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No. 219

CARCINOGENESIS BIOASSAY OF 2,6-DICHLORO-p-PHENYLENEDIAMINE

(CAS NO. 609-20-1)

IN F344 RATS AND B6C3F$_1$ MICE

(FEED STUDY)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

NTP Technical Report

on the

CARCINOGENESIS BIOASSAY

of

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NATIONAL TOXICOLOGY PROGRAM
Research Triangle Park
Box 12233
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NOTE TO THE READER

This is one in a series of experiments designed to determine whether selected chemicals produce cancer in animals. Chemicals selected for testing in the NTP carcinogenesis bioassay program are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential hazard to humans. The determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the purview of this study.

This study was initiated by the National Cancer Institute's Carcinogenesis Testing Program, now part of the National Institute of Environmental Health Sciences, National Toxicology Program.

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Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to communicate any mistakes to the Deputy Director, NTP (P.O. Box 12233, Research Triangle Park, NC 27709), so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP.

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ABSTRACT

A carcinogenesis bioassay of 2,6-dichloro-p-phenylenediamine, a chemical intermediate, was conducted in groups of 50 F344 rats and B6C3F1 mice of either sex. Male rats were fed diets containing 1,000 or 2,000 ppm 2,6-dichloro-p-phenylenediamine and female rats were fed 2,000 or 6,000 ppm for 103 weeks. Mice were fed 1,000 or 3,000 ppm of the test chemical for 103 weeks and observed for an additional 8 weeks. Controls consisted of 50 untreated rats and 50 untreated mice of each sex.

Throughout the study, mean body weights of dosed rats and mice of either sex were lower than those of the corresponding controls. A dose-related weight gain depression was particularly pronounced for rats.

Ectopic hepatocytes were observed at an increased incidence in the pancreas and nephrosis was observed in increased severity in dosed rats of either sex when compared with the corresponding controls. No increase in any tumor type was observed in treated male or female rats when compared to controls.

Increased incidences of liver tumors were observed in mice of both sexes. In male mice, the incidence of hepatocellular adenomas exhibited a significant positive dose-related trend (P=0.002), and the increased incidence of hepatocellular adenomas was statistically significant in the high-dose group (4/50, 7/50, 15/50: P=0.005). The combined incidence of hepatocellular adenomas and carcinomas showed a significant positive dose-related trend (P=0.004) and was statistically significant in the high-dose group (16/50, 19/50, 29/50: P=0.008).

In female mice, hepatocellular carcinomas exhibited a significant positive dose-related trend (P=0.025), but no single dose group had a statistically significant increased incidence of either adenomas (4/50, 4/50, 9/50; high-dose effect: P=0.12) or carcinomas (2/50, 2/50, 7/50; high-dose effect: P=0.08) alone. When the incidences of hepatocellular adenomas and carcinomas were combined (6/50, 6/50, 16/50), these data gave a positive dose-related trend (P=0.004) and were statistically significant in the high-dose group (P=0.014).

Under the conditions of this bioassay, 2,6-dichloro-p-phenylenediamine was carcinogenic for male and female B6C3F1 mice, causing increased incidences of combined hepatocellular adenomas and carcinomas, and for male B6C3F1 mice, causing an increased incidence of hepatocellular adenomas alone. 2,6-Dichloro-p-phenylenediamine was not carcinogenic for male or female F344 rats.

CONTRIBUTORS

The bioassay of 2,6-dichloro-p-phenylenediamine was conducted by Litton Bionetics, Inc., Kensington, Maryland, under a subcontract to Tracor Jitco, Inc., the prime contractor for the NCI Carcinogenesis Testing Program. The chronic study was begun in February 1977 and completed in March 1979.

The bioassay was conducted under the supervision of Dr. E. Gordon (1,2), principal investigator. Doses of the test chemical were selected by Drs. W. MacDonald (3), J. Robens (3,4), C. Cueto (5), R. Schueler (3), and E. Gordon (1,2). Mr. D. Kinsel (1) and Ms. J. Sheldon (1) were in charge of animal care, and Mr. G. North (1) supervised the preparation of the feed mixtures and collected samples of the diets for analysis. Drs. G. Parker, R. Cardy, and A. DePaoli (1), pathologists, directed the necropsies and performed the histopathologic examinations. The pathology report and selected slides were evaluated by the NCI Pathology Working Group as described in Ward et al. (1978). The diagnoses represent a consensus of contracting pathologists and the NCI Pathology Working Group with final approval by the NCI Pathology Working Group.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute, Rockville, Maryland (6). The statistical analyses were performed by Dr. J. R. Joiner (3) using methods selected for the bioassay program by Dr. J. J. Gart (7).

Chemicals used in this bioassay were analyzed at Midwest Research Institute (8), and dosed feed mixtures were analyzed by Mr. H. Paulin (1) at Litton Bionetics, Inc.

This report was prepared at Tracor Jitco (3) and reviewed by NCI. Those responsible for the report at Tracor Jitco were Dr. C. Cueto (5), Director of the Bioassay Program; Dr. S. S. Olin, Associate Director; Dr. M. A. Stedham, pathologist; Dr. A. C. Jacobs, bioscience writer; and Dr. W. D. Theriault and Ms. M. W. Glasser, technical editors.

The following scientists at NCI/NTP (7) were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. J. Fielding Douglas, Dr. Charles K. Grieshaber, Dr. Larry Hart, Dr. Joseph Haseman, Dr. James Huff, Dr. C. W. Jameson, Dr. Eugene E. McConnell, Dr. John A. Moore, Dr. Gerd Reznik, Dr. Sherman F. Stinson, Dr. R. Tennant, and Dr. Jerrold M. Ward.

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SUMMARY OF PEER REVIEW COMMENTS

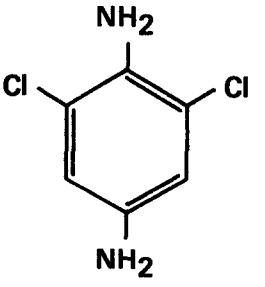
On October 15, 1980 this carcinogenesis bioassay report on 2-6-dichloro-p-phenylenediamine was peer reviewed by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts at an open meeting held in Conference Room 6, Building 31C, National Institutes of Health, Bethesda, Maryland.

Dr. Murphy, a principal reviewer for the report on the carcinogenesis bioassay of 2,6-dichloro-p-phenylenediamine, agreed with the conclusion that, under the conditions of this bioassay, this chemical was not carcinogenic for F344 rats of either sex, and caused an increased incidence of hepatocellular adenomas and an increased incidence of combined hepatocellular adenomas/carcinomas in B6C3F1 mice of either sex. In neither male nor female mice was there a significant increase in hepatocellular carcinomas alone. For males, the incidence of hepatocellular adenomas was statistically significant. Dr. Murphy noted that male rats had liver angiectasis at 28% and 24% in low- and high-dose groups compared with 6% in controls, and the unusual lesion of ectopic hepatocytes was found in male and female rats.

As a second principal reviewer, Dr. Schwetz agreed with the conclusions and with the lack of significance in mice for hepatocellular carcinomas alone; yet there was a significant trend for hepatocellular carcinomas in female mice.

Dr. Murphy moved that the report on the bioassay of 2,6-dichloro-p-phenylenediamine be accepted with additions to the conclusion and abstract indicating that the increased incidence of liver tumors in mice was based on the sum of adenomas and carcinomas, and that, taken alone, hepatocellular carcinomas were not significantly increased. Also, a note should be made in the summary that there was a reduction in weight gain to indicate the bioassay was a valid test. Dr. Schwetz seconded the motion and the report was approved unanimously by the Peer Review Panel.

I. INTRODUCTION



2,6-DICHLORO-p-PHENYLENEDIAMINE

Chemical Formula: $C_6H_6Cl_2N_2$
Molecular Weight: 177.04

2,6-Dichloro-p-phenylenediamine (CAS No. 609-20-1; C.I. 37020), a gray microcrystalline powder, is a chemical intermediate that has been considered for use as a polyurethane curative (Ong and Saxon, 1976) and as a monomer in the manufacture of polyamide fiber (Kwolek, 1970). The sole manufacturer in the United States ceased production in 1978 (Saxon, 1978).

The oral LD_{50} in male Harlan-Wistar albino rats was reported as 0.7 g/kg body weight (American Cyanamid, 1971). Dose-related depressions in weight gain and increases in liver weights were observed in 6-week old Harlan-Wistar albino rats of each sex fed diets containing 500, 2,000, or 8,000 ppm 2,6-dichloro-p-phenylenediamine for 7 days, and increased spleen weights were seen in males and females receiving the 8,000-ppm dose (American Cyanamid, 1971).

2,6-Dichloro-p-phenylenediamine is a metabolite of the herbicide-fungicide, 2,6-dichloro-4-nitroaniline, in man, rhesus monkeys, dogs, rats, and mice (Gallo et al., 1976).

At the time this bioassay was initiated, 2,6-dichloro-p-phenylenediamine was being considered as a substitute for 4,4'-methylenebis(2-chloroaniline) in polymer synthesis and increased usage of the former compound was anticipated. Apparently, 2,6-dichloro-p-phenylenediamine has not been used or further considered for this purpose.

II. MATERIALS AND METHODS

A. Chemical

The 2,6-dichloro-p-phenylenediamine (CAS No. 609-20-1) used in this study was obtained in two batches from American Cyanamid (Bound Brook, NJ). Lot No. 0005 was used for the subchronic study and the first 52 weeks of the chronic studies and Lot No. R9231-127 was used for the final 51 weeks of the chronic studies.

The results of purity and identity analyses performed at Midwest Research Institute were consistent with those expected from the structure and with literature values (Appendixes E and F). Results from thin-layer and vapor-phase chromatography indicated three unidentified minor impurities totaling less than 0.25% of the major peak for Lot No. 0005 and a single small impurity comprising 0.02% to 0.03% of the major peak for Lot No. R9231-127. 2,6-Dichloro-p-phenylenediamine was stored at 4°C.

B. Dietary Preparation

Test diets were formulated by mixing a small amount of Purina® Lab Chow (Table 1) and the required amount of 2,6-dichloro-p-phenylenediamine with a mortar and pestle and then adding this premix to the required amount of animal meal and mixing 20 minutes in a Patterson-Kelly® twin-shell blender equipped with an intensified bar. The mixture was stored in the dark at 4°C for no longer than 2 weeks. Control diets consisted of Purina® Laboratory Chow.

Results of vapor-phase chromatography performed at Midwest Research Institute indicated that 2,6-dichloro-p-phenylenediamine at 100,000 ppm in feed was stable for 2 weeks at temperatures up to 45°C (Appendix G). The mean concentrations of test chemical in selected batches of formulated diets were 1,727.6±209.7 and 6,062±218.1 ppm for samples having target concentrations of 2,000 and 6,000 ppm, respectively (Appendix H).

C. Animals

Three-week old male and female F344 rats and B6C3F1 mice were obtained from the NCI Frederick Cancer Research Center, Frederick, Maryland, observed for 2 weeks, and then assigned to test groups according to a table of random numbers.

D. Animal Maintenance

Rats and mice were housed five per cage in polycarbonate cages covered with non-woven polyester filter sheets (Table 1). Racks and filters were changed once every 2 weeks and cages, bedding, and glass water bottles equipped with stainless steel sipper tubes were replaced twice a week. Tap water acidified with hydrochloric acid to pH 2.5, and feed were available ad libitum. The stainless steel feed hoppers that contained the diets were changed once per week. The animal rooms were maintained at 22^{o}-26^{o}C and humidity was 30%-70%.

Air was filtered through AG-55 Ameriglass Roughing filters and then through HEPA-100 filters. Room air was changed 10 times per hour and fluorescent lighting provided illumination 12 hours per day.

Rats fed 2,6-dichloro-p-phenylenediamine were housed in a room in which feeding studies on 11-aminoundecanoic acid (CAS 2432-99-7) were being carried out; mice fed 2,6-dichloro-p-phenylenediamine were housed in a room in which feeding studies were being conducted on bisphenol A (CAS 80-05-7), 11-aminoundecanoic acid (CAS 2432-99-7), and caprolactam (CAS 105-60-2).

E. Repeated Dose Studies

Single-dose acute studies for 2,6-dichloro-p-phenylenediamine were not done. Repeated dose studies were conducted using groups of five F344 rats and B6C3F1 mice of each sex to determine the concentrations of 2,6-dichloro-p-phenylenediamine to be used in the subchronic studies. In the first repeated dose study, rats were fed diets containing 250-4,000 ppm

Table 1. Descriptions and Sources of Materials Used for Animal Maintenance

Item	Description	Manufacturer or Supplier
Bedding	Absorb-dri® hardwood chips	Lab Products, Inc. Garfield, NJ
Cages	Polycarbonate	Lab Products, Inc. Garfield, NJ
Feed	Ralston Purina® Laboratory Chow	Ralston Purina Richmond, IN
Filters	AG-55 Ameriglass Roughing Filter	American Air Filter Louisville, KY
Filters	HEPA-100	American Air Filter Louisville, KY
Filter Sheets	Non-woven Polyester	Snow Filtration Cincinnati, Ohio

and mice 250-2,000 ppm of the test chemical for 14 days and then killed and necropsied. Because no compound-related effects on survival or weight gain were observed for female rats or for mice of either sex (Tables 2 and 3), a second repeated dose study was undertaken using 8,000 ppm for rats and 4,000 ppm for mice.

No rats died in either repeated dose study, but mean body weight gain was depressed 75% in males fed 4,000 ppm, 93% in males fed 8,000 ppm, and 67% in females fed 8,000 ppm. Mottled lungs were observed in 3/5 female rats fed 500 ppm, 2/5 males and 3/5 females fed 4,000 ppm, and 2/5 males fed 8,000 ppm.

No mice died, and mean body weight gains were comparable among all dosed groups. Bright red lungs were observed at necropsy in 3/5 female mice fed the highest dose (4,000 ppm).

F. Subchronic Studies

Subchronic studies were conducted to determine the two concentrations to be used in the chronic studies. Groups of 12 male and 12 female rats were fed diets containing 1,000-8,000 ppm 2,6-dichloro-p-phenylenediamine and groups of 10 male and 10 female mice received 625-7,500 ppm in diets for 13 weeks (Tables 4 and 5). Animals were observed twice daily and weighed weekly. At the end of 91 days, survivors were killed, necropsies were performed on all animals, and selected tissues were taken for histopathologic analyses.

Rats: No deaths occurred during this trial. Weight gain depression was dose-related among males and only slightly decreased among females. Papillary necrosis of the kidney was found in 3/10 males, pyelonephritis in 4/10 males, and transitional cell hyperplasia in 3/10 males fed diets containing 8,000 ppm. None of these effects were observed in controls, and no compound-associated pathologic lesions were found in the female rats.

Because of papillary necrosis in the kidney and depression in weight gain, doses selected for males in the chronic study were 1,000 and 2,000 ppm 2,6-dichloro-p-phenylenediamine in feed. Doses selected for the female rats were 2,000 and 6,000 ppm.

Table 2. Doses, Survival, and Mean Body Weights of Rats Fed Diets Containing 2,6-Dichloro-p-phenylenediamine for 14 Days

Dose (ppm)	Survival (a)	Mean Body Weights (grams)			Weight Change Relative to Controls (b) (Percent)
		Initial	Final	Gain	
MALE					
0	5/5	211	251	40	
250	5/5	211	244	33	-17
500	5/5	218	250	32	-20
1,000	5/5	207	243	36	-10
2,000	5/5	217	249	32	-20
4,000	5/5	227	237	10	-75
0 (c)	5/5	236	263	27	
8,000 (c)	5/5	254	256	2	-93
FEMALE					
0	5/5	146	159	13	
250	5/5	144	159	15	+15
500	5/5	140	159	19	+46
1,000	5/5	143	156	13	0
2,000	5/5	138	152	14	+8
4,000	5/5	147	164	17	+31
0 (c)	5/5	153	165	12	
8,000 (c)	5/5	146	150	4	-67

(a) Number surviving/number per group.

(b) Weight Change Relative to Controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

(c) A second repeated-dose study conducted at two doses (0 and 8,000 ppm)

Table 3. Doses, Survival, and Mean Body Weights of Mice Fed Diets Containing 2,6-Dichloro-p-phenylenediamine for 14 Days

Dose (ppm)	Survival (a)	Mean Body Weights (grams)		
		Initial	Final	Gain
MALE				
0	5/5	23	24	+1
250	5/5	24	23	-1
500	5/5	27	24	-3
1,000	5/5	26	25	-1
1,500	5/5	25	24	-1
2,000	5/5	25	24	-1
0 (b)	5/5	25	27	+2
4000 (b)	5/5	27	28	+1
FEMALE				
0	5/5	17	18	+1
250	5/5	20	19	-1
500	5/5	19	18	-1
1,000	5/5	20	20	0
1,500	5/5	21	20	-1
2,000	5/5	19	19	0
0 (b)	5/5	19	20	+1
4,000 (b)	5/5	19	20	+1

(a) Number surviving/number per group.
(b) A second repeated dose study conducted at two doses (0 and 4,000 ppm).

Table 4. Doses, Survival, and Mean Body Weights of Rats Fed Diets Containing 2,6-Dichloro-p-phenylenediamine for 13 Weeks

Dose (ppm)	Survival (a)	Mean Body Weights (grams)			Weight Change Relative to Controls (b) (Percent)
		Initial	Final	Gain	
MALE					
0	10/10	135	307	172	
1,000	12/12	135	296	161	-6
2,000	12/12	135	268	133	-23
4,000	12/12	136	267	131	-24
6,000	12/12	136	265	129	-25
8,000	12/12	136	243	107	-38
FEMALE					
0	14/14	104	182	78	
1,000	12/12	104	181	77	-1
2,000	12/12	104	182	78	0
4,000	12/12	105	169	64	-18
6,000	12/12	104	176	72	-8
8,000	12/12	104	173	69	-12

(a) Number surviving/number per group.

(b) Weight Change Relative to Controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

Table 5. Doses, Survival, and Mean Body Weights of Mice Fed Diets Containing 2,6-Dichloro-p-phenylenediamine for 13 Weeks

Dose (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Gain	Weight Change Relative to Controls (b) (Percent)
MALE					
0	10/10	20	30	10	
625	10/10	20	28	8	-20
1,250	10/10	20	29	9	-10
2,500	10/10	20	28	8	-20
5,000	10/10	20	27	7	-30
7,500	10/10	20	27	7	-30
FEMALE					
0	10/10	17	26	9	
625	10/10	17	26	9	0
1,250	10/10	17	24	7	-22
2,500	10/10	17	23	6	-33
5,000	10/10	17	22	5	-44
7,500	10/10	17	22	5	-44

(a) Number surviving/number per group.

(b) Weight Change Relative to Controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

Mice: No deaths occurred and no compound-associated histopathologic effects were observed. Mean body weight gain depression greater than 10% occurred in all dosed mice except the females fed 625 ppm in the diet.

Because of weight gain depression in the subchronic study and concern about possible latent effects, doses selected for mice in the chronic study were 1,000 and 3,000 ppm 2,6-dichloro-p-phenylenediamine in feed.

G. Design of Chronic Studies

The test groups, doses administered, and durations of the chronic studies are shown in Table 6.

H. Clinical Examinations and Pathology

Animals were inspected twice daily. Body weights were recorded every 4 weeks. Animals that were moribund and those that survived to the end of the study were killed with CO_2 and necropsied.

Gross and microscopic examinations were performed on major tissues, major organs, and all gross lesions from killed animals and from animals found dead. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, pancreas, stomach, small intestine, large intestine, kidneys, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate and seminal vesicle or uterus, testis or ovary, brain, thymus, larynx, and esophagus.

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

Table 6. Experimental Design of Chronic Feeding Studies with 2,6-Dichloro-p-phenylendiamine in Rats and Mice

Test Group	Initial No. of Animals	2,6-Dichloro-p-phenylenediamine (ppm)	Time on Study	
			Dosed (weeks)	Observed (weeks)
MALE RATS				
Control	50	0	0	111
Low Dose	50	1,000	103	8
High Dose	50	2,000	103	8
FEMALE RATS				
Control	50	0	0	111
Low Dose	50	2,000	103	8
High Dose	50	6,000	103	8
MALE MICE				
Control	50	0	0	111
Low Dose	50	1,000	103	8
High Dose	50	3,000	103	8
FEMALE MICE				
Control	50	0	0	111
Low Dose	50	1,000	103	8
High Dose	50	3,000	103	8

I. Data Recording and Statistical Analyses

Data on this experiment were recorded in 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).

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

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

The statistical analyses of tumor incidence are intended to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for two dosed groups 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 test for inequality (Miller, 1966) requires that the P value for any comparison be less than or

equal to 0.025. When 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.

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.

Life table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was killed was entered as the time point of tumor observation. The methods of Cox and of Tarone were used for the statistical tests of the groups. The statistical tests were one-tailed.

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

The approximate 95% confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971). The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that, in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence

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

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of dosed rats of either sex were lower than those of the corresponding controls (Figure 1). By the end of the two-year study, the weight gain of high-dose male rats was 19% less than that of controls and the weight gain of high-dose female rats was 47% less than that of controls. Weight gain of low-dose rats was between that of high-dose and control animals throughout the study. No other compound-related clinical signs were observed.

B. Survival (Rats)

Estimates of the probabilities of survival for male and female rats administered 2,6-dichloro-p-phenylenediamine in feed at the concentrations of this bioassay, together with those of the control group, are shown by the Kaplan and Meier curves in Figure 2. There were no significant differences between the survival of the groups of male rats or the groups of female rats.

In male rats, 30/50 (60%) of the control group, 30/50 (60%) of the low-dose group, and 21/50 (42%) of the high-dose group lived to the end of the study at 111 weeks. In female rats, 36/50 (72%) of the control group, 32/50 (64%) of the low-dose group, and 38/50 (76%) of the high-dose group lived to the end of the study at 111 weeks.

C. Pathology (Rats)

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

A variety of neoplasms were found in both the control and compound-treated animals. There was no increased incidence of any particular type of neoplasm, or of neoplasms in general, in the dosed versus the control

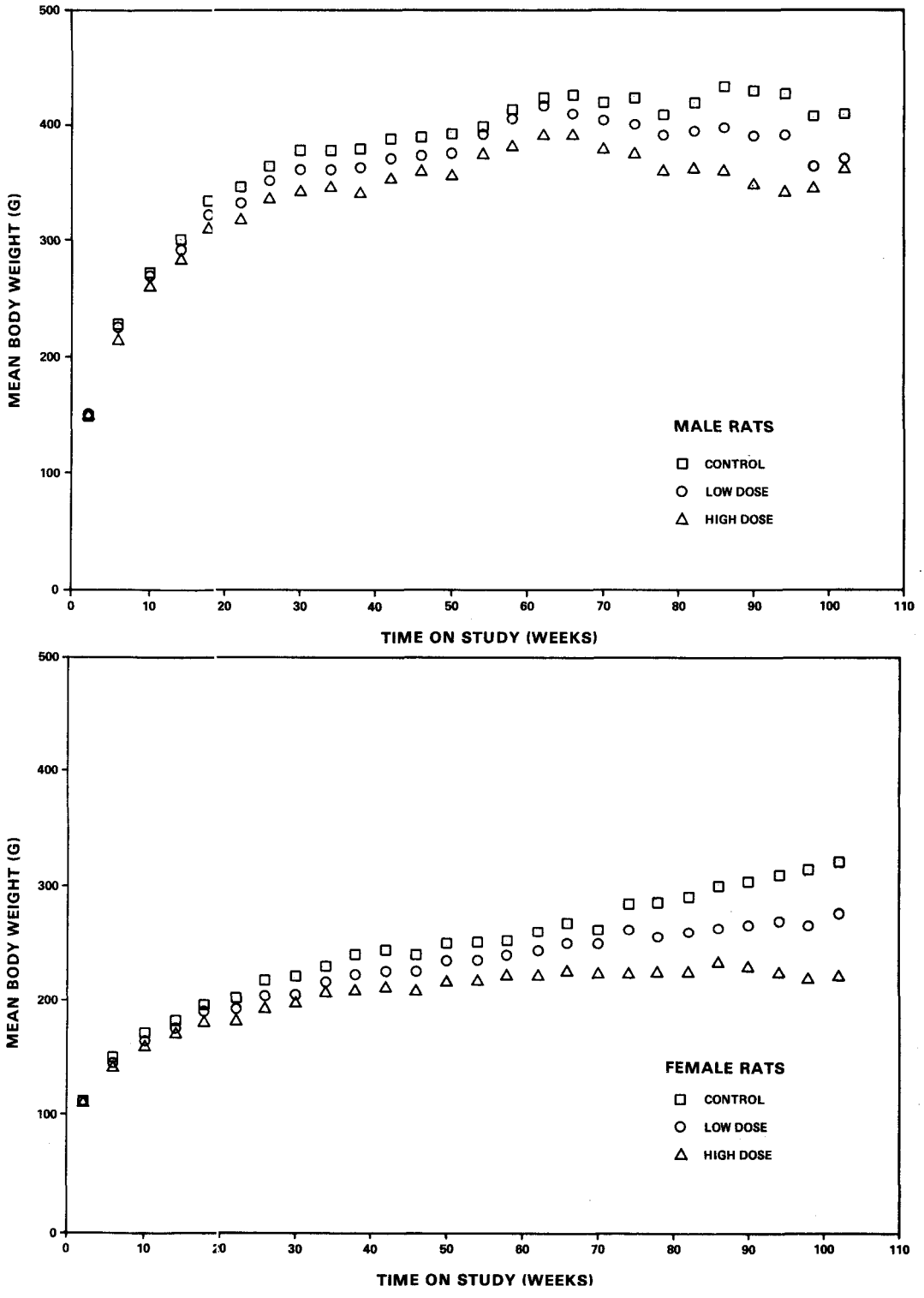


Figure 1. Growth Curves for Rats Fed Diets Containing 2,6-Dichloro-p-phenylenediamine

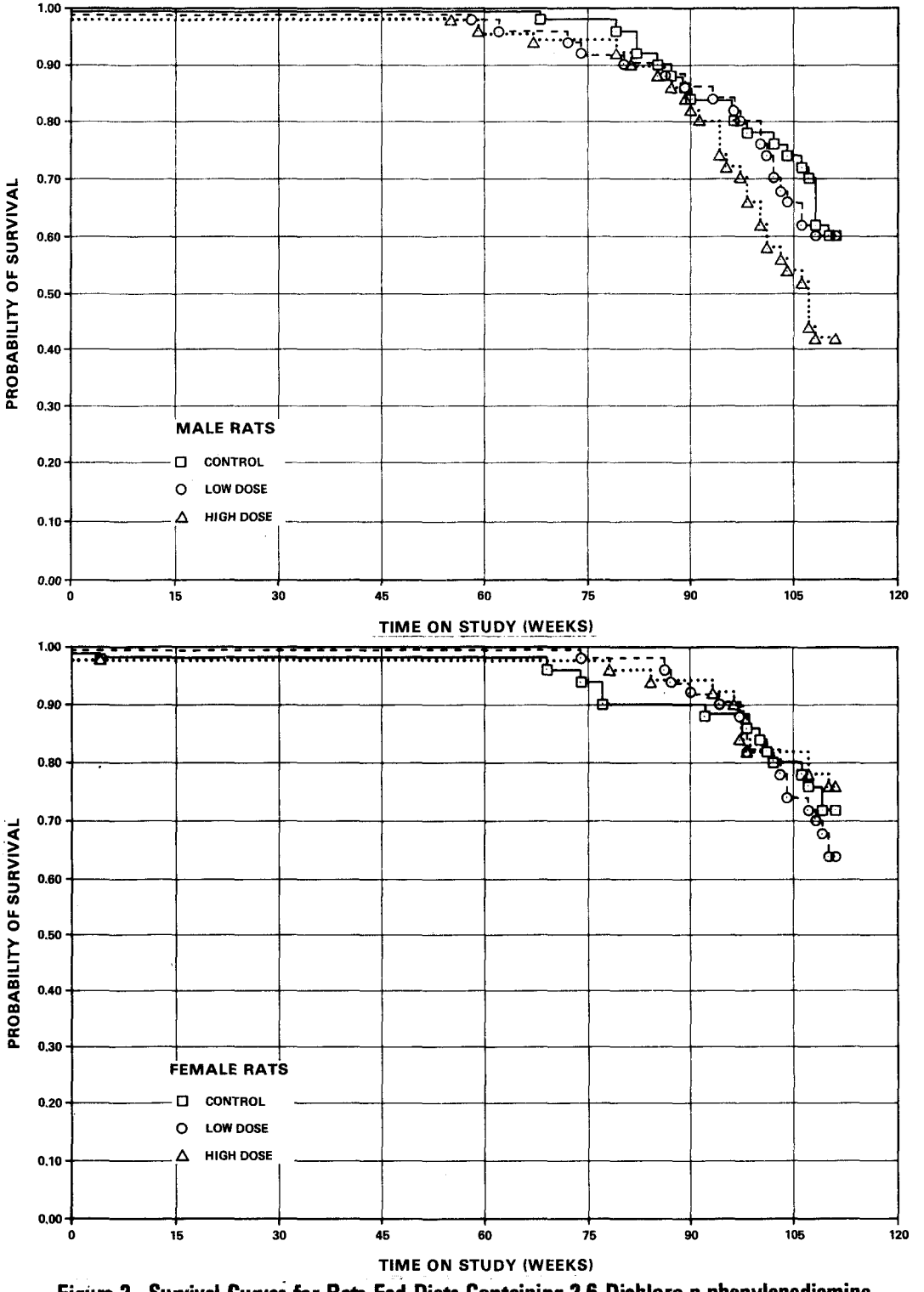


Figure 2. Survival Curves for Rats Fed Diets Containing 2,6-Dichloro-p-phenylenediamine

animals. The observed neoplasms were typical of those seen in this strain of rat.

Nonneoplastic nephropathy occurred at an increased incidence in high-dose females (control, 38/50; low dose, 35/50; high dose, 49/49) and with increased severity in dosed males and dosed females. This lesion was not apparent during macroscopic examination, although a few of the most severely affected kidneys were pitted. Microscopic examination revealed effects ranging from an accumulation of homogeneous eosinophilic material in a few cortical tubules (particularly in those near the cortico-medullary junction) to very extensive accumulations of intratubular protein, subchronic to chronic interstitial inflammation, and glomerulosclerosis of variable extent and severity.

An unusual lesion recorded as ectopic pancreas was found only in dosed rats and was in fact ectopic hepatocytes associated with the islets in the pancreas. All other nonneoplastic lesions were considered to be degenerative changes, incidental findings, or part of spontaneous disease complexes of rats. There was no discernible alteration in incidence or severity of these lesions.

Histopathologic examination indicated that there was no evidence for the carcinogenicity of 2,6-dichloro-p-phenylenediamine in F344 rats under the conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

Tables 7 and 8 contain the statistical analyses of those primary tumors that occurred in at least two animals of one group and with an incidence of at least 5% in one or more groups.

Pheochromocytomas of the adrenal in male rats were observed in decreased incidence in the low-dose group compared with the other two groups. The Fisher exact test between the low-dose group and the control group was significant ($P=0.045$), but this value of $P=0.045$ is above the value of $P=0.025$ required by the Bonferroni inequality criterion for an overall significance of $P=0.05$ when two dosed groups are compared with a common control group. Pheochromocytomas of the adrenal in female rats were not observed in significant incidence.

Islet-cell adenomas of the pancreatic islets in male rats were observed in decreased incidences in the dosed group compared with the control group. The Cochran-Armitage test for linear trend was statistically significant in the negative direction (P=0.017). The Fisher exact test between the low-dose group and the control group was significant (P=0.013). In female rats, this tumor was not observed in statistically significant proportions.

Leukemias of the hematopoietic system in female rats were observed in decreased incidence in the high-dose group. The Cochran-Armitage test for linear trend was statistically significant in the negative direction (P=0.044; control, 19/50; low dose, 25/50; high dose, 12/49). The Fisher exact tests were not significant. The incidences of this tumor were not statistically significant in male rats.

C-cell adenomas or carcinomas of the thyroid in female rats were observed in decreased incidence in the low-dose group compared with the other two groups. The Cochran-Armitage test for linear trend was not significant, but there was a departure from linear trend due to decreased incidence in the low-dose group compared with the other two dosed groups. The Fisher exact test between the low-dose group and the control group was significant (P=0.046), but this value of P=0.046 is above the value of P=0.025 required by the Bonferroni inequality criterion for an overall significance of P=0.05 when two dosed groups are compared with a common control group. In male rats, this tumor was not observed in statistically significant proportions.

Time adjusted analysis, eliminating those animals that died before 52 weeks on study, did not materially change the statistical results. Analysis by life table methods did not discern an overall trend to shorter times to observation of tumors.

The statistical conclusion was that at no site could an increase in tumor incidence be associated with the administration of the chemical. Islet-cell adenomas in male rats and C-cell tumors in female rats were observed in smaller incidence in the low-dose group than in the controls, to the extent that the upper limit of the relative risk is less than one. With these exceptions, in each of the 95% confidence intervals for relative risk the value of one is included: this indicates the absence of significant positive results. It should also be noted that each of the intervals has an upper limit greater than one indicating the theoretical possibility of tumor induction by 2,6-dichloro-p-phenylenediamine, which could not be detected under the conditions of this test.

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing 2,6-Dichloro-p-phenylenediamine (a)

Topography: Morphology	Control	Low Dose	High Dose
Subcutaneous Tissue:			
Fibroma (b)	3/50(6)	5/50(10)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.667	0.333
Lower Limit		0.344	0.006
Upper Limit		10.225	3.983
Weeks to First Observed Tumor	108	104	111
Hematopoietic System:			
Lymphoma or Leukemia (b)	23/50(46)	23/50(46)	22/50(44)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.000	0.957
Lower Limit		0.628	0.594
Upper Limit		1.593	1.538
Weeks to First Observed Tumor	79	62	85
Liver: Neoplastic			
Nodule (b)	1/50(2)	3/50(6)	4/50(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		3.000	4.000
Lower Limit		0.251	0.415
Upper Limit		154.270	192.805
Weeks to First Observed Tumor	111	111	98

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing 2,6-Dichloro-p-phenylenediamine (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Neoplastic Nodule or Hepatocellular Carcinoma (b)	1/50(2)	3/50(6)	5/50(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		3.000	5.000
Lower Limit		0.251	0.588
Upper Limit		154.270	231.346
Weeks to First Observed Tumor	111	111	98
Pituitary: Adenoma, NOS (b)	9/48(19)	9/47(19)	6/46(13)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.021	0.696
Lower Limit		0.394	0.221
Upper Limit		2.644	2.007
Weeks to First Observed Tumor	85	96	79
Adrenal: Pheochromocytoma (b)	15/50(30)	7/50(14)	9/50(18)
P Values (c),(d)	N.S.	P=0.045(N)	N.S.
Relative Risk (Control) (e)		0.467	0.600
Lower Limit		0.177	0.259
Upper Limit		1.103	1.319
Weeks to First Observed Tumor	90	100	91

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing 2,6-Dichloro-p-phenylenediamine (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-Cell			
Adenoma (b)	2/48(4)	3/47(6)	0/44(0)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.532	0.000
Lower Limit		0.184	0.000
Upper Limit		17.658	3.675
Weeks to First Observed Tumor	104	111	--
Pancreatic Islets:			
Islet Cell Adenoma (b)	6/49(12)	0/49(0)	1/48(2)
P Values (c),(d)	P=0.017(N)	P=0.013(N)	N.S.
Relative Risk (Control) (e)		0.000	0.170
Lower Limit		0.000	0.004
Upper Limit		0.625	1.327
Weeks to First Observed Tumor	90	--	100
Preputial Gland:			
Carcinoma, NOS (b)	3/50(6)	3/50(6)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.000	0.333
Lower Limit		0.140	0.006
Upper Limit		7.133	3.983
Weeks to First Observed Tumor	106	111	90

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing 2,6-Dichloro-p-phenylenediamine (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Testis: Interstitial Cell Tumor (b)	48/50(96)	47/50(94)	45/50(90)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.979	0.938
Lower Limit		0.910	0.870
Upper Limit		1.076	1.061
Weeks to First Observed Tumor	79	74	59

(a) Dosed groups received doses of 1,000 or 2,000 ppm in feed.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing 2,6-Dichloro-p-phenylenediamine (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Leukemia, NOS (b)	19/50(38)	25/50(50)	12/49(24)
P Values (c),(d)	P=0.044(N)	N.S.	N.S.
Relative Risk (Control) (e)		1.316	0.644
Lower Limit		0.808	0.323
Upper Limit		2.158	1.238
Weeks to First Observed Tumor	4	86	84
Liver: Neoplastic Nodule (b)	3/50(6)	2/50(4)	5/49(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.667	1.701
Lower Limit		0.058	0.351
Upper Limit		5.570	10.426
Weeks to First Observed Tumor	111	110	97
Liver: Neoplastic Nodule or Hepatocellular Carcinoma (b)	3/50(6)	2/50(4)	6/49(12)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.667	2.041
Lower Limit		0.058	0.464
Upper Limit		5.570	11.991
Weeks to First Observed Tumor	111	110	97

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing 2,6-Dichloro-p-phenylenediamine (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Adenoma, NOS (b)	21/48(44)	27/48(56)	27/49(55)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.286	1.259
Lower Limit		0.827	0.809
Upper Limit		2.001	1.968
Weeks to First Observed Tumor	77	98	84
Adrenal: Pheochromocytoma (b)	4/50(8)	3/49(6)	5/49(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.765	1.276
Lower Limit		0.118	0.292
Upper Limit		4.288	6.070
Weeks to First Observed Tumor	98	111	107
Thyroid: C-Cell Adenoma (b)	2/42(5)	0/47(0)	3/48(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.000	1.313
Lower Limit		0.000	0.158
Upper Limit		3.014	15.124
Weeks to First Observed Tumor	111	--	111

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing 2,6-Dichloro-p-phenylenediamine (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-Cell Adenoma or Carcinoma (b)	4/42(10)	0/47(0)	3/48(6)
P Values (c),(d)	N.S.	P=0.046(N)	N.S.
Departure from Linear Trend (f)	P=0.038		
Relative Risk (Control) (e)		0.000	0.656
Lower Limit		0.000	0.102
Upper Limit		0.961	3.663
Weeks to First Observed Tumor	111	--	111
Mammary Gland: Cystadenoma, NOS (b)	3/50(6)	1/50(2)	0/49(0)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.333	0.000
Lower Limit		0.006	0.000
Upper Limit		3.983	1.696
Weeks to First Observed Tumor	92	111	--
Mammary Gland: Fibroadenoma (b)	8/50(16)	4/50(8)	3/49(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.500	0.383
Lower Limit		0.117	0.069
Upper Limit		1.737	1.488
Weeks to First Observed Tumor	107	103	111

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing 2,6-Dichloro-p-phenylenediamine (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp (b)	10/47(21)	5/50(10)	4/48(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.470	0.392
Lower Limit		0.136	0.096
Upper Limit		1.390	1.253
Weeks to First Observed Tumor	110	111	97

(a) Dosed groups received doses of 2,000 or 6,000 ppm in feed.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of high-dose mice of either sex were lower than those of the controls (Figure 3). No other compound-related clinical signs were reported.

B. Survival (Mice)

Estimates of the probabilities of survival for male and female mice administered 2,6-dichloro-p-phenylenediamine in feed at the concentrations of this bioassay, together with those of the control group, are shown by the Kaplan and Meier curves in Figure 4. The survival in the groups of male mice were comparable. In female mice, the survival in the low-dose group was better than that in the control group. No significant compound-related linear trend was observed; however, the survival in the high-dose group was significantly shorter (P=0.033) than that in the low-dose group.

In male mice, 39/50 (78%) of the control group, 41/50 (82%) of the low-dose group, and 42/50 (84%) of the high-dose group lived to the end of the study at 111 weeks. In female mice, 40/50 (80%) of the control group, 45/50 (90%) of the low-dose group, and 35/50 (70%) of the high-dose group lived to the end of the study at 111 weeks. One high-dose female caught its head in the feeder and died. This animal was censored in the survival analysis.

C. Pathology (Mice)

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

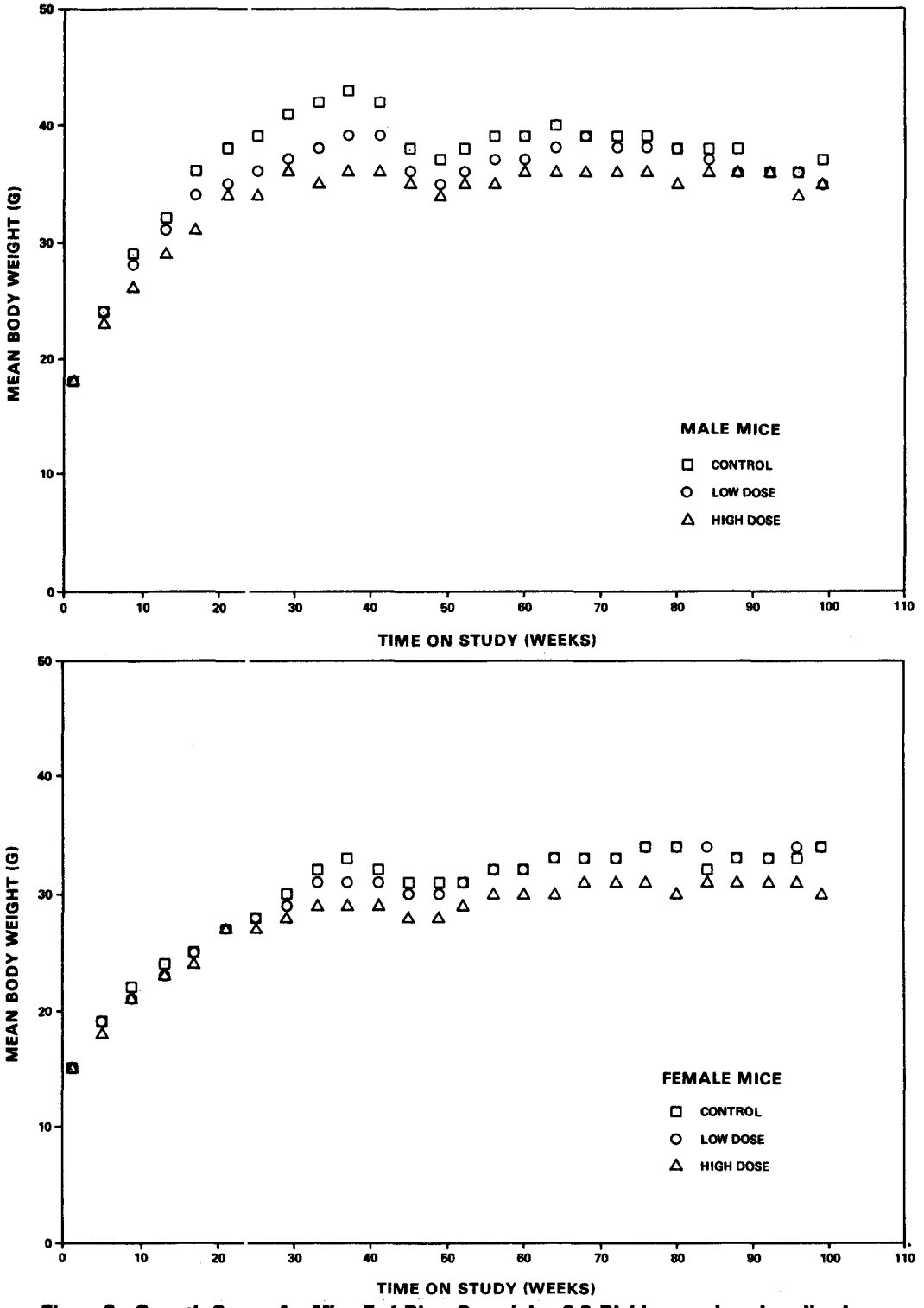


Figure 3. Growth Curves for Mice Fed Diets Containing 2,6-Dichloro-p-phenylenediamine

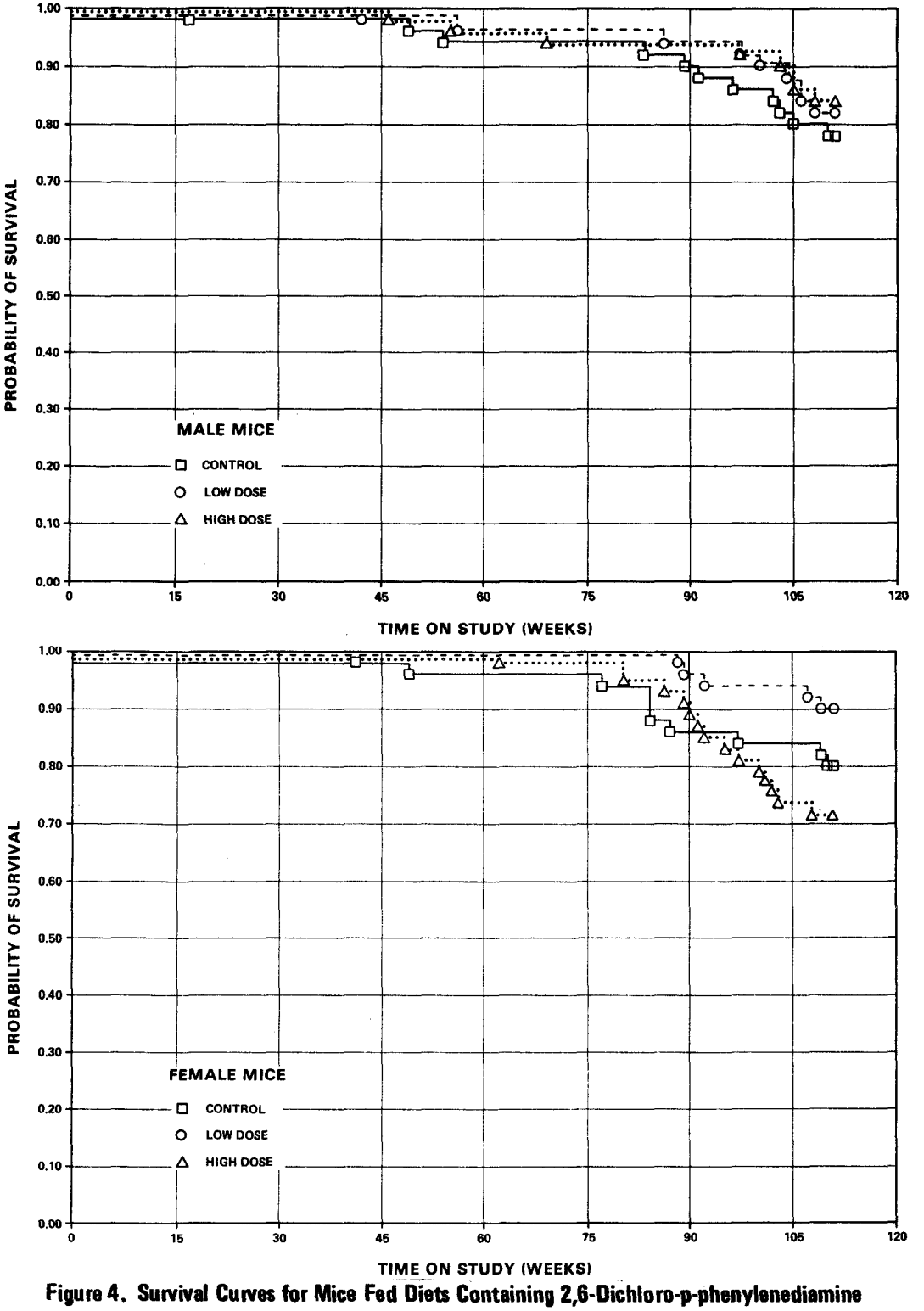


Figure 4. Survival Curves for Mice Fed Diets Containing 2,6-Dichloro-p-phenylenediamine

A variety of tumors were observed in the control groups and/or compound-treated groups. Most of these lesions are common in this strain of mouse independent of any dosing; however, the incidence of hepatocellular neoplasms was higher in dosed animals compared with the controls (Table 9).

Grossly and microscopically, these lesions were consistent with reported descriptions of hepatocellular neoplasms in the mouse. Grossly, carcinomas were irregular and generally larger than adenomas, and frequently had areas of necrosis. The tumors in high-dose male and female mice were almost always composed of large hepatocytes with eosinophilic cytoplasm, while tumors in controls had basophilic cytoplasm. Microscopically, malignancy was based on mitotic index, cellular atypia, development of a trabecular pattern, and invasion and/or metastasis. Only three mice (control males) had metastases. No toxic hepatic lesions were seen.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were encountered in animals in the dosed and control groups. Most of these nonneoplastic lesions are seen commonly in mice of this age.

Histopathologic examination indicated that, under the conditions of this bioassay, the administration of 2,6-dichloro-p-phenylenediamine to B6C3F1 mice is associated with liver tumors.

D. Statistical Analyses of Results (Mice)

Tables 10 and 11 contain the statistical analyses of those primary tumors that occurred in at least two animals in one group and with an incidence of at least 5% in one or more groups.

Hepatocellular adenomas of the liver in male mice were observed in a statistically significant positive relation (4/50, 8% in the controls; 7/50, 14% in the low-dose; and 15/50, 30% in the high-dose). The Cochran-Armitage test for linear trend was statistically significant in the positive direction ($P=0.002$). The Fisher exact test between the high-dose group and the control group was significant ($P=0.005$); no significant incidence was observed in the low-dose group, but this tumor occurred in increased incidence in that group compared with the control group. Hepatocellular adenomas or carcinomas

Table 9. Incidence of Hepatocellular Neoplasms in Mice Fed Diets Containing 2,6-Dichloro-p-phenylenediamine

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Number of Tissues Examined	50	50	50	50	50	50
Hepatocellular adenoma	4	7	15	4	4	9
Hepatocellular carcinoma	12	13	17	2	2	7
Animals with either hepatocellular adenoma or carcinoma	16	19	29	6	6	16

of the liver occurred with increased incidence (16/50, 32% in the controls; 19/50, 38% in the low-dose; and 29/50, 58% in the high-dose). The Cochran-Armitage test for linear trend is significant (P=0.004) as is the Fisher exact test of the high-dose group (P=0.008). The combined incidence in the low-dose group is higher than that in the control group but not significantly different. The historical record of the bioassay program shows 281 adenomas (7.9%) and 587 carcinomas (16.6%) for a combined incidence of 868 liver tumors in 3,543 untreated male mice (24.5%) compared with 58% observed in the high-dose group in this study. Hepatocellular carcinomas or adenomas of the liver were observed in female mice in a statistically significant positive relation (6/50, 12% in the controls; 6/50, 12% in the low-dose; 16/50, 32% in the high-dose). The Cochran-Armitage test for linear trend was significant (P=0.004) and the Fisher exact test of the high-dose was P=0.014. Historical records of the bioassay program indicate that 72 adenomas and 99 carcinomas of the liver (totaling 171/3,617, 4.7%) have been observed in untreated female mice, compared with 32% in the high-dose group in this study. Time adjusted analysis, eliminating those animals that died before 49 weeks (the week of the first observation of a liver tumor), and the life table method of analysis did not materially alter the results of the statistical analysis of the liver tumors.

Alveolar/bronchiolar adenomas of the lung in male mice occurred in decreased incidence in the dosed groups compared with the control group. The Cochran-Armitage test for linear trend was statistically significant in the negative direction (P=0.041). The P values of the Fisher exact tests are both below P=0.05 (P=0.045 in the high-dose and P=0.041 in the low-dose) and above the value of P=0.025 required by the Bonferroni inequality criterion for an overall significance of P=0.05 when two dosed groups are compared with a common control group. In female mice, this tumor was not observed in statistically significant proportions.

Life table analyses of the time to observation of tumors or time-adjusted analysis, eliminating those animals that died before 52 weeks, produced no statistical evidence of carcinogenicity other than that previously described.

In summary of the positive results observed, tumors of the liver occurred at a dose-related incidence in male and female mice.

Table 10. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing 2,6-Dichloro-p-phenylenediamine (a)

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma (b)	11/49(22)	4/50(8)	4/49(8)
P Values (c),(d)	P=0.041(N)	P=0.041(N)	P=0.045(N)
Relative Risk (Control) (e)		0.356	0.364
Lower Limit		0.088	0.090
Upper Limit		1.111	1.132
Weeks to First Observed Tumor	103	111	105
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	13/49(27)	5/50(10)	4/49(8)
P Values (c),(d)	P=0.014(N)	P=0.030(N)	P=0.015(N)
Relative Risk (Control) (e)		0.377	0.308
Lower Limit		0.114	0.078
Upper Limit		1.032	0.915
Weeks to First Observed Tumor	102	111	111
Hematopoietic System: Malignant Lymphoma, NOS (b)	2/50(4)	3/50(6)	5/50(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.500	2.500
Lower Limit		0.180	0.432
Upper Limit		17.329	25.286
Weeks to First Observed Tumor	102	100	111

Table 10. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing 2,6-Dichloro-p-phenylenediamine (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma (b)	5/50(10)	8/50(16)	8/50(16)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.600	1.600
Lower Limit		0.497	0.497
Upper Limit		5.808	5.808
Weeks to First Observed Tumor	102	100	103
Hematopoietic System:			
Lymphomas or Leukemias (b)	5/50(10)	8/50(16)	9/50(18)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.600	1.800
Lower Limit		0.497	0.586
Upper Limit		5.808	6.377
Weeks to First Observed Tumor	102	100	97
Liver: Hepatocellular Carcinoma (b)	12/50(24)	13/50(26)	17/50(34)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.083	1.417
Lower Limit		0.507	0.716
Upper Limit		2.334	2.892
Weeks to First Observed Tumor	49	111	105

Table 10. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing 2,6-Dichloro-p-phenylenediamine (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma (b)	4/50(8)	7/50(14)	15/50(30)
P Values (c),(d)	P=0.002	N.S.	P=0.005
Relative Risk (Control) (e)		1.750	3.750
Lower Limit		0.476	1.302
Upper Limit		7.682	14.451
Weeks to First Observed Tumor	111	86	105
Liver: Hepatocellular Carcinoma or Adenoma (b)	16/50(32)	19/50(38)	29/50(58)
P Values (c),(d)	P=0.004	N.S.	P=0.008
Relative Risk (Control) (e)		1.188	1.813
Lower Limit		0.659	1.107
Upper Limit		2.162	3.017
Weeks to First Observed Tumor	49	86	105

(a) Dosed groups received doses of 1,000 or 3,000 ppm in feed.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

Table 11. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing 2,6-Dichloro-p-phenylenediamine (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma (b)	17/50(34)	11/50(22)	16/50(32)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.647	0.941
Lower Limit		0.307	0.505
Upper Limit		1.308	1.746
Weeks to First Observed Tumor	77	88	86
Hematopoietic System:			
Lymphoma or Leukemia (b)	18/50(36)	12/50(24)	18/50(36)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.667	1.000
Lower Limit		0.330	0.561
Upper Limit		1.300	1.782
Weeks to First Observed Tumor	77	88	86
Circulatory System:			
Hemangiosarcoma (b)	1/50(2)	3/50(6)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		3.000	1.000
Lower Limit		0.251	0.013
Upper Limit		154.270	76.970
Weeks to First Observed Tumor	84	111	97

Table 11. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing 2,6-Dichloro-p-phenylenediamine (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	2/50(4)	2/50(4)	7/50(14)
P Values (c),(d)	P=0.025	N.S.	N.S.
Relative Risk (Control) (e)		1.000	3.500
Lower Limit		0.075	0.708
Upper Limit		13.326	33.206
Weeks to First Observed Tumor	111	111	111
Liver: Hepatocellular Adenoma (b)	4/50(8)	4/50(8)	9/50(18)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.000	2.250
Lower Limit		0.197	0.676
Upper Limit		5.083	9.394
Weeks to First Observed Tumor	111	111	97
Liver: Hepatocellular Carcinoma or Adenoma (b)	6/50(12)	6/50(12)	16/50(32)
P Values (c),(d)	P=0.004	N.S.	P=0.014
Relative Risk (Control) (e)		1.000	2.667
Lower Limit		0.287	1.091
Upper Limit		3.489	7.612
Weeks to First Observed Tumor	111	111	97

Table 11. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing 2,6-Dichloro-p-phenylenediamine (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma (b)	3/43(7)	2/48(4)	1/44(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.597	0.326
Lower Limit		0.052	0.006
Upper Limit		4.974	3.869
Weeks to First Observed Tumor	111	111	101

(a) Dosed groups received doses of 1,000 or 3,000 ppm in feed.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

V. DISCUSSION

Mean body weights of dosed rats and mice of either sex were lower than those of the controls throughout the study. A dose-related weight gain depression was particularly pronounced for rats.

Nephrosis was found in increased incidence in high-dose female rats and with increased severity in dosed male and female rats. An unusual non-neoplastic lesion, ectopic hepatocytes in the pancreas, was observed in low-dose (4/49) and high-dose (5/48) male rats and in low-dose (10/50) and high-dose (4/49) female rats but was not seen in the controls.

Liver tumors in mice were associated with the administration of 2,6-dichloro-p-phenylenediamine to the mice. In male mice, the incidence of hepatocellular adenomas exhibited a significant positive dose-related trend, and the incidence of hepatocellular adenomas was statistically significant in the high-dose group. When hepatocellular carcinomas and adenomas were combined, there was a significant positive dose-related trend and a statistically significant increase in the high-dose group. Hepatocellular carcinomas were not statistically significant in male mice. In female mice, hepatocellular carcinomas exhibited a significant positive dose-related trend, but no single dose group had a statistically significant increased incidence of either carcinomas or adenomas alone. However, the combined incidence of hepatocellular adenomas and carcinomas exhibited a positive dose-related trend and was statistically significant in the high-dose group as well as when compared with the incidences observed in historical controls of the bioassay program. The combined incidence of hepatocellular carcinomas and adenomas (4/17, 12/49, 20/46) also occurred with a dose-related trend (P=0.038) in male mice fed diets for 87 weeks containing 2,000 or 6,000 ppm of the related compound 2-chloro-p-phenylenediamine sulfate in a previous study (NCI, 1978). However, neither the high-dose nor the low-dose group had a significantly increased incidence when compared to controls. Further, 4-chloro-m-phenylenediamine was carcinogenic in male F344 rats, causing adrenal pheochromocytomas and in female B6C3F1 mice, inducing hepatocellular adenomas or carcinomas (NCI, 1978a). 4-Chloro-o-phenylenediamine was carcinogenic in

F344 rats, producing carcinomas of the urinary bladder in male and female rats and inducing hepatocellular carcinomas in male and female mice (NCI, 1978b).

VI. CONCLUSIONS

Under the conditions of this bioassay, 2,6-dichloro-p-phenylenediamine was carcinogenic for male and female B6C3F1 mice, causing increased incidences of combined hepatocelluar adenomas and carcinomas, and for male B6C3F1 mice, causing an increased incidence of hepatocellular adenomas alone. 2,6-Dichloro-p-phenylenediamine was not carcinogenic for male or female F344 rats.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS FED DIETS CONTAINING 2,6-DICHLORO-p-PHENYLENEDIAMINE

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED DIETS CONTAINING 2,6-DICHLORO-P-PHENYLENEDIAMINE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
PAPILLOMA, NOS	1 (2%)	1 (2%)	
SQUAMOUS CELL CARCINOMA	1 (2%)		1 (2%)
TRICHOEPITHELIOMA	1 (2%)		
CYSTADENOMA, NOS			1 (2%)
SARCOMA, NOS			1 (2%)
FIBROMA	3 (6%)	5 (10%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
LEUKEMIA,NOS	22 (44%)	22 (44%)	22 (44%)
#SPLEEN	(50)	(50)	(50)
LEUKEMIA,NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
NEOPLASTIC NODULE	1 (2%)	3 (6%)	4 (8%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA			1 (2%)
#DUODENUM	(50)	(48)	(49)
ADENOCARCINOMA, NOS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
TUBULAR-CELL ADENOCARCINOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(47)	(46)
ADENOMA, NOS	9 (19%)	9 (19%)	6 (13%)
#ADRENAL	(50)	(50)	(50)
CORTICAL ADENOMA	1 (2%)	1 (2%)	
PHEOCHROMOCYTOMA	15 (30%)	7 (14%)	9 (18%)
#THYROID	(48)	(47)	(44)
FOLLICULAR-CELL ADENOMA	1 (2%)	1 (2%)	1 (2%)
C-CELL ADENOMA	2 (4%)	3 (6%)	
#PANCREATIC ISLETS	(49)	(49)	(48)
ISLET-CELL ADENOMA	6 (12%)		1 (2%)
ISLET-CELL CARCINOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOMA, NOS		1 (2%)	
FIBROADENOMA	2 (4%)	2 (4%)	
*PREPUTIAL GLAND	(50)	(50)	(50)
CARCINOMA,NOS	3 (6%)	3 (6%)	1 (2%)
SQUAMOUS CELL CARCINOMA		1 (2%)	
ADENOMA, NOS	2 (4%)		
#PROSTATE	(46)	(49)	(45)
ADENOMA, NOS	2 (4%)		
#TESTIS	(50)	(50)	(50)
INTERSTITIAL-CELL TUMOR	48 (96%)	47 (94%)	45 (90%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(50)
ASTROCYTOMA		2 (4%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SACRUM	(50)	(50)	(50)
FIBROMA	1 (2%)		
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(50)
NEOPLASM, NOS, MALIGNANT	1 (2%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
MESOTHELIOMA, NOS	1 (2%)		1 (2%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	7	6	4
MORIBUND SACRIFICE	13	14	25
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	30	30	21
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	49	49	48
TOTAL PRIMARY TUMORS	127	111	95
TOTAL ANIMALS WITH BENIGN TUMORS	48	47	47
TOTAL BENIGN TUMORS	94	78	64
TOTAL ANIMALS WITH MALIGNANT TUMORS	27	28	25
TOTAL MALIGNANT TUMORS	31	30	26
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	2	3	5
TOTAL UNCERTAIN TUMORS	2	3	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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED DIETS CONTAINING 2,6-DICHLORO-P-PHENYLENEDIAMINE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(49)
TRICHOEPITHELIOMA	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(49)
SQUAMOUS CELL CARCINOMA	2 (4%)		1 (2%)
ADNEXAL ADENOMA			1 (2%)
FIBROMA		2 (4%)	1 (2%)
RESPIRATORY SYSTEM			
*NOSE	(50)	(50)	(49)
PAPILLOMA, NOS	1 (2%)		
#TRACHEA	(50)	(3)	(3)
C-CELL CARCINOMA, INVASIVE**	1 (2%)		
#LUNG	(50)	(50)	(49)
C-CELL CARCINOMA, METASTATIC	2 (4%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
LEUKEMIA,NOS	19 (38%)	25 (50%)	12 (24%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(49)
NEOPLASTIC NODULE	3 (6%)	2 (4%)	5 (10%)

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

**INVASIVE FROM THYROID

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA			1 (2%)
#PANCREAS	(50)	(50)	(49)
ACINAR-CELL ADENOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(48)	(49)
ADENOMA, NOS	21 (44%)	27 (56%)	27 (55%)
#ADRENAL	(50)	(49)	(49)
ADENOMA, NOS	1 (2%)		
CORTICAL ADENOMA	2 (4%)		1 (2%)
PHEOCHROMOCYTOMA	4 (8%)	3 (6%)	5 (10%)
GANGLIONEUROMA	1 (2%)		
#THYROID	(42)	(47)	(48)
FOLLICULAR-CELL ADENOMA	1 (2%)		
FOLLICULAR-CELL CARCINOMA		1 (2%)	
C-CELL ADENOMA	2 (5%)		3 (6%)
C-CELL CARCINOMA	2 (5%)		
PAPILLARY CYSTADENOMA, NOS			1 (2%)
#THYROID FOLLICLE	(42)	(47)	(48)
CYSTADENOMA, NOS	1 (2%)		
#PARATHYROID	(29)	(27)	(28)
PAPILLARY CYSTADENOMA, NOS			1 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(49)
ADENOMA, NOS		1 (2%)	
CYSTADENOMA, NOS	3 (6%)	1 (2%)	
FIBROADENOMA	8 (16%)	4 (8%)	3 (6%)
*CLITORAL GLAND	(50)	(50)	(49)
CARCINOMA,NOS	1 (2%)	1 (2%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#UTERUS	(47)	(50)	(48)
ENDOMETRIAL STROMAL POLYP	10 (21%)	5 (10%)	4 (8%)
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(49)
GLIOMA, NOS			1 (2%)
ASTROCYTOMA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SACRUM	(50)	(50)	(49)
FIBROMA	1 (2%)		
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	6	2	3
MORIBUND SACRIFICE	8	16	9
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	32	38
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	49	45	38
TOTAL PRIMARY TUMORS	84	74	68
TOTAL ANIMALS WITH BENIGN TUMORS	38	35	32
TOTAL BENIGN TUMORS	57	43	48
TOTAL ANIMALS WITH MALIGNANT TUMORS	23	26	14
TOTAL MALIGNANT TUMORS	24	29	15
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		
TOTAL SECONDARY TUMORS	3		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	3	2	5
TOTAL UNCERTAIN TUMORS	3	2	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 FED DIETS CONTAINING 2,6-DICHLORO-p-PHENYLENEDIAMINE

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED DIETS CONTAINING 2,6-DICHLORO-P-PHENYLENEDIAMINE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS	1 (2%)		
NEUROFIBROMA	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(49)	(50)	(49)
HEPATOCELLULAR CARCINOMA, METAST	4 (8%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	11 (22%)	4 (8%)	4 (8%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (4%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (2%)	3 (6%)	3 (6%)
MALIG.LYMPHOMA, UNDIFFER-TYPE		2 (4%)	1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)	2 (4%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
LEUKEMIA,NOS			1 (2%)
#SPLEEN	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
#MESENTERIC L. NODE	(37)	(45)	(39)
MALIGNANT LYMPHOMA, NOS	1 (3%)		1 (3%)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (3%)
*MESENTERY	(50)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
HEMANGIOMA		1 (2%)	
#LIVER	(50)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)	
#TESTIS	(49)	(49)	(50)
HEMANGIOMA	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
HEPATOCELLULAR ADENOMA	4 (8%)	7 (14%)	15 (30%)
HEPATOCELLULAR CARCINOMA	12 (24%)	13 (26%)	17 (34%)
NEUROFIBROSARCOMA			1 (2%)
#DUODENUM	(50)	(48)	(46)
ADENOCARCINOMA, NOS	2 (4%)		
#JEJUNUM	(50)	(48)	(46)
ADENOCARCINOMA, NOS			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#TESTIS	(49)	(49)	(50)
INTERSTITIAL-CELL TUMOR		1 (2%)	1 (2%)
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(50)
MENINGIOMA			1 (2%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(50)
ADENOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(50)
MESOTHELIOMA, MALIGNANT	1 (2%)		
*MESENTERY	(50)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
MESOTHELIOMA, MALIGNANT	1 (2%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	6	7	4
MORIBUND SACRIFICE	5	2	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	39	41	42
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	31	30	38
TOTAL PRIMARY TUMORS	41	36	50
TOTAL ANIMALS WITH BENIGN TUMORS	17	13	21
TOTAL BENIGN TUMORS	17	13	22
TOTAL ANIMALS WITH MALIGNANT TUMORS	19	21	25
TOTAL MALIGNANT TUMORS	24	23	28
TOTAL ANIMALS WITH SECONDARY TUMORS#	5		
TOTAL SECONDARY TUMORS	5		
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 FED DIETS CONTAINING 2,6-DICHLORO-P-PHENYLENEDIAMINE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
LEIOMYOMA	1 (2%)		
OSTEOSARCOMA			1 (2%)
NEUROFIBROMA			1 (2%)
NEUROFIBROSARCOMA		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (4%)	2 (4%)	2 (4%)
OSTEOSARCOMA, METASTATIC		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	4 (8%)	3 (6%)	5 (10%)
MALIG.LYMPHOMA, UNDIFFER-TYPE	7 (14%)	2 (4%)	2 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	4 (8%)	5 (10%)	2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	1 (2%)	2 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
LYMPHOCYTIC LEUKEMIA	1 (2%)	1 (2%)	1 (2%)
GRANULOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(49)	(50)	(50)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (2%)		
#LYMPH NODE	(46)	(45)	(48)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#MESENTERIC L. NODE	(46)	(45)	(48)
MALIGNANT LYMPHOMA, NOS			1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
*INGUINAL REGION	(50)	(50)	(50)
HEMANGIOMA			1 (2%)
#SPLEEN	(49)	(50)	(50)
HEMANGIOMA			1 (2%)
HEMANGIOSARCOMA		1 (2%)	
*SKELETAL MUSCLE	(50)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)	
#LIVER	(50)	(50)	(50)
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA	1 (2%)	1 (2%)	
*MESENTERY	(50)	(50)	(50)
HEMANGIOMA		1 (2%)	
#UTERUS	(50)	(50)	(49)
HEMANGIOSARCOMA			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
HEPATOCELLULAR ADENOMA	4 (8%)	4 (8%)	9 (18%)
HEPATOCELLULAR CARCINOMA	2 (4%)	2 (4%)	7 (14%)
*PERIANAL TISSUE	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(43)	(48)	(44)
CHROMOPHOBE ADENOMA	3 (7%)	2 (4%)	1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#THYROID	(46)	(48)	(49)
FOLLICULAR-CELL ADENOMA	2 (4%)	1 (2%)	
#PANCREATIC ISLETS	(50)	(48)	(49)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOMA, NOS		2 (4%)	1 (2%)
#UTERUS	(50)	(50)	(49)
LEIOMYOMA			1 (2%)
LEIOMYOSARCOMA	1 (2%)		
ENDOMETRIAL STROMAL POLYP		1 (2%)	
#OVARY	(47)	(49)	(42)
CARCINOMA,NOS	1 (2%)		
MUCINOUS ADENOCARCINOMA	1 (2%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(50)
ADENOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(50)	(50)	(50)
CARCINOMA, NOS, METASTATIC	1 (2%)		
ALL OTHER SYSTEMS			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATHS@	6	4	9
MORIBUND SACRIFICE	4	1	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	40	45	35
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	31	25	37
TOTAL PRIMARY TUMORS	37	33	46
TOTAL ANIMALS WITH BENIGN TUMORS	12	12	17
TOTAL BENIGN TUMORS	13	15	18
TOTAL ANIMALS WITH MALIGNANT TUMORS	22	16	25
TOTAL MALIGNANT TUMORS	24	18	28
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	1
TOTAL SECONDARY TUMORS	1	1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS FED DIETS CONTAINING 2,6-DICHLORO-p-PHENYLENEDIAMINE

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED DIETS CONTAINING 2,6-DICHLORO-P-PHENYLENEDIAMINE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
INFLAMMATION, NOS	1 (2%)		
HYPERKERATOSIS		1 (2%)	
PARAKERATOSIS		1 (2%)	
RESPIRATORY SYSTEM			
*LARYNX	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION ACTIVE CHRONIC			1 (2%)
INFLAMMATION, CHRONIC	1 (2%)	1 (2%)	1 (2%)
#TRACHEA	(3)	(4)	(4)
INFLAMMATION, SUPPURATIVE		1 (25%)	
#LUNG	(50)	(50)	(50)
CONGESTION, NOS	1 (2%)	2 (4%)	
EDEMA, NOS		1 (2%)	
HEMORRHAGE	3 (6%)	1 (2%)	5 (10%)
INFLAMMATION, INTERSTITIAL	6 (12%)	2 (4%)	2 (4%)
INFLAMMATION, SUPPURATIVE	1 (2%)		2 (4%)
INFLAMMATION, CHRONIC	9 (18%)	5 (10%)	4 (8%)
HEMOSIDEROSIS			1 (2%)
EPITHELIALIZATION		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(47)	(46)	(48)
NECROSIS, NOS		1 (2%)	
MYELOFIBROSIS			2 (4%)
HYPERPLASIA, HEMATOPOIETIC	3 (6%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#SPLEEN	(50)	(50)	(50)
HEMOSIDEROSIS		1 (2%)	1 (2%)
ATROPHY, NOS		1 (2%)	
#LYMPH NODE	(50)	(48)	(49)
HEMOSIDEROSIS		1 (2%)	
#MANDIBULAR L. NODE	(50)	(48)	(49)
CYST, NOS			1 (2%)
PLASMACYTOSIS	2 (4%)		
#MEDIASTINAL L.NODE	(50)	(48)	(49)
HEMORRHAGE			1 (2%)
MASTOCYTOSIS	1 (2%)		
#MESENTERIC L. NODE	(50)	(48)	(49)
HEMORRHAGE	1 (2%)	1 (2%)	4 (8%)
NECROSIS, FAT	1 (2%)		
#RENAL LYMPH NODE	(50)	(48)	(49)
HISTIOCYTOSIS			1 (2%)
#THYMUS	(12)	(15)	(19)
INFLAMMATION ACTIVE CHRONIC			1 (5%)
CIRCULATORY SYSTEM			
*MEDIASTINUM	(50)	(50)	(50)
PERIARTERITIS			1 (2%)
#LUNG	(50)	(50)	(50)
PERIVASCULITIS			1 (2%)
#HEART	(50)	(50)	(49)
MINERALIZATION			1 (2%)
THROMBOSIS, NOS	1 (2%)	1 (2%)	2 (4%)
THROMBUS, ORGANIZED		1 (2%)	
ENDOCARDITIS, BACTERIAL	1 (2%)		
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
FIBROSIS	40 (80%)	36 (72%)	27 (55%)
PERIARTERITIS	1 (2%)		1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MYOCARDIUM	(50)	(50)	(49)
INFLAMMATION, NOS	2 (4%)		
#ENDOCARDIUM	(50)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	
#LIVER	(50)	(50)	(50)
THROMBOSIS, NOS	1 (2%)		
#PANCREAS	(49)	(49)	(48)
PERIARTERITIS	2 (4%)		
#STOMACH	(50)	(50)	(49)
PERIARTERITIS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
CYST, NOS		1 (2%)	
CONGESTION, NOS		1 (2%)	2 (4%)
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, CHRONIC	8 (16%)	5 (10%)	3 (6%)
CHOLANGIOFIBROSIS			1 (2%)
NECROSIS, COAGULATIVE	2 (4%)	3 (6%)	3 (6%)
METAMORPHOSIS FATTY	3 (6%)		1 (2%)
CYTOPLASMIC CHANGE, NOS	3 (6%)	3 (6%)	2 (4%)
CYTOPLASMIC VACUOLIZATION		1 (2%)	
BASOPHILIC CYTO CHANGE	12 (24%)	1 (2%)	2 (4%)
GROUND-GLASS CYTO CHANGE	7 (14%)	7 (14%)	7 (14%)
EOSINOPHILIC CYTO CHANGE	6 (12%)	6 (12%)	6 (12%)
HEPATOCYTOMEGALY	1 (2%)	6 (12%)	1 (2%)
ANGIECTASIS	3 (6%)	14 (28%)	12 (24%)
#LIVER/CENTRILOBULAR	(50)	(50)	(50)
NECROSIS, NOS	1 (2%)		1 (2%)
#BILE DUCT	(50)	(50)	(50)
HYPERPLASIA, NOS	34 (68%)	30 (60%)	22 (44%)
#PANCREAS	(49)	(49)	(48)
ECTOPIA		4 (8%)	5 (10%)
DILATATION/DUCTS			2 (4%)
FIBROSIS		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
FIBROSIS, DIFFUSE	1 (2%)		
NECROSIS, FAT			2 (4%)
#PANCREATIC ACINUS	(49)	(49)	(48)
ATROPHY, NOS	15 (31%)	16 (33%)	15 (31%)
#STOMACH	(50)	(50)	(49)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, ACUTE		1 (2%)	2 (4%)
INFLAMMATION, CHRONIC		1 (2%)	
HYPERKERATOSIS			1 (2%)
#SMALL INTESTINE	(50)	(48)	(49)
INFLAMMATION, CHRONIC			1 (2%)
#DUODENUM	(50)	(48)	(49)
POLYP		1 (2%)	
#COLON	(49)	(48)	(46)
INFLAMMATION, SUPPURATIVE		2 (4%)	
NEMATODIASIS	2 (4%)	3 (6%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
MINERALIZATION			2 (4%)
CONGESTION, NOS		1 (2%)	
HEMORRHAGE	1 (2%)	1 (2%)	
INFLAMMATION, SUPPURATIVE	1 (2%)		
GLOMERULONEPHRITIS, MEMBRANOUS			1 (2%)
DEGENERATION, HYALINE	2 (4%)		
NEPHROSIS, NOS	46 (92%)	49 (98%)	47 (94%)
CYTOPLASMIC VACUOLIZATION	1 (2%)		
#KIDNEY/TUBULE	(50)	(50)	(50)
CYTOPLASMIC VACUOLIZATION	1 (2%)		
#URINARY BLADDER	(49)	(49)	(47)
HEMORRHAGE	1 (2%)	1 (2%)	
HYPERPLASIA, EPITHELIAL			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(47)	(46)
CYST, NOS	1 (2%)		5 (11%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEMORRHAGE		1 (2%)	
#ADRENAL	(50)	(50)	(50)
HEMORRHAGE			1 (2%)
NECROSIS, NOS		3 (6%)	2 (4%)
LIPOIDOSIS	1 (2%)		
#ADRENAL MEDULLA	(50)	(50)	(50)
HYPERPLASIA, NOS	1 (2%)	1 (2%)	1 (2%)
#THYROID	(48)	(47)	(44)
HYPERPLASIA, C-CELL	4 (8%)	5 (11%)	3 (7%)
#PANCREATIC ISLETS	(49)	(49)	(48)
HYPERPLASIA, NOS	1 (2%)	2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
DILATATION/DUCTS	1 (2%)	3 (6%)	
*MAMMARY DUCT	(50)	(50)	(50)
HYPERPLASIA, EPITHELIAL	1 (2%)		
*PREPUTIAL GLAND	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
#PROSTATE	(46)	(49)	(45)
INFLAMMATION, SUPPURATIVE	4 (9%)	12 (24%)	3 (7%)
INFLAMMATION ACTIVE CHRONIC	1 (2%)	1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)	1 (2%)	
FIBROSIS			1 (2%)
HYPERPLASIA, NOS	6 (13%)	6 (12%)	1 (2%)
*SEMINAL VESICLE	(50)	(50)	(50)
MINERALIZATION		1 (2%)	
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, SUPPURATIVE		1 (2%)	
#TESTIS	(50)	(50)	(50)
MINERALIZATION		1 (2%)	
HEMORRHAGE		1 (2%)	2 (4%)
INFLAMMATION, SUPPURATIVE			1 (2%)
DEGENERATION, NOS	1 (2%)	3 (6%)	2 (4%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CYTOMEGALY	1 (2%)	2 (4%)	1 (2%)
HYPOSPERMATOGENESIS	4 (8%)	3 (6%)	6 (12%)
HYPERPLASIA, INTERSTITIAL CELL	4 (8%)	2 (4%)	4 (8%)
*EPIDIDYMIS	(50)	(50)	(50)
HYPERPLASIA, NOS	1 (2%)	1 (2%)	2 (4%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(50)	(50)	(50)
MINERALIZATION			1 (2%)
FIBROSIS			1 (2%)
#BRAIN	(50)	(50)	(50)
HEMORRHAGE			2 (4%)
MALACIA		1 (2%)	
#CEREBELLUM	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, ACUTE		1 (2%)	
CATARACT	8 (16%)	7 (14%)	7 (14%)
*EYE ANTERIOR CHAMBER	(50)	(50)	(50)
HEMORRHAGE, CHRONIC	1 (2%)		
INFLAMMATION, SUPPURATIVE		1 (2%)	
*EYE POSTERIOR CHAMBE	(50)	(50)	(50)
HEMORRHAGE, CHRONIC	1 (2%)		
*SCLERA	(50)	(50)	(50)
MINERALIZATION	4 (8%)		1 (2%)
*EYE/CORNEA	(50)	(50)	(50)
MINERALIZATION		1 (2%)	
INFLAMMATION, SUPPURATIVE	1 (2%)	2 (4%)	1 (2%)
INFLAMMATION, ACUTE	8 (16%)	7 (14%)	1 (2%)
CATARACT		1 (2%)	
*EYE/RETINA	(50)	(50)	(50)
DEGENERATION, NOS	8 (16%)	7 (14%)	6 (12%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*HARDERIAN GLAND	(50)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
PORPHYRIN			1 (2%)
*EAR	(50)	(50)	(50)
INFLAMMATION ACTIVE CHRONIC		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*STERNUM	(50)	(50)	(50)
NECROSIS, NOS	9 (18%)	16 (32%)	12 (24%)
BODY CAVITIES			
*MEDIASTINUM	(50)	(50)	(50)
HEMOSIDEROSIS		2 (4%)	
*ABDOMINAL CAVITY	(50)	(50)	(50)
MINERALIZATION			1 (2%)
NECROSIS, FAT	1 (2%)		1 (2%)
*MESENTERY	(50)	(50)	(50)
NECROSIS, FAT	1 (2%)		2 (4%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
INFECTION, BACTERIAL			1 (2%)
TAIL			
INFLAMMATION, SUPPURATIVE	1		
HYPERKERATOSIS	1		
BASE OF TAIL			
EPIDERMAL INCLUSION CYST	1		
ADIPOSE TISSUE			
CONGESTION, NOS	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 FED DIETS CONTAINING 2,6-DICHLORO-P-PHENYLENEDIAMINE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*LARYNX	(50)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
#LUNG	(50)	(50)	(49)
CONGESTION, NOS	1 (2%)	2 (4%)	
HEMORRHAGE		1 (2%)	1 (2%)
INFLAMMATION, INTERSTITIAL	7 (14%)	4 (8%)	2 (4%)
INFLAMMATION, CHRONIC	7 (14%)	7 (14%)	2 (4%)
#LUNG/ALVEOLI	(50)	(50)	(49)
HISTIOCYTOSIS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(45)	(46)	(44)
NECROSIS, NOS	1 (2%)		
MYELOFIBROSIS		1 (2%)	
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	2 (5%)
#SPLEEN	(50)	(49)	(49)
MINERALIZATION	1 (2%)		
HEMORRHAGE	1 (2%)	1 (2%)	
FIBROSIS		1 (2%)	
HEMOSIDEROSIS	1 (2%)	1 (2%)	2 (4%)
LYMPHOID DEPLETION	1 (2%)		
HYPERPLASIA, RETICULUM CELL			1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIESIS	1 (2%)		
#LYMPH NODE	(50)	(49)	(48)
HYPERPLASIA, RETICULUM CELL		1 (2%)	
#MANDIBULAR L. NODE	(50)	(49)	(48)
HEMOSIDEROSIS			1 (2%)
PLASMACYTOSIS			2 (4%)
HYPERPLASIA, RETICULUM CELL			1 (2%)
#MEDIASTINAL L.NODE	(50)	(49)	(48)
HYPERPLASIA, RETICULUM CELL			1 (2%)
#MESENTERIC L. NODE	(50)	(49)	(48)
HISTIOCYTOSIS	1 (2%)	1 (2%)	1 (2%)
HYPERPLASIA, RETICULUM CELL	1 (2%)		
CIRCULATORY SYSTEM			
#HEART	(50)	(50)	(49)
MINERALIZATION	2 (4%)		
THROMBOSIS, NOS		2 (4%)	1 (2%)
INFLAMMATION, NOS	1 (2%)		2 (4%)
INFLAMMATION, CHRONIC	1 (2%)		
FIBROSIS	32 (64%)	24 (48%)	19 (39%)
ARTERIOSCLEROSIS, NOS	1 (2%)		
#AORTIC VALVE	(50)	(50)	(49)
THROMBOSIS, NOS			1 (2%)
#LIVER	(50)	(50)	(49)
THROMBOSIS, NOS		1 (2%)	1 (2%)
#UTERUS	(47)	(50)	(48)
THROMBUS, ORGANIZED		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(50)	(48)	(48)
MINERALIZATION			1 (2%)
#LIVER	(50)	(50)	(49)
ECTOPIA			1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CONGESTION, NOS		1 (2%)	1 (2%)
HEMORRHAGE	1 (2%)		
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, CHRONIC	20 (40%)	12 (24%)	11 (22%)
NECROSIS, NOS	1 (2%)		
NECROSIS, COAGULATIVE	1 (2%)		1 (2%)
METAMORPHOSIS FATTY	1 (2%)		1 (2%)
HEMOSIDEROSIS		1 (2%)	
CYTOPLASMIC CHANGE, NOS	3 (6%)	4 (8%)	1 (2%)
CYTOPLASMIC VACUOLIZATION	2 (4%)		
BASOPHILIC CYTO CHANGE	23 (46%)	2 (4%)	6 (12%)
GROUND-GLASS CYTO CHANGE		4 (8%)	2 (4%)
EOSINOPHILIC CYTO CHANGE		4 (8%)	1 (2%)
HEPATOCYTOMEGALY			1 (2%)
ANGIECTASIS		4 (8%)	1 (2%)
#BILE DUCT	(50)	(50)	(49)
HYPERPLASIA, NOS	12 (24%)	18 (36%)	13 (27%)
#PANCREAS	(50)	(50)	(49)
ECTOPIA		10 (20%)	4 (8%)
FIBROSIS			1 (2%)
#PANCREATIC ACINUS	(50)	(50)	(49)
ATROPHY, NOS	10 (20%)	19 (38%)	23 (47%)
#STOMACH	(50)	(49)	(49)
EDEMA, NOS		1 (2%)	
ULCER, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, ACUTE		1 (2%)	
ULCER, ACUTE			1 (2%)
#COLON	(48)	(48)	(49)
NEMATODIASIS	2 (4%)	2 (4%)	
*RECTUM	(50)	(50)	(49)
NEMATODIASIS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(49)
CALCULUS, NOS			1 (2%)
MINERALIZATION	4 (8%)	5 (10%)	31 (63%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CONGESTION, NOS	1 (2%)	1 (2%)	
PYELONEPHRITIS, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, CHRONIC	2 (4%)	3 (6%)	
FIBROSIS		1 (2%)	9 (18%)
FIBROSIS, FOCAL			1 (2%)
NEPHROSIS, NOS	38 (76%)	35 (71%)	49 (100%)
NECROSIS, NOS	1 (2%)		1 (2%)
METAMORPHOSIS FATTY	1 (2%)		
CYTOPLASMIC VACUOLIZATION		1 (2%)	
HYPERPLASIA, EPITHELIAL			1 (2%)
#RENAL PAPILLA	(50)	(49)	(49)
MINERALIZATION			3 (6%)
HEMORRHAGE			1 (2%)
#KIDNEY/TUBULE	(50)	(49)	(49)
PIGMENTATION, NOS		1 (2%)	
#KIDNEY/PELVIS	(50)	(49)	(49)
HYPERPLASIA, EPITHELIAL			1 (2%)
#URINARY BLADDER	(50)	(49)	(48)
EPIDERMAL INCLUSION CYST			1 (2%)
HYPERPLASIA, EPITHELIAL	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(48)	(49)
CYST, NOS	6 (13%)	4 (8%)	5 (10%)
CONGESTION, NOS		1 (2%)	
ANGIECTASIS		1 (2%)	
#ADRENAL	(50)	(49)	(49)
MINERALIZATION		1 (2%)	
CYST, NOS			3 (6%)
HEMORRHAGE	2 (4%)		1 (2%)
HEMORRHAGIC CYST		1 (2%)	
NECROSIS, NOS	2 (4%)		1 (2%)
ANGIECTASIS			1 (2%)
#THYROID	(42)	(47)	(48)
CYSTIC FOLLICLES			1 (2%)
HYPERPLASIA, C-CELL	4 (10%)	3 (6%)	1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC ISLETS	(50)	(50)	(49)
HYPERPLASIA, NOS	1 (2%)		1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(49)
DILATATION/DUCTS	2 (4%)	1 (2%)	
GALACTOCELE	2 (4%)		
CYST, NOS		1 (2%)	1 (2%)
CYSTIC DUCTS			1 (2%)
#UTERUS	(47)	(50)	(48)
CYST, NOS			1 (2%)
#UTERUS/ENDOMETRIUM	(47)	(50)	(48)
CYST, NOS			1 (2%)
HYPERPLASIA, NOS	2 (4%)	1 (2%)	
#OVARY	(48)	(49)	(48)
PAROVARIAN CYST	1 (2%)	1 (2%)	
CONGESTION, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(49)
MALACIA		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(49)
HEMORRHAGE	1 (2%)		1 (2%)
INFLAMMATION, ACUTE	1 (2%)		
CATARACT	11 (22%)	11 (22%)	13 (27%)
GROWTH ARREST		1 (2%)	
*SCLERA	(50)	(50)	(49)
MINERALIZATION	6 (12%)	4 (8%)	1 (2%)
*EYE/CORNEA	(50)	(50)	(49)
INFLAMMATION, ACUTE	19 (38%)	7 (14%)	1 (2%)
VASCULARIZATION	1 (2%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*EYE/RETINA	(50)	(50)	(49)
DEGENERATION, NOS	9 (18%)	9 (18%)	14 (29%)
*EAR	(50)	(50)	(49)
INFLAMMATION ACTIVE CHRONIC		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*STERNUM	(50)	(50)	(49)
HEMORRHAGE	1 (2%)		
NECROSIS, NOS	26 (52%)	18 (36%)	13 (27%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(49)
NECROSIS, FAT		2 (4%)	1 (2%)
*PELVIS	(50)	(50)	(49)
NECROSIS, FAT			1 (2%)
*INGUINAL REGION	(50)	(50)	(49)
NECROSIS, FAT			1 (2%)
*MESENTERY	(50)	(50)	(49)
NECROSIS, FAT	1 (2%)	1 (2%)	
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
MINERALIZATION			1
FIBROSIS			1
OMENTUM			
NECROSIS, FAT	1		
SPECIAL MORPHOLOGY SUMMARY			
NO NECROPSY PERFORMED			1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED DIETS CONTAINING 2,6-DICHLORO-p-PHENYLENEDIAMINE

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED DIETS CONTAINING 2,6-DICHLORO-P-PHENYLENEDIAMINE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(49)	(50)	(49)
CONGESTION, NOS	1 (2%)		1 (2%)
EDEMA, NOS		1 (2%)	1 (2%)
HEMORRHAGE	1 (2%)		
INFLAMMATION, INTERSTITIAL	1 (2%)		
PNEUMONIA, ASPIRATION			1 (2%)
PNEUMONIA, CHRONIC MURINE	1 (2%)	5 (10%)	3 (6%)
HEMOSIDEROSIS	1 (2%)		1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(48)	(50)	(50)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
#SPLEEN	(50)	(50)	(50)
HEMOSIDEROSIS		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (2%)	6 (12%)	
HEMATOPOIESIS		1 (2%)	
#LYMPH NODE	(37)	(45)	(39)
HYPERPLASIA, LYMPHOID		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE	(37)	(45)	(39)
HEMORRHAGE	3 (8%)	7 (16%)	4 (10%)
DEGENERATION, HYALINE	1 (3%)		
HYPERPLASIA, LYMPHOID	5 (14%)	3 (7%)	4 (10%)
CIRCULATORY SYSTEM			
#LUNG	(49)	(50)	(49)
THROMBOSIS, NOS	1 (2%)		
#HEART	(50)	(50)	(49)
PERIARTERITIS		2 (4%)	
#MYOCARDIUM	(50)	(50)	(49)
INFLAMMATION, ACUTE	1 (2%)		
INFLAMMATION, CHRONIC			1 (2%)
#ENDOCARDIUM	(50)	(50)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
*MESENTERIC ARTERY	(50)	(50)	(50)
INFLAMMATION PROLIFERATIVE		1 (2%)	
*TESTICULAR ARTERY	(50)	(50)	(50)
DEGENERATION, HYALINE		1 (2%)	
#KIDNEY	(50)	(50)	(50)
HEMANGIOMATOSIS			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(48)	(50)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		1 (2%)
#LIVER	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	
INFLAMMATION, MULTIFOCAL		1 (2%)	
INFLAMMATION, ACUTE FOCAL			1 (2%)
NECROSIS, FOCAL	1 (2%)	1 (2%)	
NECROSIS, COAGULATIVE		1 (2%)	
INFARCT, NOS			1 (2%)
FOCAL CELLULAR CHANGE	5 (10%)		3 (6%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, FOCAL		1 (2%)	1 (2%)
ANGIECTASIS	1 (2%)		
#BILE DUCT	(50)	(50)	(50)
MINERALIZATION			1 (2%)
#PANCREAS	(49)	(49)	(49)
CYSTIC DUCTS		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		
CYTOLOGIC DEGENERATION	25 (51%)	28 (57%)	19 (39%)
#PANCREATIC ACINUS	(49)	(49)	(49)
ATROPHY, NOS	2 (4%)	1 (2%)	
#ESOPHAGUS	(50)	(50)	(49)
INFLAMMATION, NOS			1 (2%)
#STOMACH	(49)	(50)	(49)
MINERALIZATION	1 (2%)		
INFLAMMATION, NOS	1 (2%)	1 (2%)	1 (2%)
INFLAMMATION, CHRONIC	1 (2%)		
#DUODENUM	(50)	(48)	(46)
ABSCESS, NOS	1 (2%)		
#LARGE INTESTINE	(50)	(47)	(48)
NEMATODIASIS	2 (4%)		
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
MINERALIZATION	2 (4%)	4 (8%)	7 (14%)
HYDRONEPHROSIS	1 (2%)		
INFLAMMATION, NOS	2 (4%)		
INFLAMMATION, FOCAL			1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR	9 (18%)	12 (24%)	5 (10%)
GLOMERULONEPHRITIS, MEMBRANOUS			1 (2%)
INFLAMMATION, CHRONIC	4 (8%)	4 (8%)	7 (14%)
INFLAMMATION, CHRONIC FOCAL	2 (4%)		1 (2%)
INFARCT, NOS			2 (4%)
INFARCT, FOCAL		4 (8%)	2 (4%)
METAPLASIA, OSSEOUS	3 (6%)	1 (2%)	1 (2%)
#KIDNEY/TUBULE	(50)	(50)	(50)
NECROSIS, NOS			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(39)	(43)	(45)
CYST, NOS			1 (2%)
#ADRENAL CORTEX	(50)	(48)	(49)
HYPERPLASIA, FOCAL	1 (2%)		1 (2%)
#THYROID	(44)	(48)	(47)
MINERALIZATION			1 (2%)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
CYSTIC DUCTS		1 (2%)	
INFLAMMATION, GRANULOMATOUS		1 (2%)	
*SEMINAL VESICLE	(50)	(50)	(50)
INFLAMMATION, CHRONIC	1 (2%)		
#TESTIS	(49)	(49)	(50)
MINERALIZATION	2 (4%)	7 (14%)	8 (16%)
CALCIFICATION, FOCAL	1 (2%)		
ATROPHY, NOS		1 (2%)	1 (2%)
*EPIDIDYMIS	(50)	(50)	(50)
MINERALIZATION		1 (2%)	
LIPOGRANULOMA			1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(50)	(50)	(50)
INFLAMMATION, NOS	5 (10%)	3 (6%)	
*CHOROID PLEXUS	(50)	(50)	(50)
INFLAMMATION, NOS	1 (2%)		1 (2%)
#BRAIN	(50)	(50)	(50)
CORPORA AMYLACEA	21 (42%)	24 (48%)	23 (46%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
INFLAMMATION, PYOGRANULOMATOUS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*MANDIBLE	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(50)
HYPERTROPHY, NOS		1 (2%)	
ALL OTHER SYSTEMS			
PERIORBITAL REGION			
ABSCESS, NOS			1
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED DIETS CONTAINING 2,6-DICHLORO-P-PHENYLENEDIAMINE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
INFLAMMATION, NOS			5 (10%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
CONGESTION, NOS		1 (2%)	
PNEUMONIA, CHRONIC MURINE	1 (2%)	9 (18%)	11 (22%)
HYPERPLASIA, FOCAL		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(49)	(50)	(50)
HYPERPLASIA, LYMPHOID	8 (16%)	8 (16%)	5 (10%)
HEMATOPOIESIS	3 (6%)	1 (2%)	1 (2%)
#LYMPH NODE	(46)	(45)	(48)
HYPERPLASIA, LYMPHOID		1 (2%)	
#SUBMANDIBULAR L.NODE	(46)	(45)	(48)
PLASMACYTOSIS			1 (2%)
#PARASTERNAL LYMPH NO	(46)	(45)	(48)
HEMORRHAGIC CYST		1 (2%)	
#MESENTERIC L. NODE	(46)	(45)	(48)
HEMORRHAGE		1 (2%)	1 (2%)
GRANULOMA, NOS	1 (2%)		
HYPERPLASIA, LYMPHOID	2 (4%)	3 (7%)	1 (2%)
#ADRENAL	(50)	(50)	(50)
HEMATOPOIESIS			1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#THYMUS	(37)	(45)	(34)
HYPERPLASIA, LYMPHOID	1 (3%)		
CIRCULATORY SYSTEM			
#MESENTERIC L. NODE	(46)	(45)	(48)
LYMPHANGIECTASIS	1 (2%)		1 (2%)
#ENDOCARDIUM	(50)	(50)	(50)
INFLAMMATION, CHRONIC			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(47)	(49)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
#LIVER	(50)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		4 (8%)
INFLAMMATION, MULTIFOCAL	1 (2%)		
INFLAMMATION, ACUTE FOCAL			1 (2%)
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL	2 (4%)		1 (2%)
METAMORPHOSIS FATTY		1 (2%)	
FOCAL CELLULAR CHANGE	2 (4%)	5 (10%)	5 (10%)
HYPERPLASIA, FOCAL		1 (2%)	
ANGIECTASIS		1 (2%)	
*GALLBLADDER	(50)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
#PANCREAS	(50)	(48)	(49)
DILATATION/DUCTS		1 (2%)	1 (2%)
CYSTIC DUCTS	1 (2%)	3 (6%)	2 (4%)
INFLAMMATION ACTIVE CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
CYTOLOGIC DEGENERATION	27 (54%)	32 (67%)	22 (45%)
#PANCREATIC ACINUS	(50)	(48)	(49)
ATROPHY, NOS	3 (6%)	4 (8%)	3 (6%)
#STOMACH	(49)	(48)	(50)
MINERALIZATION			1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, NOS		1 (2%)	3 (6%)
*ANUS	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
MINERALIZATION			1 (2%)
GLOMERULONEPHRITIS, NOS	1 (2%)		
LYMPHOCYTIC INFLAMMATORY INFILTR	8 (16%)	21 (42%)	15 (30%)
GLOMERULONEPHRITIS, MEMBRANOUS	1 (2%)		
INFLAMMATION, CHRONIC	3 (6%)	4 (8%)	4 (8%)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
NEPHROSIS, NOS		1 (2%)	
INFARCT, FOCAL		2 (4%)	3 (6%)
AMYLOIDOSIS	1 (2%)		
METAPLASIA, OSSEOUS	3 (6%)		1 (2%)
#KIDNEY/PELVIS	(50)	(50)	(50)
MINERALIZATION		1 (2%)	
#URINARY BLADDER	(50)	(48)	(47)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(43)	(48)	(44)
HEMORRHAGIC CYST	1 (2%)		
ANGIECTASIS		2 (4%)	
#ADRENAL	(50)	(50)	(50)
ANGIECTASIS			1 (2%)
#ADRENAL CORTEX	(50)	(50)	(50)
HYPERPLASIA, FOCAL	1 (2%)		1 (2%)
#THYROID	(46)	(48)	(49)
INFLAMMATION, ACUTE FOCAL			2 (4%)
HYPERPLASIA, FOLLICULAR-CELL			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
CYSTIC DUCTS		1 (2%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#UTERUS	(50)	(50)	(49)
CYST, NOS			4 (8%)
INFLAMMATION, ACUTE	1 (2%)		
#UTERUS/ENDOMETRIUM	(50)	(50)	(49)
CYST, NOS	1 (2%)	1 (2%)	
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, CYSTIC	31 (62%)	39 (78%)	26 (53%)
#ENDOMETRIAL GLAND	(50)	(50)	(49)
CYST, NOS			1 (2%)
#OVARY/OVIDUCT	(50)	(50)	(49)
INFLAMMATION, NOS	1 (2%)		
#OVARY	(47)	(49)	(42)
CYST, NOS	6 (13%)	8 (16%)	2 (5%)
PAROVARIAN CYST		3 (6%)	1 (2%)
HEMORRHAGIC CYST		1 (2%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(50)	(50)	(50)
INFLAMMATION, NOS	8 (16%)	6 (12%)	1 (2%)
*CHOROID PLEXUS	(50)	(50)	(50)
INFLAMMATION, NOS	2 (4%)	3 (6%)	2 (4%)
#BRAIN	(50)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE	1 (2%)		
CORPORA AMYLACEA	21 (42%)	20 (40%)	24 (48%)
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(50)	(50)	(50)
MINERALIZATION		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL VISCERA	(50)	(50)	(50)
INFLAMMATION, GRANULOMATOUS	1 (2%)		
*INGUINAL REGION	(50)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
*MESENTERY	(50)	(50)	(50)
NECROSIS, FAT	1 (2%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
CONGESTION, NOS			1 (2%)
NECROSIS, FAT			1 (2%)
FOOT			
INFLAMMATION, NOS			1
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

APPENDIX E

ANALYSIS OF 2,6-DICHLORO-p-PHENYLENEDIAMINE

(LOT NO. 0005)

MIDWEST RESEARCH INSTITUTE

Appendix E

Analysis of 2,6-Dichloro-p-phenylenediamine (Lot No. 0005) Midwest Research Institute

A. ELEMENTAL ANALYSIS

Element:	C	H	N	Cl
Theory:	40.70	3.42	15.83	40.05
Determined:	40.47	3.36	15.64	39.5±0.4 (δ)
	40.53	3.36	15.73	

B. WATER ANALYSIS

(Karl Fisher)
less than 0.1%

C. TITRATION OF ONE AMINO GROUP WITH PERCHLORIC ACID

99.3%±0.3 (δ) %

D. MELTING POINT

Determined	Literature Values
122°-124°C (visual, capillary)	124°-125°C (Drake et al., 1946)
119°-122°C (Dupont 900DTA)	

E. THIN-LAYER CHROMATOGRAPHY

Plates: Silica gel 60 F254
Amount Spotted: 100 and 300 μg
Ref. Standard: Aniline
Visualization: Ultraviolet, 254 and 366 nm, and visible light

System 1: Benzene:chloroform (80:20)

R_f: 0.19 (major), origin (trace, visualized at 366 nm only)

R_{st}: 0.76, origin

System 2: Methanol, 100%

R_f: 0.72 (trace, visualized at 366 nm only), 0.68 (major)

R_{st}: 1.04, 0.99

D. VAPOR-PHASE CHROMATOGRAPHY

Instrument: Tracor MT 220
Detector: Flame ionization
Column: 3% OV-17, 1.8 mm x 4 mm I.D., glass
Oven temperature program: 100°-225°C at 10°C/min
Results: Major peak and four impurities

Peak	Retention Time (min)	Retention Time (Relative to 2,6-Dichloro-p-phenylenediamine)	Area (Relative to 2,6-Dichloro-p-phenylenediamine)
1	6.1	0.68	0.02
2	6.5	0.72	0.01
3	7.4	0.83	0.01
4	9.0	1.00	100
5	10.1	1.12	0.2

E. SPECTRAL DATA

(1) Infrared

Instrument: Beckman IR-12

Cell: 1.5% KBr pellet

Results: See Figure 5

Consistent with literature spectrum (Sadtler Standard Spectra)

(2) Ultraviolet/Visible

Instrument: Cary 118

λmax (nm)	$\epsilon \times 10^{-3}$
325	3.36±0.03 (δ)
245	11.766±0.008 (δ)

Solvent: 95% ethanol

Literature Values (Sadtler Standard Spectra)

λmax (nm)	$\epsilon \times 10^{-3}$
324.5	3.57
246	11.000
208	26.6

Solvent: methanol

No maximum observed in the visible range (350-800 nm), but there was a gradual increase in absorbance toward 350 nm. Concentration: 0.3 mg/ml.

(3) Nuclear Magnetic Resonance

Instrument: Varian HA-100

Solvent: $CDCl_3$ with internal tetramethylsilane

No literature spectrum found

Assignments: See Figure 6

(a) δ 3.11-3.53 ppm (b) δ 3.77-4.28 ppm

(c) s, δ 6.65 ppm

Integration Ratios: (a + b) 4.11, (c) 2.00

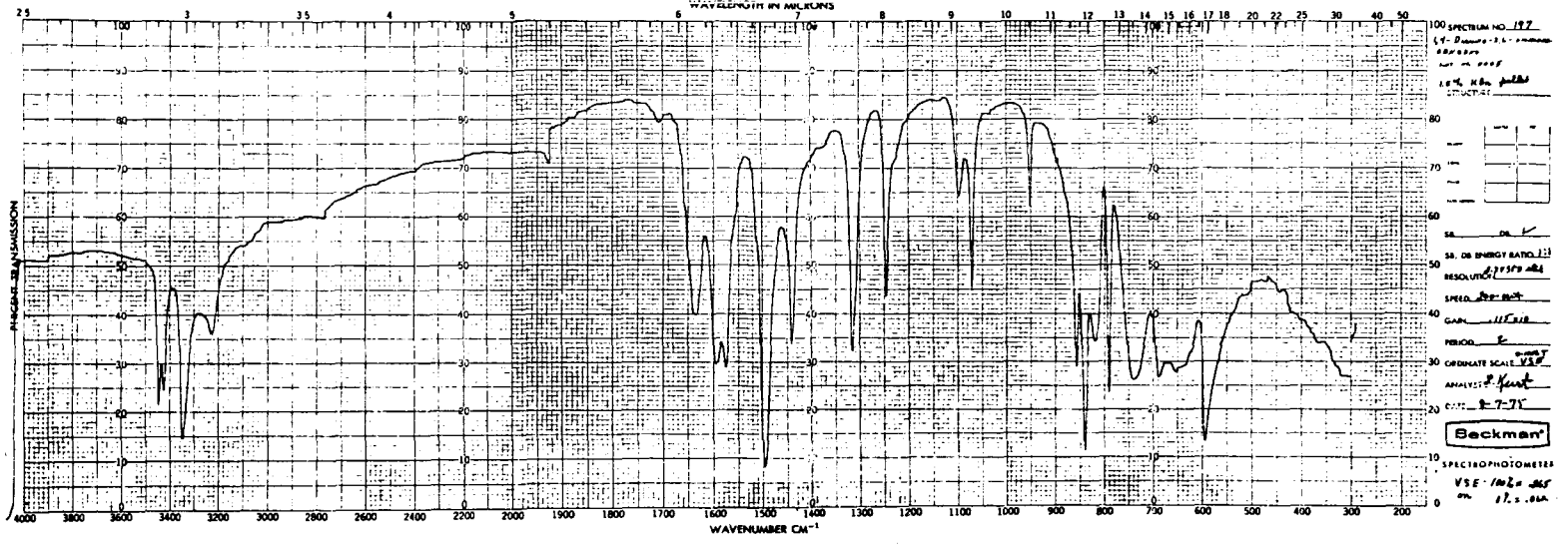

Figure 5. Infrared Absorption Spectrum of 2, 6-Dichloro-p-phenylenediamine (Lot No. 0005)

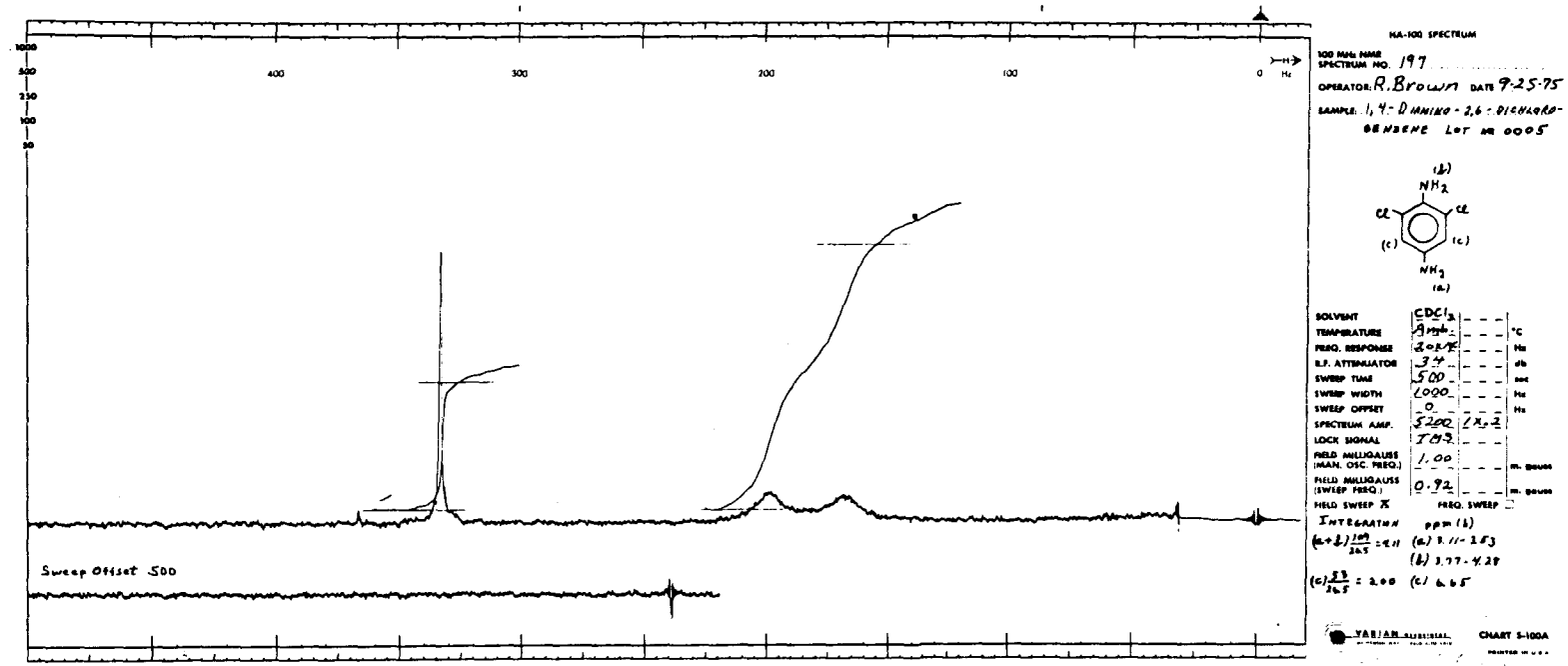

Figure 6. Nuclear Magnetic Resonance Spectrum of 2, 6-Dichloro-p-phenylenediamine (Lot No. 0005)

APPENDIX F

ANALYSIS OF 2,6-DICHLORO-p-PHENYLENEDIAMINE

(LOT NO. R9231-127)

MIDWEST RESEARCH INSTITUTE

Appendix F

Analysis of 2,6-Dichloro-p-phenylenediamine
(Lot No. R9231-127)
Midwest Research Institute

A. ELEMENTAL ANALYSIS

Element:	C	H	N	Cl
Theory:	40.70	3.43	15.83	40.05
Determined:	40.54	3.52	15.64	40.06
	40.65	3.42	15.80	40.19

B. WATER ANALYSIS

(Karl Fisher)
$0.45\% \pm 0.06(\delta)\%$

C. TITRATION OF ONE AMINO GROUP WITH PERCHLORIC ACID

$98.5\% \pm 0.3(\delta)\ \%$

D. MELTING POINT

Determined	Literature Values
124° to 125°C (visual, capillary)	124°-125°C (Drake et al., 1946)

E. THIN-LAYER CHROMATOGRAPHY

Plates: Silica Gel 60 F-254
Amount Spotted: 100 and 300 μg (10 and 30 μl of a 10.0 mg/ml solution in methanol)
Ref. Standard: Aniline, 10 μg
Visualization: UV (254 nm) and ninhydrin

System 1: Benzene:chloroform (80:20)
R_f: 0.09 (major); origin (trace, 254 nm only)
R_{st}: 0.30; origin

System 2: Methanol
R_f: 0.84 (major) (trace, visualized)
R_{st}: 1.03

F. VAPOR-PHASE CHROMATOGRAPHY

Instrument: Varian Aerograph 2400
Detector: Flame ionization
Column: 190°C
Carrier gas: Nitrogen
Oven temperature program: 100°C 3 min, 100° to 250°C at 10°C/min

1. System 1

Detector temperature: 280°C
Carrier flow rate: 30 cc/min
Column: 3% OV-17 on 80/100 Supelcoport, 1.8 m x 2 mm I.D., glass

Sample injected: A 1.0% solution (4 μl) of 2,6-dichloro-p-phenylenediamine in chloroform was injected. A 0.5% solution was injected to check for overloading.

Results: Major peak and one impurity.

Peak	Retention Time (min)	Retention Time (Relative to 2,6-Dichloro-p-phenylenediamine)	Area (Relative to 2,6-Dichloro-p-phenylenediamine)
1	7.8	0.67	0.02
2	11.6	1.00	100

2. System 2

Detector temperature: 260°C

Carrier flow rate: 50 cc/min

Column: 3% OV-1 on 80/100 Supelcoport, 1.8 m x 4 mm I.D., glass

Sample injected: A 1.0% solution (4 μl) of 2,6-dichloro-p-phenylenediamine in chloroform was injected. A 0.5% solution was injected to check for overloading.

Results: Major peak and one impurity.

Peak	Retention Time (min)	Retention Time (Relative to 2,6-Dichloro-p-phenylenediamine)	Area (Relative to 2,6-Dichloro-p-phenylenediamine)
1	7.8	0.75	0.03
2	10.4	1.00	100

G. SPECTRAL DATA

Determined	Literature Values
(1) Infrared:	
Instrument: Beckman IR-12 Cell: 1% potassium bromide pellet Results: See Figure 7	Consistent with literature spectrum (Sadtler Standard Spectra)
(2) Ultraviolet/Visible	
Instrument: Cary 118	

$\lambda max^{(nm)}$	$\epsilon \times 10^{-3}$	$\lambda max^{(nm)}$	$\epsilon \times 10^{-3}$
325	3.33±0.02(δ)	324.5	3.57
246	10.13±0.07(δ)	246	11.000
		208	26.6

No maximum from 350 to 800 nm (visible region), but a gradual increase in absorbance toward 350 nm.

Solvent: 95% ethanol	Solvent: Methanol

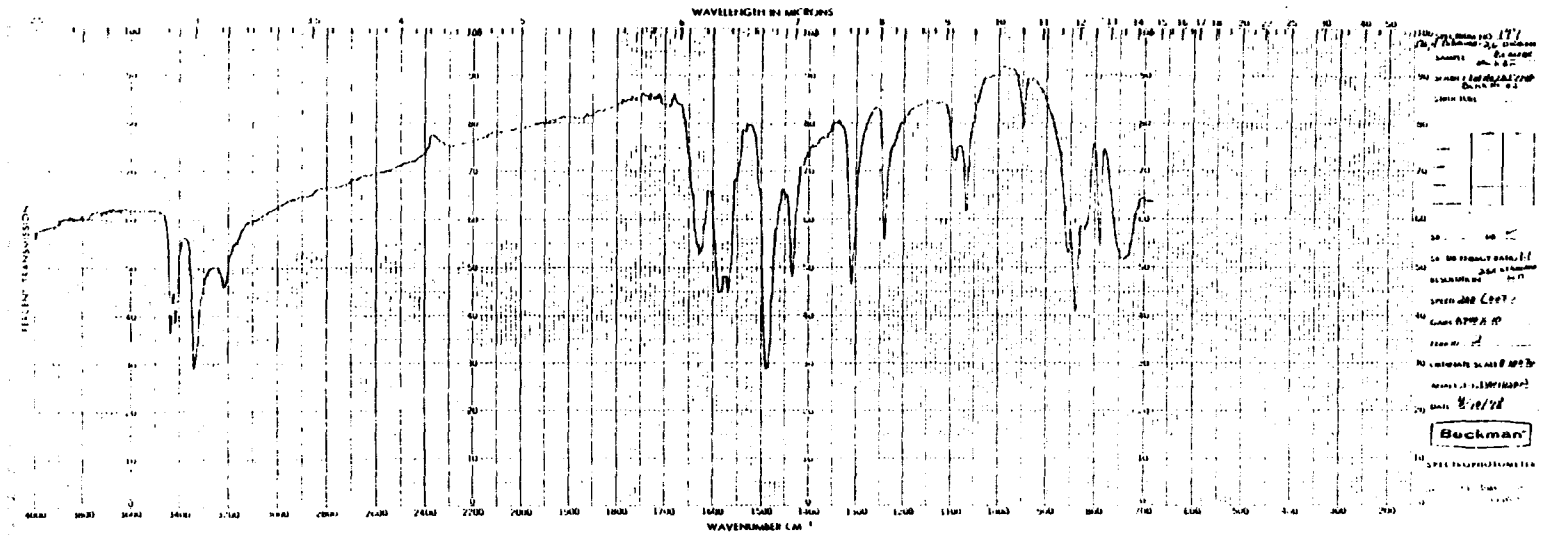

Figure 7. Infrared Absorption Spectrum of 2, 6-Dichloro-p-phenylenediamine (Lot No. R9231-127)

(3) Nuclear Magnetic Resonance

Instrument: Varian EM-360A
Solvent: $CDCl_3$ with internal tetramethylsilane

No literature spectrum found

Assignments (see Figure 8)

(a) δ 3.35 ppm

(b) δ 3.95 ppm

(c) δ 6.60 ppm

(d) δ 1.64 ppm

Integration ratios:

(a)
(b) } 3.99

(c) 2.01

(d) 0.04

The impurity peak present at 1.64 ppm is due to ammonium chloride at a concentration of 1.6% relative to 2,6-dichloro-p-phenylenediamine. This is consistent with the titration value of 98.5%$\pm$0.3%.

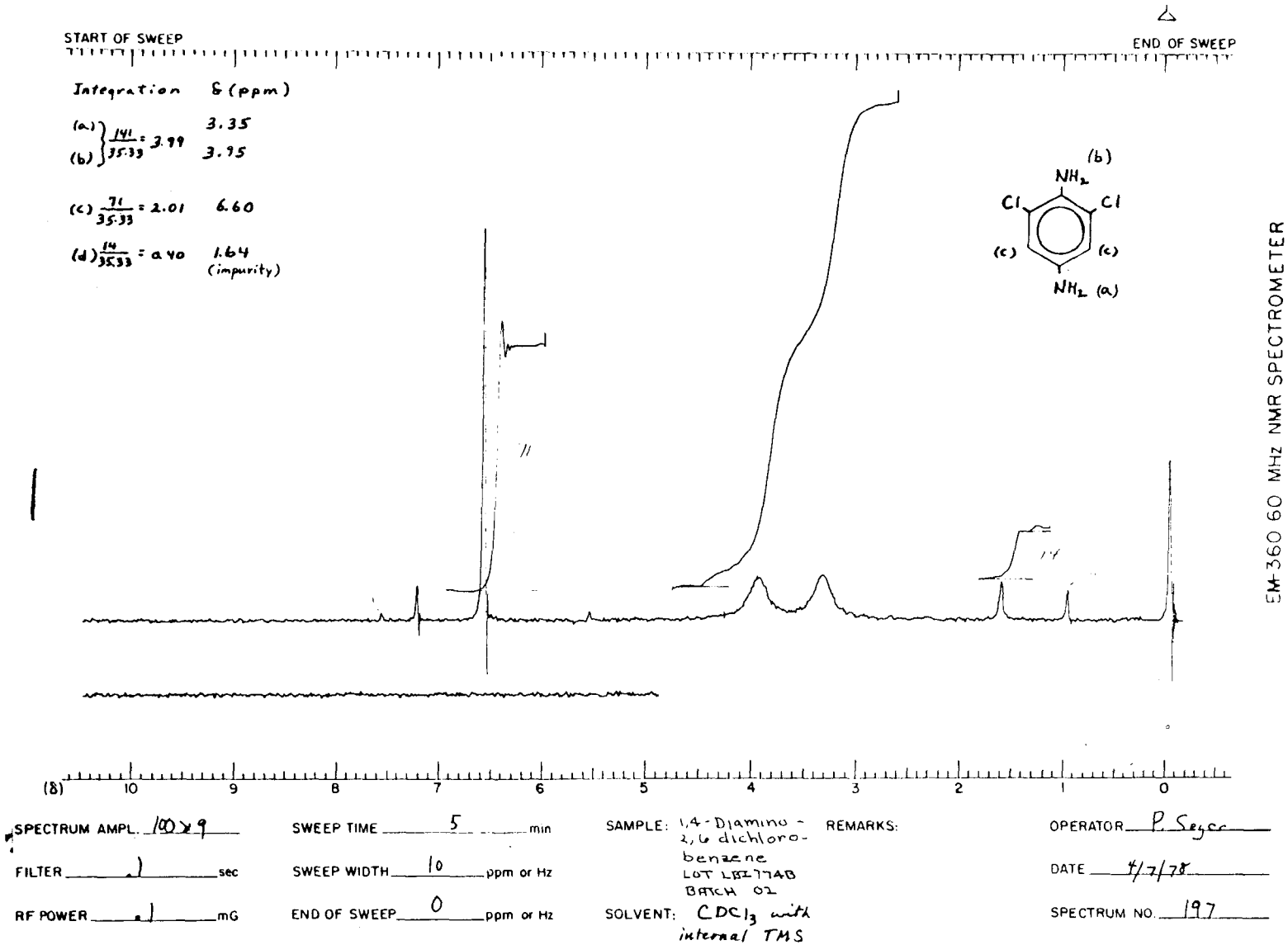

Figure 8. Nuclear Magnetic Resonance Spectrum of 2, 6-Dichloro-p-phenylenediamine (Lot No. R9231-127)

APPENDIX G

ANALYSIS OF FORMULATED DIETS FOR STABILITY OF 2,6-DICHLORO-p-PHENYLENEDIAMINE

MIDWEST RESEARCH INSTITUTE

Appendix G

Analysis of Formulated Diets for
Stability of 2,6-Dichloro-p-phenylenediamine
Midwest Research Institute

A. MIXING AND STORAGE

2,6-Dichloro-p-phenylenediamine (2.5367 g) and Wayne Lab-Blox® Rodent Feed (22.5042 g) were mixed for 15 minutes in a mortar. Samples of this 100,000 ppm mix were then removed and stored for 2 weeks at -20°, 5°, 25°, and 45°C, respectively.

B. ANALYSIS

The samples were mixed with methanol in an ultrasonic bath and triturated using a Polytron mixer. The resulting mixture was centrifuged and extracted in the same manner. The combined extracts were further diluted and analyzed by vapor-phase chromatography using the following system:

Instrument: Tracor MT-220
Column: 3% OV-1 on Supelcoport
80/100, 4 mm x 1.8 m, glass, silanized
Detection: Flame ionization
Oven temperature: 130°C, isothermal
Compound retention time: 3.31 minutes

C. RESULTS

Temperature (°C)	Average % Compound Recovered
-20	9.82±0.50
5	9.08±0.50
25	9.09±0.50
45	9.70±0.50

There were no significant differences between the samples stored at the various temperatures.

D. CONCLUSION

2,6-Dichloro-p-phenylenediamine mixed with feed is stable for 2 weeks at temperatures up to 45°C.

APPENDIX H

ANALYSES OF FORMULATED DIETS FOR CONCENTRATIONS OF 2,6-DICHLORO-p-PHENYLENEDIAMINE

LITTON BIONETICS, INC.

Appendix H

Analyses of Formulated Diets for
Concentrations of 2,6-Dichloro-p-phenylenediamine
Litton Bionetics, Inc.

A. Method

Two-gram samples, accurately weighed, were extracted by shaking for 10 minutes in an automatic shaker with two 50-ml portions of methanol. Each extraction was followed by centrifugation for 10 minutes at 1,350 rpm. The two portions of solvent were combined and mixed well. Analysis was performed by gas chromatography on a Varian Model 2100 instrument equipped with flame ionization detectors. The column used was 1.8 m x 2 mm ID glass packed with 3% OV-1 on 80/100 mesh Supelcoport. The column temperature was 120°C with a nitrogen (carrier) flow rate of 25 ml/min. Concentrations were determined by comparison with standard solutions of the test compound analyzed under the same parameters. In the case of samples containing 1,000 or 2,000 ppm of the test material, 5.0 ml of the extract were reduced to 1.0 ml in a warm water bath under nitrogen prior to analysis.

A control sample was run concurrently for each extraction.

B. Results

Theoretical Concentration in Diet (ppm)	No. of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
1,000	5	861.2	12.8	666-927
2,000	7	1,727.6	8.2	1,507-2,046
3,000	2	2,740.5	0.99	2,721-2,760
6,000	3	6,062.3	2.78	5,916-6,313

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