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**BIOASSAY OF
3,3'-DIMETHOXYBENZIDINE-
4,4'-DIISOCYANATE
FOR POSSIBLE CARCINOGENICITY**

CAS No. 91-93-0

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health



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

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DHEW Publication No. (NIH) 79-1383

REPORT ON THE BIOASSAY OF 3,3'-DIMETHOXYBENZIDINE-
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CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of 3,3'-dimethoxybenzidine-4,4'-diisocyanate conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 3,3'-dimethoxybenzidine-4,4'-diisocyanate was conducted by Litton Bionetics, Inc., Bethesda, Maryland, initially under direct contract to the NCI and currently under a sub-contract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. N. P. Page (1,2), Dr. E. K. Weisburger (1) and Dr. J. H. Weisburger (1,3). The principal investigators for the contract were Dr. F. M. Garner (4,5) and Dr. B. M. Ulland (4,5). Mr. S. Johnson (4) was the coprincipal investigator for the contract. Animal treatment and observation were supervised by Mr. R. Cypher (4), Mr. D. S. Howard (4) and Mr. H. D. Thornett (4); Mr. H. Paulin (4) analyzed dosed feed mixtures. Ms. J. Blalock (4) was responsible for data collection and assembly.

Histopathologic examinations were performed by Dr. B. C. Zook (4) at Litton Bionetics, Inc., the pathology narratives were written by Dr. B. C. Zook (4), and the diagnoses included in this report represent the interpretation of this pathologist. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (6).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (7); the statistical analysis was performed by Mr. W. W. Belew (8,9) and Mr. R. M. Helfand (8), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (10).

This report was prepared at METREK, a Division of The MITRE Corporation (8) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (8), task leader Ms. P. Walker (8), senior biologist Mr. M. Morse (8), biochemist Mr. S. C. Drill (8), chemist Dr. N. Zimmerman (8), and technical editor Ms. P. A. Miller (8). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1,11), Dr. R. A. Griesemer (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,12), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay for the possible carcinogenicity of 3,3'-dimethoxybenzidine-4,4'-diisocyanate was conducted using Fischer 344 rats and B6C3F1 mice. 3,3'-Dimethoxybenzidine-4,4'-diisocyanate was administered, at either of two concentrations, to groups of 50 male and 50 female animals of each species, with the exception of 49 high dose female rats. The compound was administered in the feed with the exception of the first 22 weeks of the rat bioassay, when it was administered by gavage. Twenty animals of each sex and species were placed on test as controls. During intubation the high and low dosages of 3,3'-dimethoxybenzidine-4,4'-diisocyanate administered to rats were 3000 and 1500 mg/kg, respectively, while the high and low concentrations administered in the feed to both rats and mice were 44,000 and 22,000 ppm, respectively. The compound was administered for a period of 78 weeks, followed by an observation period of 26 weeks for rats and 25 weeks for mice.

There was a significant positive association between the administration of 3,3'-dimethoxybenzidine-4,4'-diisocyanate and mortality in male and female rats, but not in male or female mice. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

For both sexes of rats, there was a significant positive association between dosage and the incidence of leukemia and malignant lymphoma. There was a significantly higher incidence of neoplasms of the skin, excluding skin of the ear, when dosed male rats were compared to controls. There was a significant positive association between the dosages administered and the incidences of endometrial stromal polyps in female rats. None of the statistical tests for any site in male or female mice indicated a significant positive association between compound administration and tumor incidence.

Under the conditions of this bioassay, administration of 3,3'-dimethoxybenzidine-4,4'-diisocyanate was carcinogenic to Fischer 344 rats, causing neoplasms of the skin (excluding skin of the ear) in males, endometrial stromal polyps in females, and leukemia and malignant lymphoma in both sexes. The compound was also associated with the development of a combination of squamous-cell carcinomas and sebaceous adenocarcinomas of the Zymbal's gland and skin of the ear in rats of both sexes. There was no evidence for the carcinogenicity of the compound in B6C3F1 mice.



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I. INTRODUCTION

3,3'-Dimethoxybenzidine-4,4'-diisocyanate (Figure 1) (NCI No. C02175), a dimer of o-anisidine isocyanate, was selected for bioassay by the National Cancer Institute because of the structural similarity of this compound to 3,3'-dimethoxybenzidine, a carcinogen in Fischer rats (Hadidian et al., 1968).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 4,4'-diisocyanato-3,3'-dimethoxy-1,1'-biphenyl.* It is also called dianisidine diisocyanate; isocyanic acid 3,3'-dimethoxy-4,4'-biphenylene ester; 4,4'-diisocyanato-3,3'-dimethoxybiphenyl; 3,3'-dimethoxybiphenylene-4,4'-diisocyanate; 3,3'-dimethoxy-4,4'-biphenyl diisocyanate; and 3,3'-dimethoxy-4,4'-biphenylene diisocyanate.

Several patents have been issued or applied for describing potential uses of 3,3'-dimethoxybenzidine-4,4'-diisocyanate. These include synthesis of shock-absorbing polyurea-polyurethanes, which can be used in railway car couplers (Chung, 1975); in situ manufacture of polyurethane gaskets and seals for metal pail and drum covers, aerosol mounting cups, and metal bottoms of fiber drums (Grace, 1974); synthesis of heat stable thermoplastic urethane rubbers, useful as gaskets, seals, O-rings, and wire coatings (Bonk and Shah, 1975); formulation of photocurable triazine-containing polyene-polythiol lacquers for steel

* The CAS registry number is 91-93-0.

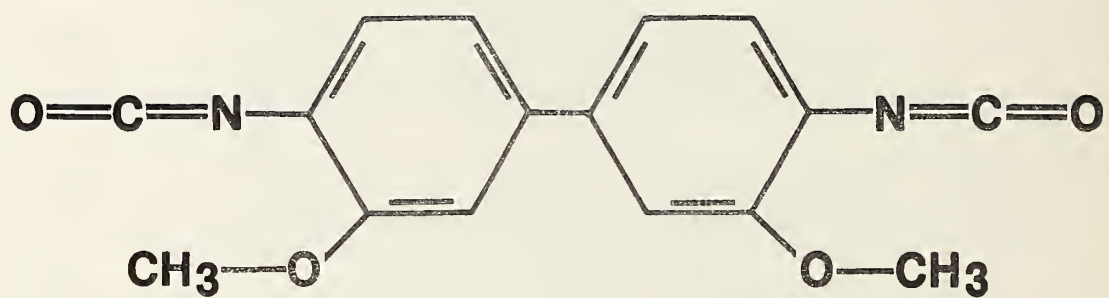


FIGURE 1
CHEMICAL STRUCTURE OF 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE

cans (Guthrie and Rendulic, 1975); and manufacture of abrasion-resistant antireflection coatings for color photographs (Chiklis, 1975); however, these uses appear to be purely experimental.

Specific production data for 3,3'-dimethoxybenzidine-4,4'-diisocyanate are not available; however, exclusion of this compound from the 1977 Directory of Chemical Producers, U.S.A. (Stanford Research Institute, 1977) implies that it is not currently produced in commercial quantities (in excess of 1000 pounds or \$1000 in value annually) in the United States.

The potential for exposure to 3,3'-dimethoxybenzidine-4,4'-diisocyanate is limited to researchers, particularly those involved in the experimental synthesis of elastomers and polymeric coatings.

II. MATERIALS AND METHODS

A. Chemicals

3,3'-Dimethoxybenzidine-4,4'-diisocyanate was purchased from Upjohn Company, Kalamazoo, Michigan. Chemical analysis was performed by Litton Bionetics, Inc., Kensington, Maryland. The experimentally determined range in melting point was 113° to 116°C. No literature value was found for comparison. Thin-layer chromatography (TLC) was performed utilizing two solvent systems (i.e., anhydrous acetone and ethyl acetate). Each plate was visualized with visible and ultraviolet light, iodine vapor, and ferricyanide-nitroprusside spray. The plate developed with acetone showed one contaminant that was less motile than the major spot, while the plate developed with ethyl acetate revealed two contaminants, one of lesser and one of greater motility than the major spot. The results of infrared (IR) and nuclear magnetic resonance (NMR) analyses were consistent with those expected based on the structure of the compound. Ultraviolet/visible (UV/VIS) spectrophotometry showed λ_{max} at 310 and 285 nm with respective molar extinction coefficients of 2.55×10^4 and 1.87×10^4 .

A second batch of the compound was purchased approximately 1.5 years later from the same manufacturer. The experimentally determined range in melting point was 109.3° to 111.3°C. TLC was performed as with the first batch. One impurity that was less motile than the major spot was observed on each plate. The results of IR and NMR analyses were consistent with those expected based on the structure

of the compound. UV/VIS analysis revealed λ_{max} at 307 and 280 nm with respective molar extinction coefficients of 2.33×10^4 and 1.67×10^4 .

Throughout this report the term 3,3'-dimethoxybenzidine-4,4'-diisocyanate is used to represent this material.

B. Dosage Preparation

Fresh solutions of 3,3'-dimethoxybenzidine-4,4'-diisocyanate in steroid suspending vehicle* (Litton Bionetics, Inc., Kensington, Maryland) were prepared on each day that intubation was performed. Excess portions of the mixtures were disposed of rather than stored. The concentration of 3,3'-dimethoxybenzidine-4,4'-diisocyanate in steroid suspending vehicle ranged from 15 to 30 percent.

C. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox[®] (Allied Mills, Inc., Chicago, Illinois). 3,3'-Dimethoxybenzidine-4,4'-diisocyanate was administered to the dosed mice as a component of the diet throughout the period of chemical administration, while it was administered to rats as a component of the diet beginning with week 23 and continuing for the duration of compound administration. (Rats were intubated with the chemical for the first 22 weeks of the bioassay.)

* Steroid suspending vehicle = 9 gm NaCl, 5 gm carboxymethylcellulose, 4 ml polysorbate 80, and 9 ml benzyl alcohol q.s. to 1 liter with distilled water.

The chemical was removed from its container and a proper amount was blended with an aliquot of the ground feed using a mortar and pestle. Once visual homogeneity was attained, the mixture was placed in a 6 kg capacity Patterson-Kelley standard model twin-shell stainless steel V-blender along with the remainder of the feed to be prepared. After 20 minutes of blending, the mixtures were placed in double plastic bags and stored in the dark at 4°C. The mixture was prepared once weekly.

Dosed feed preparations containing 23,000 and 46,000 ppm of 3,3'-dimethoxybenzidine-4,4'-diisocyanate were analyzed spectrophotometrically. The mean result immediately after preparation was 95.2 ± 4.2 percent of theoretical, including correction for the analytical method of recovery used. After ten days, at ambient room temperature, the mean result was 100.3 ± 4.4 percent of theoretical, including correction for the analytical method of recovery used.

D. Animals

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

Rats and mice were approximately 4 weeks old when received. Upon receipt, animals were examined for visible signs of disease or parasites. Obviously ill or runted animals were culled. The remaining animals were quarantined for 2 weeks prior to initiation of test. Animals which did not manifest clinical signs of disease were placed on test at this time. Animals were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

E. Animal Maintenance

All animals were housed by species in temperature- and humidity-controlled rooms. The temperature range was 22° to 26°C and the relative humidity was maintained between 45 and 55 percent. Incoming air was filtered through HEPA filters (Flanders Filters, McLean, Virginia) at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided 8 hours per day (9:00 a.m. to 5:00 p.m.).

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

bedding (Wilner Wood Products Company, Norway, Maine) was used in polycarbonate cages for the entire bioassay.

Acidulated water (pH 2.5) was supplied to animals in water bottles filled by an automated metering device that was checked daily for diluting accuracy. Water bottles were changed and washed twice weekly, and sipper tubes were washed at weekly intervals. During the period of chemical administration, dosed and control animals received treated or untreated Wayne Lab-Blox[®] meal as appropriate. The feed was supplied in hanging stainless steel hoppers which were refilled three times per week and sanitized weekly. Food and water were available ad libitum for both species.

All dosed and control rats were housed in a room with other rats receiving diets containing * N-phenyl-p-phenylenediamine hydrochloride (2198-59-6); acetylaminofluorene (53-96-3); and nitrilotriacetic acid (139-13-9).

All dosed and control mice were housed in a room with mice receiving diets containing EDTA trisodium salt (150-38-9); diaminczide (1596-84-5); N,N'-diethylthiurcāā (105-55-5); triphenyltin hydroxide (76-87-9); carbromal (75-65-6); p-quinone dioxime (105-11-3); 4-amino-2-nitrophenol (119-34-6); other mice intubated with lithocholic acid (434-13-9); and other mice receiving I.P. injections of methiodol sodium (126-31-8).

* CAS registry numbers are given in parentheses.

F. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of 3,3'-dimethoxybenzidine-4,4'-diisocyanate for administration to dosed animals in the chronic study, subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among six groups, each consisting of five males and five females. 3,3'-Dimethoxybenzidine-4,4'-diisocyanate mixed with distilled water was introduced by gavage to five of the six rat groups at dosages of 800, 1260, 2000, 3160, and 5000 mg/kg and to five of the six mouse groups at dosages of 215, 464, 1000, 2150, and 4640 mg/kg. The sixth group of each species served as a control, receiving only steroid suspending vehicle by gavage. Intubation was performed five times per week for 4 weeks, followed by a 2-week observation period to detect any delayed toxicity. Individual body weights were recorded weekly. At the end of the observation period, all survivors were sacrificed and necropsied.

At the end of the subchronic test, mean body weight gain among male rats dosed with 2000 mg/kg was 1 percent greater than the mean body weight gain of their controls, while female rats receiving the same dosage displayed a mean body weight gain 4 percent less than that of their controls. At a dosage of 3160 mg/kg, the mean body weight gain among male rats was 9 percent less than that of their controls, while female rats receiving the same dosage displayed a mean body weight gain 14 percent less than that of their controls.

The high concentration selected for administration to dosed rats in the chronic bioassay was 3000 mg/kg.

At the end of the subchronic test, mean body weight gain among male mice dosed with 4640 mg/kg was 4 percent less than the mean body weight gain of their controls, while female mice receiving the same dosage displayed a mean body weight gain 11 percent less than that of their controls. The high concentration selected for administration to dosed mice in the chronic bioassay was 44,000 ppm (equivalent to 7040 mg/kg, assuming an average food consumption of 4 grams/day and an average body weight of 25 grams).

G. Gastric Intubation

Intubation was performed for 5 consecutive days per week on a mg/kg body weight basis, utilizing the most recently observed group mean body weight as a guide for determining the dose. All rats were weighed and dosages adjusted once monthly, based on group mean body weight. Thus, although the ratio of dose to weight remained constant, the total dosage administered fluctuated with an increase or decrease in group mean body weight. Rats of each sex within a dosed group received the same dosage.

H. Experimental Design

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

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE CHRONIC BIOASSAY

	INITIAL GROUP SIZE	3,3'-DIMETHOXYBENZIDINE- 4,4'-DIISOCYANATE ^{a,b}	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
<u>MALE</u>				
VEHICLE CONTROL ^c	20	0	0	104
LOW DOSE	50	1,500 ^a 22,000 ^b 0	22 56	26
HIGH DOSE	50	3,000 ^a 44,000 ^b 0	22 56	26
<u>FEMALE</u>				
VEHICLE CONTROL ^c	20	0	0	104
LOW DOSE	50	1,500 ^a 22,000 ^b 0	22 56	26
HIGH DOSE	49	3,000 ^a 44,000 ^b 0	22 56	26

^a Dosages, given in mg/kg body weight, were administered by gavage 5 consecutive days per week. These dosages (i.e., 3000 and 1500 mg/kg) are equivalent to respective concentrations of 44,000 and 22,000 ppm (assuming average daily food consumption and body weight).

^b Concentrations given in parts per million in the feed.

^c Gavaged with steroid suspending vehicle for the first 22 weeks and subsequently transferred to the basal laboratory diet.

TABLE 2
 DESIGN SUMMARY FOR B6C3F1 MICE
 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	3,3'-DIMETHOXYBENZIDINE- 4,4'-DIISOCYANATE CONCENTRATION ^a	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
<u>MALE</u>				
CONTROL	20	0	0	103
LOW DOSE	50	22,000 0	78	25
HIGH DOSE	50	44,000 0	78	25
<u>FEMALE</u>				
CONTROL	20	0	0	103
LOW DOSE	50	22,000 0	78	25
HIGH DOSE	50	44,000 0	78	25

^aConcentrations given in parts per million.

The method of compound administration for the chronic bioassay in rats, which was begun prior to the chronic bioassay in mice, was initially intubation. Intubation was selected because it was believed that the compound was not stable in feed. After determination that the compound was stable in feed (as indicated on page 6) the method of compound administration to rats was changed to dietary and the mouse bioassay was initiated using dietary administration.

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test simultaneously. The dosages initially administered to males and females were 3000 and 1500 mg/kg, by gavage (equivalent to dietary concentrations of 44,000 and 22,000 ppm). Throughout this report those rats initially receiving the former dosage are referred to as the high dose groups and those initially receiving the latter dosage are referred to as the low dose groups. All dosed rats were administered 3,3'-dimethoxybenzidine 4,4'-diisocyanate by gavage at the dosages indicated for 22 weeks. Beginning in week 23, 3,3'-dimethoxybenzidine-4,4'-diisocyanate was mixed with the feed and administered ad libitum in the diet. All dosed rats were supplied feed containing 3,3'-dimethoxybenzidine-4,4'-diisocyanate for the remaining 56 weeks of compound administration. During this 56-week period, the high and low concentrations administered were 44,000 and 22,000 ppm, respectively. The dosed rats were observed for a period of 26 weeks after compound administration ceased.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test simultaneously. The dietary concentrations of 3,3'-dimethoxybenzidine-4,4'-diisocyanate administered to both sexes were 44,000 and 22,000 ppm. Throughout this report those mice receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups. Dosed mice were supplied with feed containing 3,3'-dimethoxybenzidine-4,4'-diisocyanate for 78 weeks followed by a 25-week observation period.

I. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. From the first day, all animals were inspected twice daily for mortality. Food consumption data were collected at monthly intervals from 20 percent of the animals in each group. Body weights of rats were recorded once a week for the first 6 weeks, every 2 weeks for the next 12 weeks, and once a month thereafter. Body weights of mice were recorded once a week for the first 6 weeks, every 2 weeks for the next 10 weeks and once a month for the remainder of the bioassay.

All moribund animals or animals that developed large, palpable masses that jeopardized their health were sacrificed. A necropsy was performed on each animal regardless of whether it died, was sacrificed when moribund, or was sacrificed at the end of the bioassay.

The animals were euthanized by carbon dioxide asphyxiation, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of all major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

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

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

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

J. Data Recording and Statistical Analyses

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

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

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

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k , are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P -value for any comparison be less than or equal to $0.05/k$. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P -values are shown.

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

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

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing

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

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

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it

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

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

Dose-related mean body weight depression was not apparent in either male or female rats. There was, however, slight mean body weight depression in dosed groups of both sexes when compared to their respective controls (Figure 2).

No abnormal clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 3,3'-dimethoxybenzidine-4,4'-diisocyanate-dosed groups are shown in Figure 3. For male rats the Tarone test for a positive association between dosage and mortality was significant ($P = 0.002$). While the Tarone test was not significant for females, the Cox tests comparing high dose to control and low dose to control indicated a significantly higher mortality among dosed rats when compared with the control group.

There were adequate numbers of male rats at risk from late-developing tumors as 80 percent (40/50) of the high dose, 88 percent (44/50) of the low dose, and 90 percent (18/20) of the control group survived on test more than 80 weeks. Thirty-two percent (16/50) of the high dose, 44 percent (22/50) of the low dose and 75 percent (15/20) of the control group survived on test until the termination of the study.

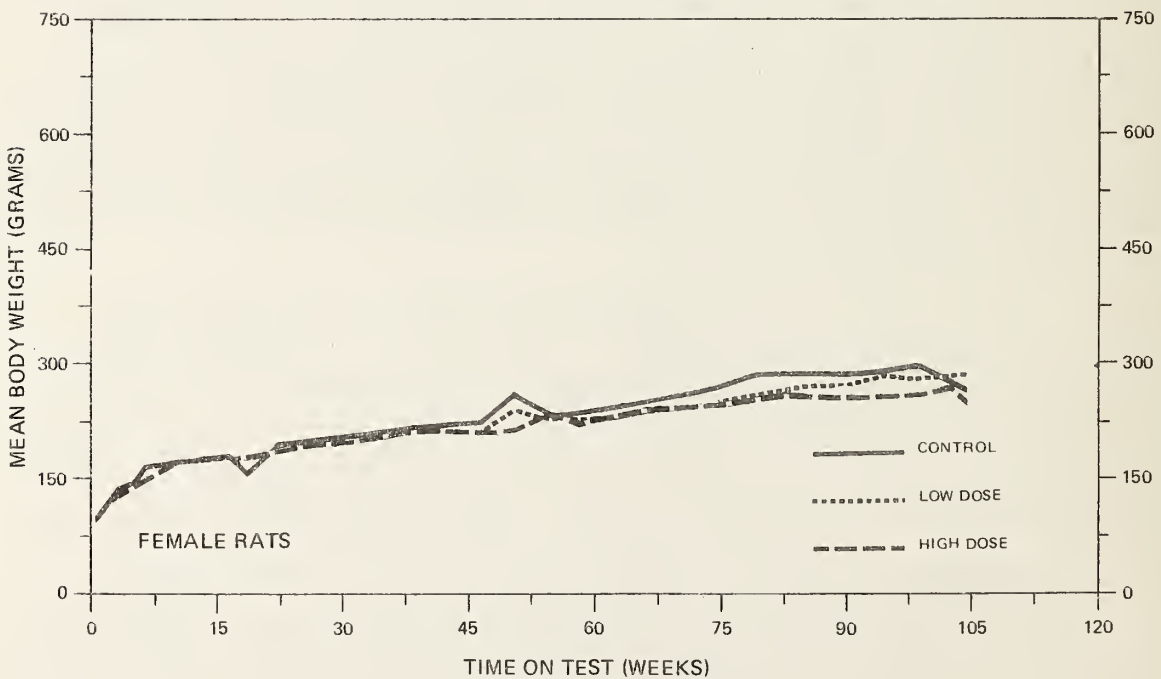
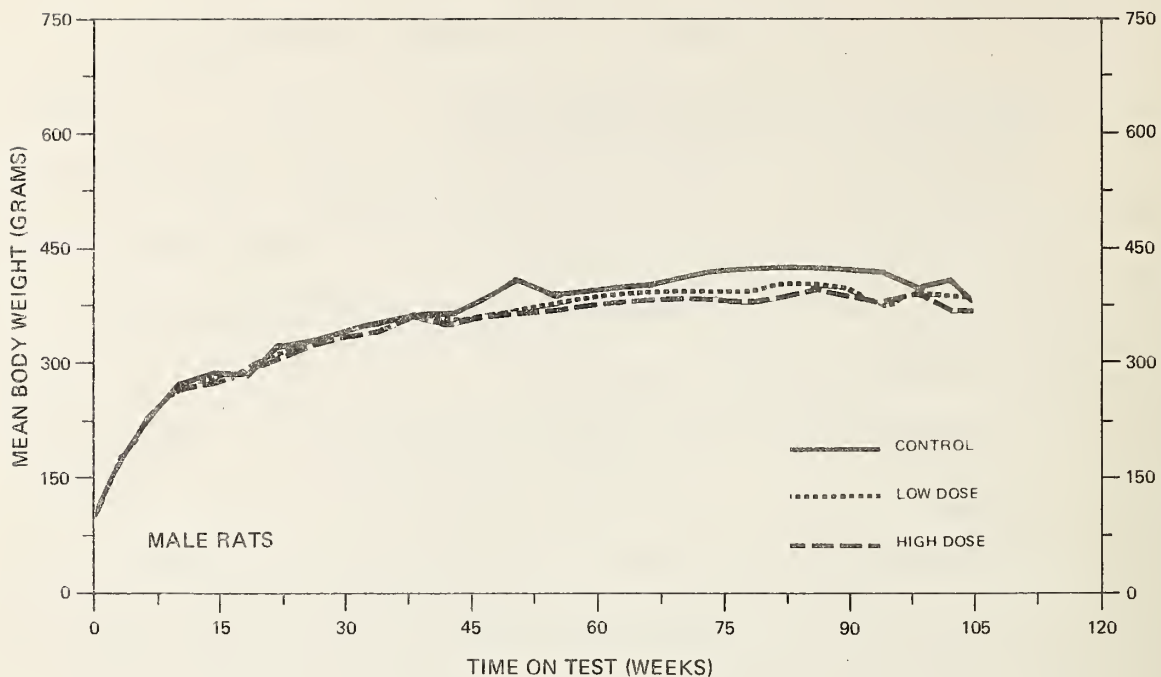


FIGURE 2
GROWTH CURVES FOR 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE CHRONIC STUDY RATS

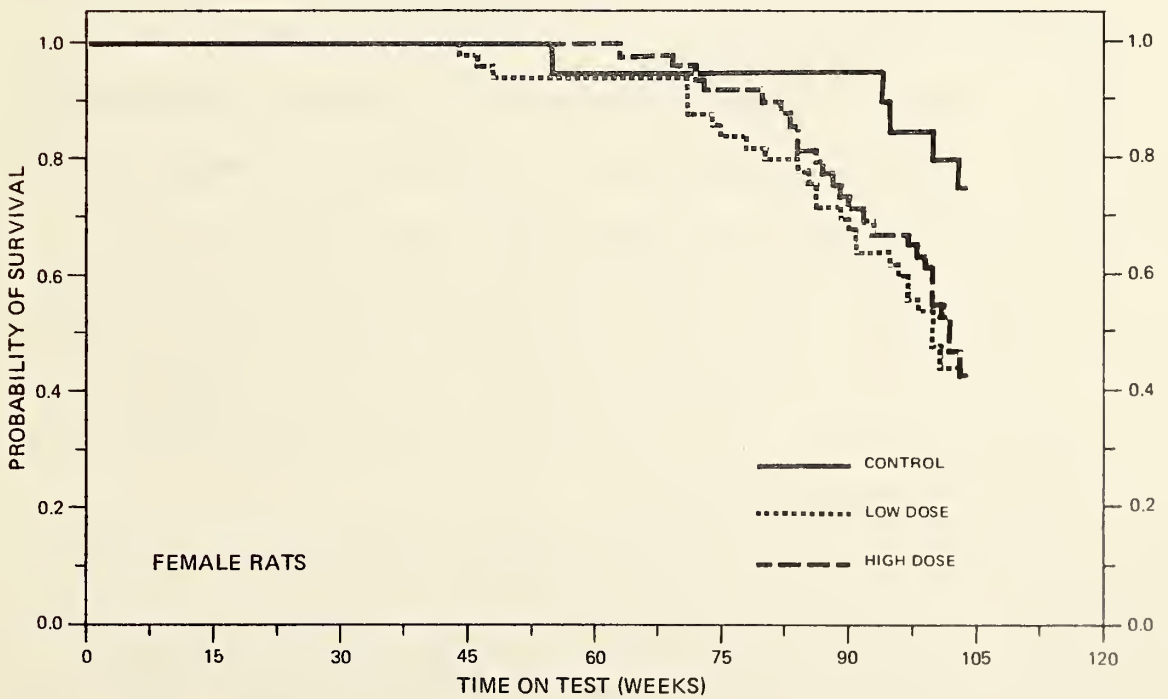
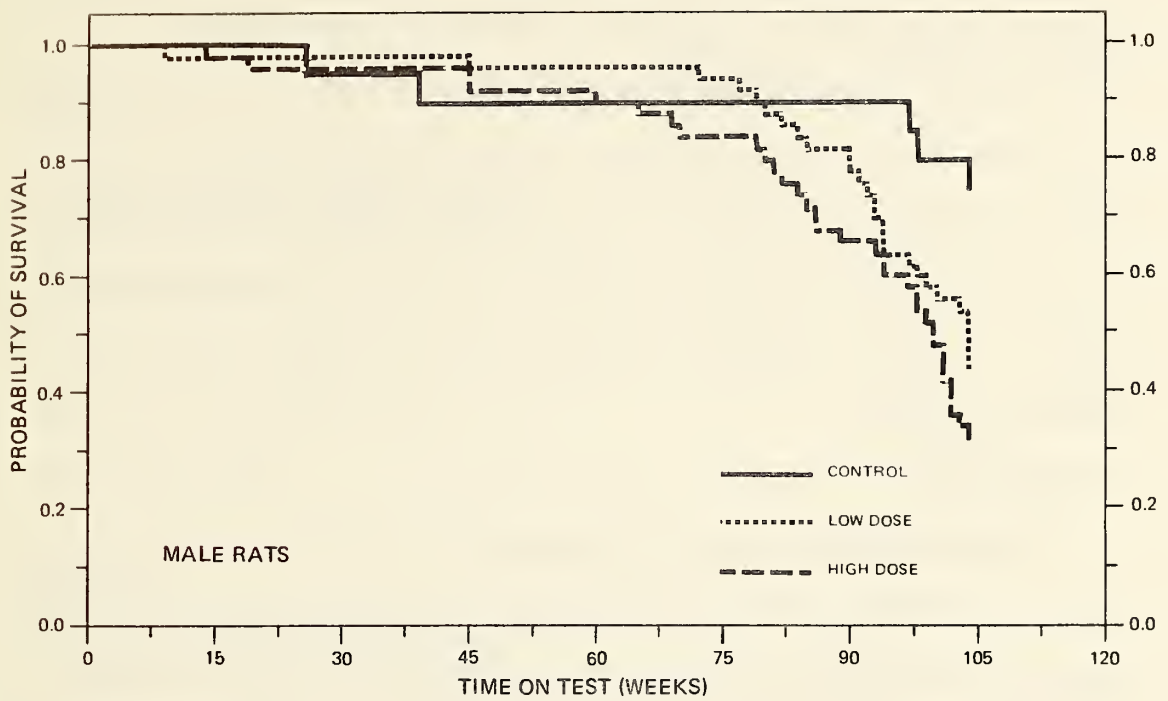


FIGURE 3
SURVIVAL COMPARISONS OF 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE CHRONIC STUDY RATS

Similarly, there were adequate numbers of female rats at risk from late-developing tumors with 90 percent (45/50) of the high dose, 80 percent (40/50) of the low dose, and 95 percent (19/20) of the control group surviving on test more than 80 weeks. At the termination of the study, 42 percent (21/50) of the high dose, 42 percent (21/50) of the low dose and 75 percent (15/20) of the control group were alive on test.

C. Pathology

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

There were increased incidences of tumors of baso-squamous epithelial origin in dosed male and female rats, when compared to their respective controls. These tumors were commonly in the skin of the head, inguinal area and back. The location of 28 tumors of the head strongly suggested that these tumors arose from the Zymbal's gland. The location and type of 17 tumors in the inguinal area suggested involvement of preputial glands. Grossly, they appeared as irregular fungating or bulging ulcerated lesions measuring about 0.5 to 3.0 cm in greatest dimension.

Microscopically, the tumors thought to arise from the Zymbal's gland were carcinomas of the sebaceous gland, squamous-cell carcinomas, and a carcinosarcoma as summarized in the following table:

	<u>Control</u>	<u>Low Dose</u>	<u>High Dose</u>
<u>MALES</u>			
<u>Number of Animals with Tissues Examined Histopathologically</u>	(20)	(50)	(50)
<u>ZYMBAL'S GLAND</u>			
Squamous-Cell Carcinoma	0	2(4%)	5(10%)
Sebaceous Adenocarcinoma	0	3(6%)	2(4%)
Carcinosarcoma	0	0	0
<u>FEMALES</u>			
<u>Number of Animals with Tissues Examined Histopathologically</u>	(20)	(50)	(48)
<u>ZYMBAL'S GLAND</u>			
Squamous-Cell Carcinoma	0	1(2%)	4(8%)
Sebaceous Adenocarcinoma	0	7(14%)	2(4%)
Carcinosarcoma	1(5%)	0	0

These and other tumors of baso-squamous origin were classified according to the apparent direction of maturation. The auditory tumors had both sebaceous and squamous differentiation as did nearly all other skin neoplasms discussed later. The Zymbal's gland tumors appeared to have the most malignant potential of any of the skin tumors. Two invaded the skull and two others metastasized to the lung.

Tumors arising from preputial and clitoral glands (i.e., 0/20, 1/50 [2 percent], and 3/50 [6 percent] in the control, low dose, and high

dose males, respectively, and 0/20, 8/50 [16 percent], and 4/48 [8 percent] in the control, low dose, and high dose females, respectively) were the most consistent of the skin tumors in that they mostly resembled sebaceous glands and as a rule had little squamous differentiation. These tumors appeared to have little malignant potential and were therefore regarded of low-grade malignancy. Additional skin tumors arose from other locations as shown in the following table:

	<u>Control</u>	<u>Low Dose</u>	<u>High Dose</u>
<u>MALES</u>			
<u>SKIN</u>			
<u>Number of Animals with Tissues Examined Histopathologically</u>	(20)	(50)	(50)
Neoplasms NOS	0	1(2%)	0
Papilloma NOS	0	0	2(4%)
Squamous-Cell Carcinoma	0	5(10%)	4(8%)
Basal-Cell Tumor	0	4(8%)	5(10%)
Trichoepithelioma	0	2(4%)	1(2%)
Sebaceous Adenoma	0	2(4%)	0
Sebaceous Adenocarcinoma	0	1(2%)	0
Keratoacanthoma	1(5%)	4(8%)	5(10%)
Carcinosarcoma	0	0	1(2%)

FEMALES

SKIN

	(20)	(50)	(48)
<u>Number of Animals with Tissues Examined Histopathologically</u>			
Neoplasms NOS	0	0	1(2%)
Papilloma NOS	0	0	0
Squamous-Cell Carcinoma	0	1(2%)	0
Basal-Cell Tumor	0	0	1(2%)
Trichoepithelioma	0	1(2%)	0
Sebaceous Adenoma	0	0	1(2%)
Sebaceous Adenocarcinoma	1(2%)	1(2%)	1(2%)
Keratoacanthoma	0	0	0
Carcinosarcoma	1(2%)	0	0

The classification of these tumors was seldom obvious. More often than not, the tumors were mixtures of basal cells often with sebaceous differentiation, squamous cells often forming keratin pearls and sometimes mimicked hair follicles. The direction of differentiation was obscured by maturation in two or more directions simultaneously. The impression was that all such skin tumors arose from similar pleuropotent cells and that the path of differentiation is variable, thus they should all be considered to have a malignant potential, however, low-grade.

Squamous-cell carcinomas were characterized by down growth of clusters of basal cells with individual cell and group keratinization, often with many keratin pearls. In some, invasion of the stroma led to a desmoplastic response. Sebaceous carcinomas were composed of lobules of basal type epithelium maturing toward sebaceous cells and often filling cystic spaces with "ghosts" of necrotic cells. Basal-cell tumors formed lobules of typical basophilic basal cells in a dense fibrous connective tissue. There were always some keratin pearls and sebaceous cells. Keratoacanthomas arose from greatly thickened surface epithelium and tended to form inverted cysts filled with laminated keratin. The cyst wall was thick, complex and contained keratin whorls and sebaceous glands, but was generally sharply circumscribed.

Leukemias were common in dosed male (i.e., 0/20, 18/50 [36 percent], and 16/50 [32 percent] in the control, low dose, and high

dose, respectively) and dosed female (i.e., 1/20 [5 percent], 8/50 [16 percent], and 12/48 [25 percent] in the control, low dose, and high dose, respectively) rats and were nearly absent in controls. The leukemias usually involved the spleen, causing splenomegaly, and less commonly involved the liver, giving it a slightly enlarged and mottled appearance.

Microscopically, in addition to the spleen and liver, the pulmonary capillaries were often filled with leukemic cells and sometimes the bone marrow sections contained leukemic cells. In all cases in which the tissues were not autolyzed, the leukemia was classified as of the undifferentiated type. The affected cells contained large nucleoli with evenly distributed chromatin. The nuclei were round with various degrees of indentation.

There was a spontaneous occurrence of a variety of other tumors in both the control and dosed groups. These lesions were of the type, incidence, and distribution often observed in aged Fischer 344 rats, and, therefore, were not attributed to compound administration.

The usual nonneoplastic lesions were seen in rats of all groups and were not considered to be compound-related.

The results of this pathologic examination indicated that 3,3'-dimethoxybenzidine-4,4'-diisocyanate caused skin and adnexal tumors in male and female rats, and may also be associated with leukemia in male and female rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 3,3'-dimethoxybenzidine-4,4'-diisocyanate-dosed groups and where such tumors were observed in at least 5 percent of the group. Due to the early mortality of a number of male rats the analyses for males have been based solely upon those males surviving at least 52 weeks or, in the event that the tumor of interest was observed earlier, at least as long as the time at which the first tumor of interest was observed.

For male rats, the Cochran-Armitage test indicated a significant ($P = 0.022$) positive association between dose and the combined incidence of leukemia or malignant lymphomas. This result was supported by significant Fisher exact test results for the low dose ($P = 0.001$) and the high dose ($P = 0.002$) groups. For females, the Cochran-Armitage test was also significant ($P = 0.007$) and was supported by a significant ($P = 0.016$) Fisher exact test comparing high dose to control. Based on these statistical results, the administration of 3,3'-dimethoxybenzidine-4,4'-diisocyanate was associated with the increased combined incidence of leukemia or malignant lymphomas in male and female Fischer 344 rats under the conditions of this bioassay.

Also in male rats, the Fisher exact tests for the incidence of skin (excluding skin of the ear) neoplasms (i.e., papilloma NOS,

TABLE 3
 TIME-ADJUSTED ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
 SPECIFIC SITES IN MALE RATS TREATED WITH 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE^{a, f}

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Skin (Excluding Skin of Ear): Squamous-Cell Carcinoma ^b	0/18(0.00)	5/48(0.10)	4/46(0.09)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.496	0.380
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	94	82
<hr/>			
Skin (Excluding Skin of Ear): Sebaceous Adenoma or Sebaceous Adenocarcinoma ^b	0/13(0.00)	3/48(0.06)	0/46(0.00)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	Infinite	---
Lower Limit	---	0.236	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	79	---
<hr/>			
Skin (Excluding Skin of Ear): Papilloma NOS, Basal-Cell Tumor, Trichoepithelioma, Sebaceous Adenoma, or Keratoacanthoma ^b	0/18(0.00)	12/48(0.25)	11/46(0.24)
P Values ^c	N.S.	P = 0.014	P = 0.018
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	1.445	1.366
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	79	60

TABLE 3 (CONTINUED)

TOPOGRAPHY: MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Skin (Excluding Skin of Ear): Squamous-Cell Carcinoma, or Sebaceous Adenocarcinoma ^b	0/18(0.00)	6/48(0.13)	4/46(0.09)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	Infinitive	Infinitive
Lower Limit	---	0.630	0.380
Upper Limit	---	Infinitive	Infinitive
Weeks to First Observed Tumor	---	94	82
Skin (Excluding Skin of Ear): Papilloma NOS, Basal-Cell Tumor, Trichoepithelioma, Sebaceous Adenoma, Keratoacanthoma, Squamous-Cell Carcinoma, or Sebaceous Adenocarcinoma ^b	0/18(0.00)	17/48(0.35)	14/46(0.30)
P Values ^c	P = 0.045	P = 0.002	P = 0.005
Departure from Linear Trend ^e	P = 0.024	---	---
Relative Risk (Control) ^d	---	Infinitive	Infinitive
Lower Limit	---	2.132	1.795
Upper Limit	---	Infinitive	Infinitive
Weeks to First Observed Tumor	---	79	60

TABLE 3 (CONTINUED)

TOPOGRAPHY: MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma ^b	0/18(0.00)	8/47(0.17)	0/43(0.00)
P Values ^c	N.S.	N.S.	N.S.
Departure from Linear Trend ^e	P = 0.002	---	---
Relative Risk (Control) ^d	---	Infinite	---
Lower Limit	---	0.919	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	79	---
Hematopoietic System: Leukemia or Malignant Lymphoma ^b			
Lung: Alveolar/Bronchiolar Adenoma ^b	0/18(0.00)	18/48(0.38)	16/46(0.35)
P Values ^c	P = 0.022	P = 0.001	P = 0.002
Departure from Linear Trend ^e	P = 0.028	---	---
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	2.270	2.083
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	72	80
Thyroid: C-Cell Adenoma or C-Cell Carcinoma ^b			
Lung: Alveolar/Bronchiolar Adenoma ^b	1/18(0.06)	3/43(0.07)	0/42(0.00)
P Values ^c	N.S.	N.S.	N.S.
Departure from Linear Trend ^e	---	1.256	0.000
Relative Risk (Control) ^d	---	0.112	0.000
Lower Limit	---	64.377	7.981
Upper Limit	---	104	---
Weeks to First Observed Tumor	104	98	---

TABLE 3 (CONTINUED)

TOPOGRAPHY; MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Preputial Gland: Sebaceous Adenocarcinoma ^b	0/18(0.00)	1/48(0.02)	3/46(0.07)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.021	0.246
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	97	94
<hr/>			
Testis: Interstitial-Cell Tumor ^b	17/18(0.94)	39/48(0.81)	36/45(0.80)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	0.860	0.847
Lower Limit	---	0.793	0.779
Upper Limit	---	1.139	1.128
Weeks to First Observed Tumor	97	77	79
<hr/>			
Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma ^b	1/18(0.06)	8/48(0.17)	3/46(0.07)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	3.000	1.174
Lower Limit	---	0.457	0.104
Upper Limit	---	129.949	60.276
Weeks to First Observed Tumor	104	104	86

TABLE 3 (CONTINUED)

TOPOGRAPHY; MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma ^{b, f}	3/16 (0.19)	7/44 (0.16)	2/34 (0.06)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	0.848	0.314
Lower Limit	---	0.231	0.029
Upper Limit	---	4.673	2.516
Weeks to First Observed Tumor	39	80	69
Adrenal: Pheochromocytoma ^b	4/18 (0.22)	3/48 (0.06)	0/45 (0.00)
P Values ^c	P = 0.003(N)	N.S.	P = 0.005(N)
Relative Risk (Control) ^d	---	0.281	0.000
Lower Limit	---	0.047	0.000
Upper Limit	---	1.530	0.425
Weeks to First Observed Tumor	104	104	---
Zymbal's Gland, Ear, and Skin of Ear: Squamous-Cell Carcinoma or Sebaceous Adenocarcinoma ^b	0/18 (0.00)	5/48 (0.10)	8/46 (0.17)
P Values ^c	P = 0.040	N.S.	N.S.
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.496	0.939
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	77	70

TABLE 3 (CONCLUDED)

TOPOGRAPHY: MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Zymbal's Gland, Ear, and Skin of Ear: Squamous-Cell Carcinoma, Sebaceous Adenocarcinoma, Keratoacanthoma, ^b Neoplasm NOS, or Basal-Cell Tumor ^b	1/18(0.06)	6/48(0.13)	9/46(0.20)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	2.250	3.522
Lower Limit	---	0.308	0.556
Upper Limit	---	101.140	150.403
Weeks to First Observed Tumor	104	77	70

^aTreated groups received doses of 1500 or 3000 mg/kg by gavage for 22 weeks followed by concentrations of 22,000 or 44,000 ppm in feed for 54 weeks.

^bNumber of tumor-bearing animals/number of animals examined at site (proportion).

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

^dThe 95% confidence interval on the relative risk of the treated group to the control group.

^eThe probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

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

TABLE 4
 ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
 SPECIFIC SITES IN FEMALE RATS TREATED WITH 3,3'-DIMETHOXYBENZIDINE-
 4,4'-DIISOCYANATE^a

TOPOGRAPHY: MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma ^b	1/20(0.05)	8/50(0.16)	15/48(0.31)
P Values ^c	P = 0.007	N.S.	P = 0.016
Relative Risk (Control) ^d	---	3.200	6.250
Lower Limit	---	0.482	1.093
Upper Limit	---	138.771	255.482
Weeks to First Observed Tumor	94	90	83
Pituitary: Chromophobe Adenoma ^b	4/18(0.22)	8/48(0.17)	8/45(0.18)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	0.750	0.800
Lower Limit	---	0.239	0.255
Upper Limit	---	3.097	3.292
Weeks to First Observed Tumor	103	71	100
Thyroid: C-Cell Adenoma or C-Cell Carcinoma ^b	0/18(0.00)	3/44(0.07)	0/41(0.00)
P Values ^c	N.S.	N.S.	N.S.
Departure from Linear Trend ^e	P = 0.049	---	---
Relative Risk (Control) ^d	---	Infinite	---
Lower Limit	---	0.258	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	100	---

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma, Adenoma NOS, or Adenocarcinoma NOS ^b	0/20(0.00)	5/50(0.10)	6/48(0.13)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.525	0.695
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	103	100
Preputial Gland: Sebaceous Adenocarcinoma or Squamous-Cell Carcinomab	0/20(0.00)	8/50(0.16)	4/48(0.08)
P Values ^c	N.S.	N.S.	N.S.
Departure from Linear Trend ^e	P = 0.043	---	---
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.952	0.402
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	71	93
Uterus: Endometrial Stromal Polyp ^b	0/20(0.00)	5/48(0.10)	10/48(0.21)
P Values ^c	P = 0.013	N.S.	P = 0.022
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.547	1.292
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	74	86

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Zymbal's Gland: Squamous-Cell Carcinoma or Sebaceous Adenocarcinoma ^b	0/20(0.00)	8/50(0.16)	6/48(0.13)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.952	0.695
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	44	72
Zymbal's Gland and Skin of Ear: Squamous-Cell Carcinoma, Sebaceous Adenocarcinoma or Trichoepithelioma ^b	0/20(0.00)	9/50(0.18)	6/48(0.13)
P Values ^c	N.S.	P = 0.039	N.S.
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	1.096	0.695
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	44	72

^aTreated groups received doses of 1500 or 3000 mg/kg by gavage for 22 weeks followed by concentrations of 22,000 or 44,000 ppm in feed for 54 weeks.

^bNumber of tumor-bearing animals/number of animals examined at site (proportion).

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

^dThe 95% confidence interval on the relative risk of the treated group to the control group.

^eThe probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

basal-cell tumor, trichoepithelioma, sebaceous adenoma, keratoacanthoma, squamous-cell carcinoma, or sebaceous adenocarcinoma) was significant when comparing both the high dose to control ($P = 0.005$) and the low dose to control ($P = 0.002$). The Cochran-Armitage test was significant ($P = 0.045$) with a significant ($P = 0.024$) departure from linear trend due to the higher incidence in the low dose group relative to the high dose group. Based on these statistical results, the administration of 3,3'-dimethoxybenzidine-4,4'-diisocyanate was associated with the increased incidence of skin neoplasms in male Fischer 344 rats under the conditions of this bioassay.

In male rats the Cochran-Armitage test indicated a significant ($P = 0.040$) positive association between dose and the incidence of squamous-cell carcinomas or sebaceous adenocarcinomas of the Zymbal's gland and the skin of the ear. While the Fisher exact tests did not support these results, the historical control data for untreated male Fischer 344 rats at this laboratory indicated only a 0.3 percent (1/300) incidence of these tumors in untreated males, indicating the rarity of occurrence of these tumors. In female rats, none of the statistical tests for these tumors at this site were significant under the Bonferroni criterion. However, as with male rats, historical control data for untreated female Fischer 344 rats at this laboratory indicate squamous-cell carcinomas and sebaceous adenocarcinomas of the Zymbal's gland and skin of the ear to be rarely occurring, with 0 percent (0/298) incidence. Based upon these results the data suggest that the administration of 3,3'-dimethoxybenzidine-4,4'-diisocyanate

was associated with the increased incidence of squamous-cell carcinomas or sebaceous adenocarcinomas of the Zymbal's gland and the skin of the ear in male and female rats.

In female rats the Cochran-Armitage test indicated a significant ($P = 0.013$) positive association between dose and the incidence of endometrial stromal polyps. The high dose to control Fisher exact test was also significant ($P = 0.022$). Based on these statistical results the administration of 3,3'-dimethoxybenzidine-4,4'-diisocyanate was associated with the increased incidence of endometrial stromal polyps.

The Cochran-Armitage test and the Fisher exact test comparing high dose to control indicated a significant negative association between dose and the incidence of adrenal pheochromocytomas in male rats. However, the historical data for this laboratory indicated an incidence of 7 percent (20/294) in male control Fischer 344 rats as compared to the higher 22 percent (4/18) observed in control male rats in this bioassay.

In summary, the statistical findings were that the administration of 3,3'-dimethoxybenzidine-4,4'-diisocyanate was associated with the increased incidence of leukemia or malignant lymphomas in male and female rats, the incidence of skin neoplasms (excluding skin of the ear) in male rats, and the incidence of endometrial stromal polyps in female rats. The data also suggest that the incidence of squamous-cell carcinomas or sebaceous adenocarcinomas of

the Zymbal's gland and the skin of the ear was associated with chemical administration to both sexes.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

Slight dose-related mean body weight depression was apparent in male mice. Female mice evidenced slight mean body weight depression in comparison to the control group (Figure 4).

No abnormal clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 3,3'-dimethoxybenzidine-4,4'-diisocyanate-dosed groups are shown in Figure 5. The Tarone test for association between dosage and mortality was not significant for either male or female mice.

The percentage of mice surviving on test is shown in Figure 6. In the male mice, despite the disappearance of 1 control in week 22, 1 low dose mouse in week 38, 3 low dose mice in week 41, and 1 low dose mouse in week 92, and the accidental sacrifice of 1 high dose mouse in week 44, there were adequate numbers at risk from late-developing tumors. Ninety-two percent (46/50) of the high dose, 80 percent (40/50) of the low dose, and 90 percent (18/20) of the control group survived on test until the termination of the study.

With 62 percent (31/50) of the high dose, 72 percent (36/50) of the low dose and 90 percent (18/20) of the control group surviving on test until termination of the study, there were adequate numbers

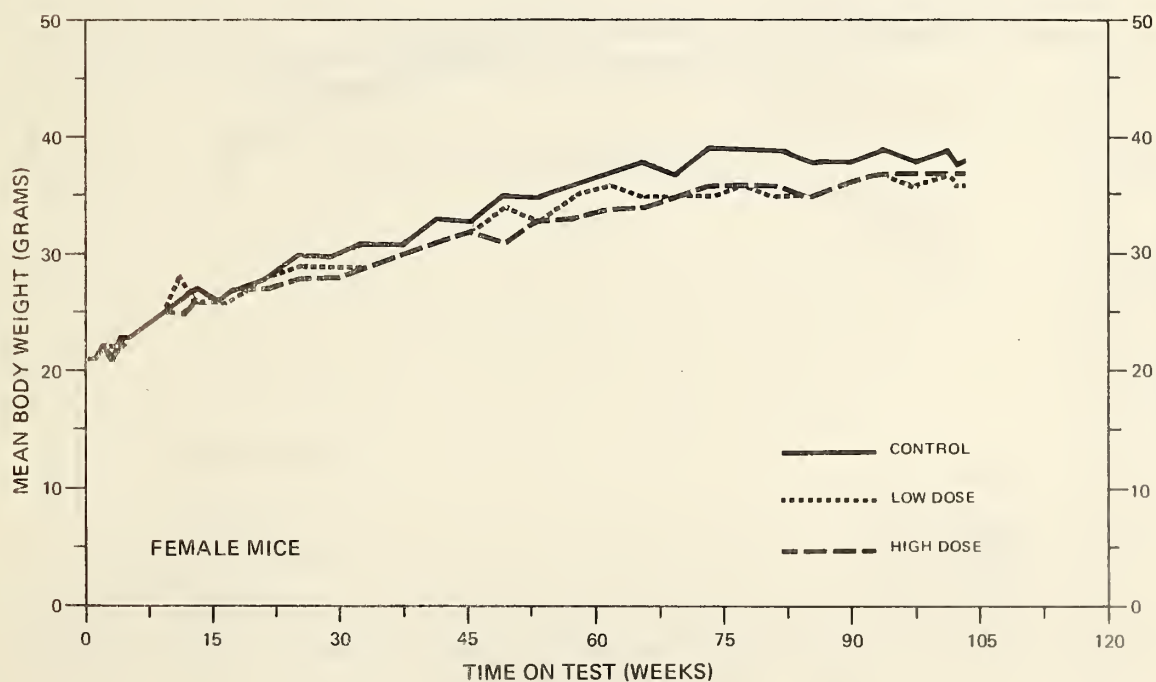
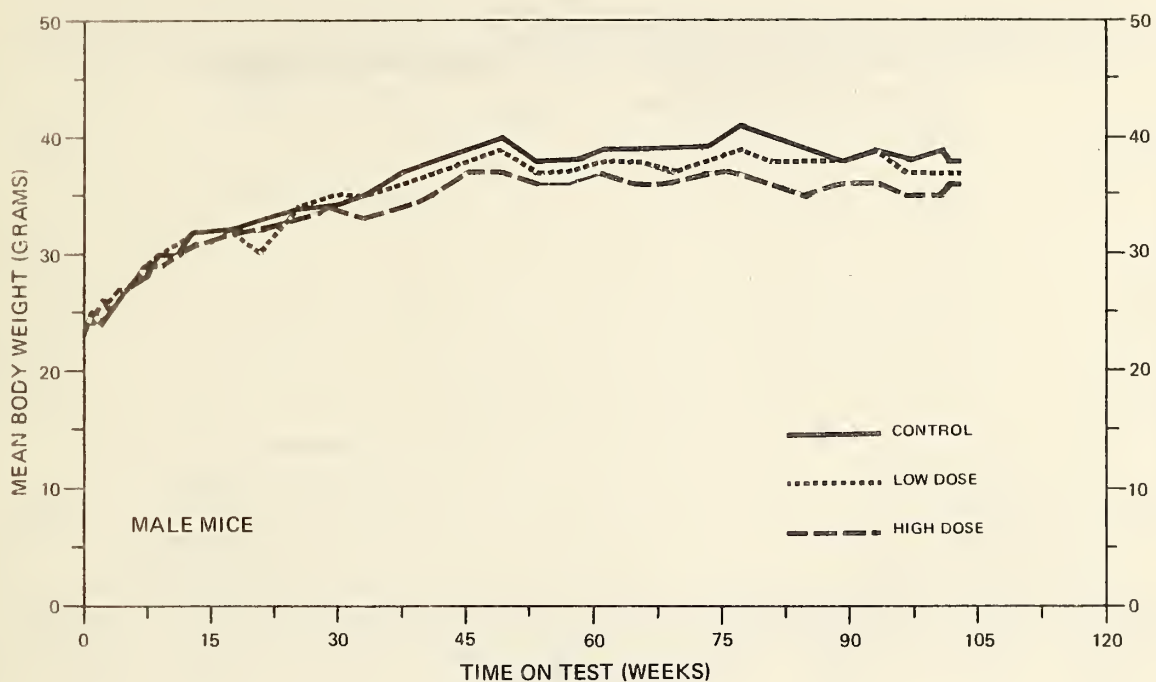


FIGURE 4
GROWTH CURVES FOR 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE CHRONIC STUDY MICE

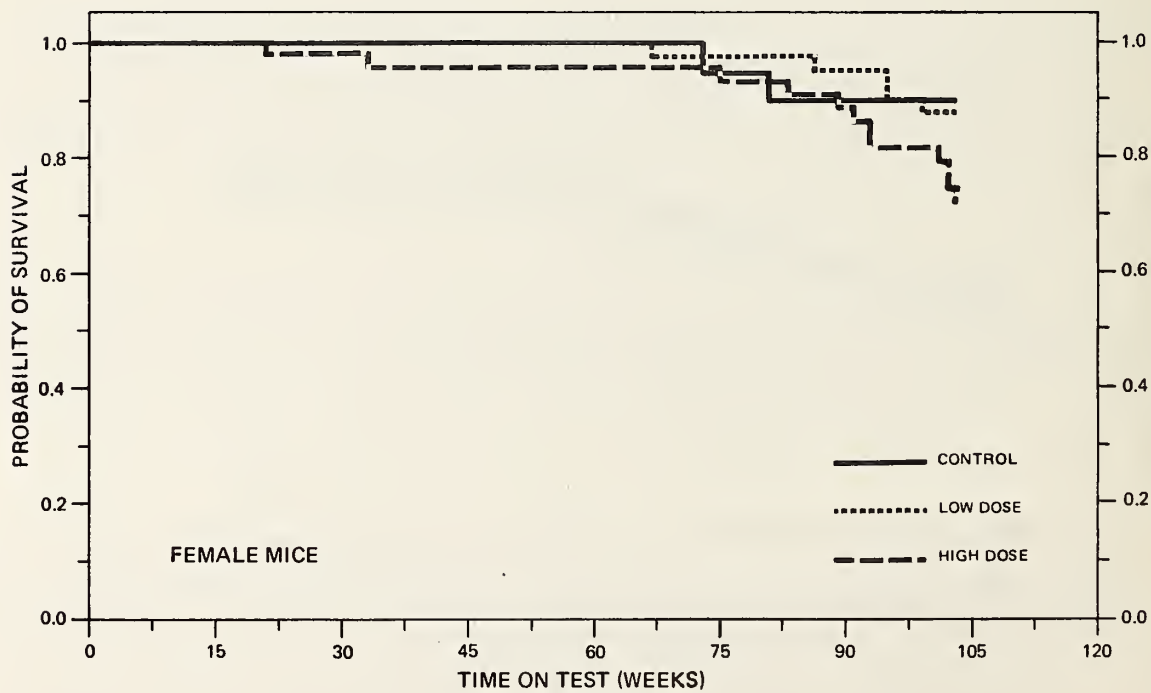
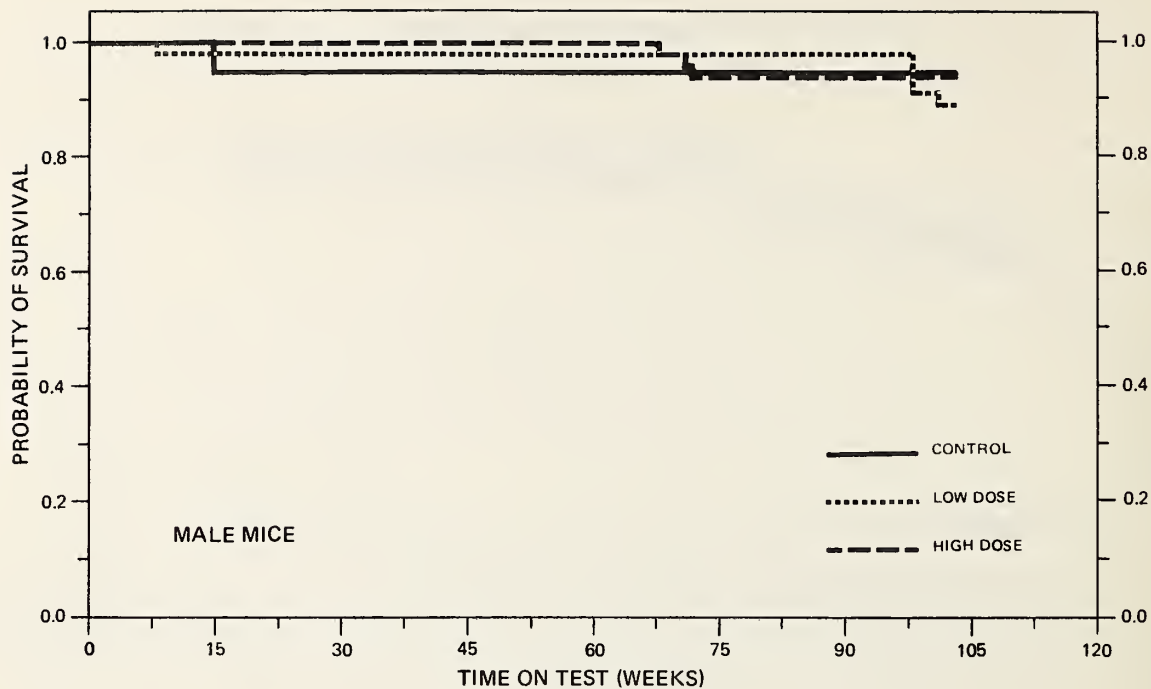


FIGURE 5
SURVIVAL PROBABILITY COMPARISONS OF 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE CHRONIC STUDY MICE

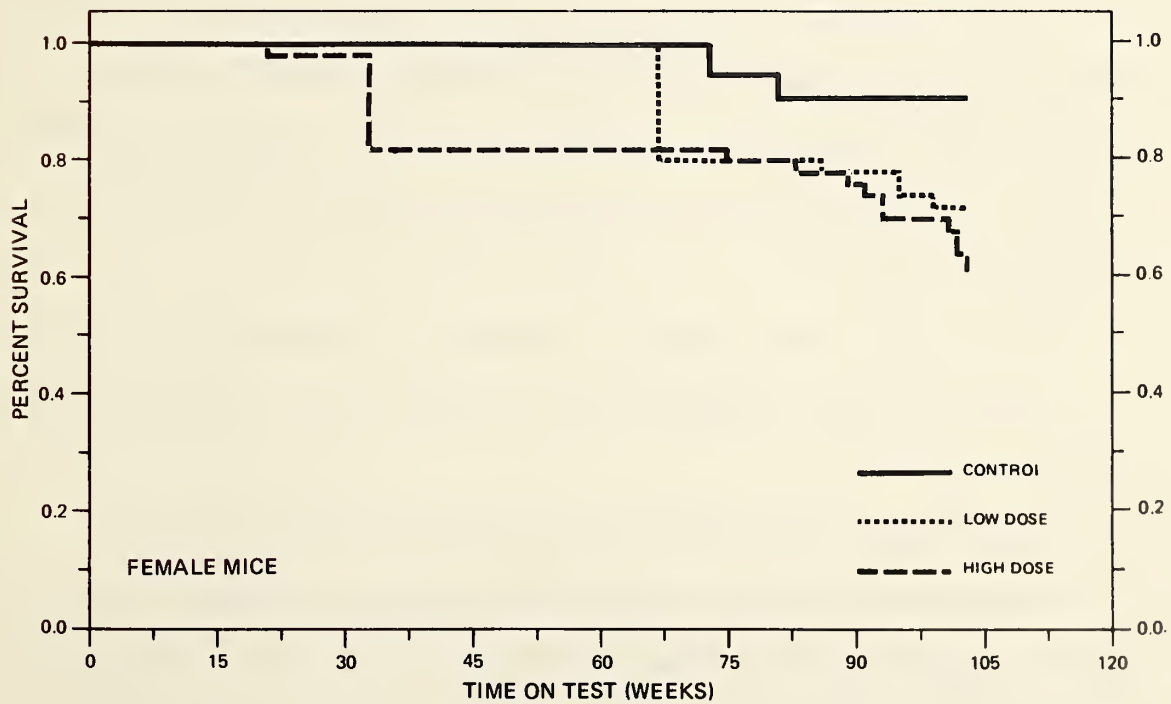
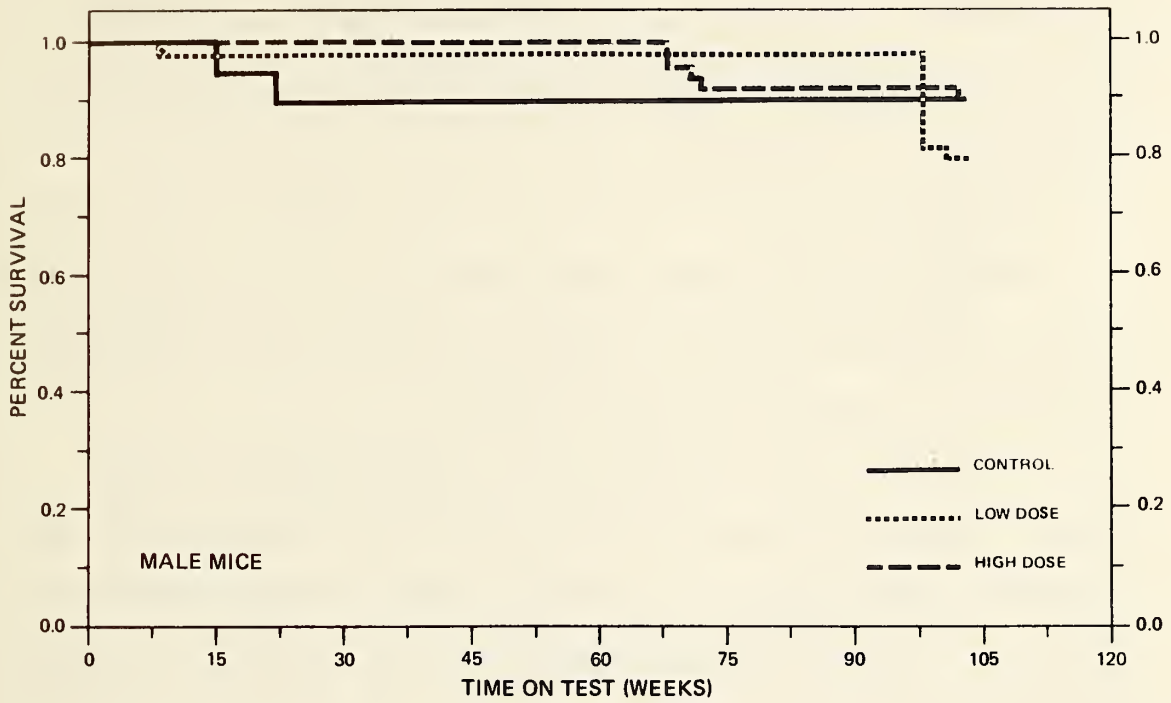


FIGURE 6
PERCENT SURVIVAL OF 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE CHRONIC STUDY MICE

of female mice at risk from late-developing tumors. Seven females from the high dose group and 9 from the low dose group were missing by week 28.

C. Pathology

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

A variety of tumors occurred in both the control and dosed groups. The type, incidence, and distribution of these lesions were similar to those expected in aged B6C3F1 mice; therefore, these lesions were considered to be spontaneous and not related to compound administration.

A few neoplasms occurred only, or with a greater frequency, in dosed groups as compared with controls. This may be noted in male mice in which benign and malignant hepatocellular neoplasms were found in 21 percent of the high dose mice compared to 5 percent of controls.

A variety of nonneoplastic, inflammatory, degenerative or fibrotic lesions occurred randomly in all groups and none appeared to be compound-related.

The results of this pathologic examination indicated that 3,3'-dimethoxybenzidine-4,4'-diisocyanate was not carcinogenic in male or female B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 3,3'-dimethoxybenzidine-4,4'-diisocyanate-dosed groups and where such tumors were observed in at least 5 percent of the group.

None of the statistical tests for any site of either male or female mice indicated a significant positive association between chemical administration and tumor incidence. Based upon these statistical results there was no evidence that 3,3'-dimethoxybenzidine-4,4'-diisocyanate was a carcinogen in B6C3F1 mice under the conditions of this bioassay.

In male mice the Cochran-Armitage test and the Fisher exact test comparing high dose to control indicated a significant negative association between dose and the incidence of interstitial-cell tumors of the testis. However, historical control data from the same laboratory indicate an incidence of 2 percent (4/266) of these tumors in untreated male B6C3F1 mice as compared with the 16 percent (3/19) in control male mice in this bioassay.

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

TABLE 5
 ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
 SPECIFIC SITES IN MALE MICE TREATED WITH 3,3'-DIMETHOXYBENZIDINE-
 4,4'-DIISOCYANATE^a

TOPOGRAPHY: MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma ^b	2/19 (0.11)	8/43 (0.19)	7/46 (0.15)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	1.767	1.446
Lower Limit	---	0.406	0.315
Upper Limit	---	16.114	13.509
Weeks to First Observed Tumor	103	103	103
Hematopoietic System: Leukemia or Malignant Lymphoma ^b	0/19 (0.00)	4/44 (0.09)	4/50 (0.08)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.418	0.368
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	98	68
Liver: Hepatocellular Carcinoma ^b	0/19 (0.00)	5/43 (0.12)	6/48 (0.13)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	Infinite	Infinite
Lower Limit	---	0.583	0.662
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	98	72

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma ^b	1/19(0.05)	8/43(0.19)	10/48(0.21)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	3.535	3.958
Lower Limit	---	0.537	0.639
Upper Limit	---	152.566	167.483
Weeks to First Observed Tumor	103	98	72
Testis: Interstitial-Cell Tumor ^b	3/19(0.16)	1/42(0.02)	0/48(0.00)
P Values ^c	P = 0.007(N)	N.S.	P = 0.020(N)
Relative Risk (Control) ^d	---	0.151	0.000
Lower Limit	---	0.003	0.000
Upper Limit	---	1.760	0.651
Weeks to First Observed Tumor	103	103	---

^aTreated groups received doses of 22,000 or 44,000 ppm in feed.

^bNumber of tumor-bearing animals/number of animals examined at site (proportion).

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

^dThe 95% confidence interval on the relative risk of the treated group to the control group.

TABLE 6
 ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
 SPECIFIC SITES IN FEMALE MICE TREATED WITH 3,3'-DIMETHOXYBENZIDINE-
 4,4'-DIISOCYANATE^a

TOPOGRAPHY: MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma ^b	3/20(0.15)	2/41(0.05)	1/40(0.03)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	0.325	0.167
Lower Limit	---	0.030	0.003
Upper Limit	---	2.651	1.945
Weeks to First Observed Tumor	103	103	103
Hematopoietic System: Leukemia or Malignant Lymphoma ^b	3/20(0.15)	9/41(0.22)	10/43(0.23)
P Values ^c	N.S.	N.S.	N.S.
Relative Risk (Control) ^d	---	1.463	1.550
Lower Limit	---	0.424	0.465
Upper Limit	---	7.728	8.072
Weeks to First Observed Tumor	103	86	33

TABLE 6 (CONCLUDED)

^aTreated groups received doses of 22,000 or 44,000 ppm in feed.

^bNumber of tumor-bearing animals/number of animals examined at site (proportion).

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

^dThe 95% confidence interval on the relative risk of the treated group to the control group.

Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by 3,3'-dimethoxybenzidine-4,4'-diisocyanate that could not be established under the conditions of this test.

V. DISCUSSION

There was a significant positive association between the dosages of 3,3'-dimethoxybenzidine-4,4'-diisocyanate administered and mortality in male rats. For female rats, mortality among the dosed groups was significantly higher than that for the controls. There were no significant positive associations between dosage and mortality among male or female mice. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Mean body weight depression was slight, but detectable, when dosed male and female mice were compared to their respective controls, indicating that the dosages administered to these animals may have approximated the maximum tolerated dosages.

For both sexes of rats, there was a significant positive association between dosage and the incidence of leukemia and malignant lymphoma. The high dose to control and the low dose to control Fisher exact comparisons for males and the high dose to control comparison for females were also significant for the incidences of these tumors. Excluding the skin of the ear in considering neoplasms of the skin (i.e., a combination of papilloma NOS, basal-cell tumor, trichoepithelioma, sebaceous adenoma, keratoacanthoma, squamous-cell carcinoma, and sebaceous adenocarcinoma) the Fisher exact comparisons were significant for both dose levels in male rats. There was a significant positive association between the dosages of the chemical administered and the incidences in female rats of endometrial stromal polyps.

The high dose to control Fisher exact comparison supported the finding. In male rats the Cochran-Armitage test provided a significant positive association between dosage and the combined incidence of squamous-cell carcinomas or sebaceous adenocarcinomas of the Zymbal's gland, ear, or the skin of the ear. For females, the association was not significant, and for both sexes the Fisher exact comparisons were not significant. The historical incidences for these tumors in Fischer 344 control rats maintained by this laboratory during the Carcinogenesis Testing Program are 1/300 (0.3 percent) and 0/298 for males and females, respectively. When compared with the incidences of these neoplasms observed in the males (i.e., 5/50 [10 percent] in the low dose and 8/50 [16 percent] in the high dose) and in the females (i.e., 8/50 [16 percent] in the low dose and 6/48 [13 percent] in the high dose) in this bioassay, it is probable that the incidences observed were related to compound administration.

None of the statistical tests for any site in male or female mice indicated a significant positive association between compound administration and tumor incidence.

Under the conditions of this bioassay, administration of 3,3'-dimethoxybenzidine-4,4'-diisocyanate was carcinogenic to Fischer 344 rats, causing neoplasms of the skin (excluding skin of the ear) in males, endometrial stromal polyps in females, and leukemia and malignant lymphoma in both sexes. The compound was also associated with the development of a combination of squamous-cell carcinomas and sebaceous adenocarcinomas of the Zymbal's gland and skin of the

ear in rats of both sexes. There was no evidence for the carcinogenicity of the compound in B6C3F1 mice.

VI. BIBLIOGRAPHY

- Armitage, P., Statistical Methods in Medical Research, Chapter 14. J. Wiley & Sons, New York, 1971.
- Berenblum, I., editor, Carcinogenicity Testing. International Union Against Cancer, Technical Report Series, Vol. 2. International Union Against Cancer, Geneva, 1969.
- Bonk, H.W., and T.M. Shah, "Polyurethanes from 3,3'-dimethyldiphenyl-4,4'-diisocyanate Polyester Diols and Bis(hydroxethyl ether) of Hydroquinone." U.S. Patent 3,899,467 (Upjohn Co.), August 12, 1975; Chemical Abstracts 83, 194863r.
- Chemical Abstracts Service, The Chemical Abstracts Service (CAS) Ninth Collective Index, Volumes 76-85, 1972-1976. American Chemical Society, Washington, D.C., 1977.
- Chiklis, C.K., "Photographic Products Containing Antireflection Layer." U.S. Public Patent Application B 354,008 (Polaroid Corp.), January 28, 1975; Chemical Abstracts 83, 35653f.
- Chung, D.A., "Polyurethane Shock Absorbers." Ger. Offen. 2,435,078 (Goodyear Tire and Rubber Co.), February 20, 1975; Chemical Abstracts 83, 44171z.
- Cox, D.R., Analysis of Binary Data, Chapters 4 and 5. Methuen and Co., Ltd., London, 1970.
- Cox, D.R., "Regression Models and Life-Tables." Journal of the Royal Statistical Society, Series "B" 34:187-220, 1972.
- Gart, J.J., "The Comparison of Proportions: A Review of Significance Tests, Confidence Limits, and Adjustments for Stratification." International Statistical Institute Review 39:148-169, 1971.
- Grace, W.R. and Co., "Polyurethane Gaskets." British Patent 1,374,745, November 20, 1974; Chemical Abstracts 83, 116347m.
- Guthrie, J.L. and F.J. Rendulic, "Photocurable Triazine-Containing Polyene-Polythiol Lacquer Composition." U.S. Patent 3,900,594 (Grace, W.R., and Co.), August 19, 1975; Chemical Abstracts 83, 195438t.

- Hadidian, Z., T.N. Frederickson, E.K. Weisburger, J.H. Weisburger, R.M. Glass, and N. Mantel, "Tests for Chemical Carcinogens. Report on the Activity of Derivatives of Aromatic Amines, Nitrosamines, Quinolines, Nitroalkanes, Amides, Epoxides, Aziridines, and Purine Antimetabolites." Journal of the National Cancer Institute 41(4):985-1025, 1968.
- Kaplan, E.L., and P. Meier, "Nonparametric Estimation from Incomplete Observations." Journal of the American Statistical Association 53:457-481, 1958.
- Linhart, M.S., J.A. Cooper, R.L. Martin, N.P. Page, and J.A. Peters, "Carcinogenesis Bioassay Data System." Computers and Biomedical Research 7:230-248, 1974.
- Miller, R.G., Simultaneous Statistical Inference. McGraw-Hill Book Co., New York, 1966.
- Saffiotti, U., R. Montesano, A.R. Sellakumar, F. Cefis, and D.G. Kaufman, "Respiratory Tract Carcinogenesis in Hamsters Induced by Different Numbers of Administration of Benzo (a) Pyrene and Ferric Oxide." Cancer Research 32:1073-1079, 1972.
- Stanford Research Institute, 1977 Directory of Chemical Producers, U.S.A. Menlo Park, California, 1977.
- Tarone, R.E., "Tests for Trend in Life-Table Analysis." Biometrika 62:679-682, 1975.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS
IN RATS TREATED WITH 3,3'-DIMETHOXYBENZIDINE-
4,4'-DIISOCYANATE

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TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED
WITH 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE

	CONTROL (VEH) 11-1095	LOW DOSE 11-1093	HIGH DOSE 11-1091
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
NEOPLASM, NOS		1 (2%)	
PAPILLOMA, NOS			2 (4%)
SQUAMOUS CELL CARCINOMA		5 (10%)	4 (8%)
BASAL-CELL TUMOR		4 (8%)	5 (10%)
TRICHOEPITHELIOMA		2 (4%)	1 (2%)
SEBACEOUS ADENOMA		2 (4%)	
SEBACEOUS ADENOCARCINOMA		1 (2%)	
KERATOACANTHOMA	1 (5%)	4 (8%)	5 (10%)
CARCINOSARCOMA			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA		1 (2%)	
FIBROSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
*LUNG	(20)	(49)	(47)
ALVEOLAR/BRONCHIOLAR ADENOMA		8 (16%)	
CARCINOSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	1 (2%)
LEUKEMIA, NOS		12 (24%)	7 (14%)
UNDIFFERENTIATED LEUKEMIA		6 (12%)	9 (18%)
*CERVICAL LYMPH NODE	(17)	(49)	(42)
CARCINOSARCOMA, METASTATIC			1 (2%)
CIRCULATORY SYSTEM			
NONE			
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS			

TABLE A1 (CONTINUED)

	CONTROL (VEH) 11-1095	LOW DOSE 11-1093	HIGH DOSE 11-1091
DIGESTIVE SYSTEM			
*LIVER	(20)	(50)	(50)
HEPATOCELLULAR ADENOMA	1 (5%)	6 (12%)	2 (4%)
HEPATOCELLULAR CARCINOMA		2 (4%)	1 (2%)
*PANCREAS	(20)	(49)	(50)
ACINAR-CELL ADENOMA		1 (2%)	
*STOMACH	(20)	(49)	(48)
PAPILLOMA, NOS		1 (2%)	
*SMALL INTESTINE	(20)	(49)	(49)
ADENOMATOUS POLYP, NOS			1 (2%)
*COLON	(19)	(47)	(46)
ADENOCARCINOMA, NOS			1 (2%)
URINARY SYSTEM			
*KIDNEY/PELVIS	(20)	(50)	(49)
TRANSITIONAL-CELL CARCINOMA	1 (5%)		
ENDOCRINE SYSTEM			
*PITUITARY	(16)	(45)	(36)
CHROMOPHOBE ADENOMA	3 (19%)	7 (16%)	2 (6%)
*ADRENAL	(20)	(50)	(49)
PHEOCHROMOCYTOMA	4 (20%)	3 (6%)	
*THYROID	(19)	(45)	(46)
FOLLICULAR-CELL ADENOMA	1 (5%)		
FOLLICULAR-CELL CARCINOMA			1 (2%)
C-CELL ADENOMA	1 (5%)	1 (2%)	
C-CELL CARCINOMA		2 (4%)	
CYSTADENOMA, NOS		1 (2%)	
*PANCREATIC ISLETS	(20)	(49)	(50)
ISLET-CELL ADENOMA	1 (5%)		1 (2%)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(20)	(50)	(50)
SEBACEOUS ADENOCARCINOMA		1 (2%)	3 (6%)

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONTINUED)

	CONTROL (VEH) 11-1095	LOW DOSE 11-1093	HIGH DOSE 11-1091
*TESTIS INTERSTITIAL-CELL TUMOR	(20) 17 (85%)	(50) 39 (78%)	(49) 36 (73%)
*EPIDIDYMHIS FIBROSARCOMA, INVASIVE	(20)	(50) 1 (2%)	(50)
NERVOUS SYSTEM			
*BRAIN SQUAMOUS CELL CARCINOMA, INVASIV GLIOMA, NOS ASTROCYTOMA OLIGODENDROGLIOMA	(19) 1 (5%)	(50)	(47) 1 (2%) 1 (2%) 1 (2%)
SPECIAL SENSE ORGANS			
*EAR SQUAMOUS CELL CARCINOMA	(20)	(50)	(50) 1 (2%)
*ZYMBAI'S GLAND SQUAMOUS CELL CARCINOMA SEBACEOUS ADENOCARCINOMA	(20)	(50) 2 (4%) 3 (6%)	(50) 5 (10%) 2 (4%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM SARCOMA, NOS	(20)	(50) 1 (2%)	(50)
*ABDOMINAL CAVITY LIPOMA	(20)	(50)	(50) 1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS CARCINOSARCOMA	(20)	(50) 1 (2%)	(50)

0 NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONCLUDED)

	CONTROL (VEH) 11-1095	LOW DOSE 11-1093	HIGH DOSE 11-1091
MESOTHELIOMA, NOS			1 (2%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH [ⓐ]	3	16	12
MORIBUND SACRIFICE	2	12	22
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	15	22	16
ANIMAL MISSING			
[ⓐ] INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	18	48	46
TOTAL PRIMARY TUMORS	31	119	95
TOTAL ANIMALS WITH BENIGN TUMORS	18	45	43
TOTAL BENIGN TUMORS	29	80	56
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	30	31
TOTAL MALIGNANT TUMORS	2	38	38
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	2
TOTAL SECONDARY TUMORS		1	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT		1	1
TOTAL UNCERTAIN TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED
WITH 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE

	CONTROL (VEH) 11-1096	LOW DOSE 11-1094	HIGH DOSE 11-1092
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALIY **	20	50	48
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(48)
NEOPLASM, NOS			1 (2%)
SQUAMOUS CELL CARCINOMA		1 (2%)	
BASAL-CELL TUMOR			1 (2%)
TRICHOEPITHELIOMA		1 (2%)	
SEBACEOUS ADENOMA			1 (2%)
SEBACEOUS ADENOCARCINOMA	1 (5%)	1 (2%)	1 (2%)
CARCINOSARCOMA	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(48)
FIBROSARCOMA			1 (2%)
LIPOMA			1 (2%)
RESPIRATORY SYSTEM			
*NASAL CAVITY	(20)	(50)	(48)
SQUAMOUS CELL CARCINOMA, INVASIV		1 (2%)	
*LUNG	(20)	(48)	(48)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	2 (4%)	1 (2%)
SEBACEOUS ADENOCARCINOMA, METAST		1 (2%)	
FIBROSARCOMA, METASTATIC			1 (2%)
CARCINOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(48)
MALIGNANT LYMPHOMA, NOS			1 (2%)
LEUKEMIA, NOS		5 (10%)	6 (13%)

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	CONTROL (VEH) 11-1096	LOW DOSE 11-1094	HIGH DOSE 11-1092
UNDIFFERENTIATED LEUKEMIA	1 (5%)	3 (6%)	6 (13%)
#BONE MARROW LEUKEMIA, NOS	(19)	(45)	(45) 1 (2%)
#SPIEEN UNDIFFERENTIATED LEUKEMIA	(20)	(48)	(48) 1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER HEPATOCELLULAR ADENOMA	(20)	(50)	(48) 2 (4%)
#PANCREAS ADENOCARCINOMA, NOS	(20)	(48) 1 (2%)	(48)
#STOMACH SQUAMOUS CELL CARCINOMA	(20)	(50) 1 (2%)	(48) 1 (2%)
#SMALL INTESTINE CYSTADENOCARCINOMA, NOS	(20)	(50)	(48) 1 (2%)
#ILEUM ADENOMATOUS POLYP, NOS	(20)	(50)	(48) 1 (2%)
#COLON ADENOCARCINOMA, NOS	(18)	(45) 1 (2%)	(47) 1 (2%)
URINARY SYSTEM			
#KIDNEY TUBULAR-CELL ADENOHA	(20)	(49) 1 (2%)	(48)
ENDOCRINE SYSTEM			
#PITUITARY NEOPLASM, NOS	(18)	(48)	(45) 1 (2%)
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE A2 (CONTINUED)

	CONTROL (VEH) 11-1096	LOW DOSE 11-1094	HIGH DOSE 11-1092
CHROMOPHOBE ADENOMA	4 (22%)	8 (17%)	8 (18%)
#ADRENAL	(20)	(49)	(47)
CORTICAL ADENOMA		1 (2%)	1 (2%)
PHOCHROMOCYTOMA	1 (5%)	1 (2%)	
#THYROID	(18)	(44)	(41)
FOLLICULAR-CELL ADENOMA		1 (2%)	
C-CELL ADENOMA		2 (5%)	
C-CELL CARCINOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(48)
ADENOMA, NOS		1 (2%)	1 (2%)
ADENOCARCINOMA, NOS			1 (2%)
CYSTADENOMA, NOS			1 (2%)
FIBROADENOMA		5 (10%)	4 (8%)
*PREPUTIAL GLAND	(20)	(50)	(48)
SQUAMOUS CELL CARCINOMA		1 (2%)	
SEBACEOUS ADENOCARCINOMA		7 (14%)	4 (8%)
*VAGINA	(20)	(50)	(48)
PAPILLOMA, NOS			1 (2%)
#UTERUS	(20)	(48)	(48)
SQUAMOUS CELL CARCINOMA		1 (2%)	
ADENOCARCINOMA, NOS		1 (2%)	
LEIOMYOMA		1 (2%)	
LEIOMYOSARCOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP		5 (10%)	10 (21%)
#UTERUS/ENDOMETRIUM	(20)	(48)	(48)
CARCINOMA, NOS	1 (5%)		
ADENOCARCINOMA, NOS			1 (2%)
#OVARY	(20)	(47)	(48)
CYSTADENOCARCINOMA, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(48)
SEBACEOUS ADENOCARCINOMA, INVASI			1 (2%)

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

TABLE A2 (CONTINUED)

	CONTROL (VEH) 11-1096	LOW DOSE 11-1094	HIGH DOSE 11-1092
EPENDYMOMA		1 (2%)	
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(20)	(50)	(48)
SQUAMOUS CELL CARCINOMA		1 (2%)	4 (8%)
SEBACEOUS ADENOCARCINOMA		7 (14%)	2 (4%)
CARCINOSARCOMA	1 (5%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(48)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
CARCINOSARCOMA		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	9	16
MORBUND SACRIFICE	2	20	12
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	15	21	21
ANIMAL MISSING			
ANIMAL DELETED (WRONG SEX)			1
@ INCLUDES AUTOLYZED ANIMALS			
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE A2 (CONCLUDED)

	CONTROL (VEH) 11-1096	LOW DOSE 11-1094	HIGH DOSE 11-1092
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	10	40	44
TOTAL PRIMARY TUMORS	11	63	69
TOTAL ANIMALS WITH BENIGN TUMORS	6	22	26
TOTAL BENIGN TUMORS	6	29	33
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	27	30
TOTAL MALIGNANT TUMORS	5	34	34
TOTAL ANIMALS WITH SECONDARY TUMORS*		5	2
TOTAL SECONDARY TUMORS		6	2
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			

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS
IN MICE TREATED WITH 3,3'-DIMETHOXYBENZIDINE-
4,4'-DIISOCYANATE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH
3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE

	CONTROL (UNTR) 22-2095	LOW DOSE 22-2093	HIGH DOSE 22-2091
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	5	
ANIMALS NECROPSIED	19	44	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	19	44	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(19)	(44)	(50)
LIPOMA		1 (2%)	
RESPIRATORY SYSTEM			
*LUNG	(19)	(43)	(46)
HEPATOCELLULAR CARCINOMA, METAST		2 (5%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	8 (19%)	7 (15%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(19)	(44)	(50)
MALIGNANT LYMPHOMA, NOS		2 (5%)	1 (2%)
MALIG. LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
LEUKEMIA, NOS		1 (2%)	
*SPLEEN	(18)	(37)	(45)
HEMANGIOSARCOMA			1 (2%)
*MESENTERIC L. NODE	(18)	(36)	(38)
MALIGNANT LYMPHOMA, NOS			1 (3%)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (3%)
*AXILLARY LYMPH NODE	(18)	(36)	(38)
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE		1 (3%)	
CIRCULATORY SYSTEM			
*HEART	(19)	(43)	(48)
HEMANGIOSARCOMA			1 (2%)

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 22-2095	LOW DOSE 22-2093	HIGH DOSE 22-2091
DIGESTIVE SYSTEM			
*LIVER	(19)	(43)	(48)
HEPATOCELLULAR ADENOMA	1 (5%)	3 (7%)	4 (8%)
HEPATOCELLULAR CARCINOMA		5 (12%)	6 (13%)
*STOMACH	(15)	(41)	(49)
ADENOMATOUS POLYP, NOS	1 (7%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
*ADRENAL	(13)	(25)	(36)
CORTICAL ADENOMA			1 (3%)
REPRODUCTIVE SYSTEM			
*TESTIS	(19)	(42)	(48)
INTERSTITIAL-CELL TUMOR	3 (16%)	1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 22-2095	LOW DOSE 22-2093	HIGH DOSE 22-2091
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH [ⓐ]	1	2	2
MORIBUND SACRIFICE		3	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	18	40	46
ANIMAL MISSING	1	5	
[ⓐ] INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	17	21
TOTAL PRIMARY TUMORS	7	22	24
TOTAL ANIMALS WITH BENIGN TUMORS	6	11	12
TOTAL BENIGN TUMORS	6	13	12
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	9	10
TOTAL MALIGNANT TUMORS	1	9	12
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	
TOTAL SECONDARY TUMORS		2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED
WITH 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE

	CONTROL (UNTR) 22-2096	LOW DOSE 22-2094	HIGH DOSE 22-2092
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		9	7
ANIMALS NECROPSIED	20	41	43
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	41	42
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE SARCOMA, NOS	(20)	(41)	(43) 1 (2%)
RESPIRATORY SYSTEM			
*LUNG ALVEOLAR/BRONCHIOLAR ADENOMA	(20) 3 (15%)	(41) 2 (5%)	(40) 1 (3%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS MALIGNANT LYMPHOMA, NOS MALIG.LYMPHOMA, LYMPHOCYTIC TYPE MALIG.LYMPHOMA, HISTIOCYTIC TYPE LEUKEMIA, NOS	(20) 2 (10%)	(41) 5 (12%) 1 (2%) 3 (7%)	(43) 3 (7%) 1 (2%) 3 (7%) 1 (2%)
*SPLEEN MALIGNANT LYMPHOMA, NOS	(18)	(41)	(41) 1 (2%)
*MESENTERIC L. NODE MALIGNANT LYMPHOMA, NOS	(20) 1 (5%)	(40)	(37)
*DUCDENUM MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	(20)	(41)	(38) 1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*SALIVARY GLAND ADENOCA/SQUAMOUS METAPLASIA	(14)	(30) 1 (3%)	(40)

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2096	LOW DOSE 22-2094	HIGH DOSE 22-2092
#LIVER	(20)	(41)	(42)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
HEPATOCELLULAR ADENOMA	1 (5%)		
HEPATOCELLULAR CARCINOMA		1 (2%)	
#CECUM	(19)	(40)	(37)
LEIOMYOMA		1 (3%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(8)	(13)	(25)
CHROMOPHOBE ADENOMA		1 (8%)	
REPRCDUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(41)	(43)
ADENOCARCINOMA, NOS		1 (2%)	1 (2%)
#UTERUS	(19)	(40)	(37)
ADENOCARCINOMA, NOS		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(41)	(43)
LIPOMA			1 (2%)
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 22-2096	LOW DOSE 22-2094	HIGH DOSE 22-2092
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH ^a	2	3	7
MORIBUND SACRIFICE		2	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	36	31
ANIMAL MISSING		9	7
^a INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	13	13
TOTAL PRIMARY TUMORS	7	17	14
TOTAL ANIMALS WITH BENIGN TUMORS	4	4	2
TOTAL BENIGN TUMORS	4	4	2
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	11	11
TOTAL MALIGNANT TUMORS	3	13	12
TOTAL ANIMALS WITH SECONDARY TUMORS [#]		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
[#] SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC
LESIONS IN RATS TREATED WITH 3,3'-DIMETHOXYBENZIDINE-
4,4'-DIISOCYANATE

TABLE CI
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE

	CONTROL (VEH) 11-1095	LOW DOSE 11-1093	HIGH DOSE 11-1091
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*TRACHEA INFLAMMATION, NOS	(20) 1 (5%)	(49)	(46)
*LUNG PNEUMONIA, ASPIRATION INFLAMMATION, SUPPURATIVE BRONCHOPNEUMONIA, ACUTE PNEUMONIA, CHRONIC MURINE INFLAMMATION, CHRONIC INFLAMMATION, CHRONIC SUPPURATIV HYPERPLASIA, ALVEOLAR EPITHELIUM	(20) 3 (15%)	(49) 1 (2%) 7 (14%) 1 (2%) 1 (2%)	(47) 2 (4%) 1 (2%) 6 (13%) 1 (2%) 1 (2%)
*LUNG/ALVEOLI PNEUMONIA, ASPIRATION	(20)	(49)	(47) 1 (2%)
HEMATOPOIETIC SYSTEM			
*SPLEEN HEMOSIDEROSIS HYPERPLASIA, NODULAR HEMATOPOIESIS	(19)	(49) 2 (4%) 1 (2%)	(49) 2 (4%) 1 (2%) 1 (2%)
*MANDIBULAR L. NODE HYPERPLASIA, PLASMA CELL	(17)	(49)	(42) 1 (2%)
*MESENTERIC L. NODE HYPERPLASIA, LYMPHOID	(17) 1 (6%)	(49)	(42)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	CONTROL (VEH) 11-1095	LOW DOSE 11-1093	HIGH DOSE 11-1091
CIRCULATORY SYSTEM			
*HEART	(19)	(48)	(46)
THROMBOSIS, NOS		1 (2%)	
INFLAMMATION, CHRONIC		2 (4%)	
*MYOCARDIUM	(19)	(48)	(46)
INFLAMMATION, CHRONIC		2 (4%)	
FIBROSIS	1 (5%)	2 (4%)	4 (9%)
FIBROSIS, DIFFUSE	1 (5%)		
DEGENERATION, NOS	2 (11%)	1 (2%)	
DIGESTIVE SYSTEM			
*LIVER	(20)	(50)	(50)
NECROSIS, FOCAL		2 (4%)	2 (4%)
NECROSIS, DIFFUSE			1 (2%)
NECROSIS, COAGULATIVE			1 (2%)
METAMORPHOSIS FATTY		4 (8%)	4 (8%)
GLYCOGENIC CELL			1 (2%)
HYPERPLASIA, NODULAR			1 (2%)
HYPERPLASTIC NODULE		1 (2%)	
HEMATOPOIESIS		1 (2%)	1 (2%)
*LIVER/HEPATOCYTES	(20)	(50)	(50)
HYPERPLASIA, FOCAL			1 (2%)
*BILE DUCT	(20)	(50)	(50)
HYPERPLASIA, NOS	1 (5%)		
*PANCREAS	(20)	(49)	(50)
FIBROSIS, FOCAL		3 (6%)	
*STOMACH	(20)	(49)	(48)
HYPERKERATOSIS			1 (2%)
*SMALL INTESTINE	(20)	(49)	(49)
ULCER, NOS			1 (2%)
*PEYERS PATCH	(20)	(49)	(49)
ABSCESS, NOS			1 (2%)
*DUCDENUM	(20)	(49)	(49)
ULCER, NOS		1 (2%)	

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL (VEH) 11-1095	LOW DOSE 11-1093	HIGH DOSE 11-1091
#LARGE INTESTINE NEMATODIASIS	(19) 7 (37%)	(47)	(46) 1 (2%)
#COLON NEMATODIASIS	(19)	(47) 14 (30%)	(46) 6 (13%)
URINARY SYSTEM			
#KIDNEY HYDRONEPHROSIS	(20) 1 (5%)	(50)	(49)
INFLAMMATION, CHRONIC GRANULOMA, NOS	13 (65%)	23 (46%) 1 (2%)	19 (39%)
PIGMENTATION, NCS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY CYST, NOS	(16)	(45) 1 (2%)	(36) 1 (3%)
HYPERPLASIA, FOCAL HYPERPLASIA, CHROMOPHOBE-CELL	2 (13%)	3 (7%)	
#ADRENAL LIPOIDOSIS	(20)	(50)	(49) 1 (2%)
#ADRENAL CORTEX HYPERPLASIA, NOS	(20)	(50) 1 (2%)	(49)
#ADRENAL MEDULLA FIBROSIS	(20)	(50) 1 (2%)	(49)
HYPERPLASIA, NOS	2 (10%)	3 (6%)	
#THYROID COLLOID CYST	(19)	(45)	(46) 1 (2%)
FOLLICULAR CYST, NOS			1 (2%)
HYPERPLASIA, C-CELL		5 (11%)	1 (2%)
#PANCREATIC ISLETS HYPERPLASIA, NOS	(20)	(49)	(50) 1 (2%)
HYPERPLASIA, ADENOMATOUS			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND MULTILOCLULAR CYST	(20)	(50) 1 (2%)	(50)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONCLUDED)

	CONTROL (VEH) 11-1095	LOW DOSE 11-1093	HIGH DOSE 11-1091
#PROSTATE	(16)	(46)	(41)
INFLAMMATION, NOS			1 (2%)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, CYSTIC			3 (7%)
*SEMINAL VESICLE	(20)	(50)	(50)
CAST, NOS	1 (5%)		
INFLAMMATION, SUPPURATIVE		1 (2%)	
HYPERPLASIA, CYSTIC			1 (2%)
#TESTIS	(20)	(50)	(49)
ATROPHY, NOS	4 (20%)	2 (4%)	
NERVUS SYSTEM			
#BRAIN	(19)	(50)	(47)
ATROPHY, NOS		2 (4%)	
ATROPHY, PRESSURE	1 (5%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*INGUINAL REGION	(20)	(50)	(50)
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
ADIPOSE TISSUE		1	2
NECROSIS, FAT			
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/HISTO PERF			1
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
MSAC: MORIBUND SACRIFICE OTHR: OTHER			

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
TREATED WITH 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE

	CONTROL (VEH) 11-1096	LOW DOSE 11-1094	HIGH DOSE 11-1092
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	48
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(48)
EPIDERMAL INCLUSION CYST	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(48)
HEMORRHAGIC CYST			1 (2%)
RESPIRATORY SYSTEM			
*LARYNX	(20)	(50)	(48)
INFLAMMATION, CHRONIC			1 (2%)
#TRACHEA	(20)	(50)	(47)
INFLAMMATION, CHRONIC SUPPURATIVE		1 (2%)	
#LUNG	(20)	(48)	(48)
PNEUMONIA, ASPIRATION		1 (2%)	
PNEUMONIA, CHRONIC MURINE	7 (35%)	16 (33%)	11 (23%)
HYPERPLASIA, ADENOMATOUS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(45)	(45)
HYPERPLASIA, NOS		1 (2%)	
#SPLEEN	(20)	(48)	(48)
HEMOSIDEROSIS		2 (4%)	2 (4%)
HYPERPLASIA, RETICULUM CFL			1 (2%)
HEMATOPOIESIS		7 (15%)	1 (2%)
#CERVICAL LYMPH NODE	(20)	(45)	(48)
INFLAMMATION, SUPPURATIVE		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL (VEH) 11-1096	LOW DOSE 11-1094	HIGH DOSE 11-1092
CIRCULATORY SYSTEM			
*MYOCARDIUM	(18)	(49)	(48)
FIBROSIS	1 (6%)	1 (2%)	2 (4%)
FIBROSIS, FOCAL			1 (2%)
DEGENERATION, NOS			1 (2%)
DIGESTIVE SYSTEM			
*LIVER	(20)	(50)	(48)
CYST, NOS		1 (2%)	
HEMORRHAGIC CYST			1 (2%)
NECROSIS, NOS	1 (5%)		
NECROSIS, FOCAL		4 (8%)	
NECROSIS, MIDZONAL		1 (2%)	
METAMORPHOSIS FATTY		4 (8%)	4 (8%)
HYPERPLASIA, NODULAR		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	
*LIVER/CENTRIOLOBULAR METAMORPHOSIS FATTY	(20)	(50) 1 (2%)	(48)
*BILE DUCT HYPERPLASIA, NOS	(20)	(50) 1 (2%)	(48)
*PANCREAS	(20)	(48)	(48)
FIBROSIS, FOCAL	2 (10%)		1 (2%)
ATROPHY, FOCAL			1 (2%)
*SMALL INTESTINE HYPERPLASIA, LYMPHOID	(20)	(50)	(48) 1 (2%)
*LARGE INTESTINE NEMATODIASIS	(18) 2 (11%)	(45) 4 (9%)	(47) 2 (4%)
*COLON NEMATODIASIS	(18) 2 (11%)	(45) 4 (9%)	(47) 3 (6%)
URINARY SYSTEM			
*KIDNEY INFLAMMATION, CHRONIC	(20) 5 (25%)	(49) 5 (10%)	(48) 14 (29%)

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (VEH) 11-1096	LOW DOSE 11-1094	HIGH DOSE 11-1092
#URINARY BLADDER HYPERPLASIA, EPITHELIAL	(12)	(36) 1 (3%)	(37)
ENDOCRINE SYSTEM			
#PITUITARY CYST, NOS	(18)	(48) 1 (2%)	(45) 2 (4%)
HYPERPLASIA, FOCAL	1 (6%)		3 (7%)
HYPERPLASIA, CHROMOPHOBE-CELL			
#ADRENAL NECROSIS, FOCAL	(20)	(49) 1 (2%)	(47)
#ADRENAL MEDULLA HYPERPLASIA, NOS	(20) 1 (5%)	(49) 1 (2%)	(47)
#THYROID HYPERPLASIA, C-CELL	(18)	(44) 3 (7%)	(41)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND HYPERPLASIA, NOS	(20)	(50)	(48) 1 (2%)
#UTERUS HYDROMETRA	(20)	(48)	(48) 1 (2%)
INFLAMMATION, SUPPURATIVE	1 (5%)	1 (2%)	1 (2%)
MYOMETRA		3 (6%)	2 (4%)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION ACUTE AND CHRONIC			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, ADENOMATOUS	1 (5%)		1 (2%)
#CERVIX UTERI INFLAMMATION ACUTE AND CHRONIC	(20)	(48)	(48) 1 (2%)
#UTERUS/ENDOMETRIUM INFLAMMATION, NOS	(20)	(48) 1 (2%)	(48) 1 (2%)
INFLAMMATION, SUPPURATIVE	2 (10%)	2 (4%)	2 (4%)
INFLAMMATION, ACUTE	1 (5%)	3 (6%)	3 (6%)
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (VEH) 11-1096	LOW DOSE 11-1094	HIGH DOSE 11-1092
INFLAMMATION, CHRONIC HYPERPLASIA, NOS		2 (4%)	1 (2%)
HYPERPLASIA, CYSTIC	1 (5%)	4 (8%)	6 (13%)
#OVARY/OVIDUCT ABSCESS, NOS	(20)	(48)	(48) 1 (2%)
#OVARY CYST, NOS	(20) 3 (15%)	(47) 2 (4%)	(48) 3 (6%)
FOLLICULAR CYST, NOS			1 (2%)
PAROVARIAN CYST			1 (2%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
NERVOUS SYSTEM			
#BRAIN ATROPHY, PRESSURE	(20) 2 (10%)	(50) 1 (2%)	(48)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY NECROSIS, FAT	(20)	(50)	(48) 2 (4%)
*PERITONEUM NECROSIS, FAT	(20)	(50)	(48) 2 (4%)
*PLEURA GRANULOMA, NOS	(20) 1 (5%)	(50)	(48)
*MESENTERY CYST, NOS	(20)	(50)	(48) 1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS PIGMENTATION, NOS	(20)	(50) 1 (2%)	(48) 1 (2%)

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

TABLE C2 (CONCLUDED)

	CONTROL (VEH) 11-1096	LOW DOSE 11-1094	HIGH DOSE 11-1092
ADIPOSE TISSUE			
STEATITIS		1	
NECROSIS, FAT			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	1	1
AUTO/NECROPSY/HISTO PERF		1	
AUTOLYSIS/NO NECROPSY			1
MISS: MISSING		TSAC: TERMINAL SACRIFICE	
MSAC: MORIBUND SACRIFICE		OTHR: OTHER	

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC
LESIONS IN MICE TREATED WITH 3,3'-DIMETHOXYBENZIDINE-
4,4'-DIISOCYANATE

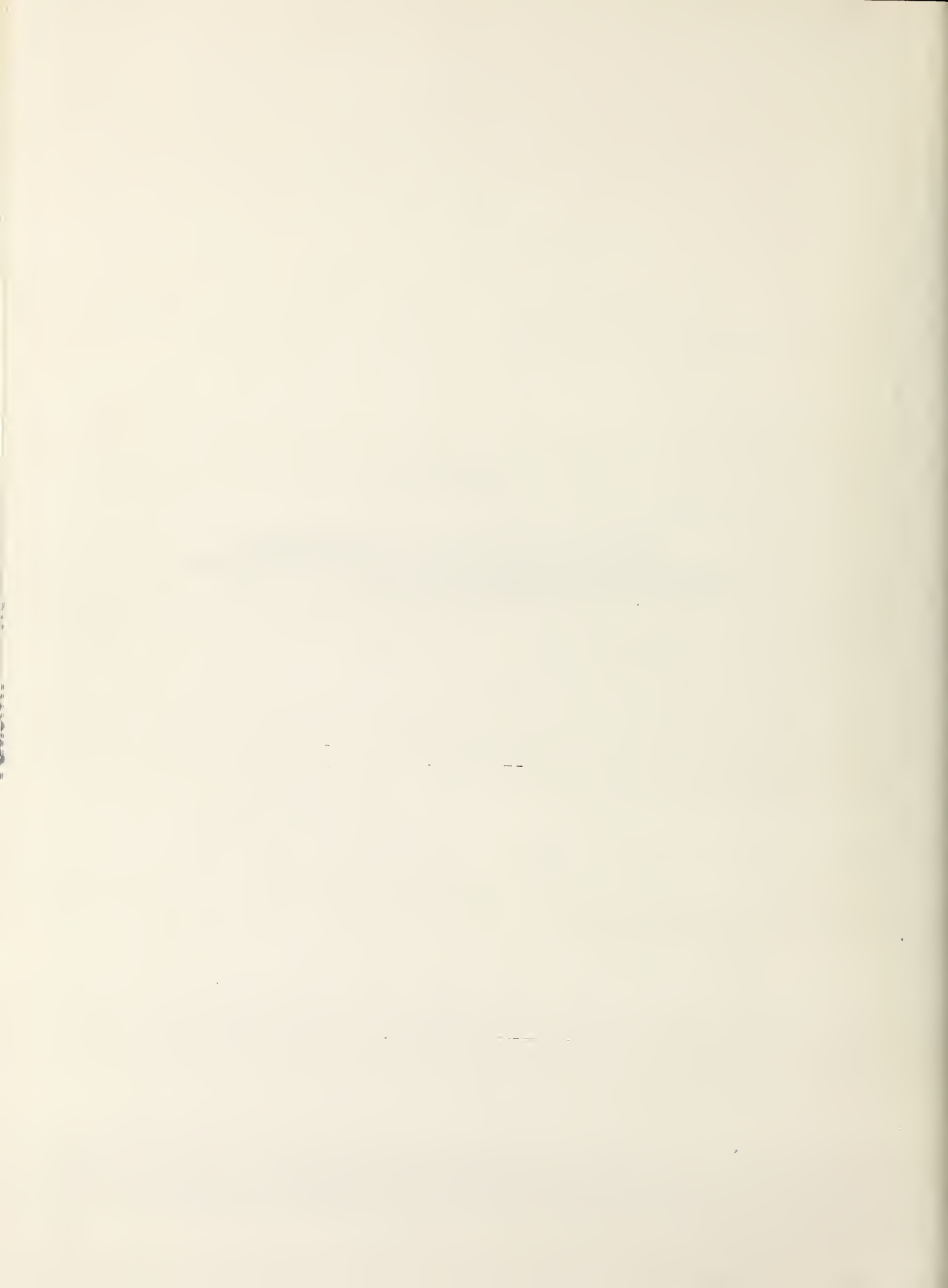


TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE

	CONTROL (UNTR) 22-2095	LOW DOSE 22-2093	HIGH DOSE 22-2091
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	5	
ANIMALS NECROPSIED	19	44	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	19	44	50
INTEGUMENTARY SYSTEM			
*SKIN	(19)	(44)	(50)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
*SUBCUT TISSUE	(19)	(44)	(50)
ABSCESS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(19)	(43)	(46)
ATELECTASIS			1 (2%)
CONGESTION, NOS	1 (5%)		
HEMORRHAGE	1 (5%)		
PNEUMONIA, LIPID	1 (5%)		
ABSCESS, NOS		1 (2%)	
PERIVASCULAR CUFFING		1 (2%)	
ALVEOLAR MACROPHAGES	1 (5%)		
HISTIOCYTOSIS		1 (2%)	2 (4%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(37)	(45)
CONGESTION, NOS		1 (3%)	
HYPERPLASIA, RETICULUM CELL		1 (3%)	
HYPERPLASIA, LYMPHOID		1 (3%)	
#LYMPH NODE	(18)	(36)	(36)
HYPERPLASIA, NOS	1 (5%)		
HYPERPLASIA, LYMPHOID		1 (3%)	
#MESENTERIC L. NODE	(18)	(36)	(38)
HYPERPLASIA, NOS	1 (5%)		
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS			

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2095	LOW DOSE 22-2093	HIGH DOSE 22-2091
HYPERPLASIA, LYMPHOID			1 (3%)
CIRCULATORY SYSTEM			
#HEART	(19)	(43)	(48)
PERIARTERITIS		2 (5%)	
CALCIFICATION, EOCAL		1 (2%)	
#MYOCARDIUM	(19)	(43)	(48)
FIBROSIS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(19)	(43)	(48)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
NECROSIS, EOCAL		2 (5%)	1 (2%)
METAMORPHOSIS EATY	1 (5%)		
NUCLEAR ENLARGEMENT		1 (2%)	
HYPERPLASIA, NODULAR			2 (4%)
HYPERPLASIA, FOCAL			1 (2%)
*BILE DUCT	(19)	(44)	(50)
CYST, NOS		1 (2%)	
#PANCREAS	(18)	(41)	(38)
INFLAMMATION, NECROTIZING			1 (3%)
ATROPHY, NOS		1 (2%)	
#STOMACH	(15)	(41)	(49)
CYST, NOS		1 (2%)	
INFLAMMATION, NOS			1 (2%)
#LARGE INTESTINE	(18)	(41)	(48)
NEMATODIASIS	1 (6%)	1 (2%)	
#COLON	(18)	(41)	(48)
NEMATODIASIS	3 (17%)	3 (7%)	3 (6%)
URINARY SYSTEM			
#KIDNEY	(19)	(42)	(50)
HYDRONEPHROSIS		2 (5%)	2 (4%)

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2095	LOW DOSE 22-2093	HIGH DOSE 22-2091
*KIDNEY/CORTEX CYST, NOS	(19)	(42)	(50) 1 (2%)
ENDOCRINE SYSTEM			
*ADRENAL CORTEX HYPERPLASIA, NOS	(13) 1 (8%)	(25)	(36)
*THYROID COLLOID CYST FIBROSIS, FOCAL	(13) 1 (8%) 1 (8%)	(36) 1 (3%)	(35) 2 (6%)
*PANCREATIC ISLETS HYPERPLASIA, NOS	(18) 1 (6%)	(41)	(38) 1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND INFLAMMATION, NOS	(19) 1 (5%)	(44)	(50)
*SEMINAL VESICLE DILATATION/DUCTS	(19)	(44) 1 (2%)	(50)
NERVOUS SYSTEM			
*BRAIN CORPORA AMYLACEA	(19) 1 (5%)	(44) 14 (32%)	(50) 9 (18%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE D1 (CONCLUDED)

	CONTROL (UNTF) 22-2095	LCW DOSE 22-2093	HIGH DOSE 22-2091
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	6	11	13
ANIMAL MISSING/NO NECROPSY	1	5	
AUTO/NECROPSY/HISTO PERF			1
AUTOLYSIS/NO NECROPSY		1	
MISS: MISSING		TSAC: TERMINAL SACRIFICE	
MSAC: MORIBUND SACRIFICE		OTHR: OTHER	

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
TREATED WITH 3,3'-DIMETHOXYBENZIDINE-4,4'-DIISOCYANATE

	CONTROL (UNTR) 22-2096	LOW DOSE 22-2094	HIGH DOSE 22-2092
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		9	7
ANIMALS NECROPSIED	20	41	43
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	41	42
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE STEATITIS	(20)	(41)	(43) 1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(41)	(40)
EMBOLISM, NOS			1 (3%)
PNEUMONIA, LIPID			1 (3%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
PNEUMONIA, CHRONIC MURINE	2 (10%)	1 (2%)	4 (10%)
GRANULOMA, NOS		1 (2%)	
HYPERPLASIA, ADENOMATOUS		1 (2%)	
HISTIOCYTOSIS		1 (2%)	
HEMATOPOIETIC SYSTEM			
*BONE MARROW HYPERPLASIA, HEMATOPOIETIC	(14)	(38)	(37) 1 (3%)
*SPLEEN	(18)	(41)	(41)
HYPERPLASIA, RETICULUM CELL		1 (2%)	1 (2%)
HYPERPLASIA, LYMPHOID	1 (6%)	2 (5%)	4 (10%)
HEMATOPOIESIS	2 (11%)		1 (2%)
*LYMPH NODE	(20)	(40)	(37)
HYPERPLASIA, NOS		1 (3%)	
HYPERPLASIA, RETICULUM CELL	1 (5%)		
HYPERPLASIA, LYMPHOID			2 (5%)
*MESENTERIC L. NODE	(20)	(40)	(37)
HYPERPLASIA, NOS	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2096	LOW DOSE 22-2094	HIGH DOSE 22-2092
#THYMUS HYPERPLASIA, LYMPHOID			(12) 1 (8%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(41)	(42)
NECROSIS, FOCAL		1 (2%)	
METAMORPHOSIS FATTY		1 (2%)	1 (2%)
NUCLEAR ENLARGEMENT		1 (2%)	
HEMATOPOIESIS			1 (2%)
#PANCREAS	(19)	(39)	(28)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (3%)	
FIBROSIS, FOCAL		1 (3%)	
#PEYERS PATCH	(20)	(41)	(38)
HYPERPLASIA, LYMPHOID		1 (2%)	
#LARGE INTESTINE	(19)	(40)	(37)
NEMATODIASIS		1 (3%)	1 (3%)
#COLON	(19)	(40)	(37)
NEMATODIASIS			2 (5%)
URINARY SYSTEM			
#KIDNEY	(19)	(41)	(42)
HYDRONEPHROSIS			2 (5%)
GLOMERULONEPHRITIS, NOS		1 (2%)	1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
AMYLOID, NOS			1 (2%)
CALCINOSIS, NOS			1 (2%)
METAPLASIA, OSSEOUS		1 (2%)	
#KIDNEY/MEDULLA	(19)	(41)	(42)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2096	LOW DOSE 22-2094	HIGH DOSE 22-2092
ENDOCRINE SYSTEM			
*ADRENAL LIPOIDOSIS	(14)	(37) 1 (3%)	(32)
*ADRENAL CORTEX HYPERPLASIA, NOS	(14)	(37)	(32) 1 (3%)
*THYROID COLLOID CYST	(12)	(36) 2 (6%)	(32)
HYPERPLASIA, FOCAL		2 (6%)	
HYPERPLASIA, FOLLICULAR-CELL			1 (3%)
*PANCREATIC ISLETS HYPERPLASIA, NOS	(19) 1 (5%)	(39) 2 (5%)	(28) 2 (7%)
REPRCDUCTIVE SYSTEM			
*VAGINA INFLAMMATION, SUPPURATIVE	(20) 2 (10%)	(41) 1 (2%)	(43) 1 (2%)
*UTERUS CYST, NOS	(19)	(40)	(37) 1 (3%)
HEMORRHAGIC CYST	1 (5%)		
INFLAMMATION, SUPPURATIVE	1 (5%)		
MYOMETRA	8 (42%)	1 (3%)	1 (3%)
*UTERINE SEROSA INFLAMMATION, FOCAL	(19)	(40) 1 (3%)	(37)
*UTERUS/ENDOMETRIUM CYST, NOS	(19)	(40) 6 (15%)	(37) 1 (3%)
INFLAMMATION, SUPPURATIVE	2 (11%)	1 (3%)	2 (5%)
HYPERPLASIA, CYSTIC	4 (21%)	19 (48%)	21 (57%)
*UTERUS/MYOMETRIUM DEGENERATION, HYALINE	(19)	(40)	(37) 1 (3%)
*OVARY/OVIDUCT INFLAMMATION, NOS	(19)	(40)	(37) 1 (3%)
*OVARY CYST, NOS	(17) 3 (18%)	(34) 7 (21%)	(22) 6 (27%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 22-2096	LOW DOSE 22-2094	HIGH DOSE 22-2092
FOLLICULAR CYST, NOS		1 (3%)	
INFLAMMATION, SUPPURATIVE			1 (5%)
ABSCISS, NOS		1 (3%)	
INFLAMMATION, CHRONIC	1 (6%)		
INFLAMMATION, GRANULOMATOUS		1 (3%)	
NERVOUS SYSTEM			
#BRAIN	(19)	(39)	(49)
CORPORA AMYLACEA	2 (11%)	8 (21%)	8 (20%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*LIGAMENT	(20)	(41)	(43)
ABSCISS, NOS		1 (2%)	
BODY CAVITIES			
*MESENTERY	(20)	(41)	(43)
NECROSIS, FAT			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(41)	(43)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		
BROAD LIGAMENT			
ABSCISS, NOS	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			3
ANIMAL MISSING/NO NECROPSY		9	7
AUTO/NECROPSY/NO HISTO			1
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

Review of the Bioassay of 3,3'-Dimethoxybenzidine-4,4'-Diisocyanate*
for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup of the
Clearinghouse on Environmental Carcinogens

August 31, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 3,3'-Dimethoxybenzidine-4,4'-Diisocyanate for carcinogenicity.

The primary reviewer said that 3,3'-Dimethoxybenzidine-4,4'-Diisocyanate induced a statistically significant increase in skin neoplasms and in leukemia in both sexes of treated rats, as well as endometrial stromal polyps in females. No evidence for the compound's carcinogenicity was found in treated mice. He noted that the compound was first administered by gavage and subsequently in the feed. The primary reviewer said that the study was acceptable and that the evidence was sufficient to conclude that 3,3'-Dimethoxybenzidine-4,4'-Diisocyanate posed a potential carcinogenic risk to man.

The secondary reviewer noted that the purity of 3,3'-Dimethoxybenzidine-4,4'-Diisocyanate was not reported. Although he agreed that the skin tumors in male rats were treatment-related, he thought that the incidence was not sufficiently high in females to draw the same conclusion from a statistical basis. He suggested that the skin tumors may have resulted through direct skin contact with the compound. The secondary reviewer agreed that the leukemia and endometrial polyps in treated rats appeared to be treatment-related.

A motion was approved unanimously that the report on the bioassay of 3,3'-Dimethoxybenzidine-4,4'-Diisocyanate be accepted as written.

Members present were:

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

* 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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