National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 41
1978

BIOASSAY OF CHLOROTHALONIL FOR POSSIBLE CARCINOGENICITY

CAS No. 1897-45-6

NCI-CG-TR-41

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

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DHEW Publication No. (NIH) 78-841

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

CONTRIBUTORS: This bioassay of chlorothalonil was conducted by Gulf South Research Institute, New Iberia, Louisiana, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., Rockville, Maryland prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by Drs. J. H. Weisburger[1,2] and R. R. Bates[1,3]; the doses were selected by Drs. T. E. Shellenberger[4,5], J. H. Weisburger, and R. R. Bates. Administration of the chemical and observation of the animals were supervised by Drs. T. E. Shellenberger and H. P. Burchfield[4], with the technical assistance of Ms. D. H. Monceaux[4], Mr. D. Broussard[4], and Mr. R. J. Wheeler[4]. Histopathologic examination was performed by Drs. E. Bernal[4] and B. Buratto[4] at Gulf South Research Institute, and the diagnoses

included in this report represent the interpretation of these pathologists.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[6]. Statistical analyses were performed by Dr. J. R. Joiner[7], using methods selected for the bioassay program by Dr. J. J. Gart[8]. Chemicals used in this bioassay were analyzed under the direction of Dr. H. P. Burchfield[4], and the results of the analyses were reviewed by Dr. S. S. Olin[7].

This report was prepared at Tracor Jitco[7] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. L. A. Campbell, Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Drs. J. F. Robens and C. H. Williams, toxicologists; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Mr. W. D. Reichardt, and Ms. L. A. Waitz, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The following other 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. Dawn G. Goodman[9], Dr. Richard A. Griesemer, Dr. Harry A. Milman, Dr. Thomas Orme, Dr. Robert A. Squire[10], Dr. Sherman Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

A bioassay of technical-grade chlorothalonil for possible carcinogenicity was conducted by administering the test chemical in the diet to Osborne-Mendel rats and B6C3F1 mice.

Groups of 50 rats of each sex were administered chlorothalonil at one of two doses for 80 weeks, then observed for 30-31 weeks. Time-weighted average doses for both males and females were 5,063 or 10,126 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls consisted of the matched-control groups combined with 55 untreated male or female rats from similar bioassays of five other test chemicals. All surviving rats were killed at 110-111 weeks.

Groups of 50 mice of each sex were administered chlorothalonil at one of two doses for 80 weeks, then observed for 11-12 weeks. Time-weighted average doses for males were 2,688 or 5,375 ppm, and for females, 3,000 or 6,000 ppm. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched-control groups combined with 50 untreated male or female mice from similar bioassays of five other test chemicals. All surviving mice were killed at 91-92 weeks.

Clinical signs that appeared with increasing frequency in dosed rats included hematuria and, from week 72 until termination of the study, bright-yellow urine. Since the dosed female mice did not have depression in mean body weights or decreased survival compared with the controls, they may have been able to tolerate a higher dose.

In rats, adenomas and carcinomas of the renal tubular epithelium occurred with a significant dose-related trend in both the males ($P = 0.030$) and the females ($P = 0.007$). These neoplasms also occurred at a higher incidence in the high-dose males ($P = 0.035$) and the high-dose females ($P = 0.016$) than in the corresponding controls (males: pooled controls 0/62, low-dose 3/46, high-dose 4/49; females: pooled controls 0/62, low-dose 1/48, high-dose

5/50). These tumors included both adenomas and carcinomas which are considered to be histogenically related. Thus these findings are interpreted as sufficient evidence for the carcinogenicity of chlorothalonil.

In mice, no tumors were found to occur at a greater incidence among dosed animals than among controls.

It is concluded that under the conditions of this bioassay, technical-grade chlorothalonil was carcinogenic to Osborne-Mendel rats, producing tumors of the kidney. Chlorothalonil was not carcinogenic for B6C3F1 mice.

TABLE OF CONTENTS

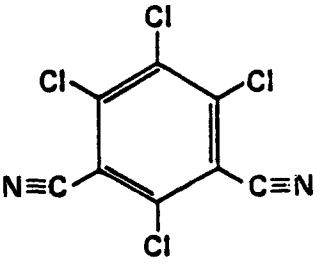
APPENDIXES

TABLES

FIGURES

I. INTRODUCTION

Chlorothalonil (CAS 1897-45-6; NCI C00102) is a broad-spectrum fungicide which has been in use in the United States since 1963. It is registered for foliar and root applications on vegetables, fruits, greenhouse plants, and turf, and as a seed treatment for cotton (EPA Pesticide Data Base, Compact Label File, 1976). Chlorothalonil is also used in formulating paints and stains for mildew resistance.



Chlorothalonil

Chlorothalonil was one of a series of pesticides selected for study in the Carcinogenesis Testing Program because of the potential for long-term human exposure to the chemical during agricultural or industrial application or to residues in food products.

II. MATERIALS AND METHODS

A. Chemical

Chlorothalonil (2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile) was obtained from Diamond Shamrock Chemical Co., Agricultural Chemicals Division, Cleveland, Ohio, in two batches of technical-grade product for the chronic studies. These batches of chlorothalonil (Daconil 2787®) were used for all the chronic studies, except for a 2-week period 6 months into the study when rats received analytical-grade chlorothalonil (99.7%) rather than technical grade.

Analyses at Gulf South Research Institute confirmed the identity of the technical- and analytical-grade batches (melting range; elemental analysis; and infrared, ultraviolet, and nuclear magnetic resonance spectra). No attempt was made to identify or quantitate impurities.

Samples of both batches were returned to Diamond Shamrock for analysis after the bioassay was complete. It was found that these batches were typical of the current product and that all impurities were within Diamond Shamrock's current specifications for technical chlorothalonil. According to the Diamond Shamrock analyses, the first batch consisted of 98.50% chlorothalonil, 1.24% pentachlorobenzonitrile (CAS 20925-85-3), 0.06% other

tetrachlorodicyanobenzene isomers, and smaller quantities of other partially chlorinated dicyanobenzenes. The second batch consisted of 98.0% chlorothalonil, 0.6% pentachlorobenzonitrile, 1.2% other tetrachlorodicyanobenzene isomers, and again smaller quantities of other partially chlorinated dicyanobenzenes (Diamond Shamrock, 1975, 1977).

The chemical was stored at 4°C in the original glass container.

B. Dietary Preparation

All diets were formulated using finely ground Wayne® Lab Blox (Allied Mills, Inc., Chicago, Ill.) to which was added the required amount of chlorothalonil for each dietary concentration. A given amount of the test chemical was first hand-mixed with an approximately equal amount of feed. This mixture was then added slowly with mechanical mixing to a larger quantity of feed to give the desired concentration of the material. Acetone (Mallinckrodt Inc., St. Louis, Mo.) and corn oil (Louana®, Opelousas Refinery Co., Opelousas, La.) were then added to the feed, each in an amount corresponding to 2% of the final weight of feed. The diets were mixed mechanically for not less than 25 minutes to assure homogeneity of the mixture and evaporation of the acetone. Formulated diets were stored at approximately 17°C until used, but no longer than one week.

The stability of chlorothalonil in feed was tested by determining the concentration of the chemical in formulated diets at intervals over a 7-day period. Diets containing 5,000 or 10,000 ppm chlorothalonil showed no change on standing at ambient temperature for this period. Extraction and analytical procedures are outlined in Appendix G.

As a quality control test for the accuracy of preparation of the diets, the concentration of chlorothalonil was determined in different batches of formulated diets during the chronic study. The results are summarized in Appendix G. At each dietary concentration, the mean of the analytical concentrations for the checked samples was within 1.5% of the theoretical concentration, and the coefficient of variation was never more than 5.7%. Thus, the evidence indicates that the formulated diets were prepared accurately.

C. Animals

Rats and mice of each sex, obtained through contracts of the Division of Cancer Treatment, National Cancer Institute, were used in these bioassays. The rats were of the Osborne-Mendel strain obtained from Bâttelle Memorial Institute, Columbus, Ohio, and the mice were B6C3F1 hybrids obtained from A. R. Schmidt, Madison, Wisconsin. On arrival at the laboratory, all animals

were quarantined (rats for 7 days, mice for 15 days) and then assigned to control or dosed groups.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature range was 22-24°C, and the relative humidity was maintained at 40-70%. The air entering each room was passed through fiberglass filters and was changed 10-12 times per hour. Fluorescent lighting provided illumination 10 hours per day. Food and water were available ad libitum.

The rats were housed individually in hanging galvanized steel mesh cages, and the mice were housed in plastic cages with filter bonnets (Lab Products, Inc., Garfield, N.J.), five per cage for females, and two or three per cage for males. Initially, rats were transferred every week to clean cages; later in the study, cages were changed every 2 weeks. Mice were transferred every week to clean cages with filter bonnets; bedding used for the mice was Absorb-Dri® (Lab Products, Inc., Garfield, N. J.). For rats, absorbent sheets under the cages were changed three times per week. Feeder jars and water bottles were changed and sterilized three times per week.

Cages for control and dosed mice were placed on separate racks in the same room. Animal racks for both species were rotated later-

ally once per week; at the same time each cage was changed to a different position in the row within the same column. Rats administered chlorothalonil in feed, along with their matched controls, were housed in a room by themselves. Control mice were housed in the same room as the respective dosed mice. Mice administered chlorothalonil were maintained in the same room as mice being administered the following chemicals in the feed:

(CAS 133-90-4) chloramben
(CAS 1918-02-1) picloram
(CAS 72-20-8) endrin

E. Subchronic Studies

Subchronic feeding studies were conducted with rats and mice to determine the two concentrations (hereinafter referred to as "low doses" and "high doses") to be administered in the chronic studies. In these subchronic studies chlorothalonil was added to the animal feed in twofold increasing concentrations, ranging from 1,250 to 20,000 ppm for rats and from 1,250 to 40,000 ppm for mice. Dosed and control groups each consisted of five male and five female animals. The chemical was provided in feed to dosed groups for 6 weeks, followed by 2 weeks of observation.

During week 1, the mean weight gains in male and female rats receiving 20,000 ppm chlorothalonil were 10 and 40%, respectively, of those of the controls. By weeks 3 and 4, these

groups had weight gains generally comparable to those of the controls. Male and female rats receiving 10,000 ppm and male rats receiving 5,000 ppm had lesser initial depressions in mean weight gains than rats receiving 20,000 ppm. The low and high doses for each sex of rats were set at 10,000 and 20,000 ppm for the chronic studies.

Male and female mice receiving 40,000 or 20,000 ppm lost 2 to 6 g of mean body weight during the first 2 to 3 weeks of the study; and those animals receiving 10,000 ppm had lesser weight gains than controls during this period. Three males receiving 40,000 ppm died during week 6; and one female receiving 40,000 ppm died during week 3. The low and high doses for male and female mice were set at 10,000 and 20,000 ppm for the chronic studies.

F. Chronic Studies

The test groups, doses administered, and times on study of the chronic feeding studies are shown in tables 1 and 2.

Since the numbers of animals in the matched-control groups were small, pooled-control groups also were used for statistical comparisons. Matched controls from the current bioassay of chlorothalonil were combined with matched controls from bioassays of malathion (CAS 121-75-5), tetrachlorvinphos (CAS 961-11-5), toxaphene (CAS 8001-35-2), lindane (CAS 58-89-9), and endrin (CAS

Table 1. Chlorothalonil Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals[a]	Chloro-thalonil in Diet[b] (ppm)	Time on Study: Dosed[c] (weeks)	Time on Study: Observed[d] (weeks)	Time-Weighted Average Dose[e] (ppm)
MALE					
Matched-Control	10	0		110	
Low-Dose	50	10,000	1		
		5,000	79		5,063
		0		30	
High-Dose	50	20,000	1		
		10,000	79		10,126
		0		31	
FEMALE					
Matched-Control	10	0		110	
Low-Dose	50	10,000	1		
		5,000	79		5,063
		0		30-31	
High-Dose	50	20,000	1		
		10,000	79		10,126
		0		30-31	

[a]All animals were 35 days of age when placed on study.

[b]Doses of chlorothalonil were lowered after 1 week on study since, based on the pattern of mortality, changes in body weight, and the general condition of the animals in this and other carcinogen bioassays, it was believed that excessive mortality would occur before termination of the study.

[c]All animals were placed on study on the same day.

[d]When diets containing chlorothalonil were discontinued, all animals and their matched controls were fed control diets (2% corn oil added).

[e]Time-weighted average dose = $\frac{\Sigma(\text{dose in ppm} \times \text{no. of weeks at that dose})}{\Sigma(\text{no. of weeks receiving each dose})}$

Table 2. Chlorothalonil Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals[a]	Chlorothalonil in Diet[b] (ppm)	Time on Study: Dosed[c] (weeks)	Time on Study: Observed[d] (weeks)	Time-Weighted Average Dose[e] (ppm)
MALE					
Matched-Control	10	0		91	
Low-Dose	50	10,000	2	11-12	
		2,500	78		2,688
		0			
High-Dose	50	20,000	2	12	
		5,000	78		5,375
		0			
FEMALE					
Matched-Control	10	0		91	
Low-Dose	50	10,000	2	11	
		5,000	10		3,000
		2,500	68		
		0			
High-Dose	50	20,000	2	12	
		10,000	10		6,000
		5,000	68		
		0			

[a]All animals were 35 days of age when placed on study.

[b]Doses of chlorothalonil were lowered after 2 and 10 weeks on study since, based upon the pattern of mortality, changes in body weight, and the general condition of the animals in this and other carcinogen bioassays, it was believed that excessive mortality would occur before termination of the study.

[c]All animals were placed on study on the same day.

[d]When diets containing chlorothalonil were discontinued, all animals and their matched controls were fed control diets (2% corn oil added).

[e]Time-weighted average dose = $\frac{\Sigma(\text{dose in ppm} \times \text{no. of weeks at that dose})}{\Sigma(\text{no. of weeks receiving each dose})}$

72-20-8). All control animals received 2% corn oil in the feed. The pooled controls for statistical tests using rats consisted of 65 males and 65 females; using mice, 60 males and 60 females. The bioassays on chemicals other than chlorothalonil were also conducted at Gulf South Research Institute and overlapped the chlorothalonil study by at least 1 year. The matched-control groups of rats for the different test chemicals which comprised the pooled controls were of the same strain and from the same supplier and were examined by the same pathologists. The other control groups of mice which comprised the pooled controls were of the same strain and were examined by the same pathologists, but were received from a different supplier.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, weighed at regular intervals, and palpated for masses at each weighing. Animals that were moribund at the time of clinical examination were killed and necropsied.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart,

salivary gland, liver, gallbladder (mice), pancreas, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, and brain. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were used when indicated for more definitive diagnosis.

A few tissues from some animals were not examined, particularly from those animals that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. 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 statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and 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 ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a 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 ($P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

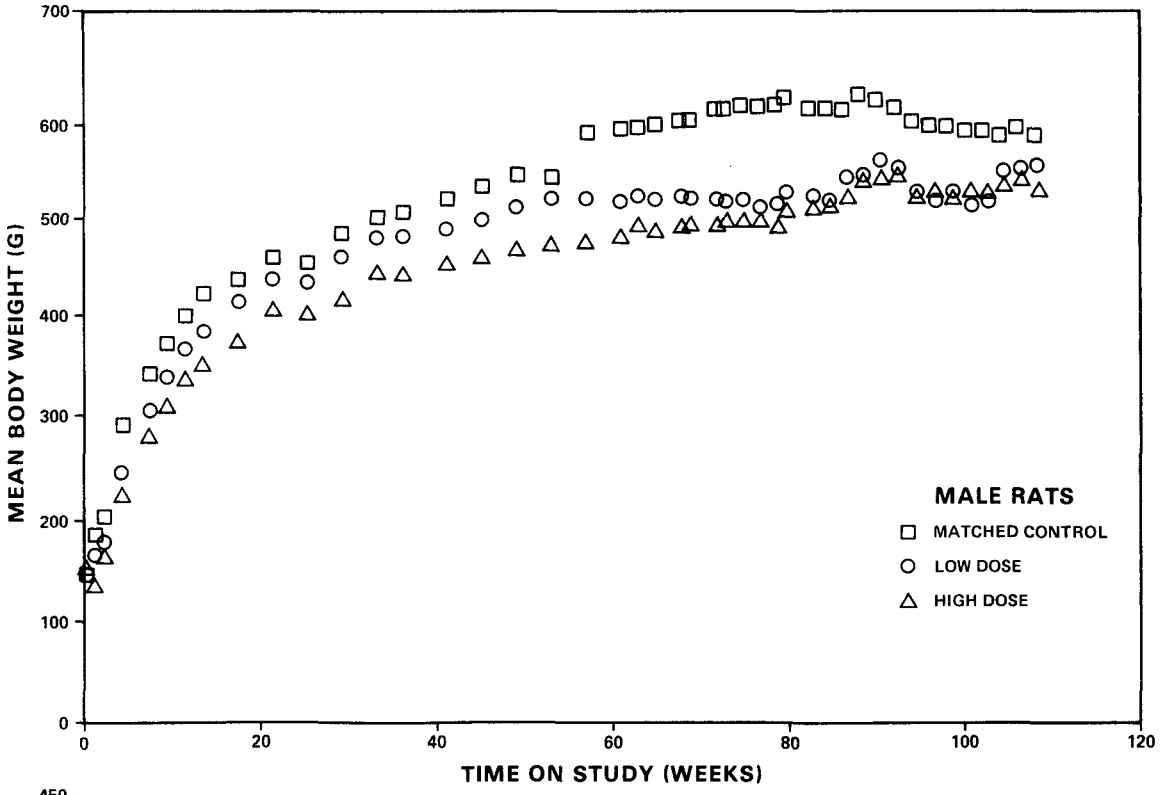
III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

The mean body weights of the rats fed chlorothalonil were consistently lower than those of the matched controls, and the data indicate that the effects were dose related for both sexes (figure 1). Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation.

During the first year of the study, some animals had rough hair coats; however, the dosed animals were generally comparable to the controls in appearance and behavior. A few animals showed evidence of an eye infection which was diagnosed as viral conjunctivitis.

Clinical signs in all dosed groups were noted at a low or moderate incidence during the first half of the second year, and with gradually increasing frequency during the second half of that year. These signs included loss of weight (figure 1), decreased food consumption, rough hair coats, pale mucous membranes, ataxia, tachypnea, epistaxis, dermatitis, hematuria, hyperactivity, and vaginal bleeding. Several nodular masses developed as abscesses that, in some cases, ruptured and exuded a



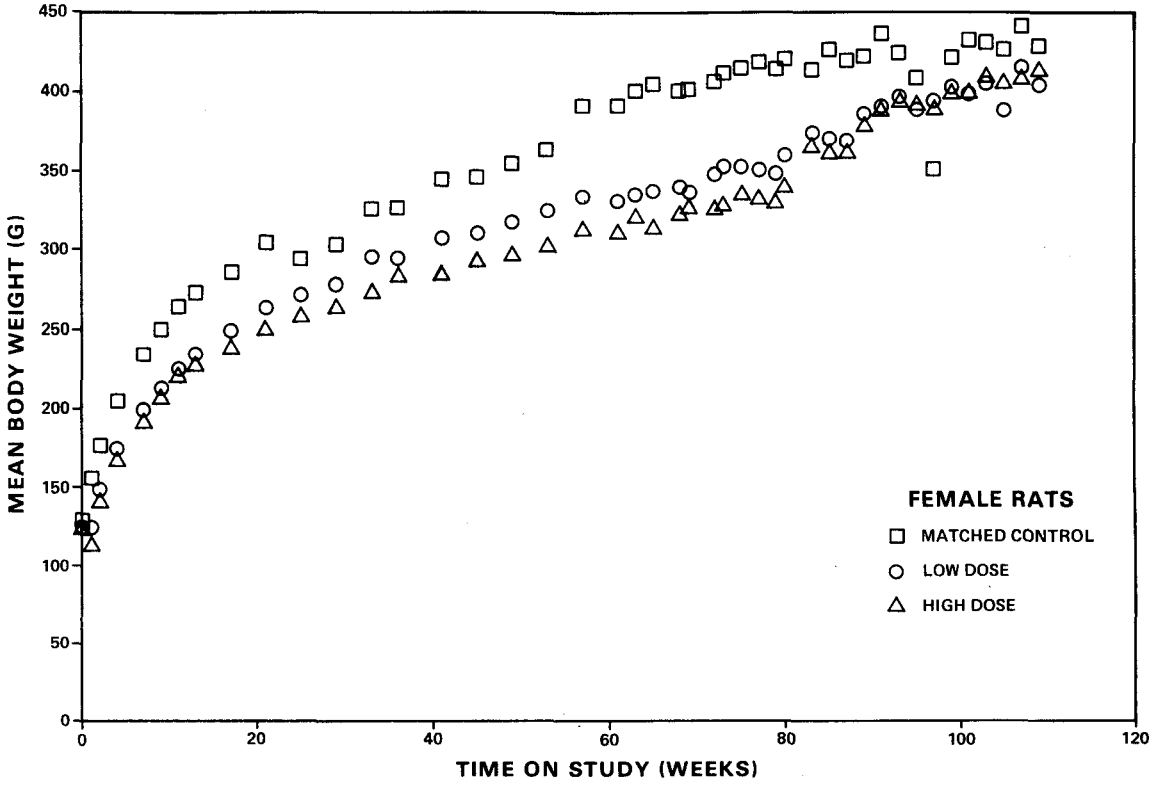


Figure 1. Growth Curves For Rats Fed Chlorothalonil In The Diet

purulent discharge. Beginning at week 72, all dosed animals had discolored hair coats and bright-yellow urine.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats fed chlorothalonil in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2. In male and female rats, the results of the Tarone test for positive dose-related trend in mortality are not statistically significant ($P > 0.05$), but the rates of survival of the dosed females decreased after week 60. There is a significant ($P = 0.002$) decrease in survival of the dosed males when compared with those in the pooled controls used in this study. In female rats, the survival in the dosed groups are comparable with that in the pooled controls ($P = 0.061$).

In male rats, all 49 of the high-dose animals, all 50 of the low-dose animals, and all 10 of the matched controls survived beyond week 52 on study. Neither the high-dose animals that died before week 68 nor the low-dose animals that died before week 86 had tumors. While the rates of survival of the low- and high-dose males showed little difference from one another, there is a steep decline in survival in either dosed group after week 75 compared with the control group.

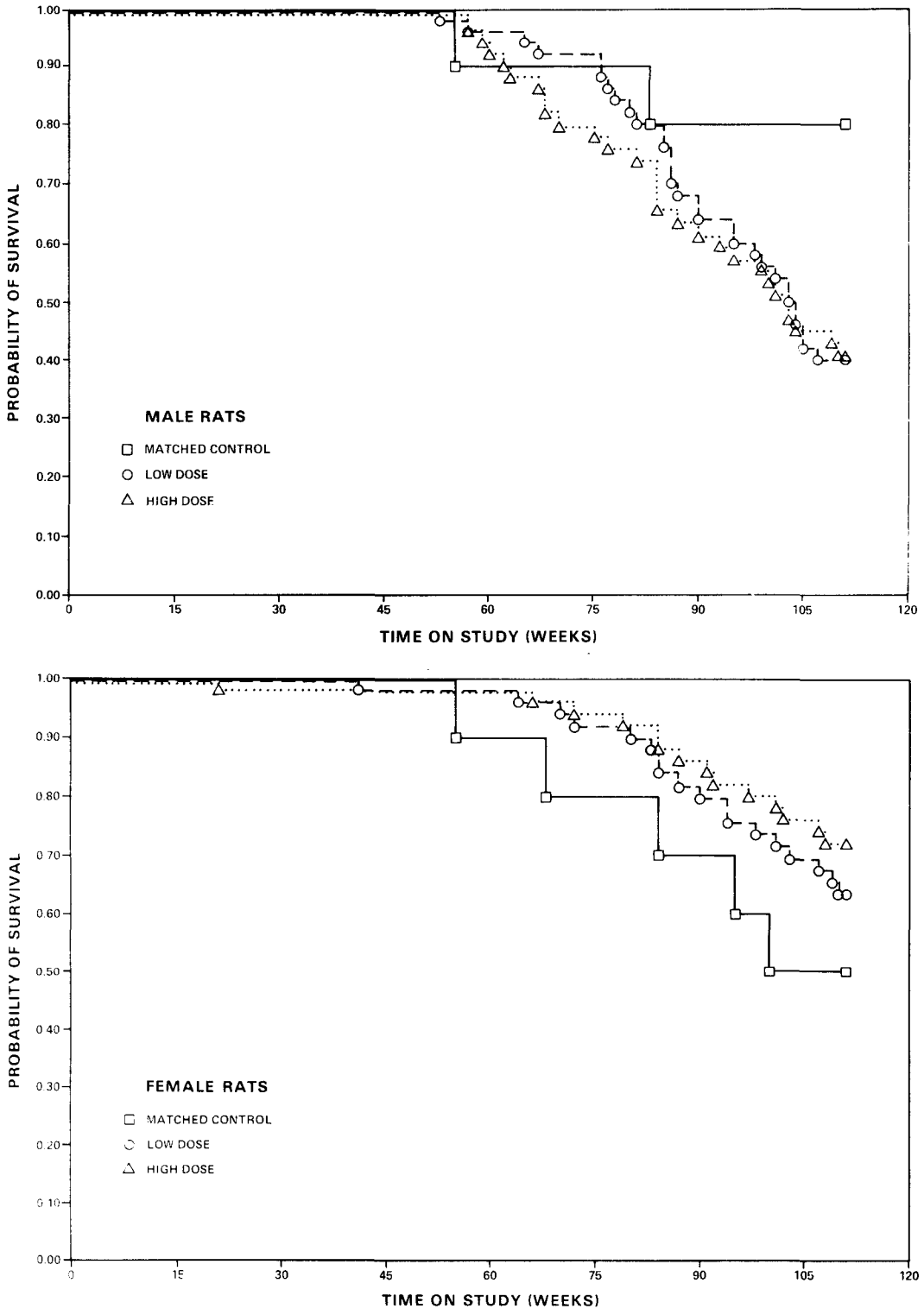


Figure 2. Survival Curves For Rats Fed Chlorothalonil In The Diet

Forty-nine out of fifty (98%) of the high-dose females, 48/49 (98%) of the low-dose females, and all 10 of the female matched controls survived beyond week 52 on study.

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.

The majority of neoplastic and nonneoplastic lesions were not unusual findings, and their incidences in animals of the dosed groups were comparable to those in the controls. Therefore, these lesions are considered to have occurred spontaneously. In some instances, the pathologic changes were found only in either dosed or control rats; except for renal tumors the frequency of these findings was, however, within normal range for the Osborne-Mendel rats. Medial calcification of arteries and parathyroid hyperplasia occurred with a relatively high incidence in dosed rats. These lesions are often associated with chronic inflammatory renal disease, however, and since they are commonly observed in aging rats regardless of any treatment, it is unlikely that chlorothalonil was the cause of induction.

Neoplasms of the tubular epithelium of the kidney were observed in dosed but not in control rats; these tumors occurred as follows:

	Males			Females		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Carcinoma	0/10	1/46	3/49	0/10	1/48	2/50
Adenoma	0/10	2/46	1/49	0/10	0/48	3/50
Total	0/10	3/46	4/49	0/10	1/48	5/50

Renal tumors are rarely observed in other control Osborne-Mendel rats at this laboratory. The relatively high incidence in this bioassay of tubular-cell renal tumors in dosed rats indicates a compound-related effect. In addition, a transitional-cell carcinoma, a carcinosarcoma, a liposarcoma, and a hamartoma were recorded in low-dose male rats.

Based on the histopathologic evaluation, the results indicate that chlorothalonil induced renal neoplasms in the rats 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 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.

The results of the Cochran-Armitage test on the incidence of male rats with carcinoma, tubular-cell adenocarcinoma, adenoma, adenocarcinoma, or papillary adenoma of the kidney are significant (P = 0.030) using the pooled controls. The Fisher exact comparison of the incidence in the high-dose group with that in the pooled-control group shows a P value of 0.035, which is above the 0.025 level for significance when the Bonferroni inequality criterion is used for multiple comparison. Historical records of this bioassay program at this laboratory indicate an incidence of tubular-cell adenomas in male rats of 3/240 (1.25%) with no other renal tumors occurring.

In females, the results of the Cochran-Armitage test on the incidence of animals with either adenomas or tubular-cell adenomas of the kidney are significant (P = 0.028) using the pooled controls, but the results of the Fisher exact test are not significant. The results of the Cochran-Armitage test on the incidence of female rats with adenomas, carcinomas, tubular-cell adenomas, or tubular-cell adenocarcinomas are significant (P = 0.007), and the results of the Fisher exact test show that the incidence in the high-dose group is significantly higher than that in the pooled controls (P = 0.016). Historical records

indicate none of the above tumors were seen in 235 female control rats at this laboratory. Overall, the statistical tests indicate a dose association of this chemical with the kidney tumors in both sexes of rats at the doses administered in this bioassay.

In male rats, the results of the Cochran-Armitage test for positive dose-related trend in the incidence of malignant fibrous histiocytomas of the subcutaneous tissue in the integumentary system are significant ($P = 0.030$) using the pooled controls; however, the results of the Fisher exact test for the comparisons of the incidences in the dosed groups with those in the control groups are not significant ($P > 0.05$). Statistical tests on the incidence of these tumors in female rats are not significant.

In female rats, a negative result is observed in the incidence of chromophobe adenoma of the pituitary.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of dosed male mice were lower than those of the controls and were dose related throughout most of the bioassay (figure 3). There was little difference among control and dosed groups of female mice. Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation.

During the first year of the study, the dosed animals were generally comparable to the controls in appearance and behavior. A few animals had alopecia and were hyperexcitable.

Clinical signs were noted at a low incidence in all dosed groups of both males and females during the second year of the study and included alopecia, loss of weight, rough hair coats, abdominal distention, and nodular masses, many of which were located in the lower abdomen. On many of the low- and high-dose males, nodules located in the inguinal area appeared intermittently during the last year of the study. Several males also intermittently had swollen testes. Beginning at week 62 and persisting until termination of the study, a majority of the low- and high-dose males were hyperexcitable.

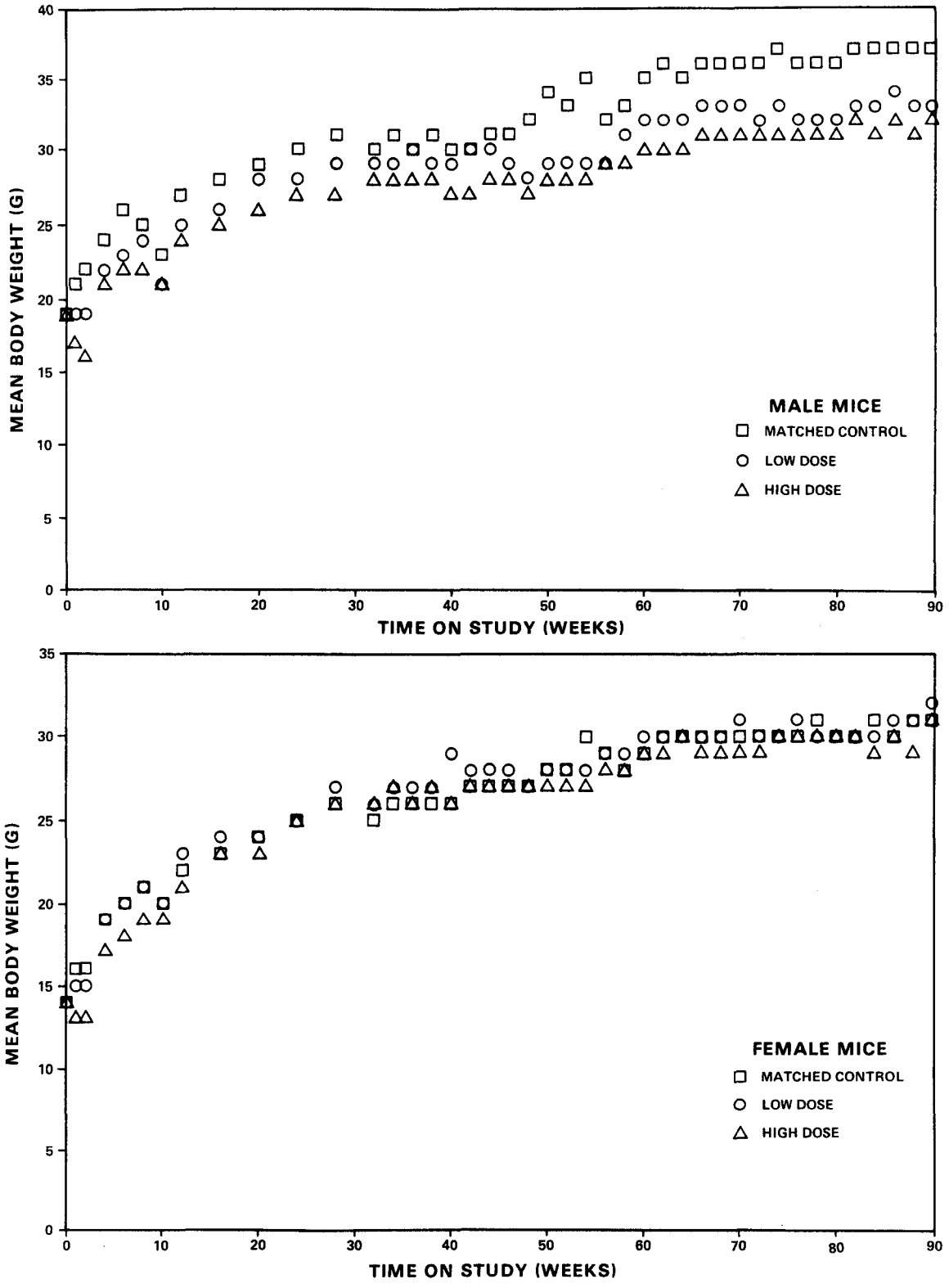


Figure 3. Growth Curves For Mice Fed Chlorothalonil In The Diet

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice fed chlorothalonil in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 4. The result of the Tarone test for positive dose-related trend in mortality is not statistically significant in either sex. In each sex the rates of survival of the dosed groups are comparable to those of the respective pooled controls.

In male mice, all 50 of the high-dose group, 42/50 (84%) of the low-dose group, and 9/10 (90%) of the controls were still alive at week 91. In female mice, 41/50 (82%) of the high-dose group, 47/50 (94%) of the low-dose group, and 7/10 (70%) of the controls were still alive at week 91. Sufficient numbers of mice of each sex were at risk for the development of 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.

The lesions observed in mice were not as numerous or of various types as were those in rats. The distribution and frequency of

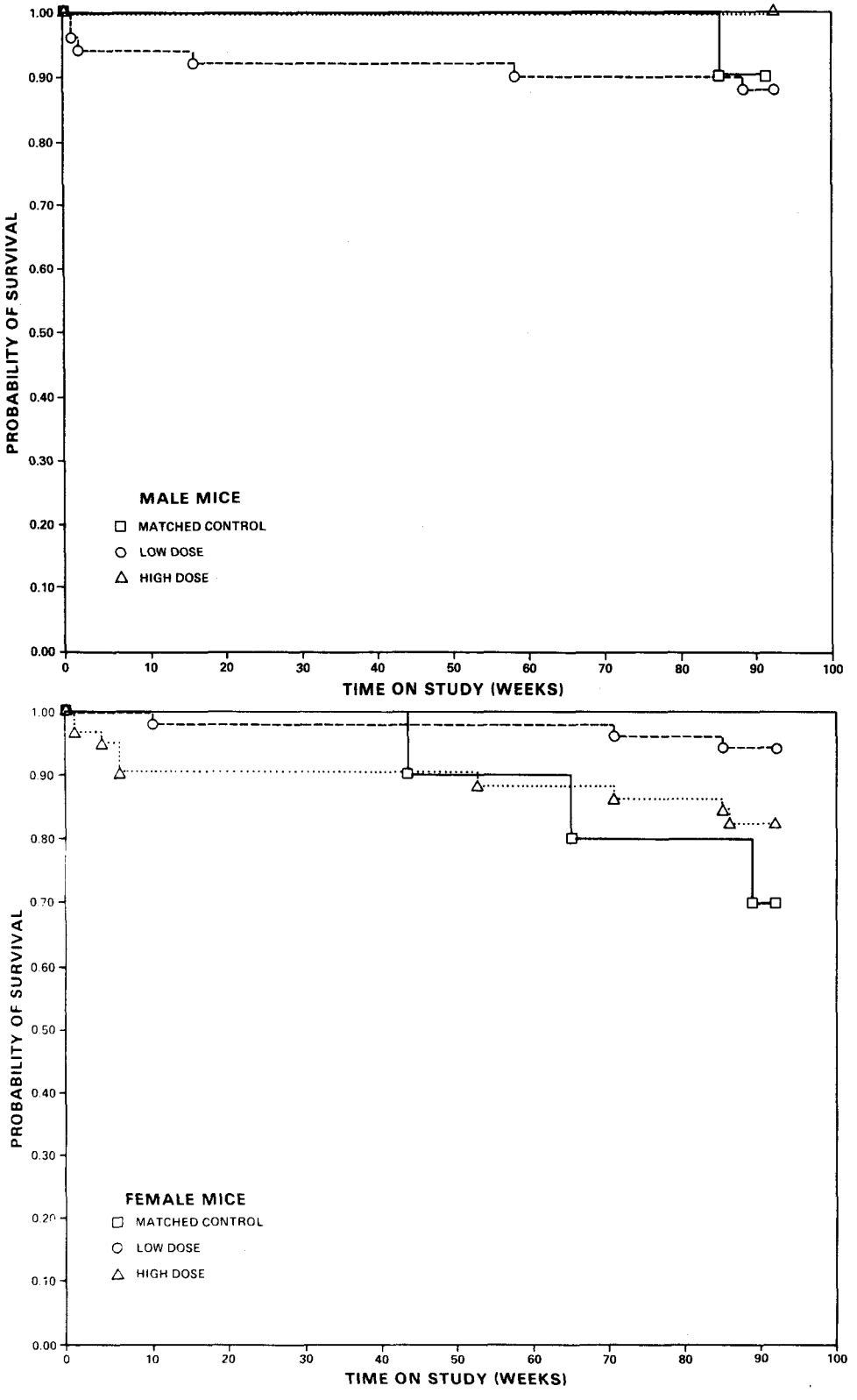


Figure 4. Survival Curves For Mice Fed Chlorothalonil In The Diet

these pathologic changes among the dosed and control mice indicate no relationship with the exposure to chlorothalonil.

Based on the histopathologic examination, tumors were not related to administration of chlorothalonil in the B6C3F1 hybrid mouse 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.

The results of the Cochran-Armitage test for positive dose-related trend and of the Fisher exact test for direct comparisons of the incidences in the control and dosed groups are not significant in either sex.

In male mice, a significant trend in the negative direction is observed in the incidence of alveolar/bronchiolar adenomas of the lung when the matched-control, low-dose, and high-dose groups are used, because the incidence in the matched-control group exceeds the incidences in the dosed groups. There is also a negative association indicated by the incidences of hepatocellular carcinoma when the incidence in pooled controls is used. These

negative associations cannot be explained by differential survival.

In each of the 95% confidence intervals 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 chlorothalonil which could not be detected under the conditions of this bioassay.

V. DISCUSSION

Chlorothalonil is a chlorinated phthalonitrile of low acute oral toxicity (oral LD_{50} in rats > 10,000 mg/kg, Spencer, 1973; oral LD_{50} in female ICR mice 6,000 mg/kg, Yoshikawa and Kawai, 1966). In this bioassay, Osborne-Mendel rats fed chlorothalonil were comparable to the controls in appearance and behavior during the first year of the study. In the second year of the study, hematuria, vaginal bleeding, abscesses, and rough hair coats were observed with increasing frequency, particularly among the dosed rats. From week 72 until termination of the study, discolored hair and bright-yellow urine were noted only among dosed rats. A majority of dosed male mice were hyperexcitable from week 62 until termination of the study. Mean body weights of rats of each sex and of male mice were consistently lower than those of the controls throughout the study. Since the dosed female mice did not have a depression in mean weight or decreased survival compared with controls, they may have been able to tolerate a higher dose.

Survival rates of the dosed rats decreased sharply after week 60, but sufficient numbers of rats were at risk for late-developing tumors. Survival of the mice was higher than that of the rats.

In rats, neoplasms of the renal tubular epithelium occurred with a significant dose-related trend in both the males (P = 0.030) and the females (P = 0.007) and also occurred at a higher incidence in the high-dose males (P = 0.035) and the high-dose females (P = 0.016) than in the corresponding controls (males: pooled controls 0/62, low-dose 3/46 [7%], high- dose 4/49 [8%]; females: pooled controls 0/62, low-dose 1/48 [2%], high-dose 5/50 [10%]). These tumors included both adenomas and carcinomas which are considered to be histogenically related. Only adenomas have been observed among historical-control Osborne-Mendel rats at this laboratory (males 3/240 [1.3%], females 0/235). Thus the tumors are rare and their incidence in the present study is interpreted as sufficient evidence for the carcinogenicity of chlorothalonil.

In mice, no tumors were found to occur at a significantly greater incidence among dosed animals than among controls.

It is concluded that under the conditions of this bioassay, technical-grade chlorothalonil was carcinogenic to Osborne-Mendel rats, producing tumors of the kidney. Chlorothalonil was not carcinogenic for B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS FED CHLOROTHALONIL IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED CHLOROTHALONIL IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	47	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	47	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(10)	(47)	(49)
FIBROMA		1 (2%)	
FIBROUS HISTIOCYTOMA, MALIGNANT			3 (6%)
RESPIRATORY SYSTEM			
#LUNG	(10)	(44)	(47)
FIBROUS HISTIOCYTOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(10)	(46)	(49)
HEMANGIOSARCOMA			1 (2%)
ANGIOMA	1 (10%)		
HAMARTOMA			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*ORAL MUCOUS MEMBRANE	(10)	(47)	(49)
FIBROMA	1 (10%)		
URINARY SYSTEM			
#KIDNEY	(10)	(46)	(49)
CARCINOMA,NOS			2 (4%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TRANSITIONAL-CELL CARCINOMA		1 (2%)	
ADENOCARCINOMA, NOS			1 (2%)
PAPILLARY ADENOMA		1 (2%)	
TUBULAR-CELL ADENOMA		1 (2%)	1 (2%)
TUBULAR-CELL ADENOCARCINOMA		1 (2%)	
LIPOSARCOMA		1 (2%)	
CARCINOSARCOMA		1 (2%)	
† HAMARTOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(46)	(46)
CARCINOMA,NOS	1 (11%)		
ADENOCARCINOMA, NOS		1 (2%)	1 (2%)
CHROMOPHOBE ADENOMA		3 (7%)	5 (11%)
CHROMOPHOBE CARCINOMA		2 (4%)	
#ADRENAL	(10)	(45)	(49)
GANGLIONEUROBLASTOMA			1 (2%)
#THYROID	(9)	(44)	(45)
PAPILLARY ADENOMA	1 (11%)		
FOLLICULAR-CELL ADENOMA		2 (5%)	3 (7%)
C-CELL ADENOMA			1 (2%)
#PANCREATIC ISLETS	(9)	(45)	(49)
ISLET-CELL ADENOMA		2 (4%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(47)	(49)
ADENOMA, NOS		1 (2%)	
#TESTIS	(9)	(45)	(48)
INTERSTITIAL-CELL TUMOR			1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(10)	(45)	(49)
MENINGIOMA		1 (2%)	
#BRAIN	(10)	(45)	(49)
GRANULAR-CELL TUMOR, MALIGNANT			1 (2%)
SPECIAL SENSE ORGANS			
NONE			

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

† This is considered to be a benign form of the malignant mixed tumor of the kidney and consists of lipocytes, tubular structures, and fibroblasts in varying proportions.

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*TUNICA VAGINALIS	(10)	(47)	(49)
MESOTHELIOMA, NOS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@		7	7
MORIBUND SACRIFICE	2	23	22
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	8	20	20
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	19	16
TOTAL PRIMARY TUMORS	4	21	22
TOTAL ANIMALS WITH BENIGN TUMORS	3	10	9
TOTAL BENIGN TUMORS	3	12	12
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	8	10
TOTAL MALIGNANT TUMORS	1	8	10
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED CHLOROTHALONIL IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	48	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	48	50
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(48)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
*SUBCUT TISSUE	(10)	(48)	(50)
FIBROMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(9)	(47)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (2%)
CORTICAL CARCINOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(48)	(50)
LYMPHOCYTIC LEUKEMIA			1 (2%)
#THYMUS		(1)	
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (100%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(10)	(48)	(50)
ADENOMA, NOS		1 (2%)	
#PAROTID GLAND	(10)	(48)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(10)	(48)	(50)
NEOPLASTIC NODULE		3 (6%)	2 (4%)
URINARY SYSTEM			
#KIDNEY	(10)	(48)	(50)
CARCINOMA,NOS		1 (2%)	1 (2%)
ADENOMA, NOS			1 (2%)
TUBULAR-CELL ADENOMA			2 (4%)
TUBULAR-CELL ADENOCARCINOMA			1 (2%)
LIPOMA		1 (2%)	1 (2%)
#URINARY BLADDER	(9)	(47)	(47)
CARCINOMA, NOS, METASTATIC			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(48)	(49)
CHROMOPHOBE ADENOMA	2 (20%)	1 (2%)	6 (12%)
#ADRENAL	(10)	(48)	(50)
CORTICAL ADENOMA	1 (10%)		
CORTICAL CARCINOMA		1 (2%)	
#THYROID	(7)	(45)	(41)
FOLLICULAR-CELL ADENOMA			1 (2%)
FOLLICULAR-CELL CARCINOMA		2 (4%)	1 (2%)
C-CELL ADENOMA		1 (2%)	
#PANCREATIC ISLETS	(9)	(48)	(50)
ISLET-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(48)	(50)
ADENOMA, NOS		2 (4%)	1 (2%)
PAPILLARY ADENOCARCINOMA			1 (2%)
FIBROMA		3 (6%)	1 (2%)
FIBROADENOMA	1 (10%)	8 (17%)	4 (8%)
#UTERUS	(9)	(47)	(49)
CARCINOMA,NOS			1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ADENOCARCINOMA, NOS			1 (2%)
SARCOMA, NOS			1 (2%)
LEIOMYOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP	1 (11%)	2 (4%)	2 (4%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(10)	(48)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT			2 (4%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@		7	2
MORIBUND SACRIFICE	5	11	12
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	5	31	36
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	5	20	28
TOTAL PRIMARY TUMORS	5	29	35
TOTAL ANIMALS WITH BENIGN TUMORS	5	15	21
TOTAL BENIGN TUMORS	5	19	23
TOTAL ANIMALS WITH MALIGNANT TUMORS		6	10
TOTAL MALIGNANT TUMORS		7	10
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		3	2
TOTAL UNCERTAIN TUMORS		3	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE FED CHLOROTHALONIL IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED CHLOROTHALONIL IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	48	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(9)	(46)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (11%)	4 (9%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(9)	(44)	(50)
HEMANGIOMA		1 (2%)	
#KIDNEY	(9)	(45)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(46)	(50)
HEPATOCELLULAR CARCINOMA	2 (20%)	1 (2%)	1 (2%)
#STOMACH	(8)	(46)	(50)
SQUAMOUS CELL PAPILLOMA		1 (2%)	
SQUAMOUS CELL CARCINOMA		1 (2%)	
URINARY SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#ADRENAL	(9)	(45)	(50)
CORTICAL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@	1	5	
MORIBUND SACRIFICE		1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		2	
TERMINAL SACRIFICE	9	42	50
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	3	9	1
TOTAL PRIMARY TUMORS	3	10	1
TOTAL ANIMALS WITH BENIGN TUMORS	1	6	
TOTAL BENIGN TUMORS	1	7	
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	3	1
TOTAL MALIGNANT TUMORS	2	3	1
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 FED CHLOROTHALONIL IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	49	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	47
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(10)	(49)	(47)
FIBROMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(46)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (10%)	1 (2%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(49)	(47)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
LYMPHOCYTIC LEUKEMIA		1 (2%)	
#SPLEEN	(9)	(48)	(45)
HEMANGIOMA	1 (11%)		1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#STOMACH	(10)	(49)	(46)
PAPILLOMA, NOS		1 (2%)	
SQUAMOUS CELL CARCINOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY	(10)	(49)	(46)
TUBULAR-CELL ADENOMA			1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#ADRENAL	(10)	(49)	(44)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
#PANCREATIC ISLETS	(9)	(49)	(44)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(49)	(47)
ADENOMA, NOS	1 (10%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(10)	(49)	(47)
PAPILLARY ADENOMA	1 (10%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(10)	(49)	(47)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
ALL OTHER SYSTEMS			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@		1	5
MORIBUND SACRIFICE	3	2	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	7	47	41
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	3	6	8
TOTAL PRIMARY TUMORS	4	6	8
TOTAL ANIMALS WITH BENIGN TUMORS	3	3	4
TOTAL BENIGN TUMORS	4	3	4
TOTAL ANIMALS WITH MALIGNANT TUMORS		3	4
TOTAL MALIGNANT TUMORS		3	4
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS FED CHLOROTHALONIL IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED CHLOROTHALONIL IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	47	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	47	49
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(47)	(49)
ULCER, NOS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(10)	(44)	(47)
EMPHYSEMA, NOS		1 (2%)	
EDEMA, NOS	1 (10%)		
HEMORRHAGE	1 (10%)		
INFLAMMATION, CHRONIC			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(10)	(45)	(49)
MYELOFIBROSIS		1 (2%)	
#SPLEEN	(10)	(46)	(49)
CONGESTION, NOS			2 (4%)
HEMORRHAGE			1 (2%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
ANGIECTASIS		1 (2%)	
HYPOPLASIA, LYMPHOID			1 (2%)
#CERVICAL LYMPH NODE	(9)	(42)	(46)
INFLAMMATION, NOS	1 (11%)		
CIRCULATORY SYSTEM			
#HEART	(8)	(44)	(45)
CALCIFICATION, FOCAL		1 (2%)	
*ARTERY	(10)	(47)	(49)
PERIARTERITIS		1 (2%)	2 (4%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MEDIAL CALCIFICATION		6 (13%)	5 (10%)
*AORTA	(10)	(47)	(49)
MEDIAL CALCIFICATION		3 (6%)	2 (4%)
*MESENTERIC ARTERY	(10)	(47)	(49)
THROMBOSIS, NOS		1 (2%)	
*TESTICULAR ARTERY	(10)	(47)	(49)
INFLAMMATION, NOS		3 (6%)	
DIGESTIVE SYSTEM			
#LIVER	(10)	(46)	(48)
DEGENERATION, BALLOONING		1 (2%)	
DEGENERATION PARENCHYMATOUS	6 (60%)		
METAMORPHOSIS FATTY	1 (10%)	7 (15%)	6 (13%)
ANGIECTASIS		2 (4%)	
*BILE DUCT	(10)	(47)	(49)
HYPERPLASIA, NOS		1 (2%)	
#PANCREAS	(9)	(45)	(49)
PERIARTERITIS		4 (9%)	4 (8%)
#PANCREATIC ACINUS	(9)	(45)	(49)
ATROPHY, NOS		1 (2%)	
#STOMACH	(9)	(45)	(47)
MINERALIZATION		1 (2%)	
CALCIFICATION, FOCAL		7 (16%)	2 (4%)
#LARGE INTESTINE		(1)	(2)
INFLAMMATION, HEMORRHAGIC			1 (50%)
#COLON		(1)	(2)
INFLAMMATION, NOS			1 (50%)
#CECUM		(1)	(2)
INFLAMMATION, CHRONIC		1 (100%)	
URINARY SYSTEM			
#KIDNEY	(10)	(46)	(49)
INFLAMMATION, NOS		3 (7%)	1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, INTERSTITIAL	5 (50%)		
INFLAMMATION, CHRONIC	2 (20%)	33 (72%)	36 (73%)
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(46)	(46)
CYST, NOS	1 (11%)	2 (4%)	
MULTILOCULAR CYST		6 (13%)	2 (4%)
ANGIECTASIS		1 (2%)	
#ADRENAL	(10)	(45)	(49)
METAMORPHOSIS FATTY		1 (2%)	1 (2%)
ANGIECTASIS		2 (4%)	
#THYROID	(9)	(44)	(45)
HYPERPLASIA, C-CELL			1 (2%)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	1 (2%)
#PARATHYROID	(3)	(32)	(35)
HYPERPLASIA, NOS		7 (22%)	5 (14%)
HYPERPLASIA, EPITHELIAL		1 (3%)	1 (3%)
REPRODUCTIVE SYSTEM			
#PROSTATE	(9)	(44)	(47)
INFLAMMATION, NOS		2 (5%)	
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, ACUTE SUPPURATIVE	1 (11%)		
#TESTIS	(9)	(45)	(48)
EDEMA, NOS	1 (11%)	2 (4%)	
PERIARTERITIS		1 (2%)	
ATROPHY, NOS	3 (33%)	17 (38%)	10 (21%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*MESENTERY	(10)	(47)	(49)
PERIARTERITIS		8 (17%)	6 (12%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(10)	(47)	(49)
PERIARTERITIS		1 (2%)	1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
NC LESION REPORTED	1	2	4
AUTOLYSIS/NO NECROPSY		3	

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED CHLOROTHALONIL IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	48	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	48	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(9)	(47)	(50)
CALCIFICATION, METASTATIC	1 (11%)		
#LUNG/ALVEOLI	(9)	(47)	(50)
EMPHYSEMA, NOS	1 (11%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(10)	(47)	(50)
INFLAMMATION, CHRONIC			1 (2%)
HEMOSIDEROSIS	1 (10%)		
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(9)	(47)	(50)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
*ARTERY	(10)	(48)	(50)
MEDIAL CALCIFICATION		1 (2%)	
*AORTA	(10)	(48)	(50)
ARTERIOSCLEROSIS, NOS	1 (10%)		
*CORONARY ARTERY	(10)	(48)	(50)
MEDIAL CALCIFICATION		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(10)	(48)	(50)
FIBROSIS, FOCAL			1 (2%)
DEGENERATION PARENCHYMATOUS	2 (20%)		
DEGENERATION, GRANULAR	1 (10%)		
NECROSIS, FOCAL	1 (10%)		
METAMORPHOSIS FATTY	1 (10%)	1 (2%)	1 (2%)
ANGIECTASIS		5 (10%)	1 (2%)
#LIVER/CENTRILOBULAR	(10)	(48)	(50)
METAMORPHOSIS FATTY	2 (20%)		
*BILE DUCT	(10)	(48)	(50)
HYPERPLASIA, NOS		2 (4%)	
#PANCREAS	(9)	(48)	(50)
PERIARTERITIS		2 (4%)	2 (4%)
#STOMACH	(10)	(48)	(49)
CALCIFICATION, METASTATIC	1 (10%)		
HYPERPLASIA, NOS		1 (2%)	
#GASTRIC MUCOSA	(10)	(48)	(49)
CALCIFICATION, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(10)	(48)	(50)
HYDRONEPHROSIS			1 (2%)
GLOMERULONEPHRITIS, NOS	1 (10%)		
INFLAMMATION, INTERSTITIAL	2 (20%)		
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC	1 (10%)	13 (27%)	6 (12%)
FIBROSIS, FOCAL		1 (2%)	
#URINARY BLADDER	(9)	(47)	(47)
HYPERPLASIA, EPITHELIAL		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(48)	(49)
HEMORRHAGE	1 (10%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANGIECTASIS			2 (4%)
#ADRENAL	(10)	(48)	(50)
NECROSIS, HEMORRHAGIC			1 (2%)
ANGIECTASIS		1 (2%)	6 (12%)
#ADRENAL CORTEX	(10)	(48)	(50)
ANGIECTASIS	1 (10%)		
#THYROID	(7)	(45)	(41)
HYPERPLASIA, C-CELL	1 (14%)	2 (4%)	2 (5%)
HYPERPLASIA, FOLLICULAR-CELL			1 (2%)
#PARATHYROID	(5)	(26)	(26)
HYPERPLASIA, NOS	1 (20%)	2 (8%)	1 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(48)	(50)
HYPERPLASIA, NOS	1 (10%)		
ADENOSIS	1 (10%)		
#UTERUS/ENDOMETRIUM	(9)	(47)	(49)
HYPERPLASIA, CYSTIC		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(10)	(48)	(50)
PERIARTERITIS		3 (6%)	3 (6%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(10)	(48)	(50)
PERIARTERITIS		2 (4%)	1 (2%)
MEDIAL CALCIFICATION		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	8	11
AUTOLYSIS/NO NECROPSY		1	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED CHLOROTHALONIL IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED CHLOROTHALONIL IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	48	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(9)	(46)	(50)
ATELECTASIS	1 (11%)		
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM			1 (2%)
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(46)	(50)
HYPERPLASIA, NODULAR	1 (10%)	2 (4%)	3 (6%)
#STOMACH	(8)	(46)	(50)
EROSION			1 (2%)
URINARY SYSTEM			
NONE			

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#THYROID	(9)	(43)	(49)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	36	44
AUTO/NECROPSY/NO HISTO		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 FED CHLOROTHALONIL IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	49	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	47
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(46)
EDEMA, NOS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(9)	(48)	(45)
HEMATOPOIESIS			1 (2%)
#SPLENIC FOLLICLES	(9)	(48)	(45)
HYPERPLASIA, NOS	1 (11%)		
#MESENTERIC L. NODE	(10)	(44)	(43)
INFLAMMATION, GRANULOMATOUS	1 (10%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(49)	(46)
INFLAMMATION, ACUTE DIFFUSE			1 (2%)
INFLAMMATION, ACUTE NECROTIZING		1 (2%)	
INFARCT, FOCAL			1 (2%)
HYPERPLASIA, NODULAR	1 (10%)		1 (2%)
URINARY SYSTEM			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#THYROID	(9)	(45)	(44)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(49)	(47)
HYPERPLASIA, NOS			1 (2%)
#UTERUS/ENDOMETRIUM	(10)	(48)	(43)
HYPERPLASIA, CYSTIC	2 (20%)		
#OVARY	(10)	(49)	(42)
HEMORRHAGIC CYST	1 (10%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	41	37
AUTOLYSIS/NO NECROPSY		1	3

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS FED CHLOROTHALONIL IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Chlorothalonil in the Diet[a]

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Integumentary System:				
Malignant Fibrous Histiocytoma of the Subcutaneous Tissue[b]	0/59 (0)	0/10 (0)	0/47 (0)	3/49 (6)
P Values[c,d]	P = 0.030	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			--	Infinite
Lower Limit			--	0.721
Upper Limit			--	Infinite
Relative Risk (Matched Control)[f]			--	Infinite
Lower Limit			--	0.136
Upper Limit			--	Infinite
Weeks to First Observed Tumor	--	--	--	87
Pituitary: Chromophobe Adenoma or Carcinoma[b]	6/56 (11)	0/9 (0)	5/46 (11)	5/46 (11)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			1.014	1.014
Lower Limit			0.260	0.260
Upper Limit			3.722	3.722
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.280	0.280
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	103	87

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Chlorothalonil in the Diet[a]

(continued)

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Adenoma[b]	4/55 (7)	0/9 (0)	2/44 (5)	3/45 (7)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			0.625	0.917
Lower Limit			0.059	0.140
Upper Limit			4.132	5.125
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.068	0.136
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	101	84
Kidney: Carcinoma, NOS, Adenocarcinoma or Tubular-cell Adenocarcinoma[b]	0/62 (0)	0/10 (0)	1/46 (2)	3/49 (6)
P Values[c,d]	P = 0.044	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.072	0.757
Upper Limit			Infinite	Infinite
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.013	0.136
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	110	84

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Chlorothalonil in the Diet[a]

(continued)

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Kidney: Papillary Adenoma or Tubular-cell Adenoma[b]	0/62 (0)	0/10 (0)	2/46 (4)	1/49 (2)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.397	0.068
Upper Limit			Infinite	Infinite
Relative Risk (Matched Control)[f]			Infintie	Infinite
Lower Limit			0.071	0.012
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	103	95
Kidney: Carcinoma, NOS, Tubular-cell Adenoma, Tubular-cell Adenocarcinoma, Adenocarcinoma, or Papillary Adenoma[b]	0/62 (0)	0/10 (0)	3/46 (7)	4/49 (8)
P Values[c,d]	P = 0.030	N.S.	N.S.	P = 0.035**
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.807	1.167
Upper Limit			Infinite	Infinite
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.145	0.211
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	103	84

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Chlorothalonil in the Diet[a]

(continued)

[a]Dosed groups received time-weighted average doses of 5,063 or 10,126 ppm.

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

[c]Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when $P < 0.05$; 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 (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; 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 $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the specified control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Chlorothalonil in the Diet[a]

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	9/56 (16)	2/10 (20)	1/48 (2)	6/49 (12)
P Values[c,d]	N.S.	N.S.	P = 0.015**(N)	N.S.
Departure from Linear Trend[e]	P = 0.024	P = 0.025		
Relative Risk (Pooled Control)[f]			0.130	0.762
Lower Limit			0.003	0.239
Upper Limit			0.882	2.215
Relative Risk (Matched Control)[f]			0.104	0.612
Lower Limit			0.002	0.141
Upper Limit			1.883	5.791
Weeks to First Observed Tumor	--	100	94	102
Thyroid: Follicular-cell Adenoma or Carcinoma[b]	0/58 (0)	0/7 (0)	2/45 (4)	2/41 (5)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.380	0.417
Upper Limit			Infinite	Infinite
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.054	0.059
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	111	111

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Chlorothalonil in the Diet[a]

(continued)

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Liver: Neoplastic Nodule[b]	1/59 (2)	0/10 (0)	3/48 (6)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			3.688	2.360
Lower Limit			0.307	0.126
Upper Limit			189.405	136.426
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.139	0.065
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	84	11
Kidney: Carcinoma, NOS, or Tubular-Cell Adenocarcinoma[b]	0/62 (0)	0/10 (0)	1/48 (2)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.069	0.365
Upper Limit			Infinite	Infinite
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.012	0.065
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	107	91

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Chlorothalonil in the Diet[a]

(continued)

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Kidney: Adenoma, NOS, or Tubular-Cell Adenoma[b]	0/62 (0)	0/10 (0)	0/48 (0)	3/50 (6)
P Values[c,d]	P = 0.028	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			--	Infinite
Lower Limit			--	0.742
Upper Limit			--	Infinite
Relative Risk (Matched Control)[f]			--	Infinite
Lower Limit			--	0.134
Upper Limit			--	Infinite
Weeks to First Observed Tumor	--	--	--	97
Kidney: Adenoma, Carcinoma, NOS, Tubular-cell Adenoma, or Tubular-cell Adenocarcinoma[b]	0/62 (0)	0/10 (0)	1/48 (2)	5/50 (10)
P Values[c,d]	P = 0.007	N.S.	N.S.	P = 0.016**
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.069	1.556
Upper Limit			Infinite	Infinite
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.012	0.281
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	107	91

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Chlorothalonil in the Diet[a]

(continued)

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	9/64 (14)	1/10 (10)	8/48 (17)	4/50 (8)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			1.185	0.569
Lower Limit			0.428	0.135
Upper Limit			3.188	1.903
Relative Risk (Matched Control)[f]			1.667	0.800
Lower Limit			0.279	0.097
Upper Limit			72.240	38.616
Weeks to First Observed Tumor	--	68	72	72
Mammary Gland: Fibroma[b]	1/64 (2)	0/10 (0)	3/48 (6)	1/50 (2)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			4.000	1.280
Lower Limit			0.333	0.017
Upper Limit			205.455	98.511
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.139	0.012
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	101	111

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Chlorothalonil in the Diet[a]

(continued)

[a]Dosed groups received time-weighted average doses of 5,063 or 10,126 ppm.

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

[c]Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when $P < 0.05$; 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 (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; 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 $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the specified control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE FED CHLOROTHALONIL IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Chlorothalonil in the Diet[a]

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	4/58 (7)	1/9 (11)	4/46 (9)	0/50 (0)
P Values[c,d]	N.S.	P = 0.040(N)	N.S.	N.S.
Relative Risk (Pooled Control)[f]			1.261	0.000
Lower Limit			0.247	0.000
Upper Limit			6.400	1.253
Relative Risk (Matched Control)[f]			0.783	0.000
Lower Limit			0.097	0.000
Upper Limit			37.702	3.374
Weeks to First Observed Tumor	--	91	91	--
Liver: Hepatocellular Carcinoma[b]	9/59 (15)	2/10 (20)	1/46 (2)	1/50 (2)
P Values[c,d]	P = 0.005(N)	N.S.	P = 0.022**(N)	P = 0.016**(N)
Departure from Linear Trend[e]		P = 0.041		
Relative Risk (Pooled Control)[f]			0.143	0.131
Lower Limit			0.003	0.003
Upper Limit			0.969	0.895
Relative Risk (Matched Control)[f]			0.109	0.100
Lower Limit			0.002	0.002
Upper Limit			1.963	1.810
Weeks to First Observed Tumor	--	85	92	92

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Chlorothalonil in the Diet[a]

(continued)

[a]Dosed groups received time-weighted averages doses of 2,688 or 5,375 ppm.

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

[c]Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when $P < 0.05$; 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 (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; 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 $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the specified control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Chlorothalonil in the Diet[a]

Topography: Morphology	Pooled Control	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	3/58 (5)	1/10 (10)	2/49 (4)	3/46 (7)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Pooled Control)[f]			0.789	1.261
Lower Limit			0.068	0.176
Upper Limit			6.598	8.980
Relative Risk (Matched Control)[f]			0.408	0.652
Lower Limit			0.025	0.063
Upper Limit			23.619	33.512
Weeks to First Observed Tumor	--	91	91	92

[a]Dosed groups received time-weighted average doses of 3,000 or 6,000 ppm.

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

[c]Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when $P < 0.05$; 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 (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; 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 $P < 0.05$ for any comparison.

[f]The 95% confidence interval of the relative risk between each dosed group and the specified control group.

APPENDIX G

ANALYSIS OF FORMULATED DIETS FOR CONCENTRATIONS OF CHLOROTHALONIL

APPENDIX G

Analysis of Formulated Diets for

Concentrations of Chlorothalonil

A 10-g sample of the dosage mixture to be analyzed was shaken with 125 ml benzene at room temperature for 16 hours, then filtered through Celite with benzene washes, and reduced in volume to 10 ml. After appropriate dilutions, the solution was quantitatively analyzed for chlorothalonil by gas-liquid chromatography (electron capture detector, 10% DC-200 on Gas Chrom Q column). Recoveries were checked with spiked samples, and external standards were used for calibration.

Theoretical Concentrations in Diet (ppm)	No. of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
2,500	22	2,506	5.4%	2,271-2,835
5,000	33	5,016	5.7%	4,375-5,648
10,000	30	10,110	4.3%	9,500-10,960
20,000	2	19,700	2.2%	19,400-20,000

Review of the Bioassay of Chlorothalonil* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

June 29, 1978

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

The reviewer said that the compound was carcinogenic in treated rats but "was not clearly carcinogenic" in treated mice. The reviewer was particularly critical of the limited number of matched control animals. She pointed out the occurrence of certain tumors in the treated rats and mice that may have taken on greater significance had the control groups been larger. Other deficiencies noted by the reviewer included the use of different batches of Chlorothalonil and poor animal survival. Despite the shortcomings, she said that the kidney tumors in both the treated male and female rats appeared to be biologically significant.

In subsequent discussions, a staff pathologist described the criteria for classifying kidney tumors, particularly with respect to differentiating between benign and malignant neoplasms. He noted greater incidences of renal toxicity and hypoparathyroidism observed in treated animals than in controls and he suggested that Chlorothalonil was probably a renal toxin. A representative of the manufacturer of

Chlorothalonil said that the compound had been shown to produce renal toxicity, which was probably exerted through an indirect mechanism.

A motion was made that the report on the bioassay of Chlorothalonil be accepted as written. The motion was approved without objection.

Clearinghouse Members present:

Arnold L. Brown (Chairman), Mayo Clinic
Paul Nettesheim, National Institute of Environmental Health Sciences
Verne Ray, Pfizer Medical Research Laboratory
Verald K. Rowe, Dow Chemical U.S.A.
Michael B. Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center

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

☆U.S. GOVERNMENT PRINTING OFFICE: 1978-260-899/3185

NCI-CG-TR-41

DHEW Publication No. (NIH) 78-841

1978