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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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REPORT ON THE BIOASSAY OF 4-CHLORO-O-PHENYLENEDIAMINE
FOR POSSIBLE CARCINOGENICITY

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

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

CONTRIBUTORS: This bioassay of 4-chloro-o-phenylenediamine was conducted by Mason Research Institute, Worcester, Massachusetts, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. E. Smith (3) and Dr. A. Handler (3). Animal treatment and observation were supervised by Mr. G. Wade (3) and Ms. E. Zepp (3). Chemical analysis was performed by Midwest Research Institute (4) and the analytical results were reviewed by Dr. N. Zimmerman (5).

Histopathologic examinations were performed by Dr. A. S. Krishna Murthy (3) and Dr. A. Russfield (3) at the Mason Research Institute, and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (6).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (7); the statistical analysis was performed by Mr. W. W. Belew (5) and Dr. A. Chu (7), using methods selected for the Bioassay Program by Dr. J. J. Gart (8).

This report was prepared at METREK, a Division of The MITRE Corporation (5) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (5), the task leader, Dr. M. R. Kornreich (5), the senior biologist, Ms. P. Walker (5) and the technical editor, Ms. P. A. Miller (5). The final report was reviewed by members of the participating organizations.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of the NCI: Dr. J. J. Gart (8), Mr. J. Nam (8), Dr. H. M. Pettigrew (8), and Dr. R. E. Tarone (8).

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1), Dr. R. A. Griesemer (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,9), and Dr. J. M. Ward (1).

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SUMMARY

A bioassay for possible carcinogenicity of technical-grade 4-chloro-o-phenylenediamine was conducted using Fischer 344 rats and B6C3F1 mice. 4-Chloro-o-phenylenediamine was administered in the feed, at either of two concentrations, to groups of 49 or 50 male and 50 female animals of each species. For male and female rats, the high and low time-weighted average dietary concentrations of 4-chloro-o-phenylenediamine were 1.0 and 0.5 percent, respectively. For male and female mice, the high and low time-weighted average dietary concentrations were 1.4 and 0.7 percent, respectively. After a 78-week period of chemical administration, observation of the rats continued for up to an additional 28 weeks and observation of the mice continued for up to an additional 18 weeks. Fifty animals of each species and sex were placed on test as controls for the chronic bioassay.

There was a statistically significant positive association between increased dosage and accelerated mortality in female rats and male mice; however, survival among all groups was adequate for meaningful statistical analysis of late-developing tumors.

In male and female rats receiving the test chemical a significantly increased incidence of neoplasms of the urinary bladder occurred. Neoplastic nodules in the liver and tumors of the forestomach may also have been related to administration of the chemical. A significantly increased incidence of hepatocellular carcinomas occurred in chemically treated male and female mice.

It is concluded that under the conditions of this bioassay 4-chloro-o-phenylenediamine was carcinogenic in Fischer 344 rats and B6C3F1 mice, inducing tumors of the urinary bladder and forestomach in both sexes of rats and hepatocellular carcinomas in both sexes of mice.

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I. INTRODUCTION

4-Chloro-o-phenylenediamine (NCI No. C03292), an aromatic amine used as an intermediate in dye production, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer reported among dye manufacturing industry workers (Anthony and Thomas, 1970; Wynder et al., 1963). Aromatic amines are one of several classes of chemicals thought to contribute to the increased cancer risk in this industry (Wynder et al., 1963). The apparent lack of chronic toxicity data for 4-chloro-o-phenylenediamine was an additional factor in its selection for testing.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 4-chloro-1,2-benzenediamine.* It is also known as 4-chloro-1,2-diaminobenzene and Ursol Olive 6G (C.I. [Colour Index] 76015).

4-Chloro-o-phenylenediamine is used in the production of the dye C.I. Vat Brown 22, as an oxidation base (Society of Dyers and Colourists, 1956), and in the synthesis of substituted benzimidazothioxanthenoisoquinolinone dyes for polyester fibers (Kadhim and Peters, 1974). 4-Chloro-o-phenylenediamine has also been used to synthesize experimental drugs tested for prolongation of barbiturate narcosis in mice, such as 4-methyl-7-chloro-2,3-dihydro-1H-1,5-benzodiazepin-2-one

*The CAS registry number is 95-83-0.

(Stolyarchuk et al., 1975) and substituted carbomethoxyaminobenzimidazoles, which possess anthelmintic activity in mice, sheep, and dogs (Actor and Pagano, 1975).

Specific production figures for 4-chloro-o-phenylenediamine are not available; however, the inclusion of the compound in Synthetic Organic Chemicals, United States Production and Sales, 1975 (U.S. International Trade Commission, 1977) implies an annual commercial production in excess of 1000 pounds or $1000 in value. C.I. Vat Brown 22 is not produced in commercial quantities (U.S. International Trade Commission, 1977).

Since 4-chloro-o-phenylenediamine is used solely as an intermediate in synthesis, serious risk of exposure is limited to workers in the dye and chemical industries and those involved in pharmaceutical research.

II. MATERIALS AND METHODS

A. Chemicals

Technical-grade 4-chloro-o-phenylenediamine was purchased from Carroll Products, Wood River Junction, Rhode Island and chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri. The experimentally determined melting point (69° to 72°C) suggested a compound of fairly high purity due to its proximity to the literature value (76°C) and narrow range. Elemental analysis was also quite close to the theoretical. Thin-layer chromatography utilizing two systems (ethyl acetate and benzene:methanol) and visualized with ultraviolet light and salicylaldehyde revealed the presence of two impurities. Vapor phase chromatography also showed the presence of two impurities. Nonaqueous titration of the amine function was approximately 96 percent of the theoretical. It should be emphasized that this test yields a definition of maximum purity and may not be indicative of the actual purity of the compound due to the presence of other amines. Infrared analysis was not inconsistent with the structure of the compound. Ultraviolet analysis yielded λ_{max} at 247 and 304 nm with respective ϵ values of 6860 and 3830. Values reported in the literature were λ_{max} at 247 and 302.5 nm with respective ϵ values of 6725 and 3220. Examination of the lower λ_{max} indicated an approximate purity of 99 percent, assuming no other impurities absorbed in this region. The value for the upper λ_{max} was higher than the literature value and this was probably due to the

presence of impurities absorbing in this region. However, the evidence does suggest a compound of relatively high purity.

Throughout this report the term 4-chloro-o-phenylenediamine is used to represent this technical-grade material.

B. Dietary Preparation

The basal laboratory diet for both treated and control animals was Wayne Lab-Blox® (Allied Mills, Inc., Chicago, Illinois). 4-Chloro-o-phenylenediamine was administered to the treated animals as a component of the diet. The compound was mixed in the diet using a 6 kg capacity Patterson-Kelly stainless steel twin-shell V-blender. The mixtures were prepared once weekly, placed in double plastic bags, and stored in the dark at 4°C.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. Fischer 344 rats and B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Animals of both species were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Treated and control rats arrived in separate shipments, and mice placed in treated groups also arrived in separate shipments from their controls.

Upon arrival, a random sample of animals from each shipment was examined for nematode infestation and other signs of disease. The remaining animals were quarantined for 2 weeks prior to initiation of test. Animals were assigned to groups and distributed among cages so

that average body weight per cage was approximately equal for a given sex and species.

D. Animal Maintenance

All animals were housed by species in rooms having a temperature range of 23° to 34°C and a range in relative humidity of 10 to 85 percent. Incoming air was filtered through Tri-Dek® 15/40 denier Dacron® filters (Tri-Dim Filter Corp., Hawthorne, New Jersey), providing six changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

Rats were housed five per cage by sex. During the quarantine period and for the first 14 months of study, rats were housed in galvanized-steel wire-mesh cages suspended above newpapers. Newspapers were replaced daily and cages and racks washed weekly. For the remainder of the study, rats were housed in suspended solid-bottom polycarbonate cages equipped with disposable nonwoven fiber filter sheets. Corncob bedding (SAN-I-CEL®, Paxton Processing Company, Paxton, Illinois) or hardwood chip bedding (Aspen bedding, American Excelsior Company, Baltimore, Maryland) and clean cages were provided twice weekly. Stainless steel racks were cleaned once every two weeks, and disposable filters were replaced at that time.

Mice were housed by sex in solid-bottom polycarbonate cages fitted with stainless steel lids and nonwoven fiber filter bonnets. Animals were housed ten per cage for the first 12 months of study and five per cage thereafter. Cages, lids, and bedding were changed

three times a week while mice populations were ten per cage and twice a week when the cage populations were reduced to five. Hardwood chip bedding (Ab-sorb-dri®, Wilner Wood Products Company, Norway, Maine) was used during quarantine and the first month of test. Corncob bedding (SAN-I-CEL®) was used for the next 11 months, then replaced by Bed-o-Cobs® (The Andersons Cob Division, Maumee, Ohio) for the next 8 months. Aspen bedding was used for the remainder of the test. Once every two weeks, filters for mouse cages were changed and pipe cage racks were cleaned.

Water was available from 250 ml polycarbonate water bottles fitted with rubber stoppers and stainless steel sipper tubes. Both were replaced twice weekly when animals were housed five per cage, and three times per week when cage populations were ten. Water for rats was supplied as needed between changes. Tap water (chlorinated to 1 ppm at Worcester City Water Department) was used for all animals. Food and water were available ad libitum.

Pelleted Wayne Lab-Blox® was supplied on cage floors during the quarantine period. During the chemical administration phase of study, Wayne Lab-Blox® meal containing the appropriate concentration of the chemical was available to all treated animals. Control animals had untreated meal available. While rats were housed in wire-mesh cages, meal was dispensed in Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc., Woburn, Massachusetts) equipped with stainless steel baffles. While in polycarbonate cages, rats received meal from

stainless steel gangstyle food hoppers (Scientific Cages, Inc., Bryan, Texas). Mice were fed from Alpine® feed cups for the first 14 months of study and from gangstyle hoppers for the remainder of the study. Gangstyle hoppers were changed once per week and Alpine® feed cups twice per week.

Treated and control rats were housed in a room with other rats receiving diets treated with* p-cresidine (120-71-8); 1H-benzotriazole (95-14-7); 2,3,5,6-tetrachloro-4-nitroanisole (2438-88-2); 4-chloro-m-phenylenediamine (5131-60-2); and acetylaminofluorene (53-96-3).

Treated mice were housed in a room with other mice receiving diets treated with acetylaminofluorene (53-96-3); 4-chloro-m-phenylenediamine (5131-60-2); 1H-benzotriazole (95-14-7); fenaminosulf (140-56-7); cupferron (135-20-6); o-anisidine hydrochloride (134-29-0); and p-anisidine hydrochloride (20265-97-8). Control mice were in a room with other mice receiving diets treated with acetylaminofluorene (53-96-3); fenaminosulf (140-56-7); and p-cresidine (120-71-8).

E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of 4-chloro-o-phenylenediamine for administration to treated animals in the chronic bioassay, subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among five groups, each consisting of five males and five females. 4-Chloro-o-phenylenediamine was incorporated into the basal laboratory

*CAS registry numbers are given in parentheses.

diet and supplied ad libitum to four of the five rat groups and four of the five mouse groups in concentrations of 0.03, 0.1, 1.0, and 3.0 percent. The fifth group of each species served as a control, receiving only the basal laboratory diet. The dosed dietary preparations were administered for a period of 8 weeks. Individual body weights were recorded at weekly intervals throughout the study. Food consumption was recorded during weeks 1, 4, and 7. All survivors were sacrificed and necropsied at the end of the subchronic test.

A dosage inducing no mortality, no gross abnormalities, and no mean group body weight depression in excess of 30 percent relative to controls in either sex was to be selected as the initial high concentration for the chronic bioassay.

No gross pathology was observed in any of the animals. Deaths were recorded in all groups receiving 3.0 percent 4-chloro-o-phenylenediamine but not in any other group. Mean body weight depression was approximately 71, 80, 85, and 88 percent in the male rats, female rats, male mice, and female mice, respectively, receiving 1.0 percent of the compound in their feed. The initial high concentration selected for administration to rats and mice in the chronic bioassay was 1.0 percent. A concentration of 2.0 percent, however, was utilized for the mouse bioassay.

F. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, concentrations administered, duration of

treated and untreated observation periods, and the time-weighted average concentrations) are summarized in Tables 1 and 2.

The low dose, high dose, and control rats were all approximately 6 weeks old at the time they were placed on test, but control rats were placed on test one week after dosed rats. The high and low concentrations of 4-chloro-o-phenylenediamine administered to both sexes were 1.0 and 0.5 percent, respectively. At the end of the 78-week dosing period, observation continued for up to an additional 28 weeks.

The low dose, high dose, and control mice were all approximately 6 weeks old at the time they were placed on test, but control mice were placed on test one week after dosed mice. The high and low concentrations of 4-chloro-o-phenylenediamine initially administered to both sexes were 2.0 and 1.0 percent, respectively. In week 34 the concentrations administered to high and low dose mice were lowered to 1.0 and 0.5 percent, respectively, due to high mortality and excess weight depression in high dose rats. At the end of the 78-week dosing period, observation continued for up to an additional 18 weeks.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. From the first day, all animals were inspected twice daily for mortality. Body weights were recorded twice weekly for the first 12 weeks of the study and at monthly intervals thereafter. Food consumption, for two cages from each group, was monitored for seven consecutive days once a month for the first nine months of

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
4-CHLORO-O-PHENYLENEDIAMINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	4-CHLORO-O-PHENYLENEDIAMINE CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	50	0	0	105
LOW DOSE	49	0.5 0	78	27
HIGH DOSE	50	1.0 0	78	28
FEMALE				
CONTROL	50	0	0	106
LOW DOSE	50	0.5 0	78	28
HIGH DOSE	50	1.0 0	78	28

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
4-CHLORO-O-PHENYLENEDIAMINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	4-CHLORO-O-PHENYLENEDIAMINE CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION[a]
MALE					
CONTROL	50	0	0	97	0
LOW DOSE	50	1.0 0.5 0	33 45	 17	0.7
HIGH DOSE	50	2.0 1.0 0	33 45	 18	1.4
FEMALE					
CONTROL	50	0	0	97	0
LOW DOSE	50	1.0 0.5 0	33 45	 18	0.7
HIGH DOSE	50	2.0 1.0 0	33 45	 18	1.4

[a] Time-weighted average concentration = $\frac{\Sigma(\text{concentration X weeks received})}{\Sigma(\text{weeks receiving chemical})}$

the bioassay and for three consecutive days each month thereafter. The presence of tissue masses and lesions was determined by monthly observation and palpation of each animal.

A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized by carbon dioxide inhalation, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of major tissues, organs, or gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

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

Tissues for which slides were prepared were preserved in 10 percent buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination. An occasional section was subjected to special staining techniques for more definitive diagnosis.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized,

or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were placed on experiment in each group.

H. Data Recording and Statistical Analyses

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

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on

survival used the method of Cox (1972) for testing two groups for equality and used Tarone's (1975) extensions of Cox's methods for testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality

(Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

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

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

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

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

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses.

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

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

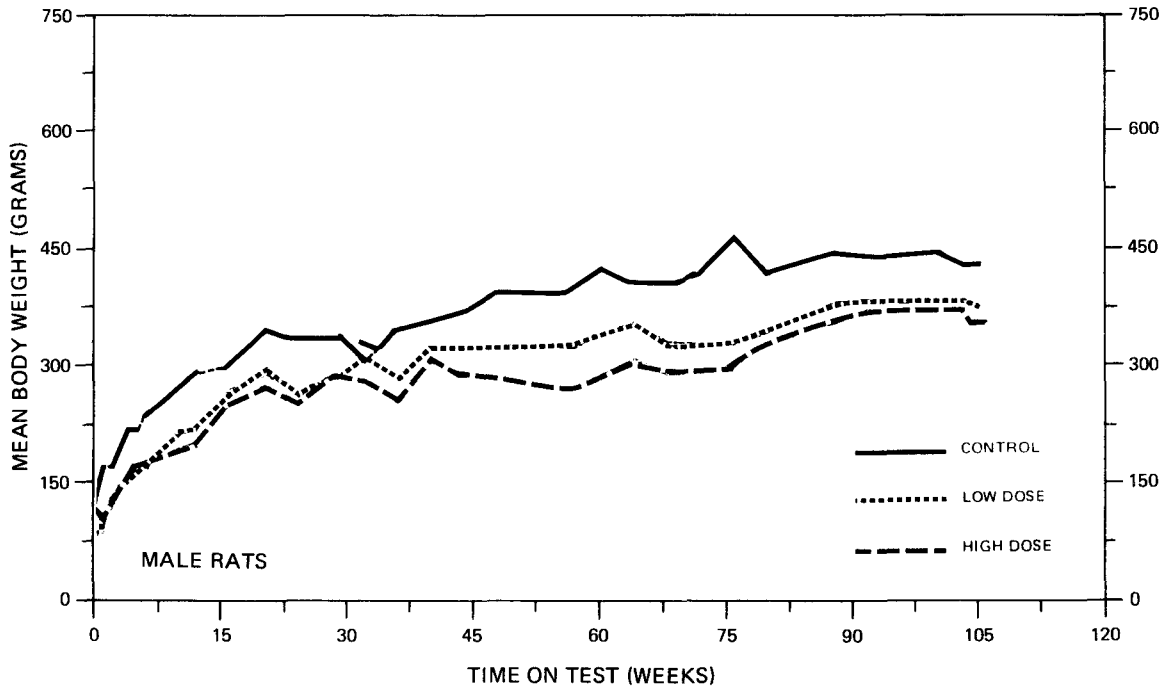
Distinct dose-related mean body weight depression was apparent among treated male and female rats from week 10 until the end of the bioassay (Figure 1).

Two low dose males had palpable scrotal masses, one high dose male had a firm abdominal mass, and four low dose females had subcutaneous masses. One low dose female displayed eye discoloration and in another, an exudate from the left ear was observed. Among controls, three females had firm subcutaneous masses, two males had scrotal masses, one male had a subcutaneous nodular mass, one male had a mass at the base of the tail, and several control males exhibited discoloration of the eyes.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 4-chloro-o-phenylenediamine-dosed groups are shown in Figure 2.

For males the Tarone test did not indicate a statistically significant positive association between dose and mortality. The actual survival was adequate for meaningful statistical analysis despite the sacrifice of five high dose and five control rats in week 78, as 56 percent (28/50) of the high dose, 80 percent (39/49) of the low dose, and 64 percent (32/50) of the control rats survived until the end of the study.



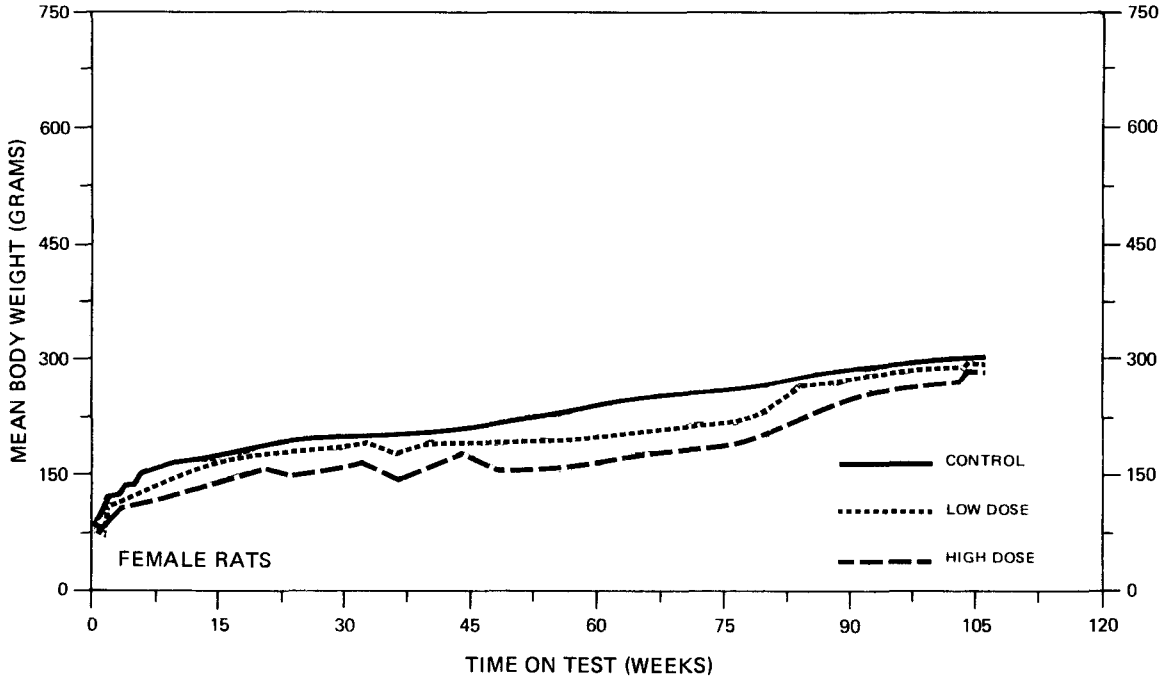
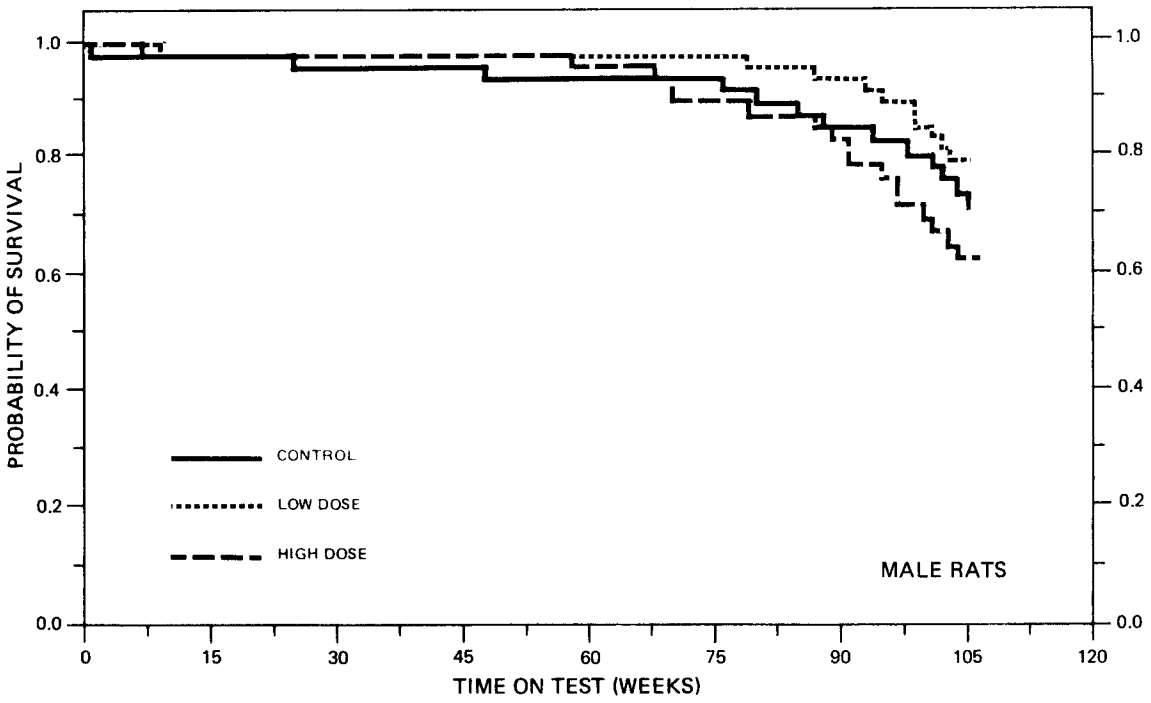


FIGURE 1
GROWTH CURVES FOR 4-CHLORO-O-PHENYLENEDIAMINE CHRONIC STUDY RATS



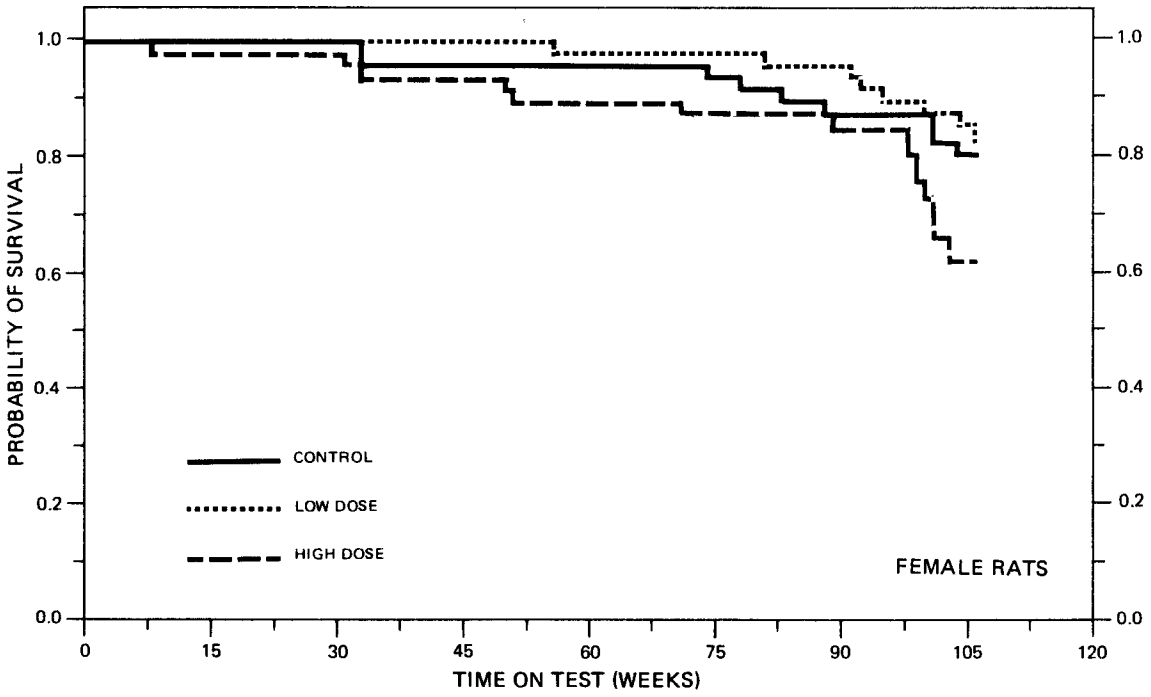


FIGURE 2
SURVIVAL COMPARISONS OF 4-CHLORO-O-PHENYLENEDIAMINE CHRONIC STUDY RATS

For females the Tarone test indicated a significant association between increased dosage and accelerated mortality. The actual survival was adequate for meaningful statistical analysis despite the sacrifice of five high dose and five control rats in week 78, as 54 percent (27/50) of the high dose, 84 percent (42/50) of the low dose, and 72 percent (36/50) of the control rats survived until the end of the study.

C. Pathology

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

As shown in Tables A1 and A2, the incidence of neoplasms in treated rats was higher than in controls. Neoplastic nodules of the liver and tumors of the forestomach and urinary bladder were the predominant neoplasms that occurred in treated rats.

The neoplastic nodules of the liver were found in 10 treated rats (4/47 low dose males, 4/48 high dose males, and 2/46 high dose females) but in none of the control rats.

Neoplasms of the forestomach developed in 9 high dose rats (4/48 males and 5/46 females). Five of these tumors were diagnosed as squamous-cell papillomas and three as squamous-cell carcinomas. A squamous-cell carcinoma of the stomach had metastasized to the liver and lung in one rat.

A spectrum of changes occurred in the urinary bladders of treated rats ranging from transitional-cell hyperplasia to transitional-cell papilloma to transitional-cell carcinoma as summarized in the following table:

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Number of Animals with Tissues Examined Histopathologically	48	42	49	47	46	45
Papilloma NOS	0	0	2	0	1	0
Papillomatosis	0	0	0	0	0	2
Transitional-Cell Papilloma	0	8	5	0	9	8
Transitional-Cell Carcinoma	0	7	18	0	4	22
Papillary Carcinoma	0	0	0	0	1	0
Squamous-Cell Carcinoma	0	0	3	0	0	0
Adenocarcinoma NOS	0	0	1	0	0	0

A focal increase of cells in the urinary bladder epithelium was considered to be transitional-cell hyperplasia. A polypoid mass covered with markedly cellular transitional-cell epithelium with a delicate fibrovascular septa was diagnosed as transitional-cell papilloma. This tumor was neither extensive nor infiltrating. Cells in this growth were much like those in the normal bladder epithelium. Transitional-cell carcinomas were characterized as solitary or multiple papillary tumors which grew into the lumen of the bladder. Tumor cells were arranged in villi or as syncitia. There was a pleomorphism in shape and size of cells. Mitotic figures were numerous. Squamous metaplasia had occurred in a few tumors. Some of the tumors were

infiltrating. Emboli of tumor cells were occasionally recognized in blood vessels or lymphatics. In two rats, this tumor had metastasized to lymph nodes and the lung or spleen. Squamous-cell carcinoma of the urinary bladder was diagnosed in 3/49 high dose male rats.

Except for hydronephrosis of the kidney in 1/47 low dose and 6/48 high dose males and 8/47 high dose females, there were no other compound-related nonneoplastic lesions.

In conclusion, 4-chloro-o-phenylenediamine is considered to be carcinogenic to Fischer 344 rats for the following reasons: tumors of the urinary bladder occurred in 45 male and 47 female treated rats, but in none of the controls, and neoplastic nodules of the liver and tumors of the forestomach were observed in treated rats but not in the control rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of tumor in either sex where at least two such tumors were observed in at least one of the control or 4-chloro-o-phenylenediamine-dosed groups and where such tumors were observed in at least 5 percent of the group.

Neoplasms were noted in large numbers of the urinary bladders of treated rats of both sexes: 0/48 control, 15/42 low dose, and 30/49 high dose male rats had a urinary bladder tumor, as did 0/47 control, 15/46 low dose, and 32/45 high dose female rats. For male rats the

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 4-CHLORO-O-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	3/48(0.06)	1/47(0.02)	1/50(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.340	0.320
Lower Limit	---	0.007	0.006
Upper Limit	---	4.058	3.822
Weeks to First Observed Tumor	105	105	91
Lung: Alveolar/Bronchiolar Adenoma[b]	3/48(0.06)	5/47(0.11)	4/48(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.702	1.333
Lower Limit	---	0.354	0.237
Upper Limit	---	10.411	8.665
Weeks to First Observed Tumor	105	105	91
Hematopoietic System:Leukemia or Malignant Lymphoma[b]	7/48(0.15)	4/47(0.09)	4/50(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.584	0.549
Lower Limit	---	0.133	0.125
Upper Limit	---	2.135	2.011
Weeks to First Observed Tumor	80	93	95

TABLE 3 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma[b]	10/45(0.22)	5/39(0.13)	4/42(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.577	0.429
Lower Limit	---	0.168	0.106
Upper Limit	---	1.678	1.358
Weeks to First Observed Tumor	102	101	106
Adrenal: Pheochromocytoma[b]	4/46(0.09)	5/46(0.11	5/48(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.250	1.198
Lower Limit	---	0.286	0.276
Upper Limit	---	5.928	5.687
Weeks to First Observed Tumor	78	105	106
Thyroid: Follicular-Cell Adenoma or Follicular-Cell Carcinoma[b]	0/43(0.00)	0/40(0.00)	3/40(0.08)
P Values[c]	P = 0.034	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.647
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	97

TABLE 3 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	5/43(0.12)	1/40(0.03)	0/40(0.00)
P Values[c]	P = 0.013(N)	N.S.	P = 0.033(N)
Relative Risk (Control)[d]	---	0.215	0.000
Lower Limit	---	0.005	0.000
Upper Limit	---	1.806	0.846
Weeks to First Observed Tumor	94	105	---
Liver: Neoplastic Nodule[b]	0/48(0.00)	4/47(0.09)	4/48(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.948	0.929
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	101	78
Urinary Bladder: Squamous-Cell Carcinoma[b]	0/48(0.00)	0/42(0.00)	3/49(0.06)
P Values[c]	P = 0.042	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.592
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	89

TABLE 3 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Urinary Bladder: Transitional-Cell Carcinoma[b]	0/48(0.00)	7/42(0.17)	18/49(0.37)
P Values[c]	P < 0.001	P = 0.004	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	2.229	5.642
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	99	70
Urinary Bladder: Papilloma NOS, Transitional-Cell Papilloma, or Transitional-Cell Carcinoma[b]	0/48(0.00)	15/42(0.36)	25/49(0.51)
P Values[c]	P < 0.001	P < 0.001	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	5.407	8.070
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	99	58
Testis: Interstitial-Cell Tumor[b]	37/48(0.77)	33/45(0.73)	40/47(0.85)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.951	1.104
Lower Limit	---	0.741	0.889
Upper Limit	---	1.225	1.338
Weeks to First Observed Tumor	78	93	78

TABLE 3 (Concluded)

[a]Treated groups received time-weighted average doses of 0.5 or 1.0 percent in feed.

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

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

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

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 4-CHLORO-O-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	0/50(0.00)	5/49(0.10)	0/47(0.00)
P Values[c]	N.S.	P = 0.027	N.S.
Departure from Linear Trend[e]	P = 0.002	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	1.282	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	105	---
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	4/50(0.08)	6/49(0.12)	5/49(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.531	1.276
Lower Limit	---	0.386	0.292
Upper Limit	---	6.952	6.068
Weeks to First Observed Tumor	101	92	89
Stomach: Squamous-Cell Papilloma[b]	0/49(0.00)	0/49(0.00)	3/46(0.07)
P Values[c]	P = 0.033	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.638
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	78

TABLE 4 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Carcinoma[b]	0/43(0.00)	1/45(0.02)	3/44(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.052	0.593
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	101
Thyroid: Follicular-Cell Adenoma or Follicular-Cell Carcinoma[b]	0/43(0.00)	2/45(0.04)	4/44(0.09)
P Values[c]	P = 0.038	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.285	0.910
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	78
Mammary Gland: Fibroadenoma[b]	6/50(0.12)	10/49(0.20)	0/49(0.00)
P Values[c]	P = 0.041(N)	N.S.	P = 0.014(N)
Departure from Linear Trend[e]	P = 0.008	---	---
Relative Risk (Control)[d]	---	1.701	0.000
Lower Limit	---	0.609	0.000
Upper Limit	---	5.266	0.637
Weeks to First Observed Tumor	105	105	---

TABLE 4 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Urinary Bladder: Papillary Carcinoma or Transitional-Cell Carcinoma[b]	0/47(0.00)	5/46(0.11)	22/45(0.49)
P Values[c]	P < 0.001	P = 0.026	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	1.292	7.517
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	78
Urinary Bladder: Papillary Carcinoma, Transitional-Cell Carcinoma, Papilloma NOS, Papillomatosis, or Transitional-Cell Papilloma[b]	0/47(0.00)	15/46(0.33)	32/45(0.71)
P Values[c]	P < 0.001	P < 0.001	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	4.828	11.322
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	78
Pituitary: Adenoma[b]	16/40(0.40)	15/38(0.39)	7/38(0.18)
P Values[c]	P = 0.030(N)	N.S.	P = 0.032(N)
Relative Risk (Control)[d]	---	0.987	0.461
Lower Limit	---	0.534	0.182
Upper Limit	---	1.808	1.038
Weeks to First Observed Tumor	101	105	101

TABLE 4 (Concluded)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Adrenal: Pheochromocytoma[b]	6/48(0.13)	2/47(0.04)	4/46(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.340	0.696
Lower Limit	---	0.035	0.153
Upper Limit	---	1.799	2.741
Weeks to First Observed Tumor	105	105	99

[a]Treated groups received time-weighted average doses of 0.5 or 1.0 percent in feed.

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

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

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

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

Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dosage and the incidence of transitional-cell carcinomas. The Fisher exact tests confirmed these findings with significant comparisons of both high dose ($P < 0.001$) and low dose ($P = 0.004$) to control. Additionally, when incidences were combined so that the numerator represented male rats with either a transitional-cell carcinoma, a transitional-cell papilloma, or a papilloma NOS, the Cochran-Armitage test indicated a significant ($P < 0.001$) dose-response association. Again, the Fisher exact test confirmed these findings with significant comparisons for both high dose ($P < 0.001$) and low dose ($P < 0.001$).

Results for the female rats were quite similar. Additionally, when incidences were combined so that the numerator represented an animal with either a papillary carcinoma, a transitional-cell carcinoma, a papilloma NOS, a papillomatosis, or a transitional-cell papilloma of the urinary bladder, the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dosage and incidence. The Fisher exact tests confirmed this with significant ($P < 0.001$) comparisons of both high dose and low dose to control. When incidences were combined so that the numerator represented female rats with either a papillary carcinoma or a transitional-cell carcinoma of the urinary bladder, the Cochran-Armitage test indicated a significant ($P < 0.001$) association between dosage and incidence. The Fisher exact test comparing high dose to control confirmed this with a

significant ($P < 0.001$) result; the Fisher exact test comparing low dose to control had a probability level of $P = 0.026$, a marginal result which was not significant under the Bonferroni criterion.

Based upon these statistical results the administration of 4-chloro-o-phenylenediamine was associated with an increased incidence of urinary bladder tumors in both male and female rats.

The Cochran-Armitage test indicated a significant positive association between dosage and the incidences of follicular-cell thyroid tumors ($P = 0.034$) in males and of squamous-cell papillomas of the stomach ($P = 0.033$) and follicular-cell thyroid tumors ($P = 0.038$) in females. These results were not supported, however, by the findings of the Fisher exact tests. For alveolar/bronchiolar adenomas in females the Fisher exact test comparing low dose to control showed a probability level of $P = 0.027$, a marginal result which was not significant under the Bonferroni criterion.

The possibility of a negative association between dosage and incidence was observed for mammary fibroadenomas in females. In historical data compiled by Mason Research Institute for the NCI Bioassay Program 115/585 (20 percent) untreated female Fischer 344 rats had this neoplasm.

For C-cell thyroid neoplasms in the males and for pituitary adenomas in the females, the Cochran-Armitage test indicated a significant negative association between dosage and incidence. The Fisher exact tests, however, were not significant.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

Compound-related mean body weight depression was observed in male and female mice (Figure 3).

No adverse clinical signs were observed among treated or control mice of either sex.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 4-chloro-o-phenylenediamine-dosed groups are shown in Figure 4.

For male mice the Tarone test indicated a significant positive association between dosage and mortality. The actual survival, however, was adequate for meaningful statistical analysis despite the sacrifice of five high dose and five control mice in week 78, as 70 percent (35/50) of the high dose, 84 percent (42/50) of the low dose, and 84 percent (42/50) of the control mice survived until the end of the study.

For female mice no significant positive association between dose and mortality was detected. The actual survival was adequate for meaningful statistical analysis despite the sacrifice of five high dose and five control mice in week 78, as 78 percent (39/50) of the high dose, 88 percent (44/50) of the low dose, and 72 percent (36/50) of the control mice survived until the end of the study.

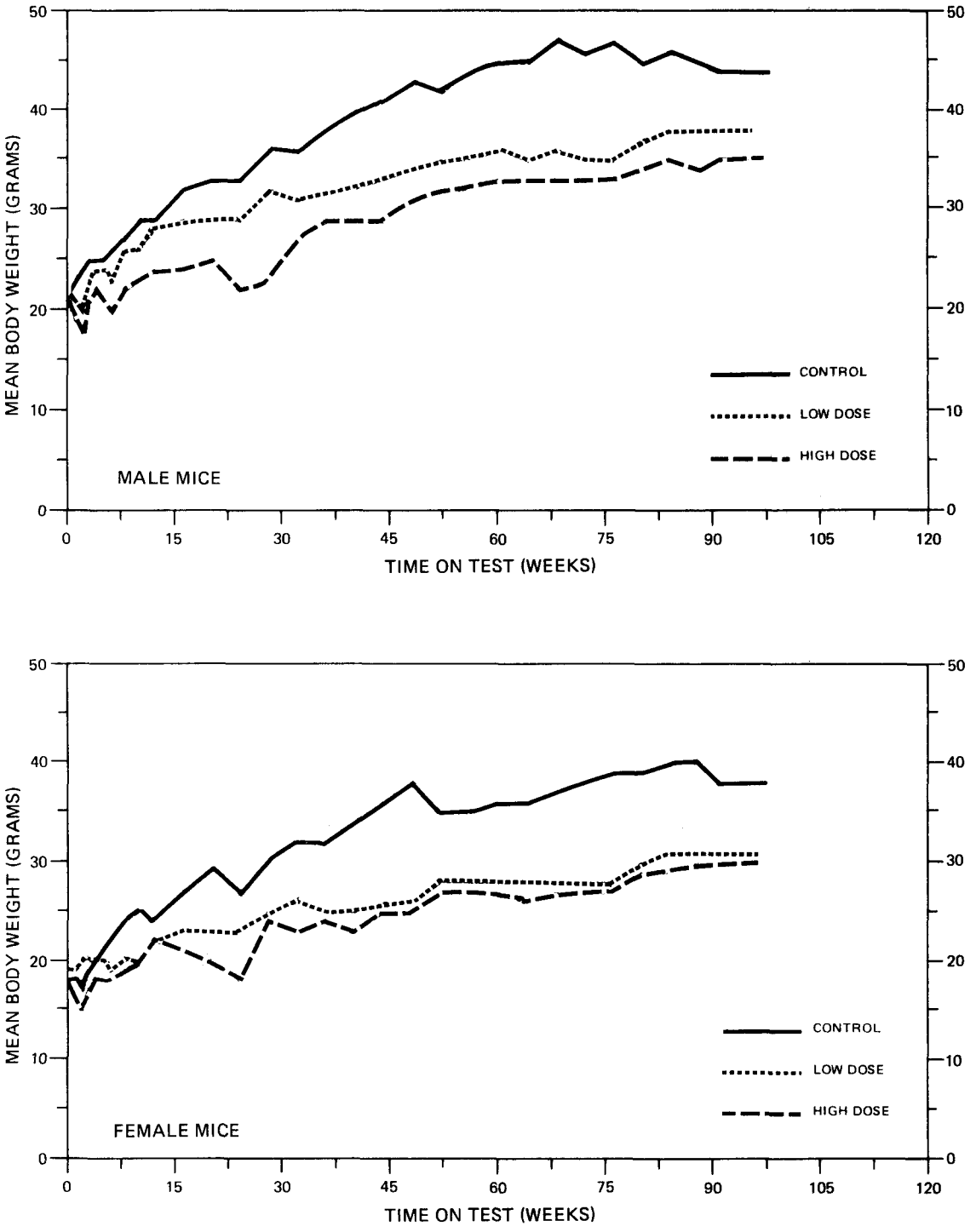
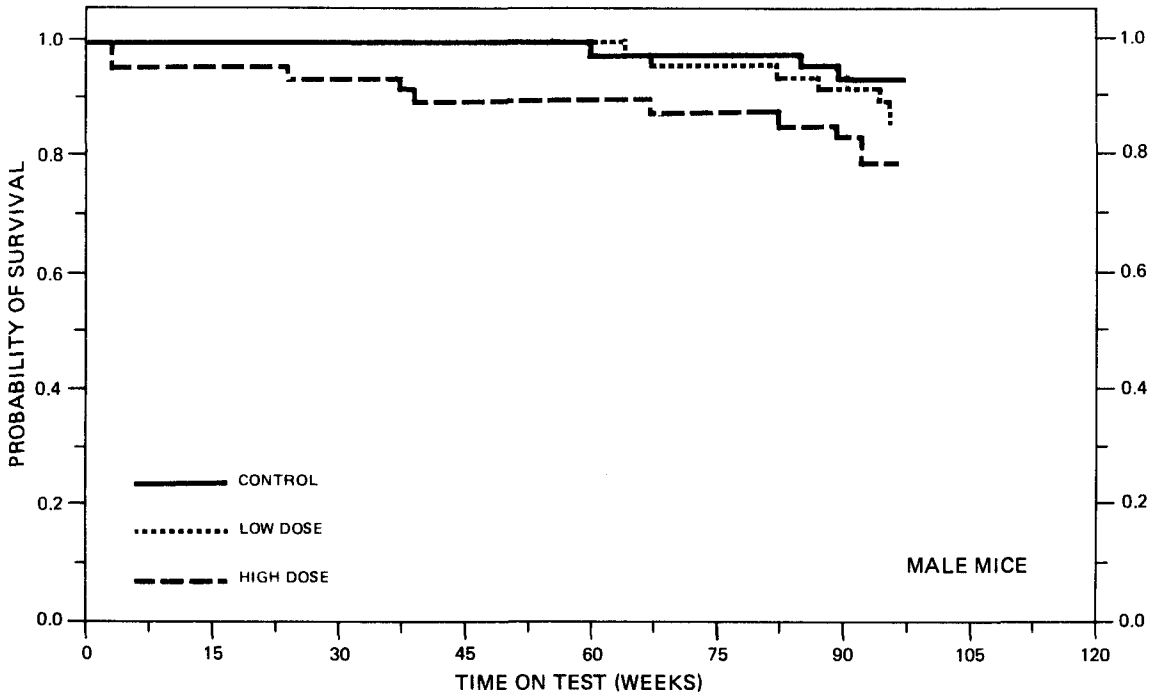


FIGURE 3
GROWTH CURVES FOR 4-CHLORO-O-PHENYLENEDIAMINE CHRONIC STUDY MICE



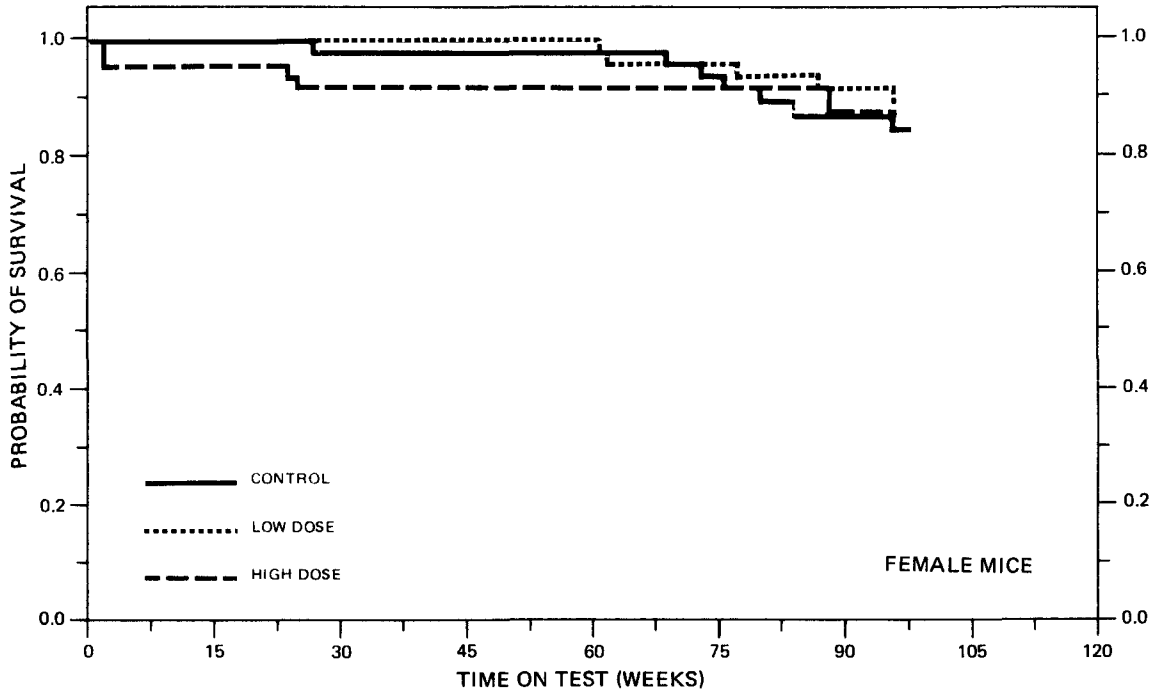


FIGURE 4
SURVIVAL COMPARISONS OF 4-CHLORO-O-PHENYLENEDIAMINE CHRONIC STUDY MICE

C. Pathology

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

The incidence of neoplasms in mice treated with 4-chloro-o-phenylenediamine was higher than that in control mice. Hepatocellular neoplasms accounted for most of the neoplasms in treated mice, and their distribution is summarized in the following table:

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Number of Animals with Tissues Examined Histopathologically	50	49	47	46	48	47
Hepatocellular Carcinoma	10	18	26*	0	4	6
Hepatocellular Adenoma or Carcinoma	15	28	34	0	11	10

*Metastatic to lung (1).

The hepatocellular neoplasms occurred in more male than female mice (control or treated). A small circumscribed area comprised of large hepatocytes with eosinophilic cytoplasm and vesicular nuclei and loss of lobular architecture was considered to be a hepatocellular adenoma. Hepatocellular carcinomas had replaced a part of or a whole lobe of the liver. The normal lobular architecture was distorted. Transformed

hepatocytes were large. Cytoplasm of the cells was eosinophilic; in some cells it was vacuolated and suggested fatty metamorphosis. Pleomorphism in nuclear size was evident. There were a few mitotic figures. A hepatocellular carcinoma metastasized to the lung in one high dose male mouse.

A variety of nonneoplastic lesions were present in treated and control mice. The only compound-related nonneoplastic lesion was hyperplasia of the gall bladder epithelium which occurred in eight treated mice.

The results of this histopathologic examination indicate that there was a compound-related increase in the incidence of hepatocellular neoplasms in male and female mice.

D. Statistical Analyses of Results

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

Significant numbers of liver neoplasms were observed in both male and female mice. For males the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dosage and the incidence of hepatocellular carcinomas. The significance ($P < 0.001$) of the Fisher exact test comparing high dose to control confirmed this

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH 4-CHLORO-O-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	2/50(0.04)	5/48(0.10)	2/47(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.604	1.064
Lower Limit	---	0.451	0.080
Upper Limit	---	26.304	14.153
Weeks to First Observed Tumor	97	87	96
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	7/50(0.14)	9/48(0.19)	7/47(0.15)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.339	1.064
Lower Limit	---	0.483	0.344
Upper Limit	---	3.894	3.278
Weeks to First Observed Tumor	97	87	78
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	4/50(0.08)	4/49(0.08)	3/47(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.020	0.798
Lower Limit	---	0.200	0.122
Upper Limit	---	5.183	4.463
Weeks to First Observed Tumor	97	94	82

TABLE 5 (Continued)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	10/50(0.20)	18/49(0.37)	26/47(0.55)
P Values[c]	P < 0.001	N.S.	P < 0.001
Relative Risk (Control)[d]	---	1.837	2.766
Lower Limit	---	0.900	1.475
Upper Limit	---	3.968	5.519
Weeks to First Observed Tumor	60	82	39
Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma[b]	15/50(0.30)	28/49(0.57)	34/47(0.72)
P Values[c]	P < 0.001	P = 0.006	P < 0.001
Relative Risk (Control)[d]	---	1.905	2.411
Lower Limit	---	1.140	1.518
Upper Limit	---	3.246	3.817
Weeks to First Observed Tumor	60	82	39
Thyroid: Follicular-Cell Adenoma or Follicular-Cell Carcinoma[b]	2/39(0.05)	2/46(0.04)	3/41(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.848	1.427
Lower Limit	---	0.064	0.174
Upper Limit	---	11.248	16.339
Weeks to First Observed Tumor	97	95	96

TABLE 5 (Concluded)

[a]Treated groups received time-weighted average doses of 0.7 or 1.4 percent in feed.

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

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

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

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 4-CHLORO-O-PHENYLENEDIAMINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	3/46(0.07)	1/48(0.02)	0/45(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.319	0.000
Lower Limit	---	0.006	0.000
Upper Limit	---	3.809	1.694
Weeks to First Observed Tumor	97	96	---
Lung: Alveolar/Bronchiolar Adenoma or Alveolar/Bronchiolar Carcinoma[b]	4/46(0.09)	2/48(0.04)	3/45(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.479	0.767
Lower Limit	---	0.045	0.118
Upper Limit	---	3.171	4.275
Weeks to First Observed Tumor	97	96	96
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	6/47(0.13)	11/48(0.23)	3/48(0.06)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.029	---	---
Relative Risk (Control)[d]	---	1.795	0.490
Lower Limit	---	0.669	0.083
Upper Limit	---	5.438	2.148
Weeks to First Observed Tumor	69	87	88

TABLE 6 (Concluded)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	0/46(0.00)	4/48(0.08)	6/47(0.13)
P Values[c]	P = 0.014	N.S.	P = 0.014
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.891	1.570
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	95	78
Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma[b]	0/46(0.00)	11/48(0.23)	10/47(0.21)
P Values[c]	P = 0.003	P < 0.001	P = 0.001
Departure from Linear Trend[e]	P = 0.048	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	3.325	3.043
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	95	78

[a]Treated groups received time-weighted average doses of 0.7 or 1.4 percent in feed.

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

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

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

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

finding. When incidences were combined so that the numerator represented a male with either a hepatocellular carcinoma or a hepatocellular adenoma, the results were stronger: The Fisher exact test comparing low dose to control ($P = 0.006$), the high dose comparison ($P < 0.001$), and the Cochran-Armitage test ($P < 0.001$) were all significant.

For females the Cochran-Armitage test also indicated a significant ($P = 0.014$) positive association between dosage and the incidence of hepatocellular carcinomas. The comparison of high dose to control ($P = 0.014$) confirmed these findings. When incidences were combined so that the numerator represented females with either a hepatocellular carcinoma or a hepatocellular adenoma, the Cochran-Armitage test was again significant ($P = 0.003$). The departure from linear trend was significant ($P = 0.048$), primarily because of the elevated incidences observed in both dosed groups. The Fisher exact tests were significant ($P \leq 0.001$) for both high and low dose females.

Based on these results the statistical conclusion is that there was a positive association between the administration of 4-chloro-o-phenylenediamine and the incidence of liver tumors in mice of both sexes.

V. DISCUSSION

Survival among all groups in this bioassay was adequate for meaningful statistical analysis of late-developing tumors.

In rats, a broad spectrum of neoplasms of the urinary bladder was observed among treated males and females (i.e., the incidence of total tumors of the bladder was 0/48 control males, 15/42 low dose males, 30/49 high dose males, 0/47 control females, 15/46 low dose females, and 32/45 high dose females). These tumors included primarily transitional-cell papillomas and transitional-cell carcinomas. For males, when incidences were grouped so that the numerator represented rats with a papilloma NOS or transitional-cell papilloma of the bladder, the Cochran-Armitage test indicated a significant positive association between dosage and incidence. This association was substantiated by significant results of the low dose to control and high dose to control Fisher exact comparisons. When the incidences were grouped so that the numerator represented males with bladder carcinomas (i.e., squamous-cell carcinoma, transitional-cell carcinoma, or adenocarcinoma NOS) the Cochran-Armitage test and both Fisher exact comparisons were significantly positive. In the females, when the numerator of the incidence represented those rats with papilloma NOS, papillomatosis, or transitional-cell papilloma, the Cochran-Armitage test indicated a significant positive association between dosage and the incidence of these bladder tumors. In addition, the high dose and low dose to control Fisher exact tests confirmed the

relationship. When the numerator of the incidence represented females with papillary carcinomas or transitional-cell carcinomas, the Cochran-Armitage test and the high dose to control Fisher exact comparison were significantly positive.

Two types of unusual tumors were detected in the forestomach of high dose male and female rats (i.e., squamous-cell papillomas in 2/48 [4 percent] high dose males and 3/46 [7 percent] high dose females and squamous-cell carcinomas in 2/48 [4 percent] high dose males and 1/46 [2 percent] high dose females). Although these tumors were not present in statistically significant incidences they do occur with sufficient rarity to be of concern and are considered in this study to be related to administration of the test compound. This is further supported by the finding of nonneoplastic proliferative lesions of the stomach in several of the treated animals.

In mice hepatocellular carcinomas occurred in 10/50 (20 percent), 18/49 (37 percent), and 26/47 (55 percent), of the control, low dose, and high dose males, respectively, and in 0/46, 4/48 (8 percent), and 6/48 (13 percent) of the control, low dose, and high dose females, respectively. In both sexes statistical analysis of these incidences indicated a significant positive association between dosage and tumor incidence and this association was confirmed by the high dose to control Fisher exact comparison. When incidences were combined so that the numerator represented those animals having either hepatocellular carcinomas or hepatocellular adenomas, for both males and females,

the low dose to control Fisher exact comparison was significant in addition to the Cochran-Armitage test and the high dose to control Fisher exact comparison. There were no other neoplasms occurring at statistically significant incidences in male or female mice.

It is concluded that under the conditions of this bioassay 4-chloro-o-phenylenediamine was carcinogenic in Fischer 344 rats and B6C3F1 mice, inducing tumors of the urinary bladder and forestomach in both sexes of rats and hepatocellular carcinomas in both sexes of mice.

VI. BIBLIOGRAPHY

Actor, P.P., and J.F. Pagano, "5(6)-Butyl- and propoxy-2-carbomethoxy-benzimidazoles." U.S. Reissue 28:403 (Smithkline Corporation) Chemical Abstracts 84, 4950s, April 1975.

Anthony, H.M., and G.M. Thomas, "Tumors of the Urinary Bladder: An Analysis of the Occupations of 1,030 Patients in Leeds, England." Journal of the National Cancer Institute 45:879-895, 1970.

Armitage, P., Statistical Methods in Medical Research, Chapter 14. J. Wiley & Sons, New York, 1971.

Berenblum, I., editor, Carcinogenicity Testing. International Union Against Cancer, Technical Report Series, Vol. 2. International Union Against Cancer, Geneva, 1969.

Chemical Abstracts Service. The Chemical Abstracts Service (CAS) Ninth Collective Index, Volumes 76-85, 1972-1976. American Chemical Society, Washington, D.C., 1977.

Cox, D.R., Analysis of Binary Data, Chapters 4 and 5. Methuen and Co., Ltd., London, 1970.

Cox, D.R., "Regression Models and Life-Tables." Journal of the Royal Statistical Society, Series "B" 34:187-220, 1972.

Gart, J.J., "The Comparison of Proportions: A Review of Significance Tests, Confidence Limits, and Adjustments for Stratification." International Statistical Institute Review 39:148-169, 1971.

Kadhim, A.M., and A.T. Peters, "New Intermediates and Dyes for Synthetic Polymer Fibers. Substituted Benzimidazothioxanthenoisoquinolinones for Polyester Fibers." Journal of the Society of Dyers and Colourists 90(6):199-202; Chemical Abstracts 83, 61612m, 1974.

Kaplan, E.L., and P. Meier, "Nonparametric Estimation from Incomplete Observations." Journal of the American Statistical Association 53:457-481, 1958.

Linhart, M.S., J.A. Cooper, R.L. Martin, N.P. Page, and J.A. Peters, "Carcinogenesis Bioassay Data System." Computers and Biomedical Research 7:230-248, 1974.

Miller, R.G., Simultaneous Statistical Inference. McGraw-Hill Book Co., New York, 1966.

Saffiotti, U., R. Montesano, A.R. Sellakumar, F. Cefis, and D.G. Kaufman, "Respiratory Tract Carcinogenesis in Hamsters Induced by Different Numbers of Administration of Benzo (a) Pyrene and Ferric Oxide." Cancer Research 32:1073-1079, 1972.

Society of Dyers and Colourists, Colour Index, 2nd edition, Volume 3. Yorkshire, England, 1956.

Stolyarchuk, A.A., Y.N. Furman, V.L. Pikalov, Z.F. Solomko, and S.V. Tkachenko, "Synthesis and Pharmacological Study of 2,3 Dihydro-1H-1,5-benzodiazepin-2-ones." Khimiko-Farmatsevtichi cheskii Zhurnal 9(8):19-21; Chemical Abstracts 84, 9925g, 1975.

Tarone, R.E., "Tests for Trend in Life-Table Analysis." Biometrika 62:679-682, 1975.

U.S. International Trade Commission, Synthetic Organic Chemicals, United States Production and Sales, 1975. USITC publication 804, U.S. Government Printing Office, Washington, D.C., 1977.

Wynder, E.L., J. Onderdonk, and N. Mantel, 1963. "An Epidemiological Investigation of Cancer of the Bladder." Cancer 16:1388-1407, 1963.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 4-CHLORO-O-PHENYLENEDIAMINE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED
WITH 4-CHLORO-O-PHENYLENEDIAMINE

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0205	HIGH DOSE 01-0210
ANIMALS INITIALLY IN STUDY	50	@49	50
ANIMALS NECROPSIED	48	47	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	48	47	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(48)	(47)	(50)
FIBROMA	3 (6%)	1 (2%)	1 (2%)
FIBROSARCOMA	2 (4%)		
FIBROADENOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(48)	(47)	(48)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
TRANSITIONAL-CELL CARCINOMA, MET			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (6%)	5 (11%)	4 (8%)
FIBROSARCOMA, METASTATIC	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(48)	(47)	(50)
MALIGNANT LYMPHOMA, NOS	1 (2%)	2 (4%)	
LEUKEMIA,NOS		1 (2%)	
UNDIFFERENTIATED LEUKEMIA	2 (4%)		1 (2%)
MYELOMONOCYTIC LEUKEMIA		1 (2%)	3 (6%)
#SPLEEN	(48)	(46)	(48)
TRANSITIONAL-CELL CARCINOMA, MET		1 (2%)	
MYELOMONOCYTIC LEUKEMIA	4 (8%)		
#LYMPH NODE	(43)	(40)	(39)
TRANSITIONAL-CELL CARCINOMA, MET		1 (3%)	1 (3%)
CIRCULATORY SYSTEM			
NONE			

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
***EXCLUDES PARTIALLY AUTOLYZED ANIMALS*
@ NOTE: 50 ANIMALS WERE INITIALLY IN STUDY BUT ONE WAS FOUND TO BE A FEMALE IN A MALE GROUP AND WAS DELETED.

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0205	HIGH DOSE 01-0210
DIGESTIVE SYSTEM			
#LIVER	(48)	(47)	(48)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
NEOPLASTIC NODULE		4 (9%)	4 (8%)
#STOMACH	(48)	(46)	(48)
SQUAMOUS CELL PAPILLOMA			2 (4%)
SQUAMOUS CELL CARCINOMA			2 (4%)
#COLON	(42)	(43)	(35)
ADENOMATOUS POLYP, NOS			1 (3%)
URINARY SYSTEM			
#URINARY BLADDER	(48)	(42)	(49)
NEOPLASM, NOS			1 (2%)
PAPILLOMA, NOS			2 (4%)
SQUAMOUS CELL CARCINOMA			3 (6%)
TRANSITIONAL-CELL PAPILLOMA		8 (19%)	5 (10%)
TRANSITIONAL-CELL CARCINOMA		7 (17%)	18 (37%)
ADENOCARCINOMA, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(39)	(42)
ADENOMA, NOS	10 (22%)	5 (13%)	4 (10%)
#ADRENAL	(46)	(46)	(48)
PHEOCHROMOCYTOMA	4 (9%)	5 (11%)	5 (10%)
#ADRENAL MEDULLA	(46)	(46)	(48)
GANGLIONEUROMA			1 (2%)
#THYROID	(43)	(40)	(40)
FOLLICULAR-CELL ADENOMA			2 (5%)
FOLLICULAR-CELL CARCINOMA			1 (3%)
C-CELL ADENOMA	3 (7%)	1 (3%)	
C-CELL CARCINOMA	2 (5%)		
#PARATHYROID	(25)	(28)	(22)
ADENOMA, NOS	1 (4%)		
#PANCREATIC ISLETS	(44)	(43)	(41)
ISLET-CELL ADENOMA		2 (5%)	1 (2%)

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0205	HIGH DOSE 01-0210
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(48)	(47)	(50)
ADENOMA, NOS		1 (2%)	
*TESTIS	(48)	(45)	(47)
INTERSTITIAL-CELL TUMOR	37 (77%)	33 (73%)	40 (85%)
NERVOUS SYSTEM			
#BRAIN	(46)	(46)	(48)
OSTEOSARCOMA, METASTATIC	1 (2%)		
ASTROCYTOMA		2 (4%)	
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(48)	(47)	(50)
SEBACEOUS ADENOCARCINOMA		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*SKULL	(48)	(47)	(50)
OSTEOSARCOMA	1 (2%)		
BODY CAVITIES			
*BODY CAVITIES	(48)	(47)	(50)
MESOTHELIOMA, NOS	1 (2%)	2 (4%)	
*ABDOMINAL CAVITY	(48)	(47)	(50)
TRANSITIONAL-CELL CARCINOMA, MET		1 (2%)	
ALL OTHER SYSTEMS			
NONE			

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

TABLE A1 (CONCLUDED)

	CONTROL(UNTR) 01-0220	LOW DOSE 01-0205	HIGH DOSE 01-0210
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	8	5	10
MORIBUND SACRIFICE	5	5	7
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	32	39	28
ANIMAL MISSING			
ANIMAL DELETED (WRONG SEX)		1	
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	42	42	46
TOTAL PRIMARY TUMORS	74	81	103
TOTAL ANIMALS WITH BENIGN TUMORS	40	41	43
TOTAL BENIGN TUMORS	61	61	69
TOTAL ANIMALS WITH MALIGNANT TUMORS	11	13	26
TOTAL MALIGNANT TUMORS	12	14	29
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	1	2
TOTAL SECONDARY TUMORS	2	3	4
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	5	5
TOTAL UNCERTAIN TUMORS	1	6	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 TREATED
WITH 4-CHLORO-O-PHENYLENEDIAMINE

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0205	HIGH DOSE 02-0210
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	50	49	48
INTEGUMENTARY SYSTEM			
*SUBCUTIISSUE	(50)	(49)	(49)
SARCOMA, NOS	1 (2%)		
LEIOMYOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(47)
ALVEOLAR/BRONCHIOLAR ADENOMA		5 (10%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(49)
MALIGNANT LYMPHOMA, NOS	2 (4%)	1 (2%)	
UNDIFFERENTIATED LEUKEMIA		2 (4%)	
MYELOMONOCYTIC LEUKEMIA		3 (6%)	5 (10%)
#SPLEEN	(50)	(48)	(46)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
MYELOMONOCYTIC LEUKEMIA	2 (4%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(46)
NEOPLASTIC NODULE			2 (4%)
#STOMACH	(49)	(49)	(46)
SQUAMOUS CELL PAPILLOMA			3 (7%)

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

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0205	HIGH DOSE 02-0210
SQUAMOUS CELL CARCINOMA			1 (2%)
BASAL-CELL CARCINOMA			1 (2%)
URINARY SYSTEM			
#URINARY BLADDER	(47)	(46)	(45)
PAPILLOMA, NOS		1 (2%)	
PAPILLARY CARCINOMA		1 (2%)	
PAPILLOMATOSIS			2 (4%)
TRANSITIONAL-CELL PAPILLOMA		9 (20%)	8 (18%)
TRANSITIONAL-CELL CARCINOMA		4 (9%)	22 (49%)
ENDOCRINE SYSTEM			
#PITUITARY	(40)	(38)	(38)
ADENOMA, NOS	16 (40%)	15 (39%)	7 (18%)
CHROMOPHOBE ADENOMA	1 (3%)		
#ADRENAL	(48)	(47)	(46)
PHEOCHROMOCYTOMA	6 (13%)	2 (4%)	4 (9%)
#ADRENAL CORTEX	(48)	(47)	(46)
LIPOMA		1 (2%)	
#THYROID	(43)	(45)	(44)
FOLLICULAR-CELL ADENOMA		1 (2%)	1 (2%)
FOLLICULAR-CELL CARCINOMA		1 (2%)	3 (7%)
C-CELL CARCINOMA		1 (2%)	2 (5%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(49)
ADENOMA, NOS			2 (4%)
ADENOCARCINOMA, NOS	1 (2%)		
FIBROADENOMA	6 (12%)	10 (20%)	
*CLITORAL GLAND	(50)	(49)	(49)
ADENOMA, NOS		1 (2%)	
#UTERUS	(48)	(49)	(46)
ADENOCARCINOMA, NOS	1 (2%)		
FIBROMA	2 (4%)	1 (2%)	
ENDOMETRIAL STROMAL POLYP	2 (4%)	2 (4%)	1 (2%)

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

TABLE A2 (CONTINUED)

	CONTROL(UNTR) 02-0220	LOW DOSE 02-0205	HIGH DOSE 02-0210
ENDOMETRIAL STROMAL SARCOMA	2 (4%)		
NERVOUS SYSTEM			
#BRAIN	(50)	(49)	(46)
ASTROCYTOMA			1 (2%)
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(50)	(49)	(49)
SQUAMOUS CELL CARCINOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
SITE UNKNOWN			
SQUAMOUS CELL CARCINOMA			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	7	6	8
MORIBUND SACRIFICE	2	2	9
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	42	27
ANIMAL MISSING			1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL(UNTR) 02-0220	LOW DOSE 02-0205	HIGH DOSE 02-0210
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	27	35	37
TOTAL PRIMARY TUMORS	42	62	67
TOTAL ANIMALS WITH BENIGN TUMORS	23	27	20
TOTAL BENIGN TUMORS	33	48	28
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	13	30
TOTAL MALIGNANT TUMORS	9	14	37
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			2
TOTAL UNCERTAIN TUMORS			2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 4-CHLORO-O-PHENYLENEDIAMINE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED
WITH 4-CHLORO-O-PHENYLENEDIAMINE

	CONTROL (UNTR) 05-0220	LOW DOSE 05-0210	HIGH DOSE 05-0215
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	50	49	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	50	49	47
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(49)	(47)
LEICMYOSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(48)	(47)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	5 (10%)	4 (8%)	5 (11%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (4%)	5 (10%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(47)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		2 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE		2 (4%)	
#SPLEEN	(50)	(47)	(44)
SARCOMA, NOS		1 (2%)	
HEMANGIOMA	1 (2%)		
HEMANGIOSARCOMA	2 (4%)	1 (2%)	
#MESENTERIC L. NODE	(44)	(44)	(35)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
#LIVER	(50)	(49)	(47)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
CIRCULATORY SYSTEM			
NONE			

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

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 05-0220	LOW DOSE 05-0210	HIGH DOSE 05-0215
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(47)
HEPATOCELLULAR ADENOMA	5 (10%)	10 (20%)	8 (17%)
HEPATOCELLULAR CARCINOMA	10 (20%)	18 (37%)	26 (55%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(42)	(45)	(41)
PHEOCHROMOCYTOMA		1 (2%)	
#THYROID	(39)	(46)	(41)
FOLLICULAR-CELL ADENOMA	1 (3%)	2 (4%)	3 (7%)
FOLLICULAR-CELL CARCINOMA	1 (3%)		
REPRODUCTIVE SYSTEM			
#TESTIS	(50)	(49)	(46)
INTERSTITIAL-CELL TUMOR			1 (2%)
EMBRYONAL CARCINOMA	1 (2%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(49)	(47)
ADENOMA, NOS		1 (2%)	
PAPILLARY ADENOMA		1 (2%)	1 (2%)
CYSTADENOMA, NOS	1 (2%)		
PAPILLARY CYSTADENOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 05-0220	LOW DOSE 05-0210	HIGH DOSE 05-0215
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	3	6	7
MORIBUND SACRIFICE		1	3
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	42	42	35
ANIMAL MISSING		1	
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	22	37	39
TOTAL PRIMARY TUMORS	33	48	51
TOTAL ANIMALS WITH BENIGN TUMORS	12	17	18
TOTAL BENIGN TUMORS	13	19	19
TOTAL ANIMALS WITH MALIGNANT TUMORS	16	25	31
TOTAL MALIGNANT TUMORS	20	29	32
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH 4-CHLORO-O-PHENYLENEDIAMINE

	CONTROL (UNTR) 06-0220	LOW DOSE 06-0210	HIGH DOSE 06-0215
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	2	1	
ANIMALS NECROPSIED	47	48	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	47	48	48
INTEGUMENTARY SYSTEM			
*SKIN	(47)	(48)	(48)
KERATOACANTHOMA	1 (2%)		
*SUBCUT TISSUE	(47)	(48)	(48)
HEMANGIOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(46)	(48)	(45)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)	1 (2%)	3 (7%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (7%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(47)	(48)	(48)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
MALIG.LYMPHOMA, UNDIFFER-TYPE		2 (4%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	3 (6%)	5 (10%)	
UNDIFFERENTIATED LEUKEMIA		1 (2%)	1 (2%)
#SPLEEN	(45)	(48)	(48)
HEMANGIOMA		1 (2%)	1 (2%)
HEMANGIOSARCOMA		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (4%)		
#LYMPH NODE	(38)	(46)	(38)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#MESENTERIC L. NODE	(38)	(46)	(38)
MALIGNANT LYMPHOMA, NOS			1 (3%)
#PEYERS PATCH	(44)	(46)	(46)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (4%)	1 (2%)

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0220	LOW DOSE 06-0210	HIGH DOSE 06-0215
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(46)	(48)	(47)
HEPATOCELLULAR ADENOMA		7 (15%)	4 (9%)
HEPATOCELLULAR CARCINOMA		4 (8%)	6 (13%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(33)	(34)	(35)
ADENOMA, NOS	1 (3%)	1 (3%)	
#ADRENAL/CAPSULE	(40)	(45)	(47)
ADENOMA, NOS	1 (3%)		
#ADRENAL MEDULLA	(40)	(45)	(47)
NEUROBLASTOMA		1 (2%)	
#THYROID	(29)	(46)	(39)
FOLLICULAR-CELL ADENOMA	1 (3%)	1 (2%)	1 (3%)
#PANCREATIC ISLETS	(45)	(47)	(42)
ISLET-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(45)	(45)	(44)
LEIOMYOSARCOMA			1 (2%)
HEMANGIOMA			1 (2%)
#OVARY	(40)	(45)	(38)
PAPILLARY ADENOMA	1 (3%)		1 (3%)
GRANULOSA-CELL TUMOR			1 (3%)
NERVOUS SYSTEM			
NONE			

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

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0220	LOW DOSE 06-0210	HIGH DOSE 06-0215
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(47)	(48)	(48)
PAPILLARY ADENOMA		1 (2%)	
CYSTADENOMA, NOS			2 (4%)
PAPILLARY CYSTADENOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	4	5	5
MORIBUND SACRIFICE	3		1
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	44	39
ANIMAL MISSING	2	1	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2 (CONCLUDED)

	CONTROL(UNTR) 06-0220	LOW DOSE 06-0210	HIGH DOSE 06-0215
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	24	21
TOTAL PRIMARY TUMORS	15	31	26
TOTAL ANIMALS WITH BENIGN TUMORS	5	10	12
TOTAL BENIGN TUMORS	6	12	15
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	15	10
TOTAL MALIGNANT TUMORS	9	19	10
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 4-CHLORO-O-PHENYLENEDIAMINE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH 4-CHLORO-O-PHENYLENEDIAMINE

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0205	HIGH DOSE 01-0210
ANIMALS INITIALLY IN STUDY	50	@49	50
ANIMALS NECROPSIED	48	47	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	48	47	49
INTEGUMENTARY SYSTEM			
*SKIN	(48)	(47)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)	2 (4%)	
*SUBCUT TISSUE	(48)	(47)	(50)
ABSCESS, NOS	3 (6%)		
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(48)	(47)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
#TRACHEA	(29)	(46)	(47)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, ACUTE/CHRONIC		2 (4%)	
#LUNG/BRONCHUS	(48)	(47)	(48)
BRONCHIECTASIS		3 (6%)	
INFLAMMATION, NOS		1 (2%)	2 (4%)
INFLAMMATION, FOCAL		1 (2%)	1 (2%)
#LUNG	(48)	(47)	(48)
CONGESTION, NOS		2 (4%)	
INFLAMMATION, INTERSTITIAL		1 (2%)	2 (4%)
PNEUMONIA, CHRONIC MURINE		1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)	2 (4%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(48)	(46)	(48)
CONGESTION, NOS		1 (2%)	
ABSCESS, NOS		1 (2%)	
INFARCT, NOS	1 (2%)	1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS
@ NOTE: 50 ANIMALS WERE INITIALLY IN STUDY BUT ONE WAS FOUND TO BE A FEMALE IN A MALE

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0205	HIGH DOSE 01-0210
HEMOSIDEROSIS		9 (20%)	6 (13%)
LYMPHOCYTOSIS			1 (2%)
HYPERPLASIA, HEMATOPOIETIC			1 (2%)
HYPERPLASIA, ERYTHROID			1 (2%)
HEMATOPOIESIS		16 (35%)	10 (21%)
#LYMPH NODE	(43)	(40)	(39)
DEGENERATION, CYSTIC		1 (3%)	
HYPERPLASIA, NOS			2 (5%)
#THYMUS	(32)	(21)	(24)
HYPERPLASIA, NOS	1 (3%)		
CIRCULATORY SYSTEM			
#HEART/VENTRICLE	(48)	(47)	(47)
THROMBOSIS, NOS		1 (2%)	
#MYOCARDIUM	(48)	(47)	(47)
INFLAMMATION, INTERSTITIAL		1 (2%)	3 (6%)
FIBROSIS		18 (38%)	14 (30%)
#ENDOCARDIUM	(48)	(47)	(47)
ENDOCARDITIS, BACTERIAL		1 (2%)	
*ARTERY	(48)	(47)	(50)
MINERALIZATION			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(48)	(47)	(48)
FIBROSIS SEPTAL LIVER			1 (2%)
NECROSIS, FOCAL		1 (2%)	4 (8%)
NECROSIS, COAGULATIVE		1 (2%)	2 (4%)
METAMORPHOSIS FATTY	2 (4%)	8 (17%)	5 (10%)
HYPERPLASIA, FOCAL	3 (6%)	3 (6%)	10 (21%)
HEMATOPOIESIS		1 (2%)	
#LIVER/PERIPORTAL	(48)	(47)	(48)
FIBROSIS		1 (2%)	
*BILE DUCT	(48)	(47)	(50)
INFLAMMATION, FOCAL		1 (2%)	
HYPERPLASIA, NOS		6 (13%)	3 (6%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0205	HIGH DOSE 01-0210
#PANCREAS	(44)	(43)	(41)
INFLAMMATION, NOS	2 (5%)	11 (26%)	9 (22%)
FIBROSIS			1 (2%)
DEGENERATION, CYSTIC			2 (5%)
#STOMACH	(48)	(46)	(48)
MINERALIZATION			1 (2%)
INFLAMMATION, NOS		3 (7%)	1 (2%)
ABSCESS, NOS		1 (2%)	
HYPERPLASIA, BASAL CELL		7 (15%)	4 (8%)
HYPERKERATOSIS		2 (4%)	5 (10%)
ACANTHOSIS		11 (24%)	8 (17%)
#PEYERS PATCH	(46)	(45)	(46)
HYPERPLASIA, NOS		6 (13%)	3 (7%)
#COLON	(42)	(43)	(35)
PARASITISM		3 (7%)	2 (6%)
URINARY SYSTEM			
#KIDNEY	(48)	(47)	(48)
HYDRONEPHROSIS		1 (2%)	6 (13%)
GLOMERULONEPHRITIS, NOS		20 (43%)	10 (21%)
PYELONEPHRITIS, NOS		24 (51%)	34 (71%)
ABSCESS, NOS		1 (2%)	
FIBROSIS, DIFFUSE		5 (11%)	
NEPHROPATHY	35 (73%)		
HYPERPLASIA, TUBULAR CELL		1 (2%)	
HYPERPLASIA, EPITHELIAL			5 (10%)
#KIDNEY/MEDULLA	(48)	(47)	(48)
MINERALIZATION		1 (2%)	8 (17%)
HEMORRHAGE			2 (4%)
#KIDNEY/GLOMERULUS	(48)	(47)	(48)
NECROSIS, FOCAL		1 (2%)	
#KIDNEY/TUBULE	(48)	(47)	(48)
MINERALIZATION			2 (4%)
NECROSIS, FOCAL		1 (2%)	
#URINARY BLADDER	(48)	(42)	(49)
HYPERPLASIA, EPITHELIAL		3 (7%)	6 (12%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0205	HIGH DOSE 01-0210
HYPERPLASIA, PAPILLARY		1 (2%)	4 (8%)
METAPLASIA, SQUAMOUS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(39)	(42)
CYST, NOS		1 (3%)	
NECROSIS, HEMORRHAGIC		1 (3%)	
HYPERPLASIA, FOCAL	1 (2%)	8 (21%)	3 (7%)
#ADRENAL	(46)	(46)	(48)
HYPERPLASIA, NOS		1 (2%)	
#ADRENAL CORTEX	(46)	(46)	(48)
HYPERTROPHY, FOCAL		1 (2%)	
#ADRENAL MEDULLA	(46)	(46)	(48)
HYPERPLASIA, NOS	1 (2%)		1 (2%)
HYPERPLASIA, FOCAL	3 (7%)	1 (2%)	1 (2%)
#THYROID	(43)	(40)	(40)
FOLLICULAR CYST, NOS		1 (3%)	
HYPERPLASIA, C-CELL		3 (8%)	1 (3%)
#THYROID FOLLICLE	(43)	(40)	(40)
DEGENERATION, NOS			1 (3%)
PIGMENTATION, NOS		2 (5%)	4 (10%)
#PARATHYROID	(25)	(28)	(22)
HYPERPLASIA, NOS		2 (7%)	2 (9%)
#PANCREATIC ISLETS	(44)	(43)	(41)
HYPERPLASIA, NOS	1 (2%)	2 (5%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(48)	(47)	(50)
GALACTOCELE			1 (2%)
#PROSTATE	(45)	(44)	(44)
INFLAMMATION, NOS		8 (18%)	4 (9%)
*SEMINAL VESICLE	(48)	(47)	(50)
ATROPHY, NOS		1 (2%)	

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0205	HIGH DOSE 01-0210
#TESTIS	(48)	(45)	(47)
MINERALIZATION	1 (2%)	4 (9%)	4 (9%)
ATROPHY, NOS	4 (8%)	6 (13%)	8 (17%)
ATROPHY, FOCAL		2 (4%)	
HYPERPLASIA, INTERSTITIAL CELL	3 (6%)	4 (9%)	2 (4%)
#TESTIS/TUBULE	(48)	(45)	(47)
MINERALIZATION		2 (4%)	2 (4%)
*EPIDIDYMIS	(48)	(47)	(50)
ABSCESS, NOS	1 (2%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(48)	(47)	(50)
CATARACT	1 (2%)		
*EYE/RETINA	(48)	(47)	(50)
ATROPHY, NOS	2 (4%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM			
MINERALIZATION		1	
NECROSIS, FAT		2	2
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/HISTO PERF	1	1	
AUTO/NECROPSY/NO HISTO			1
AUTOLYSIS/NO NECROPSY	2	2	

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

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
TREATED WITH 4-CHLORO-O-PHENYLENEDIAMINE

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0205	HIGH DOSE 02-0210
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	50	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(50)	(49)	(49)
INFLAMMATION, NOS	1 (2%)		
#LUNG/BRONCHUS	(50)	(49)	(47)
INFLAMMATION, NOS		3 (6%)	
INFLAMMATION, SUPPURATIVE			1 (2%)
#LUNG	(50)	(49)	(47)
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, INTERSTITIAL	1 (2%)	6 (12%)	2 (4%)
ABSCESS, NOS	2 (4%)		1 (2%)
PNEUMONIA, CHRONIC MURINE	1 (2%)		1 (2%)
INFLAMMATION, GRANULOMATOUS			1 (2%)
GRANULOMA, NOS	1 (2%)		
HYPERPLASIA, EPITHELIAL		1 (2%)	4 (9%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(48)	(46)
HEMOSIDEROSIS		22 (46%)	1 (2%)
ATROPHY, NOS		1 (2%)	
HEMATOPOIESIS	7 (14%)	20 (42%)	9 (20%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(50)	(49)	(47)
INFLAMMATION, INTERSTITIAL		1 (2%)	

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0205	HIGH DOSE 02-0210
FIBROSIS		5 (10%)	4 (9%)
#ENDOCARDIUM	(50)	(49)	(47)
INFLAMMATION, NOS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(46)
FIBROSIS SEPTAL LIVER		1 (2%)	1 (2%)
NECROSIS, FOCAL		3 (6%)	1 (2%)
NECROSIS, COAGULATIVE			1 (2%)
METAMORPHOSIS FATTY	4 (8%)	1 (2%)	1 (2%)
BASOPHILIC CYTO CHANGE			1 (2%)
HYPERPLASIA, FOCAL	9 (18%)	23 (47%)	22 (48%)
ANGIECTASIS		1 (2%)	
#LIVER/CENTRILOBULAR	(50)	(49)	(46)
NECROSIS, NOS		1 (2%)	
#LIVER/PERIPORTAL	(50)	(49)	(46)
FIBROSIS		1 (2%)	1 (2%)
*BILE DUCT	(50)	(49)	(49)
INFLAMMATION, NOS		2 (4%)	
HYPERPLASIA, NOS		10 (20%)	1 (2%)
#PANCREAS	(46)	(44)	(45)
INFLAMMATION, NOS		10 (23%)	8 (18%)
#STOMACH	(49)	(49)	(46)
DEGENERATION, NOS		1 (2%)	
HYPERPLASIA, PAPILLARY	1 (2%)		
HYPERPLASIA, BASAL CELL		7 (14%)	6 (13%)
HYPERKERATOSIS		1 (2%)	4 (9%)
ACANTHOSIS		4 (8%)	6 (13%)
#PEYERS PATCH	(47)	(48)	(45)
HYPERPLASIA, NOS		6 (13%)	
#COLON	(40)	(40)	(36)
PARASITISM		1 (3%)	1 (3%)
URINARY SYSTEM			
#KIDNEY	(49)	(49)	(47)
HYDRONEPHROSIS			8 (17%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0205	HIGH DOSE 02-0210
CYST, NOS		1 (2%)	
GLOMERULONEPHRITIS, NOS	1 (2%)	35 (71%)	4 (9%)
PYELONEPHRITIS, NOS		6 (12%)	38 (81%)
INFLAMMATION, ACUTE			1 (2%)
NEPHROPATHY	18 (37%)		
DEGENERATION, NOS			1 (2%)
HYPERPLASIA, EPITHELIAL			1 (2%)
#KIDNEY/MEDULLA	(49)	(49)	(47)
MINERALIZATION			1 (2%)
#URINARY BLADDER	(47)	(46)	(45)
HYPERPLASIA, EPITHELIAL		4 (9%)	2 (4%)
HYPERPLASIA, PAPILLARY			2 (4%)
ENDOCRINE SYSTEM			
#PITUITARY	(40)	(38)	(38)
MINERALIZATION	1 (3%)		
HEMORRHAGE		2 (5%)	
HYPERPLASIA, FOCAL		2 (5%)	
#ADRENAL	(48)	(47)	(46)
THROMBOSIS, NOS		1 (2%)	
#ADRENAL CORTEX	(48)	(47)	(46)
HYPERTROPHY, FOCAL		1 (2%)	
#ADRENAL MEDULLA	(48)	(47)	(46)
HYPERPLASIA, NOS			1 (2%)
#THYROID	(43)	(45)	(44)
ULTIMOBRANCHIAL CYST		1 (2%)	
FOLLICULAR CYST, NOS		1 (2%)	
HYPERPLASIA, C-CELL	1 (2%)	1 (2%)	
#PARATHYROID	(31)	(22)	(30)
HYPERPLASIA, NODULAR	1 (3%)		
#PANCREATIC ISLETS	(46)	(44)	(45)
HYPERPLASIA, NOS			2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(49)
GALACTOCELE	2 (4%)	5 (10%)	5 (10%)

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0205	HIGH DOSE 02-0210
HYPERPLASIA, NOS	1 (2%)	3 (6%)	
*CLITORAL GLAND	(50)	(49)	(49)
METAPLASIA, SQUAMOUS		1 (2%)	
#UTERUS	(48)	(49)	(46)
HYDROMETRA		1 (2%)	
ABSCESS, NOS	1 (2%)		1 (2%)
NECROSIS, NOS	1 (2%)		
#UTERUS/ENDOMETRIUM	(48)	(49)	(46)
INFLAMMATION, NOS	1 (2%)	1 (2%)	2 (4%)
ABSCESS, NOS		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
#OVARY/OVIDUCT	(48)	(49)	(46)
INFLAMMATION, NOS		1 (2%)	
#OVARY	(49)	(49)	(47)
CYST, NOS		1 (2%)	
INFLAMMATION, NOS	1 (2%)		
DEGENERATION, CYSTIC	2 (4%)		
HYPERPLASIA, NOS		2 (4%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(50)	(49)	(46)
INFLAMMATION, ACUTE			1 (2%)
SPECIAL SENSE ORGANS			
*EYE	(50)	(49)	(49)
CATARACT		1 (2%)	1 (2%)
*EYE/CORNEA	(50)	(49)	(49)
INFLAMMATION, HEMORRHAGIC			2 (4%)
*EYE/RETINA	(50)	(49)	(49)
ATROPHY, NOS		1 (2%)	
*HARDERIAN GLAND	(50)	(49)	(49)
INFLAMMATION, CHRONIC		1 (2%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0205	HIGH DOSE 02-0210
BODY CAVITIES			
*PLEURA	(50)	(49)	(49)
HYPERPLASIA, NOS			1 (2%)
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, FAT			2
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	11		1
ANIMAL MISSING/NO NECROPSY			1
AUTO/NECROPSY/HISTO PERF	1		1
AUTO/NECROPSY/NO HISTO			1
AUTOLYSIS/NO NECROPSY		1	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 4-CHLORO-O-PHENYLENEDIAMINE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH 4-CHLORO-O-PHENYLENEDIAMINE

	CONTROL (UNTR) 05-0220	LOW DOSE 05-0210	HIGH DOSE 05-0215
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	50	49	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	50	49	47
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(47)
INFLAMMATION, NECROTIZING			1 (2%)
*SUBCUT TISSUE	(50)	(49)	(47)
ABSCESS, NOS	1 (2%)		1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
RESPIRATORY SYSTEM			
#TRACHEA	(28)	(44)	(40)
HYPERPLASIA, FOCAL		1 (2%)	
#LUNG/BRONCHUS	(50)	(48)	(47)
INFLAMMATION, NOS	1 (2%)	7 (15%)	1 (2%)
#LUNG/BRONCHIOLE	(50)	(48)	(47)
INFLAMMATION, NOS		2 (4%)	
#LUNG	(50)	(48)	(47)
INFLAMMATION, INTERSTITIAL		13 (27%)	3 (6%)
PERIVASCULITIS		1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(47)	(44)
HYPERPLASIA, NOS		6 (13%)	2 (5%)
HYPERPLASIA, HEMATOPOIETIC			2 (5%)
HYPERPLASIA, ERYTHROID			2 (5%)
HYPERPLASIA, LYMPHOID		2 (4%)	
HEMATOPOIESIS		3 (6%)	4 (9%)
#LYMPH NODE	(44)	(44)	(35)
INFLAMMATION, NOS		1 (2%)	

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0220	LOW DOSE 05-0210	HIGH DOSE 05-0215
HYPERPLASIA, NOS			3 (9%)
RETICULOCYTOSIS		2 (5%)	1 (3%)
HYPERPLASIA, LYMPHOID			1 (3%)
HEMATOPOIESIS		2 (5%)	1 (3%)
#MESENTERIC L. NODE	(44)	(44)	(35)
HEMATOPOIESIS	1 (2%)		
CIRCULATORY SYSTEM			
*CARDIOVASCULAR SYSTE	(50)	(49)	(47)
PERIVASCULITIS		1 (2%)	
#HEART/VENTRICLE	(50)	(45)	(46)
MELANIN		4 (9%)	11 (24%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(47)
INFLAMMATION, INTERSTITIAL		1 (2%)	
NECROSIS, FOCAL		2 (4%)	1 (2%)
NECROSIS, COAGULATIVE	1 (2%)		
METAMORPHOSIS FATTY		3 (6%)	2 (4%)
HYPERPLASTIC NODULE		5 (10%)	7 (15%)
HYPERPLASIA, FOCAL		3 (6%)	
HYPERPLASIA, HEMATOPOIETIC			1 (2%)
HEMATOPOIESIS			1 (2%)
*GALLBLADDER	(50)	(49)	(47)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
HYPERPLASIA, PAPILLARY			1 (2%)
#PANCREAS	(50)	(44)	(43)
INFLAMMATION, NOS		2 (5%)	1 (2%)
#PANCREATIC ACINUS	(50)	(44)	(43)
DEGENERATION, CYSTIC		1 (2%)	
ATROPHY, NOS	1 (2%)		
HYPERTROPHY, FOCAL	1 (2%)		
#STOMACH	(50)	(44)	(42)
INFLAMMATION, NOS		1 (2%)	
HYPERPLASIA, NOS			2 (5%)
HYPERKERATOSIS			4 (10%)

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0220	LOW DOSE 05-0210	HIGH DOSE 05-0215
ACANTHOSIS		3 (7%)	2 (5%)
#GASTRIC MUCOSA	(50)	(44)	(42)
DEGENERATION, NOS			1 (2%)
#PEYERS PATCH	(50)	(45)	(44)
HYPERPLASIA, NOS		4 (9%)	2 (5%)
#COLON	(44)	(43)	(36)
PARASITISM		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(47)
HYDRONEPHROSIS	1 (2%)	1 (2%)	1 (2%)
GLOMERULONEPHRITIS, NOS		10 (21%)	6 (13%)
INFLAMMATION, NOS			2 (4%)
INFLAMMATION, INTERSTITIAL		6 (13%)	8 (17%)
FIBROSIS, DIFFUSE		1 (2%)	2 (4%)
METAMORPHOSIS FATTY			1 (2%)
HYPERPLASIA, TUBULAR CELL		1 (2%)	
#URINARY BLADDER	(50)	(49)	(44)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
HYPERPLASIA, EPITHELIAL		2 (4%)	1 (2%)
METAPLASIA, SQUAMOUS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(34)	(34)	(32)
CYST, NOS		1 (3%)	
HYPERPLASIA, FOCAL		2 (6%)	2 (6%)
#ADRENAL/CAPSULE	(42)	(45)	(41)
NODULE		1 (2%)	
#ADRENAL CORTEX	(42)	(45)	(41)
HYPERTROPHY, FOCAL			2 (5%)
HYPERPLASIA, NOS		3 (7%)	2 (5%)
HYPERPLASIA, FOCAL		1 (2%)	
#THYROID	(39)	(46)	(41)
FOLLICULAR CYST, NOS		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0220	LOW DOSE 05-0210	HIGH DOSE 05-0215
HYPERPLASIA, FOLLICULAR-CELL			1 (2%)
#PARATHYROID	(17)	(27)	(19)
HYPERPLASIA, NOS		3 (11%)	
#PANCREATIC ISLETS	(50)	(44)	(43)
INFLAMMATION, NOS	3 (6%)		
HYPERPLASIA, ADENOMATOUS	1 (2%)		
REPRODUCTIVE SYSTEM			
*PENIS	(50)	(49)	(47)
CALCULUS, NOS			1 (2%)
INFLAMMATION, ACUTE DIFFUSE			1 (2%)
*PREPUTIAL GLAND	(50)	(49)	(47)
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, NOS		1 (2%)	
#PROSTATE	(41)	(43)	(41)
HYPERPLASIA, FOCAL			2 (5%)
#TESTIS	(50)	(49)	(46)
ATROPHY, NOS	1 (2%)		
ATROPHY, FOCAL		1 (2%)	
#TESTIS/TUBULE	(50)	(49)	(46)
MINERALIZATION	2 (4%)		
NERVOUS SYSTEM			
#BRAIN	(50)	(48)	(46)
GRANULOMA, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 05-0220	LOW DOSE 05-0210	HIGH DOSE 05-0215
BODY CAVITIES			
*PERITONEUM	(50)	(49)	(47)
INFLAMMATION, NOS			1 (2%)
*PLEURA	(50)	(49)	(47)
GRANULOMA, NOS		1 (2%)	
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, FAT		1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	22	1	
ANIMAL MISSING/NO NECROPSY		1	
AUTC/NECROPSY/HISTO PERF		1	2
AUTOLYSIS/NO NECROPSY			3

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

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
TREATED WITH 4-CHLORO-O-PHENYLENEDIAMINE

	CONTROL (UNTR) 06-0220	LOW DOSE 06-0210	HIGH DOSE 06-0215
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	2	1	
ANIMALS NECROPSIED	47	48	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	47	48	48
INTEGUMENTARY SYSTEM			
*SUBCUTIS TISSUE	(47)	(48)	(48)
INFLAMMATION, NOS	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(46)	(48)	(45)
INFLAMMATION, INTERSTITIAL		1 (2%)	
INFARCT, NOS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(39)	(43)	(45)
OSTEOSCLEROSIS		4 (9%)	3 (7%)
#SPLEEN	(45)	(48)	(48)
ATROPHY, NOS			1 (2%)
HYPERPLASIA, NOS		4 (8%)	5 (10%)
RETICULOCYTOSIS		1 (2%)	1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
HEMATOPOIESIS	1 (2%)	9 (19%)	4 (8%)
#LYMPH NODE	(38)	(46)	(38)
INFLAMMATION, NOS		1 (2%)	
HYPERPLASIA, NOS		2 (4%)	
RETICULOCYTOSIS			1 (3%)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	1 (3%)
HYPERPLASIA, LYMPHOID		1 (2%)	
#THYMUS	(30)	(36)	(28)
HYPERPLASIA, LYMPHOID		2 (6%)	
CIRCULATORY SYSTEM			
#HEART	(45)	(48)	(47)
INFLAMMATION, CHRONIC			1 (2%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0220	LOW DOSE 06-0210	HIGH DOSE 06-0215
#HEART/VENTRICLE	(45)	(48)	(47)
MELANIN		7 (15%)	3 (6%)
#MYOCARDIUM	(45)	(48)	(47)
FIBROSIS, FOCAL			1 (2%)
*ARTERY	(47)	(48)	(48)
PERIVASCULITIS		1 (2%)	1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(46)	(48)	(47)
FIBROSIS			1 (2%)
NECROSIS, FOCAL		6 (13%)	2 (4%)
NECROSIS, COAGULATIVE			1 (2%)
METAMORPHOSIS FATTY	1 (2%)	3 (6%)	1 (2%)
HYPERPLASTIC NODULE		5 (10%)	7 (15%)
HYPERPLASIA, FOCAL		1 (2%)	4 (9%)
*GALLBLADDER	(47)	(48)	(48)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL		2 (4%)	
HYPERPLASIA, PAPILLARY		3 (6%)	1 (2%)
#PANCREATIC ACINUS	(45)	(47)	(42)
DEGENERATION, NOS		1 (2%)	
#STOMACH	(46)	(46)	(46)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, FOCAL		1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)	2 (4%)
HYPERKERATOSIS		2 (4%)	5 (11%)
ACANTHOSIS		7 (15%)	7 (15%)
#PEYERS PATCH	(44)	(46)	(46)
NECROSIS, NOS			1 (2%)
HYPERPLASIA, NOS		2 (4%)	3 (7%)
URINARY SYSTEM			
#KIDNEY	(46)	(48)	(48)
GLOMERULONEPHRITIS, NOS	1 (2%)	1 (2%)	3 (6%)
INFLAMMATION, INTERSTITIAL			3 (6%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0220	LOW DOSE 06-0210	HIGH DOSE 06-0215
INFLAMMATION, CHRONIC			1 (2%)
#URINARY BLADDER	(45)	(47)	(42)
HYPERPLASIA, EPITHELIAL			4 (10%)
METAPLASIA, SQUAMOUS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(33)	(34)	(35)
HYPERPLASIA, FOCAL		1 (3%)	2 (6%)
#ADRENAL CORTEX	(40)	(45)	(47)
NODULE			1 (2%)
HYPERTROPHY, FOCAL			1 (2%)
HYPERPLASIA, NOS		10 (22%)	4 (9%)
#THYROID	(29)	(46)	(39)
FOLLICULAR CYST, NOS		1 (2%)	
HYPERPLASIA, FOCAL			1 (3%)
HYPERPLASIA, PAPILLARY		1 (2%)	
#PARATHYROID	(13)	(24)	(21)
MELANIN			2 (10%)
HYPERPLASIA, NOS			1 (5%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(47)	(48)	(48)
HYPERPLASIA, NOS	1 (2%)		
#UTERUS	(45)	(45)	(44)
HYDROMETRA		7 (16%)	3 (7%)
METAPLASIA, SQUAMOUS		1 (2%)	
#UTERUS/ENDOMETRIUM	(45)	(45)	(44)
INFLAMMATION, NOS		1 (2%)	1 (2%)
HYPERPLASIA, NOS		17 (38%)	18 (41%)
HYPERPLASIA, CYSTIC		7 (16%)	3 (7%)
#OVARY/OVIDUCT	(45)	(45)	(44)
INFLAMMATION, NOS		1 (2%)	
DEGENERATION, NOS	1 (2%)	1 (2%)	
HYPERPLASIA, PAPILLARY			1 (2%)
#OVARY	(40)	(45)	(38)
MINERALIZATION		1 (2%)	

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

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 06-0220	LOW DOSE 06-0210	HIGH DOSE 06-0215
CYST, NOS		2 (4%)	1 (3%)
CYSTIC FOLLICLES			2 (5%)
HEMORRHAGE		2 (4%)	1 (3%)
INFLAMMATION, CHRONIC		1 (2%)	
DEGENERATION, NOS			1 (3%)
DEGENERATION, CYSTIC		3 (7%)	1 (3%)
HYPERPLASIA, GRANULOSA-CELL		1 (2%)	3 (8%)
#OVARY/FOLLICLE	(40)	(45)	(38)
HEMORRHAGE			1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(47)	(48)	(48)
HYPERPLASIA, PAPILLARY		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	30	3	4
ANIMAL MISSING/NO NECROPSY	2	1	
AUTO/NECROPSY/HISTO PERF	1		
AUTOLYSIS/NO NECROPSY	1	1	2

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

Review of the Bioassay of 4-Chloro-o-phenylenediamine* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

March 6, 1978

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

The primary reviewer agreed with the conclusion in the report that 4-Chloro-o-phenylenediamine was carcinogenic in both sexes of treated rats and mice, under the conditions of test. Tumors of the urinary bladder and forestomach were induced in the rats and hepatocellular carcinomas in the mice. He opined that the increased incidence of liver neoplastic nodules in treated male rats could be biologically significant, although a lesser number were found in females. The primary reviewer said that the results were particularly significant since the bladder tumors are similar to ones induced in humans by certain aromatic amine carcinogens.

The secondary reviewer was critical of the selection of the dose levels used in the chronic study. However, he concurred with the conclusion that 4-Chloro-o-phenylenediamine was carcinogenic in the treated animals, under the conditions of test. He added that the substance could pose a carcinogenic risk to humans.

The primary reviewer moved that the report on the bioassay of 4-Chloro-o-phenylenediamine be accepted as written. The motion was seconded and approved unanimously.

Members present were

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology
Arnold Brown, Mayo Clinic
Lawrence Garfinkel, American Cancer Society
E. Cuyler Hammond, American Cancer Society
Joseph Highland, Environmental Defense Fund
Henry Pitot, University of Wisconsin Medical Center
George Roush, Jr., Monsanto Company
Sheldon Samuels, Industrial Union Department, AFL-CIO
Michael Shimkin, University of California at San Diego
John Weisburger, American Health Foundation
Sidney Wolfe, Health Research Group

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

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