CAS 72-56-0)	p,p'-ethyl-DDD
(CAS 20941-65-5)	ethyl tellurac
(CAS 298-00-0)	methyl parathion
(CAS 85-44-9)	phthalic anhydride
(CAS 51-03-6)	piperonyl butoxide
(CAS 86-06-2)	2,4,6-trichlorophenol

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses (MTD's) of azobenzene, on the basis of which two concentrations (referred to in this report as "low" and "high" doses) were selected for administration in the chronic studies. Groups of five rats of each sex and five mice of each sex were fed diets containing azobenzene at one of several doses, and groups of five control animals of each species and sex were administered basal diet only. The test chemical was administered for 7 weeks, followed by 1 week of additional observation. Each animal was weighed twice per week. Table 1 shows the survival of animals in each dose group at the end of the study and the week on study when the last death occurred; the table also shows the mean body weights of each dosed group at week 7, expressed as percentages of mean body weights of controls. At the end of the

Table 1. Azobenzene Subchronic Feeding Studies in Rats and Mice

Dose (ppm)	Male			Female		
	Survival (a)	Week on Study When Last Death Occurred	Mean Weight at Week 7 as % of Control	Survival (a)	Week on Study When Last Death Occurred	Mean Weight at Week 7 as % of Control
Rats						
500	5/5		88	5/5		96
700	5/5		75	5/5		83
1,000	5/5		73	5/5		69
2,200	5/5		33	0/5	6	
4,600	0/5	2		0/5	2	
Mice						
500	5/5		88	5/5		91
700	5/5		86	5/5		91
1,000	5/5		91	5/5		91
2,200	4/5	5	89	5/5		91
4,600	5/5		66	5/5		67

(a) Number surviving/number in group.

subchronic studies, all animals were killed using CO_2 and necropsied.

Groups of male and female rats receiving doses of 2,200 or 4,600 ppm were observed during clinical examination to be emaciated. At necropsy, the groups of rats receiving the four highest doses had slightly enlarged livers. Histopathologic changes due to administration of the azobenzene were noted in the kidneys and livers of male and female rats dosed at 1,000 or 2,200 ppm. In these animals the proximal convoluted tubules of the kidney contained generally moderate amounts of granular, yellowish-brown, intracytoplasmic pigment. Trace to moderate amounts of centrilobular cytoplasmic vacuolation of hepatocytes, suggestive of lipidosis, occurred in six male rats. Trace to very small amounts of bile stasis were noted in the livers of both males and females. The hepatic changes indicated mild injury. Pigmentation of renal tubules was considered to represent accumulation of lipofuscin in association with mild degenerative changes in the tubular epithelium.

No clinical signs were observed during examination of male and female mice in the groups dosed at 4,600 ppm. At necropsy the groups of mice receiving the four highest doses had enlarged spleens and mesenteric nodes. Trace to very slight stasis of the

bile, trace to very slight granular intracytoplasmic pigmentation of the proximal convoluted tubular epithelium, and slight pigmentation of the splenic red pulp were noted in all animals examined.

Ten percent depression in body weight was the major criterion for estimation of MTD's. The doses that were required to produce this response were determined by the following procedure: first, least square regressions of mean body weights versus days on study were used to estimate mean body weights of each of the dosed groups at day 49. Next, probits of the percent weights of corresponding control groups were plotted against the logarithms of the doses, and least squares regressions fitted to the data were used to estimate the doses required to induce 10% depression in weight.

Based on the data thus obtained, the low and high doses for chronic studies using male and female rats were set at 200 and 400 ppm; using male mice, 200 and 400 ppm; and using female mice, 400 to 800 ppm.

F. Chronic Studies

The test groups, doses administered, and durations of the chronic feeding studies are shown in tables 2 and 3. Due to excessive weight depression in the dosed female mice, doses for the low- and high-dose groups were reduced to 100 and 400 ppm, respectively, after week 38.

G. Clinical and Pathologic Examinations

All animals were observed twice daily. Observations for sick, tumor-bearing, and moribund animals were recorded daily. Clinical examination and palpation for masses were performed each month, and the animals were weighed at least once per month. Moribund animals and animals that survived to the end of the bioassay were killed using CO_2 and necropsied.

The pathologic evaluation consisted of gross and microsocopic examination of major tissues, major organs, and all gross lesions. The tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone marrow (femur), spleen,

Table 2. Azobenzene Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals (a)	Azobenzene in Diet (b) (ppm)	Time on Study (weeks)
Male			
Matched-Control	20	0	106
Low-Dose	50	200	106
High-Dose	50	400	105
Female			
Matched-Control	20	0	106
Low-Dose	50	200	106
High-Dose	50	400	105-106

(a) All animals were 6 weeks of age when placed on study.

(b) Test and control diets were provided ad libitum 7 days per week.

Table 3. Azobenzene Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals (a)	Azobenzene in Diet (b) (ppm)	Time on Study (weeks)	Time-Weighted Average Dose (c) (ppm)
Male				
Matched-Control	20	0	106	
Low-Dose	50	200	105	
High-Dose	50	400	105	
Female				
Matched-Control	20	0	106	
Low-Dose	50	400	38	
		100	68	208
High-Dose	50	800	38	
		400	67	545

(a) All animals were 6 weeks of age when placed on study.

(b) Test and control diets were provided *ad libitum* 7 days per week.

(c) Time-weighted average dose = $\frac{\Sigma(\text{dose in ppm} \times \text{no. of weeks at that dose})}{\Sigma(\text{no. of weeks receiving each dose})}$

lymph nodes (mesenteric and submandibular), thymus, heart, salivary glands (parotid, sublingual, and submaxillary), liver, pancreas, esophagus, stomach (glandular and nonglandular), small and large intestines, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, mammary gland, uterus, ovary, brain (cerebrum and cerebellum), and all tissue masses. Peripheral blood smears also were made for all animals, whenever possible.

Necropsies were also performed on all animals found dead, unless precluded in whole or in part by autolysis or cannibalization. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and

individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

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

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

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

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

narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

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

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

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

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

of unity represent the condition of a larger proportion in the dosed group than in the control.

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

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of dosed male and female rats were lower than those of corresponding controls and were dose related throughout the bioassay (figure 1). Fluctuation in the growth curves may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. Other clinical signs, such as corneal opacity and tissue masses, occurred at low incidences and were common to dosed and control groups.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered azobenzene in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2. In male rats, the result of the Tarone test for positive dose-related trend in mortality is significant (P less than 0.001). An indicated departure from linear trend is observed (P less than 0.001) because the low-dose animals survived longer than the control

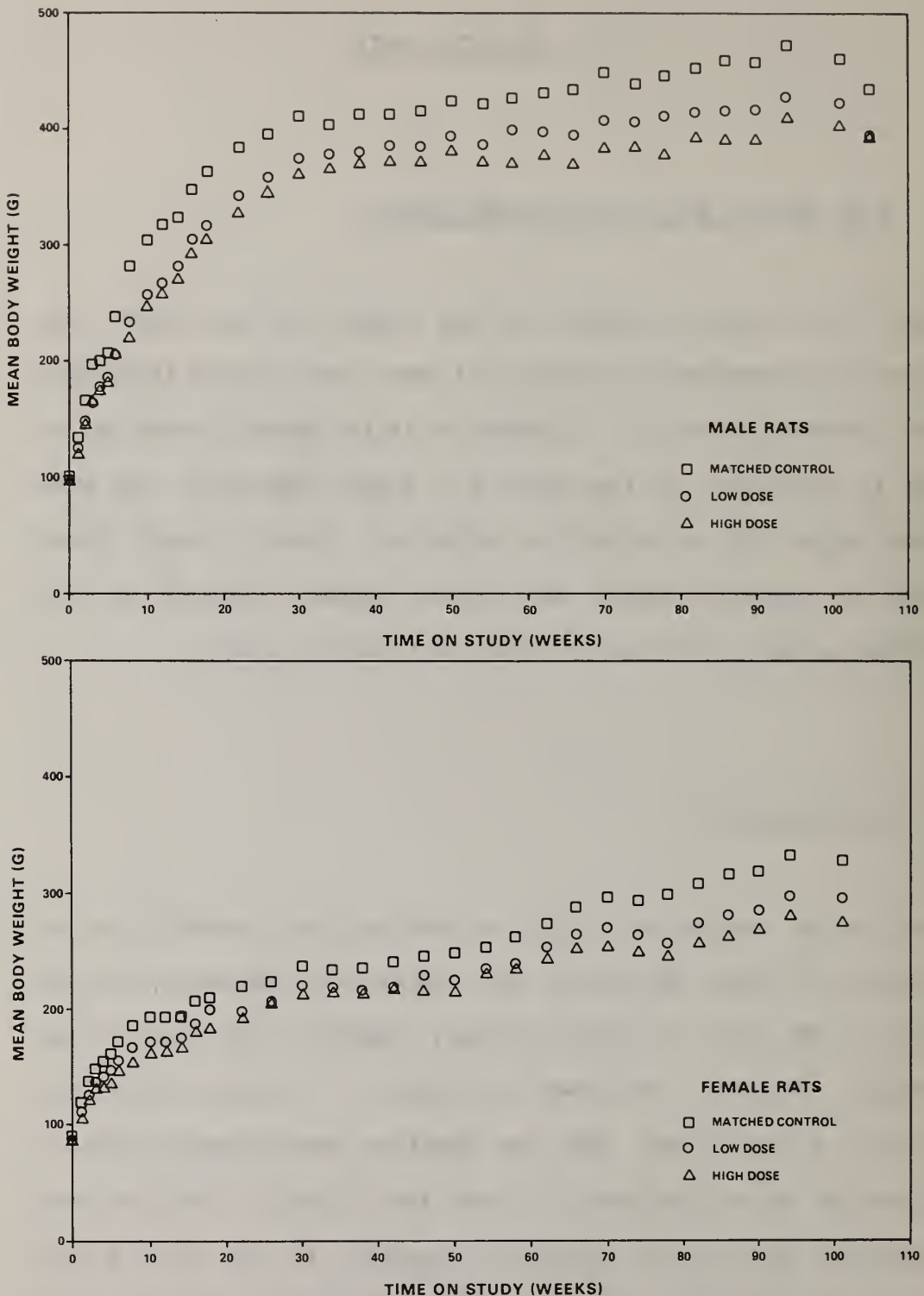


Figure 1. Growth Curves for Rats Administered Azobenzene in the Diet

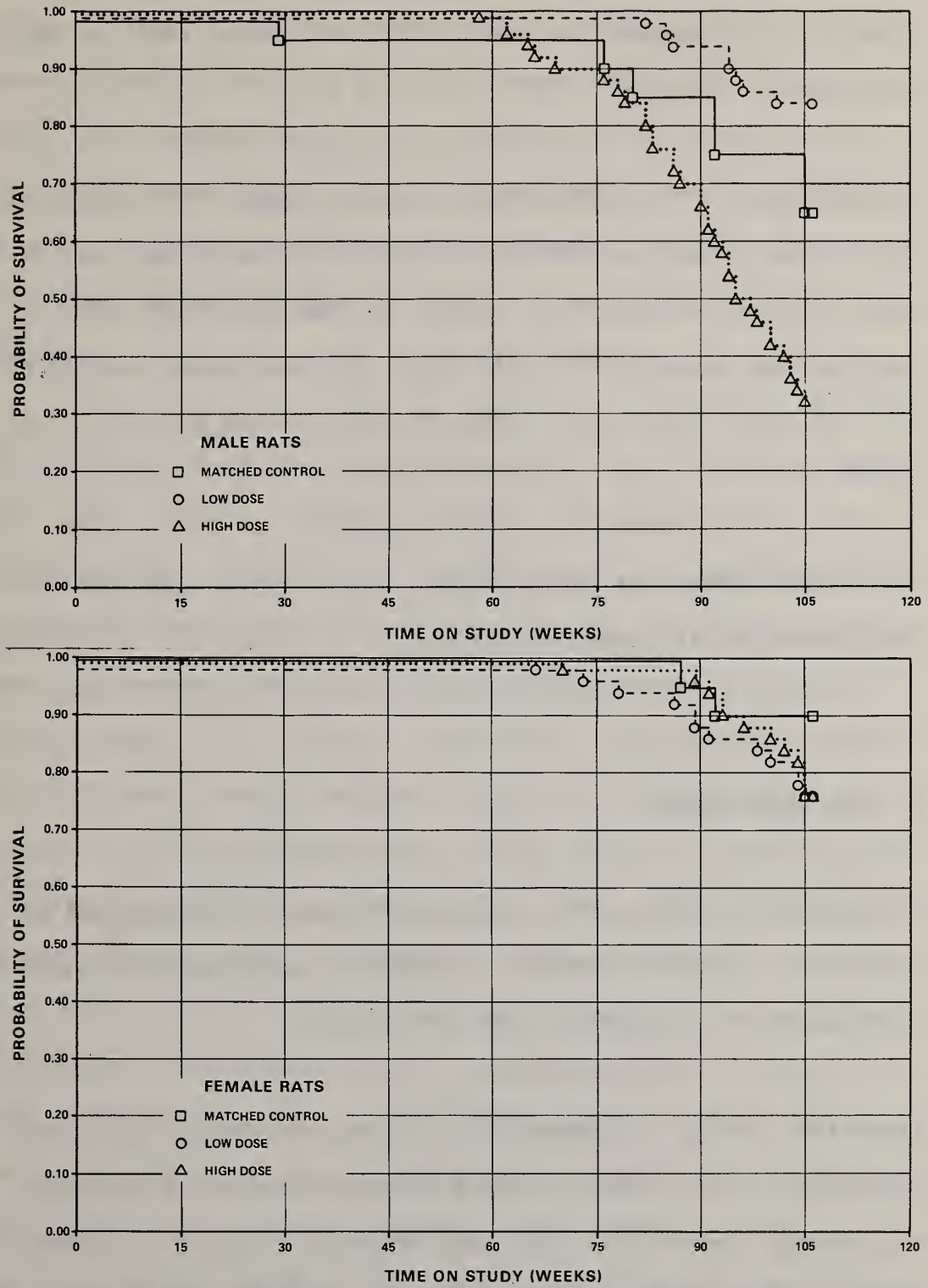


Figure 2. Survival Curves for Rats Administered Azobenzene in the Diet

animals. In females, the result of the Tarone test is not significant.

In male rats, 35/50 (70%) of the high-dose group, 47/50 (94%) of the low-dose group, and 17/20 (85%) of the control group lived at least as long as week 90 on study. In females, 48/50 (96%) of the high-dose group, 44/50 (88%) of the low-dose group, and 19/20 (95%) of the control group lived at least as long as week 90 on study.

Sufficient numbers of rats of each sex were at risk for the development of late-appearing tumors.

C. Pathology (Rats)

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

Long-term feeding of azobenzene to male and female rats was associated with a marked increase in the frequency of malignant mesenchymal tumors involving the abdominal viscera including mesentery and omentum. None of these neoplasms were seen in

control animals. These tumors often spread throughout the abdominal cavity involving multiple tissues and organs to such an extent that determination of the primary site was precluded. The spectrum of mesenchymal malignancies included a striking array of forms, from bizarre undifferentiated sarcomas to relatively well-differentiated fibrosarcomas, osteogenic sarcomas, and vascular neoplasms.

The abdominal organ most constantly involved was the spleen, and the most commonly observed tumors of the spleen were fibrosarcomas. Some were characterized by proliferating anaplastic spindle cells forming broad sheets, the occurrence of tumor giant cells, the presence of numerous bizarre mitoses, and large areas of ischemic necrosis. Others were more differentiated, having varying amounts of collagen, and were composed of proliferating spindle cells growing in sheets and intersecting bundles. Occasionally, these tumors contained mature adipose tissue. Osteogenic sarcomas observed were all well-differentiated and contained large amounts of bone, often trabecular, and osteoid tissue. Hemangiosarcomas observed in dosed animals varied from solid tumors composed of proliferating sheets of spindle cells containing a myriad of cleft-like structures or vascular channels with varying numbers of erythrocytes to massive cavernous blood-filled tumors with

thick fibrotic walls and intersected by traberculae lined by neoplastic cells. A well-differentiated hemangiopericytoma, with a typical "whorling" pattern of proliferating pericytes was also observed in a dosed animal.

A large number of sarcomas were observed involving multiple organs of the abdominal cavity. These probably represent extensions of primary splenic neoplasms. Often, nearly every abdominal organ and tissue, including scrotal fat, was affected, testifying to the extreme invasiveness of these tumors.

A wide variety of other neoplasms were observed in all groups, but there was no clear-cut relationship of these neoplasms to azobenzene exposure.

Nonproliferative lesions associated with long-term dietary intake of azobenzene were observed in several instances. Increased amounts of hemosiderin were deposited in the spleen, liver, and renal tubular epithelium of dosed female rats. Chronic capsulitis of the spleen was observed in all dosed groups of males and females, but particularly in the females. This was characterized by a cystic papillary proliferation of serosal cells, thickening of the capsule, and focal collections of mononuclear cells and mineral deposits.

Several low-dose male and female rats had unusual accumulations of mature-appearing adipose tissue within the spleen, and several females had varying degrees of fibrosis of the splenic pulp. These changes may be within the spectrum of proliferative lesions already discussed.

A wide variety of lesions previously found in aged F344 rats occurred in all groups without relationship to administration of the test chemical.

Based on the histopathologic examination, azobenzene was carcinogenic (sarcomagenic) to F344 rats, being associated with a high incidence of malignant mesenchymal tumors that were not observed in control animals, under the conditions of this bioassay. The striking array of splenic proliferative lesions suggests that azobenzene may have an effect on primitive reticular cells that are the precursors of the various differentiated components.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that occurred in at

least two animals of one group and at an incidence of at least 5% in one or more than one group.

The result of the Cochran-Armitage test for positive dose-related trend in the incidence of fibrosarcoma of the spleen is significant in both male (P = 0.020) and female (P = 0.012) rats, although the results of the Fisher exact test are not significant. The historical records for the rats maintained as controls at this laboratory show an incidence of tumors of 1/285 (0.4%) in males and 0/285 in females. Using the incidence of 1/285 as a parameter and assuming a binomial distribution, the probability level of obtaining 7 or more such tumors out of 49 or 50 animals is less than 0.001.

In male rats, the result of the Cochran-Armitage test for the incidence of fibrosarcoma of multiple organs is significant (P less than 0.001). An indicated departure from linear trend is observed (P = 0.030), due to the steep increase in the incidence of tumors in the high-dose group. The Fisher exact test shows that the incidence in the high-dose group is significantly higher (P = 0.007) than that in the control group. The statistical conclusion is that the incidence of fibrosarcoma of multiple organs in male rats is associated with the administration of

azobenzene. No such tumor is observed at a significant incidence in females.

In female rats, the result of the Cochran-Armitage test for the incidence of osteosarcoma of the spleen is significant (P = 0.041), but the results of the Fisher exact test are not significant. The historical records of this laboratory show no such tumor among 285 control F344 female rats. Using 1/285 as a parameter and assuming a binomial distribution, the probability level of obtaining 5 such tumors out of 50 animals is less than 0.001.

When tests are performed using the incidences of animals with any type of sarcoma in the abdominal cavity, the P values for dose-related trend and for significance of direct comparisons of high-dose and control groups are less than 0.001 for both male and female rats.

Significant results in the negative direction are observed in the incidences of adenomas of the pituitary and of interstitial-cell tumors of the testis in male rats. The increased incidence in the negative direction may be due to the earlier mortality of the high-dose animals.

In summary, the incidences of male and female animals with sarcomas in the abdominal cavity are related to the administration of azobenzene.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of dosed male and female mice were lower than those of corresponding controls, and for female mice were dose related throughout the bioassay (figure 3). Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to greater variation. Other clinical signs such as alopecia, corneal opacity, and tissue masses occurred at low incidences and were common to both dosed and control groups.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered azobenzene in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 4. The result of the Tarone test for positive dose-related trend in mortality is not significant in male mice, but is significant (P less than 0.001) in females.

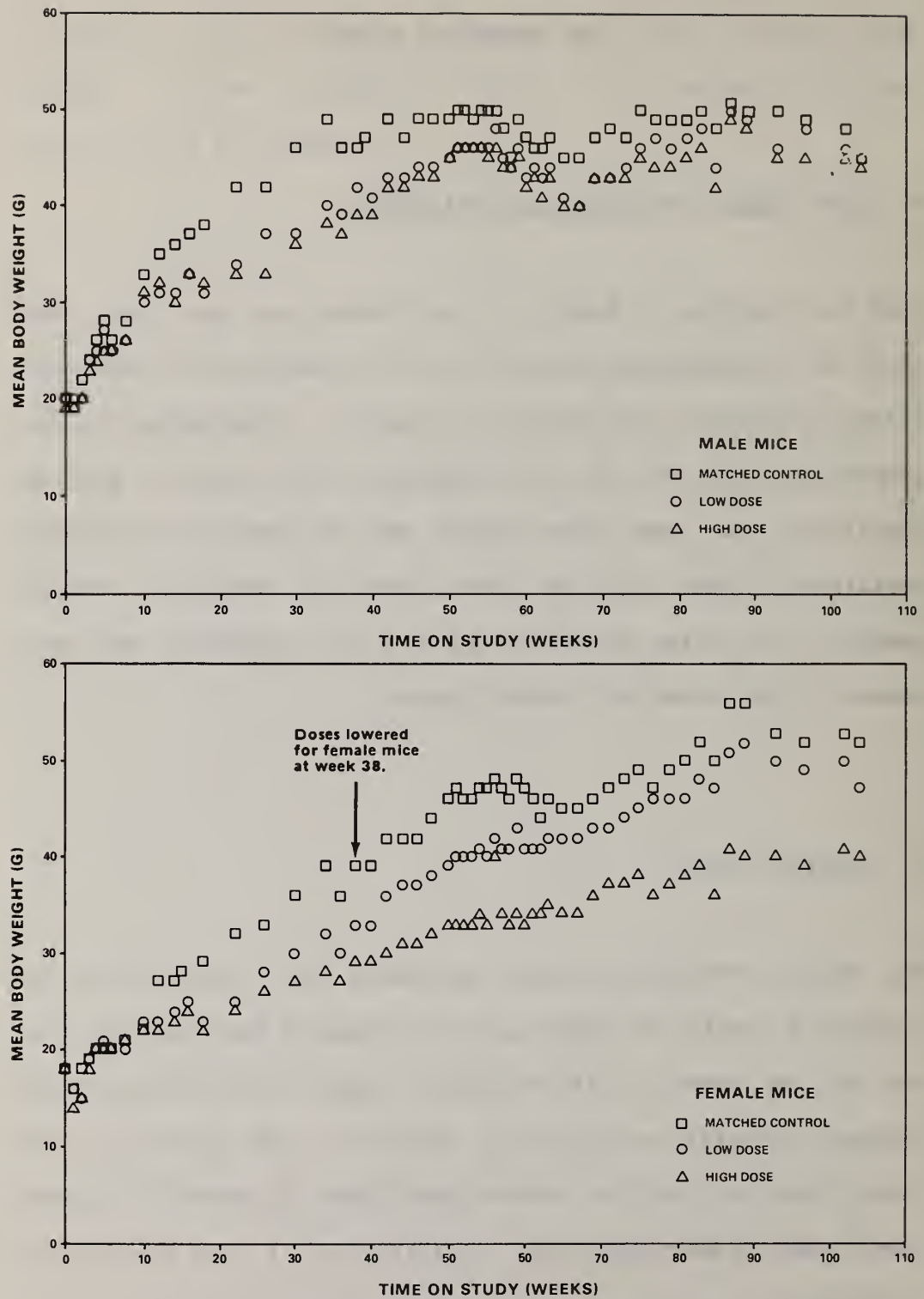


Figure 3. Growth Curves for Mice Administered Azobenzene in the Diet

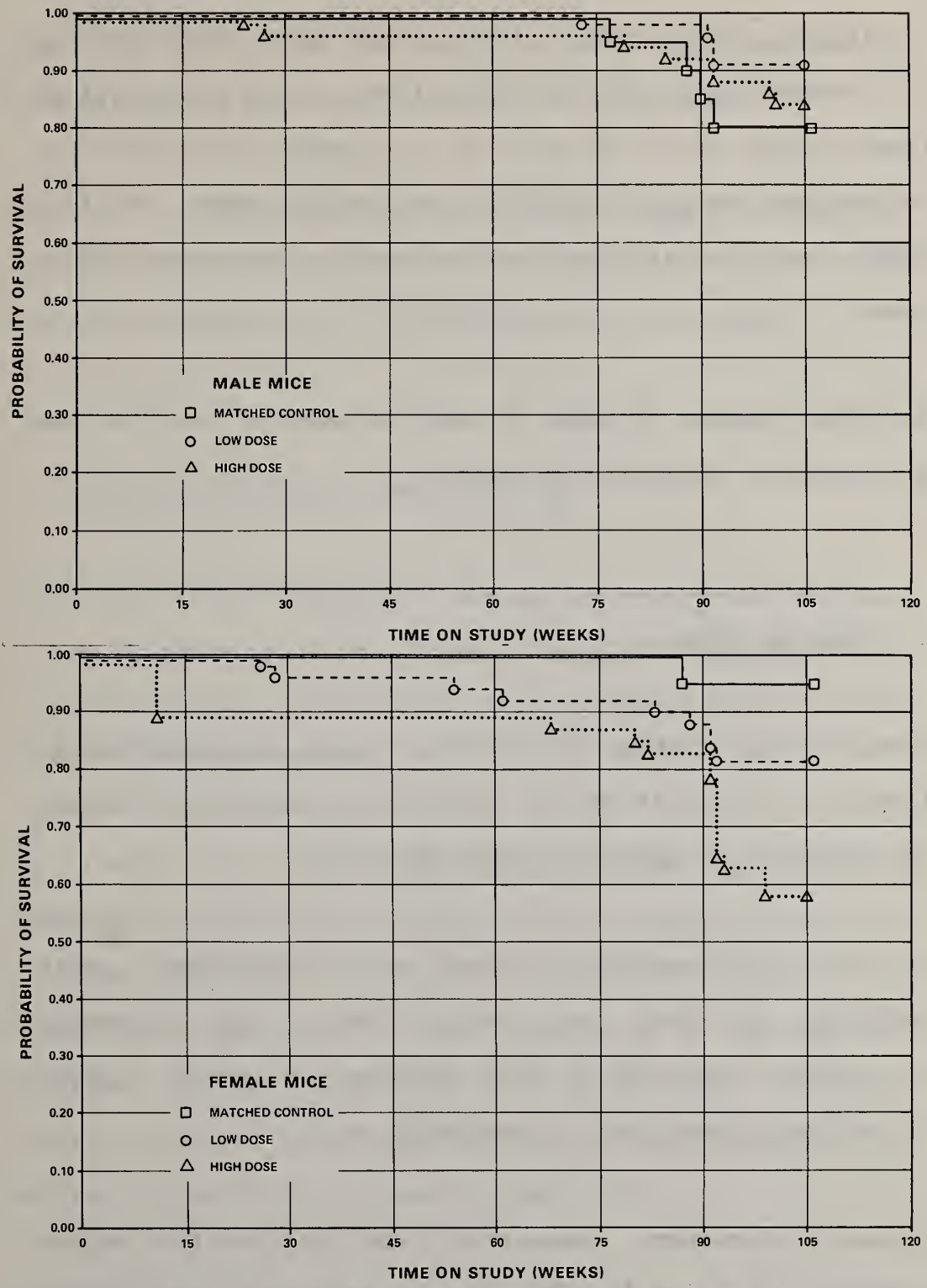


Figure 4. Survival Curves for Mice Administered Azobenzene in the Diet

In male mice, 46/50 (92%) of the high-dose group, 49/50 (98%) of the low-dose group, and 18/20 (90%) of the control group lived at least as long as week 90 on study. In females, 37/50 (74%) of the high-dose group, 43/50 (86%) of the low-dose group, and 19/20 (95%) of the control group lived at least as long as week 90 on study.

Sufficient numbers of mice of each sex were at risk for the development of late-appearing tumors.

C. Pathology (Mice)

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

A variety of neoplasms occurred with approximately equal frequency in dosed and control mice. The incidence, distribution, and nature of these neoplasms are similar to those of neoplasms commonly seen in aged B6C3F1 mice.

Several inflammatory, degenerative, and proliferative lesions commonly seen in aged B6C3F1 mice occurred with approximately

equal frequency in dosed and control animals. The occurrence of these lesions was unrelated to exposure to azobenzene.

Based on the histopathologic examination, changes related to administration of azobenzene were not observed in B6C3F1 mice receiving azobenzene under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

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

The results of the Cochran-Armitage test for positive dose-related trend in the incidence of tumors and the results of the Fisher exact test comparing the incidence of tumors in the control group with that in each dosed group in the positive direction are not significant in either sex. However, significant results in the negative direction are observed in the combined incidence of liver tumors in male mice.

In each of the 95% confidence intervals for relative risk, shown in the tables, the value of one or less than one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals, except that for the combined incidence of liver tumors in the high-dose male mice, has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by azobenzene, which could not be detected under the conditions of this test.

V. DISCUSSION

Mean body weights of the dosed rats and mice of each sex were lower than those of corresponding controls, and were generally dose related throughout the bioassay. Mortality was dose related in the male rats and the female mice, but was not significantly affected in the female rats and male mice. Survival was 70% or greater at week 90 on study in all dosed and control groups of each species and sex; thus, sufficient numbers of animals were at risk in all groups for the development of late-appearing tumors.

In rats, a large number of animals had various types of sarcomas of the spleen and other abdominal organs. Fibrosarcomas of the spleen occurred at incidences that were dose related in both male ($P = 0.020$) and female ($P = 0.012$) rats, although in direct comparisons incidences of the tumors in individual dosed groups were not significantly higher than those in corresponding controls (males: controls 0/20, low-dose 2/49, high-dose 7/49; females: controls 0/20, low-dose 1/50, high-dose 7/50). The incidence of fibrosarcomas of the spleen in historical-control male F344 rats at this laboratory is 1/285, and in female F344 rats it is 0/285; using 1/285 as a parameter and assuming a binomial distribution, the probability that the occurrence of 7

such tumors in 49 male or 50 female high-dose rats in the present bioassay was due to chance is less than 0.001.

Fibrosarcomas of multiple organs (organs other than the spleen) occurred at incidences that were dose related (P less than 0.001) in the male rats, and in a direct comparison the incidence in the high-dose group was significantly higher (P = 0.007) than that in the control group (controls 0/20, low-dose 0/49, high-dose 13/50). In females, fibrosarcomas of multiple organs occurred only in one high-dose animal.

Osteosarcomas of the spleen occurred at incidences that were dose related (P = 0.041) in the female rats, although in direct comparisons the incidences in the individual dosed groups were not significantly higher than that in the control group (controls 0/20, low-dose 1/50, high-dose 5/50). The incidence of osteosarcomas of the spleen in historical-control female F344 rats at this laboratory is 0/285; using 1/285 as a parameter and assuming a binomial distribution, the probability that the occurrence of 5 such tumors in the 50 high-dose female rats of the present bioassay was due to chance is less than 0.001. In males, osteosarcomas of the spleen occurred in one low-dose male and one high-dose male, but in no control males. Osteosarcomas also occurred in multiple organs in three additional high-dose

females and in three additional high-dose males, but in no controls of either sex.

When all types of sarcomas of the abdominal cavity were combined, the incidences of such tumors in both male and female rats were dose related (P less than 0.001); and in direct comparisons the incidences of these tumors in the high-dose groups of males and females were significantly higher (P less than 0.001) than those in the corresponding controls (males: controls 0/20, low-dose 5/49, high-dose 31/49; females: controls 0/20, low-dose 5/50, high-dose 21/50).

No tumors occurred in the male or female mice at incidences that were significantly higher in the dosed groups than in the corresponding controls.

Essentially no evidence of carcinogenicity of azobenzene for rats or mice was obtained in early work carried out from 1936 to 1952 (Hartwell, 1963; Eldredge and Luck, 1952; Spitz et al., 1950), and the compound has generally been considered by cancer investigators not to be carcinogenic. In the work of Innes et al. (International Agency for Research on Cancer, 1975; Innes et al., 1969; NTIS, 1968), however, it was reported that when azobenzene was administered at 21.5 mg/kg body weight by stomach

tube for 3 weeks, then in the diet at 56 ppm for 18 months, to hybrid mice (B6C3F1 and B6AKF1), an elevated incidence of hepatomas ($P = 0.01$) was observed in the male B6C3F1 hybrids; nevertheless, additional evaluation was proposed. The observation of an increased incidence of tumors of the liver in B6C3F1 mice in the study by Innes et al. was not confirmed by the results of the present bioassay. Damage to the spleen of the dosed F344 rats, characterized by hemosiderosis and capsulitis, occurred in the present bioassay; similar damage was reported for Wistar rats fed azobenzene in the diet in previous studies (Smith et al., 1943).

It is concluded that under the conditions of this bioassay, azobenzene was carcinogenic (sarcomagenic) for F344 rats, inducing various types of sarcomas in the spleen and other abdominal organs of both males and females. The test chemical was not carcinogenic for B6C3F1 mice of either sex.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED AZOBENZENE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED AZOBENZENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(49)	(50)
BASAL-CELL CARCINOMA		1 (2%)	1 (2%)
FIBROMA		2 (4%)	1 (2%)
FIBROSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)		
C-CELL CARCINOMA, METASTATIC			1 (2%)
OSTEOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
MALIG.LYMPHOMA, UNDIFFER-TYPE			2 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MONOCYTIC LEUKEMIA	2 (10%)	1 (2%)	5 (10%)
#SPLEEN	(20)	(49)	(49)
NEOPLASM, NOS, MALIGNANT			1 (2%)
SARCOMA, NOS		1 (2%)	2 (4%)
FIBROSARCOMA		2 (4%)	7 (14%)
HEMANGIOSARCOMA		1 (2%)	4 (8%)
OSTEOSARCOMA		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
NEOPLASTIC NODULE		1 (2%)	2 (4%)
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)
HEMANGIOSARCOMA			1 (2%)
OSTEOSARCOMA, METASTATIC			1 (2%)
#PANCREAS	(20)	(47)	(48)
HEMANGIOSARCOMA			1 (2%)
#COLON	(20)	(47)	(50)
MUCINOUS CYSTADENOCARCINOMA		1 (2%)	
MUCINOUS ADENOCARCINOMA	1 (5%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(49)	(49)
ADENOMA, NOS	4 (20%)	2 (4%)	
CHROMOPHOBE ADENOMA		2 (4%)	3 (6%)
#ADRENAL	(20)	(49)	(50)
PHEOCHROMOCYTOMA	1 (5%)	1 (2%)	1 (2%)
#THYROID	(20)	(49)	(48)
C-CELL CARCINOMA		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(50)
FIBROADENOMA		2 (4%)	
*PREPUTIAL GLAND	(20)	(49)	(50)
SQUAMOUS CELL CARCINOMA	1 (5%)		
#TESTIS	(20)	(48)	(49)
INTERSTITIAL-CELL TUMOR	17 (85%)	41 (85%)	31 (63%)
NERVOUS SYSTEM			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EAR CANAL	(20)	(49)	(50)
KERATOACANTHOMA	1 (5%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(49)	(50)
HEMANGIOSARCOMA			1 (2%)
*MESENTERY	(20)	(49)	(50)
SARCOMA, NOS			1 (2%)
OSTEOSARCOMA, METASTATIC		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(49)	(50)
SARCOMA, NOS			2 (4%)
FIBROSARCOMA			13 (26%)
MESOTHELIOMA, MALIGNANT		1 (2%)	
OSTEOSARCOMA			3 (6%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	5	4	29
MORIBUND SACRIFICE	2	4	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	41	16
ANIMAL MISSING		1	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	45	47
TOTAL PRIMARY TUMORS	29	63	87
TOTAL ANIMALS WITH BENIGN TUMORS	19	45	34
TOTAL BENIGN TUMORS	23	51	36
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	11	42
TOTAL MALIGNANT TUMORS	6	11	49
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	2
TOTAL SECONDARY TUMORS		2	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	2
TOTAL UNCERTAIN TUMORS		1	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED AZOBENZENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA		2 (4%)	1 (2%)
FIBROADENOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
MIXED TUMOR, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
GRANULOCYTIC LEUKEMIA		2 (4%)	1 (2%)
MONOCYTIC LEUKEMIA	1 (5%)	3 (6%)	
#SPLEEN	(20)	(50)	(50)
FIBROSARCOMA		1 (2%)	7 (14%)
HEMANGIOSARCOMA		1 (2%)	4 (8%)
HEMANGIOPERICYTOMA, MALIGNANT			1 (2%)
OSTEOSARCOMA		1 (2%)	5 (10%)
#SPLENIC CAPSULE	(20)	(50)	(50)
FIBROSARCOMA		1 (2%)	
#LYMPH NODE	(20)	(50)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
NEOPLASTIC NODULE			2 (4%)
HEPATOCELLULAR CARCINOMA		1 (2%)	
#JEJUNUM	(20)	(50)	(50)
SARCOMA, NOS		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(49)	(50)
ADENOMA, NOS	4 (20%)	7 (14%)	7 (14%)
CHROMOPHOBE ADENOMA	1 (5%)	1 (2%)	
#ADRENAL	(20)	(50)	(50)
PHEOCHROMOCYTOMA			1 (2%)
#THYROID	(20)	(50)	(48)
FOLLICULAR-CELL ADENOMA		1 (2%)	
FOLLICULAR-CELL CARCINOMA		1 (2%)	
C-CELL CARCINOMA	1 (5%)	3 (6%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
UNDIFFERENTIATED CARCINOMA	1 (5%)		
ADENOMA, NOS		1 (2%)	1 (2%)
ADENOCARCINOMA, NOS	1 (5%)		
MIXED TUMOR, MALIGNANT			1 (2%)
FIBROADENOMA	3 (15%)	5 (10%)	3 (6%)
*CLITORAL GLAND	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	1 (2%)
#UTERUS	(20)	(50)	(50)
CARCINOMA,NOS		1 (2%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOMETRIAL STROMAL POLYP	2 (10%)	5 (10%)	1 (2%)
NERVOUS SYSTEM			
#CEREBRUM	(20)	(50)	(50)
ASTROCYTOMA	1 (5%)	1 (2%)	
MENINGIOMA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
OSTEOSARCOMA			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
FIBROSARCOMA			1 (2%)
OSTEOSARCOMA			3 (6%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	9	9
MORIBUND SACRIFICE		3	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	38	38
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	10	32	33
TOTAL PRIMARY TUMORS	15	43	45
TOTAL ANIMALS WITH BENIGN TUMORS	9	19	13
TOTAL BENIGN TUMORS	10	23	16
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	18	24
TOTAL MALIGNANT TUMORS	5	20	27
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			2
TOTAL UNCERTAIN TUMORS			2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED AZOBENZENE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED AZOBENZENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	2
ANIMALS NECROPSIED	20	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	48
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(49)	(48)
SARCOMA, NCS			1 (2%)
FIBROSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(47)
HEPATOCELLULAR CARCINOMA, METAST	1 (5%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)	3 (6%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(48)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (5%)	2 (4%)	1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (10%)		3 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (4%)	2 (4%)
#SPLEEN	(20)	(49)	(47)
HEMANGIOSARCOMA	1 (5%)	2 (4%)	2 (4%)
#MESENTERIC L. NODE	(20)	(48)	(47)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
#LIVER	(20)	(49)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		
*MESENTERY	(20)	(49)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
#THYMUS	(12)	(40)	(40)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (3%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(48)
NEOPLASTIC NODULE	6 (30%)	8 (16%)	
HEPATOCELLULAR CARCINOMA	3 (15%)	10 (20%)	2 (4%)
HEMANGIOSARCOMA			3 (6%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(20)	(48)	(45)
PHEOCHROMOCYTOMA			3 (7%)
SARCOMA, NOS		1 (2%)	
#THYROID	(18)	(49)	(47)
PAPILLARY ADENOCARCINOMA		1 (2%)	
FOLLICULAR-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	4	5
MORIBUND SACRIFICE	2		3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	45	40
ANIMAL MISSING		1	2
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	25	20
TOTAL PRIMARY TUMORS	16	31	23
TOTAL ANIMALS WITH BENIGN TUMORS	2	3	5
TOTAL BENIGN TUMORS	2	3	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	8	19	17
TOTAL MALIGNANT TUMORS	8	20	18
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		
TOTAL SECONDARY TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	6	8	
TOTAL UNCERTAIN TUMORS	6	8	
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 ADMINISTERED AZOBENZENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	7
ANIMALS NECROPSIED	20	47	38
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	47	38
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(47)	(38)
HEMANGIOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(46)	(36)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)	2 (4%)	2 (6%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(47)	(38)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (5%)	1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (10%)	1 (2%)	4 (11%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (10%)	2 (4%)	1 (3%)
UNDIFFERENTIATED LEUKEMIA		1 (2%)	
GRANULOCYTIC LEUKEMIA		1 (2%)	2 (5%)
#SPLEEN	(20)	(47)	(38)
HEMANGIOSARCOMA		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(47)	(36)
NEOPLASM, NOS, METASTATIC		1 (2%)	
HEPATOCELLULAR CARCINOMA		2 (4%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMANGIOSARCOMA			1 (3%)
ANGIOSARCOMA			1 (3%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(42)	(30)
ADENOMA, NOS	2 (11%)	1 (2%)	
#ADRENAL	(20)	(47)	(37)
PHEOCHROMOCYTOMA		2 (4%)	1 (3%)
#THYROID	(20)	(45)	(35)
FOLLICULAR-CELL ADENOMA	1 (5%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(47)	(38)
ADENOMA, NOS			1 (3%)
#UTERUS	(20)	(47)	(37)
HEMANGIOSARCOMA		1 (2%)	
#OVARY	(19)	(46)	(37)
GRANULOSA-CELL CARCINOMA			1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(20)	(47)	(38)
ADENOMA, NOS		3 (6%)	1 (3%)
MUSCULOSKELETAL SYSTEM			
*ABDOMINAL MUSCLE	(20)	(47)	(38)
HEMANGIOSARCOMA		1 (2%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*MEDIASTINUM	(20)	(47)	(38)
GRANULOSA-CELL CARCINOMA, METAST			1 (3%)
*PERITONEUM	(20)	(47)	(38)
SARCOMA, NOS		1 (2%)	
*MESENTERY	(20)	(47)	(38)
HEMANGIOSARCOMA		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(47)	(38)
HEMANGIOSARCOMA		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATHS	1	9	16
MORIBUND SACRIFICE			3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	19	40	23
ANIMAL MISSING		1	7

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	20	12
TOTAL PRIMARY TUMORS	10	23	15
TOTAL ANIMALS WITH BENIGN TUMORS	4	8	5
TOTAL BENIGN TUMORS	5	8	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	13	8
TOTAL MALIGNANT TUMORS	5	15	10
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		1	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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS ADMINISTERED AZOBENZENE IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED AZOBENZENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(49)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
HYPERKERATOSIS	1 (5%)	1 (2%)	
*SUBCUT TISSUE	(20)	(49)	(50)
HEMORRHAGE			1 (2%)
HEMATOMA, NOS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(20)	(49)	(50)
BRONCHIECTASIS			1 (2%)
#LUNG	(20)	(49)	(50)
BRONCHOPNEUMONIA, NOS			1 (2%)
INFLAMMATION, NOS	1 (5%)	1 (2%)	1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(49)	(49)
CONGESTION, NOS			1 (2%)
FIBROSIS		1 (2%)	
METAMORPHOSIS FATTY		3 (6%)	
LYMPHOID DEPLETION			1 (2%)
HYPERPLASIA, FOCAL			1 (2%)
HYPERPLASIA, MESOTHELIAL			1 (2%)
HYPERPLASIA, RETICULUM CELL			3 (6%)
#SPLENIC CAPSULE	(20)	(49)	(49)
INFLAMMATION, NOS		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, CHRONIC		11 (22%)	10 (20%)
SIDEROSIS			1 (2%)
#SPLENIC RED PULP	(20)	(49)	(49)
HYPERPLASIA, NOS		4 (8%)	1 (2%)
#MANDIBULAR L. NODE	(20)	(49)	(50)
HEMORRHAGE			1 (2%)
SIDEROSIS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(20)	(49)	(50)
THROMBOSIS, NOS		1 (2%)	
FIBROSIS	9 (45%)	14 (29%)	2 (4%)
FIBROSIS, DIFFUSE		1 (2%)	
DEGENERATION, NOS		1 (2%)	3 (6%)
#MYOCARDIUM	(20)	(49)	(50)
INFLAMMATION, FOCAL			1 (2%)
FIBROSIS	1 (5%)	1 (2%)	2 (4%)
DEGENERATION, NOS	2 (10%)		2 (4%)
#ENDOCARDIUM	(20)	(49)	(50)
FIBROELASTOSIS, NOS		1 (2%)	
*PANCREATIC ARTERY,	(20)	(49)	(50)
INFLAMMATION, CHRONIC	1 (5%)		
DIGESTIVE SYSTEM			
#PAROTID GLAND	(20)	(49)	(50)
INFLAMMATION, NOS		2 (4%)	1 (2%)
INFLAMMATION, NECROTIZING	4 (20%)	1 (2%)	1 (2%)
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
#SUBMAXILLARY GLAND	(20)	(49)	(50)
INFLAMMATION, NOS		4 (8%)	
INFLAMMATION, NECROTIZING	3 (15%)	2 (4%)	
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, ACUTE/CHRONIC		2 (4%)	1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC			1 (2%)
ATROPHY, NOS			1 (2%)
REGENERATION, NOS			1 (2%)
#LIVER	(20)	(49)	(50)
INFLAMMATION, NECROTIZING			1 (2%)
GRANULOMA, NOS		11 (22%)	3 (6%)
FIBROSIS			1 (2%)
CHOLANGIOFIBROSIS	11 (55%)	35 (71%)	13 (26%)
CIRRHOSIS, NOS			1 (2%)
NECROSIS, NOS		1 (2%)	3 (6%)
METAMORPHOSIS FATTY		1 (2%)	2 (4%)
FOCAL CELLULAR CHANGE		3 (6%)	4 (8%)
#LIVER/CENTRILOBULAR	(20)	(49)	(50)
CYTOPLASMIC VACUOLIZATION			1 (2%)
MEGALOCYTOSIS			1 (2%)
#BILE DUCT	(20)	(49)	(50)
HYPERPLASIA, NOS		1 (2%)	3 (6%)
HYPERPLASIA, FOCAL		1 (2%)	
#PANCREAS	(20)	(47)	(48)
PERIARTERITIS		4 (9%)	2 (4%)
ATROPHY, NOS		4 (9%)	2 (4%)
#STOMACH	(20)	(49)	(50)
INFLAMMATION, CHRONIC		1 (2%)	2 (4%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
NEPHROPATHY	15 (75%)	34 (69%)	14 (28%)
INFARCT, NOS			1 (2%)
PIGMENTATION, NOS			1 (2%)
#KIDNEY/CORTEX	(20)	(49)	(50)
CYST, NOS			1 (2%)
#KIDNEY/TUBULE	(20)	(49)	(50)
PIGMENTATION, NOS			2 (4%)
#URINARY BLADDER	(20)	(47)	(47)
INFLAMMATION, ACUTE		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(49)	(49)
HEMORRHAGIC CYST		1 (2%)	
HYPERPLASIA, NOS	1 (5%)	3 (6%)	4 (8%)
HYPERPLASIA, FOCAL	1 (5%)	3 (6%)	
#ADRENAL	(20)	(49)	(50)
METAMORPHOSIS FATTY		1 (2%)	1 (2%)
#ADRENAL CORTEX	(20)	(49)	(50)
NECROSIS, NOS			1 (2%)
#ADRENAL MEDULLA	(20)	(49)	(50)
HYPERPLASIA, NOS		7 (14%)	1 (2%)
#THYROID	(20)	(49)	(48)
COLLOID CYST		1 (2%)	
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, C-CELL	1 (5%)	2 (4%)	
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(20)	(49)	(50)
HYPERPLASIA, CYSTIC		1 (2%)	
#PROSTATE	(20)	(47)	(47)
INFLAMMATION, ACUTE		8 (17%)	
#TESTIS	(20)	(48)	(49)
GRANULOMA, SPERMATIC		1 (2%)	
PERIARTERITIS		12 (25%)	
ATROPHY, NOS	1 (5%)	6 (13%)	1 (2%)
HYPERPLASIA, INTERSTITIAL CELL	3 (15%)	2 (4%)	2 (4%)
NERVOUS SYSTEM			
#CEREBRUM	(20)	(49)	(50)
GLIOSIS		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(20)	(49)	(50)
HEMORRHAGIC CYST			1 (2%)
*MESENTERY	(20)	(49)	(50)
INFARCT, NOS			1 (2%)
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC		1	
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY		1	

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED AZOBENZENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
BRONCHOPNEUMONIA, NOS			4 (8%)
INFLAMMATION, NOS			3 (6%)
INFLAMMATION, FOCAL			1 (2%)
PNEUMONIA, LIPID			1 (2%)
INFLAMMATION, CHRONIC	1 (5%)		
GRANULOMA, NOS			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(50)	(50)
HEMORRHAGE			1 (2%)
HEMATOMA, NOS		1 (2%)	
HEMATOMA, ORGANIZED			1 (2%)
FIBROSIS		1 (2%)	2 (4%)
PERIARTERITIS			1 (2%)
DEGENERATION, NOS			2 (4%)
METAMORPHOSIS FATTY		5 (10%)	5 (10%)
PIGMENTATION, NOS		4 (8%)	9 (18%)
HEMOSIDEROSIS	2 (10%)		3 (6%)
HEMATOPOIESIS			1 (2%)
#SPLENIC CAPSULE	(20)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	2 (4%)
INFLAMMATION, CHRONIC		29 (58%)	31 (62%)
#SPLENIC RED PULP	(20)	(50)	(50)
FIBROSIS			1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
PIGMENTATION, NOS		1 (2%)	1 (2%)
HYPERPLASIA, NOS		2 (4%)	
#LYMPH NODE	(20)	(50)	(50)
HEMORRHAGE			1 (2%)
HYPERPLASIA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(50)
FIBROSIS	6 (30%)	3 (6%)	11 (22%)
#MYOCARDIUM	(20)	(50)	(50)
FIBROSIS		1 (2%)	
DEGENERATION, NOS		2 (4%)	
DIGESTIVE SYSTEM			
#PAROTID GLAND	(20)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	1 (2%)
INFLAMMATION, NECROTIZING	1 (5%)	1 (2%)	2 (4%)
INFLAMMATION, ACUTE/CHRONIC		2 (4%)	
INFLAMMATION, CHRONIC		2 (4%)	
#SUBMAXILLARY GLAND	(20)	(50)	(50)
INFLAMMATION, NOS			7 (14%)
INFLAMMATION, NECROTIZING	1 (5%)	7 (14%)	4 (8%)
INFLAMMATION, ACUTE	1 (5%)		1 (2%)
INFLAMMATION, ACUTE/CHRONIC	2 (10%)	1 (2%)	1 (2%)
INFLAMMATION, CHRONIC		2 (4%)	3 (6%)
#LIVER	(20)	(50)	(50)
GRANULOMA, NOS		16 (32%)	15 (30%)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
CHOLANGIOFIBROSIS	3 (15%)	17 (34%)	2 (4%)
CIRRHOSIS, NOS	1 (5%)		
HEPATITIS, TOXIC		1 (2%)	
NECROSIS, NOS		1 (2%)	2 (4%)
NECROSIS, FOCAL		1 (2%)	
NECROSIS, COAGULATIVE	1 (5%)		
METAMORPHOSIS FATTY	1 (5%)	2 (4%)	1 (2%)
PIGMENTATION, NOS			11 (22%)
FOCAL CELLULAR CHANGE	3 (15%)	7 (14%)	3 (6%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LIVER/CENTRILOBULAR	(20)	(50)	(50)
NECROSIS, NOS			1 (2%)
#LIVER/HEPATOCYTES	(20)	(50)	(50)
MEGALOCYTOSIS	1 (5%)		
#PANCREAS	(20)	(50)	(50)
ATROPHY, NOS		1 (2%)	1 (2%)
#STOMACH	(20)	(50)	(50)
ULCER, NOS	1 (5%)		
INFLAMMATION, CHRONIC		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
PYELONEPHRITIS, NOS			1 (2%)
NEPHROPATHY	2 (10%)	4 (8%)	3 (6%)
PIGMENTATION, NOS		27 (54%)	2 (4%)
#KIDNEY/TUBULE	(20)	(50)	(50)
PIGMENTATION, NOS	1 (5%)		5 (10%)
#URINARY BLADDER	(20)	(50)	(50)
HYPERPLASIA, EPITHELIAL		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(49)	(50)
CYST, NOS	1 (5%)	1 (2%)	
HEMORRHAGIC CYST	1 (5%)		
HYPERPLASIA, NOS		2 (4%)	1 (2%)
HYPERPLASIA, FOCAL	2 (10%)	2 (4%)	5 (10%)
#ADRENAL	(20)	(50)	(50)
HEMORRHAGE			1 (2%)
NECROSIS, NOS	1 (5%)		
HYPERPLASIA, FOCAL			1 (2%)
#ADRENAL CORTEX	(20)	(50)	(50)
METAMORPHOSIS FATTY			1 (2%)
HYPERPLASIA, NOS		2 (4%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#ADRENAL MEDULLA	(20)	(50)	(50)
HYPERPLASIA, NOS		1 (2%)	6 (12%)
#THYROID	(20)	(50)	(48)
COLLOID CYST	1 (5%)	1 (2%)	
HYPERPLASIA, C-CELL	2 (10%)	4 (8%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
CYST, NOS	1 (5%)		
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, CYSTIC	1 (5%)		
*PREPUTIAL GLAND	(20)	(50)	(50)
HYPERPLASIA, CYSTIC			1 (2%)
#UTERUS	(20)	(50)	(50)
FIBROSIS	1 (5%)	1 (2%)	2 (4%)
#UTERUS/ENDOMETRIUM	(20)	(50)	(50)
INFLAMMATION, VESICULAR		2 (4%)	
#OVARY	(20)	(50)	(50)
CYST, NOS	1 (5%)		
#OVARY/MEDULLA	(20)	(50)	(50)
HYPERPLASIA, NOS	1 (5%)	1 (2%)	7 (14%)
NERVOUS SYSTEM			
#CEREBELLUM	(20)	(50)	(50)
HEMORRHAGE		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
STEATITIS			1 (2%)
NECROSIS, FAT			1 (2%)
*MESENTERY	(20)	(50)	(50)
PERIARTERITIS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPECIOGY SUMMARY			
NO LESION REPORTED	3		
AUTO/NECROPSY/HISTO PERF		1	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED AZOBENZENE IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED AZOBENZENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	2
ANIMALS NECROPSIED	20	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(49)	(48)
HYPERPLASIA, CYSTIC	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(20)	(49)	(47)
HYPERPLASIA, LYMPHOID		3 (6%)	
#LUNG	(20)	(49)	(47)
INFLAMMATION, FOCAL	1 (5%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(49)	(47)
ATROPHY, NOS			2 (4%)
HYPERPLASIA, LYMPHOID	2 (10%)	4 (8%)	1 (2%)
HEMATOPOIESIS	1 (5%)	1 (2%)	
#LYMPH NODE	(20)	(48)	(47)
HYPERPLASIA, LYMPHOID			15 (32%)
#SUBMANDIBULAR L.NODE	(20)	(48)	(47)
HYPERPLASIA, LYMPHOID	1 (5%)		
#CERVICAL LYMPH NODE	(20)	(48)	(47)
METAMORPHOSIS FATTY	1 (5%)		
HYPERPLASIA, LYMPHOID	1 (5%)	1 (2%)	
#PANCREATIC L.NODE	(20)	(48)	(47)
HYPERPLASIA, LYMPHOID		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE	(20)	(48)	(47)
ATROPHY, NOS	2 (10%)	3 (6%)	
HYPERPLASIA, LYMPHOID	1 (5%)	3 (6%)	
HEMATOPOIESIS	1 (5%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(48)	(46)
FIBROSIS		1 (2%)	
FIBROSIS, FOCAL		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(48)	(47)
INFLAMMATION, NOS		1 (2%)	
#SUBMAXILLARY GLAND	(19)	(48)	(47)
INFLAMMATION, NOS	6 (32%)	16 (33%)	
#LIVER	(20)	(49)	(48)
THROMBOSIS, NOS	1 (5%)		
HEMORRHAGIC CYST	1 (5%)		
INFLAMMATION ACUTE PUSTULAR		1 (2%)	
INFLAMMATION, CHRONIC FOCAL	1 (5%)		
INFARCT, NOS	1 (5%)	1 (2%)	
BASOPHILIC CYTO CHANGE			1 (2%)
FOCAL CELLULAR CHANGE	1 (5%)	5 (10%)	
MEGALOCYTOSIS			1 (2%)
HYPERPLASTIC NODULE	1 (5%)		1 (2%)
HYPERPLASIA, LYMPHOID		2 (4%)	
#LIVER/CENTRILOBULAR	(20)	(49)	(48)
BILE STASIS			1 (2%)
CYTOPLASMIC VACUOLIZATION			1 (2%)
#LIVER/KUPFFER CELL	(20)	(49)	(48)
HYPERPLASIA, NOS	1 (5%)		
#STOMACH	(20)	(49)	(47)
ULCER, NOS			1 (2%)
ABSCESS, NOS			1 (2%)
#PEYERS PATCH	(20)	(49)	(47)
HYPERPLASIA, LYMPHOID		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(46)
PYELONEPHRITIS, NOS		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
GLOMERULONEPHRITIS, CHRONIC	1 (5%)		
HYPERPLASIA, LYMPHOID	6 (30%)	17 (35%)	
#URINARY BLADDER	(20)	(49)	(45)
HYPERPLASIA, LYMPHOID		4 (8%)	
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(45)	(38)
CYST, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
#PROSTATE	(20)	(48)	(46)
INFLAMMATION, CHRONIC	1 (5%)	1 (2%)	
*SCROTUM	(20)	(49)	(48)
NECROSIS, FAT	1 (5%)		
NERVOUS SYSTEM			
#CEREBRUM	(20)	(49)	(46)
CALCIFICATION, NOS	12 (60%)	17 (35%)	
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(20)	(49)	(48)
INFLAMMATION, NECROTIZING			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
MUSCULOSKELETAL SYSTEM			
*MUSCLE OF BACK	(20)	(49)	(48)
INFLAMMATION, CHRONIC	1 (5%)		

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(49)	(48)
NECROSIS, FAT	1 (5%)		
INFARCT, NOS		1 (2%)	
*MESENTERY	(20)	(49)	(48)
NECROSIS, FAT	1 (5%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	2	10
ANIMAL MISSING/NO NECROPSY		1	2
AUTO/NECROPSY/HISTO PERF			1

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED AZOBENZENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	7
ANIMALS NECROPSIED	20	47	38
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	47	38
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(20)	(46)	(36)
HYPERPLASIA, LYMPHOID		10 (22%)	
#LUNG	(20)	(46)	(36)
HEMORRHAGE		1 (2%)	
INFLAMMATION, NOS			1 (3%)
HYPERPLASIA, FOCAL	1 (5%)		
HYPERPLASIA, LYMPHOID	6 (30%)	2 (4%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(46)	(37)
SIDEROSIS			1 (3%)
#SPLEEN	(20)	(47)	(38)
HEMOSIDEROSIS			6 (16%)
HYPERPLASIA, LYMPHOID	7 (35%)	16 (34%)	4 (11%)
HEMATOPOIESIS	7 (35%)	8 (17%)	
#LYMPH NODE	(20)	(47)	(35)
HYPERPLASIA, LYMPHOID	1 (5%)		8 (23%)
#SUBMANDIBULAR L.NODE	(20)	(47)	(35)
HYPERPLASIA, LYMPHOID		1 (2%)	
#CERVICAL LYMPH NODE	(20)	(47)	(35)
HYPERPLASIA, NOS		1 (2%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, LYMPHOID	4 (20%)		
#MESENTERIC L. NODE	(20)	(47)	(35)
ATROPHY, NOS	1 (5%)	2 (4%)	
HYPERPLASIA, NOS		1 (2%)	
LYMPHOCYTOSIS		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (5%)	2 (4%)	
#THYMUS	(16)	(41)	(28)
ATROPHY, NOS	1 (6%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(45)	(35)
INFLAMMATION, NOS	2 (11%)	1 (2%)	
#SUBMAXILLARY GLAND	(19)	(45)	(35)
INFLAMMATION, NOS	7 (37%)	14 (31%)	
#LIVER	(20)	(47)	(36)
INFLAMMATION, NECROTIZING		1 (2%)	1 (3%)
NECROSIS, NOS			1 (3%)
NECROSIS, FOCAL	1 (5%)	1 (2%)	
METAMORPHOSIS FATTY			4 (11%)
FOCAL CELLULAR CHANGE	1 (5%)	2 (4%)	
MEGALOCYTOSIS			1 (3%)
HYPERPLASIA, LYMPHOID	1 (5%)	9 (19%)	
#LIVER/CAUDATE LOBE	(20)	(47)	(36)
INFARCT, NOS			1 (3%)
#PANCREAS	(20)	(47)	(34)
INFLAMMATION, NOS	1 (5%)	1 (2%)	
INFLAMMATION, ACUTE		1 (2%)	
#STOMACH	(20)	(47)	(37)
HEMORRHAGE			1 (3%)
URINARY SYSTEM			
#KIDNEY	(20)	(47)	(38)
INFLAMMATION, CHRONIC	1 (5%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, LYMPHOID	10 (50%)	13 (28%)	
#KIDNEY/CORTEX	(20)	(47)	(38)
CYST, NOS			1 (3%)
#KIDNEY/TUBULE	(20)	(47)	(38)
PIGMENTATION, NOS			1 (3%)
#URINARY BLADDER	(20)	(47)	(36)
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, LYMPHOID	5 (25%)	10 (21%)	
#U.BLADDER/SUBMUCOSA	(20)	(47)	(36)
HYPERPLASIA, LYMPHOID	1 (5%)		
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(42)	(30)
HYPERPLASIA, FOCAL		1 (2%)	
#ADRENAL CORTEX	(20)	(47)	(37)
DEGENERATION, NOS		1 (2%)	
#ADRENAL MEDULLA	(20)	(47)	(37)
HYPERPLASIA, NOS			2 (5%)
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(47)	(37)
HYDROMETRA			2 (5%)
HEMORRHAGE		1 (2%)	
HEMORRHAGIC CYST		1 (2%)	1 (3%)
HYPERPLASIA, LYMPHOID		1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(47)	(37)
INFLAMMATION, NOS			11 (30%)
INFLAMMATION, VESICULAR	8 (40%)	27 (57%)	4 (11%)
#OVARY/PAROVARIAN	(20)	(47)	(37)
INFARCT, NOS	2 (10%)		
#OVARY	(19)	(46)	(37)
CYST, NOS	1 (5%)	9 (20%)	2 (5%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
THROMBOSIS, NOS		2 (4%)	1 (3%)
HEMORRHAGIC CYST	1 (5%)		1 (3%)
NERVOUS SYSTEM			
#CEREBRUM	(20)	(47)	(36)
CALCIFICATION, NOS	13 (65%)	12 (26%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(47)	(38)
NECROSIS, FAT		1 (2%)	
INFARCT, NOS			1 (3%)
HYPERPLASIA, LYMPHOID		1 (2%)	
*MESENTERY	(20)	(47)	(38)
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, FAT	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			2
ANIMAL MISSING/NO NECROPSY		1	7
AUTOLYSIS/NO NECROPSY		2	5

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED AZOBENZENE IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Azobenzene in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma (b)	1/20 (5)	0/49 (0)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.000	1.200
Lower Limit		0.000	0.106
Upper Limit		7.624	61.724
Weeks to First Observed Tumor	105	--	65
Hematopoietic System:			
Monocytic Leukemia (b)	2/20 (10)	1/49 (2)	5/50 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.204	1.000
Lower Limit		0.004	0.184
Upper Limit		3.754	10.007
Weeks to First Observed Tumor	80	94	78

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Azobenzene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma or Leukemia (b)	3/20 (15)	1/49 (2)	8/50 (16)
P Values (c,d)	N.S.	N.S.	N.S.
Departure From Linear Trend (e)	P = 0.022		
Relative Risk (f)		0.136	1.067
Lower Limit		0.003	0.295
Upper Limit		1.599	5.813
Weeks to First Observed Tumor	80	94	65
Liver: Hepatocellular Carcinoma			
or Neoplastic Nodule (b)	0/20 (0)	1/49 (2)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.023	0.250
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	106	91

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Azobenzene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Adenoma, NOS (b)	4/20 (20)	2/49(4)	0/49 (0)
P Values (c,d)	P = 0.002 (N)	N.S.	P = 0.006 (N)
Relative Risk (f)		0.204	0.000
Lower Limit		0.020	0.000
Upper Limit		1.323	0.435
Weeks to First Observed Tumor	105	106	--
Pituitary: Chromophobe Adenoma (b)	0/20 (0)	2/49 (4)	3/49 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.125	0.255
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	86	90

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Azobenzene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor (b)	17/20 (85)	41/48 (85)	31/49 (63)
P Values (c,d)	P = 0.012 (N)	N.S.	N.S.
Relative Risk (f)		1.005	0.744
Lower Limit		0.836	0.607
Upper Limit		1.335	1.083
Weeks to First Observed Tumor	76	82	82
Spleen: Fibrosarcoma (b)	0/20 (0)	2/49 (4)	7/49 (14)
P Values (c,d)	P = 0.020	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.125	0.826
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	106	86

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Azobenzene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangiosarcoma (b)	0/20 (0)	1/49 (2)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.023	0.386
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	106	82
Multiple Organs: Fibrosarcoma (b)	0/20 (0)	0/49 (0)	13/50 (26)
P Values (c,d)	P less than 0.001	--	P = 0.007
Departure From Linear Trend (e)	P = 0.030		
Relative Risk (f)		--	Infinite
Lower Limit		--	1.674
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	76

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Azobenzene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Multiple Organs: Osteosarcoma (b)	0/20 (0)	0/49 (0)	3/50 (6)
P Values (c,d)	N.S.	--	N.S.
Relative Risk (f)		--	Infinite
Lower Limit		--	0.250
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	97
Abdominal Cavity:			
Sarcoma (b,g)	0/20 (0)	6/49 (12)	31/49 (63)
P Values (c,d)	P less than 0.001	N.S.	P less than 0.001
Departure from Linear Trend (e)	P = 0.027		
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.680	4.415
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	106	76

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Azobenzene in the Diet (a)

(continued)

(a) Dosed groups received 200 or 400 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

(g) Malignant neoplasm, NOS; sarcoma, NOS; fibrosarcoma; hemangiosarcoma; osteosarcoma; or mesothelioma.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Azobenzene in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System:			
Monocytic Leukemia (b)	1/20 (5)	3/50 (6)	0/50 (0)
P Values, (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.200	0.000
Lower Limit		0.106	0.000
Upper Limit		61.724	7.475
Weeks to First Observed Tumor	106	89	--
Hematopoietic System :			
Lymphoma or Leukemia (b)	1/20 (5)	6/50 (12)	1/50 (2)
P Values, (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.400	0.400
Lower Limit		0.325	0.005
Upper Limit		108.021	30.802
Weeks to First Observed Tumor	106	89	106

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Azobenzene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Adenoma, NOS (b)	4/20 (20)	7/49 (14)	7/50 (14)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.714	0.700
Lower Limit		0.211	0.207
Upper Limit		3.052	2.994
Weeks to First Observed Tumor	106	106	105
Thyroid: C-cell Carcinoma (b)	1/20 (5)	3/50 (6)	1/48 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.200	0.417
Lower Limit		0.106	0.006
Upper Limit		61.724	32.058
Weeks to First Observed Tumor	106	104	100

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Azobenzene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland:			
Fibroadenoma (b)	3/20 (15)	5/50 (10)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.667	0.400
Lower Limit		0.147	0.060
Upper Limit		4.014	2.802
Weeks to First Observed Tumor	106	106	105
Uterus: Endometrial Stromal			
Polyp (b)	2/20 (10)	5/50 (10)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.000	0.200
Lower Limit		0.184	0.004
Upper Limit		10.007	3.681
Weeks to First Observed Tumor	106	106	100

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Azobenzene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Spleen: Fibrosarcoma (b)	0/20 (0)	1/50 (2)	7/50 (14)
P Values (c,d)	P = 0.012	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.022	0.809
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	106	91
Spleen: Osteosarcoma (b)	0/20 (0)	1/50 (2)	5/50 (10)
P Values (c,d)	P = 0.041	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.022	0.525
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	106	106

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Azobenzene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Spleen: Hemangiosarcoma (b)	0/20 (0)	1/50 (2)	4/50 (8)
P Values, (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.022	0.386
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	106	93
Multiple Organs: Osteosarcoma (b)	0/20 (0)	0/50 (0)	3/50 (6)
P Values, (c,d)	N.S.	--	N.S.
Relative Risk (f)		--	Infinite
Lower Limit		--	0.250
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	102

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Azobenzene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Abdominal Cavity:			
Sarcoma (b,g)	0/20 (0)	5/50 (10)	21/50 (42)
P Values (c,d)	P less than 0.001	N.S.	P less than 0.001
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.525	2.840
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	106	91

(a) Dosed groups received 200 or 400 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

(g) Fibrosarcoma, hemangiosarcoma, malignant hemagiopericytoma, osteosarcoma, or sarcoma, NOS.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED AZOBENZENE IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Azobenzene in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	2/20 (10)	4/49 (8)	1/47 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.816	0.213
Lower Limit		0.131	0.004
Upper Limit		8.603	3.909
Weeks to First Observed Tumor	106	92	85
Hematopoietic System: Lymphoma (b)	4/20 (20)	5/49 (10)	8/48 (17)
P Values (c,d)	N. S.	N.S.	N.S.
Relative Risk (f)		0.510	0.833
Lower Limit		0.126	0.261
Upper Limit		2.367	3.459
Weeks to First Observed Tumor	106	92	105

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Azobenzene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangiosarcoma (b)	1/20 (5)	2/49 (4)	5/48 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.816	2.083
Lower Limit		0.046	0.259
Upper Limit		47.195	96.358
Weeks to First Observed Tumor	106	105	100
Liver: Hepatocellular Carcinoma (b)	3/20 (15)	10/49 (20)	2/48 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.361	0.278
Lower Limit		0.406	0.025
Upper Limit		7.138	2.278
Weeks to First Observed Tumor	106	91	105

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Azobenzene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Neoplastic Nodule (b)	8/20 (40)	16/49 (33)	2/48 (4)
P Values (c,d)	P less than 0.001 (N)	N.S.	P = 0.001 (N)
Relative Risk (f)		0.816	0.104
Lower Limit		0.412	0.012
Upper Limit		1.896	0.469
Weeks to First Observed Tumor	106	91	105
Adrenal: Pheochromocytoma (b)	0/20 (0)	0/48 (0)	3/45 (7)
P Values (c,d)	N.S.	--	N.S.
Relative Risk (f)		--	Infinite
Lower Limit		--	0.278
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	105

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Azobenzene in the Diet (a)

(continued)

(a) Dosed groups received 200 or 400 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Azobenzene in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma (b)	2/20 (10)	2/46 (4)	2/36 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.435	0.556
Lower Limit		0.034	0.044
Upper Limit		5.721	7.244
Weeks to First Observed Tumor	106	88	105
Hematopoietic System: Lymphoma or Leukemia (b)	5/20 (25)	6/47 (13)	7/38 (18)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.511	0.737
Lower Limit		0.152	0.238
Upper Limit		1.916	2.626
Weeks to First Observed Tumor	87	61	80

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Azobenzene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangiosarcoma (b)	0/20 (0)	4/47 (9)	1/38 (3)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.411	0.029
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	88	105
Pituitary: Adenoma, NOS (b)	2/19 (11)	1/42 (2)	0/30 (0)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.226	0.000
Lower Limit		0.004	0.000
Upper Limit		4.137	2.096
Weeks to First Observed Tumor	106	106	--

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Azobenzene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Harderin Gland: Adenoma, NOS (b)	0/20 (0)	3/47 (6)	1/38 (3)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.266	0.029
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	106	105

(a) Dosed groups received 208 or 545 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

Review of the Bioassay of Azobenzene* for Carcinogenicity
by the Data EvaluationRisk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

December 13, 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 on the Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, and State health officials. 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 Azobenzene.

The primary reviewer for the report on the bioassay of Azobenzene agreed that the compound was carcinogenic in treated rats. After commenting on the conditions of test, he said that the bioassay was properly designed and conducted. Based on the results of the study, he concluded that Azobenzene may pose a carcinogenic risk to humans.

The secondary reviewer said that the study was straightforward and that she agreed with the conclusion in the report. It was moved that the report on the bioassay of Azobenzene be accepted as written. The motion was seconded and approved without objection.

Clearinghouse Members Present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center
Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Verald K. Rowe, Dow Chemical USA
Michael Shimkin, University of California at San Diego

Louise Strong, University of Texas Health Sciences Center
Kenneth Wilcox, Michigan State Health Department

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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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