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BIOASSAY OF 4,4'-METHYLENEBIS-(N,N-DIMETHYL)BENZENAMINE FOR POSSIBLE CARCINOGENICITY

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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BIOASSAY OF

4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20205

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REPORT ON THE BIOASSAY OF
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE
FOR POSSIBLE CARCINOGENICITY

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

FOREWORD: This report presents the results of the bioassay of 4,4'-methylenebis(N,N-dimethyl)benzenamine 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 4,4'-methylenebis(N,N-dimethyl)benzenamine was conducted by Litton Bionetics, Inc., Kensington, Maryland, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

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

Histopathologic examinations were performed at Litton Bionetics, Inc. (4). The rat pathology narrative was written by Dr. A. S. Krishna Murthy (4), the mouse pathology narrative was written by Dr. S. Wyand (4), and the diagnoses included in this report represent the interpretation of these pathologists. 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. R. M. Helfand (8) and Dr. J. P. Dirkse, III (9) 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. R. A. Griesemer (1), Dr. T. E. Hamm (1), Dr. W. V. Hartwell (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. A. R. Patel (1), 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 4,4'-methylenebis-(N,N-dimethyl)benzenamine was conducted using Fischer 344 rats and B6C3F1 mice. 4,4'-Methylenebis(N,N-dimethyl)benzenamine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of 4,4'-methylenebis(N,N-dimethyl)benzenamine were, respectively, 750 and 375 ppm for rats and 2500 and 1250 ppm for mice. The compound was administered for 59 weeks to rats and for 78 weeks to mice. The period of compound administration was followed by an observation period of 45 weeks for rats and 13 weeks for mice.

There were no significant positive associations between the concentrations of 4,4'-methylenebis(N,N-dimethyl)benzenamine administered and mortality among rats or mice of either sex. Adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors. There was slight dose-related mean body weight depression among female rats, the mean body weight of high dose male rats was slightly less than that for controls, and the mean body weights of dosed mice were significantly lower than their controls, indicating that the concentrations of 4,4'-methylenebis-(N,N-dimethyl)benzenamine administered to these animals in this bioassay may have approximated the maximum tolerated concentrations.

For both male and female rats, there was a significant positive association between the concentrations of 4,4'-methylenebis(N,N-dimethyl)benzenamine administered and the incidences of follicular-cell carcinomas of the thyroid (i.e., 1/18, 4/50, and 21/46 in the control, low dose, and high dose males, respectively; and 0/20, 3/46, and 23/45 in the control, low dose, and high dose females, respectively). The high dose to control Fisher exact comparisons were also significant for each sex.

Liver neoplasms were observed among male and female mice. There were elevated incidences of hepatocellular adenomas in dosed mice when compared to controls (i.e., 2/20, 3/50, and 16/48 in control, low dose, and high dose males, respectively; and 1/19, 18/49, and 22/48 in control, low dose, and high dose females). The incidences of hepatocellular carcinomas in dosed mice did not differ greatly from those in controls (i.e., 3/20, 9/50, and 6/48 in control, low dose, and high dose males, respectively; and 0/19, 1/49, and 1/48 in control, low dose, and high dose females). Among both sexes of mice, there was a significant positive association between the concentrations of the chemical administered and the incidences of a combination of hepatocellular adenomas and hepatocellular carcinomas. For

male mice, the Fisher exact comparisons were not significant; however, for females, both the high dose to control and the low dose to control comparisons were significant.

In both sexes of both species nonneoplastic proliferative lesions of the thyroid occurred in dosed animals but not in any of the controls.

Under the conditions of this bioassay, 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic in Fischer 344 rats, inducing thyroid follicular-cell carcinomas in both males and females. Administration of the compound was carcinogenic in female B6C3F1 mice, inducing liver neoplasms. There was no conclusive evidence that 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic in male B6C3F1 mice.

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I. INTRODUCTION

4,4'-Methylenebis(N,N-dimethyl)benzenamine (Figure 1) (NCI No. C01990), a bicyclic aromatic amine and an intermediate in dye manufacture, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer observed among dye manufacturing industry workers (Anthony and Thomas, 1970; Wynder et al., 1963). The carcinogenicity of the structurally similar compounds 4,4'-methylenebis(2-chloroaniline) and 4,4'-methylenebis(2-methylaniline) (International Agency for Research on Cancer, 1974) was an additional factor in the selection of 4,4'-methylenebis(N,N-dimethyl)benzenamine for testing.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 4,4'-methylenebis(N,N-dimethyl) benzenamine.* It is also called 4,4'-methylenebis(N,N-dimethyl) aniline; tetramethyldiaminodiphenylmethane; 4,4'-bis(dimethylamino) diphenylmethane; tetra base; methane base; Michler's base; Michler's hydride; and Michler's methane.

4,4'-Methylenebis(N,N-dimethyl)benzenamine is an intermediate in the synthesis of at least 5 dyes: C.I. (Colour Index) Basic Yellow 2, C.I. Solvent Yellow 34, Methylene Red, C.I. Basic Orange 14, and C.I. Solvent Orange 15 (Society of Dyers and Colourists, 1956).

*The CAS registry number is 101-61-1.

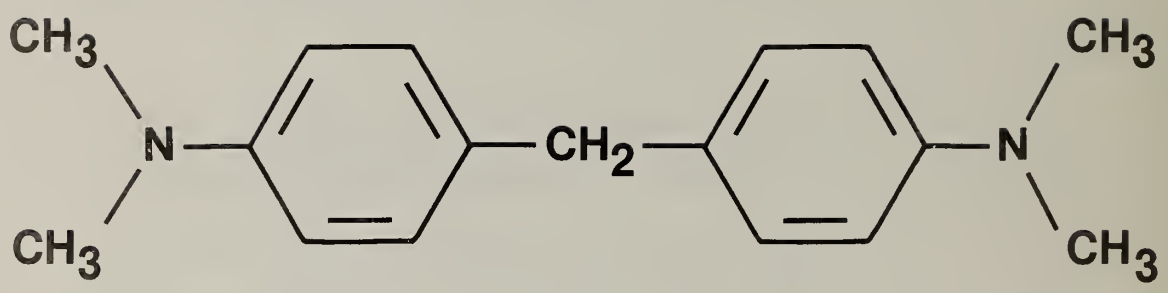

FIGURE 1
CHEMICAL STRUCTURE OF 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

4,4'-Methylenebis(N,N-dimethyl)benzenamine hydrochloride is used as an analytical reagent for the determination of lead (Windholz, 1976).

Specific production data for 4,4'-methylenebis(N,N-dimethyl) benzenamine are not available; however, this compound is produced in commercial quantities (in excess of 1000 pounds or $1000 in value annually) by four U.S. companies (Stanford Research Institute, 1977). C.I. Basic Yellow 2 and C.I. Solvent Yellow 34, for which 4,4'-methylenebis(N,N-dimethyl)benzenamine is utilized as an intermediate, are also produced in commercial quantities in the United States (U.S. International Trade Commission, 1977).

The potential for exposure to 4,4'-methylenebis(N,N-dimethyl) benzenamine is greatest for workers in the dye and chemical manufacturing industries.

II. MATERIALS AND METHODS

A. Chemicals

Technical-grade 4,4'-methylenebis(N,N-dimethyl)benzenamine was purchased from E.I. duPont de Nemours & Company, Wilmington, Delaware. Chemical analysis was performed by Litton Bionetics, Inc., Kensington, Maryland. The experimentally determined melting point range, 85° to 87°C, was compared to the manufacturer's specification of 88°C. The results of infrared and nuclear magnetic resonance analyses were consistent with those expected based on the structure of the compound. Thin-layer chromatography was performed utilizing two solvent systems. Each plate was visualized with visible and ultraviolet light, iodine vapor, and ferric chloride-potassium ferricyanide spray. In all cases only one spot was observed.

Throughout this report, the term 4,4'-methylenebis(N,N-dimethyl) benzenamine is used to represent this technical-grade material.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® meal (Allied Mills, Inc., Chicago, Illinois). 4,4'-Methylenebis(N,N-dimethyl)benzenamine was administered to the dosed animals as a component of the diet.

The chemical was removed from its container and a proper amount was blended with an aliquot of the 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.

C. Animals

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

Rats and mice, approximately 4 weeks old when received, were examined and any obviously ill or runted animals were killed. The remaining animals were quarantined for 2 weeks prior to initiation of test. Animals which did not manifest clinical signs of disease were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenance

Animals were housed by species in rooms maintained at 22° to 26°C and 45 to 55 percent relative humidity. 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.).

Animal species were housed in separate rooms. Rats were housed four per cage by sex and mice were housed five per cage by sex. Polycarbonate cages (Lab Products, Inc., Garfield, New Jersey), which were suspended from aluminum racks, were used for dosed and control animals. 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 (Ab-sorb-dri® hardwood chip bedding, Wilner Wood Products Company, Norway, Maine) were provided twice weekly.

Acidulated water (pH 2.5) was supplied to animals in water bottles which were changed and washed twice weekly. 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.

Dosed and control rats were housed in a room with other rats receiving diets containing* p-quinone dioxime (105-11-3); and NTA

*CAS registry numbers are given in parentheses.

trisodium salt (5064-31-3); and other rats intubated with styrene (100-42-5).

Dosed and control mice were housed in a room with other mice receiving diets containing Michler's ketone (90-94-8); 2-nitro-p-phenylenediamine (5307-14-2); p-chloroaniline (106-47-8); 5-chloro-o-toluidine (95-79-4); N-phenyl-p-phenylenediamine hydrochloride (2198-59-6); 1-phenyl-2-thiourea (103-85-5); trimethylthiourea (2489-77-2); dibutyltin diacetate (1067-33-0); and 3-chloro-p-toluidine (95-74-9).

E. Selection of Initial Concentrations

To establish the concentrations of 4,4'-methylenebis(N,N-dimethyl)benzenamine for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Rats were distributed among six groups, each consisting of five males and five females. 4,4'-Methylenebis(N,N-dimethyl)benzenamine was incorporated into the basal laboratory diet and supplied ad libitum to five of the six rat groups in concentrations of 145, 315, 680, 1466 and 3155 ppm. The remaining rat group served as a control group, receiving only the basal laboratory diet.

Mice were distributed among eleven groups, each consisting of five males and five females. 4,4'-Methylenebis(N,N-dimethyl)benzenamine was incorporated into the basal laboratory diet and supplied ad libitum to nine of the eleven mouse groups in concentrations of 370, 550, 810, 1180, 1740, 3750, 5500, 8080 and 11,830 ppm. The two

remaining mouse groups served as control groups, receiving only the basal laboratory diet.

The dosed dietary preparations were administered for a period of 4 weeks, followed by a 2-week observation period during which all animals were fed the basal laboratory diet. Individual body weights were recorded twice weekly throughout the study. Upon termination of the study all survivors were euthanized and necropsied.

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

RAT SUBCHRONIC STUDY RESULTS

	Mean Body Weight Gain (%)*		Survival**		Observation of Rough Hair and Arched Backs**	
ppm	Males	Females	Males	Females	Males	Females
3155	-135	-61	5/5	5/5	5/5	0/5
1466	-107	-49	5/5	5/5	0/5	0/5
680	- 37	-11	5/5	5/5	0/5	0/5
315	- 13	- 1	5/5	5/5	0/5	0/5
145	- 26	+ 6	5/5	5/5	0/5	0/5
0	--	--	5/5	5/5	0/5	0/5

The high concentration selected for administration to dosed rats in the chronic bioassay was 750 ppm.

*+ is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.

**Number of animals observed/number of animals originally in group.

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

MOUSE SUBCHRONIC STUDY RESULTS

ppm	Mean Body Weight Gain (%)*		Survival	
	Males	Females	Males	Females
11,830	- 2	- 1	5/5	5/5
8,080	+ 3	- 2	5/5	5/5
5,500	-12	- 5	5/5	5/5
3,750	- 4	- 4	5/5	5/5
1,740	- 2	-10	5/5	5/5
1,180	- 4	- 8	5/5	5/5
810	- 1	+ 2	5/5	5/5
550	+ 6	- 8	5/5	5/5
370	+ 3	- 4	5/5	5/5
0	--	--	10/10	10/10

No other clinical abnormalities which could be attributed to administration of the compound were observed. The high concentration selected for administration to dosed mice in the chronic bioassay was 2500 ppm.

F. Experimental Design

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

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*+ is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.

**Number of animals observed/number of animals originally in group.

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE
FEEDING EXPERIMENT

	INITIAL GROUP SIZE	4,4'-METHYLENEBIS (N,N-DIMETHYL)BENZENAMINE CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	20	0		104
LOW DOSE	50	375	59	
		0		45
HIGH DOSE	50	750	59	
		0		45
FEMALE				
CONTROL	20	0		104
LOW DOSE	50	375	59	
		0		45
HIGH DOSE	50	750	59	
		0		45

[a]Concentrations given in parts per million.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE
FEEDING EXPERIMENT

	INITIAL GROUP SIZE	4,4'-METHYLENEBIS (N,N-DIMETHYL)BENZENAMINE CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	20	0		91
LOW DOSE	50	1250	78	
		0		13
HIGH DOSE	50	2500	78	
		0		13
FEMALE				
CONTROL	20	0		91
LOW DOSE	50	1250	78	
		0		13
HIGH DOSE	50	2500	78	
		0		13

[a]Concentrations given in parts per million.

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed rats were supplied with diets containing 750 and 375 ppm 4,4'-methylenebis(N,N-dimethyl)benzenamine for 59 weeks followed by a 45-week observation period, when no test chemicals were used. Throughout this report those rats 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.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed mice were supplied with diets containing 2500 and 1250 ppm 4,4'-methylenebis-(N,N-dimethyl)benzenamine for 78 weeks followed by a 13-week observation period, when no test chemicals were used. 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.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment and body weights were recorded once a week for the first 6 weeks, every 2 weeks for the next 12 weeks, and at monthly intervals thereafter. All animals were inspected twice daily.

All moribund animals, animals that developed large, palpable masses that jeopardized their health, or animals that survived until the end of the bioassay were euthanized using carbon dioxide.

Necropsies were immediately performed on these animals and on all animals found dead during the bioassay. Gross and microscopic examinations were performed on all major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

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

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

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System

(Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

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

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

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which

that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

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

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test

determined if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

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

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed

and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

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

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower

limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

High dose male rats evidenced slight mean body weight depression in comparison with their controls after week 30. Slight dose-related mean body weight depression was apparent in female rats after week 35 (Figure 2).

No other clinical signs were recorded.

B. Survival

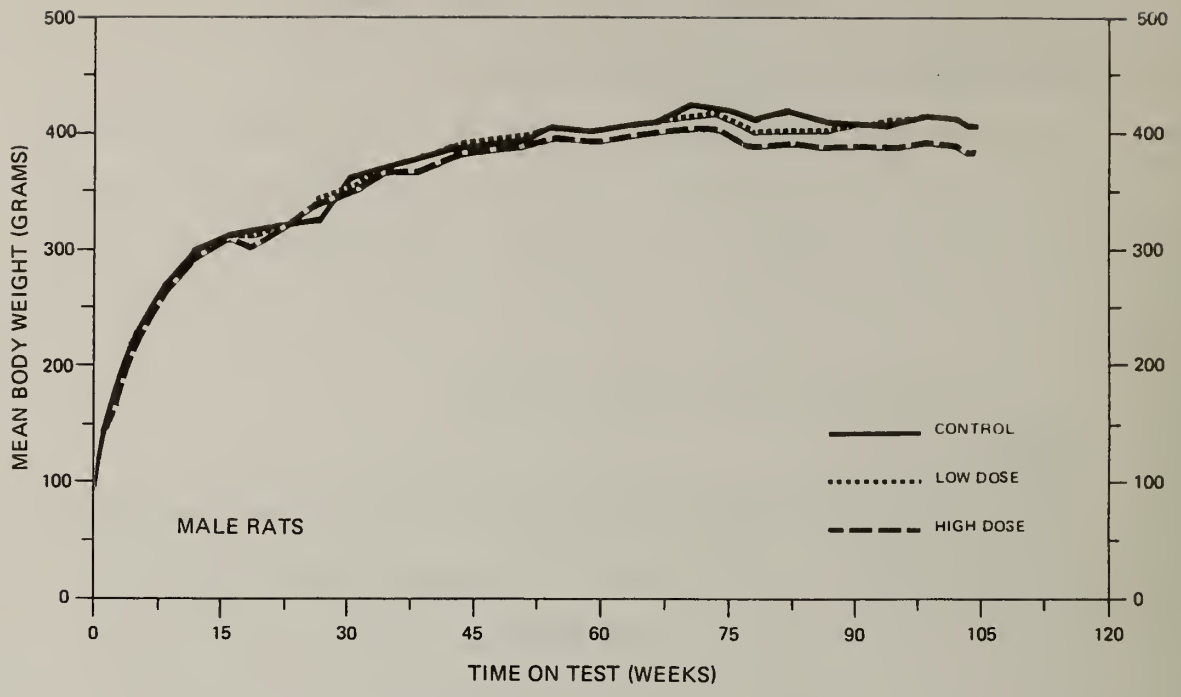
The estimated probabilities of survival for male and female rats in the control and 4,4'-methylenebis(N,N-dimethyl)benzenamine-dosed groups are shown in Figure 3. The Tarone test for association between dosage and mortality was not significant for either males or females.

There were adequate numbers of male rats at risk from late-developing tumors, as 88 percent (44/50) of the high dose, 78 percent (39/50) of the low dose, and 80 percent (16/20) of the controls survived on test for at least 104 weeks.

There were adequate numbers of female rats at risk from late-developing tumors, as 74 percent (37/50) of the high dose, 82 percent (41/50) of the low dose, and 85 percent (17/20) of the controls survived on test for at least 104 weeks.

C. Pathology

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



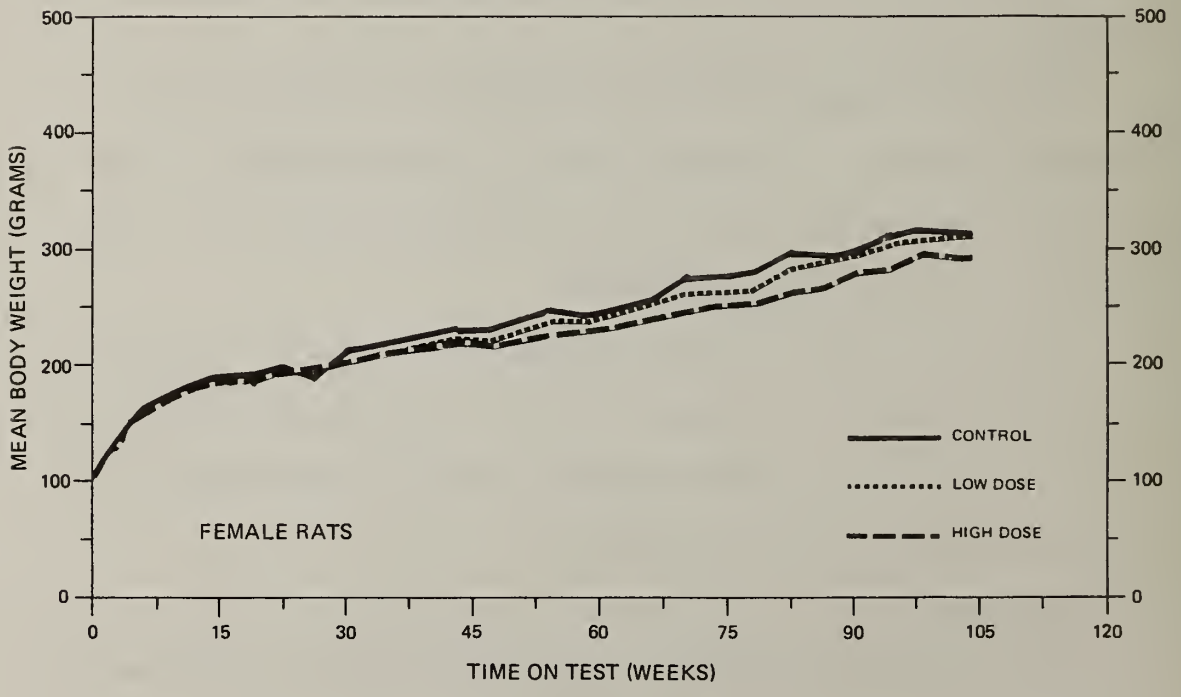
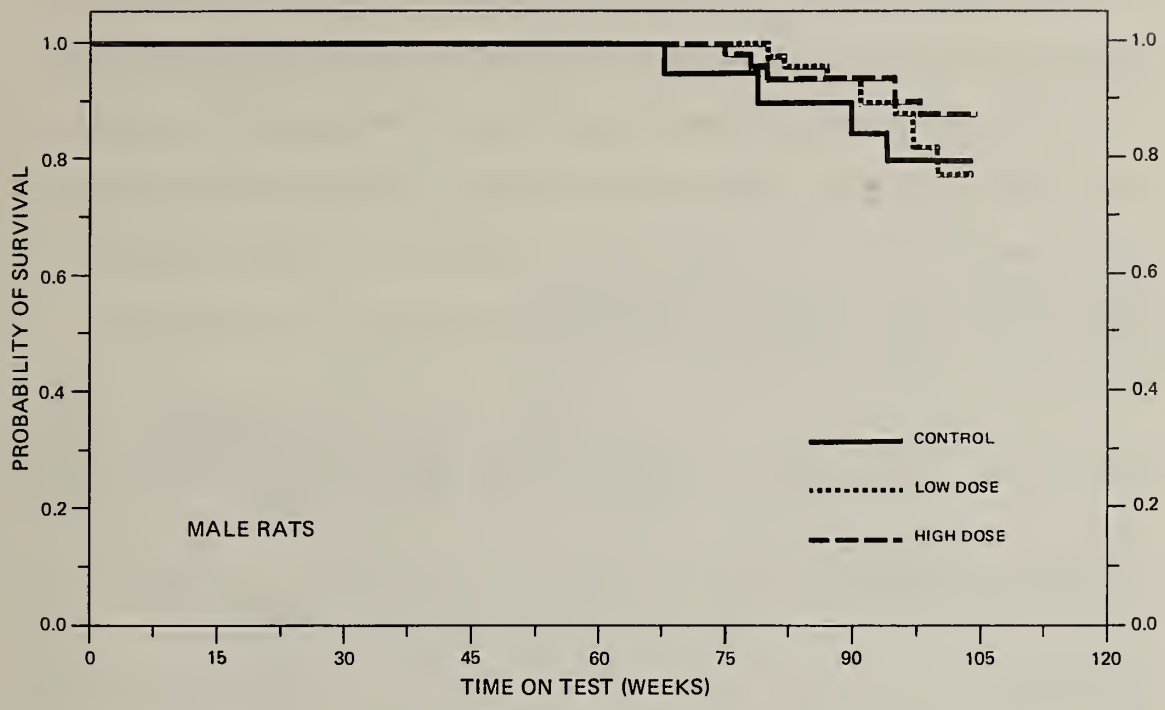


FIGURE 2
GROWTH CURVES FOR 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE
CHRONIC STUDY RATS



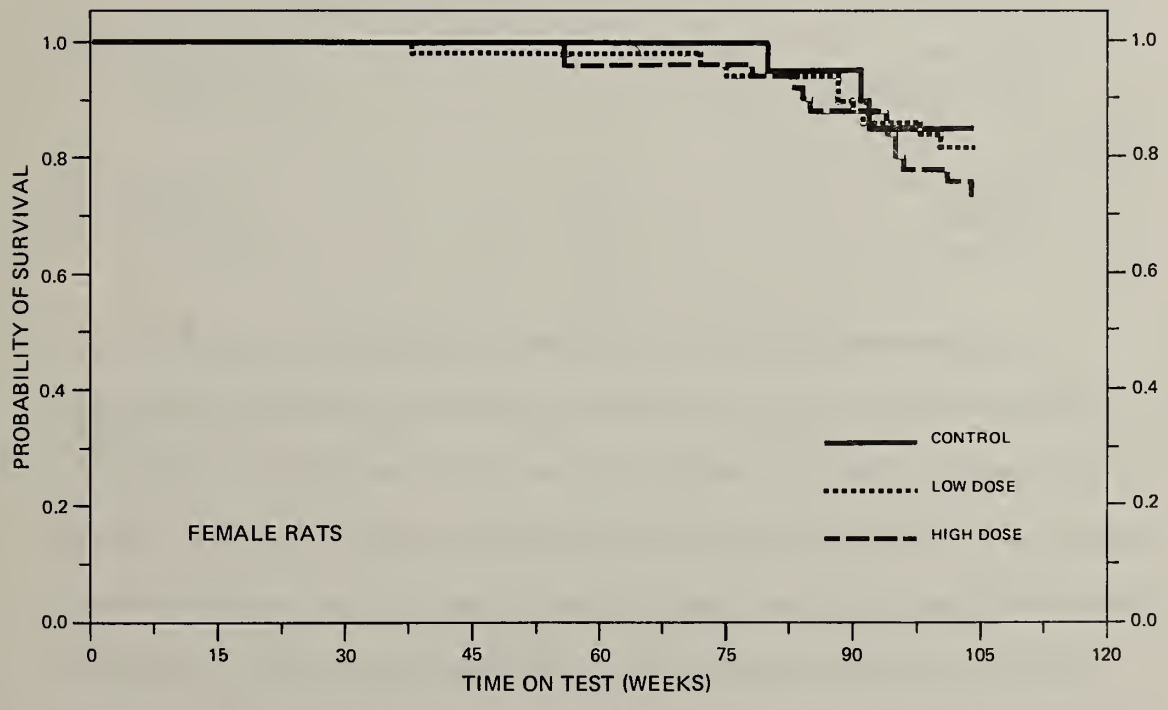


FIGURE 3
SURVIVAL COMPARISONS OF 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE CHRONIC STUDY RATS

The occurrence of follicular neoplasms of the thyroid gland, both of male and female rats, was related to the administration of 4,4'-methylenebis(N,N-dimethyl)benzenamine. The number of animals with follicular cysts, hyperplasia, adenoma, carcinoma, and C-cell hyperplasia was also increased. The incidences of these neoplastic and nonneoplastic lesions are summarized in the following table:

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
No. of Animals with Thyroids Examined Histopathologically	(18)	(50)	(46)	(20)	(46)	(45)
Follicular:						
Cyst	0	4	3	0	3	1
Hyperplasia	0	0	7	0	1	5
Adenoma	0	0	13	0	1	13
Carcinoma	1	4	21	0	3	23
Neoplasm NOS	0	0	1	0	0	1
C-Cell:						
Hyperplasia	0	5	1	0	1	1
Adenoma	2	0	3	2	1	1
Carcinoma	0	2	1	0	1	0

A markedly distended follicle with eosinophilic or pale colloid and normal epithelium was considered a follicular cyst. Follicular hyperplasia was either localized or diffuse. Irregular papillary ingrowths of the epithelium resulted in follicles of varying sizes. The follicular cells were large, and the nuclei were hyperchromatic.

Follicular neoplasms of the thyroid gland were generally well-vascularized. Many arteries, particularly those in the capsule, were

sclerotic and occluded. The fibrous capsule was prominent in animals with this neoplasm. Some of the morphologic features of these neoplasms (adenoma and carcinoma) are recorded in the following table:

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
No. of Animals with Thyroids Examined Histopathologically	(18)	(50)	(46)	(20)	(46)	(45)
Follicular:						
Adenoma	0	0	13	0	1	13
Carcinoma	1	4	21	0	3	23
Lobes Involved:						
One	1	4	21	0	4	9
Both	0	1	13	0	0	27
Infiltration:						
Capsule	1	4	21	0	3	22
Blood Vessel	0	0	2	0	0	6
Compression:						
Trachea	0	0	6	0	0	5
Esophagus	0	0	1	0	0	2

Follicular adenoma involved a part or an entire lobe of the gland and compressed the adjacent areas of tissue. Although circumscribed, not all adenomas were encapsulated. The morphologic features of the adenomas were different from either normal or hyperplastic tissue, in that they were cellular with piling of cells in some areas. Both macro- and micro-follicular variants were recognized. The follicular pattern was common in many adenomas, and the

papillary pattern in others. The epithelial cells were cuboidal or columnar. Cytoplasm was basophilic, and the nuclei were hyperchromatic. Mitotic figures were infrequent. Infiltration of the tumor into the capsule or blood vessels, together with distortion of architecture and back-to-back cellular arrangement, were considered the cardinal features of a follicular carcinoma. The carcinoma either involved one or both lobes and compressed the trachea or esophagus in a few animals.

Both follicular and follicular-papillary arrangements of cells were common in many tumors. The colloid was eosinophilic in large follicles and unstained in others. A pleomorphism in follicular size was evident. Back-to-back arrangement of cells with scant stroma was found in many areas. The cells were either columnar or cuboidal with a basophilic cytoplasm and hyperchromatic nuclei. Mitotic figures were not numerous.

Areas of necrosis and cystic degeneration were seen in many tumors. Foci of mineralization, clumps of golden-brown pigment, and cholesterol clefts were additional features in areas of degeneration. In a few tumors, the stroma was hyalinized and there was a stromal reaction (as evidenced by proliferating fibroblasts). Squamous metaplasia was noticed in one tumor. Follicular carcinoma in one high dose rat had metastasized to the lung.

Hyperplasia and neoplasia of the C-cells of the thyroid gland occurred both in control and dosed rats, and probably was not compound-related.

The nonneoplastic lesions in the dosed rats did not appear to be related to the feeding of the chemical.

Based on the results of this pathology examination, 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic to male and female Fischer 344 rats, inducing follicular-cell neoplasms of the thyroid gland under the conditions of this bioassay.

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 4,4'-methylenebis(N,N-dimethyl)benzenamine-dosed groups and where such tumors were observed in at least 5 percent of the group.

For male rats, the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and the incidence of follicular-cell carcinomas of the thyroid. This was supported by a significant ($P = 0.002$) positive high dose to control Fisher exact comparison. The test for departure from linear trend was also significant at this site. The Cochran-Armitage test also indicated a significant ($P < 0.001$) positive association between dose and the combined incidence of follicular-cell carcinomas or follicular-cell

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	2/20(0.10)	1/50(0.02)	0/50(0.00)
P Values[c]	P = 0.035(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.200	0.000
Lower Limit	---	0.004	0.000
Upper Limit	---	3.681	1.345
Weeks to First Observed Tumor	68	97	---
Liver: Hepatocellular Carcinoma[b]	2/19(0.11)	0/50(0.00)	0/49(0.00)
P Values[c]	P = 0.023(N)	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.036	---	---
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	1.278	1.303
Weeks to First Observed Tumor	104	---	---
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	3/19(0.16)	5/50(0.10)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.633	0.388
Lower Limit	---	0.141	0.058
Upper Limit	---	3.807	2.710
Weeks to First Observed Tumor	104	104	98

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	2/19(0.11)	12/47(0.26)	14/48(0.29)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.426	2.771
Lower Limit	---	0.625	0.739
Upper Limit	---	21.038	23.650
Weeks to First Observed Tumor	104	97	80
Adrenal: Pheochromocytoma[b]	1/20(0.05)	3/47(0.06)	5/48(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.277	2.083
Lower Limit	---	0.112	0.259
Upper Limit	---	65.563	96.358
Weeks to First Observed Tumor	104	82	104
Thyroid: Follicular-Cell Carcinoma[b]	1/18(0.06)	4/50(0.08)	21/46(0.46)
P Values[c]	P < 0.001	N.S.	P = 0.002
Departure from Linear Trend[e]	P = 0.031	---	---
Relative Risk (Control)[d]	---	1.440	8.217
Lower Limit	---	0.159	1.532
Upper Limit	---	69.469	326.674
Weeks to First Observed Tumor	104	95	95

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Carcinoma or Follicular-Cell Adenoma[b]	1/18(0.06)	4/50(0.08)	34/46(0.74)
P Values[c]	P < 0.001	N.S.	P < 0.001
Departure from Linear Trend[e]	P < 0.001	---	---
Relative Risk (Control)[d]	---	1.440	13.304
Lower Limit	---	0.159	2.653
Upper Limit	---	69.469	496.812
Weeks to First Observed Tumor	104	95	80
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	2/18(0.11)	2/50(0.04)	4/46(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.360	0.783
Lower Limit	---	0.029	0.127
Upper Limit	---	4.740	8.213
Weeks to First Observed Tumor	90	104	104
Testis: Interstitial-Cell Tumor[b]	20/20(1.00)	47/49(0.96)	45/49(0.92)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.959	0.918
Lower Limit	---	0.000	0.000
Upper Limit	---	1.111	1.250
Weeks to First Observed Tumor	68	80	78

TABLE 3 (CONCLUDED)

[a]Treated groups received doses of 375 or 750 ppm in feed.

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

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

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

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

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	1/20(0.05)	3/49(0.06)	1/50(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.224	0.400
Lower Limit	---	0.108	0.005
Upper Limit	---	62.958	30.802
Weeks to First Observed Tumor	104	104	104
Liver: Neoplastic Nodule[b]	0/20(0.00)	0/49(0.00)	4/47(0.09)
P Values[c]	P = 0.036	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.411
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	104
Pituitary: Chromophobe Adenoma[b]	11/20(0.55)	18/47(0.38)	21/48(0.44)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.696	0.795
Lower Limit	---	0.410	0.482
Upper Limit	---	1.354	1.507
Weeks to First Observed Tumor	80	91	84

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Carcinoma[b]	0/20(0.00)	3/46(0.07)	23/45(0.51)
P Values[c]	P < 0.001	N.S.	P < 0.001
Departure from Linear Trend[e]	P = 0.021	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.272	3.492
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	104	83
Thyroid: Follicular-Cell Carcinoma or Follicular-Cell Adenoma[b]	0/20(0.00)	4/46(0.09)	36/45(0.80)
P Values[c]	P < 0.001	N.S.	P < 0.001
Departure from Linear Trend[e]	P = 0.001	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.420	5.720
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	104	83
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	2/20(0.10)	2/46(0.04)	1/45(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.435	0.222
Lower Limit	---	0.034	0.004
Upper Limit	---	5.721	4.077
Weeks to First Observed Tumor	104	104	104

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma[b]	1/20(0.05)	5/50(0.10)	8/50(0.16)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.000	3.200
Lower Limit	---	0.249	0.482
Upper Limit	---	92.596	138.771
Weeks to First Observed Tumor	104	104	101
Mammary Gland: Fibroadenoma, Adenoma NOS or Adenocarcinoma NOS[b]	1/20(0.05)	7/50(0.14)	9/50(0.18)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.800	3.600
Lower Limit	---	0.403	0.561
Upper Limit	---	123.407	154.106
Weeks to First Observed Tumor	104	90	85
Uterus: Endometrial Stromal Polyp[b]	1/20(0.05)	3/49(0.06)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.224	1.224
Lower Limit	---	0.108	0.108
Upper Limit	---	62.958	62.958
Weeks to First Observed Tumor	104	104	104

TABLE 4 (CONCLUDED)

[a]Treated groups received doses of 375 or 750 ppm in feed.

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

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

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

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

adenomas of the thyroid. The Fisher exact high dose to control comparison was significant ($P < 0.001$), as was the test for departure from linear trend.

In female rats, the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and the incidence of follicular-cell carcinomas of the thyroid. This was supported by a significant ($P < 0.001$) positive Fisher exact test comparing the high dose group to the control group. The test for departure from linear trend was also significant. As in males, the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and the combined incidence of follicular-cell carcinomas or follicular-cell adenomas of the thyroid. This was supported by a significant ($P < 0.001$) positive high dose to control Fisher exact comparison. The test for departure from linear trend was also significant at this site. Also in female rats, the Cochran-Armitage test indicated a significant ($P = 0.036$) positive association between dose and the incidence of neoplastic nodules of the liver. This result, however, was not supported by either of the Fisher exact tests.

Based on these statistical results, the administration of 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic to both male and female Fischer 344 rats under the conditions of this bioassay, causing both follicular-cell carcinomas of the thyroid and a combination of follicular-cell carcinomas or follicular-cell adenomas of the thyroid in rats of both sexes.

In male rats, the Cochran-Armitage test indicated a significant negative association both between dose and the incidence of fibromas of the subcutaneous tissue and between dose and the incidence of hepatocellular carcinomas. In the latter case, the test for departure from linear trend was also significant.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

The mean body weights of dosed male mice were considerably lower than that of the controls after week 30. Dose-related mean body weight depression was apparent in female mice after week 30 (Figure 4).

No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 4,4'-methylenebis(N,N-dimethyl)benzenamine-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, but the test for departure from linear trend was significant for males.

There were adequate numbers of male mice at risk from late-developing tumors, as 100 percent (50/50) of the high dose, 94 percent (47/50) of the low dose and 100 percent (20/20) of the controls survived on test until the termination of the study.

There were adequate numbers of female mice at risk from late-developing tumors, as 84 percent (42/50) of the high dose, 92 percent (46/50) of the low dose and 90 percent (18/20) of the controls survived on test until the termination of the study. Two high dose females and one low dose female were missing in week 14.

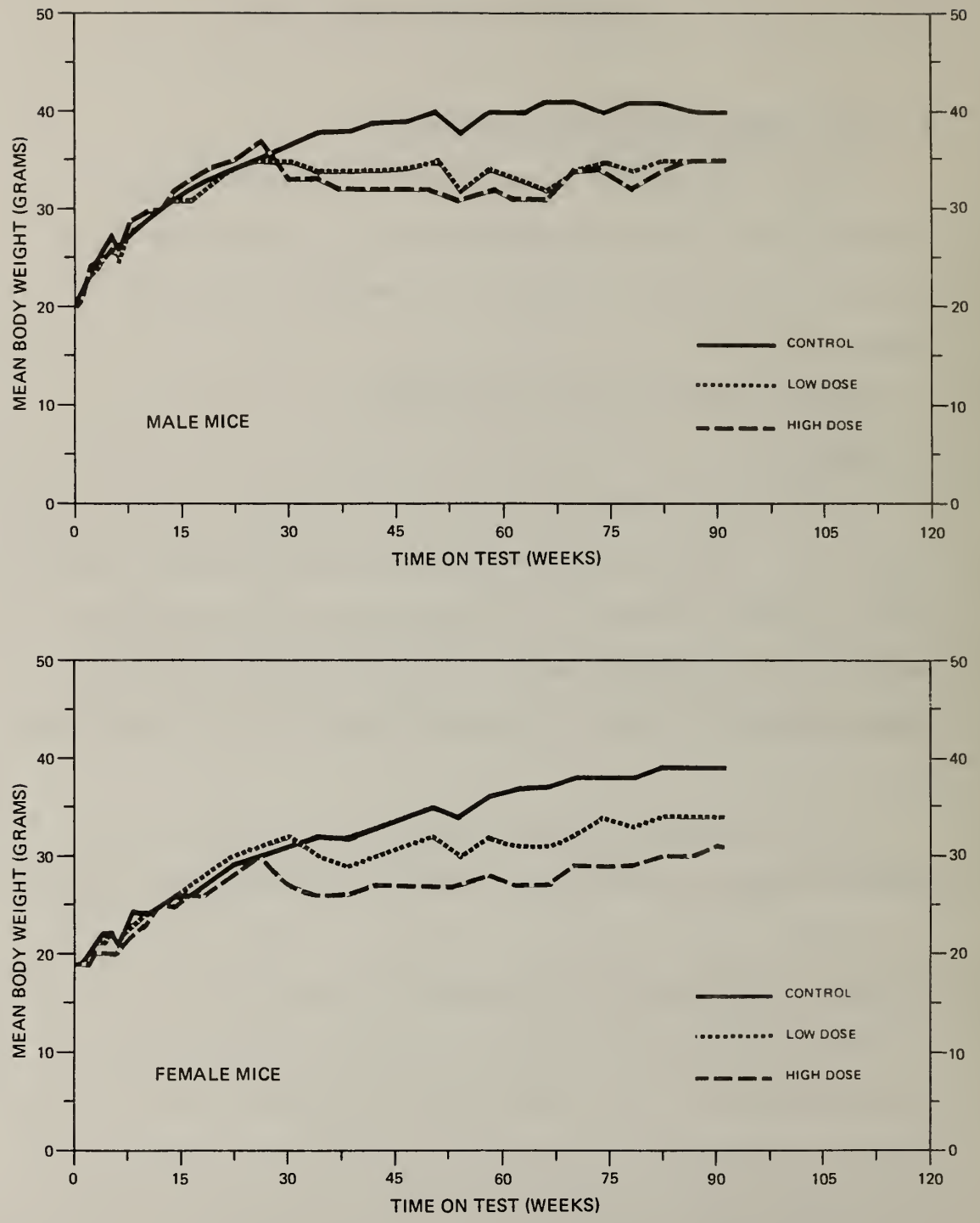
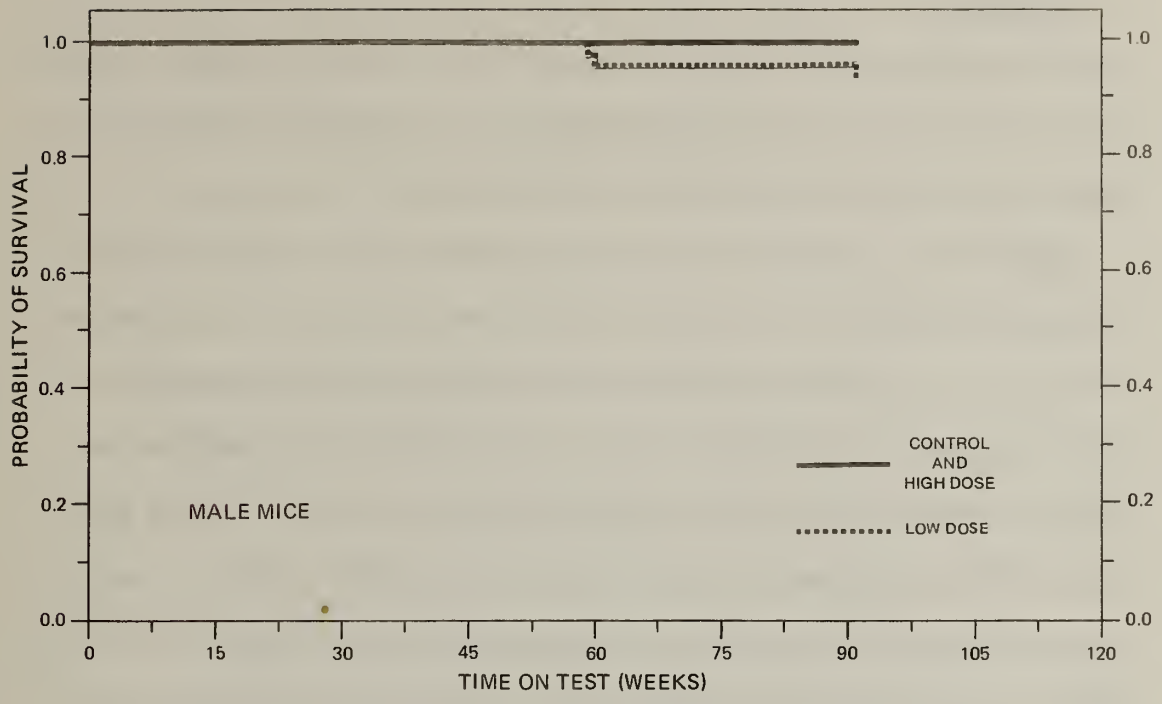


FIGURE 4
GROWTH CURVES FOR 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE
CHRONIC STUDY MICE



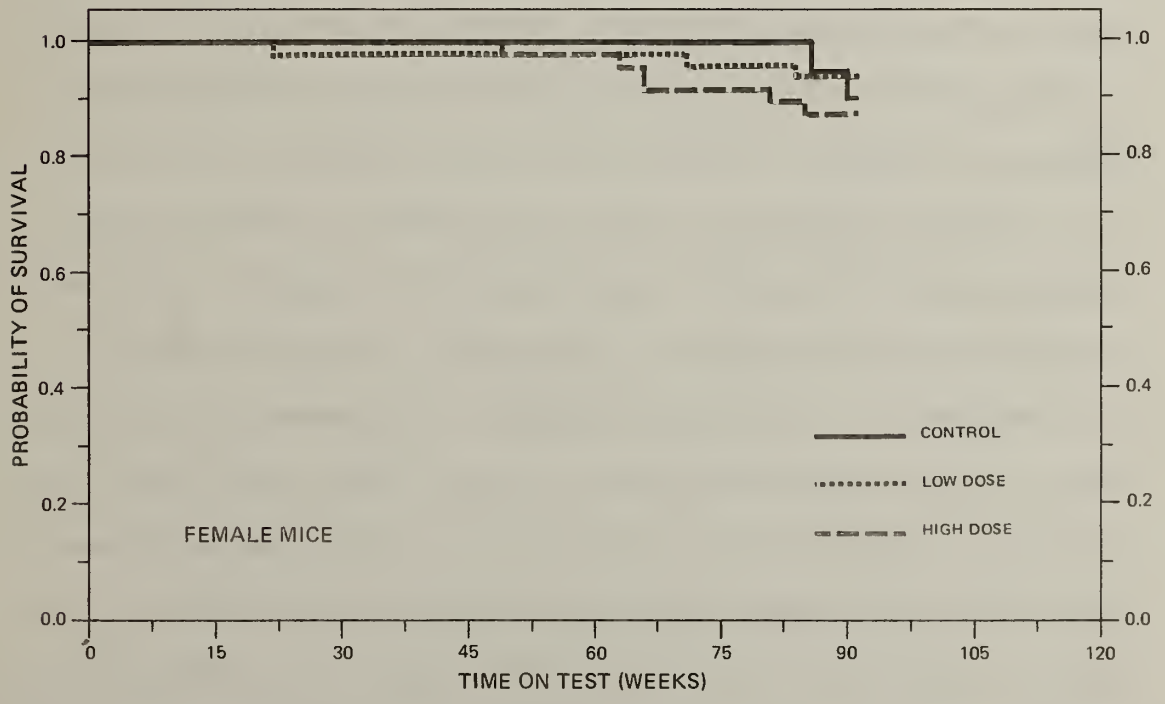


FIGURE 5
SURVIVAL COMPARISONS OF 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE
CHRONIC STUDY MICE

C. Pathology

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

A variety of neoplasms was seen in both control and dosed mice. The majority appeared unrelated to chemical administration. However, there was an increased incidence of hepatocellular adenomas (i.e., 2/20 [10 percent], 3/50 [6 percent], and 16/48 [33 percent] in control, low dose, and high dose males, respectively, and 1/19 [5 percent], 18/49 [37 percent], and 22/48 [46 percent] in control, low dose, and high dose females) or hepatocellular carcinomas (i.e., 3/20 [15 percent], 9/50 [18 percent], and 6/48 [13 percent] in control, low dose, and high dose males, respectively, and 0/19, 1/49 [2 percent], and 1/48 [2 percent] in control, low dose, and high dose females) in dosed male and female mice. As indicated, most of the liver neoplasms observed in females were adenomas. Hepatocellular adenomas were usually small, roughly spherical expanding nodules of well-differentiated basophilic, eosinophilic, and vacuolated cells which compressed the adjacent parenchyma. Eosinophilic and vacuolated cells were larger than normal. Mitotic figures were rare. Some tumors contained a few cells with hyaline cytoplasmic droplets. Hepatocellular carcinomas were mostly solid tumors with areas of trabecular formation. Variable morphologic areas were common within the same tumor. Cytoplasmic vacuolation and hyaline inclusions,

along with small hyperbasophilic and large eosinophilic cells, occurred. Mitotic figures were often plentiful. Hepatocytes arranged in papillary formations and extensive tumor necrosis were also seen. Foci of cytoplasmic change occurred in an elevated incidence in the livers of 6/49 (12 percent) low dose and 7/48 (15 percent) high dose female mice, a finding which is consistent with the increased incidence of hepatic tumors induced by the test compound.

Nonneoplastic proliferative lesions induced in mice were found in the thyroid. Thyroid papillary hyperplasia occurred in 10/45 (22 percent) low dose male, 13/49 (27 percent) high dose male, 7/43 (16 percent) low dose female, and 6/43 (14 percent) high dose female mice. Adenomatous goiter occurred in 4/45 (9 percent) low dose male, 22/49 (45 percent) high dose male, 25/43 (58 percent) low dose female, and 35/43 (81 percent) high dose female mice. Two high dose males were diagnosed as having colloid goiter, a stage of adenomatous goiter, which would make the total goiter incidence 24/49 (49 percent) for this group. None of the types of lesions discussed above were observed in male or female controls. Papillary thyroid hyperplasia was characterized by enlarged follicles with hyperplastic papillae projecting into their lumen. This change was usually focal, involving a few follicles, or had a patchy distribution throughout the thyroid. In some cases, the papillary hyperplasias resembled papillary adenomas. However, there was no connective tissue capsule and lesions were usually multifocal, labular or diffuse.

In adenomatous goiter there was diffuse distention of follicles with colloid, often with considerable variation in follicular size. The goiters in female mice were more severe than those in males. Some thyroids had other lesions such as hemosiderin deposition, sloughed follicular cells, calcified debris in the colloid, and stromal fibrosis.

Other inflammatory and degenerative lesions were seen which commonly occur in aging mice of this strain. These occurred in approximately equal numbers compared to controls and thus were not considered compound-related.

Based on the results of this pathology examination, 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic to B6C3F1 mice, inducing hepatocellular neoplasms under the conditions of this bioassay. The compound also induced hyperplasias of thyroid follicular cells in male and female B6C3F1 mice.

D. Statistical Analyses of Results

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

For male mice, the Cochran-Armitage test indicated a significant ($P = 0.025$) positive association between dose and the combined

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	1/20(0.05)	6/49(0.12)	7/50(0.14)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.449	2.800
Lower Limit	---	0.332	0.403
Upper Limit	---	110.166	123.407
Weeks to First Observed Tumor	91	91	91
All Sites: Hemangioma or Hemangiosarcoma[b]	0/20(0.00)	5/50(0.10)	5/50(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.525	0.525
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	91
Liver: Hepatocellular Carcinoma[b]	3/20(0.15)	9/50(0.18)	6/48(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.200	0.833
Lower Limit	---	0.346	0.204
Upper Limit	---	6.408	4.799
Weeks to First Observed Tumor	91	91	91

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	5/20(0.25)	12/50(0.24)	22/48(0.46)
P Values[c]	P = 0.025	N.S.	N.S.
Relative Risk (Control)[d]	---	0.960	1.833
Lower Limit	---	0.376	0.822
Upper Limit	---	3.124	5.424
Weeks to First Observed Tumor	91	91	91

[a]Treated groups received doses of 1250 or 2500 ppm in feed.

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

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

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

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	0/20(0.00)	3/48(0.06)	0/47(0.00)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.046	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.261	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	91	---
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	5/20(0.25)	6/49(0.12)	7/48(0.15)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.490	0.583
Lower Limit	---	0.146	0.187
Upper Limit	---	1.842	2.109
Weeks to First Observed Tumor	86	84	81
All Sites: Hemangioma or Hemangiosarcoma[b]	0/20(0.00)	1/49(0.02)	3/48(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.023	0.261
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	85

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	1/19(0.05)	19/49(0.39)	23/48(0.48)
P Values[c]	P = 0.002	P = 0.005	P = 0.001
Relative Risk (Control)[d]	---	7.367	9.104
Lower Limit	---	1.343	1.704
Upper Limit	---	296.332	360.488
Weeks to First Observed Tumor	91	84	91

[a]Treated groups received doses of 1250 or 2500 ppm in feed.

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

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

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

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

incidence of hepatocellular carcinomas or hepatocellular adenomas. However, neither of the Fisher exact comparisons were significant. No other statistical tests for tumor incidence were significant at any site in male mice.

In female mice, the Cochran-Armitage test indicated a significant (P = 0.002) positive association between dose and the combined incidence of hepatocellular carcinomas or hepatocellular adenomas. This result was supported both by a significant (P = 0.001) positive high dose to control Fisher exact comparison and by a significant (P = 0.005) positive low dose to control comparison. No other statistical tests were significant at any site in female mice.

Based on these statistical results, the administration of 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic to female B6C3F1 mice, causing a combination of hepatocellular carcinomas or hepatocellular adenomas under the conditions of this bioassay.

V. DISCUSSION

There were no significant positive associations between the concentrations of 4,4'-methylenebis(N,N-dimethyl)benzenamine administered and mortality among rats or mice of either sex. The compound was administered for 59 weeks to rats and for 78 weeks to mice. Adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors. There was slight dose-related mean body weight depression among female rats, the mean body weight of high dose male rats was slightly less than that for controls, and the mean body weights of dosed mice were significantly lower than their controls, indicating that the concentrations of 4,4'-methylenebis(N,N-dimethyl)benzenamine administered to these animals in this bioassay may have approximated the maximum tolerated concentrations.

For both male and female rats, there was a significant positive association between the concentrations of 4,4'-methylenebis(N,N-dimethyl)benzenamine administered and the incidences of follicular-cell carcinomas of the thyroid (i.e., 1/18, 4/50, and 21/46 in the control, low dose, and high dose males, respectively; and 0/20, 3/46, and 23/45 in the control, low dose, and high dose females, respectively). The high dose to control Fisher exact comparisons were also significant for each sex. These associations were apparent even though the compound was administered to the dosed rats for only 59 weeks instead of the usual 78 weeks.

Liver neoplasms were observed among male and female mice. There were elevated incidences of hepatocellular adenomas in dosed mice when compared to controls (i.e., 2/20, 3/50, and 16/48 in control, low dose, and high dose males, respectively; and 1/19, 18/49, and 22/48 in control, low dose, and high dose females). The incidences of hepatocellular carcinomas in dosed mice did not differ greatly from those in controls (i.e., 3/20, 9/50, and 6/48 in control, low dose, and high dose males, respectively; and 0/19, 1/49, and 1/48 in control, low dose, and high dose females). Among both sexes of mice, there was a significant positive association between the concentrations of the chemical administered and the incidences of a combination of hepatocellular adenomas and hepatocellular carcinomas. For male mice, the Fisher exact comparisons were not significant; however, for females, both the high dose to control and the low dose to control comparisons were significant.

In both sexes of both species, nonneoplastic proliferative lesions of the thyroid occurred in dosed animals but not in any of the controls. This may represent a toxic lesion in both species which is associated with cancer of the thyroid in the rat but not in the mouse.

Under the conditions of this bioassay, 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic in Fischer 344 rats, inducing thyroid follicular-cell carcinomas in both males and females. Administration of the compound was carcinogenic in female B6C3F1 mice,

inducing liver neoplasms. There was no conclusive evidence that 4,4'-methylenebis(N,N-dimethyl)benzenamine was carcinogenic in male B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 4,4'-METHYLENEBIS (N,N-DIMETHYL)BENZENAMINE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL (UNTR) 11-1015	LOW DOSE 11-1013	HIGH DOSE 11-1011
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
BASAL-CELL CARCINOMA	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
SARCOMA, NOS	1 (5%)		
FIBROMA	2 (10%)	1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKEMIA,NOS	1 (5%)	1 (2%)	
*HEMATOPOIETIC SYSTEM	(20)	(50)	(50)
NEOPLASM, NOS		2 (4%)	
#SPLEEN	(18)	(48)	(47)
HEMANGIOMA			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(50)	(49)
NEOPLASTIC NODULE	1 (5%)	5 (10%)	3 (6%)

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1015	LOW DOSE 11-1013	HIGH DOSE 11-1011
HEPATOCELLULAR CARCINOMA	2 (11%)		
#STOMACH	(18)	(49)	(47)
SQUAMOUS CELL PAPILLOMA	1 (6%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(47)	(48)
CHROMOPHOBE ADENOMA	2 (11%)	12 (26%)	14 (29%)
#ADRENAL	(20)	(47)	(48)
NEOPLASM, NOS			1 (2%)
PHEOCHROMOCYTOMA	1 (5%)	3 (6%)	5 (10%)
#THYROID	(18)	(50)	(46)
NEOPLASM, NOS			1 (2%)
FOLLICULAR-CELL ADENOMA			13 (28%)
FOLLICULAR-CELL CARCINOMA	1 (6%)	4 (8%)	21 (46%)
C-CELL ADENOMA	2 (11%)		3 (7%)
C-CELL CARCINOMA		2 (4%)	1 (2%)
#THYROID FOLLICLE	(18)	(50)	(46)
NEOPLASM, NOS			1 (2%)
CYSTADENOMA, NOS		1 (2%)	
#PARATHYROID	(10)	(28)	(10)
ADENOMA, NOS		1 (4%)	
#PANCREATIC ISLETS	(16)	(48)	(43)
ISLET-CELL ADENOMA	1 (6%)	1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(20)	(50)	(50)
ADENOMA, NOS	1 (5%)	1 (2%)	
#TESTIS	(20)	(49)	(49)
INTERSTITIAL-CELL TUMOR	20 (100%)	47 (96%)	45 (92%)

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1015	LOW DOSE 11-1013	HIGH DOSE 11-1011
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(48)
ASTROCYTOMA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*TUNICA VAGINALIS	(20)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	2 (4%)
ALL OTHER SYSTEMS			
SITE UNKNOWN			
NEOPLASM, NOS			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	10	4
MORIBUND SACRIFICE	1	1	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	39	44
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 11-1015	LOW DOSE 11-1013	HIGH DOSE 11-1011
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	50	50
TOTAL PRIMARY TUMORS	38	85	114
TOTAL ANIMALS WITH BENIGN TUMORS	20	50	48
TOTAL BENIGN TUMORS	31	68	83
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	9	22
TOTAL MALIGNANT TUMORS	6	9	22
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	8	7
TOTAL UNCERTAIN TUMORS	1	8	9
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL (UNTR) 11-1016	LOW DOSE 11-1014	HIGH DOSE 11-1012
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(50)
SARCOMA, NOS			1 (2%)
FIBROMA	1 (5%)		
LEIOMYOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	3 (6%)	1 (2%)
FOLLICULAR-CELL CARCINOMA, METAS			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
LEUKEMIA,NOS	1 (5%)	2 (4%)	1 (2%)
#MESENTERIC L. NODE	(15)	(43)	(45)
SARCOMA, NOS	1 (7%)		
CIRCULATORY SYSTEM *			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(47)
NEOPLASTIC NODULE			4 (9%)

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1016	LOW DOSE 11-1014	HIGH DOSE 11-1012
LEIOMYOSARCOMA, INVASIVE		1 (2%)	
#STOMACH	(19)	(49)	(49)
LEIOMYOSARCOMA		1 (2%)	
#PEYERS PATCH	(19)	(49)	(46)
SARCOMA, NOS	1 (5%)		
#COLON	(18)	(43)	(42)
SARCOMA, NOS			1 (2%)
URINARY SYSTEM			
#URINARY BLADDER	(13)	(38)	(38)
TRANSITIONAL-CELL PAPILLOMA			1 (3%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(47)	(48)
CHROMOPHOBE ADENOMA	11 (55%)	18 (38%)	21 (44%)
#ADRENAL	(20)	(49)	(50)
CORTICAL ADENOMA		1 (2%)	
PHEOCHROMOCYTOMA			1 (2%)
#THYROID	(20)	(46)	(45)
NEOPLASM, NOS			1 (2%)
FOLLICULAR-CELL ADENOMA		1 (2%)	13 (29%)
FOLLICULAR-CELL CARCINOMA		3 (7%)	23 (51%)
C-CELL ADENOMA	2 (10%)	1 (2%)	1 (2%)
C-CELL CARCINOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOMA, NOS		1 (2%)	2 (4%)
ADENOCARCINOMA, NOS		1 (2%)	
FIBROADENOMA	1 (5%)	5 (10%)	8 (16%)
*CLITORAL GLAND	(20)	(50)	(50)
ADENOMA, NOS	1 (5%)	2 (4%)	1 (2%)
#UTERUS	(20)	(49)	(49)
ADENOMA, NOS		1 (2%)	

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1016	LOW DOSE 11-1014	HIGH DOSE 11-1012
ENDOMETRIAL STROMAL POLYP	1 (5%)	3 (6%)	3 (6%)
NERVOUS SYSTEM			
*BRAIN	(20)	(49)	(50)
ASTROCYTOMA			1 (2%)
SPECIAL SENSE ORGANS			
*EYELID	(20)	(50)	(50)
SARCOMA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(20)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	
ALL OTHER SYSTEMS			
SITE UNKNOWN			
CARCINOMA,NOS	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	8	7
MORIBUND SACRIFICE		1	6
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	41	37
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 11-1016	LOW DOSE 11-1014	HIGH DOSE 11-1012
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	17	33	44
TOTAL PRIMARY TUMORS	22	48	85
TOTAL ANIMALS WITH BENIGN TUMORS	16	29	36
TOTAL BENIGN TUMORS	18	36	52
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	11	25
TOTAL MALIGNANT TUMORS	4	11	28
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT		1	5
TOTAL UNCERTAIN TUMORS		1	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 4,4'-METHYLENEBIS (N,N-DIMETHYL)BENZENAMINE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL(UNTR) 22-2015	LOW DOSE 22-2013	HIGH DOSE 22-2011
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	6 (12%)	7 (14%)
HEMANGIOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	1 (2%)
#MESENTERIC L. NODE	(15)	(43)	(44)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(48)
HEPATOCELLULAR ADENOMA	2 (10%)	3 (6%)	16 (33%)
HEPATOCELLULAR CARCINOMA	3 (15%)	9 (18%)	6 (13%)
SARCOMA, NOS			1 (2%)
HEMANGIOMA		2 (4%)	4 (8%)
HEMANGIOSARCOMA		2 (4%)	

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

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 22-2015	LOW DOSE 22-2013	HIGH DOSE 22-2011
*PANCREAS	(20)	(49)	(48)
HEMANGIOSARCOMA		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
*THYROID	(18)	(45)	(49)
FOLLICULAR-CELL ADENOMA	1 (6%)	1 (2%)	
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)	

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

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 22-2015	LOW DOSE 22-2013	HIGH DOSE 22-2011
ADIPOSE TISSUE			
HEMANGIOMA			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@		3	
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	20	47	50
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	24	31
TOTAL PRIMARY TUMORS	7	27	37
TOTAL ANIMALS WITH BENIGN TUMORS	4	10	24
TOTAL BENIGN TUMORS	4	12	28
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	14	9
TOTAL MALIGNANT TUMORS	3	15	9
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL (UNTR) 22-2016	LOW DOSE 22-2014	HIGH DOSE 22-2012
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	2
ANIMALS NECROPSIED	20	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*LUNG	(20)	(48)	(47)
ALVEOLAR/BRONCHIOLAR ADENOMA		3 (6%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(48)
MALIGNANT LYMPHOMA, NOS	5 (25%)	3 (6%)	5 (10%)
LYMPHOCYTIC LEUKEMIA		1 (2%)	
*MEDIASTINUM	(20)	(49)	(48)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
*SPLEEN	(19)	(49)	(47)
HEMANGIOSARCOMA			1 (2%)
MALIG. LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
*LIVER	(19)	(49)	(48)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
*ILEUM	(20)	(48)	(44)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2016	LOW DOSE 22-2014	HIGH DOSE 22-2012
DIGESTIVE SYSTEM			
#LIVER	(19)	(49)	(48)
HEPATOCELLULAR ADENOMA	1 (5%)	18 (37%)	22 (46%)
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)
HEMANGIOMA			1 (2%)
HEMANGIOSARCOMA			1 (2%)
#HEPATIC CAPSULE	(19)	(49)	(48)
CORTICAL CARCINOMA, METASTATIC		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(18)	(44)	(38)
CORTICAL CARCINOMA		1 (2%)	
PHEOCHROMOCYTOMA			1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(48)
ADENOCARCINOMA, NOS		1 (2%)	
#UTERUS	(19)	(49)	(47)
LEIOMYOSARCOMA			1 (2%)
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(20)	(49)	(48)
PAPILLARY CYSTADENOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 22-2016	LOW DOSE 22-2014	HIGH DOSE 22-2012
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	2	5
MORIBUND SACRIFICE		1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	46	42
ANIMAL MISSING		1	2
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	26	30
TOTAL PRIMARY TUMORS	6	31	37
TOTAL ANIMALS WITH BENIGN TUMORS	1	21	25
TOTAL BENIGN TUMORS	1	22	25
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	9	10
TOTAL MALIGNANT TUMORS	5	9	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 4,4'-METHYLENEBIS (N,N-DIMETHYL)BENZENAMINE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL (UNTR) 11-1015	LOW DOSE 11-1013	HIGH DOSE 11-1011
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(20)	(49)	(50)
INFLAMMATION, NOS			3 (6%)
#LUNG	(20)	(49)	(50)
INFLAMMATION, NOS		3 (6%)	
PNEUMONIA, CHRONIC MURINE		1 (2%)	2 (4%)
REACTION, FOREIGN BODY			4 (8%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(48)	(47)
INFARCT, NOS	1 (6%)		
HEMATOPOIESIS		1 (2%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(19)	(50)	(50)
DEGENERATION, NOS	4 (21%)	8 (16%)	5 (10%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(18)	(47)	(49)
HYPERPLASIA, FOCAL		1 (2%)	
#LIVER	(19)	(50)	(49)
INFLAMMATION, FOCAL		1 (2%)	

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-1015	LOW DOSE 11-1013	HIGH DOSE 11-1011
NECROSIS, FOCAL		1 (2%)	2 (4%)
METAMORPHOSIS FATTY		2 (4%)	4 (8%)
BASOPHILIC CYTO CHANGE	2 (11%)	9 (18%)	11 (22%)
CLEAR-CELL CHANGE		1 (2%)	5 (10%)
#PANCREATIC ACINUS	(16)	(48)	(43)
ATROPHY, NOS	1 (6%)	2 (4%)	2 (5%)
#ILEUM	(18)	(48)	(44)
INFLAMMATION, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(19)	(49)	(49)
NEPHROPATHY	16 (84%)	45 (92%)	39 (80%)
DEGENERATION, CYSTIC		1 (2%)	
#URINARY BLADDER	(14)	(41)	(40)
INFLAMMATION, NOS			1 (3%)
ENDOCRINE SYSTEM			
#ADRENAL MEDULLA	(20)	(47)	(48)
HYPERPLASIA, NOS		2 (4%)	2 (4%)
HYPERPLASIA, FOCAL			2 (4%)
#THYROID	(18)	(50)	(46)
FOLLICULAR CYST, NOS		4 (8%)	3 (7%)
HYPERPLASIA, C-CELL		5 (10%)	1 (2%)
HYPERPLASIA, FOLLICULAR-CELL			7 (15%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
GALACTOCELE		1 (2%)	
*PREPUTIAL GLAND	(20)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
NECROSIS, NOS	1 (5%)		
#TESTIS	(20)	(49)	(49)
ATROPHY, NOS	1 (5%)	3 (6%)	2 (4%)

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 11-1015	LOW DOSE 11-1013	HIGH DOSE 11-1011
HYPERPLASIA, INTERSTITIAL CELL			2 (4%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, FAT		1	
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL (UNTR) 11-1016	LOW DOSE 11-1014	HIGH DOSE 11-1012
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
PNEUMONIA, CHRONIC MURINE			1 (2%)
REACTION, FOREIGN BODY	1 (5%)		3 (6%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(17)	(40)	(40)
MYELOFIBROSIS		1 (3%)	
#SPLEEN	(20)	(49)	(47)
HEMATOPOIESIS	2 (10%)	6 (12%)	2 (4%)
CIRCULATORY SYSTEM			
#HEART	(20)	(49)	(50)
PERIVASCULITIS	1 (5%)		
#MYOCARDIUM	(20)	(49)	(50)
DEGENERATION, NOS	1 (5%)	3 (6%)	2 (4%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(47)
INFLAMMATION, NOS	1 (5%)		

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1016	LOW DOSE 11-1014	HIGH DOSE 11-1012
CHOLANGIOFIBROSIS			1 (2%)
NECROSIS, FOCAL	1 (5%)		1 (2%)
NECROSIS, CENTRAL		1 (2%)	
METAMORPHOSIS FATTY	2 (10%)	1 (2%)	2 (4%)
BASOPHILIC CYTO CHANGE	13 (65%)	38 (78%)	23 (49%)
CLEAR-CELL CHANGE		1 (2%)	
HEMATOPOIESIS		1 (2%)	
#STOMACH	(19)	(49)	(49)
HYPERPLASIA, BASAL CELL		1 (2%)	
ACANTHOSIS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(49)
NEPHROPATHY	5 (25%)	26 (53%)	19 (39%)
ENDOCRINE SYSTEM			
#ADRENAL MEDULLA	(20)	(49)	(50)
HYPERPLASIA, FOCAL	1 (5%)		
#THYROID	(20)	(46)	(45)
FOLLICULAR CYST, NOS		3 (7%)	1 (2%)
HYPERPLASIA, C-CELL		1 (2%)	1 (2%)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	5 (11%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
GALACTOCELE	3 (15%)	5 (10%)	1 (2%)
*CLITORAL GLAND	(20)	(50)	(50)
NECROSIS, NOS		1 (2%)	
#UTERUS	(20)	(49)	(49)
HYDROMETRA		1 (2%)	
PYOMETRA		1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(49)	(49)
HYPERPLASIA, CYSTIC		1 (2%)	

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

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 11-1016	LOW DOSE 11-1014	HIGH DOSE 11-1012
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(50)
HEMORRHAGE		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, FAT		2	2
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	2
AUTO/NECROPSY/HISTO PERF			1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 4,4'-METHYLENEBIS (N,N-DIMETHYL)BENZENAMINE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH
4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL (UNTR) 22-2015	LOW DOSE 22-2013	HIGH DOSE 22-2011
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
HYPERPLASIA, NOS	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(50)
ABSCESS, NOS	1 (5%)	1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
GRANULOMA, FOREIGN BODY			1 (2%)
#LUNG/ALVEOLI	(20)	(49)	(50)
HEMORRHAGE		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(50)	(47)
ERYTHROPOIESIS		1 (2%)	
#MESENTERIC L. NODE	(15)	(43)	(44)
CONGESTION, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(48)
INFLAMMATION, ACUTE FOCAL		1 (2%)	

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2015	LOW DOSE 22-2013	HIGH DOSE 22-2011
CALCIFICATION, FOCAL			1 (2%)
CYTOPLASMIC VACUOLIZATION			1 (2%)
BASOPHILIC CYTO CHANGE		1 (2%)	1 (2%)
MEGALOCYTOSIS			1 (2%)
HYPERTROPHY, FOCAL		1 (2%)	
#LIVER/CENTRILOBULAR	(20)	(50)	(48)
METAMORPHOSIS FATTY			1 (2%)
HYPERTROPHY, NOS			1 (2%)
#LIVER/KUPFFER CELL	(20)	(50)	(48)
HYPERPLASIA, FOCAL			1 (2%)
#BILE DUCT	(20)	(50)	(48)
INFLAMMATION, CHRONIC FOCAL	1 (5%)		
#STOMACH	(20)	(50)	(48)
INFLAMMATION, ACUTE	1 (5%)	2 (4%)	
#PEYERS PATCH	(20)	(48)	(50)
HYPERPLASIA, LYMPHOID			2 (4%)
#COLON	(19)	(49)	(49)
NEMATODIASIS		1 (2%)	
URINARY SYSTEM			
#KIDNEY/GLOMERULUS	(20)	(50)	(48)
SCLEROSIS	1 (5%)		
#URINARY BLADDER	(17)	(47)	(48)
INFLAMMATION, ACUTE FOCAL			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
HYPERPLASIA, EPITHELIAL			2 (4%)
ENDOCRINE SYSTEM			
#THYROID	(18)	(45)	(49)
GOITER COLLOID			2 (4%)
GOITER ADENOMATOUS		4 (9%)	22 (45%)
HYPERPLASIA, FOCAL		1 (2%)	1 (2%)
HYPERPLASIA, PAPILLARY		9 (20%)	12 (24%)

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

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 22-2015	LOW DOSE 22-2013	HIGH DOSE 22-2011
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	2 (4%)
#THYROID FOLLICLE	(18)	(45)	(49)
DISTENTION			1 (2%)
HYPERTROPHY, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(20)	(50)	(50)
CYST, NOS		2 (4%)	
#TESTIS/TUBULE	(19)	(50)	(49)
CYST, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
63 MSAC 100 222012			CEREBRAL CORTEX THYROID UTERUS/ENDOMETRIUI

* ORGAN WITH MULTIPLE TUMORS OR MULTIPLE ORGANS WITH IDENTICAL TUMORS

DISPOSITION CODES:

ACCK: ACCIDENTALLY KILLED	NATD: NATURAL DEATH
AUTO: AUTOLYSIS	SSAC: SCHEDULED SACRIFICE
MISS: MISSING	TSAC: TERMINAL SACRIFICE
MSAC: MORIBUND SACRIFICE	OTHR: OTHER

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH 4,4'-METHYLENEBIS(N,N-DIMETHYL)BENZENAMINE

	CONTROL(UNTR) 22-2016	LOW DOSE 22-2014	HIGH DOSE 22-2012
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	2
ANIMALS NECROPSIED	20	49	48
ANIMALS EXAMINED HISTOPATHCLOGICALLY**	20	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(20)	(48)	(47)
INFLAMMATION, CHRONIC	1 (5%)		
#LUNG/BRONCHIOLE	(20)	(48)	(47)
BRONCHIOLECTASIS	1 (5%)		
#LUNG	(20)	(48)	(47)
ATELECTASIS	1 (5%)		
CONGESTION, ACUTE PASSIVE		1 (2%)	
PERIVASCULITIS	1 (5%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(49)	(47)
HYPERPLASIA, LYMPHOID		1 (2%)	2 (4%)
HEMATOPOIESIS			1 (2%)
#MEDIASTINAL L.NODE	(17)	(43)	(36)
HYPERPLASIA, NOS		1 (2%)	
#PANCREATIC L.NODE	(17)	(43)	(36)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
#MESENTERIC L. NODE	(17)	(43)	(36)
INFLAMMATION, ACUTE/CHRONIC			1 (3%)
INFLAMMATION, GRANULOMATOUS		1 (2%)	

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2016	LOW DOSE 22-2014	HIGH DOSE 22-2012
HYPERPLASIA, NOS			1 (3%)
HYPERPLASIA, LYMPHOID		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(18)	(46)	(37)
INFLAMMATION, CHRONIC			1 (3%)
#LIVER	(19)	(49)	(48)
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY	1 (5%)		
BASOPHILIC CYTO CHANGE		4 (8%)	
EOSINOPHILIC CYTO CHANGE		2 (4%)	7 (15%)
#BILE DUCT	(19)	(49)	(48)
INFLAMMATION, CHRONIC	1 (5%)		
HYPERPLASIA, FOCAL			1 (2%)
#PANCREAS	(17)	(49)	(48)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
#STOMACH	(20)	(49)	(47)
INFLAMMATION, CHRONIC			1 (2%)
#GASTRIC MUCOSA	(20)	(49)	(47)
NECROSIS, FOCAL	1 (5%)		
#PEYERS PATCH	(20)	(48)	(44)
HYPERPLASIA, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(19)	(49)	(48)
HYDRONEPHROSIS			3 (6%)
INFLAMMATION, INTERSTITIAL		1 (2%)	
PERIVASCULITIS			1 (2%)
#URINARY BLADDER	(16)	(41)	(41)
INFLAMMATION, CHRONIC		1 (2%)	7 (17%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2016	LOW DOSE 22-2014	HIGH DOSE 22-2012
INFLAMMATION, CHRONIC FOCAL	1 (6%)	5 (12%)	2 (5%)
#U.BLADDER/MUSCULARIS	(16)	(41)	(41)
INFLAMMATION, CHRONIC FOCAL	1 (6%)		
ENDOCRINE SYSTEM			
#THYROID	(16)	(43)	(43)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
GOITER ADENOMATOUS		25 (58%)	35 (81%)
HYPERPLASIA, PAPILLARY		7 (16%)	6 (14%)
#THYROID FOLLICLE	(16)	(43)	(43)
DISTENTION		2 (5%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(19)	(49)	(47)
HYDROMETRA	1 (5%)	3 (6%)	
ANGIECTASIS		1 (2%)	
#UTERUS/ENDOMETRIUM	(19)	(49)	(47)
INFLAMMATION, SUPPURATIVE	2 (11%)		7 (15%)
HYPERPLASIA, CYSTIC	5 (26%)	28 (57%)	18 (38%)
#OVARY	(17)	(37)	(34)
CYST, NOS	6 (35%)	7 (19%)	2 (6%)
PAROVARIAN CYST		1 (3%)	
HEMORRHAGIC CYST			1 (3%)
ANGIECTASIS			1 (3%)
NERVOUS SYSTEM			
#CEREBRAL CORTEX	(20)	(48)	(41)
PERIVASCULAR CUFFING			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 22-2016	LOW DOSE 22-2014	HIGH DOSE 22-2012
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	1	1
ANIMAL MISSING/NO NECROPSY		1	2
AUTO/NECROPSY/HISTO PERF			1

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

Review of the Bioassay of 4,4'-Methylenebis(N,N-dimethyl)Benzenamine*
for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

October 25, 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, and State health officials. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 4,4'-Methylenebis(N,N-dimethyl) Benzenamine for carcinogenicity.

The primary reviewer said that 4,4'-Methylenebis(N,N-dimethyl)benzenamine caused a dose-related incidence of follicular-cell carcinomas of the thyroid in treated rats and a significant number of hepatocellular tumors in treated mice. After briefly describing the experimental design, he said that the study was well-conducted and supported the conclusion given in the report. The primary reviewer said that 4,4'-Methylenebis(N,N-dimethyl)benzenamine should be considered as a potential carcinogen for humans.

The secondary reviewer questioned if the incidence of hepatocellular adenomas in the high dose treated male mice was statistically significant. A Program staff member noted that the response was only marginal when compared with historical control animals. The secondary reviewer suggested that it would be worth pointing out in the report summary the thyroid follicular hyperplasia observed in treated mice.

There was no objection to a recommendation that the report on the bioassay of 4,4'-Methylenebis(N,N-dimethyl)benzenamine be accepted as written.

Clearinghouse Members Present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center
Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Kenneth Wilcox, Michigan State Health Department

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

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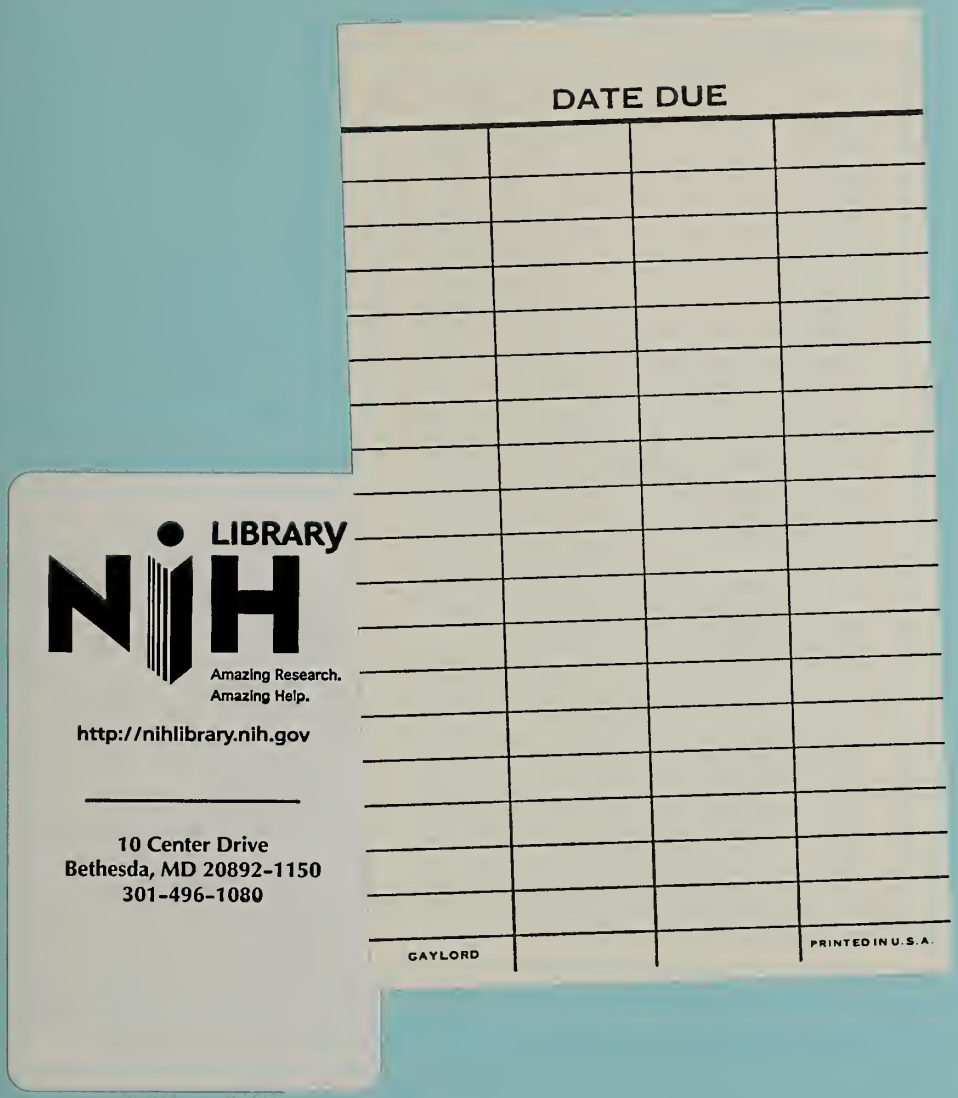

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