National Cancer Institute
CARCINOGENESIS
Technical Report Series
NO. 158
1979

BIOASSAY OF (2-CHLOROETHYL) TRIMETHYLAMMONIUM CHLORIDE (CCC) FOR POSSIBLE CARCINOGENICITY

CAS No. 999-81-5
NCI-CG-TR-158

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

BIOASSAY OF

(2-CHLOROETHYL)TRIMETHYLAMMONIUM CHLORIDE

FOR POSSIBLE CARCINOGENICITY

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

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

DHEW Publication No. (NIH) 79-1714

BIOASSAY OF
(2-CHLOROETHYL)TRIMETHYLAMMONIUM CHLORIDE
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 (2-chloroethyl)trimethylammonium chloride conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. A positive result demonstrates that the test chemical is carcinogenic for animals under the conditions of the test and indicates that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic in animals requires a wider analysis.

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

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

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (3). Statistical analyses were performed by Dr. J. R. Joiner (4) and Ms. P. L. Yong (4), using methods selected for the bioassay program by Dr. J. J. Gart (5).

The chemicals used in this bioassay were analyzed at FCRC by Dr. W. Zielinsky, and the chemical analyses were reviewed and approved by Dr. W. Lijinsky.

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

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

---

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

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

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

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

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

SUMMARY

A bioassay of (2-chloroethyl)trimethylammonium chloride for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F1 mice.

Groups of 50 rats of each sex were administered either 1,500 or 3,000 ppm of the compound for 108 weeks, and 50 mice of each sex were administered 500 or 2,000 ppm for 102 weeks. Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the end of the period of administration of the test chemical.

Mean body weights of dosed rats and mice were lower than those of corresponding controls for part or all of the bioassay, except for the dosed male mice, whose mean body weights were essentially the same as those of the corresponding controls. Survival was not affected significantly in any of the dosed groups of rats or mice and was at least 64% in every dosed or control group of each species at the end of the bioassay. Sufficient numbers of dosed and control rats and mice of each sex were at risk for the development of late-appearing tumors. Since there was virtually no decrease in mean body weight in dosed male mice and only a slight decrease in female mice, and since there were no other toxic signs and no dose-related mortality, the animals may have been able to tolerate higher doses.

No tumors occurred in the rats or mice of either sex at incidences that could be associated with administration of the test chemical.

It is concluded that under the conditions of this bioassay, (2-chloroethyl)trimethylammonium chloride was not carcinogenic for F344 rats or B6C3F1 mice of either sex.

TABLE OF CONTENTS

		Page
I.	Introduction	1
II.	Materials and Methods	3
	A. Chemical	3
	B. Dietary Preparation	3
	C. Animals	4
	D. Animal Maintenance	5
	E. Subchronic Studies	7
	F. Chronic Studies	10
	G. Clinical and Pathologic Examinations	10
	H. Data Recording and Statistical Analyses	13
III.	Results - Rats	19
	A. Body Weights and Clinical Signs (Rats)	19
	B. Survival (Rats)	19
	C. Pathology (Rats)	22
	D. Statistical Analyses of Results (Rats)	23
IV.	Results - Mice	25
	A. Body Weights and Clinical Signs (Mice)	25
	B. Survival (Mice)	25
	C. Pathology (Mice)	28
	D. Statistical Analyses of Results (Mice)	29
V.	Discussion	31
VI.	Bibliography	35

APPENDIXES

Appendix A	Summary of the Incidence of Neoplasms in Rats Administered CCC in the Diet	37
Table A1	Summary of the Incidence of Neoplasms in Male Rats Administered CCC in the Diet	39
Table A2	Summary of the Incidence of Neoplasms in Female Rats Administered CCC in the Diet	43

		Page
Appendix B	Summary of the Incidence of Neoplasms in Mice Administered CCC in the Diet	47
Table B1	Summary of the Incidence of Neoplasms in Male Mice Administered CCC in the Diet	49
Table B2	Summary of the Incidence of Neoplasms in Female Mice Administered CCC in the Diet	52
Appendix C	Summary of the Incidence of Nonneoplastic Lesions in Rats Administered CCC in the Diet..	57
Table C1	Summary of the Incidence of Nonneoplastic Lesions in Male Rats Administered CCC in the Diet	59
Table C2	Summary of the Incidence of Nonneoplastic Lesions in Female Rats Administered CCC in the Diet	66
Appendix D	Summary of the Incidence of Nonneoplastic Lesions in Mice Administered CCC in the Diet	73
Table D1	Summary of the Incidence of Nonneoplastic Lesions in Male Mice Administered CCC in the Diet	75
Table D2	Summary of the Incidence of Nonneoplastic Lesions in Female Mice Administered CCC in the Diet	79
Appendix E	Analyses of the Incidence of Primary Tumors in Rats Administered CCC in the Diet	83
Table E1	Analyses of the Incidence of Primary Tumors in Male Rats Administered CCC in the Diet	85
Table E2	Analyses of the Incidence of Primary Tumors in Female Rats Administered CCC in the Diet	91
Appendix F	Analyses of the Incidence of Primary Tumors in Mice Administered CCC in the Diet	95

Page

Table F1 Analyses of the Incidence of Primary Tumors in Male Mice Administered CCC in the Diet 97

Table F2 Analyses of the Incidence of Primary Tumors in Female Mice Administered CCC in the Diet 100

TABLES

Table 1 CCC Subchronic Feeding Studies in Rats and Mice 9

Table 2 CCC Chronic Feeding Studies in Rats 11

Table 3 CCC Chronic Feeding Studies in Mice 12

FIGURES

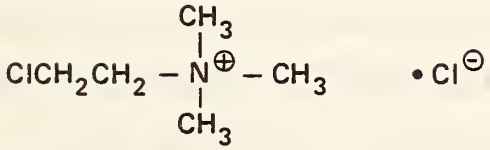
Figure 1 Growth Curves for Rats Administered CCC in the Diet 20

Figure 2 Survival Curves for Rats Administered CCC in the Diet 21

Figure 3 Growth Curves for Mice Administered CCC in the Diet 26

Figure 4 Survival Curves for Mice Administered CCC in the Diet 27

I. INTRODUCTION



(2-Chloroethyl) trimethylammonium chloride

(2-Chloroethyl)trimethylammonium chloride (CAS 999-81-5; NCI C02960) is a plant growth regulator, or dwarfing agent, used on poinsettias and azaleas in the United States (Meister, 1977), and on several food crops, specifically cereal grains, grapes, and pears in Europe (Vettorazzi, 1977; WHO/FAO, 1973). It has been marketed as Cyclocel® since 1959, and is known by the common names chlormequat, chlorocholine chloride, or CCC (Spencer, 1973). The term CCC will be used in this report.

The acute oral LD_{50} of CCC, has been reported to be in the range of 215 to 1,020 mg/kg in mice and 330 to 750 mg/kg in rats (WHO/FAO, 1973). Hennighausen and Tiefenbach (1975) found that 500 mg/kg of CCC given orally killed 21/40 male mice (strain not specified).

Acutely toxic doses of CCC cause lacrimation, salivation, and intestinal motility, and although these signs of toxicity of CCC in mammals resemble those of anticholinesterase agents, the chemical does not inhibit cholinesterase. These effects are produced by stimulation at muscarinic receptors and are partially antagonized by low doses of atropine, a cholinergic blocking agent which specifically blocks muscarinic receptors. Lethal doses cause respiratory failure that is due to neuromuscular blockage and that is unaffected by atropine treatment (Hennighausen et al., 1974; Hennighausen and Tiefenbach, 1975).

NCI initiated long-term tests with CCC in the early 1960's as part of an effort to assess the carcinogenic potential of chemicals that were of concern to public health because of their industrial importance or widespread use in the environment. In these chronic tests, some animals developed hepatomas, but these could not clearly be associated with the administration of the test chemical (Innes et al., 1969). Because these studies were preliminary, the chemical was selected for study in the Carcinogenesis Testing Program using expanded protocols.

II. MATERIALS AND METHODS

A. Chemical

CCC ($C_5H_{13}Cl_2N$) was obtained as technical-grade nonformulated material from American Cyanamid Co. The material was a yellow-white crystalline solid made by reacting ethylene dichloride with trimethylamine. The compound had a stated technical-grade purity of 97 to 98%. The effluent from high-pressure liquid chromatography using a refractive index detector contained three components of which 90% was CCC. Elemental analysis showed 36.4% carbon, 8.5% hydrogen, and 8.2% nitrogen (theoretical: 38.0% carbon, 8.2% hydrogen, and 8.9% nitrogen). The test material had a melting point of 240°C with decomposition (literature: 245°C with decomposition).

The CCC was stored at 7°C until used.

B. Dietary Preparation

Test diets containing CCC were prepared fresh every 1 to 1-1/2 weeks in 6- to 12-kg batches at appropriate doses. A known

weight of the chemical was first mixed with an equal weight of autoclaved Wayne® Sterilizable Lab Meal with 4% fat (Allied Mills, Inc., Chicago, Ill.), using a mortar and pestle. The mixing was continued with second and third additions of feed, and final mixing was performed with the remaining quantity of feed for a minimum of 15 minutes in a Patterson-Kelly twin-shell blender with an intensifer bar.

The diets were stored at 7°C in plastic bags until used.

C. Animals

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

21 g, averaging at least 18.5 g. Individual animals were identified by ear punch.

D. Animal Maintenance

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

The contaminated bedding was disposed of through an enclosed vacuum line that led to a holding tank from which the bedding was fed periodically into an incinerator. The cages were sanitized twice per week and the feed hoppers twice per month at 82 to

88°C in a tunnel-type cagewasher (Industrial Washing Corp., Mataway, N.J.), using the detergents, Clout® (Pharmacal Research Laboratories, Greenwich, Conn.) or Oxford D'Chlor (Oxford Chemicals, Atlanta, Ga.). The glass bottles and sipper tubes were sanitized at 82 to 88°C in a tunnel-type bottle washer (Consolidated Equipment Supply Co., Mercersburg, Pa.) three times per week, using a Calgen Commercial Division detergent (St. Louis, Mo.). The racks for the cages were sanitized at or above 82°C in a rack washer (Consolidated Equipment Supply Co.) once per month, using the Calgen Commercial Division detergent, and the filter paper was changed at the same time.

The animal rooms were maintained at 22 to 24°C and 45 to 55% relative humidity. Incoming air was passed through a filter of 65% efficiency and a bag filter of 95% efficiency at the intake and was expelled without recirculation through a "Z"-type roughing filter of 30% efficiency and a bag system of 90 to 95% efficiency at the exhaust (American Air Filters, Louisville, Ky., Mine Safety Appliances, Pittsburgh, Pa.). The room air was changed 15 times per hour. The air pressure was maintained negative to a clean hallway and positive to a return hallway. Fluorescent lighting was provided automatically on a 12-hour-per-day cycle.

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

(CAS 86-06-2) 2,4,6-trichlorophenol
(CAS 51-03-6) piperonyl butoxide

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

(CAS 156-62-7) calcium cyanamide
(CAS 95-80-7) 2,4 diaminotoluene
(CAS 19010-66-3) lead dimethyldithiocarbamate
(CAS 86-30-6) N-nitrosodiphenylamine
(CAS 88-96-0) phthalamide
(CAS 120-62-7) piperonyl sulfoxide
(CAS 137-17-7) 2,4,5-trimethylaniline

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses (MTD's) of CCC, on the basis of which two concentrations (referred to in this report as "low" and "high" doses) were selected for administration in the chronic studies. Groups of five rats and five mice of each sex were administered feed containing CCC at one of several doses for 7 weeks followed by 1 week of observation, and groups of five control animals of

each species and sex were administered basal diet only. Each animal was weighed twice per week.

Table 1 shows the survival of animals in each dosed group at the end of the course of chemical administration, and the mean body weights of dosed animals at week 7 expressed as percentages of mean body weights of the controls.

At the end of the subchronic studies, all animals were killed using CO_2 and necropsied. Clinical observations exclusive of weight and microscopic examination, showed no dose-related changes for male or female rats dosed at 3,150 or 6,800 ppm nor for male or female mice dosed at 10,000 ppm.

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

Table 1. CCC Subchronic Feeding Studies in Rats and Mice

Dose (ppm)	Male		Female	
	Survival (a)	Mean Weight at Week 7 as % of Control	Survival (a)	Mean Weight at Week 7 as % of Control
RATS				
3,150	5/5	85	5/5	88
4,600	5/5	83	5/5	81
6,800	5/5	79	5/5	68
10,000	4/5	45	4/5	49
14,700	0/5		0/5	
MICE				
1,200	5/5	86	5/5	79
2,500	5/5	78	5/5	85
4,000	5/5	81	5/5	66
5,000	5/5	77	5/5	56
7,000	5/5	74	5/5	68
10,000	5/5	70	5/5	53
20,000	2/5	60	2/5	59

(a) Number surviving/number in group.

The low and high doses for rats were set at 1,500 and 3,000 ppm; for mice, 500 and 2,000 ppm.

F. Chronic Studies

The test groups, doses administered, and durations of the chronic feeding studies are shown in tables 2 and 3.

G. Clinical and Pathologic Examinations

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

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions. The tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined

Table 2. CCC Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals (a)	CCC in Diet (b) (ppm)	Time on Study (weeks)
Male			
Matched-Control	20	0	108
Low-Dose	50	1,500	108
High-Dose	50	3,000	108
Female			
Matched-Control	20	0	108
Low-Dose	50	1,500	108
High-Dose	50	3,000	108

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

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

Table 3. CCC Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals (a)	CCC in Diet (b) (ppm)	Time on Study (weeks)
Male			
Matched-Control	20	0	102
Low-Dose	50	500	102
High-Dose	50	2,000	102
Female			
Matched-Control	20	0	102
Low-Dose	50	500	102
High-Dose	50	2,000	102

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

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

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

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

H. Data Recording and Statistical Analyses

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

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

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P 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 is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the 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) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for a number of dosed groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the

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

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

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

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

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

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

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

III. RESULTS - RATS

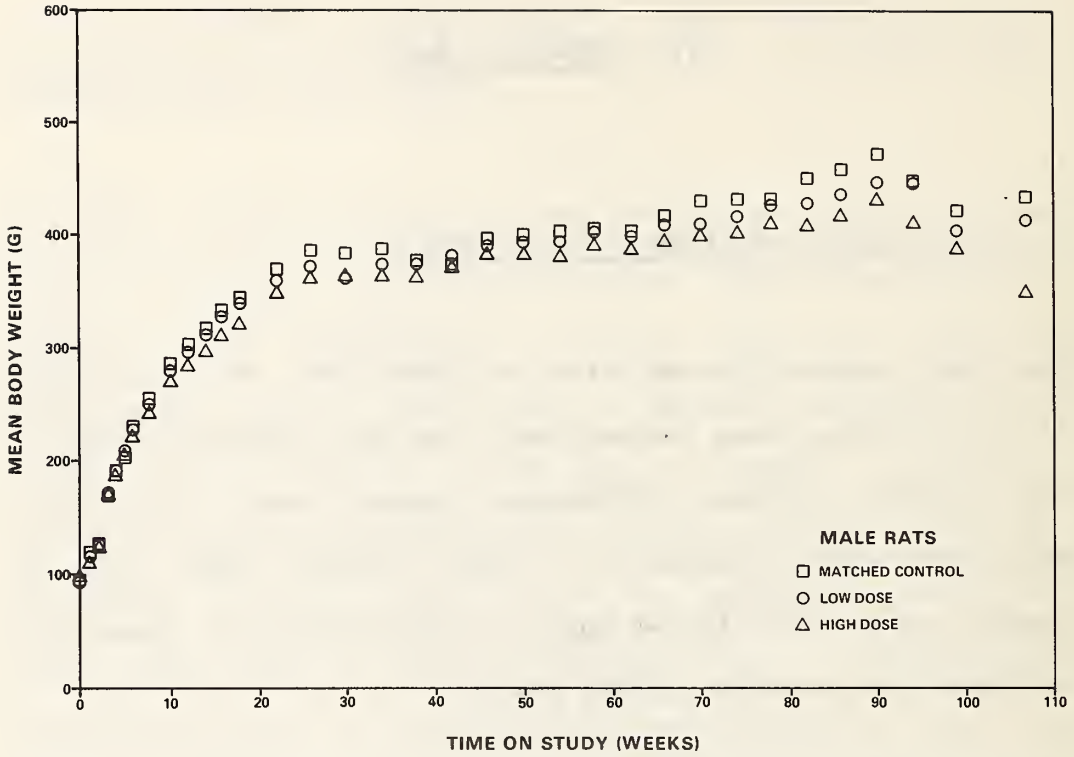
A. Body Weights and Clinical Signs (Rats)

Mean body weights of dosed male and female rats were lower than those of corresponding controls and were dose related throughout the bioassay, although differences between dosed and control males were slight (figure 1). Other clinical signs, such as corneal opacity and tissue masses, were observed at comparable incidences in dosed and control groups.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered CCC in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2. The result of the Tarone test for dose-related trend in mortality is not significant in either sex.

In male rats, 32/50 (64%) of the high-dose group, 37/50 (74%) of the low-dose group, and 14/20 (70%) of the control group lived to



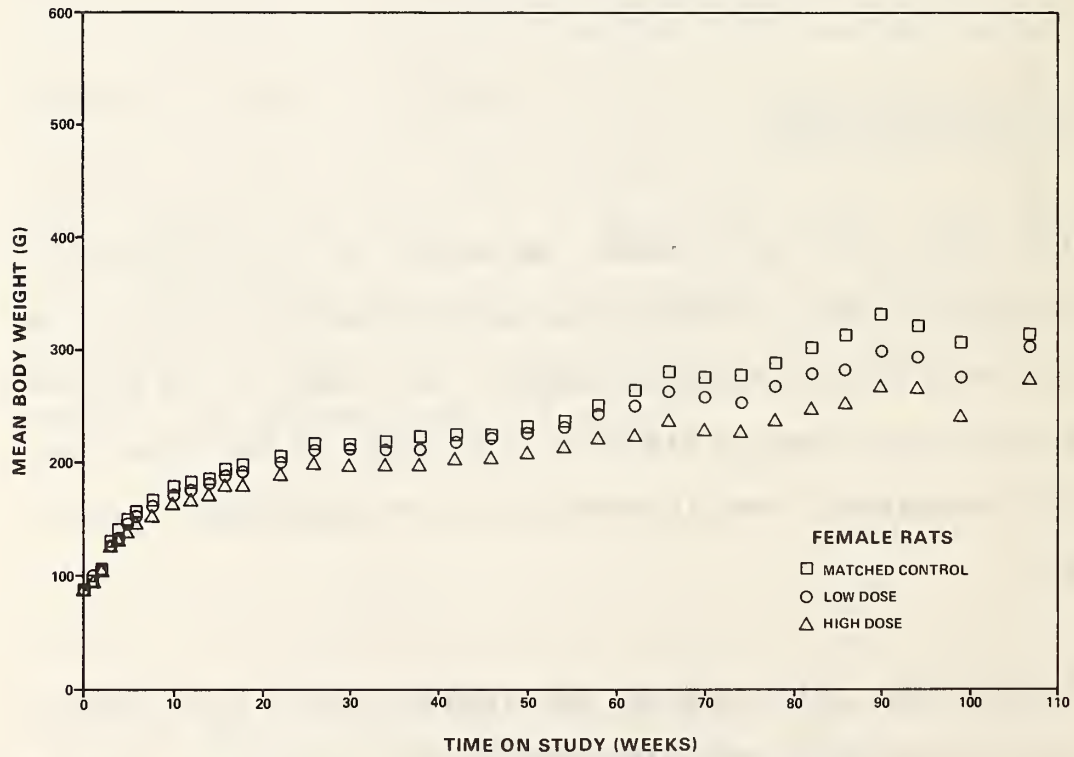


Figure 1. Growth Curves for Rats Administered CCC in the Diet

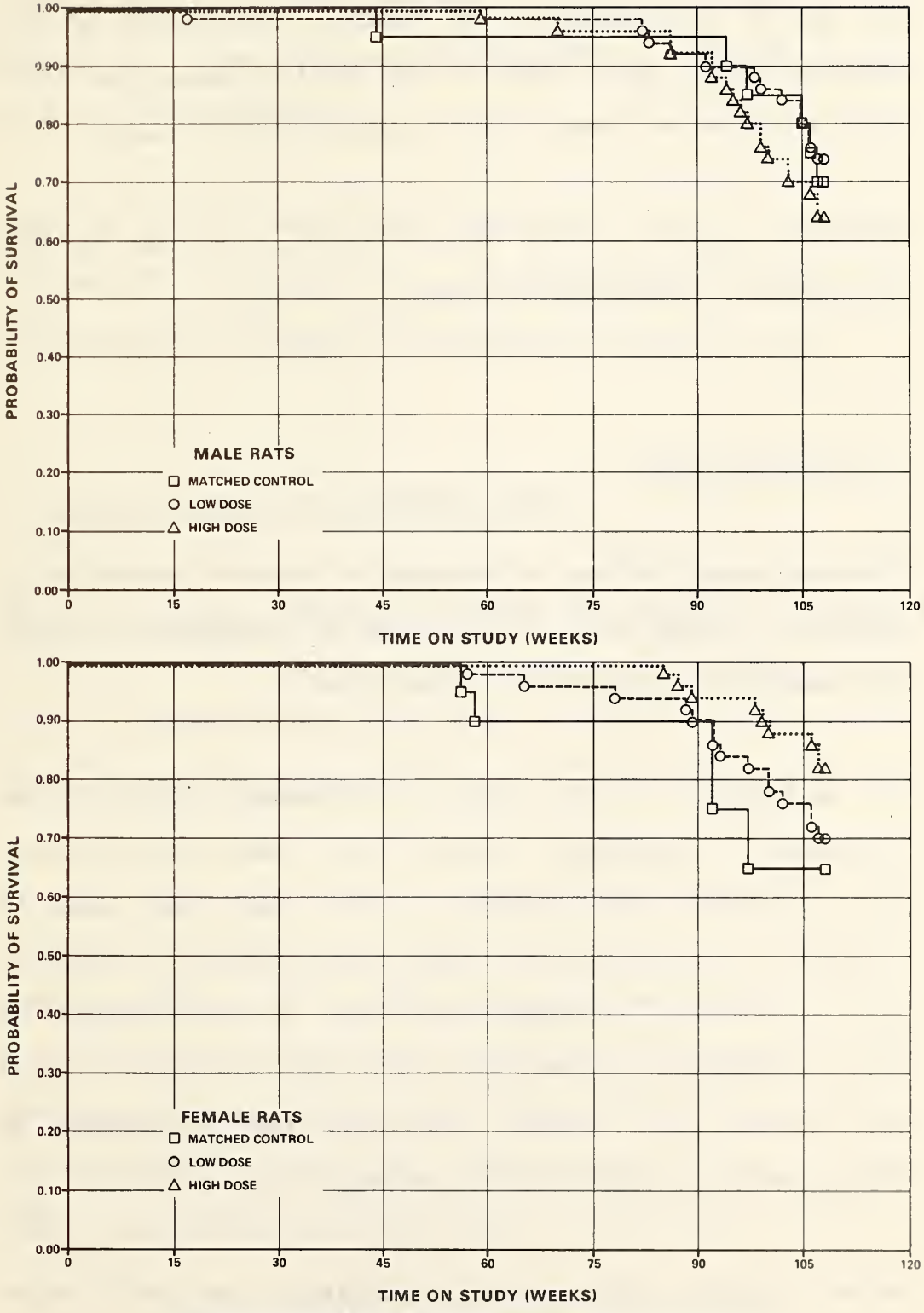


Figure 2. Survival Curves for Rats Administered CCC in the Diet

the end of the bioassay. In females, 41/50 (82%) of the high-dose group, 35/50 (70%) of the low-dose group, and 13/20 (65%) of the control group lived to the end of the bioassay.

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

C. Pathology (Rats)

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

From an inspection of the numerical differences in the incidences of leukemia or malignant lymphoma in the female rats (controls 3/20, low-dose 11/50, high-dose 14/50), one could infer an increase in neoplasia in the animals receiving CCC. There was also an apparent dose-related increase in the incidence of islet-cell adenomas of the pancreas of the male rats (controls 0/18, low-dose 1/47, high-dose 7/45). No islet-cell adenomas of the pancreas were seen in any of the female rats.

Several chronic inflammatory, degenerative, or proliferative

lesions frequently seen in aged F344 rats occurred with approximately equal frequency and severity in each sex of the dosed and control animals.

Based on the histopathologic examination, there was no clear evidence of carcinogenicity in F344 rats due to the administration of CCC under the conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidence of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In male rats, the result of the Cochran-Armitage test for positive dose-related trend in the incidence of islet-cell adenoma is significant (P = 0.023), but the results of the Fisher exact test are not significant. The historical records of this laboratory show an incidence of 16/416 (4%) in male controls, with incidences in individual control groups as high as 3/16 (19%) or 2/19 (11%) to as low as 0/20.

The incidences of female rats with lymphoma or leukemia are 3/20 (15%) in the control group, 11/50 (22%) in the low-dose group, and 14/50 (28%) in the high-dose groups. The results of the Cochran-Armitage test and the Fisher exact test are not significant. The historical records of this laboratory show an incidence of 42/420 (10%) in female controls with incidences in individual control groups as high as 4/20 (20%) or 3/20 (15%) to as low as 0/20.

Significant results in the negative direction are observed in the incidence of C-cell tumors of the thyroid in male rats and in the incidence of fibroadenomas of the mammary gland in female rats.

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

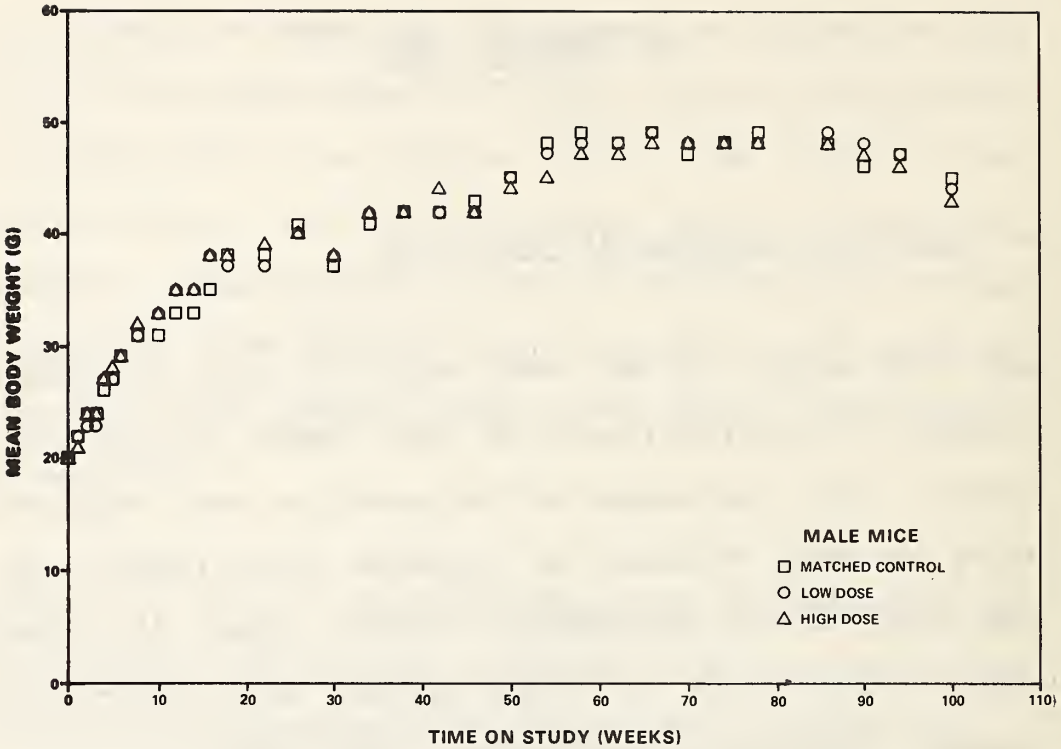
IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the dosed male mice were essentially unaffected by administration of the test chemical throughout the bioassay. Mean body weights of the female mice were unaffected during the first 40 weeks, but thereafter were slightly lower than those of the corresponding controls (figure 3). Other clinical signs, such as tissue masses, were observed at comparable incidences in the dosed and control groups.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered CCC in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 4. In male mice, the result of the Tarone test for dose-related trend in mortality is not significant. An indicated departure from linear trend ($P = 0.014$) is observed, because the control animals did not survive as long as the dosed animals. The result of the Cox test between



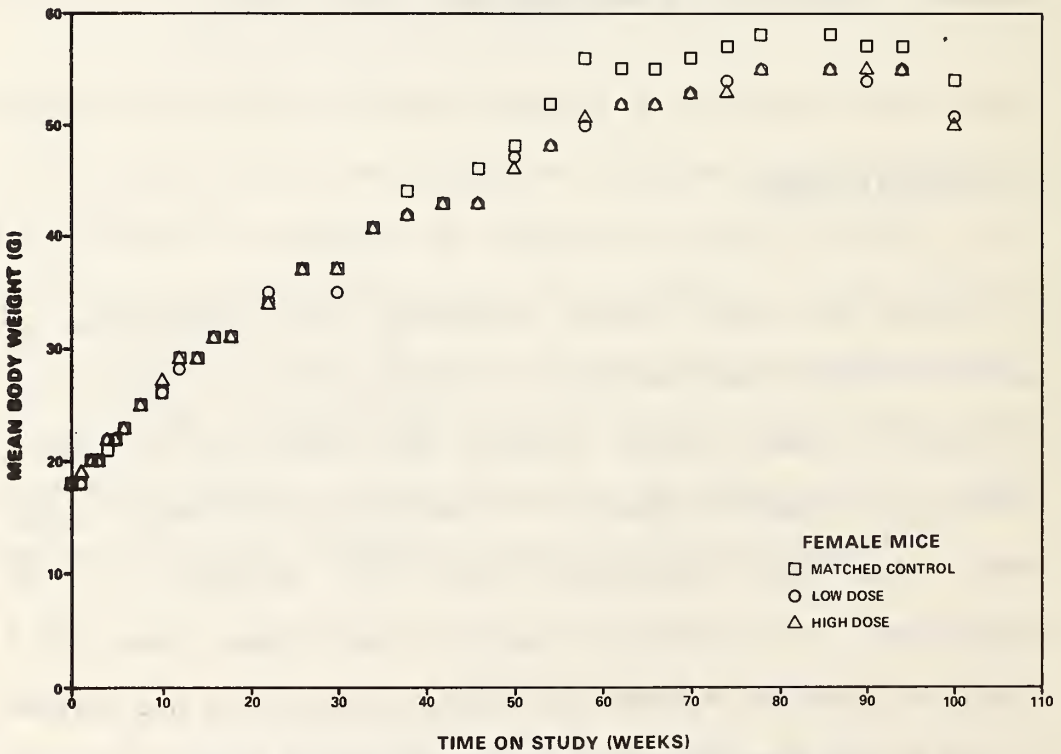


Figure 3. Growth Curves for Mice Administered CCC in the Diet

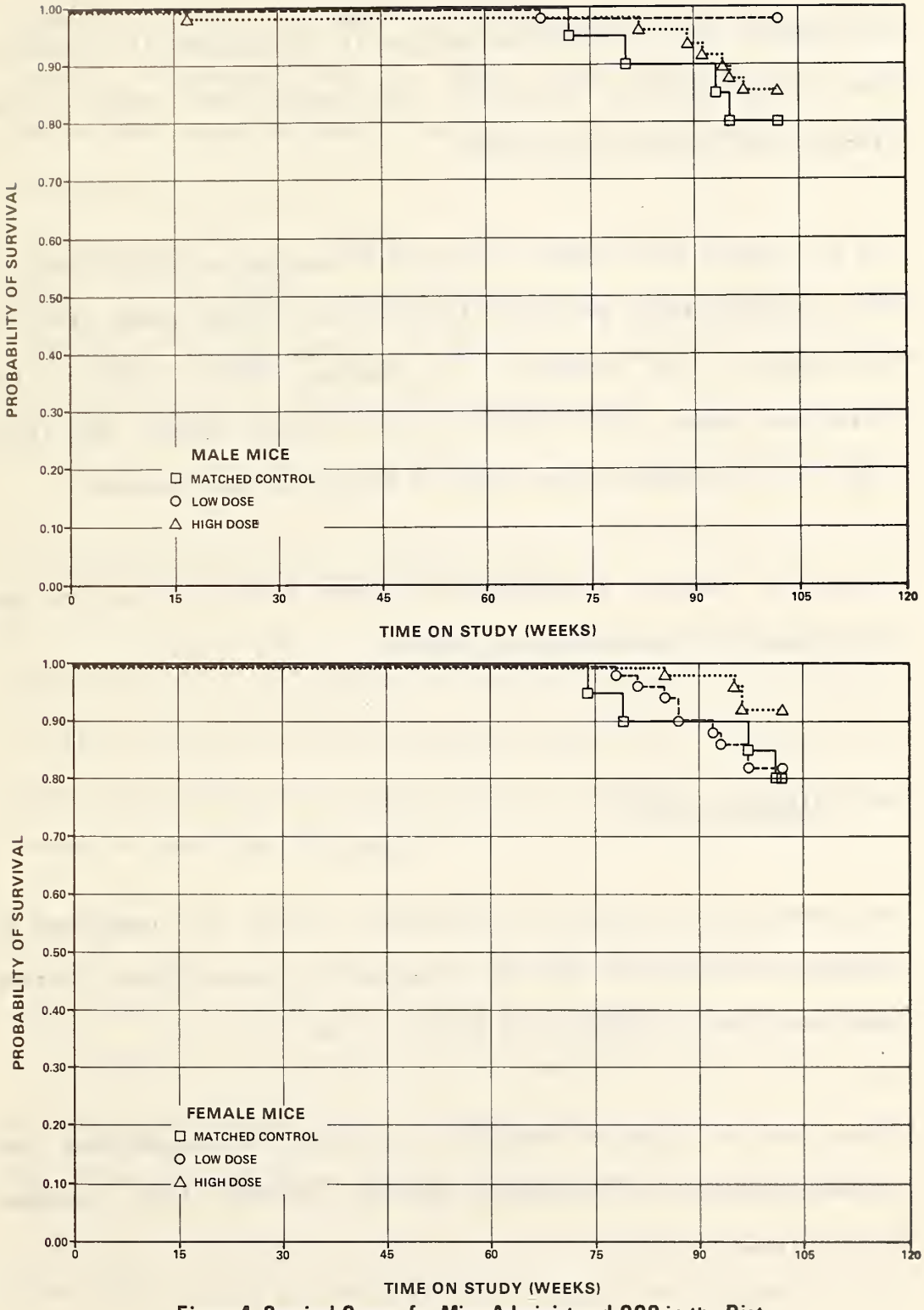


Figure 4. Survival Curves for Mice Administered CCC in the Diet

the control and the low-dose groups is significant (P = 0.034), but in the negative direction. In females, the result of the Tarone test is not significant.

In male mice, 42/50 (84%) of the high-dose group, 49/50 (98%) of the low-dose group, and 16/20 (80%) of the control group lived to the end of the bioassay. In females, 46/50 (92%) of the high-dose group, 41/50 (82%) of the low-dose group, and 16/20 (80%) of the control group lived to the end of the bioassay.

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

C. Pathology (Mice)

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

There was a slightly increased incidence of hemangiomas and hemangiosarcomas in the dosed females (controls 1/20, low-dose 4/50, high-dose 5/50).

Several chronic inflammatory, degenerative, or proliferative lesions frequently seen in aged B6C3F1 mice occurred with approximate equal frequency and severity in the dosed and control animals.

Based on the histopathologic examination, there was no clear evidence of carcinogenicity in B6C3F1 mice due to administration of CCC under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

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

In male mice, the result of the Cochran-Armitage test for dose-related trend in the incidence of hepatocellular carcinoma is significant ($P = 0.036$), but the results of the Fisher exact test are not significant. In female mice, a slightly increased incidence of hemangiomas and hemangiosarcomas is not significant.

Significant trends in the negative direction are observed in the

incidences of lymphoma and of cortical adenoma of the adrenal in male mice and of adenoma of the pituitary in females.

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

V. DISCUSSION

Mean body weights of the dosed rats and mice were lower than those of corresponding controls for part or all of the bioassay, except for the dosed male mice, whose mean body weights were essentially unaffected by administration of the test chemical. Survival was not affected significantly in any of the dosed groups of rats or mice and was 64% or greater in both dosed and control groups of each species at the end of the bioassay. Sufficient numbers of rats and mice of each sex were at risk for the development of late-appearing tumors. Since there was virtually no decrease in mean body weight in dosed male mice and only a slight decrease in female mice and since there were no other toxic signs and no dose-related mortality, the mice may have been able to tolerate higher doses.

Islet-cell adenomas of the pancreas occurred in the male rats at incidences that were dose related ($P = 0.023$), but in direct comparisons the incidences in the individual dosed groups were not significantly higher than those in the control group (controls 0/19, low-dose 2/47, high-dose 7/49). In female rats, lymphoma or leukemia occurred in a higher percentage of dosed than control animals (controls 3/20, or 15%, low-dose 11/50, or

22%, and high-dose 14/50, or 28%). The results of the statistical analyses were not, however, significant. Hepatocellular carcinomas occurred in the male mice at incidences that were dose related (P = 0.036), but in direct comparisons the incidences in the individual dosed groups were not significantly higher than that in the control group (controls 7/20, low-dose 13/50, high-dose 23/49). Thus, the occurrence of pancreatic tumors in the dosed male rats, lymphoma or leukemia in the dosed female rats, and liver tumors in the dosed male mice cannot clearly be related to administration of the test chemical. No tumors occurred in the female mice at incidences that were significant either for positive dose-related trend or for greater incidences in dosed groups than in control groups.

In previous long-term feeding studies of CCC, administration of 1,000 ppm for 78 weeks to CFLP mice caused no adverse effect on the survival and only about 6% decrease in body weight gained; an incidence of benign lung tumors of 20/52 in the dosed males was higher than that of 10/51 in the controls, but was considered to be within the normal range under the conditions of the test (WHO/FAO, 1973). In other long-term feeding studies in mice, administration of CCC at 21.5 mg/kg by stomach tube for 4 weeks, then in the diet at 65 ppm for 18 months, to B6C3F1 and B6AKF1 hybrids led to incidences of hepatomas in 5/18 males of each

hybrid compared with incidences of 6/257 and 7/240 in the corresponding controls (Innes et al., 1969; WHO/FAO 1973). When rats of unidentified strain were administered 500 or 1,000 ppm CCC in the diet for 2 years, they showed no signs of toxicity or histopathologic abnormalities attributable to the test chemical (WHO/FAO, 1973).

It is concluded that under the conditions of this bioassay, CCC was not carcinogenic for F344 rats or B6C3F1 mice of either sex.

VI. BIBLIOGRAPHY

Armitage, P., Statistical Methods in Medical Research, John Wiley & Sons, Inc., New York, 1971, pp. 362-365.

Berenblum, I., ed., Carcinogenicity Testing, A Report of the Panel on Carcinogenicity of the Cancer Research Commission of the UICC, Vol. 2, International Union Against Cancer, Geneva, 1969.

Cox, D. R., Regression models and life tables. J. R. Statist. Soc. B 34(2):187-220, 1972.

Cox, D. R., Analysis of Binary Data, Methuen & Co., Ltd., London, 1970, pp.48-52.

Gart, J. J., The comparison of proportions: A review of significance tests, confidence limits and adjustments for stratification. Rev. Int. Statist. Inst. 39(2):148-169, 1971.

Hennighausen, G. and Tiefenbach, B., The toxicological and pharmacological properties of chlorocholine chloride. In: Developmental and Genetic Aspects of Drug and Environmental Toxicity. Proceedings of the European Society of Toxicology, Vol. 16, American Elsevier Publishing Co., Inc., New York, 1975, pp. 300-302.

Hennighausen, G., Tiefenbach, B., and Dietrich, C., Untersuchungen über toxikologische und pharmakologische Eigenschaften von Chlorcholinchlorid und N,N-Dimethyl-(2-bromäthyl)-hydraziniumbromid. Acta biol. med. germ. 33:89-98, 1974.

Innes, J. R. M., Ulland, B. M., Valerio, M. G., Petrucelli, L., Fishbein, L., Hart, E. R., Pallotta, A. J., Bates, R. R., Falk, H. L., Gart, J. J., Klein, M., Mitchell, I., and Peters, J., Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. J. Natl. Cancer Inst. 42(6):1101-1114.

Kaplan, E. L., and Meier, P., Nonparametric estimation from incomplete observations. J. Amer. Statist. Assoc. 53:457-481, 1958.

Linhart, M. S., Cooper, J. A., Martin, R. L., Page, N. P., and Peters, J. A., Carcinogenesis bioassay data system. Comp. and Biomed. Res. 7:230-248, 1974.

Meister, R. T., ed., Chlormequat chloride. In: 1977 Farm Chemicals Handbook, Meister Publishing Co., Willoughby, Ohio, 1977, p. D60.

Miller, R. G., Jr., Simultaneous Statistical Inference, McGraw-Hill Book Co., New York, 1966, pp. 6-10.

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F., and Kaufman, D. G., Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo (a) pyrene and ferric oxide. Cancer Res. 32:1073-1081, 1972.

Spencer, E. Y., Chlormequat. In: Guide to the Chemicals Used in Crop Protection, University of Western Ontario, London, Ontario, 1973, p. 104.

Tarone, R. E., Tests for trend in life table analysis. Biometrika 62:679-682, 1975.

Vettorazzi, G., State of the art of the toxicological evaluation carried out by the Joint FAO/WHO Expert Committee on Pesticides Residues. III. Miscellaneous pesticides used in agriculture and public health. Residues Review 66:137-184, 1977.

WHO/FAO, Chlormequat. In: 1972 Evaluations of Some Pesticide Residues in Food - The Monographs, World Health Organization, Geneva, 1973, pp.103-130.

APPENDIX A

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

TABLE A1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
BASAL-CELL TUMOR		1 (2%)	
TRICHOEPITHELIOMA	1 (5%)	2 (4%)	
FIBROMA			2 (4%)
*SUBCUT TISSUE	(20)	(50)	(50)
SARCOMA, NOS	1 (5%)		1 (2%)
FIBROMA	1 (5%)	1 (2%)	3 (6%)
LIPOMA			2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
CARCINOMA, NOS, METASTATIC		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		3 (6%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	5 (25%)	5 (10%)	11 (22%)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (5%)		
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		2 (4%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (4%)	1 (2%)
MONOCYTIC LEUKEMIA		1 (2%)	
#SPLEEN	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#THYMUS	(7)	(36)	(43)
CARCINOMA,NOS		1 (3%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FIBROSARCOMA			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(50)
HEPATOCELLULAR CARCINOMA	1 (5%)	2 (4%)	2 (4%)
#PANCREAS	(19)	(47)	(49)
ACINAR-CELL ADENOMA		1 (2%)	1 (2%)
#STOMACH	(20)	(49)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
TUBULAR-CELL ADENOMA			1 (2%)
LIPOSARCOMA	1 (5%)		
#KIDNEY/CAPSULE	(20)	(49)	(50)
SARCOMA, NOS, METASTATIC	1 (5%)		
#URINARY BLADDER	(19)	(49)	(47)
TRANSITIONAL-CELL CARCINOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(49)	(47)
CHROMOPHOBE ADENOMA	6 (30%)	11 (22%)	16 (34%)
CHROMOPHOBE CARCINOMA		4 (8%)	
#ADRENAL	(20)	(49)	(50)
CARCINOMA, NOS, METASTATIC		1 (2%)	
CORTICAL CARCINOMA		1 (2%)	
PHEOCHROMOCYTOMA	1 (5%)	3 (6%)	
#THYROID	(20)	(48)	(50)
FOLLICULAR-CELL ADENOMA			1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FOLLICULAR-CELL CARCINOMA			2 (4%)
C-CELL ADENOMA	3 (15%)	7 (15%)	
C-CELL CARCINOMA		1 (2%)	
CYSTADENOMA, NOS		1 (2%)	
#PANCREATIC ISLETS	(19)	(47)	(49)
ISLET-CELL ADENOMA		2 (4%)	7 (14%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
FIBROADENOMA		1 (2%)	
*PREPUTIAL GLAND	(20)	(50)	(50)
ADENOMA, NOS		1 (2%)	
#TESTIS	(20)	(49)	(49)
INTERSTITIAL-CELL TUMOR	17 (85%)	42 (86%)	38 (78%)
LIPOMA			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(49)
OSTEOSARCOMA		1 (2%)	
OLIGODENDROGLIOMA			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*BODY CAVITIES	(20)	(50)	(50)
MESOTHELIOMA, NOS			1 (2%)
*PERITONEUM	(20)	(50)	(50)
FIBROSARCOMA			1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*PLEURA	(20)	(50)	(50)
CARCINOMA,NOS	1 (5%)		
MESOTHELIOMA, NOS	1 (5%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
FIBROSARCOMA			1 (2%)
MESOTHELIOMA, MALIGNANT	1 (5%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	11	11
MORIBUND SACRIFICE	3	2	7
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	14	37	32
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	47	49
TOTAL PRIMARY TUMORS	41	98	98
TOTAL ANIMALS WITH BENIGN TUMORS	18	45	47
TOTAL BENIGN TUMORS	29	76	72
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	17	20
TOTAL MALIGNANT TUMORS	11	22	25
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	
TOTAL SECONDARY TUMORS	1	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1		1
TOTAL UNCERTAIN TUMORS	1		1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
PAPILLOMA, NOS		1 (2%)	
SQUAMOUS CELL CARCINOMA		1 (2%)	
TRICHOEPITHELIOMA		1 (2%)	
KERATOACANTHOMA			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA			1 (2%)
FIBROSARCOMA			1 (2%)
HEMANGIOSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#TRACHEA	(20)	(48)	(49)
ADENOCARCINOMA, NOS	1 (5%)		
#LUNG	(20)	(50)	(49)
ADENOCARCINOMA, NOS, METASTATIC	1 (5%)		
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		5 (10%)	13 (26%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
LEUKEMIA,NOS	1 (5%)		
MONOCYTIC LEUKEMIA	1 (5%)	1 (2%)	
*BLOOD	(20)	(50)	(50)
LEUKEMIA,NOS		2 (4%)	
LYMPHOCYTIC LEUKEMIA		1 (2%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#SPLEEN	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)	1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#MANDIBULAR L. NODE	(20)	(49)	(49)
ADENOCARCINOMA, NOS, METASTATIC	1 (5%)		
#THYMUS	(12)	(38)	(38)
CARCINOMA,NOS			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
HEPATOCELLULAR CARCINOMA			1 (2%)
#CECUM	(18)	(50)	(50)
ADENOMATOUS POLYP, NOS			1 (2%)
URINARY SYSTEM			
#URINARY BLADDER	(19)	(50)	(50)
TRANSITIONAL-CELL CARCINOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(49)	(49)
CARCINOMA,NOS		1 (2%)	
ADENOMA, NOS			1 (2%)
CHROMOPHOBE ADENOMA	5 (25%)	22 (45%)	20 (41%)
CHROMOPHOBE CARCINOMA	1 (5%)	2 (4%)	1 (2%)
#ADRENAL	(20)	(50)	(50)
CORTICAL ADENOMA			1 (2%)
PHEOCHROMOCYTOMA			1 (2%)
#THYROID	(20)	(49)	(49)
CARCINOMA,NOS			1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ADENOMA, NOS			1 (2%)
C-CELL ADENOMA		3 (6%)	2 (4%)
C-CELL CARCINOMA	1 (5%)	1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOMA, NOS			1 (2%)
CYSTADENOMA, NOS	1 (5%)		
FIBROMA	1 (5%)		
FIBROADENOMA	4 (20%)	7 (14%)	2 (4%)
#UTERUS	(20)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
LEIOMYOMA		1 (2%)	
LEIOMYOSARCOMA	1 (5%)		1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(50)
CHROMOPHOBE CARCINOMA, INVASIVE		1 (2%)	
CHROMOPHOBE CARCINOMA, METASTATI			1 (2%)
ASTROCYTOMA	1 (5%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	8	7
MORIBUND SACRIFICE	5	7	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	35	41
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	41	37
TOTAL PRIMARY TUMORS	19	54	55
TOTAL ANIMALS WITH BENIGN TUMORS	8	30	27
TOTAL BENIGN TUMORS	11	37	33
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	17	19
TOTAL MALIGNANT TUMORS	8	17	22
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	1
TOTAL SECONDARY TUMORS	2	1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE
ADIMINISTERED CCC IN THE DIET

TABLE B1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(49)
HEMANGIOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)	3 (6%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (10%)	7 (14%)	3 (6%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS	3 (15%)	7 (14%)	2 (4%)
#MESENTERIC L. NODE	(20)	(50)	(49)
HEMANGIOMA		1 (2%)	
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#KIDNEY	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#THYMUS	(18)	(43)	(44)
THYMOMA, MALIGNANT		1 (2%)	
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(49)
HEPATOCELLULAR CARCINOMA	7 (35%)	13 (26%)	23 (47%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMANGIOSARCOMA		1 (2%)	
#ESOPHAGUS	(20)	(41)	(44)
PAPILLOMA, NOS	1 (5%)		
#STOMACH	(20)	(50)	(49)
SQUAMOUS CELL CARCINOMA			1 (2%)
ADENOMATOUS POLYP, NOS		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(20)	(48)	(49)
CORTICAL ADENOMA	2 (10%)	1 (2%)	
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	1	7
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	16	49	42
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	29	29
TOTAL PRIMARY TUMORS	17	38	32
TOTAL ANIMALS WITH BENIGN TUMORS	4	6	3
TOTAL BENIGN TUMORS	5	6	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	28	26
TOTAL MALIGNANT TUMORS	12	32	29
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
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 ADMINISTERED CCC IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
RHABDOMYOSARCOMA			1 (2%)
HEMANGIOMA	1 (5%)		
HEMANGIOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)	2 (4%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	7 (35%)	8 (16%)	10 (20%)
*BLOOD	(20)	(50)	(50)
LEUKEMIA,NOS	1 (5%)		2 (4%)
#BONE MARROW	(19)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)	
#SPLEEN	(19)	(48)	(50)
HEMANGIOSARCOMA		2 (4%)	1 (2%)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#LUNG	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(19)	(49)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
*MESENTERY	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#KIDNEY	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#THYMUS	(16)	(41)	(46)
MALIGNANT LYMPHOMA, NOS		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(47)	(50)
CYSTADENOMA, NOS			1 (2%)
#LIVER	(19)	(49)	(50)
HEPATOCELLULAR CARCINOMA	4 (21%)	7 (14%)	4 (8%)
#DUODENUM	(18)	(45)	(50)
HEMANGIOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(49)	(48)
ADENOMA, NOS	2 (13%)	2 (4%)	
#ADRENAL	(19)	(49)	(50)
CORTICAL ADENOMA		1 (2%)	
#THYROID	(19)	(47)	(50)
FOLLICULAR-CELL ADENOMA	1 (5%)	1 (2%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOCARCINOMA, NOS			1 (2%)
#UTERUS	(20)	(46)	(50)
ADENOCARCINOMA, NOS			1 (2%)
LEIOMYOMA			1 (2%)
#OVARY	(20)	(47)	(50)
CYSTADENOMA, NOS	1 (5%)		
GRANULOSA-CELL TUMOR		2 (4%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
LEIOMYOSARCOMA			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
HEMANGIOSARCOMA			2 (4%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	9	4
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	41	46
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	25	26
TOTAL PRIMARY TUMORS	18	30	34
TOTAL ANIMALS WITH BENIGN TUMORS	3	5	4
TOTAL BENIGN TUMORS	5	5	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	11	19	24
TOTAL MALIGNANT TUMORS	13	23	30
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
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 C

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

TABLE C1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
HEMATOMA, NOS			1 (2%)
HEMORRHAGIC CYST			1 (2%)
STEATITIS		1 (2%)	
FIBROSIS		1 (2%)	
RESPIRATORY SYSTEM			
#TRACHEA	(20)	(46)	(50)
INFLAMMATION, CHRONIC			1 (2%)
#LUNG/BRONCHUS	(20)	(49)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR	11 (55%)	13 (27%)	
#LUNG	(20)	(49)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR		24 (49%)	39 (78%)
INFLAMMATION, INTERSTITIAL		1 (2%)	1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
HEMATOPOIETIC SYSTEM			
*BLOOD	(20)	(50)	(50)
MONOCYTOSIS		2 (4%)	
LEUKOPENIA, NOS	1 (5%)	1 (2%)	
HYPERPLASIA, NEUTROPHILIC		2 (4%)	1 (2%)
#BONE MARROW	(20)	(49)	(50)
HYPERPLASIA, GRANULOCYTIC		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#SPLEEN	(20)	(49)	(50)
CONGESTION, NOS	2 (10%)		2 (4%)
INFARCT, NOS			1 (2%)
HEMOSIDEROSIS		3 (6%)	3 (6%)
HYPERPLASIA, RETICULUM CELL			1 (2%)
HYPERPLASIA, LYMPHOID	1 (5%)		
HEMATOPOIESIS	10 (50%)	29 (59%)	20 (40%)
MYELOPOIESIS		1 (2%)	
#LYMPH NODE	(20)	(49)	(50)
INFLAMMATION, NECROTIZING		1 (2%)	
HYPERPLASIA, DIFFUSE	1 (5%)		
HYPERPLASIA, LYMPHOID			1 (2%)
#MANDIBULAR L. NODE	(20)	(49)	(50)
CYST, NOS		1 (2%)	2 (4%)
CONGESTION, NOS			1 (2%)
EDEMA, NOS			1 (2%)
HEMORRHAGE			1 (2%)
FIBROSIS			1 (2%)
PERIARTERITIS			1 (2%)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, DIFFUSE	2 (10%)		
PLASMACYTOSIS	6 (30%)		12 (24%)
ERYTHROPHAGOCYTOSIS			1 (2%)
HYPERPLASIA, RETICULUM CELL			1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#MEDIASTINAL L.NODE	(20)	(49)	(50)
PIGMENTATION, NOS	1 (5%)		
#MESENTERIC L. NODE	(20)	(49)	(50)
CYST, NOS			2 (4%)
EDEMA, NOS	1 (5%)	1 (2%)	1 (2%)
PIGMENTATION, NOS	1 (5%)		
HYPERPLASIA, RETICULUM CELL		1 (2%)	
#THYMUS	(7)	(36)	(43)
CONGESTION, NOS		1 (3%)	
CIRCULATORY SYSTEM			
#HEART	(20)	(49)	(50)
THROMBUS, MURAL		1 (2%)	1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, SUPPURATIVE	1 (5%)		
FIBROSIS		27 (55%)	40 (80%)
FIBROSIS, FOCAL	11 (55%)	16 (33%)	
#HEART/ATRIUM	(20)	(49)	(50)
THROMBOSIS, NOS	1 (5%)		
#MYOCARDIUM	(20)	(49)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
*PULMONARY ARTERY	(20)	(50)	(50)
HYPERTROPHY, NOS			8 (16%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(49)	(50)
INFLAMMATION, CHRONIC			1 (2%)
#LIVER	(20)	(49)	(50)
CONGESTION, NOS			1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)	1 (2%)	
INFLAMMATION, NECROTIZING		1 (2%)	
CHOLANGIOFIBROSIS	11 (55%)	41 (84%)	37 (74%)
CIRRHOSIS, NOS	2 (10%)		3 (6%)
CIRRHOSIS, PORTAL			1 (2%)
DEGENERATION, HYDROPIC		1 (2%)	
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL		5 (10%)	
AMYLOIDOSIS	1 (5%)		
METAMORPHOSIS FATTY	2 (10%)	9 (18%)	9 (18%)
LIPOIDOSIS			1 (2%)
BASOPHILIC CYTO CHANGE	1 (5%)	13 (27%)	3 (6%)
CLEAR-CELL CHANGE		2 (4%)	1 (2%)
MEGALOCYTOSIS	1 (5%)	5 (10%)	2 (4%)
LEUKEMOID REACTION		2 (4%)	1 (2%)
#LIVER/CENTRILOBULAR	(20)	(49)	(50)
DEGENERATION, HYDROPIC		1 (2%)	
#LIVER/HEPATOCYTES	(20)	(49)	(50)
METAMORPHOSIS FATTY	1 (5%)		
#BILE DUCT	(20)	(49)	(50)
INFLAMMATION, CHRONIC FOCAL	1 (5%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NOS	1 (5%)		
#PANCREAS	(19)	(47)	(49)
CONGESTION, NOS			1 (2%)
FIBROSIS			3 (6%)
FIBROSIS, FOCAL	1 (5%)	4 (9%)	3 (6%)
PERIARTERITIS	1 (5%)		1 (2%)
ATROPHY, NOS	1 (5%)	6 (13%)	3 (6%)
#PANCREATIC ACINUS	(19)	(47)	(49)
FIBROSIS, FOCAL		3 (6%)	
HYPERPLASIA, NOS			1 (2%)
#STOMACH	(20)	(49)	(50)
ULCER, NOS		1 (2%)	1 (2%)
ULCER, FOCAL	1 (5%)		
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, NECROTIZING		1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE		1 (2%)	
GRANULOMA, FOREIGN BODY		1 (2%)	
ULCER, PERFORATED			1 (2%)
FIBROSIS, FOCAL			1 (2%)
#GASTRIC MUCOSA	(20)	(49)	(50)
DILATATION, NOS			1 (2%)
#ILEUM	(19)	(49)	(49)
FIBROSIS, FOCAL			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
CAST, NOS	13 (65%)	42 (86%)	38 (76%)
PYELONEPHRITIS SUPPURATIVE			2 (4%)
ABSCESS, NOS	1 (5%)	1 (2%)	
INFLAMMATION, CHRONIC		43 (88%)	40 (80%)
INFLAMMATION, CHRONIC FOCAL	9 (45%)		
INFLAMMATION, CHRONIC DIFFUSE	1 (5%)		
INFLAMMATION, PYOGRANULOMATOUS		1 (2%)	
PERIARTERITIS			1 (2%)
DEGENERATION, HYALINE	1 (5%)		
HYPERPLASIA, TUBULAR CELL			1 (2%)
#KIDNEY/TUBULE	(20)	(49)	(50)
METAMORPHOSIS FATTY		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
PIGMENTATION, NOS	3 (15%)		
HEMOSIDEROSIS		1 (2%)	
#URINARY BLADDER	(19)	(49)	(47)
CALCULUS, NOS			1 (2%)
INFLAMMATION, NECROTIZING			1 (2%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(49)	(47)
CYST, NOS	2 (10%)		4 (9%)
ANGIECTASIS	2 (10%)		1 (2%)
#ADRENAL	(20)	(49)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY	1 (5%)	2 (4%)	
ANGIECTASIS	4 (20%)	18 (37%)	28 (56%)
#ADRENAL CORTEX	(20)	(49)	(50)
METAMORPHOSIS FATTY		2 (4%)	1 (2%)
#ADRENAL MEDULLA	(20)	(49)	(50)
HYPERPLASIA, NODULAR	1 (5%)		
#THYROID	(20)	(48)	(50)
HYPERPLASIA, C-CELL		4 (8%)	1 (2%)
#PANCREATIC ISLETS	(19)	(47)	(49)
HYPERPLASIA, NOS	1 (5%)		1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
DILATATION/DUCTS		8 (16%)	12 (24%)
CYST, NOS			1 (2%)
*PREPUTIAL GLAND	(20)	(50)	(50)
CYST, NOS		1 (2%)	
#PROSTATE	(9)	(37)	(47)
INFLAMMATION, SUPPURATIVE	1 (11%)	2 (5%)	2 (4%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE		1 (3%)	
FIBROSIS			1 (2%)
HYPERPLASIA, NODULAR	1 (11%)		
HYPERPLASIA, NOS			2 (4%)
*SEMINAL VESICLE	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE		2 (4%)	1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE		1 (2%)	
FIBROSIS, FOCAL		1 (2%)	
#TESTIS	(20)	(49)	(49)
GRANULOMA, SPERMATIC	1 (5%)		1 (2%)
CYTOMEGALY		1 (2%)	1 (2%)
ATROPHY, NOS	1 (5%)	10 (20%)	13 (27%)
ASPERMATOGENESIS		1 (2%)	
#TESTIS/TUBULE	(20)	(49)	(49)
MINERALIZATION			1 (2%)
DEGENERATION, HYALINE	1 (5%)		
*EPIDIDYMIS	(20)	(50)	(50)
INFLAMMATION, CHRONIC			1 (2%)
FIBROSIS, DIFFUSE		1 (2%)	
NECROSIS, FAT		1 (2%)	
NERVOUS SYSTEM			
#CEREBRUM	(20)	(49)	(49)
ABSCESS, NOS			1 (2%)
#BRAIN	(20)	(49)	(49)
HYDROCEPHALUS, NOS		1 (2%)	
HYDROCEPHALUS, INTERNAL		1 (2%)	
HEMORRHAGE			1 (2%)
NECROSIS, NOS			2 (4%)
#BRAIN/THALAMUS	(20)	(49)	(49)
HEMORRHAGE			1 (2%)
*SPINAL CORD	(20)	(50)	(50)
HEMORRHAGE			1 (2%)
NECROSIS, NOS			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EYE	(20)	(50)	(50)
CATARACT	1 (5%)		
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(50)	(50)
INFLAMMATION, FOCAL	1 (5%)		
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
STEATITIS		1 (2%)	
*PERITONEUM	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE	1 (5%)		
INFLAMMATION, GRANULOMATOUS			1 (2%)
*MESENTERY	(20)	(50)	(50)
STEATITIS		1 (2%)	
PERIARTERITIS		6 (12%)	
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKEMOID REACTION			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/NO HISTO		1	

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

TABLE C2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
INFLAMMATION, CHRONIC SUPPURATIV		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
NECROSIS, FAT			1 (2%)
HYPERPLASIA, NOS			1 (2%)
RESPIRATORY SYSTEM			
#TRACHEA	(20)	(48)	(49)
INFLAMMATION, NOS			4 (8%)
#LUNG/BRONCHUS	(20)	(50)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR	18 (90%)		
#LUNG	(20)	(50)	(49)
HEMORRHAGE			1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR		42 (84%)	42 (86%)
INFLAMMATION, INTERSTITIAL		2 (4%)	
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
CHOLESTEROL DEPOSIT		1 (2%)	
HEMATOPOIETIC SYSTEM			
*BLOOD	(20)	(50)	(50)
CYTOPLASMIC VACUOLIZATION	1 (5%)		
CYTOMEGALY	1 (5%)		
LEUKOCYTOSIS, NOS			1 (2%)
LEUKOCYTOSIS, NEUTROPHILIC	2 (10%)		
LYMPHOCYTOSIS		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
LEUKOPENIA, NOS		1 (2%)	
ERYTHROBLASTOSIS		1 (2%)	
HYPERPLASIA, NEUTROPHILIC	1 (5%)	2 (4%)	2 (4%)
#SPLEEN	(20)	(50)	(50)
CONGESTION, NOS		1 (2%)	
INFARCT, NOS	1 (5%)		
HEMOSIDEROSIS	5 (25%)	39 (78%)	34 (68%)
HYPERPLASIA, RETICULUM CELL		1 (2%)	3 (6%)
HEMATOPOIESIS	14 (70%)	38 (76%)	36 (72%)
#LYMPH NODE	(20)	(49)	(49)
CONGESTION, NOS		1 (2%)	
PIGMENTATION, NOS		1 (2%)	
PLASMACYTOSIS		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	
#MANDIBULAR L. NODE	(20)	(49)	(49)
CYST, NOS			2 (4%)
CONGESTION, NOS	1 (5%)	1 (2%)	1 (2%)
EDEMA, NOS		1 (2%)	
PIGMENTATION, NOS			1 (2%)
HEMOSIDEROSIS			1 (2%)
HYPERPLASIA, NOS		1 (2%)	
PLASMACYTOSIS	11 (55%)	14 (29%)	20 (41%)
HYPERPLASIA, LYMPHOID		2 (4%)	
#MEDIASTINAL L.NODE	(20)	(49)	(49)
CONGESTION, NOS	1 (5%)	1 (2%)	
PIGMENTATION, NOS	1 (5%)	20 (41%)	5 (10%)
#MESENTERIC L. NODE	(20)	(49)	(49)
CONGESTION, NOS		2 (4%)	
EDEMA, NOS		1 (2%)	
PIGMENTATION, NOS		4 (8%)	
HYPERPLASIA, LYMPHOID		1 (2%)	
#THYMUS	(12)	(38)	(38)
HYPERPLASIA, NOS		1 (3%)	
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(49)
FIBROSIS		41 (82%)	37 (76%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FIBROSIS, FOCAL	13 (65%)		
METAMORPHOSIS FATTY		1 (2%)	
HEMOSIDEROSIS	1 (5%)		
#HEART/ATRIUM	(20)	(50)	(49)
THROMBOSIS, NOS		1 (2%)	
*PULMONARY ARTERY	(20)	(50)	(50)
HYPERTROPHY, NOS			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(50)	(49)
CYSTIC DUCTS			1 (2%)
INFLAMMATION, NOS			2 (4%)
FIBROSIS, FOCAL		1 (2%)	
#LIVER	(20)	(50)	(50)
CONGESTION, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE	1 (5%)		
GRANULOMA, NOS		2 (4%)	
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
FIBROSIS, FOCAL		1 (2%)	
NODULE	1 (5%)		
CHOLANGIOFIBROSIS	6 (30%)	25 (50%)	24 (48%)
METAMORPHOSIS FATTY	1 (5%)	3 (6%)	6 (12%)
BASOPHILIC CYTO CHANGE	14 (70%)	40 (80%)	38 (76%)
CLEAR-CELL CHANGE		3 (6%)	
MEGALOCYTOSIS		6 (12%)	3 (6%)
HYPERPLASIA, NODULAR	1 (5%)		
#LIVER/CENTRILOBULAR	(20)	(50)	(50)
METAMORPHOSIS FATTY	1 (5%)		
#BILE DUCT	(20)	(50)	(50)
INFLAMMATION, CHRONIC	1 (5%)		
HYPERPLASIA, NOS	1 (5%)		
#PANCREAS	(19)	(50)	(50)
FIBROSIS		6 (12%)	3 (6%)
FIBROSIS, FOCAL	2 (11%)	1 (2%)	
FIBROSIS, DIFFUSE			1 (2%)
PERIARTERITIS		1 (2%)	
ATROPHY, NOS	2 (11%)	4 (8%)	2 (4%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#STOMACH	(19)	(50)	(50)
CYST, NOS		1 (2%)	
ULCER, NOS			1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE	1 (5%)		
#SMALL INTESTINE	(18)	(49)	(50)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
#COLON	(18)	(50)	(50)
NEMATODIASIS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
CAST, NOS	10 (50%)	22 (44%)	18 (36%)
HYDRONEPHROSIS	1 (5%)		
PYELONEPHRITIS, NOS			1 (2%)
INFLAMMATION, CHRONIC	2 (10%)	26 (52%)	28 (56%)
INFLAMMATION, CHRONIC FOCAL	12 (60%)		
PIGMENTATION, NOS			1 (2%)
HEMOSIDEROSIS		1 (2%)	
#KIDNEY/TUBULE	(20)	(50)	(50)
PIGMENTATION, NOS		1 (2%)	
#KIDNEY/PELVIS	(20)	(50)	(50)
CALCULUS, NOS		1 (2%)	
#URINARY BLADDER	(19)	(50)	(50)
HEMORRHAGE		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(49)	(49)
CYST, NOS	2 (10%)	3 (6%)	16 (33%)
HEMORRHAGE			1 (2%)
HEMORRHAGIC CYST	1 (5%)	1 (2%)	
ANGIECTASIS	2 (10%)		
#ADRENAL	(20)	(50)	(50)
FIBROSIS		1 (2%)	
NECROSIS, CORTICAL			1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
METAMORPHOSIS FATTY		3 (6%)	1 (2%)
HYPERPLASIA, NODULAR		2 (4%)	
ANGIECTASIS	7 (35%)	28 (56%)	26 (52%)
#ADRENAL CORTEX	(20)	(50)	(50)
MINERALIZATION		1 (2%)	
CYST, NOS			1 (2%)
CONGESTION, NOS			1 (2%)
METAMORPHOSIS FATTY		1 (2%)	1 (2%)
HYPERPLASIA, NODULAR		2 (4%)	
HYPERPLASIA, NOS		1 (2%)	2 (4%)
#ADRENAL MEDULLA	(20)	(50)	(50)
ANGIECTASIS			1 (2%)
#THYROID	(20)	(49)	(49)
INFLAMMATION, NOS			1 (2%)
HYPERPLASIA, C-CELL	2 (10%)	8 (16%)	10 (20%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
DILATATION/DUCTS	8 (40%)	31 (62%)	20 (40%)
CYST, NOS	1 (5%)		
ANGIECTASIS			1 (2%)
*MAMMARY DUCT	(20)	(50)	(50)
RETENTION OF CONTENT		1 (2%)	
#UTERUS	(20)	(50)	(50)
PYOMETRA			1 (2%)
NECROSIS, FAT			1 (2%)
POLYP, INFLAMMATORY	2 (10%)	4 (8%)	6 (12%)
#CERVIX UTERI	(20)	(50)	(50)
FIBROSIS		1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(50)	(50)
CYST, NOS		1 (2%)	2 (4%)
MULTILOCULAR CYST	1 (5%)		
#OVARY	(20)	(50)	(50)
CYST, NOS	1 (5%)	2 (4%)	3 (6%)
CORPUS LUTEUM CYST			1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CORPUS LUTEUM	17 (85%)	41 (82%)	43 (86%)
#OVARY/FOLLICLE	(20)	(50)	(50)
ATRESIA		2 (4%)	
NERVOUS SYSTEM			
#CEREBRAL VENTRICLE	(20)	(50)	(50)
HEMORRHAGE		1 (2%)	
#BRAIN	(20)	(50)	(50)
HYDROCEPHALUS, NOS		1 (2%)	
HYDROCEPHALUS, INTERNAL		1 (2%)	
HEMORRHAGE		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(20)	(50)	(50)
PERIARTERITIS		1 (2%)	
*MESENTERY	(20)	(50)	(50)
FIBROSIS	1 (5%)		
NECROSIS, FAT	1 (5%)		
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
NECROSIS, FAT		1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1		

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

APPENDIX D

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

TABLE D1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(49)
ABSCESS, NOS			2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR	2 (10%)		1 (2%)
INFLAMMATION, INTERSTITIAL		1 (2%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*BLOOD	(20)	(50)	(49)
LEUKOPENIA, NOS	1 (5%)		
#BONE MARROW	(20)	(50)	(49)
HYPERPLASIA, RETICULUM CELL			2 (4%)
#SPLEEN	(20)	(50)	(48)
HYPERPLASIA, RETICULUM CELL		1 (2%)	5 (10%)
HYPERPLASIA, LYMPHOID	1 (5%)		1 (2%)
#MESENTERIC L. NODE	(20)	(50)	(49)
CONGESTION, NOS	9 (45%)	15 (30%)	17 (35%)
INFLAMMATION, CHRONIC		1 (2%)	
FIBROSIS		2 (4%)	
PLASMACYTOSIS		1 (2%)	
MEGAKARYOCYTOSIS			1 (2%)
HYPERPLASIA, RETICULUM CELL		6 (12%)	8 (16%)
HYPERPLASIA, LYMPHOID		2 (4%)	1 (2%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(19)	(50)	(49)
INFLAMMATION, CHRONIC FOCAL			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(50)	(49)
FIBROSIS, FOCAL	1 (5%)		
#LIVER	(20)	(50)	(49)
INFLAMMATION, CHRONIC FOCAL			2 (4%)
NECROSIS, FOCAL	1 (5%)		
INFARCT, NOS	1 (5%)		
METAMORPHOSIS FATTY	4 (20%)	4 (8%)	6 (12%)
ANGIECTASIS		1 (2%)	
#PANCREAS	(20)	(48)	(48)
METAMORPHOSIS FATTY		2 (4%)	
ATROPHY, FOCAL		1 (2%)	
#PANCREATIC ACINUS	(20)	(48)	(48)
ATROPHY, NOS		2 (4%)	
#STOMACH	(20)	(50)	(49)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
#PEYERS PATCH	(20)	(50)	(48)
HYPERPLASIA, RETICULUM CELL		2 (4%)	1 (2%)
HYPERPLASIA, LYMPHOID			1 (2%)
#DUODENUM	(20)	(50)	(48)
DIVERTICULOSIS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(49)
HYDRONEPHROSIS		1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR	2 (10%)	2 (4%)	7 (14%)
INFLAMMATION, CHRONIC		2 (4%)	3 (6%)
INFLAMMATION, CHRONIC FOCAL	1 (5%)		2 (4%)
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(48)	(49)
CYST, NOS	1 (6%)		1 (2%)
#THYROID	(19)	(47)	(47)
FIBROSIS, FOCAL			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ATROPHY, NOS			1 (2%)
HYPERPLASIA, FOLLICULAR-CELL	1 (5%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
INFLAMMATION, NOS			1 (2%)
*SEMINAL VESICLE	(20)	(50)	(49)
DILATATION, NOS		2 (4%)	1 (2%)
#TESTIS	(20)	(50)	(49)
ATROPHY, FOCAL	1 (5%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(49)
NECROSIS, FAT	2 (10%)	1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	6	6

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
AUTC/NECROPSY/HISTO PERF			1
AUTOLYSIS/NO NECROPSY			1

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

TABLE D2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(50)
HEMORRHAGIC CYST		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR	4 (20%)	3 (6%)	5 (10%)
INFLAMMATION, INTERSTITIAL		1 (2%)	
HEMOSIDEROSIS			1 (2%)
HEMATOPOIETIC SYSTEM			
*BLOOD	(20)	(50)	(50)
HYPERPLASIA, NEUTROPHILIC		1 (2%)	1 (2%)
#SPLEEN	(19)	(48)	(50)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	2 (4%)
HYPERPLASIA, RETICULUM CELL			2 (4%)
HYPERPLASIA, LYMPHOID	6 (32%)	7 (15%)	1 (2%)
#LYMPH NODE	(19)	(47)	(50)
HEMORRHAGIC CYST		1 (2%)	
PLASMACYTOSIS		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	
#MANDIBULAR L. NODE	(19)	(47)	(50)
CONGESTION, NOS	1 (5%)		
HYPERPLASIA, RETICULUM CELL		1 (2%)	
HYPERPLASIA, LYMPHOID			1 (2%)
#MESENTERIC L. NODE	(19)	(47)	(50)
CONGESTION, NOS		2 (4%)	1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMORRHAGIC CYST			1 (2%)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
HYPERPLASIA, RETICULUM CELL		4 (9%)	3 (6%)
HYPERPLASIA, LYMPHOID	2 (11%)		2 (4%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(49)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR	4 (21%)	10 (20%)	5 (10%)
INFLAMMATION, ACUTE FOCAL	1 (5%)		
ABSCESS, NOS		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU		2 (4%)	
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY	2 (11%)	5 (10%)	2 (4%)
ANGIECTASIS		1 (2%)	1 (2%)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
MYELOPOIESIS			1 (2%)
#PANCREAS	(19)	(46)	(47)
ANGIECTASIS			1 (2%)
#STOMACH	(19)	(46)	(49)
INFLAMMATION, ACUTE FOCAL	1 (5%)		
#SMALL INTESTINAL SUB	(18)	(45)	(50)
DIVERTICULOSIS		1 (2%)	
#PEYERS PATCH	(18)	(45)	(50)
HYPERPLASIA, RETICULUM CELL		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (6%)		
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
HYDRONEPHROSIS			2 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)	10 (20%)	4 (8%)
INFLAMMATION, INTERSTITIAL	1 (5%)		
INFLAMMATION, CHRONIC FOCAL			3 (6%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC DIFFUSE			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(49)	(48)
HEMORRHAGIC CYST		1 (2%)	
#ADRENAL	(19)	(49)	(50)
HYPERPLASIA, NODULAR	1 (5%)		
#ADRENAL CORTEX	(19)	(49)	(50)
METAMORPHOSIS FATTY	1 (5%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(46)	(50)
HEMORRHAGIC CYST	1 (5%)		
POLYP, INFLAMMATORY		1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(46)	(50)
DILATATION, NOS	6 (30%)	20 (43%)	20 (40%)
#OVARY	(20)	(47)	(50)
MINERALIZATION		1 (2%)	
CYST, NOS	2 (10%)	11 (23%)	13 (26%)
HEMORRHAGIC CYST	1 (5%)	1 (2%)	2 (4%)
FIBROSIS		1 (2%)	
NECROSIS, FAT	2 (10%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
ABSCESS, NOS		1 (2%)	
*PERITONEUM	(20)	(50)	(50)
INFLAMMATION, ACUTE DIFFUSE		1 (2%)	
INFLAMMATION, CHRONIC DIFFUSE		1 (2%)	
*MESENTERY	(20)	(50)	(50)
NECROSIS, FAT	4 (20%)	6 (12%)	3 (6%)
ANGIECTASIS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		2	3

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

APPENDIX E

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Fibroma (b)	1/20(5)	1/50(2)	5/50(10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.400	2.000
Lower Limit		0.005	0.249
Upper Limit		30.802	92.596
Weeks to First Observed Tumor	108	108	95
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	0/20(0)	4/49(8)	2/50(4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.394	0.123
Upper Limit		Infinite	Infinte
Weeks to First Observed Tumor	--	108	108

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia (b)	6/20(30)	10/50(20)	13/50(26)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.667	0.867
Lower Limit		0.264	0.372
Upper Limit		1.989	2.463
Weeks to First Observed Tumor	108	82	92
Pituitary: Chromophobe Carcinoma (b)	0/20(0)	4/49(8)	0/47(0)
P Values (c,d)	N.S.	N.S.	--
Departure From Linear Trend (e)	P = 0.022		
Relative Risk (f)		Infinite	--
Lower Limit		0.394	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	108	--

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Carcinoma or Adenoma (b)	6/20(30)	15/49(31)	16/47(34)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.020	1.135
Lower Limit		0.455	0.514
Upper Limit		2.828	3.101
Weeks to First Observed Tumor	108	108	70
Adrenal: Pheochromocytoma (b)	1/20(5)	3/49(6)	0/50(0)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.224	0.000
Lower Limit		0.108	0.000
Upper Limit		62.958	7.475
Weeks to First Observed Tumor	105	108	--

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Carcinoma or Adenoma (b)	0/20(0)	0/48(0)	3/50(6)
P Values (c,d)	N.S.	--	N.S.
Relative Risk (f)		--	Infinite
Lower Limit		--	0.250
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	99
Thyroid C-cell Carcinoma or Adenoma (b)	3/20(15)	8/48(17)	0/50(0)
P Values (c,d)	P = 0.011 (N)	N.S.	P = 0.021 (N)
Relative Risk (f)		1.111	0.000
Lower Limit		0.308	0.000
Upper Limit		6.043	0.659
Weeks to First Observed Tumor	108	105	--

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pancreatic Islets: Islet-cell Adenoma (b)	0/19(0)	2/47(4)	7/49(14)
P Values (c,d)	P = 0.023	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.124	0.787
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	108	108
Testis: Interstitial-cell Tumor (b)	17/20(85)	42/49(86)	38/49(78)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.008	0.912
Lower Limit		0.840	0.755
Upper Limit		1.337	1.265
Weeks to First Observed Tumor	94	83	86

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

(continued)

(a) Dosed groups received 1,500 or 3,000 ppm.

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

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

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

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

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

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Leukemia or Lymphoma (b)	3/20(15)	11/50(22)	14/50(28)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.467	1.867
Lower Limit		0.450	0.609
Upper Limit		7.594	9.359
Weeks to First Observed Tumor	58	89	89
Pituitary: Chromophobe Carcinoma or Adenoma (b)	6/20(30)	24/49(49)	21/49(43)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.633	1.429
Lower Limit		0.799	0.683
Upper Limit		4.204	3.757
Weeks to First Observed Tumor	108	78	99

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma or Adenoma (b)	1/20(5)	4/49(8)	2/49(4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.633	0.816
Lower Limit		0.179	0.046
Upper Limit		78.704	47.195
Weeks to First Observed Tumor	108	78	108
Mammary Gland: Fibroadenoma (a)	4/20(20)	7/50(14)	2/50(4)
P Values (c,d)	P = 0.027 (N)	N.S.	N.S.
Relative Risk (f)		0.700	0.200
Lower Limit		0.207	0.020
Upper Limit		2.994	1.297
Weeks to First Observed Tumor	92	65	108

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

(continued)

(a) Dosed groups received 1,500 or 3,000 ppm.

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

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

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

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

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

APPENDIX F

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	2/20(10)	7/50(14)	3/49(6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.400	0.612
Lower Limit		0.303	0.078
Upper Limit		13.138	6.996
Weeks to First Observed Tumor	80	102	102
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	4/20(20)	9/50(18)	5/49(10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.900	0.510
Lower Limit		0.294	0.126
Upper Limit		3.660	2.367
Weeks to First Observed Tumor	80	102	102

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma (b)	3/20(15)	10/50(20)	2/49(4)
P Values (c,d)	P = 0.019 (N)	N.S.	N.S.
Relative Risk (f)		1.333	0.272
Lower Limit		0.398	0.025
Upper Limit		7.002	2.233
Weeks to First Observed Tumor	93	68	94
Liver: Hepatocellular Carcinoma (b)	7/20(35)	13/50(26)	23/49(47)
P Values (c,d)	P = 0.036	N.S.	N.S.
Relative Risk (f)		0.743	1.341
Lower Limit		0.338	0.693
Upper Limit		1.927	3.159
Weeks to First Observed Tumor	72	102	82

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Cortical adenoma (b)	2/20(10)	1/48(2)	0/49(0)
P Values (c,d)	P = 0.048 (N)	N.S.	N.S.
Relative Risk (f)		0.208	0.000
Lower Limit		0.004	0.000
Upper Limit		3.830	1.372
Weeks to First Observed Tumor	102	102	--

(a) Dosed groups received 500 or 2,000 ppm.

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

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

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

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

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveloar/Bronchiolar Carcinoma or Adenoma (b)	1/20(5)	3/49(6)	2/50(4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.224	0.800
Lower Limit		0.108	0.045
Upper Limit		62.958	46.273
Weeks to First Observed Tumor	101	102	102
Hematopoietic System: Lymphoma or Leukemia (b)	7/20(35)	10/50(20)	15/50(30)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.571	0.857
Lower Limit		0.239	0.405
Upper Limit		1.560	2.169
Weeks to First Observed Tumor	74	85	85

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangioma or Hemangiosarcoma (b)	1/20(5)	4/50(8)	5/50(10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.600	2.000
Lower Limit		0.175	0.249
Upper Limit		77.169	92.596
Weeks to First Observed Tumor	102	81	102
Liver: Hepatocellular Carcinoma (b)	4/19(21)	7/49(14)	4/50(8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.679	0.380
Lower Limit		0.202	0.081
Upper Limit		2.892	1.880
Weeks to First Observed Tumor	102	102	102

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Adenoma, NOS(b)	2/16(13)	2/49(4)	0/48(0)
P Values (c,d)	P = 0.031 (N)	N.S.	N.S.
Relative Risk (f)		0.327	0.000
Lower Limit		0.026	0.000
Upper Limit		4.287	1.118
Weeks to First Observed Tumor	102	102	--

(a) Dosed groups received 500 or 2,000 ppm.

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

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

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

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

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

Review of the Bioassay of (2-Chloroethyl) Trimethylammonium Chloride* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

December 13, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute on the Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, and State health officials. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of (2-Chloroethyl) Trimethylammonium Chloride.

The reviewer for the report on the bioassay of (2-Chloroethyl) Trimethylammonium Chloride agreed with the conclusion that the compound was not carcinogenic under the conditions of test. After a brief description of the experimental design, he noted the lack of data on the stability and content of the compound in the diet mix and the inadequate number of matched controls. He opined that these shortcomings probably did not affect the conclusion reached. Based on the results of the bioassay, the reviewer said that (2-Chloroethyl) Trimethylammonium Chloride would not appear to pose a carcinogenic hazard to human beings.

A discussion ensued on the possible significance of the lung infiltrates observed among treated rats. A Program staff pathologist mentioned that the finding was common in aged rats, although different nomenclature may be used to report it.

It was moved that the report on the bioassay of (2-Chloroethyl) Trimethylammonium Chloride be accepted as written. The motion was seconded and approved without objection.

Clearinghouse Members Present:

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

---

* 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.

☆U.S. GOVERNMENT PRINTING OFFICE: 1979-281-217/3013

DHEW Publication No. (NIH) 79-1714