National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 33
1978

BIOASSAY OF TETRACHLORVINPHOS FOR POSSIBLE CARCINOGENICITY

CAS No. 961-11-5

NCI-CG-TR-33

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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Carcinogen Bioassay and Program Resources Branch
Carcinogenesis Program
Division of Cancer Cause and Prevention
National Cancer Institute
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DHEW Publication No. (NIH) 78-833

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CONTRIBUTORS: This report presents the results of the bioassay of tetrachlorvinphos for possible carcinogenicity, conducted for the Carcinogen Bioassay and Program Resources Branch, Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), Bethesda, Maryland. The bioassay 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., prime contractor for the NCI carcinogenesis bioassay 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. Animal treatment and observation were supervised by Drs. T. E. Shellenberger and H. P. Burchfield[4], with the technical assistance of Ms. D. H. Monceaux[4] and Mr. D. Broussard[4]. Histopathology 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 and the analytical results were reviewed by Dr. S. S. Olin[7].

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SUMMARY

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

Groups of 50 rats of each sex were administered tetrachlorvinphos at one of two doses for 80 weeks, then observed for 31 additional weeks. Time-weighted average doses were either 4,250 or 8,500 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 45 untreated male and 45 untreated female rats from similar bioassays of four other test chemicals. All surviving rats were killed at 111 weeks.

Groups of 50 mice of each sex were administered tetrachlorvinphos at one of two doses, either 8,000 or 16,000 ppm, for 80 weeks, then observed for 12 additional weeks. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched controls combined with 40 untreated male and 40 untreated female mice from similar bioassays of four other test chemicals. All surviving mice were killed at 90-92 weeks.

The mean body weights of the treated rats and mice were generally lower than those of the matched controls; however, the mortality rate was affected adversely by tetrachlorvinphos only in the male rats. Survival of all groups of rats and mice was adequate for meaningful statistical analyses of the incidence of tumors, except for a matched-control group of female rats for which the survival was abnormally low.

In rats, C-cell adenoma of the thyroid showed a significant dose-related trend in the females, using pooled controls (controls 1/46, low-dose 2/50, high-dose 7/46, P = 0.013), and by direct comparison, an increased incidence in the high-dose group (P = 0.027). High incidences of C-cell hyperplasia in treated

males and females further indicated a chemical-related effect on proliferative lesions of the thyroid. Cortical adenoma of the adrenal also showed a significant dose-related trend in the females, using pooled controls (controls 0/50, low-dose 2/49, high-dose 5/50, P = 0.017), and by direct comparison, an increased incidence in the high-dose group (P = 0.022). Hemangioma of the spleen occurred in male rats at a significantly higher incidence in the low-dose group than in the pooled controls (controls 0/52, low-dose 4/48, P = 0.049); however, neither the incidence in the high-dose group (0/47) nor the test result for dose-related trend was statistically significant.

In mice, hepatocellular carcinoma in males showed a highly significant dose-related trend, using either matched controls (controls 0/9, low-dose 36/50, high-dose 40/50, P < 0.001) or pooled controls (controls 5/49, P < 0.001). This finding was supported by direct comparisons of low- and high-dose groups of males with matched- or pooled-control groups, which showed highly significant increases in incidences of the tumor in the treated groups in all instances (P < 0.001). In females, the incidence of hepatocellular carcinoma was not significant; however, the incidence of neoplastic nodule was significantly higher in both the low- and high-dose groups than in the pooled controls (controls 1/48, low-dose 14/49, P < 0.001; high-dose 9/47, P = 0.007), using pooled controls for tests for both doses. Because of this higher incidence in the low-dose group than in the high-dose group, there was a significant departure from linear trend (P = 0.006).

Granulomatous lesions of the liver were found in high proportions in both treated rats and treated mice, but none were found in matched controls.

It is concluded that under the conditions of this bioassay, the administration of technical-grade tetrachlorvinphos in Osborne-Mendel rats was associated with proliferative lesions of the C cells of the thyroid and cortical adenomas of the adrenal in females. In female B6C3F1 mice, the incidence of neoplastic nodule of the liver was associated with treatment, and in male mice tetrachlorvinphos was carcinogenic, causing hepatocellular carcinoma of the liver.

TABLE OF CONTENTS

TABLES

FIGURES

I. INTRODUCTION

Tetrachlorvinphos (CAS 961-11-5; NCI C00168), which is the generic name for 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate, is an organophosphorous pesticide introduced in 1966 by Shell Development Company (Whetstone et al., 1966). It is registered for use against various pests of fruits, vegetables, ornamental plants, forest trees, and livestock, and for use on agricultural premises, agricultural equipment, and recreational areas (EPA Compendium, 1973). Tetrachlorvinphos was selected for testing in the carcinogenesis program because of its extensive use on food crops and livestock and because there was a lack of chronic toxicity studies of the chemical.

II. MATERIALS AND METHODS

A. Chemical

The material tested was technical-grade tetrachlorvinphos obtained in one batch from Shell Chemical Company, San Ramon, California, for use in the chronic study. As synthesized by the Perkow reaction with trimethyl phosphite and 2,4,5-α, α-pentachloroacetophenone, the compound consists of α- and β-isomers in the ratio of 1 to 9, the former being removed by crystallization (Eto, 1974). The technical product, Gardona®, contains 98% β-isomer, in which the chlorine and phosphate groups are cis. Minimum purity was 94%, according to the manufacturer's specification. It was stored at 4°C in the original glass container.

Chemical and physical analyses on the test material were performed at Gulf South Research Institute. Elemental analysis (C, H, Cl, P) was correct for $C_{10}H_9Cl_4O_4P$, the molecular formula of tetrachlorvinphos. Infrared, nuclear magnetic resonance, and mass spectra and thin-layer chromatographic patterns compared well with those of analytical-grade tetrachlorvinphos (99.5%). No attempt was made to identify or quantitate impurities.

After completion of the bioassay, Shell Oil Company also analyzed the batch used for the chronic study and found it to be 98.0%

pure by quantitative infrared analysis, with 0.014% volatiles. Thus, the chemical retained its purity during the bioassay.

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 tetrachlorvinphos 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 chemical. 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 1 week.

The stability of tetrachlorvinphos in feed was tested by determining the concentration of the material in formulated diets at intervals over a 7-day period. Diets containing 8,000 ppm tetrachlorvinphos showed no change on standing at ambient temperature for this period.

As a quality control test on the accuracy of preparation of the diets, the concentration of tetrachlorvinphos 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 samples tested was within 1.5% of the theoretical concentration, and the coefficient of variation was never more than 5.3%. Thus, the evidence indicates that the formulated diets were prepared accurately.

C. Animals

Rats and mice of both sexes, 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 Battelle Memorial Institute, Columbus, Ohio, and the mice were B6C3F1 hybrids obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. On arrival at the laboratory, all animals were quarantined for an acclimation period (rats for 6 days, mice for 12 days) and were then assigned to control and treated 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 in each room was changed 10-12 times per hour. Fluorescent lighting provided illumination 10 hours per day. Food and water were supplied *ad libitum*.

The rats were housed individually in hanging galvanized steel mesh cages, and the mice were housed in plastic cages with filter bonnets, five per cage for females, and two or three per cage for males. Initially, rats were transferred once per week to clean cages; later in the study, cages were changed every 2 weeks. Mice were transferred once per 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 treated mice were placed on separate racks in the same room. Animal racks for both species were rotated laterally once per week; at the same time, each cage was changed to a different position in the row within the same column. Rats receiving tetrachlorvinphos, along with their matched controls, were housed in a room by themselves. Mice receiving tetrachlorvinphos were maintained in a room housing mice administered dieldrin (CAS 60-57-1) or malathion (CAS 121-75-5), together with their respective matched controls.

E. Subchronic Studies

Subchronic studies were conducted to determine the maximum tolerated doses of technical-grade tetrachlorvinphos, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the chronic studies. In these subchronic studies, tetrachlorvinphos was added to the animal feed in twofold increasing concentrations, ranging from 500 to 8,000 ppm for Osborne-Mendel rats and from 2,000 to 32,000 ppm for B6C3F1 mice. Treated and matched-control groups each consisted of five male and five female animals. The chemical was provided in the feed to the treated groups for 6 weeks, followed by observation for 2 weeks. A second study with rats was conducted at dietary concentrations ranging from 4,000 to 32,000 ppm.

In both male and female rats, weight depression was apparent at 8,000 and 16,000 ppm during the first weeks. Later these animals appeared to adapt to the test chemical, and gains in weight of the treated rats approached those of the controls. There were no deaths in the male rats. One female rat receiving 16,000 ppm died. The low and high doses for rats were set at 8,000 and 16,000 ppm for the chronic studies.

In mice, males receiving 8,000 ppm or higher initially lost weight; females receiving 16,000 or 32,000 ppm also lost weight at the beginning of the study. Both males and females generally gained or maintained weight during the remainder of the study. No deaths occurred in either sex at any dose tested. The low and high doses for mice were set at 8,000 and 16,000 ppm for the chronic studies.

F. Designs of Chronic Studies

The designs of the chronic 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 studies on tetrachlorvinphos were combined with matched controls from studies performed on malathion, toxaphene (CAS 8001-35-2), endrin (CAS 72-20-8), and lindane (CAS 58-89-9). The pooled controls for statistical tests using rats consisted of 55 males and 55 females; using mice, 50 males and 50 females. Studies on chemicals other than tetrachlorvinphos were conducted at Gulf South Research Institute and overlapped the tetrachlorvinphos study by at least 1 year. The matched-control groups for the different test chemicals were of the same strain and from the same supplier, and they were examined by the same pathologists.

Table 1. Design of Tetrachlorvinphos Chronic Feeding Studies in Rats

Sex and Treatment Group	Initial No. of Animals[a]	Tetrachlorvinphos in Diet[b] (ppm)	Time on Study Treated (weeks)	Time on Study Untreated[c] (weeks)	Time-Weighted Average Dose[d] (ppm)
Male					
Matched-Control	10	0		111	
Low-Dose	50	8,000	5		
		4,000	75		4,250
		0		31	
High-Dose	50	16,000	5		
		8,000	75		8,500
		0		31	
Female					
Matched-Control	10	0		111	
Low-Dose	50	8,000	5		
		4,000	75		4,250
		0		31	
High-Dose	50	16,000	5		
		8,000	75		8,500
		0		31	

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

[b]Doses were lowered at 5 weeks on study, since it was believed that the pattern of deaths, weight gains, and the general condition of the animals in this and other studies indicated that excessive mortality might occur before the end of the study.

[c]When diets containing tetrachlorvinphos were discontinued, treated animals and matched controls were fed control diets (2% corn oil added).

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

Table 2. Design of Tetrachlorvinphos Chronic Feeding Studies in Mice

Sex and Treatment Group	Initial No. of Animals[a]	Tetrachlorvinphos in Diet (ppm)	Time on Study: Treated (Weeks)	Time on Study: Untreated[b] (Weeks)
Male				
Matched-Control	10	0		90-92
Low-Dose	50	8,000	80	
		0		12
High-Dose	50	16,000	80	
		0		12
Female				
Matched-Control	10	0		90-92
Low-Dose	50	8,000	80	
		0		12
High-Dose	50	16,000	80	
		0		12

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

[b]When diets containing tetrachlorvinphos were discontinued, treated animals and matched controls were fed control diets (2% corn oil added).

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 utilized 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 experiment in each group.

H. Data Recording and Statistical Analyses

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

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and 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 treated animals at each dose level. When results for a number of treated groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) 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 animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

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

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

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

The mean body weights of the rats fed tetrachlorvinphos were lower than those of the matched controls throughout most of the 2-year study (figure 1). The data indicate a dose-related effect on the weights of the males.

The treated male rats were generally comparable to the controls in appearance and behavior during the entire study. However, a majority of low-dose and high-dose females had wet and urine-stained hair coats on their ventral surfaces beginning at week 7; this condition persisted in these groups until termination of the study. At week 28, convulsions were observed in one high-dose female.

During the first half of the second year, a moderate incidence of clinical signs including pale mucous membranes, alopecia, rough and discolored hair coats, dyspnea, hematuria, and vaginal bleeding was observed in both groups. These signs increased during the second half of the year.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats receiving tetrachlorvinphos at

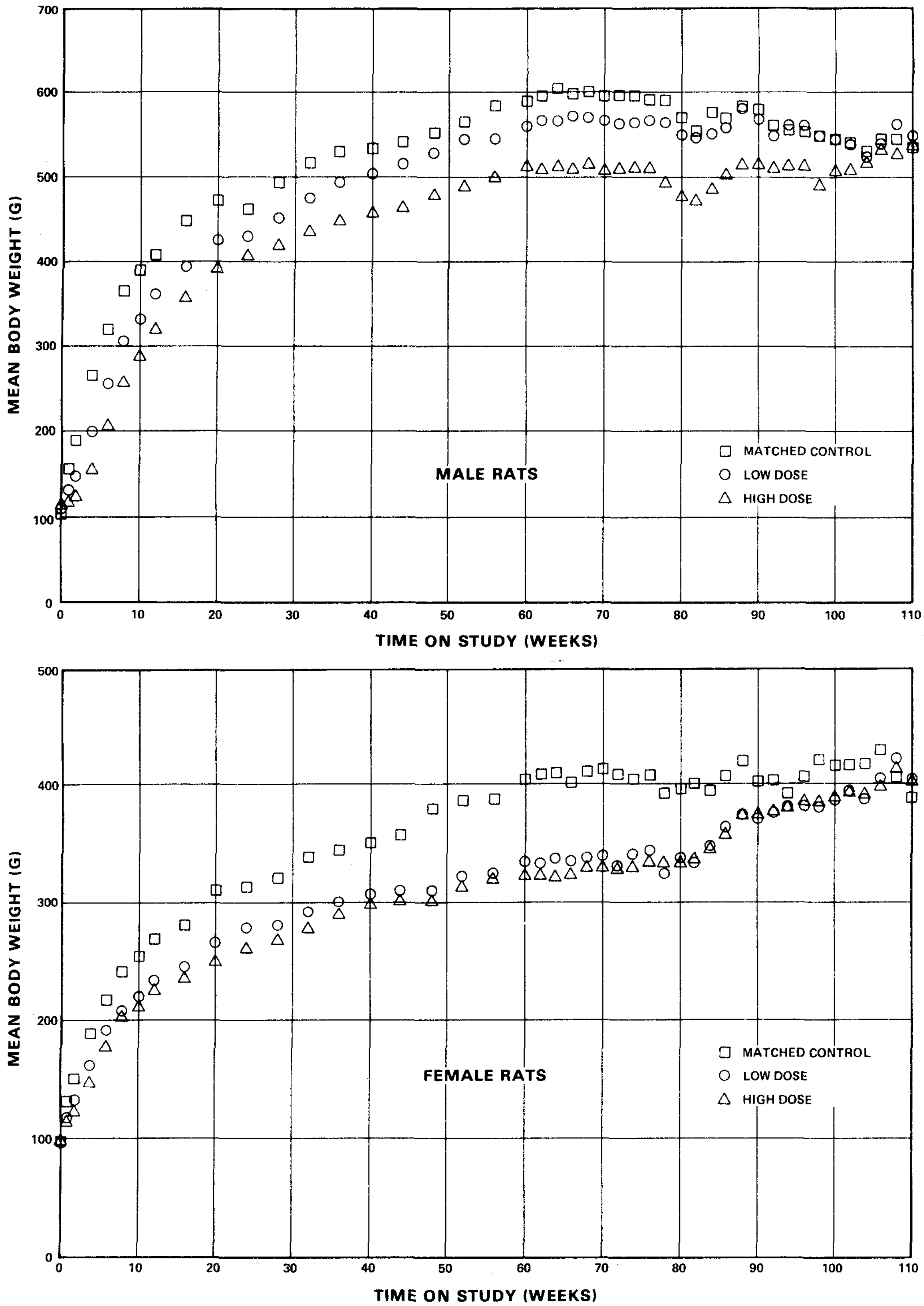

Figure 1. Growth Curves for Rats Fed Tetrachlorvinphos in the Diet

the doses used in this experiment, together with those of the matched controls, are shown in figure 2.

In male rats, the Tarone test for positive dose-related trend in mortality over the period of the study had a probability level of 0.010, and only 48% of the high-dose group survived to the end of the study. Survival in the low-dose and matched-control groups of males was higher than that in the high-dose group, with 72% of the low-dose and 80% of the matched-control groups living to the end of the study, while only 48% of the high-dose group survived. Early deaths in the high-dose males were not associated with tumors.

In female rats, the Tarone test for positive dose-related trend in mortality over the period had a probability level greater than 0.05. Survival in the controls was the lowest among the three groups, with only 40% of the controls living to termination of the study, while 82% of the high-dose and 84% of the low-dose groups lived to the end of the study. A sufficient number of treated animals survived for meaningful statistical analyses of the incidence of tumors.

C. <u>Pathology (Rats)</u>

Histopathologic findings on neoplasms in rats are summarized in

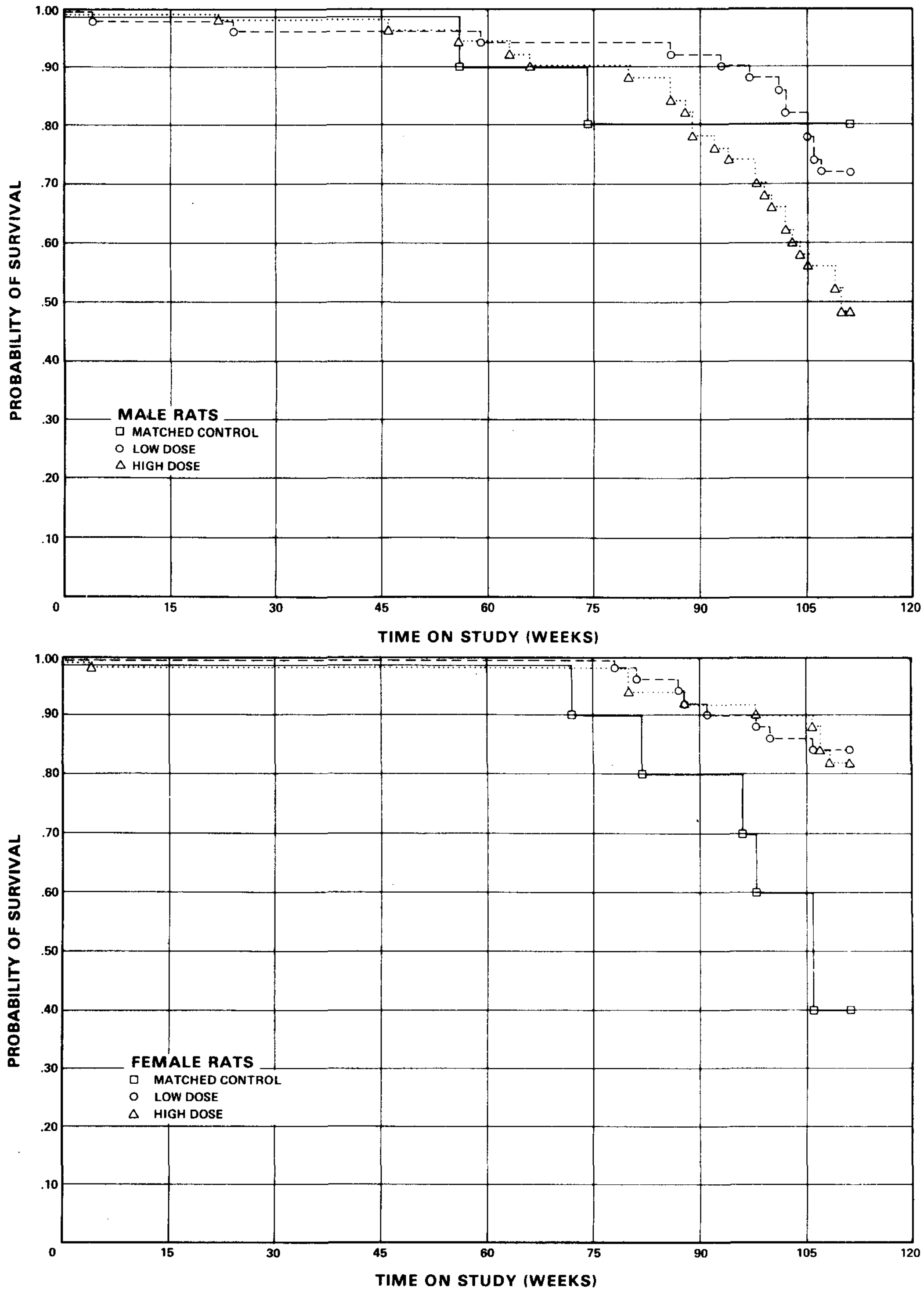

Figure 2. Survival Curves for Rats Fed Tetrachlorvinphos in the Diet

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

There was a spontaneous and random occurrence of a variety of neoplasms in both the control and treated groups. Some types of neoplasms occurred only, or with greater frequency, in rats of treated groups compared with controls. These lesions, however, are not uncommon in this strain of rat independent of any treatment.

In addition to the neoplastic lesions, a large number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the control and treated groups (Appendix C). For the most part, these nonneoplastic lesions were similar to those commonly seen in aged rats; however, more proliferative changes occurred in the thyroid glands of treated animals than in the thyroid glands of the matched controls. The incidences of these lesions were as follows:

	MALES			FEMALES		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Number of Tissues Examined	(10)	(45)	(45)	(9)	(50)	(46)
Thyroid						
C-cell Hyperplasia	0	18	8	0	7	16
C-cell Adenoma	1	2	3	1	2	7
C-cell Carcinoma	0	0	1	0	0	0
Follicular-cell Hyperplasia	1	15	14	1	12	12
Follicular-cell Adenoma	0	1	0	0	0	1
Follicular-cell Carcinoma	0	3	2	0	0	0

The C-cell adenomas in the control and treated rats were generally small proliferative nodular lesions which were composed of well differentiated C cells with much cytoplasm, uniform regular nuclei and few mitotic figures. C-cell hyperplasia was mostly a unilateral change which appeared grossly as a slight enlargement of the affected lobe, with a pale-yellow discoloration. Microscopically, there was a fairly uniform, diffuse increase of parafollicular cells ("C" cells) scattered between thyroid follicles. These cells had pale, finely granular cytoplasm and distinct cytoplasmic membranes. Nuclei were round and open with some basophilic granules and distinct nuclear membranes.

Follicular-cell hyperplasia was bilateral on several occasions and appeared grossly as tiny nodular alterations on the thyroid surface. Microscopically, these lesions were quite variable: multifocal and cystic or having inward papillary projections of variable thickness. Follicular epithelial cells lining the projecting fronds were quite regular in appearance. Degenerative changes were few, if any. Colloid production was not a feature.

The etiology of the proliferative thyroid lesions in these rats is somewhat equivocal. The incidence of C-cell hyperplasia in low-dose males was more than double that in the high-dose males; the reverse was true in the females, where the incidence in the high-dose group was more than twice that in the low-dose group. Nevertheless, there was a rather large number of animals with the lesion in the treated groups and none in the matched controls. The incidence of follicular-cell hyperplasia seems significant in both the treated and control groups, suggesting spontaneous occurrence. In actual proportions, however, the treated rats had as much as a threefold increase in this change over the controls. The increased incidence of both of these hyperplastic thyroid lesions in rats suggests that these changes may be chemical related. There did not, however, appear to be an increased incidence of tumors of either cell type, based on matched controls.

Several adenomas of the adrenal cortex occurred among treated animals. These adrenal adenomas in both male and female rats were composed of well differentiated cells with abundant eosinophilic cytoplasm, and commonly, there was sinusoidal dilatation and hemorrhage in the tumors.

Granulomatous lesions of the liver in rats occurred in 2/50 (4%) low-dose and 14/46 (30%) high-dose males, and in 10/49 (20%) low-dose and 38/49 (78%) high-dose females, but in no matched-control animals of either sex. The micrograulomas seen in the livers of the rats were randomly and sparsely scattered about the parenchyma; they appeared as microscopic foci not exceeding 50 microns in diameter that were made up of a collection of histiocytes and lymphocytes. Gross changes were not evident in these livers. Special stains for microorganisms were used in the livers from a few animals of each sex and group, including controls. These included McManus Periodic Acid Schiff (PAS) and acid-fast stains, and all were negative. The microgranulomatous inflammatory foci in the livers of treated rats seem dose related for both males and females, with a greater incidence in the females than in the males.

The results of this histopathologic study indicate that tetrachlorvinphos is responsible in Osborne-Mendel rats for the induction of granulomatous disease in the liver under the conditions of this study.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 of Appendix E contain the statistical analyses of the incidences of those specific primary tumors that were observed in at least 5% of one or more treated groups of either sex.

In male rats, although the Cochran-Armitage test result for positive dose-related trend in proportions for hemangioma of the spleen is not significant at the 0.05 level, there is a significant departure from linear trend due to the higher incidence in the low-dose group than in the high-dose group. The Fisher exact test shows that the incidence in the low-dose group is significantly higher than that in the pooled controls (P = 0.049). No such tumor was observed in female rats. The results of the test are inconclusive, however, in that the dose association is apparent in only one treated group, and the level of significance is above the Bonferroni criterion of the 0.025 level necessary to establish an error rate of 0.05 throughout the experiment.

In female rats, the Cochran-Armitage test result for positive dose-related trend in proportions of animals for cortical adenoma of the adrenal is significant (P = 0.017), using the pooled controls. In addition, the Fisher exact test shows a significantly higher incidence of this tumor in the high-dose group (P = 0.022) when compared with the pooled controls. The historical record for this bioassay program of this strain of female rats at this laboratory for the incidence of cortical adenoma is 3/240 (1.25%). Using this value as the true parameter of the binomial distribution representing the probability of spontaneous tumors (Fears, 1977), the probability of the occurrence of five or more tumors in the 50 high-dose animals is 0.0004, a significant result. The results of tests on the incidence of this tumor in males are not statistically significant.

In the analyses of C-cell adenoma of the thyroid in female rats, the Cochran-Armitage test for positive dose-related trend has a probability level of 0.013, using the pooled controls. A positive finding is also established by the Fisher exact test, which shows that the incidence in the high-dose group is significantly higher than that in the pooled controls (P = 0.027), implying that the incidence of C-cell adenoma of the thyroid in female rats may be related to treatment. The historical record for this bioassay program of this strain of female rats at this laboratory for the incidence of C-cell adenoma of the thyroid is 8/240 (3.33%). Using this value as the true parameter of the binomial distribution representing the probability of spontaneous tumors, the probability of the occurrence of seven or more tumors in the 46 high-dose animals is 0.0008, a significant result. The statistical conclusion is that an effect has been observed in the high-dose female rats. No C-cell carcinoma was observed in females, and the statistical test results on the combined incidence of C-cell adenoma and carcinoma of the thyroid in males are not statistically significant.

In the analyses of chromophobe adenoma of the pituitary in female rats, except for the probability level of 0.021 shown by the Cochran-Armitage test using the matched controls, no other statistical test results are significant in the positive direction.

Although the high-dose males died early, time-adjusted analyses were not significant, due to the low incidence of tumors in the high-dose males. There are no other incidences of specific tumors that have statistical significance. When tumors at a single site are grouped (as in follicular-cell adenoma and carcinoma of the thyroid in male rats), the incidences of the individual components of the grouping are not included in tables E1 and E2 unless they occur in adequate proportions for meaningful statistical analyses; however, a list of the incidences of each type of tumor is provided in tables A1 and A2 of Appendix A.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

The mean body weights of the mice fed tetrachlorvinphos were lower than those of the matched controls throughout the 2-year study (figure 3). The data show dose-related effects on the weights in both the male and female mice.

During the first year of the study, the treated animals were generally comparable to the controls in appearance and behavior. A few animals had generalized alopecia. At week 60, a majority of the high-dose males and high-dose females had rough hair coats, which persisted until termination of the study. Other clinical signs appeared in both treated and control groups, including alopecia, rough hair coats, hyperactivity, tachypnea, and abdominal distention. One low-dose female was observed to have convulsions periodically during the second year of the study.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice receiving tetrachlorvinphos at the doses used in this experiment, together with those of the matched controls, are shown in figure 4. In both sexes, the

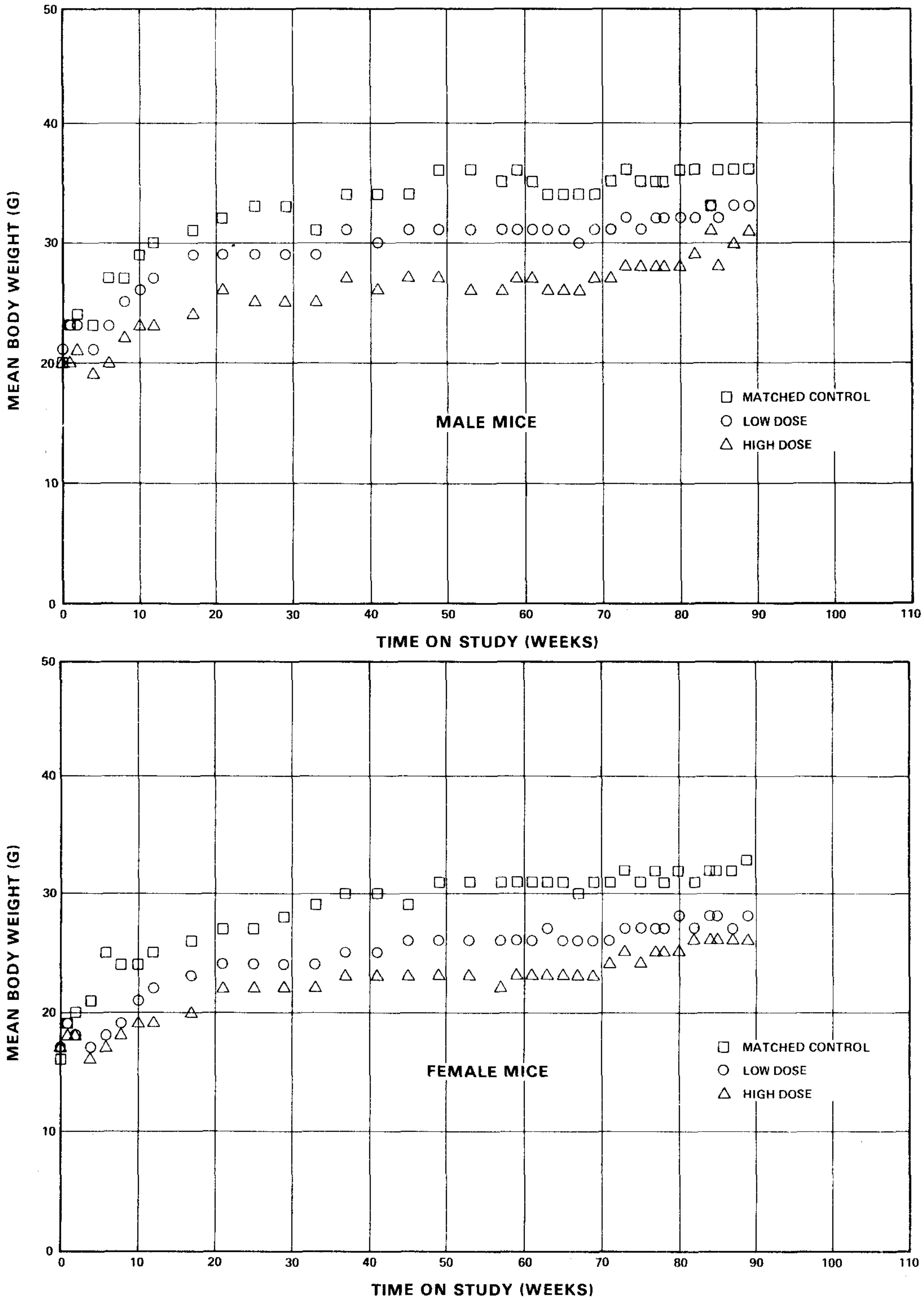

Figure 3. Growth Curves for Mice Fed Tetrachlorvinphos in the Diet

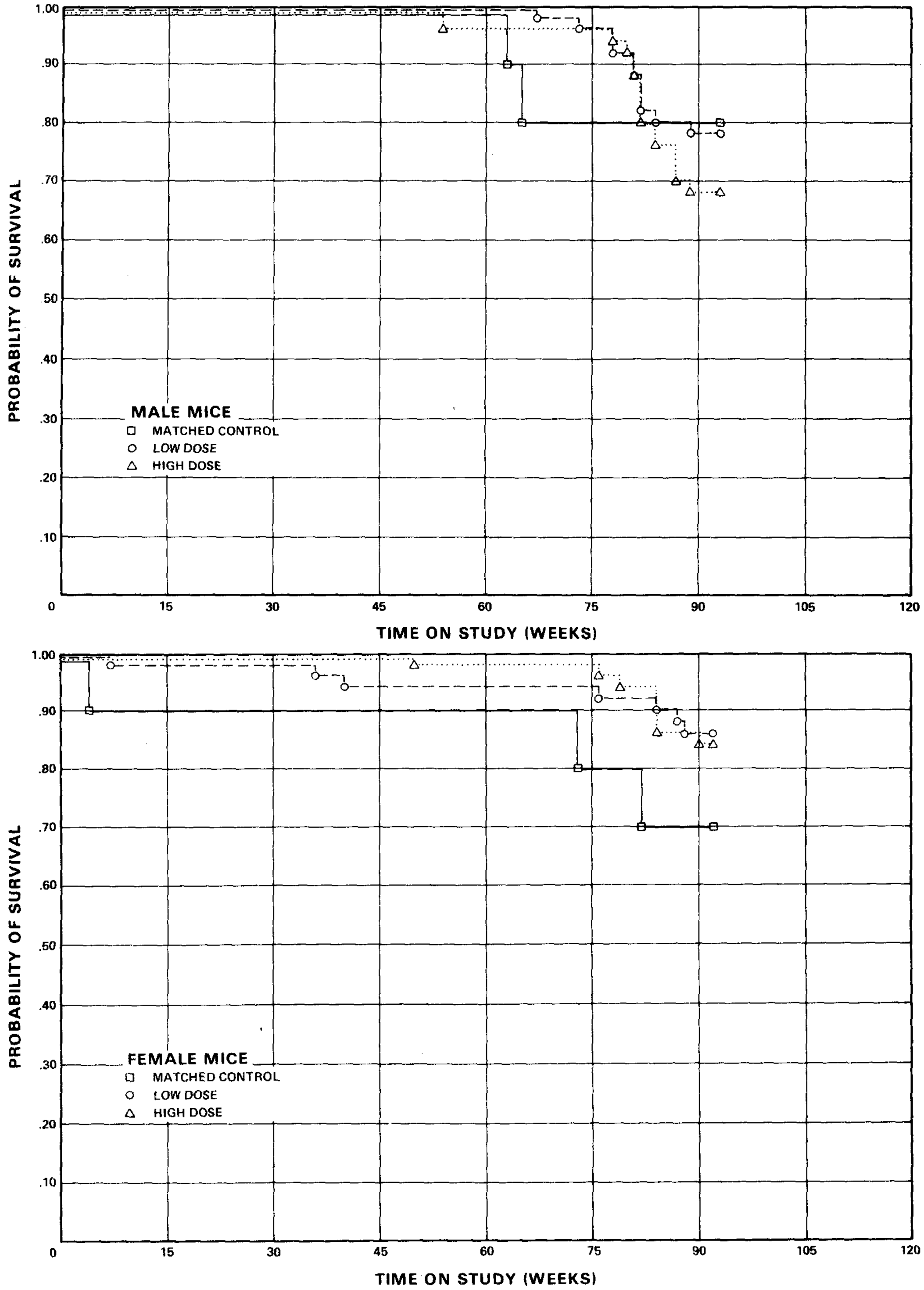

Figure 4. Survival Curves for Mice Fed Tetrachlorvinphos in the Diet

Tarone test results for dose-related trend in mortality over the period are not statistically significant. Eighty percent of the controls, 78% of the low-dose males, and 68% of the high-dose males lived to termination of the study.

In the females, the survival rate was relatively lower in the controls than in the treated groups. Seventy percent of the controls, 86% of the low-dose females, and 84% of the high-dose females lived to the end of the study. Sufficient numbers of animals of both sexes survived to provide meaningful statistical analyses of the incidence of late-developing 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.

With the exception of hepatocellular carcinomas and neoplastic nodules, the neoplasms listed in Appendix B appeared with approximately equal frequency in treated and control mice, or appeared in insignificant numbers. Hepatocellular carcinomas and other pertinent lesions of the liver are listed below:

	MALES			FEMALES		
	Matched Control	Low Dose	High Dose	Matched Control	Low Dose	High Dose
Number of Tissues Examined	(9)	(50)	(50)	(9)	(49)	(47)
Liver						
Hepatocellular carcinoma	0	36	40	0	5	2
Neoplastic nodules	0	11	2	0	14	9
Granulomatous inflammation	0	50	49	0	48	47

The gross appearance of the livers of the treated mice which had hepatocellular carcinoma was markedly altered. Many livers had multinodular growths of small caliber throughout their parenchyma. For the most part, these were tan-red and variegated in pattern with occasional irregular areas of necrosis. The neoplastic nodules were generally single or few in number, seldom larger than 0.5 centimeter in diameter, pale tan, and homogeneous on a cut surface. Granulomatous lesions were often intermingled with the above changes, confusing the gross picture. In cases where granulomatous change existed alone, livers were essentially normal in size, but rubbery in consistency and pale brown. In a few instances, no gross changes were evident.

Microscopically, the hepatocellular carcinomas were mostly cellular, pleomorphic-appearing masses of hepatic-like cells devoid of any architectural arrangement, infiltrating the adjoining parenchyma. The number of liver carcinomas within the treated groups of male mice suggests a chemical-related sex predilection for this group. The neoplastic nodules were commonly expanding, well-delineated lesions of regular-appearing liver cells arranged in thickened trabecular and sheet-like growth patterns. Small bile-duct structures were evident within these nodular growths. The neoplastic nodules seen in the mice were considered to be similar to those seen in rats as described by Squire and Levitt (1975).

Fewer nonneoplastic lesions (Appendix D) occurred in the mice than in the rats, and except for a granulomatous inflammatory reaction, the lesions were of commonly encountered types. This granulomatous inflammatory reaction occurred in the livers of nearly all of the treated mice, but not in the controls.

The granulomatous foci were numerous and randomly distributed throughout the liver parenchyma, with the frequent exception of areas of malignant change. These granulomatous foci were generally 30 to 70 microns in diameter and consisted of aggregations of histiocytes and lymphocytes, with occasional Langhans'-type giant cells. Connective tissue formation was not a histologic feature. The extent of granuloma formation was markedly more severe in the mice than in the rats. Special stains for microorganisms were used on the livers from a few animals of each sex and group, including controls. These included PAS and acid-fast stains, and all were negative.

Widespread microgranuloma formation in the livers of treated mice and the complete absence of this lesion in the control animals implicate tetrachlorvinphos as the inciting cause. The fact that the majority of the mice lived until termination of the study suggests a lesion of slow progression, resulting from extended toxic reaction.

The results of this histopathologic study indicate that tetrachlorvinphos is responsible in B6C3F1 mice for the induction of hepatocellular carcinomas, neoplastic nodules, and granulomatous foci in the liver under the conditions of this study.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those specific primary tumors that were observed in at least 5% of one or more treated groups of either sex.

The Cochran-Armitage test results for positive dose-related trend in proportions of male mice for hepatocellular carcinoma are significant ($P < 0.001$), using either matched controls or pooled controls. Also, there are significant departures from linear trend, with a probability level of 0.003 using the matched controls, and a probability level of 0.002 using the pooled controls. These departures from linearity are due to the steep increases in incidences in the treated groups. The Fisher exact test results for the comparison of the proportions between treated and control groups are also significant ($P < 0.001$). All of these statistical tests imply a carcinogenic effect of tetrachlorvinphos on the liver in male mice at the doses used in this experiment. The incidence of neoplastic nodule of the liver in low-dose male mice shows a significant result ($P = 0.024$) by the Fisher exact test when compared with the pooled controls; however, neither the Cochran-Armitage test result nor the Fisher exact comparison of the incidence in the high-dose group with that in the controls is significant. When the occurrences of neoplastic nodule and hepatocellular carcinoma are grouped, the statistical tests show significant results; all of the tests have probability levels of less than or equal to 0.001. The incidence of hepatocellular carcinoma, rather than neoplastic nodule, is primarily responsible for the significance of these grouped results in the male mice.

In female mice the reverse is true; neoplastic nodule occurs in significant proportion, but not hepatocellular carcinoma. The Cochran-Armitage test for a positive dose-related trend in the proportions of neoplastic nodule of the liver has a probability level of 0.018 using the pooled controls. There is an indicated departure from linear trend (P = 0.047 using the matched controls, P = 0.006 using the pooled controls), due to the higher incidence in the low-dose group than in the high-dose group. Moreover, the Fisher exact test shows significantly higher incidences in the low-dose (P < 0.001) and the high-dose (P = 0.007) groups when compared with the pooled controls. The statistical conclusion is that neoplastic nodule of the liver in female mice is associated with tetrachlorvinphos at the doses used in this experiment.

When the liver tumors (neoplastic nodule and hepatocellular carcinoma) in female mice are grouped, the Cochran-Armitage test has a probability level of 0.030 using the pooled controls, with indicated departures from linearity (P = 0.010 using the matched controls, P = 0.002 using the pooled controls), due to the higher proportion in the low-dose group than in the high-dose group. The Fisher exact test shows that the incidence in the low-dose group is significantly higher than that in either the matched controls (P = 0.020) or the pooled controls (P < 0.001), and the incidence in the high-dose group is significantly higher than that in the pooled controls (P = 0.019). The significance of this grouped incidence is accounted for by the incidence of neoplastic nodule, and not by that of hepatocellular carcinoma. There are no other specific incidences of tumors in mice of either sex for which the statistical test results are significant in the positive direction.

V. DISCUSSION

Tetrachlorvinphos is a member of the organophosphorus group of pesticides that function as neurotoxins by inhibiting cholinesterase (Eto, 1974). The neurotoxicity of tetrachlorvinphos, however, is low in mammals, due to its low solubility in water and in organic solvents, with consequent slow penetration to target areas (Whetstone et al., 1966). In the present bioassay, only one high-dose female rat and one low-dose female mouse showed neurotoxic manifestations.

Tetrachlorvinphos is readily detoxified in mammals by metabolic processes involving hydrolysis, reduction, oxidation, and glucuronide formation to yield a variety of products that are excreted mainly in the urine (Akintowa and Hutson, 1967). No reports are available on chronic studies of tetrachlorvinphos.

The toxicity of tetrachlorvinphos in the present study was manifested by lower body weights in the treated rats and mice than in the matched controls, and by granulomatous lesions of the liver in both rats and mice. Mortality rates showed a dose-related trend in the male rats, but not in the females; survival in the matched-control females was abnormally low. In mice, dose-related trends in mortality were not seen in either males or females. Except for the matched-control group of female rats, the survival of all groups of rats and mice was adequate for meaningful statistical analyses of the incidence of tumors.

In rats, the pathologist associated the presence of granulomatous lesions of the liver in both sexes with treatment by tetrachlorvinphos, and special stains showed that there were no microorganisms associated with these lesions. However, significant numbers of hepatic neoplasms in treated animals were not observed. The incidences of C-cell adenoma of the thyroid showed a significant dose-related trend in the females using pooled controls (controls 1/46, low-dose 2/50, high-dose 7/46, P = 0.013), and by direct comparison, an increased incidence in the high-dose group (P = 0.027). Additionally, hyperplasia of the C cells was observed in 7/50 low-dose and 16/46 high-dose female rats, but in no matched-control females and in only one pooled-control female. This further indicated a chemical-related effect on proliferative lesions of the thyroid. In females, there was also a significant dose-related trend in the incidence of adrenal cortical adenoma using pooled controls (controls 0/50, low-dose 2/49, high-dose 5/50, P = 0.017), and by direct comparison, an increased incidence in the high-dose group (P = 0.022). The incidence of this adenoma in the treated groups was also higher than among laboratory historical-control females (3/240).

Hemangioma of the spleen occurred at a significantly higher incidence in the low-dose males than in the corresponding pooled controls, but the association of this tumor with treatment is questionable, since there were only four tumors in the low-dose group and none in the high-dose group, and the test result for dose-related trend was not significant. No other tumor in rats showed a statistically significant incidence.

In mice, hepatocellular carcinoma in males showed a highly significant dose-related trend, using either matched or pooled controls (matched controls 0/9, pooled controls 5/49, low-dose 36/50, high-dose 40/50, $P < 0.001$). Direct comparisons of low- and high-dose groups of males with matched- or pooled-control groups showed highly significant increases in the incidences of the tumor in the treated groups in every case. In female mice, the incidence of hepatocellular carcinoma by itself was not significant. However, the incidence of neoplastic nodule alone (pooled controls 1/48, low-dose 14/49, high-dose 9/47) and in combination with that of hepatocellular carcinoma (pooled controls 3/48, low-dose 19/49, high-dose 11/47) showed significant dose-related trends and also significantly increased rates in low- and high-dose groups using pooled controls. The direct comparison of the combined incidence in the low-dose group was the only comparison with matched controls in females that was significant. There was a significant departure from linear trend for neoplastic nodule or for combined nodule and carcinoma, since greater numbers were observed in the low-dose than in the high-dose groups. In addition, granulomatous lesions of the liver were observed in all but two of the treated mice, but in none of the matched- or pooled-control animals. Special stains showed that there were no microorganisms associated with these lesions.

It is concluded that under the conditions of this bioassay, administration of technical-grade tetrachlorvinphos in Osborne-Mendel rats was associated with proliferative lesions of the C cells of the thyroid and cortical adenomas of the adrenal in females. In female B6C3F1 mice, the incidence of neoplastic nodule of the liver was associated with treatment, and in male mice tetrachlorvinphos was carcinogenic, causing hepatocellular carcinoma of the liver.

VI. BIBLIOGRAPHY

Akintowa, D. A. A. and Hutson, D. H., Metabolism of 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate in dog and rat. J. Agric. Food Chem. 15:632, 1967.

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 of Carcinogenicity of the Cancer Research Commission of 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.

Environmental Protection Agency, EPA Compendium of Registered Pesticides, U. S. Government Printing Office, Washington, D.C., 1973, III-C-29.1-III-C-29.13.

Eto, M., Organophosphorus Pesticides: Organic and Biological Chemistry, CRC Press, Cleveland, Ohio, 1974.

Fears, T. R., Tarone, R. E., and Chu, K. C., False-positive and false-negative rates for carcinogenicity screens. Cancer Res. 37:1941-1945, 1977.

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

Kaplan, E. L. and Meier, P., Nonparametric estimation from incomplete observations. J. Am. 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.

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.

Squire, R. A. and Levitt, M. H., Report on a workshop on classification of specific hepatocellular lesions in rats, Cancer Res. 35:3214-3223, 1975.

Tarone, R. E., Tests for trend in life table analysis. Biometrika 62(3):679-682, 1975.

Whetstone, R. R., Phillips, D. D., Sun, Y. P., Ward, L. F., Jr., and Shellenberger, T. E., 2-Chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate, a new insecticide with low toxicity to mammals. J. Agric. Food Chem. 14:352, 1966.

APPENDIX A

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

TABLE A1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	50	48
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(50)	(48)
FIBROUS HISTIOCYTOMA		1 (2%)	
*SUBCUT TISSUE	(10)	(50)	(48)
MYXOMA		1 (2%)	
HAMARTOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(10)	(50)	(46)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(50)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#SPLEEN	(10)	(48)	(47)
HEMANGIOMA		4 (8%)	
ANGIOMA		1 (2%)	
HAMARTOMA		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(10)	(47)	(45)
ADENOCARCINOMA, NOS		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
#LIVER	(10)	(50)	(47)
NEOPLASTIC NODULE		1 (2%)	
HEPATOCELLULAR CARCINOMA		1 (2%)	
*BILE DUCT	(10)	(50)	(48)
HAMARTOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY	(10)	(49)	(47)
TUBULAR-CELL ADENOCARCINOMA		1 (2%)	
LIPOSARCOMA			1 (2%)
HAMARTOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(43)	(37)
CHROMOPHOBE ADENOMA	4 (44%)	5 (12%)	
ACIDOPHIL ADENOMA			1 (3%)
#ADRENAL	(9)	(48)	(45)
CORTICAL ADENOMA		3 (6%)	1 (2%)
PHEOCHROMOCYTOMA	1 (11%)		
#THYROID	(10)	(45)	(45)
FOLLICULAR-CELL ADENOMA		1 (2%)	
FOLLICULAR-CELL CARCINOMA		3 (7%)	2 (4%)
C-CELL ADENOMA	1 (10%)	2 (4%)	3 (7%)
C-CELL CARCINOMA			1 (2%)
#PANCREATIC ISLETS	(10)	(47)	(46)
ISLET-CELL ADENOMA		2 (4%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(48)
CARCINOMA,NOS	1 (10%)		
LIPOMA			1 (2%)
#TESTIS	(10)	(49)	(46)
INTERSTITIAL-CELL TUMOR		1 (2%)	
*EPIDIDYMIS	(10)	(50)	(48)
LIPOMA		1 (2%)	

\# 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)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
*CRANIAL NERVE	(10)	(50)	(48)
HAMARTOMA			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(10)	(50)	(48)
MESOTHELIOMA, NOS		1 (2%)	
*TUNICA VAGINALIS	(10)	(50)	(48)
MESOTHELIOMA, NOS		1 (2%)	2 (4%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@	1	8	7
MORIBUND SACRIFICE	2	6	21
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	7	36	22
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	5	23	15
TOTAL PRIMARY TUMORS	7	33	16
TOTAL ANIMALS WITH BENIGN TUMORS	5	19	9
TOTAL BENIGN TUMORS	6	24	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	6	5
TOTAL MALIGNANT TUMORS	1	6	5
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		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

TABLE A2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	9	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(9)	(50)	(50)
LIPOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(9)	(50)	(49)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (11%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(9)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
LYMPHOCYTIC LEUKEMIA			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(8)	(49)	(50)
NEOPLASTIC NODULE		2 (4%)	
*BILE DUCT	(9)	(50)	(50)
BILE DUCT CARCINOMA			1 (2%)
HAMARTOMA		2 (4%)	2 (4%)
URINARY SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(44)	(45)
CARCINOMA,NOS			2 (4%)
ADENOMA, NOS		2 (5%)	
CHROMOPHOBE ADENOMA		1 (2%)	7 (16%)
#ADRENAL	(9)	(49)	(50)
CARCINOMA,NOS		2 (4%)	
CORTICAL ADENOMA		2 (4%)	5 (10%)
#THYROID	(9)	(50)	(46)
FOLLICULAR-CELL ADENOMA			1 (2%)
C-CELL ADENOMA	1 (11%)	2 (4%)	7 (15%)
#PANCREATIC ISLETS	(9)	(48)	(49)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(9)	(50)	(50)
FIBROMA	1 (11%)	1 (2%)	1 (2%)
FIBROADENOMA	3 (33%)	4 (8%)	3 (6%)
#UTERUS	(9)	(47)	(47)
ENDOMETRIAL STROMAL POLYP	2 (22%)	2 (4%)	1 (2%)
#OVARY	(8)	(49)	(48)
PAPILLARY ADENOMA			1 (2%)
PAPILLARY CYSTADENOMA, NOS			1 (2%)
GRANULOSA-CELL TUMOR		1 (2%)	2 (4%)
NERVOUS SYSTEM			
#BRAIN	(9)	(48)	(49)
GRANULAR-CELL TUMOR, BENIGN			1 (2%)
SPECIAL SENSE ORGANS			
*EAR CANAL	(9)	(50)	(50)
LEIOMYOMA	1 (11%)		
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(9)	(50)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT			1 (2%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@	1	2	1
MORIBUND SACRIFICE	6	7	10
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	3	41	39
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	20	24
TOTAL PRIMARY TUMORS	9	23	38
TOTAL ANIMALS WITH BENIGN TUMORS	7	16	23
TOTAL BENIGN TUMORS	8	17	31
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	3	5
TOTAL MALIGNANT TUMORS	1	3	5
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
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 TETRACHLORVINPHOS IN THE DIET

TABLE B1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	9	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(8)	(49)	(50)
CARCINOMA,NOS	1 (13%)		
#LUNG	(8)	(49)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		3 (6%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(9)	(50)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
GRANULOCYTIC LEUKEMIA	1 (11%)		
#LYMPH NODE	(8)	(43)	(38)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(9)	(50)	(50)
NEOPLASTIC NODULE		11 (22%)	2 (4%)
HEPATOCELLULAR CARCINOMA		36 (72%)	40 (80%)
HEMANGIOMA		1 (2%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(9)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	1 (2%)
TUBULAR-CELL ADENOCARCINOMA			1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL	(8)	(47)	(50)
CORTICAL CARCINOMA		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	10	50	50
NATURAL DEATH@	2		1
MORIBUND SACRIFICE		11	15
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	8	39	34
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	47	42
TOTAL PRIMARY TUMORS	2	56	45
TOTAL ANIMALS WITH BENIGN TUMORS		2	2
TOTAL BENIGN TUMORS		2	2
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	38	40
TOTAL MALIGNANT TUMORS	2	43	41
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT		11	2
TOTAL UNCERTAIN TUMORS		11	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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED TETRACHLORVINPHOS IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	@10	50	&50
ANIMALS NECROPSIED	9	49	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	49	47
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(9)	(49)	(47)
UNDIFFERENTIATED CARCINOMA			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA		4 (8%)	5 (11%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(9)	(49)	(47)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#MESENTERIC L. NODE	(9)	(39)	(42)
FIBROUS HISTIOCYTOMA			1 (2%)
#LIVER	(9)	(49)	(47)
MALIGNANT LYMPHOMA, NOS	1 (11%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(9)	(49)	(47)
NEOPLASTIC NODULE		14 (29%)	9 (19%)
HEPATOCELLULAR CARCINOMA		5 (10%)	2 (4%)
HEMANGIOMA		2 (4%)	

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

@ 10 ANIMALS WERE INITIALLY IN STUDY BUT ONE WAS DELETED WHEN FOUND TO BE A MALE ANIMAL IN A FEMALE GROUP.
& 50 ANIMALS WERE INITIALLY IN STUDY BUT ONE WAS DELETED WHEN FOUND TO BE A MALE ANIMAL IN A FEMALE GROUP.

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(9)	(49)	(46)
TUBULAR-CELL ADENOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(5)	(45)	(39)
CHROMOPHOBE ADENOMA		1 (2%)	
#THYROID	(9)	(46)	(41)
FOLLICULAR-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(9)	(49)	(47)
ADENOCARCINOMA, NOS		1 (2%)	
#UTERUS	(8)	(47)	(39)
ADENOCARCINOMA, NOS			1 (3%)
LEIOMYOMA			1 (3%)
#OVARY	(8)	(47)	(44)
GRANULOSA-CELL TUMOR			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@		2	2
MORIBUND SACRIFICE	2	4	6
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	7	44	41
ANIMAL MISSING			
ANIMAL DELETED/WRONG SEX	1		1

@ INCLUDES AUTOLYZED ANIMALS

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	1	25	19
TOTAL PRIMARY TUMORS	1	30	22
TOTAL ANIMALS WITH BENIGN TUMORS		8	6
TOTAL BENIGN TUMORS		8	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	8	4
TOTAL MALIGNANT TUMORS	1	8	4
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		14	10
TOTAL UNCERTAIN TUMORS		14	10
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 TETRACHLORVINPHOS IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED TETRACHLORVINPHOS

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	50	48
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(50)	(48)
GRANULOMA, NOS		1 (2%)	
GRANULATION, TISSUE		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(10)	(50)	(46)
CONGESTION, NOS		1 (2%)	
EDEMA, NOS			1 (2%)
CALCIFICATION, METASTATIC		2 (4%)	
#LUNG/ALVEOLI	(10)	(50)	(46)
EMPHYSEMA, NOS			1 (2%)
CALCIFICATION, METASTATIC		1 (2%)	2 (4%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(10)	(49)	(46)
HYPERPLASIA, NOS		1 (2%)	
#SPLEEN	(10)	(48)	(47)
FIBROSIS, FOCAL		1 (2%)	
HEMOSIDEROSIS		1 (2%)	
#MEDIASTINAL L.NODE	(9)	(42)	(41)
INFLAMMATION, CHRONIC		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(10)	(49)	(47)
THROMBUS, ORGANIZED		1 (2%)	
FIBROSIS, FOCAL			1 (2%)
CALCIFICATION, METASTATIC		3 (6%)	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
#MYOCARDIUM	(10)	(49)	(47)
FIBROSIS		1 (2%)	
FIBROSIS, FOCAL		8 (16%)	6 (13%)
FIBROSIS, DIFFUSE			1 (2%)
CALCIFICATION, METASTATIC			2 (4%)
#ENDOCARDIUM	(10)	(49)	(47)
FIBROSIS		1 (2%)	
FIBROSIS, FOCAL		1 (2%)	
*AORTA	(10)	(50)	(48)
MEDIAL CALCIFICATION		2 (4%)	9 (19%)
*CORONARY ARTERY	(10)	(50)	(48)
MEDIAL CALCIFICATION		1 (2%)	3 (6%)
*LINGUAL ARTERY	(10)	(50)	(48)
MEDIAL CALCIFICATION		1 (2%)	1 (2%)
*SPLENIC ARTERY	(10)	(50)	(48)
FIBROSIS, FOCAL		1 (2%)	
MEDIAL CALCIFICATION		1 (2%)	2 (4%)
*MESENTERIC ARTERY	(10)	(50)	(48)
MEDIAL CALCIFICATION		2 (4%)	3 (6%)
DIGESTIVE SYSTEM			
#LIVER	(10)	(50)	(47)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
INFLAMMATION, GRANULOMATOUS		2 (4%)	17 (36%)
GRANULOMA, NOS		1 (2%)	
FIBROSIS, FOCAL		1 (2%)	
CIRRHOSIS, NOS		1 (2%)	
NECROSIS, FOCAL		1 (2%)	
METAMORPHOSIS FATTY		4 (8%)	15 (32%)
CALCIFICATION, NOS		1 (2%)	
HEMOSIDEROSIS		1 (2%)	
FOCAL CELLULAR CHANGE		2 (4%)	1 (2%)
*BILE DUCT	(10)	(50)	(48)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
HYPERPLASIA, FOCAL		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
#PANCREAS	(10)	(47)	(46)
PERIARTERITIS		2 (4%)	2 (4%)
ATROPHY, NOS			1 (2%)
#PANCREATIC ACINUS	(10)	(47)	(46)
ATROPHY, NOS		3 (6%)	
ATROPHY, FOCAL		1 (2%)	
#STOMACH	(10)	(49)	(43)
CALCIFICATION, METASTATIC		2 (4%)	1 (2%)
#GASTRIC MUCOSA	(10)	(49)	(43)
ULCER, NOS		1 (2%)	
EROSION		2 (4%)	
NECROSIS, FOCAL	1 (10%)		
CALCIFICATION, METASTATIC		1 (2%)	7 (16%)
#GASTRIC SUBMUCOSA	(10)	(49)	(43)
EDEMA, NOS			1 (2%)
#CECUM	(7)	(40)	(24)
INFLAMMATION, ACUTE NECROTIZING			1 (4%)
URINARY SYSTEM			
#KIDNEY	(10)	(49)	(47)
THROMBOSIS, NOS			1 (2%)
INFLAMMATION, CHRONIC	5 (50%)	32 (65%)	34 (72%)
CALCIFICATION, METASTATIC		1 (2%)	1 (2%)
HYPERPLASIA, FOCAL		2 (4%)	
METAPLASIA, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(43)	(37)
CYST, NOS		3 (7%)	
MULTIPLE CYSTS	1 (11%)		
CONGESTION, NOS		2 (5%)	1 (3%)
HEMORRHAGE		5 (12%)	
DEGENERATION, CYSTIC		1 (2%)	
HYPERPLASIA, FOCAL		3 (7%)	1 (3%)
ANGIECTASIS	1 (11%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#ADRENAL	(9)	(48)	(45)
HEMORRHAGE		5 (10%)	2 (4%)
DEGENERATION, CYSTIC		5 (10%)	2 (4%)
CALCIFICATION, METASTATIC		1 (2%)	
CYTOLOGIC DEGENERATION		1 (2%)	
#ADRENAL CORTEX	(9)	(48)	(45)
HEMORRHAGE		2 (4%)	
DEGENERATION, NOS			1 (2%)
DEGENERATION, CYSTIC		1 (2%)	1 (2%)
METAMORPHOSIS FATTY		3 (6%)	1 (2%)
HYPERPLASIA, FOCAL		4 (8%)	4 (9%)
#ADRENAL MEDULLA	(9)	(48)	(45)
HYPERPLASIA, FOCAL			1 (2%)
#THYROID	(10)	(45)	(45)
CYSTIC FOLLICLES		6 (13%)	
ATROPHY, NOS		1 (2%)	
HYPERPLASIA, C-CELL		18 (40%)	8 (18%)
HYPERPLASIA, FOLLICULAR-CELL	1 (10%)	15 (33%)	14 (31%)
#THYROID FOLLICLE	(10)	(45)	(45)
ATROPHY, NOS		1 (2%)	
#PARATHYROID	(5)	(26)	(33)
HYPERPLASIA, NOS		2 (8%)	7 (21%)
HYPERPLASIA, SECONDARY			1 (3%)
HYPERPLASIA, DIFFUSE		2 (8%)	4 (12%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(48)
NECROSIS, FAT			1 (2%)
#PROSTATE	(10)	(46)	(46)
DILATATION, NOS		1 (2%)	
OBSTRUCTION, NOS		1 (2%)	
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL			1 (2%)
#TESTIS	(10)	(49)	(46)
PERIARTERITIS			2 (4%)
PERIVASCULITIS		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
DEGENERATION, NOS		5 (10%)	
ATROPHY, NOS	1 (10%)	15 (31%)	20 (43%)
ATROPHY, FOCAL		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(10)	(50)	(47)
HYDROCEPHALUS, NOS		1 (2%)	1 (2%)
#OLFACTORY BULB	(10)	(50)	(47)
GLIOSIS			1 (2%)
SPECIAL SENSE ORGANS			
*EYE/CONJUNCTIVA	(10)	(50)	(48)
INFLAMMATION, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
*FEMUR	(10)	(50)	(48)
OSTEOPOROSIS			4 (8%)
FIBROUS OSTEODYSTROPHY		2 (4%)	1 (2%)
BODY CAVITIES			
*MESENTERY	(10)	(50)	(48)
PERIARTERITIS		1 (2%)	5 (10%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1		2
AUTO/NECROPSY/HISTO PERF		1	1
AUTOLYSIS/NO NECROPSY			2

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

TABLE C2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	9	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(9)	(50)	(49)
BRONCHOPNEUMONIA NECROTIZING		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(8)	(49)	(48)
INFARCT, FOCAL			1 (2%)
HEMOSIDEROSIS	1 (13%)		1 (2%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(9)	(50)	(50)
FIBROSIS, FOCAL			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(8)	(49)	(50)
HEMORRHAGE		1 (2%)	
INFLAMMATION, GRANULOMATOUS		10 (20%)	38 (76%)
DEGENERATION, CYSTIC		1 (2%)	
METAMORPHOSIS FATTY	1 (13%)		2 (4%)
FOCAL CELLULAR CHANGE		2 (4%)	
ANGIECTASIS	3 (38%)		
*BILE DUCT	(9)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL		2 (4%)	
HYPERPLASIA, FOCAL			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
#PANCREAS	(9)	(48)	(49)
HYPERPLASTIC NODULE			1 (2%)
#ESOPHAGUS		(6)	(9)
MEGAESOPHAGUS		1 (17%)	
#STOMACH	(9)	(48)	(46)
ULCER, ACUTE		1 (2%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(9)	(50)	(50)
INFLAMMATION, CHRONIC		6 (12%)	11 (22%)
METAPLASIA, NOS		1 (2%)	1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(44)	(45)
CYST, NOS		1 (2%)	
CONGESTION, NOS		2 (5%)	2 (4%)
HEMORRHAGE		1 (2%)	
HYPERPLASIA, NOS		2 (5%)	
HYPERPLASIA, FOCAL		1 (2%)	1 (2%)
#ADRENAL	(9)	(49)	(50)
HEMORRHAGE		5 (10%)	5 (10%)
DEGENERATION, CYSTIC		2 (4%)	5 (10%)
#ADRENAL CORTEX	(9)	(49)	(50)
NODULE			1 (2%)
DEGENERATION, CYSTIC		10 (20%)	2 (4%)
METAMORPHOSIS FATTY			1 (2%)
HYPERPLASIA, FOCAL		4 (8%)	2 (4%)
#THYROID	(9)	(50)	(46)
CYSTIC FOLLICLES			1 (2%)
ATROPHY, NOS			1 (2%)
HYPERPLASIA, C-CELL		7 (14%)	16 (35%)
HYPERPLASIA, FOLLICULAR-CELL	1 (11%)	12 (24%)	12 (26%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(9)	(50)	(50)
DEGENERATION, CYSTIC		1 (2%)	
HYPERPLASIA, NOS	1 (11%)	8 (16%)	4 (8%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*OVARY	(8)	(49)	(48)
FOLLICULAR CYST, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	4	3
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 TETRACHLORVINPHOS IN THE DIET

TABLE D1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	9	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(8)	(49)	(50)
INFLAMMATION, CHRONIC			1 (2%)
#LUNG	(8)	(49)	(50)
ATELECTASIS			1 (2%)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (13%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(8)	(49)	(50)
INFLAMMATION, CHRONIC		2 (4%)	1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	
#MESENTERIC L. NODE	(8)	(43)	(38)
INFLAMMATION, NOS			1 (3%)
NECROSIS, FOCAL			1 (3%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(8)	(49)	(50)
INFLAMMATION, INTERSTITIAL		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(9)	(50)	(50)
INFLAMMATION, GRANULOMATOUS		50 (100%)	49 (98%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
FOCAL CELLULAR CHANGE			1 (2%)
*BILE DUCT	(9)	(50)	(50)
DILATATION, NOS		1 (2%)	
#PANCREATIC DUCT	(8)	(48)	(50)
DILATATION, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(9)	(50)	(50)
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
HYPERPLASIA, TUBULAR CELL			1 (2%)
METAPLASIA, OSSEOUS			1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(8)	(47)	(47)
HYPERPLASIA, FOLLICULAR-CELL		2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
*COAGULATING GLAND	(9)	(50)	(50)
RETENTION FLUID			1 (2%)
*VAS DEFERENS	(9)	(50)	(50)
DILATATION, NOS			1 (2%)
NERVOUS SYSTEM			
*CHOROID PLEXUS	(9)	(50)	(50)
METAPLASIA, SQUAMOUS		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*FEMUR	(9)	(50)	(50)
GRANULOMA, NOS			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
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5		
AUTO/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 FED TETRACHLORVINPHOS IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	@10	50	&50
ANIMALS NECROPSIED	9	49	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	49	47
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(9)	(49)	(47)
INFLAMMATION, FOCAL		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(9)	(46)	(47)
ABSCESS, NOS			1 (2%)
INFLAMMATION, CHRONIC		2 (4%)	
HYPERPLASIA, LYMPHOID		5 (11%)	3 (6%)
#LYMPH NODE	(9)	(39)	(42)
INFLAMMATION, CHRONIC		1 (3%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(9)	(48)	(47)
INFLAMMATION, INTERSTITIAL			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(9)	(49)	(47)
INFLAMMATION, GRANULOMATOUS		48 (98%)	47 (100%)
ANGIECTASIS		1 (2%)	
*BILE DUCT	(9)	(49)	(47)
DILATATION, NOS		1 (2%)	1 (2%)

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

@ 10 ANIMALS WERE INITIALLY IN STUDY BUT ONE WAS DELETED WHEN FOUND TO BE A MALE ANIMAL IN A FEMALE GROUP.

& 50 ANIMALS WERE INITIALLY IN STUDY BUT ONE WAS DELETED WHEN FOUND TO BE A MALE ANIMAL IN A FEMALE GROUP.

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#PANCREAS	(9)	(45)	(47)
NECROSIS, FAT			1 (2%)
#PANCREATIC DUCT	(9)	(45)	(47)
DILATATION, NOS		3 (7%)	
#PANCREATIC ACINUS	(9)	(45)	(47)
ATROPHY, NOS		3 (7%)	
URINARY SYSTEM			
#KIDNEY	(9)	(49)	(46)
HYDRONEPHROSIS			5 (11%)
INFLAMMATION, CHRONIC			1 (2%)
#KIDNEY/CORTEX	(9)	(49)	(46)
ATROPHY, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(5)	(45)	(39)
HYPERPLASIA, NOS		1 (2%)	
ANGIECTASIS		2 (4%)	
#THYROID	(9)	(46)	(41)
HYPERPLASIA, C-CELL		1 (2%)	
HYPERPLASIA, FOLLICULAR-CELL		2 (4%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(8)	(47)	(39)
HYDROMETRA			1 (3%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#UTERUS/ENDOMETRIUM	(8)	(47)	(39)
HYPERPLASIA, FOCAL			1 (3%)
HYPERPLASIA, CYSTIC	3 (38%)	2 (4%)	
#OVARY	(8)	(47)	(44)
DISTENTION			1 (2%)
FOLLICULAR CYST, NOS	1 (13%)		1 (2%)
INFLAMMATION, NOS		1 (2%)	7 (16%)

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, SUPPURATIVE	1 (13%)		2 (5%)
INFLAMMATION, CHRONIC			2 (5%)
ATROPHY, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4		
AUTO/NECROPSY/HISTO PERF		1	
AUTOLYSIS/NO NECROPSY		1	2

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

APPENDIX E

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

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

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Thyroid: Follicular-cell Carcinoma[b]	0/10 (0)	1/46 (2)	3/45 (7)	2/45 (4)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.149	0.072
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			3.067	2.044
Lower Limit			0.260	0.111
Upper Limit			155.684	115.984
Weeks to First Observed Tumor	--	--	111	111
Thyroid: Follicular-cell Adenoma or Carcinoma[b]	0/10 (0)	4/46 (9)	4/45 (9)	2/45 (4)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.229	0.072
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			1.022	0.511
Lower Limit			0.202	0.048
Upper Limit			5.160	3.362
Weeks to First Observed Tumor	--	--	111	111

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Thyroid: C-cell Adenoma[b]	1/10 (10)	2/46 (4)	2/45 (4)	3/45 (7)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.444	0.667
Lower Limit			0.027	0.065
Upper Limit			25.233	34.664
Relative Risk (Pooled Control)[f]			1.022	1.533
Lower Limit			0.077	0.184
Upper Limit			13.502	17.729
Weeks to First Observed Tumor	111	--	111	111
Thyroid: C-cell Adenoma or Carcinoma[b]	1/10 (10)	2/46 (4)	2/45 (4)	4/45 (9)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.444	0.889
Lower Limit			0.027	0.108
Upper Limit			25.233	43.482
Relative Risk (Pooled Control)[f]			1.022	2.044
Lower Limit			0.077	0.309
Upper Limit			13.502	21.921
Weeks to First Observed Tumor	111	--	111	111

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	4/9 (44)	6/46 (13)	5/43 (12)	0/37 (0)
P Values[c,d]	P < 0.001(N)	P = 0.033(N)	P = 0.037*(N)	P = 0.023**(N) P < 0.001*(N)
Relative Risk (Matched Control)[f]			0.262	0.000
Lower Limit			0.084	0.000
Upper Limit			1.139	0.250
Relative Risk (Pooled Control)[f]			0.891	0.000
Lower Limit			0.231	0.000
Upper Limit			3.255	0.769
Weeks to First Observed Tumor	74	--	107	--
Adrenal: Cortical Adenoma[b]	0/9 (0)	2/52 (4)	3/48 (6)	1/45 (2)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.127	0.012
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			1.625	0.578
Lower Limit			0.195	0.010
Upper Limit			18.563	10.753
Weeks to First Observed Tumor	--	--	111	110

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Spleen: Hemangioma[b]	0/10 (0)	0/52 (0)	4/48 (8)	0/47 (0)
P Values[c,d]	N.S.	N.S.	P = 0.049**	N.S.
Departure from Linear Trend[e]		P = 0.004		
Relative Risk (Matched Control)[f]			Infinite	--
Lower Limit			0.215	--
Upper Limit			Infinite	--
Relative Risk (Pooled Control)[f]			Infinite	--
Lower Limit			1.004	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor	--	--	111	--

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

(continued)

[a]Treated groups received time-weighted average doses of 4,250 or 8,500 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 treated group is the probability level for the Fisher exact test for the comparison of that treated 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 treated 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 treated group and the specified control group.

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

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Thyroid: Follicular-cell Adenoma[b]	0/9 (0)	0/46 (0)	0/50 (0)	1/46 (2)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			--	Infinite
Lower Limit			--	0.011
Upper Limit			--	Infinite
Relative Risk (Pooled Control)[f]			--	Infinite
Lower Limit			--	0.054
Upper Limit			--	Infinite
Weeks to First Observed Tumor	--	--	--	98
Thyroid: C-cell Adenoma[b]	1/9 (11)	1/46 (2)	2/50 (4)	7/46 (15)
P Values[c,d]	N.S.	P = 0.013	N.S.	P = 0.027**
Relative Risk (Matched Control)[f]			0.360	1.370
Lower Limit			0.023	0.224
Upper Limit			20.996	60.637
Relative Risk (Pooled Control)[f]			1.840	7.000
Lower Limit			0.100	0.954
Upper Limit			107.069	307.988
Weeks to First Observed Tumor	111	--	111	88

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Neoplastic Nodule[b]	0/8 (0)	1/53 (2)	2/49 (4)	0/49 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	--
Lower Limit			0.055	--
Upper Limit			Infinite	--
Relative Risk (Pooled Control)[f]			2.163	0.000
Lower Limit			0.117	0.000
Upper Limit			124.171	20.103
Weeks to First Observed Tumor	--	--	111	--
Pituitary: Chromophobe Adenoma[b]	0/9 (0)	9/46 (20)	1/44 (2)	7/45 (16)
P Values[c,d]	P = 0.021	N.S.	P = 0.010**(N)	N.S.
Departure from Linear Trend[e]		P = 0.012		
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.012	0.441
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			0.116	0.795
Lower Limit			0.003	0.274
Upper Limit			0.785	2.192
Weeks to First Observed Tumor	--	--	111	107

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Adrenal: Cortical Adenoma[b]	0/9 (0)	0/50 (0)	2/49 (4)	5/50 (10)
P Values[c,d]	N.S.	P = 0.017	N.S.	P = 0.022**
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.061	0.257
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.301	1.258
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	111	107
Mammary Gland: Fibroadenoma[b]	3/9 (33)	8/54 (15)	4/50 (8)	3/50 (6)
P Values[c,d]	P = 0.044(N)	N.S.	N.S.	P = 0.040*(N)
Relative Risk (Matched Control)[f]			0.240	0.180
Lower Limit			0.056	0.032
Upper Limit			1.467	1.212
Relative Risk (Pooled Control)[f]			0.540	0.405
Lower Limit			0.126	0.073
Upper Limit			1.888	1.585
Weeks to First Observed Tumor	82	--	111	90

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

(continued)

[a]Treated groups received time-weighted average doses of 4,250 or 8,500 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 treated group is the probability level for the Fisher exact test for the comparison of that treated 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 treated 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 treated group and the specified control group.

APPENDIX F

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

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

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	0/8 (0)	3/47 (6)	1/49 (2)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.010	0.054
Upper limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			0.320	0.627
Lower Limit			0.006	0.054
Upper Limit			3.834	5.234
Weeks to First Observed Tumor	--	--	93	82
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/8 (0)	0/47 (0)	3/49 (6)	0/50 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]		P = 0.014		
Relative Risk (Matched Control)[f]			Infinite	--
Lower Limit			0.113	--
Upper Limit			Infinite	--
Relative Risk (Pooled Control)[f]			Infinite	--
Lower Limit			0.413	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor	--	--	93	--

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	0/8 (0)	3/47 (6)	4/49 (8)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.175	0.054
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			1.279	0.627
Lower Limit			0.229	0.054
Upper Limit			8.319	5.234
Weeks to First Observed Tumor	--	--	93	82
Liver: Neoplastic Nodule[b]	0/9 (0)	3/49 (6)	11/50 (22)	2/50 (4)
P Values[c,d]	N.S.	N.S.	P = 0.024**	N.S.
Departure from Linear Trend[e]		P = 0.002		
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.678	0.060
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			3.593	0.653
Lower Limit			1.024	0.057
Upper Limit			18.934	5.471
Weeks to First Observed Tumor	--	--	93	93

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	0/9 (0)	5/49 (10)	36/50 (72)	40/50 (80)
P Values[c,d]	$P < 0.001$	$P < 0.001$	$P < 0.001$** $P < 0.001$*	$P < 0.001$** $P < 0.001$*
Departure from Linear Trend[e]	$P = 0.003$	$P = 0.002$		
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			2.485	2.795
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			7.056	7.840
Lower Limit			3.176	3.622
Upper Limit			19.491	20.198
Weeks to First Observed Tumor	--	--	67	78

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Neoplastic Nodule or Hepatocellular Carcinoma[b]	0/9 (0)	8/49 (16)	47/50 (94)	42/50 (84)
P Values[c,d]	P = 0.001	P < 0.001	P < 0.001** P < 0.001*	P < 0.001** P < 0.001*
Departure from Linear Trend[e]	P < 0.001	P < 0.001		
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			3.425	2.956
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			5.758	5.145
Lower Limit			3.429	2.866
Upper Limit			8.321	9.455
Weeks to First Observed Tumor	--	--	67	78

[a]Treated groups received doses of 8,000 or 16,000 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 treated group is the probability level for the Fisher exact test for the comparison of that treated 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.

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

(continued)

[d]A negative trend (N) indicates a lower incidence in a treated 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 treated group and the specified control group.

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

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	0/9 (0)	1/48 (2)	4/49 (8)	5/47 (11)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.192	0.274
Upper limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			3.918	5.106
Lower Limit			0.405	0.602
Upper Limit			188.830	238.482
Weeks to First Observed Tumor	--	--	92	84
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/9 (0)	1/48 (2)	1/49 (2)	0/47 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	--
Lower Limit			0.011	--
Upper Limit			Infinite	--
Relative Risk (Pooled Control)[f]			0.980	0.000
Lower Limit			0.013	0.000
Upper Limit			75.635	38.589
Weeks to First Observed Tumor	--	--	92	--

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	0/9 (0)	2/48 (4)	5/49 (10)	5/47 (11)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.262	0.274
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			2.449	2.553
Lower Limit			0.424	0.444
Upper Limit			24.674	25.945
Weeks to First Observed Tumor	--	--	92	84
Liver: Neoplastic Nodule[b]	0/9 (0)	1/48 (2)	14/49 (29)	9/47 (19)
P Values[c,d]	N.S.	P = 0.018	P < 0.001**	P = 0.007**
Departure from Linear Trend[e]	P = 0.047	P = 0.006		
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.909	0.571
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			13.714	9.191
Lower Limit			2.238	1.351
Upper Limit			569.159	391.340
Weeks to First Observed Tumor	--	--	84	90

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	0/9 (0)	2/48 (4)	5/49 (10)	2/47 (4)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.262	0.063
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			2.449	1.021
Lower Limit			0.424	0.077
Upper Limit			24.764	13.550
Weeks to First Observed Tumor	--	--	92	93

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Neoplastic Nodule or Hepatocellular Carcinoma[b]	0/9 (0)	3/48 (6)	19/49 (39)	11/47 (23)
P Values[c,d]	N.S.	P = 0.030	P < 0.001** P = 0.020*	P = 0.019**
Departure from Linear Trend[e]	P = 0.010	P = 0.002		
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			1.274	0.722
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			6.204	3.745
Lower Limit			2.004	1.068
Upper Limit			30.490	19.601
Weeks to First Observed Tumor	--	--	84	90

[a]Treated groups received doses of 8,000 or 16,000 ppm.

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

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

(continued)

[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 treated group is the probability level for the Fisher exact test for the comparison of that treated 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 treated 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 treated group and the specified control group.

APPENDIX G

ANALYSIS OF FORMULATED DIETS FOR CONCENTRATIONS OF TETRACHLORVINPHOS

APPENDIX G

Analysis of Formulated Diets for Concentrations of Tetrachlorvinphos

A 100-g sample of the diet mixture was shaken with 100 ml hexane at room temperature for 16 hours, then filtered through Celite with hexane washes, and reduced in volume to 10 ml. After appropriate dilutions, the solution was quantitatively analyzed for tetrachlorvinphos by gas-liquid chromatography (electron-capture detector, 5% QF-1 on Chromosorb W 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)
4,000	23	4,015	4.5%	3,591-4,296
8,000	28	7,993	5.1%	7,060-8,610
16,000	26	15,760	5.3%	13,500-17,280

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

November 28, 1977

The Clearinghouse on Environmental Carcinogens was established in May, 1976 under the authority of the National Cancer Act of 1971 (P.L. 92-218). The purpose of the Clearinghouse is to advise on the National Cancer 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, 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 organic 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 NCI bioassay reports on chemicals studied for carcinogenicity. In this context, below is the edited excerpt from the minutes of the Subgroup's meeting at which Tetrachlorvinphos was reviewed.

The primary reviewer noted that Tetrachlorvinphos induced neoplastic changes in the livers of the treated mice. He was uncertain, however, as to the interpretation of the changes in view of the controversial nature of the lesions. He said that if the diagnoses of the liver lesions are accepted as given in the report, the incidence of hepatocellular carcinomas was dose-related. In regard to the finding of an increased incidence of cortical adenomas of the adrenal in treated female rats, the reviewer said that he had difficulty in evaluating this type of lesion. He added that the adenomas were statistically significant when compared to the historical control animals. The reviewer was most skeptical of the significance of the dose-related trend in thyroid C-cell adenomas in treated female rats. He noted that thyroid proliferative lesions were found in almost all of the rats.

In commenting on the pathology findings, an NCI staff pathologist said that the mouse liver lesions were reexamined and the diagnoses of hepatocellular carcinomas confirmed. In regard to the thyroid lesions, he said that the staff was confident that they were treatment-related.

A consultant to Shell Oil Company discussed the views of Shell regarding the Tretrachlorvinphos study. He said that consultant pathologists to Shell have reviewed the mouse liver lesions and found no increase in the incidence of hepatocellular carcinomas among the treated animals. He also noted that at the same time the Tetrachlorvinphos study was underway, Dieldrin and Malathion were being tested in the same room. He suggested that, through cross-contamination, these compounds may have acted as potentiators of toxicity in the Tetrachlorvinphos treated mice, resulting in an increase of hepatocellular carcinomas over and above the baseline incidence. He mentioned other variables that could have affected the findings. In conclusion, he briefly described the ongoing Shell-sponsored study.

A motion was made that Tetrachlorvinphos induced hepatocellular carcinomas in mice under the conditions of test. The motion was seconded and approved unanimously.

Members present were:

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology
Lawrence Garfinkel, American Cancer Society
Henry C. Pitot, University of Wisconsin Medical Center
George Roush, Jr., Monsanto Company
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
John H. Weisburger, American Health Foundation

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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 260-899/3010 1-3

DHEW Publication No. (NIH) 78-833