National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 9
1977

BIOASSAY OF HEPTACHLOR FOR POSSIBLE CARCINOGENICITY

CAS No. 76-44-8

NCI-CG-TR-9

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

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HEPTACHLOR

FOR POSSIBLE CARCINOGENICITY

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DHEW Publication No. (NIH) 77-809

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Carcinogenesis Program, Division of Cancer Cause and Prevention

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SUMMARY

A bioassay of technical-grade heptachlor for possible carcinogenicity was conducted by administering the test material in feed to Osborne-Mendel rats and B6C3F1 mice. Groups of 50 rats of each sex were administered low or high concentrations of the heptachlor for 80 weeks, then observed for 30 weeks. Doses for females were first increased, but because of toxic effects the doses were then reduced twice for both male and female rats during the remaining course of the tests. Time-weighted average doses used for the male rats were 38.9 and 77.9 ppm; for the females, 25.7 and 51.3 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls consisted of the matched-control groups combined with 50 untreated male and 50 untreated female rats from similar bioassays of five other compounds. All surviving rats were killed at 110-111 weeks.

Groups of 50 mice of each sex were administered the test material at low or high concentrations for 80 weeks, then observed for 10 weeks. The low- and high-dose groups were tested at different calendar times, but a concurrent control group was started with each. Because of toxic effects, doses were reduced once for the males at 17-18 weeks after the initiation of tests; twice for the females, at 17 and 30 weeks, after the initiation of tests. The time-weighted average doses used for the male mice were 6.1 and 13.8 ppm; for the females, 9 and 18 ppm. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched-control groups combined with 90 untreated male and 70 untreated female mice from similar bioassays of five other compounds. All surviving mice were killed at 90-91 weeks.

The effects of heptachlor on body weights and other clinical signs in rats and mice indicated that the dosages used were near the maximum permissible. This was evident in that average body weights of rats treated with high doses were consistently lower than those of untreated controls, while body weights of low-dose rats were unaffected. Body weights of mice given either high or low doses showed little or no differences from those of control mice; however, other adverse clinical signs were found in high-dose mice, predominantly in the females.

The effects of heptachlor on survival rates indicated that mortality was dose-related for both female rats and female mice, but not for males of either species. However, a substantial proportion of all groups of animals survived to an age at which tumors could be expected to appear.

In mice, hepatocellular carcinoma showed a highly significant dose-related trend in both males (matched controls 5/19, low dose 11/46, high dose 34/47, $P = 0.001$) and females (control 2/10, low dose 3/47, high dose 30/42, $P < 0.0001$). When pooled controls were used for the comparison, the significance of the trend in males increased to $P < 0.0001$. Comparably high levels of significance were attained when the data were subjected to life-table adjustment. No other tumors were found in mice in sufficient numbers to justify analysis.

In marked contrast to the findings observed in mice, no hepatic tumors were observed in rats administered heptachlor. There was significant statistical evidence for the induction of proliferative lesions of follicular cells of the thyroid in treated female rats, but this finding was discounted because the rates of incidence were comparatively low and are known to be variable in control rat populations.

It is concluded that under the conditions of this bioassay, heptachlor is carcinogenic for the liver in mice.

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I. INTRODUCTION

Heptachlor is a member of the cyclodiene group of chlorinated insecticides (aldrin, dieldrin, endrin, chlordane, heptachlor, and endosulfan) that were developed in the 15 years following World War II. It was registered as a commercial pesticide in 1952 for foliar, soil, and structure applications and for malarial control programs (EPA, 1972; Casarett and Doull, 1975); after 1960 it was used primarily in soil applications against agricultural pests and to a lesser extent against termites.

On November 18, 1974, the EPA issued a notice of intent to cancel the registration and use of pesticides containing heptachlor, except for subsurface ground insertion against termites and for dipping of nonfood plants (EPA, 1974). On December 24, 1975, the EPA issued an order that suspended the registered uses of products containing heptachlor against ticks and chiggers, against pests of home, garden, lawn, and turf, as an ingredient in shelf paper, and (effective August 1, 1976) as a control against cutworms on corn (EPA, 1976). Exempted from this order was the use of heptachlor for treatment of seeds, control of ants on Hawaiian pineapples, and control of the narcissus bulb fly.

The persistence of heptachlor in the environment has been well documented. Human exposure occurs as a result of mobilization of

the compound in crops, water, and atmosphere. Zero tolerance was established for residues on 16 vegetables, 4 fruits, and 12 field crops used for animal consumption; also, for residues in meat and milk (EPA, 1972). Tolerance of 0.1 ppm was set for four additional vegetables. An acceptable daily human intake of 0.005 mg/kg was established for heptachlor and its epoxide (FAO/WHO, 1967). The average daily intake of both compounds from 1965 to 1970 was 0.00003 mg/kg, well below the acceptable daily intake (Duggan and Corneliussen, 1972). When assays for heptachlor were performed on tissue samples in 1970, mean concentrations of 0.08 ppm were detected in 96% of 3,451 hospital patients tested. Similar observations were made in 1971 and 1972, with some individual concentrations ranging as high as 1.68 ppm. Heptachlor has also been shown to be transported across the placenta and to pass into breast milk (EPA, 1974).

In 1969 the National Cancer Institute (NCI) was requested to review the biological and environmental data on pesticides by the Secretary's Commission on Pesticides and their Relationship to Environmental Health, a committee of the Department of Health, Education and Welfare. This review pointed out the need for an evaluation of the carcinogenicity of several pesticides, including heptachlor (Secretary's Commission on Pesticides, 1969). Because of known biological effects of low doses of heptachlor over extended periods of time (FAO/WHO, 1967), the persistence of

the compound in the environment, and the probability of continued human exposure, this insecticide was selected by the NCI for inclusion in the carcinogen bioassay program.

II. MATERIALS AND METHODS

A. Chemical

The material tested was technical-grade heptachlor obtained from Velsicol Chemical Corporation (Chicago, Ill.) in one batch for use in the chronic study. Heptachlor is made by allylic chlorination of chlordene, the Diels-Alder adduct of hexachlorocyclopentadiene and cyclopentadiene. According to the manufacturer, a typical analysis of the technical-grade product is 73% heptachlor, 22% *trans*-chlordane, and 5% nonachlor. Analysis of the bioassay batch at Gulf South Research Institute by melting point, elemental analysis, and spectroscopic and chromatographic techniques confirmed the identity of the material.

After completion of the bioassay, this batch was reanalyzed at Midwest Research Institute. Gas-liquid chromatographic (glc) comparison with the sample that was used for the bioassay showed the two samples to be essentially the same. All major components were present in similar proportions, and all minor components at a level > 0.1% appeared in both samples. Using authentic proportions of each component for standardization, it was determined by glc that the technical-grade heptachlor contained approximately 72 $\pm$ 3% heptachlor, 18% *trans*-chlordane, 2% *cis*-

chlordane, 2% nonachlor, 1% chlordene 0.2% hexachlorobutadiene, and smaller amounts of 10-15 other compounds.

B. Dietary Preparation

All diets were formulated weekly using Wayne® Lab Blox Meal (Allied Mills, Inc., Chicago, Ill.) to which was added the required amount of heptachlor for each dietary concentration. Small amounts of acetone (Mallinckrodt Chemical Works, St. Louis, Mo.) were used as an aid to uniform dispersion of the test compound in the feed. The diets were mixed mechanically to assure homogeneity and to allow for evaporation of the acetone. Corn oil equal to 2% of the final weight of feed was then added, primarily as a dust suppressant. Diets for control animals were the same as those for treated animals except for the absence of heptachlor. The corn oil (Louana®) was produced by Opelousas Refinery Co., Opelousas, La. Formulated diets were stored at approximately 17°C until used, but no longer than 1 week. Water and the formulated diets were made available *ad libitum* to the experimental animals and were replaced three times per week.

The stability of heptachlor in feed was checked by analyzing formulated diets for the concentration of heptachlor at intervals over a 7-day period. Diets containing 5 and 20 ppm heptachlor showed no change on standing at ambient temperature for this period.

Theoretical heptachlor concentrations in formulated diets were checked analytically at intervals during the chronic study to assess the accuracy of the diet preparation and the homogeneity of the mixtures. Results are summarized in Appendix G. At each dietary concentration, ranging from 5 to 160 ppm, the mean of the analytical concentrations for the samples checked was within 10% of the theoretical concentration, and the coefficient of variation was not more than 6.9% at any level. Thus, the evidence indicates that the formulated diets were accurately prepared and were homogeneous.

C. Animals

Rats and mice of both sexes, obtained through contracts of the Division of Cancer Treatment, NCI, were used in these tests. The rats were the Osborne-Mendel strain procured from Battelle Memorial Institute, Columbus, Ohio, and the mice were B6C3F1 hybrids obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. Upon arrival at the laboratory, all animals were quarantined for 14 days as a laboratory acclimation period, and randomly selected and assigned to cages and treatment groups.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. Incoming air was filtered through fiberglass air condi-

tioner filters that were changed monthly. The total air in each room was changed 10-12 times per hour. Fluorescent lighting provided illumination 10 hours per day. The rats were housed individually in hanging galvanized steel-mesh cages; the mice, in plastic cages equipped with filter caps, five mice per cage for females and two or three per cage for males. Initially, rats were transferred weekly to clean cages; later in the study, clean cages were provided biweekly. Mice were transferred weekly to clean cages with filter bonnets. Fresh bedding (Absorb-Dri®, Lab Products) was provided two times a week for male mice and three times a week for females. Food (Wayne® Lab Blox Meal) and water were consumed ad libitum. Feeder jars and water bottles were changed and sterilized three times per week. Animal racks were rotated laterally for both species at weekly intervals.

Rats receiving heptachlor, along with their controls, were housed in a room by themselves. Mice receiving heptachlor were maintained in a room housing mice administered chlordane, toxaphene, and chlordecone. Cages for control and treated mice were placed on separate racks in the same room.

E. Subchronic Studies

Feeding studies were conducted with rats and mice to estimate the maximum tolerated doses of heptachlor, on the basis of which high and low concentrations (hereinafter referred to as "high doses"

and "low doses") were determined for administration in the chronic studies. The low doses given in the chronic studies were 1/2 of the high doses. In the subchronic studies, heptachlor was provided in feed to groups of five male and five female rats and mice for 6 weeks, followed by a 2-week period of observation. Twofold increasing concentrations, from 20 ppm through 320 ppm, were used. Weights of animals and consumption of feed were measured weekly, and deaths were noted.

With rats given 320 ppm, two out of five males and all of five females died. At 80 or 160 ppm, no males died, and their average weekly gain in weight and consumption of feed were similar to those of untreated controls. Four of five females died, however, when given 160 ppm. At 80 ppm, the treated females showed less gain in weight than the controls for the first week of treatment, with comparable gains thereafter. Based on these findings, low and high doses of 80 and 160 ppm, respectively, were selected for the chronic studies in the male rats; 40 and 80 ppm, for the females.

With mice fed 80 ppm, all males died during the second and the third weeks, and two of the females died during the sixth week, while those mice fed concentrations of only 20 or 40 ppm showed no deaths and no adverse effects with respect to either body weight or feed consumption. The concentrations of 20 and 40 ppm

were selected, therefore, as low and high doses for chronic studies in both male and female mice.

F. Design of Chronic Studies

Designs of the chronic studies with rats and mice, including test and matched-control groups, are shown in tables 1 and 2. When tests with the rats were initiated at doses indicated by the subchronic studies, toxic effects were observed with the males, requiring reduction of the dose after 31 weeks and again after 45 weeks; with the female rats, the dose was increased at 22 weeks, but development of toxicity required subsequent reductions in dose at 31 and 45 weeks. When tests with mice were initiated at doses indicated by the subchronic studies, high mortality resulted in the males, and the original high-dose group of males was discarded. The males receiving 20 ppm then became the high-dose group, and the study was continued, keeping the original 10 control males. A new low-dose group of male mice was started at 10 ppm, along with an additional group of 10 male controls. The total number of male controls thus became 20. Due to further appearance of toxic effects, doses for males were reduced once, at 17-18 weeks, and doses for females were reduced twice, at 17 weeks and again at 30 weeks.

Since the matched-control groups for both rats (10 males, 10 females) and mice (20 males, 20 females) were small, pooled-

Table 1. Design of Heptachlor Chronic Feeding Studies in Rats

Sex and Treatment Group	Initial No. of Animals[a]	Heptachlor in Diet (ppm)	Time on Study Treated (weeks)	Time on Study Untreated[b] (weeks)	Time-Weighted Average Dose[c] (ppm)
MALE					
Matched-Control	10	0	0	111	
Low-Dose	50	80	31		38.9
		20	14		
		10	35		
		0	0	30	
High-Dose	50	160	31		77.9
		40	14		
		20	35		
		0	0	30	
FEMALE					
Matched-Control	10	0	0	111	
Low-Dose	50	40	22		25.7
		60	9		
		20	14		
		10	35		
		0	0	30	
High-Dose		80	22		
		120	9		51.3
		40	14		
		20	35		
		0	0	30	

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

[b]When diets containing heptachlor were discontinued, treated male rats and their matched controls were fed plain feed diets (without corn oil) for 11 weeks, then control diets (2% corn oil added) for an additional 18 weeks; treated female rats and their matched controls were fed plain feed diets for 9.5 weeks, then control diets for an additional 20 weeks.

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

Table 2. Design of Heptachlor Chronic Feeding Studies in Mice

Sex and Treatment Group	Initial No. of Animals[a]	Heptachlor in Diet (ppm)	Time on Study: Treated (weeks)	Time on Study: Untreated[b] (weeks)	Time-Weighted Average Dose[c] (ppm)
MALE					
Matched-Control	20[d]	0	0	90-91	
Low-Dose	50	10	18		6.1
		5	62		
		0	0	10	
High-Dose	50	20	17		13.8
		10	50		
		0	0	10	
FEMALE					
Matched-Control	10	0	0	90-91	
Low-Dose	50	20	17		9.0
		10	13		
		5	50		
		0	0	10	
High-Dose	50	40	17		18.0
		20	13		
		10	50		
		0	0	10	

[a]All animals were 35 days of age when placed on test.
[b]When diets containing heptachlor were discontinued, mice received control diet (2% corn oil added) until termination.
[c]Time-weighted average dose = $\frac{\Sigma(\text{dose in ppm x no. of days at that dose})}{\Sigma(\text{no. of days receiving each dose})}$
[d]Initially 10 males were placed on test as matched controls; however, when the study was restarted, 10 additional male animals were placed on test as matched controls.

control groups were formed by combining matched controls from similar bioassays of other compounds with the matched controls for heptachlor. The periods during which the bioassays of the different compounds were performed overlapped one another for at least a year. For rats, the animals that comprised the pooled-control groups consisted of groups of 10 male and 10 female controls taken from tests performed on aldrin, dieldrin, chlordane, heptachlor, dichlorvos, and dimethoate; this gave pooled-control groups containing 60 males and 60 females, respectively. For mice, the animals that comprised the pooled-control groups consisted of groups of 20 male and 20 female controls taken from tests performed on dieldrin and chlordane, 20 male and 10 female controls from tests on aldrin and heptachlor, and 10 male and 10 female controls from tests on dichlorvos and dimethoate; this gave pooled-control groups containing 100 males and 80 females, respectively. All treated and pooled-control animals were placed on study as weanlings at 35 days of age except for the matched-control rats for dichlorvos. Because dichlorvos was the last compound of this series to be bioassayed, there were slight differences in the dichlorvos matched-control rats that were pooled for use as controls in the heptachlor study: (1) half of the animals of each sex were started on test at 43 days of age and half at 36 days of age; (2) they were obtained from the Charles River

Breeding Laboratories, Inc. and were the progeny (third generation) of a group of Osborne-Mendel rats which were purchased from the Battelle Memorial Institute, Columbus, Ohio. Thus there was probably no significant genetic drift that might influence the incidence of tumors.

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. Those animals appearing moribund at the time of clinical examination were killed and necropsied.

The pathologic evaluation consisted of gross and microscopic examination of all major tissues, organs, or gross lesions. The following tissues and organs were taken from killed animals and, where feasible, from animals found dead: skin, mammary gland, brain, pituitary, mandibular nodes, salivary glands, thyroid, parathyroid, trachea, lung, heart, stomach, small intestine, large intestine, pancreas, adrenal, kidney, liver, spleen, urinary bladder, prostate or uterus, testis or ovary, and bone. Tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, routinely stained with hematoxylin and eosin, and examined histopathologically. An occasional section was subjected to special staining techniques for more definitive diagnosis.

A few tissues were not examined for some animals, particularly for those that showed early deaths. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically, varies and does not necessarily represent the number of animals that were placed on experiment in each group.

H. Data Recording and Statistical Analyses

Pertinent data for 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, animal 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.

Survival curves were computed using standard life-table methods (e.g., Kaplan and Meier [1958] or Armitage [1971]). Deaths which were labeled accidental or scheduled sacrifice were excluded from the numerator but not the denominator, i.e., they were treated as censored observations. All other deaths were counted as uncen-

sored observations in the numerator. Statistical tests of differences between groups were computed using the methods of Cox (1972). When two groups were to be compared, the method explicitly given by Cox was employed. When three groups were compared, an extension by Tarone (1975) of Cox's method was used; this was a test for linear trend in survival rate among the control, low-dose, and high-dose groups. In all instances the P value was given for a one-tailed test. Unless otherwise noted, the P value was given in terms of a positive relation to dose. If there was a significant departure from the linear relation, this was so noted. Combined tests on groups treated at different times were carried out by methods formally equivalent to those of Mantel (1963).

The incidence of neoplastic or nonneoplastic lesions is given as the proportion of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals examined pathologically at that site (denominator). For the organs and tissues in which most of the lesions appeared, the denominators included only those animals for which such sites were examined histologically. For tissues that required gross observation for detection of lesions (e.g., skin or mammary tumors), for lesions that appeared at several sites (e.g., lymphomas), or for tissues that were examined histologically only

when lesions were detected grossly, the denominators consisted of the numbers of animals necropsied.

The analysis of tumor incidence took two forms: (a) comparisons of the number of animals with a given tumor as a proportion of those examined for that type; (b) comparisons of the groups with regard to both the number of animals with tumors and the times (in weeks) at which all of the examined animals died.

In the first analyses, the exact (or conditional) test for proportions was used as given by Cox (1970). For the comparison of two groups, this was simply the Fisher exact test. When three groups were compared, this was the exact test for a linear trend in the logistic scale. All tests were one-tailed and, unless otherwise noted, in the direction of a positive relationship to dose. If there was a significant departure from linearity, this was so noted. Combined tests over groups run at different times were also performed using exact methods.

For some of the important tumor sites, the exact analysis was applied in an additional way. This analysis eliminated from the denominators for any of the groups being compared all animals which died or were killed at a time before the first animal was found to have a tumor at that site.

The second analysis of tumor incidence used life-table methods.

Curves of the proportion surviving without tumor being observed were computed using life-table methods (e.g., Saffiotti et al., 1972). The times at which animals were killed were entered as the last time point of tumor observation. Cox's methods of comparing these curves were used for two groups, and Tarone's extension to testing for linear trend was used for three groups. All tests of tumor incidence using life-table methods were one-tailed and in the direction of a positive dose relationship unless otherwise noted. If there was a significant departure from linearity this is so noted. Combined tests on groups treated at different times were carried out using methods formally equivalent to Mantel (1963).

All P values are given on a per comparison basis rather than on an experiment-wise basis. If the latter is desired, one may utilize the Bonferroni inequality and multiply any given P value by the total number of comparisons of interest to arrive at an experiment-wise P value (Wilks, 1962).

Analyses that were applied to the comparisons including pooled controls were similar to those used in the comparisons involving matched controls.

The original data were converted by computer program from days on study to weeks on study for the individual animal data. Since the weeks on study were given in whole integers only, it was

possible for animals dying or being killed 1 to 4 days apart to be reported as a week apart. These small possible discrepancies were not of major importance in the statistical analyses except when deaths of a large number of animals were involved in a given week. This could happen at the termination of the test. The animals in one dose group could be sacrificed 4 days later than the others. Thus their time on study differs by 1 week. These were all grouped to one time interval in the statistical tests.

III. RESULTS - RATS

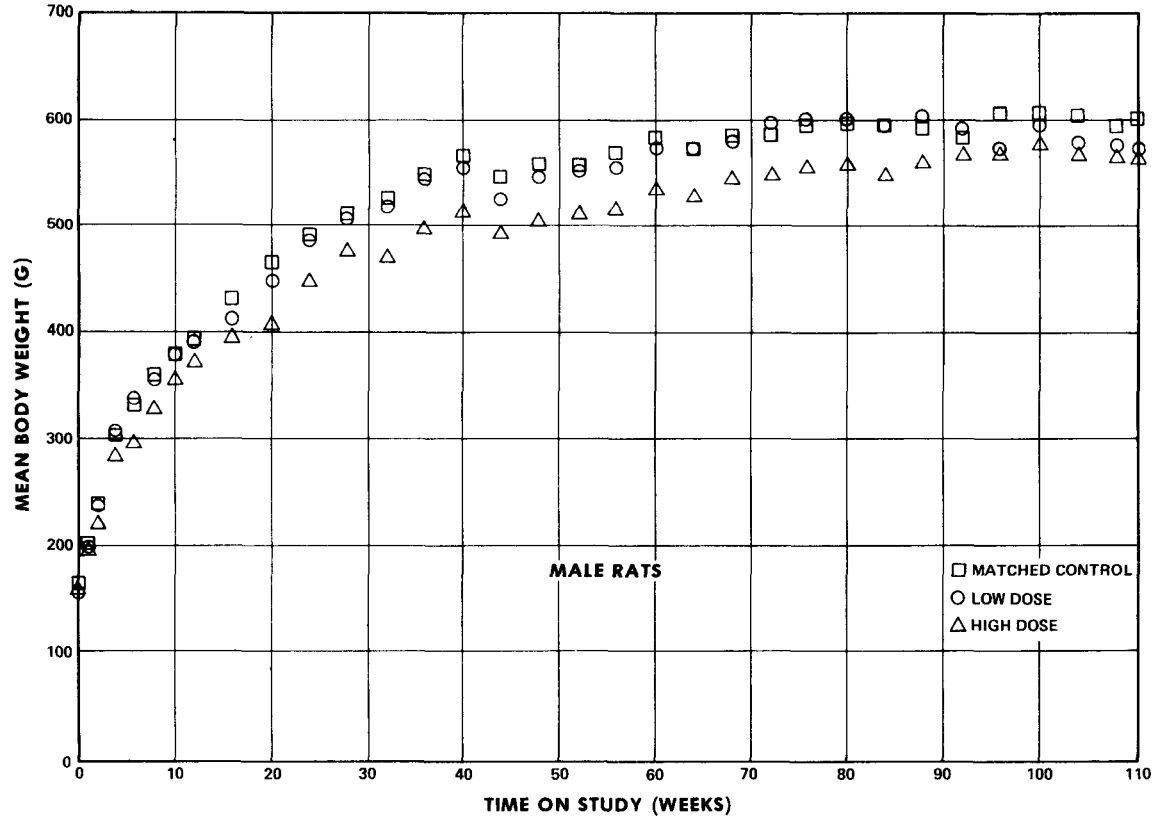
A. Body Weights and Clinical Signs (Rats)

Average body weights of high-dose male rats were consistently lower than those of untreated controls; there was less effect in high-dose females. Low doses had, however, essentially no effect on body weights (figure 1).

Adverse clinical signs in the treated and control groups were noted at a low or moderate incidence during the first year and gradually increased in frequency during the second year of study. These signs in individual animals included loss of weight, rough and discolored hair coats, and palpable masses. After 80 weeks vaginal bleeding was observed in some females (both high- and low-dose). Several palpable subcutaneous masses in the abdominal region, probably mammary tumors, were noted in the low-dose females. Surviving animals at termination of the study (111 weeks) were generally in a poor physical condition.

B. Survival (Rats)

Curves showing the probability of survival of treated and control rats are presented in figure 2. Although a positive dose-relation was observed for mortality in male rats, the result was not statistically significant ($P = 0.17$). For female rats,



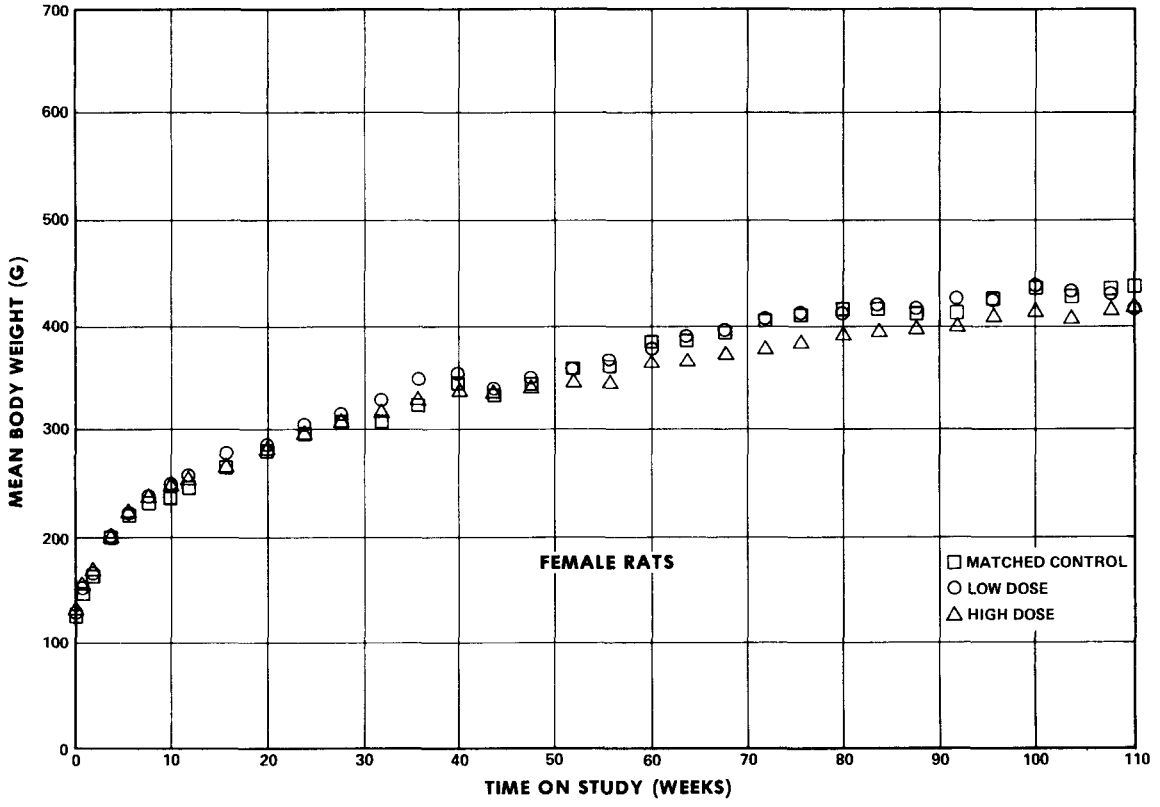
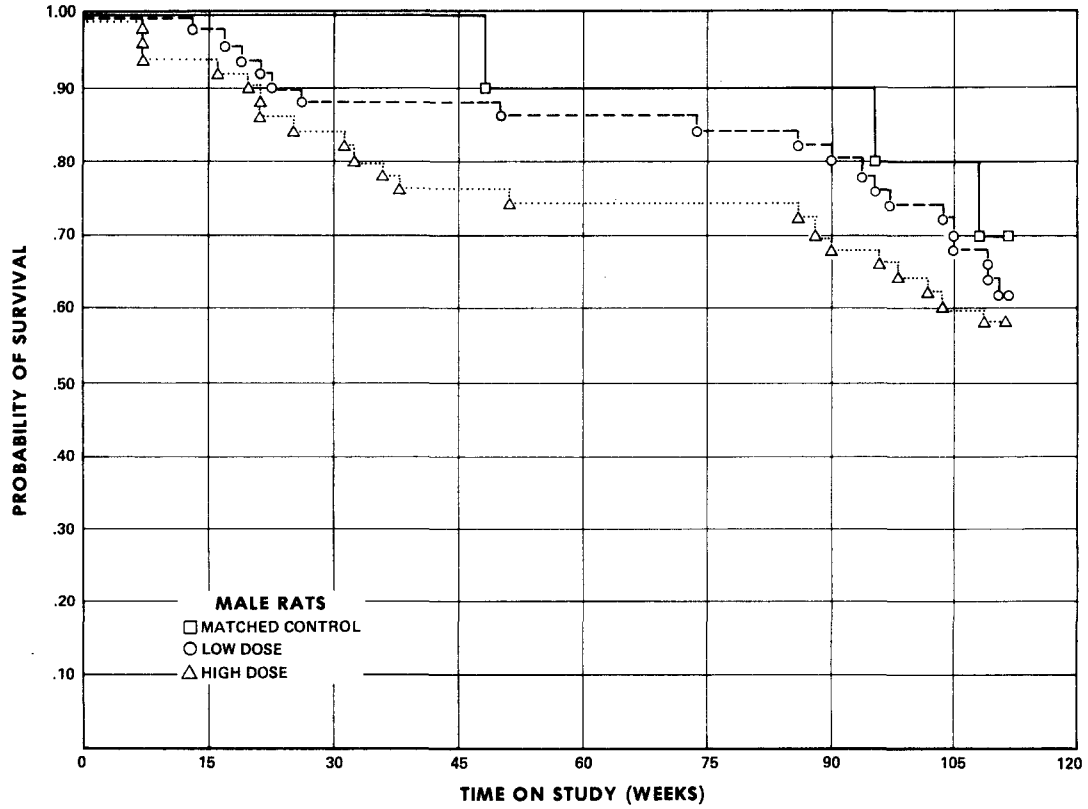


Figure 1. Growth Curves for Rats Fed Heptachlor in the Diet



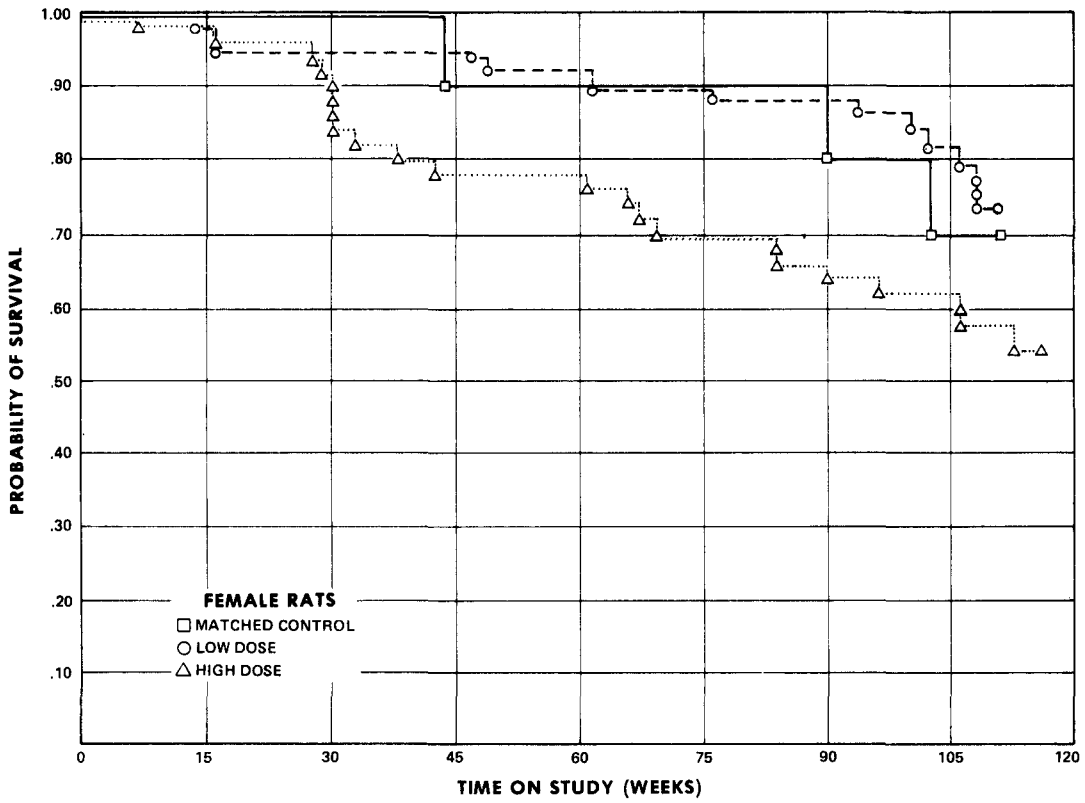


Figure 2. Survival Curves for Rats Fed Heptachlor in the Diet

however, the linear trend test for mortality was significantly positive (P = 0.04). This was due mainly to the difference in mortality between the low- and high-dose animals.

C. Pathology (Rats)

Histopathologic findings are tabulated in Appendix A, tables A1-A8, covering neoplasms and other proliferative lesions, and in Appendix C, tables C1 and C2, covering nonneoplastic lesions.

Numerous inflammatory, degenerative, and proliferative lesions commonly seen in aged rats occurred with approximately equal frequency in drug-treated and control animals. These included aggregates of alveolar macrophages in the lungs, pericholangitis and biliary hyperplasia, chronic nephritis with tubular dilatation and epithelial hyperplasia of the renal pelvis, chronic cystitis with varying degrees of hyperplasia of the urinary vesical epithelium, subacute to chronic prostatitis, and atrophy of seminiferous epithelium of the testes.

Table A2 summarizes the incidence of proliferative lesions of the thyroid. Both follicular-cell and C-cell lesions were observed. Areas of follicular-cell hyperplasia were characterized by follicles with larger lumens than normal, lined by follicular epithelial cells which were either stratified to form an irregular epithelial border several cells thick, or projecting

into the lumen by papillary infolding of simple cuboidal or columnar epithelial cells which were usually larger and had more basophilic cytoplasm than the surrounding normal follicular cells. Compression of adjacent tissue was minimal other than that caused by distention of the follicular lumen. Follicular architecture (size and shape of lumen, thickness of the layer of follicular cells, and amount of stroma) was heterogeneous and resembled somewhat the adjacent normal thyroid. As a result, the lesion blended in gradually with the adjacent normal tissue, especially when compression was minimal. There was no evidence of encapsulation by connective tissue. In some cases, multiple foci of hyperplasia were present.

In comparison with those lesions classified as hyperplastic, follicular-cell adenomas of the thyroid (Bloodworth, 1968; Meissner and Warren, 1968) characteristically were more distinctly circumscribed, had some evidence of connective-tissue encapsulation, and had a follicular architecture more distinct from adjacent normal thyroid parenchyma. Follicular-cell adenomas were usually single.

Follicular-cell carcinomas were characterized by a variable mixture of solid masses of follicular cells and numerous closely packed small follicles. In both patterns the follicular cells were basophilic with hyperchromatic nuclei, and numerous mitotic

figures were present. Evidence of connective tissue and/or inflammatory response and necrosis was often present. No metastasis of a follicular-cell carcinoma occurred.

Proliferative C-cell lesions presented a spectrum of histologic characteristics ranging from very minimal increases in numbers of C-cells interspersed among normal thyroid follicles, with no compression or distortion of follicular architecture, to grossly visible tumors that invaded adjacent tissue and in one case metastasized to the lung. This spectrum of lesions was easy to classify at each end (minimal hyperplasia and overt carcinoma), but differentiation between severe hyperplasia and adenoma and between adenoma and carcinoma was difficult. The following criteria were used in classifying proliferative lesions of C-cells in these rats

A. Hyperplasia

1. Increased numbers of C-cells are interspersed among follicles but are not compressing follicles or distorting follicle architecture.

2. Proliferating C-cells are polyhedral to spherical with pale eosinophilic cytoplasm and spherical nucleus usually centrally located.

B. Adenoma

1. A discrete mass of C-cells widely separates follicles, although some isolated single follicles may be present within the mass.

2. Spindling or lengthening of C-cells to form interlacing bundles may be present.

3. An increase in basophilia of C-cells may be present.

4. No invasion of thyroid capsule, adjacent tissue, or lymphatics is present.

5. Encapsulation is rare.

C. Carcinoma

1. Invasion of thyroid capsule, adjacent tissue, or vessels is present.

2. Metastasis may be present.

3. Spindling and increased basophilia of neoplastic C-cells are usually prominent features in carcinoma, but are not necessary for diagnosis of malignancy.

4. Increased mitotic activity is rare.

The incidence of other endocrine neoplasms is summarized in table A3. Pituitary adenomas occurred frequently in all groups; neoplasms of adrenal, parathyroid, and pancreatic islets occurred infrequently.

Table A4 summarizes the incidence of primary neoplasms of the digestive system. These included a number of hepatic lesions classified as neoplastic nodules, which consisted of nodules of enlarged hepatocytes compressing adjacent tissue, and similar in microscopic appearance to nodules produced experimentally in rat livers by known carcinogens. Such nodules have recently been defined morphologically and given the designation of neoplastic

nodules (Squire and Levitt, 1975); as such, they have been categorized and coded as neoplasms when observed in this study.

Table A5 summarizes the incidence of neoplasms of the reproductive system and mammary gland. Endometrial stromal polyps represented the most frequently occurring neoplasms of the reproductive tract. Numerous mammary fibroadenomas, some of which were multiple, were observed in test and control females.

Several other neoplasms occurred infrequently in various tissues from test and control rats; the incidence of these neoplasms is summarized in tables A6, A7, and A8. Neoplasms similar to the malignant fibrous histiocytomas observed in this study (table A8) have been described previously (NCI, Bioassay of Chlordane for Possible Carcinogenicity).

There was a distinct increase in the incidence of follicular-cell neoplasms, including both adenomas and carcinomas in high-dose female rats (15/38 [39%] versus pooled controls (3/58 [5%]) and matched controls(1/9 [11%]); a smaller increase in the total incidence of follicular-cell neoplasms was present in low-dose males (11/38 [29%]) versus pooled control 4/51 [8%] and matched controls (1/8 [13%]). The biologic significance of these increases is difficult to assess. Experimental induction of thyroid hyperplasia in rats and birds by chronic exposure to chlorinated hydrocarbons has been described in the literature

(Moriarty, 1975); however, no thyroid neoplasms induced by heptachlor were mentioned in the studies cited. The data in the present study, although somewhat suggestive, do not, in the judgment of the pathologist, appear to be sufficient to indicate clearly a carcinogenic effect of heptachlor on the thyroid follicular cells of rats.

There were other instances where neoplasms occurred only in test animals, or with increased frequency when compared to control groups. However, the nature, incidence, and severity of the lesions observed provide no clear evidence of carcinogenic effect of heptachlor in rats.

D. Statistical Analyses of Results (Rats)

Statistical analyses of neoplasms in rats fed heptachlor are given in Appendix E, tables E1-E12. No hepatocellular carcinomas were found among the 187 heptachlor-treated animals whose livers were examined (table E1). When hepatocellular carcinoma was combined with neoplastic nodules, a significant linear trend still was not found in either sex by either statistical test. These findings were confirmed by comparisons using the pooled controls (table E2).

The data for proliferative thyroid lesions were divided into follicular-cell and C-cell types. Several follicular-cell

carcinomas were found among the treated male and female rats, but none of the findings were statistically significant (table E3). When carcinoma was combined with adenoma, a significantly positive linear trend was found among the female rats by each test (P = 0.002 and P = 0.001). This result was not confirmed in the male animals. Comparisons with pooled controls for the females yielded significant results for carcinomas and confirmed the results when adenomas were added (table E4). In male rats the low-dose group had significantly more carcinomas and/or adenomas than the pooled controls; however, here the high dose and pooled controls had the same percentages.

A few C-cell thyroid carcinomas were found among the treated male rats, but the results were not significant (table E5). Only one C-cell thyroid carcinoma was found among the female rats; this was in the control group. The life-table adjusted analyses could not be validly applied here because of the small number of tumors. The exact test was more appropriate, and it yielded a nonsignificant result (P = 0.10). When the carcinoma was combined with adenoma, the results for the male rats remained nonsignificant, but the results for the females were significant in the negative direction by both tests. The pooled comparisons for male rats, in general, paralleled the results with matched controls. Significantly negative trends were found for the com-

parison involving pooled female controls for the carcinoma-only criterion (table E6).

The results of analyses of thyroid lesions were ambiguous in that the various criteria for female rats yielded (a) a significantly positive linear dose effect for follicular-cell lesions, and (b) a significantly negative linear dose effect for C-cell lesions. The significantly positive relations for the follicular-cell tumors reached higher degrees of significance for females (P = 0.002 and 0.001 for the two matched-control tests), while the significantly negative relationships (P = 0.046 and 0.03) for the C-cell tumors would be discounted on the basis of experiment-wise error rates.

No carcinomas of the pituitary were found in any of the 149 treated animals or the 13 matched-control animals whose pituitary glands were examined microscopically (table E7). When the carcinoma was combined with adenoma, still no significant effect was found. The pooled controls yielded only one adenocarcinoma and one carcinoma among the male rats and none among the female rats; therefore, no analyses were carried out for this category of tumors. A significantly negative result was found for female rats for the combination of adenocarcinoma, carcinoma, and adenoma (table E8), but this did not reach high levels of signi-

ficance except for one comparison involving trend among male rats (P = 0.001).

When different categories of mammary tumors were considered together, a large number of mammary tumors were found in the female rats. No significant differences were found, however, between treated animals and matched controls (table E9). In the pooled-control analysis (table E10), only the low-dose group yielded a positive significant result by one criterion.

With respect to tumors of the uterus, two endometrial stromal sarcomas were found in the high-dose female group (table E11). This result was not significant. When polyps were added to the criteria, no significance was found. Tests using pooled controls yielded similar results (table E12).

IV. RESULTS - MICE

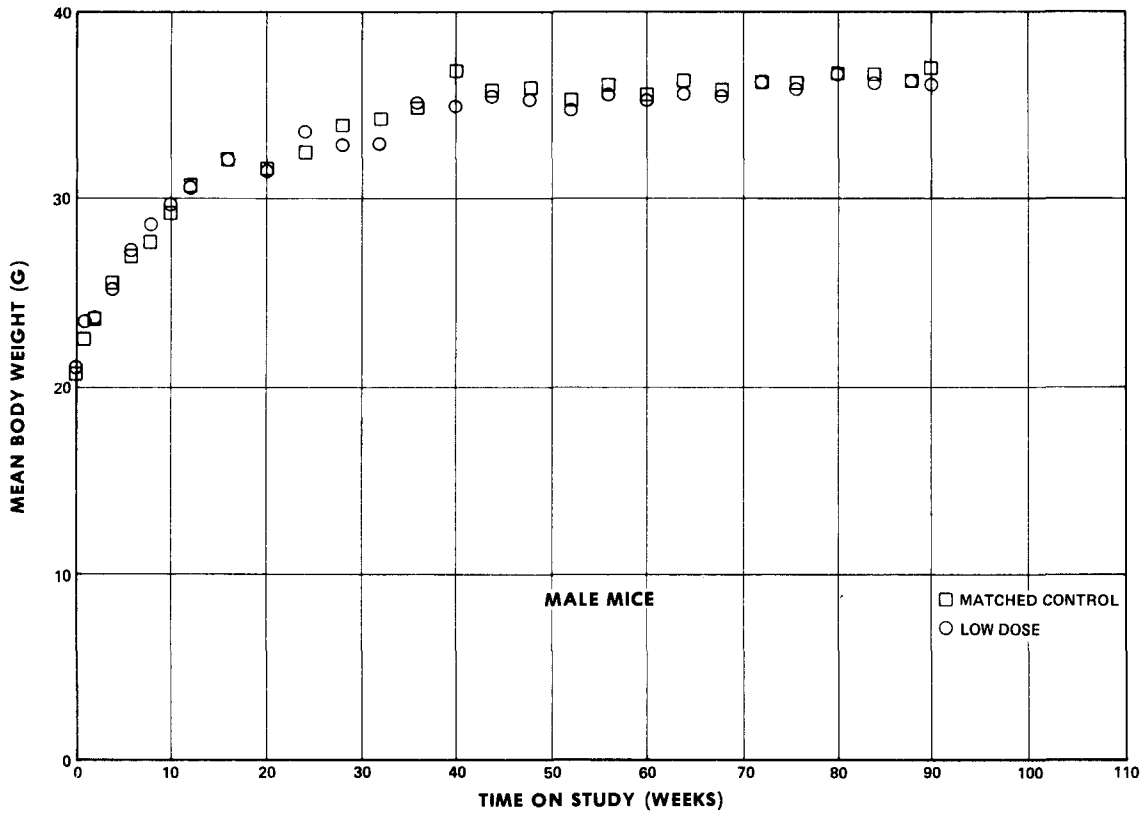
A. Body Weights and Clinical Signs (Mice)

There were no perceptible differences between the average body weights of the treated mice and their corresponding controls (figures 3 and 4).

During the first year of study, the appearance and behavior of the treated and control mice were generally comparable. Alopecia (generalized and/or localized) and sores on the body were noted in a number of mice in the treated and control groups. After 52 weeks of treatment, bloating or abdominal distention and alopecia were the predominant observations in the high-dose females. Rough coats, alopecia, sores, and palpable masses were noted in an increasing number of mice in the treated and control groups during the remainder of the study.

B. Survival (Mice)

Curves for the probability of survival of treated and control mice are shown in figures 5 and 6. For male mice there were no significant differences in survival between the high-dose group and its control or the low-dose group and its control. In fact, in each case the control groups had survival rates slightly lower than those of the treated groups. In the female mice there is a



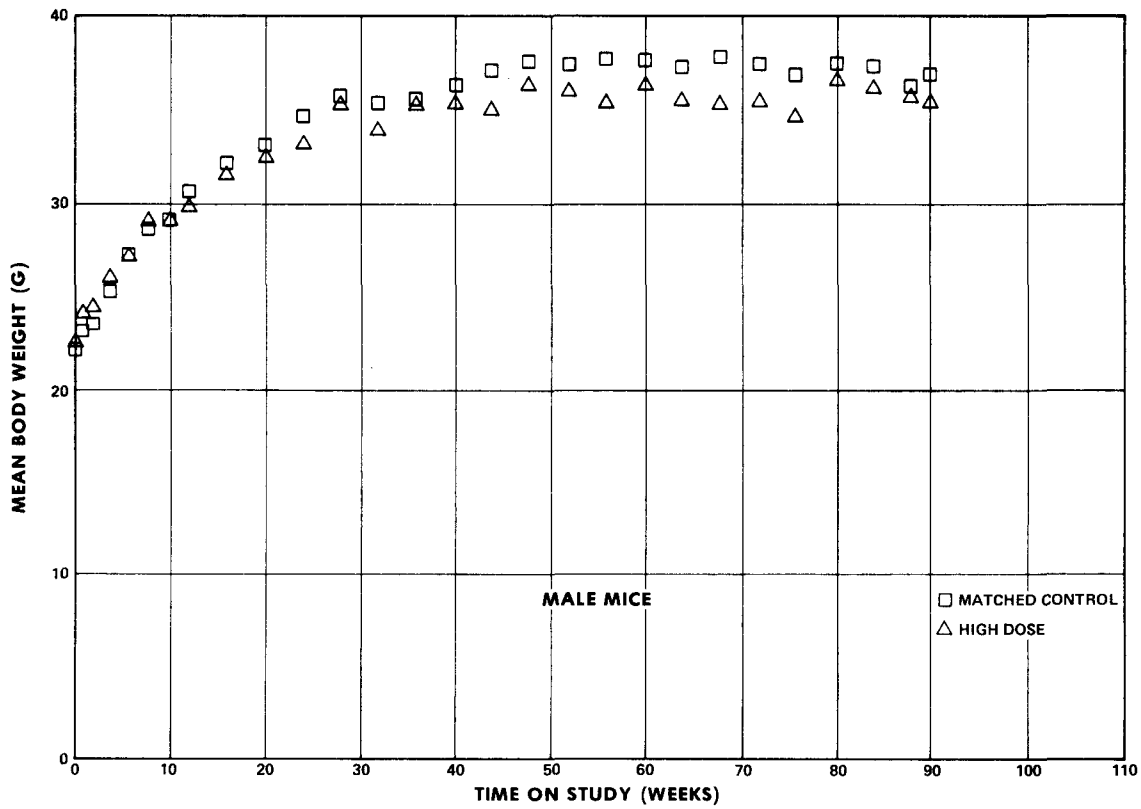


Figure 3. Growth Curves for Male Mice Fed Heptachlor in the Diet

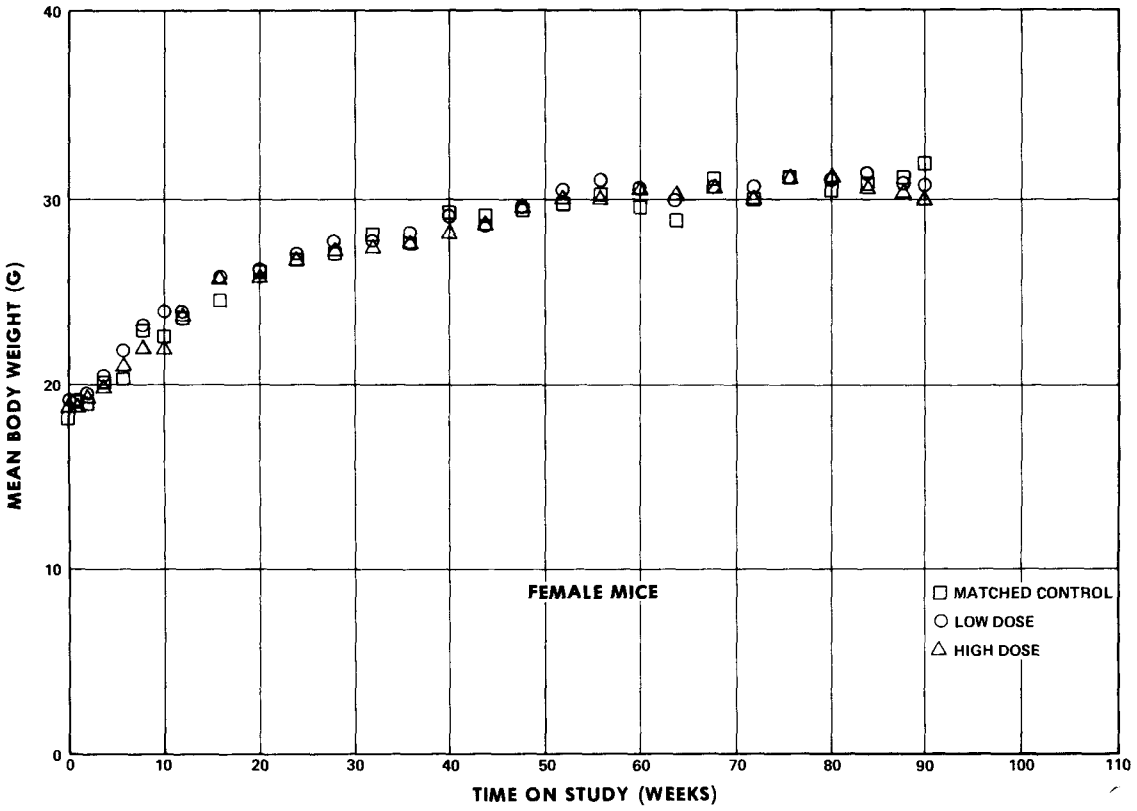
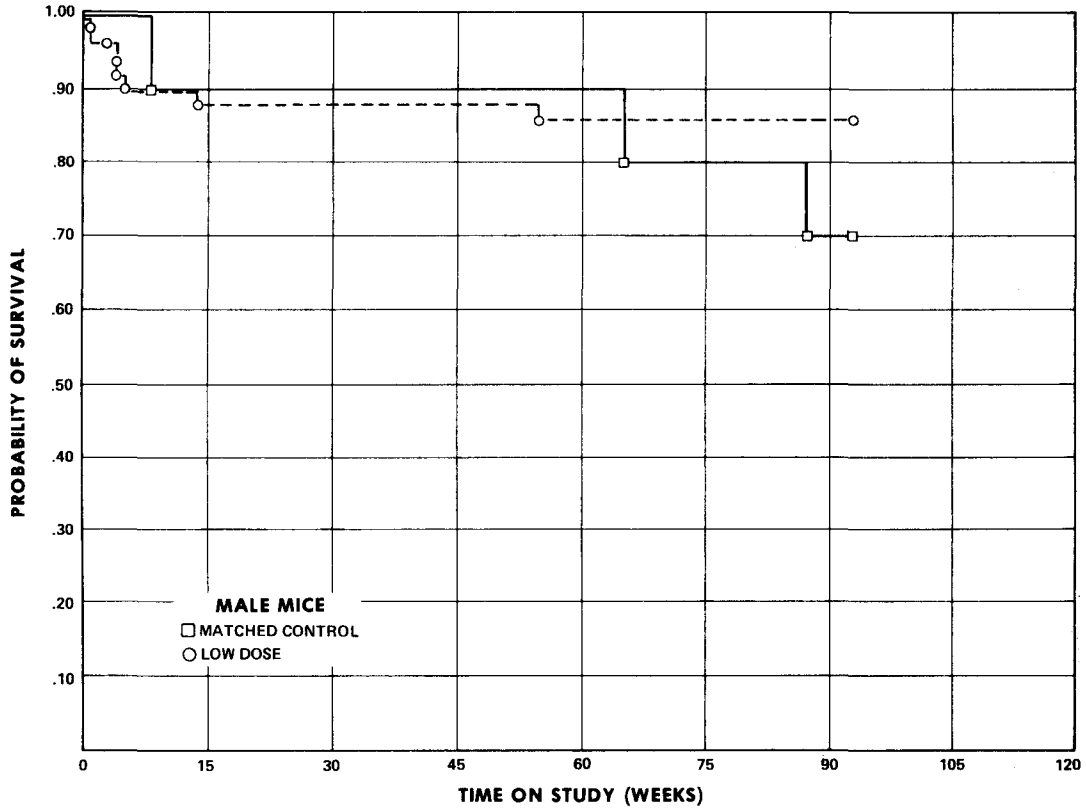


Figure 4. Growth Curves for Female Mice Fed Heptachlor in the Diet



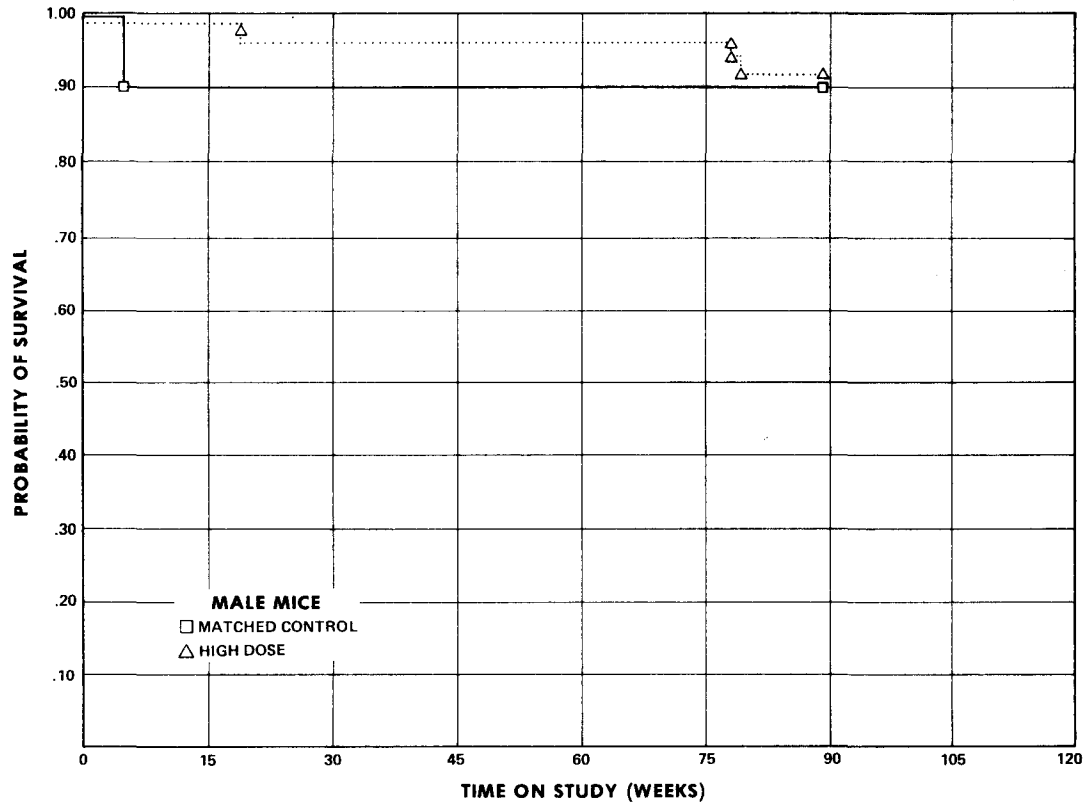


Figure 5. Survival Curves for Male Mice Fed Heptachlor in the Diet

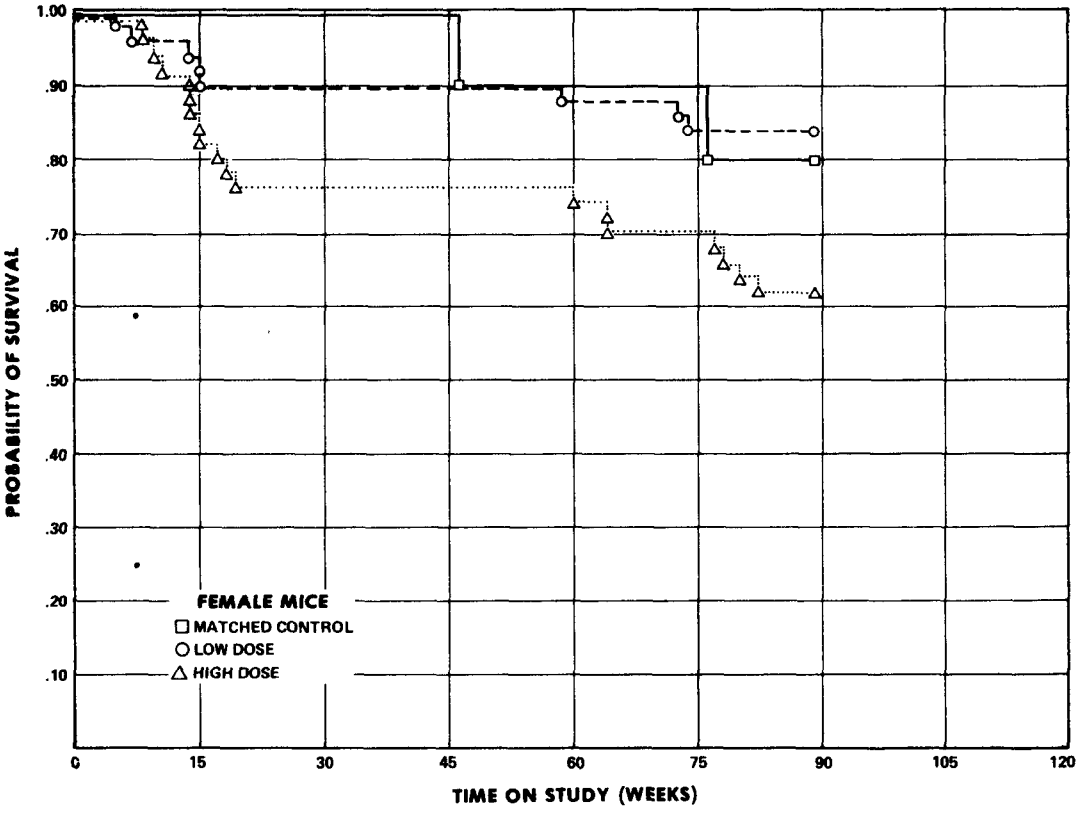


Figure 6. Survival Curves for Female Mice Fed Heptachlor in the Diet

significant positive linear trend (P = 0.02), which is mainly a reflection of the difference between the high- and low-dose survivorship. This may indicate that heptachlor was toxic at the high dose in female mice.

C. Pathology (Mice)

Histopathologic findings are summarized in Appendix B, tables B1-B3, covering neoplasms and other proliferative lesions, and in Appendix D, tables D1 and D2, covering nonneoplastic lesions.

The most frequently occurring neoplasm was hepatocellular carcinoma; the incidence of this and other proliferative hepatocytic lesions is summarized in table B2. The morphology of those lesions classified as hepatocellular carcinoma varied widely. Some were present as one or more discrete nodules containing solid cords and nests of well-differentiated but hyperbasophilic hepatocytes with an increased nuclear:cytoplasmic ratio. These lesions appeared to have enlarged by expansion, with distinct compression but no obvious invasion of adjacent normal hepatic parenchyma. Other hepatic neoplasms appeared as very large masses which had completely replaced one or more hepatic lobes and were composed of large, anaplastic hepatocytes, forming confluent sheets, papillae, and pseudoacini, with large foci of necropsis and complete loss of normal lobular architecture. The morphologic appearance of the majority of

hepatocellular carcinomas fell somewhere between these two extremes. Pulmonary metastasis of hepatocellular carcinoma was observed in one low-dose male mouse.

Hepatic lesions that were classified as nodular hyperplasia consisted of small nodules of proliferating hepatocytes, which compressed adjacent hepatic parenchyma but which did not have sufficient abnormality of cellular morphology or lobular architecture to warrant diagnosis of neoplasia.

There was a striking increase in the incidence of hepatocellular carcinoma in mice fed the high-dose concentration of hepatachlor when compared with control groups: (34/47 [72%] in high-dose males, 17/92 [18%] in pooled-control males, and 5/19 [26%] in matched-control males; 30/42 [71%] in high-dose females, 3/78 [4%] in pooled-control females, and 2/10 [20%] in matched-control females). There was no apparent increase in the incidence of this tumor in low-dose mice. Nevertheless, it seems apparent that hepatchlor fed at the higher dosage concentration used in this study induced a distinct increase in the incidence of hepatocellular carcinoma in mice.

There was a low incidence of other type of neoplasms involving various tissues (table B3), with no striking differences in incidence between test and control groups.

Suppurative inflammation involving the ovaries, oviducts, and endometrium, in some cases associated with cystic hyperplasia of the endometrium, occurred frequently in both test and control female mice. There was a low incidence of other inflammatory, degenerative, and nonneoplastic proliferative lesions in test and control mice of both sexes (Appendix D).

D. Statistical Analyses of Results (Mice)

Statistical analyses of neoplasms in mice fed heptachlor are given in Appendix F, tables F1-F4.

The small size of the control group notwithstanding, a highly significant difference in hepatocellular carcinoma (table F1) was found between the high-dose male group and its control by the two simple proportion analyses (P = 0.001 or P = 0.0007) and by the life-table adjusted analysis (P = 0.002). The low-dose male group had a somewhat smaller percentage of animals with hepatocellular carcinoma animals than the male controls, which appear to have had an unusually large number of tumors. However, this difference is not significant by any of the tests, although the life-table adjusted test approaches a significantly negative result (P = 0.05+).

Comparison of the incidence in treated male mice with the pooled controls reinforced the positive findings (table F2). The pooled

controls showed a lower proportion of hepatocellular carcinomas than the low-dose group, although not significantly lower. Statistical tests on hepatocellular carcinoma in the high-dose group yielded smaller P values using pooled controls than using matched controls (table F2).

In female mice highly significant linear trends ($P < 0.0001$) were found for hepatocellular carcinoma by all tests which were applied (table F3). This effect reflected mainly the difference between the high- and low-dose groups, since the data departed significantly from a linear trend. However, when the incidence in the high-dose group was compared directly to that in the matched control, significance was still found by the simple proportion analyses ($P < 0.005$). The pooled-control analyses reinforced these results (table F4).

At no other site was a sufficient number of animals with tumors found to warrant statistical analysis.

V. DISCUSSION

Heptachlor is a member of the organochlorine group of pesticides classed as neurotoxins, which in high doses produce CNS stimulation; however, neither the hyperexcitability observed in bioassays of endrin nor the tremors observed with chlordane were reported in the present study with heptachlor.

The effects of heptachlor on body weights and other clinical signs in rats and mice indicated that the dosages used were near the maximum permissible. This was evident in that average body weights of rats treated with high doses were consistently lower than those of untreated controls, while body weights of low-dose rats were unaffected. Adverse clinical signs in treated rats included rough and discolored hair coats and, in females, vaginal bleeding. Body weights of mice given either low or high doses showed little or no differences from those of control mice; however, adverse clinical signs of alopecia and abdominal distention were noted, predominantly in high-dose females. Doses were reduced during the course of the tests in an effort to minimize toxic effects.

The effects of heptachlor on survival rates indicated that in both male and female rats, mortality was affected more adversely by the high dose than by the low dose, but that a statistically

significant dose-related trend was found only in the females. Survival in male mice was unaffected by either low or high doses, but survival in female mice showed a statistically significant dose-related decrease, due mainly to the effect of the high dose. The fact that greater toxicity was observed in the high-dose females than in the high-dose males may have been due to the higher total dose received by the females. A substantial proportion of all groups survived, however, to an age at which tumors could be expected to appear.

Hepatocellular carcinoma was induced at a highly significant rate of incidence in mice given the high dose of heptachlor (Appendix F, tables F1 and F2), using the Fisher exact test either with matched or pooled controls. At the low dose, the incidence of proliferative lesions of the liver was significant only in male mice, using pooled controls, and then only when carcinoma was combined with nodular hyperplasia. Dose-related trends, however, were highly significant for both males and females, using matched controls for comparison; when pooled controls were used, the significance of the trend in the males increased to the very high levels shown by the females. Comparably high levels of significance of dose-related trends were also attained when hepatocellular carcinoma was combined with nodular hyperplasia or when the data were subjected to life-table adjustment.

In contrast to findings with mice, no hepatocellular carcinomas were induced by heptachlor in rats (Appendix E, tables E1 and E2). Further, the incidence of proliferative lesions of the liver in rats did not become statistically significant when hepatocellular carcinoma was combined with neoplastic nodules or when life-table adjustment was applied to the data.

A statistically significant dose-related trend in proliferative follicular-cell lesions of the thyroid was found, however, in the rats (table E3). Although the trends for follicular-cell carcinoma alone were not significant in either males or females, the trend for follicular-cell carcinoma combined with adenoma was significant for the females. The trend for follicular-cell lesions remained significant when pooled controls (table E4) were used instead of matched controls and when the data were subjected to life-table adjustment. The male rats showed a higher incidence of follicular-cell lesions in the low-dose animals than in the controls, while the incidence in the high-dose animals was similar to that in the controls (tables E1 and E4). C-cell carcinomas and/or adenomas showed a significantly negative dose-related trend in female rats, using matched controls (table E5). In the judgment of the pathologist, the nature, incidence, and severity of the proliferative thyroid lesions were not sufficient to indicate clearly a carcinogenic effect of heptachlor in rats.

The metabolism of heptachlor in the rat consists first of transformation to heptachlor epoxide, which is stored in body fat (Davidow and Radomski, 1953; O'Brien, 1967). The epoxide has been found in extremely low concentrations in the urine of the general human population (Cueto and Biros, 1967). Dehydrochlorination of the epoxide, followed by hydroxylation and double-bond rearrangement, produces a metabolite that is the principle form in which heptachlor is excreted in the feces (Matsumura and Nelson, 1970).

Unpublished reports on long-term feeding of rats with diets containing heptachlor and/or heptachlor epoxide were reviewed in an IARC monograph (1974). In three of five such studies, rats received diets containing 12.5 ppm of the test material for at least 2 years but showed no increase in tumor incidence attributable to treatment. In a fourth study, only an increase in liver weight was reported for rats receiving 10 and 20 ppm heptachlor. In a fifth study with rats, the incidences of tumors in the animals given 0.5 to 10 ppm (65/114 [57%] in males; 92/114 [81%] in females) were greater than in controls (8/23 [35%] in males; 13/24 [54%] in females), with most tumors located in endocrine organs and with liver tumors appearing in 7 males and 12 females but not in the controls. In a study with C3Heb/Fe/J mice, the feeding of heptachlor or heptachlor epoxide at a level

of 10 ppm in the diet has been reported to bring about an increase in liver tumors including carcinomas (EPA, 1974).

In the present bioassay, heptachlor was hepatocarcinogenic in mice but not in rats. In rats, heptachlor induced a statistically significant increase in proliferative lesions of thyroid follicular cells. The relationship of these lesions to administration of the compound is not clear, since the spontaneous incidence of the lesions has varied throughout the bioassay program.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS AND OTHER PROLIFERATIVE LESIONS IN RATS FED HEPTACHLOR IN THE DIET

TABLE A1

SUMMARY OF THE DISPOSITION OF TISSUES

	MALE RATS				FEMALE RATS			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
Animals Initially in Study	60	10	50	50	60	10	49[a]	50
Animals Necropsied	58	10	45	50	60	10	48	48
Animals Examined Histopathologically	58	10	45	49	60	10	48	48

[a]One animal in this group was found to be a male at necropsy; data from this animal was not included in this report.

TABLE A2

PROLIFERATIVE LESIONS OF THE THYROID[a]

	MALE RATS				FEMALE RATS			
	Pooled Control (51)	Matched Control (8)	Low Dose (38)	High Dose (38)	Pooled Control (58)	Matched Control (9)	Low Dose (43)	High Dose (38)
Follicular-cell Carcinoma	1	0	4	0	1	1	2	5
Follicular-cell Adenoma	3	1	7	3	2	0	1	10
C-cell Carcinoma	1	0	3	1	5	1	0	0
C-cell Adenoma	3	0	4	1	7	2	7	3
Follicular-cell Hyperplasia	0	0	2	2	4	1	4	4
C-cell Hyperplasia	29	3	13	21	30	4	21	14

[a]Numbers in parentheses represent the numbers of tissues examined microscopically.

TABLE A3

OTHER NEOPLASMS OF THE ENDOCRINE SYSTEM[a]

	MALE RATS				FEMALE RATS			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
ADRENAL	(55)	(9)	(42)	(41)	(56)	(9)	(45)	(40)
Pheochromocytoma	1	1	1	0	0	0	0	1
Cortical Carcinoma	1	1	0	0	0	0	0	0
Cortical Adenoma	2	0	1	1	0	0	0	2
PITUITARY	(48)	(7)	(42)	(34)	(52)	(6)	(39)	(34)
Adenoma	14	4	8	8	23	3	9	8
PANCREATIC ISLETS	(52)	(9)	(40)	(42)	(60)	(10)	(41)	(41)
Carcinoma	0	0	0	0	0	0	0	1
Adenoma	1	0	2	1	1	0	3	0
PARATHYROID	(38)	(3)	(22)	(25)	(38)	(5)	(32)	(21)
Adenoma	2	0	0	0	0	0	1	0

[a]Numbers in parentheses represent the numbers of tissues examined microscopically.

TABLE A4

NEOPLASMS OF THE DIGESTIVE SYSTEM[a]

	MALE RATS				FEMALE RATS			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
LIVER	(58)	(10)	(44)	(49)	(59)	(10)	(48)	(46)
Cholangiocarcinoma	0	0	1	0	0	0	0	0
Neoplastic Nodule	2	1	3	6	5	1	9	5
SALIVARY GLAND	(56)	(9)	(42)	(44)	(60)	(10)	(47)	(42)
Carcinoma, N.O.S.	0	0	0	1	0	0	0	0
Fibroma	0	0	0	0	0	0	1	0

[a]Numbers in parentheses represent the numbers of tissues examined microscopically.

TABLE A5

NEOPLASMS OF THE REPRODUCTIVE SYSTEM AND MAMMARY GLAND[a]

	MALE RATS				FEMALE RATS			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
UTERUS	---	---	---	---	(56)	(10)	(43)	(41)
Endometrial Stromal Sarcoma					0	0	0	2
Endometrial Stromal Polyp					6	2	7	6
OVARY	---	---	---	---	(58)	(9)	(45)	(43)
Cystadenocarcinoma					0	0	1	0
Granulosa-cell Tumor					1	0	2	0
MAMMARY GLAND	(58)[b]	(10)[b]	(45)[b]	(50)[b]	(60)[b]	(10)[b]	(48)[b]	(48)[b]
Carcinoma	0	0	1	0	2	1	1	1
Adenoma	0	0	0	0	1	1	4	1
Fibrosarcoma	0	0	0	1	0	0	0	0

TABLE A5

NEOPLASMS OF THE REPRODUCTIVE SYSTEM AND MAMMARY GLAND[a]

(Continued)

	MALE RATS				FEMALE RATS			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
MAMMARY GLAND (Continued)	(58)[b]	(10)[b]	(45)[b]	(50)[b]	(60)[b]	(10)[b]	(48)[b]	(48)[b]
Sarcoma, N.O.S.	0	0	0	0	0	0	1	0
Fibroadenoma	0	0	0	0	8	1	10	5
Fibroma	1	0	3	0	1	0	3	1
Lipoma	0	0	1	0	0	0	0	0

[a]Numbers in parentheses represent the numbers of tissues examined microscopically.

[b]The adjacent number in parentheses represents the number of animals necropsied in that group, rather than the number of tissues examined microscopically.

TABLE A6

NEOPLASMS OF THE CARDIOVASCULAR SYSTEM[a]

	MALE RATS				FEMALE RATS			
	Pooled Control (58)[b]	Matched Control (10)[b]	Low Dose (45)[b]	High Dose (50)[b]	Pooled Control (60)[b]	Matched Control (10)[b]	Low Dose (48)[b]	High Dose (48)[b]
Hemangiosarcoma	3	0	0	1	0	0	1	0
HEART	(58)	(10)	(44)	(49)	(57)	(10)	(48)	(44)
Sarcoma, N.O.S.	2	0	0	0	1	0	2	0
Fibroma	1	1	0	0	0	0	0	0

[a]Numbers in parentheses represent the numbers of tissues examined microscopically.

[b]The adjacent number in parentheses represents the number of animals necropsied in that group, rather than the number of tissues examined microscopically.

TABLE A7

NEOPLASMS OF THE HEMATOPOIETIC SYSTEM[a]

	MALE RATS				FEMALE RATS			
	Pooled Control (58)[b]	Matched Control (10)[b]	Low Dose (45)[b]	High Dose (50)[b]	Pooled Control (60)[b]	Matched Control (10)[b]	Low Dose (48)[b]	High Dose (48)[b]
Monocytic Leukemia	0	0	0	0	1	1	0	0
Thymoma	0	0	0	1	0	0	0	0

[a]Numbers in parentheses represent the numbers of tissues examined microscopically.

[b]The adjacent number in parentheses represents the number of animals necropsied in that group, rather than the number of tissues examined microscopically.

TABLE A8

MISCELLANEOUS NEOPLASMS[a]

	MALE RATS				FEMALE RATS			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
SUBCUTIS	(58)[b]	(10)[b]	(45)[b]	(50)[b]	(60)[b]	(10)[b]	(48)[b]	(48)[b]
Malignant Fibrous Histiocytoma	1	0	1	0	0	0	0	0
Fibrosarcoma	0	0	1	0	0	0	0	1
Leiomyoma	0	0	1	0	0	0	0	0
Fibroma	0	0	0	1	1	1	0	0
LUNG	(58)	(10)	(44)	(47)	(58)	(10)	(47)	(46)
Adenoma	0	0	1	0	0	0	0	0
BRAIN	(57)	(10)	(43)	(47)	(59)	(10)	(48)	(46)
Meningioma	0	0	0	0	0	0	1	0

[a]Numbers in parentheses represent the numbers of tissues examined microscopically.

[b]The adjacent number in parentheses represents the number of animals necropsied in that group, rather than the number of tissues examined microscopically.

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS AND OTHER PROLIFERATIVE LESIONS IN MICE FED HEPTACHLOR IN THE DIET

TABLE B1

SUMMARY OF THE DISPOSITION OF TISSUES

	MALE MICE				FEMALE MICE			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
Animals Initially in Study	100	20	50	48	80	10	50	50
Animals Necropsied	92	20	47	47	79	10	48	45
Animals Examined Histopathologically	92	19	46	47	79	10	48	43

TABLE B2

PROLIFERATIVE LESIONS OF THE LIVER[a]

	MALE MICE				FEMALE MICE			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
LIVER	(92)	(19)	(46)	(47)	(78)	(10)	(47)	(42)
Hepatocellular Carcinoma	17	5	11	34	3	2	3	30
Nodular Hyperplasia	3	1	9	6	2	0	3	0
Diffuse Hyperplasia	3	0	1	3	1	0	0	0
Hepatocytomegaly	0	0	1	0	1	0	0	1

[a]Numbers in parentheses represent the numbers of tissues examined microscopically.

TABLE B3

MISCELLANEOUS NEOPLASMS[a]

	MALE MICE				FEMALE MICE			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM	(92)[b]	(20)[b]	(47)[b]	(47)[b]	(79)[b]	(10)[b]	(48)[b]	(45)[b]
Malignant Lymphoma[c]	5	1	0	0	8	2	4	1
LUNG	(91)	(19)	(46)	(47)	(79)	(10)	(47)	(42)
Alveolar/Bronchiolar Adenoma	6	1	1	7	0	0	1	1
UTERUS	--	--	--	--	(78)	(10)	(47)	(37)
Adenocarcinoma	--	--	--	--	0	0	1	0
OVARY	--	--	--	--	(73)	(8)	(45)	(35)
Luteoma	--	--	--	--	1	1	0	0
THYROID	(79)	(14)	(32)	(36)	(72)	(8)	(33)	(22)
Follicular-cell Adenoma	1	0	0	0	1	0	1	0

TABLE B3

MISCELLANEOUS NEOPLASMS[a]

(Continued)

	MALE MICE				FEMALE MICE			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
PITUITARY	(70)	(12)	(16)	(19)	(58)	(5)	(20)	(12)
Adenoma	0	0	0	0	0	0	1	0
ADRENAL CORTEX	(88)	(17)	(41)	(44)	(78)	(9)	(39)	(36)
Carcinoma		0	0	0		0	1	0

[a]Numbers in parentheses represent the numbers of tissues examined microscopically.

[b]The adjacent number in parentheses represents the number of animals necropsied in that group, rather than the number of tissues examined microscopically.

[c]For purposes of this summary, the following lesions are grouped under "Malignant Lymphoma". Lymphosarcoma (of any site or multiple sites), lymphocytic lymphosarcomas, and reticulum cell sarcoma.

APPENDIX C

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

TABLE C1

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10 (100%)	45 (100%)	50 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	45	50
ANIMALS WITH NON-TUMOR PATHOLOGY	9 (90%)	43 (96%)	44 (88%)
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM *	2 (20%)	16 (36%)	18 (36%)
TRACHEA		1	
INFLAMMATION CHRONIC		1	
LUNG	2	15	18
EMPHYSEMA		1	
EDEMA			1
INFLAMMATION		2	
INFLAMMATION FOCAL		1	
INFLAMMATION CHRONIC			1
ALVEOLAR MACROPHAGES	2	15	18
LUNG/ALVEOLI			1
INFLAMMATION FOCAL			1
CIRCULATORY SYSTEM	5 (50%)	14 (31%)	16 (32%)
MYOCARDIUM	5	14	16
INFLAMMATION FOCAL	1		
INFLAMMATION INTERSTITIAL		1	2
FIBROSIS	4	14	15
FIBROSIS FOCAL			1
CALCIFICATION		1	
DIGESTIVE SYSTEM	9 (90%)	39 (87%)	34 (68%)
SALIVARY GLAND	1		
DILATATION/DUCTS	1		

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C1 MALE RATS: NONNEOPLASTIC LESIONS (CONT.)

	CONTROL	LOW DOSE	HIGH DOSE
LIVER	7	20	21
CONGESTION		3	
HEMORRHAGE	1		
PERIARTERITIS			1
METAMORPHOSIS FATTY		2	4
HEPATOCYTOMEGALY	4	18	17
ANGIECTASIS	4	4	8
BILE DUCT	8	32	25
DILATATION		2	
INFLAMMATION	8	18	21
FIBROSIS	2	4	8
HYPERPLASIA	5	26	19
PANCREAS	1	6	5
FIBROSIS		5	3
PERIARTERITIS	1	1	2
PANCREATIC ACINUS	2	1	3
ATROPHY	2	1	3
STOMACH	2		
ULCER	1		
NECROSIS FOCAL	1		
GASTRIC MUCOSA	1	1	1
CALCIFICATION		1	1
CALCIFICATION DYSTROPHIC	1		
SMALL INTESTINE		1	
HEMORRHAGE		1	
INFLAMMATION		1	
URINARY SYSTEM *	8 (80%)	39 (87%)	39 (78%)
KIDNEY	8	39	39
INFLAMMATION CHRONIC	8	39	39
METAMORPHOSIS FATTY		1	
KIDNEY/PELVIS		5	5
HYPERPLASIA EPITHELIAL		5	5
URINARY BLADDER	1	5	2
INFLAMMATION CHRONIC	1		
HYPERPLASIA EPITHELIAL	1	5	2

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C1 MALE RATS: NONNEOPLASTIC LESIONS (CONT.)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM *	7 (70%)	27 (60%)	29 (58%)
PITUITARY	5	6	6
CYST	1	2	2
CONGESTION			1
HYPERPLASIA CHROMOPHOBE-CELL		1	1
ANGIECTASIS	4	3	3
ADRENAL			1
CYTOMEGALY			1
ADRENAL CORTEX	1	5	7
CYTOMEGALY	1	5	7
THYROID	3	17	26
ULTIMOBRANCHIAL CYST			2
CYSTIC FOLLICLES		3	4
HEMORRHAGE			1
HYPERPLASIA C-CELL	3	13	21
HYPERPLASIA FOLLICULAR-CELL		2	1
PARATHYROID	1	2	1
HYPERPLASIA	1	2	1
PANCREATIC ISLETS			1
HYPERPLASIA			1
HEMATOPOIETIC SYSTEM	1 (10%)	6 (13%)	4 (8%)
SPLEEN	1	5	4
CONGESTION		2	
HYPEREMIA		1	
INFLAMMATION CHRONIC		2	
INFLAMMATION FOCAL GRANULOMATOUS			1
HEMOSIDEROSIS	1		3
LYMPHOID HYPERPLASIA		1	
HEMATOPOIESIS		2	
CERVICAL LYMPH NODE		2	
DILATATION		1	
LYMPHOID HYPERPLASIA		1	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C1 MALE RATS: NONNEOPLASTIC LESIONS (CONT.)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS *		9 (20%)	10 (20%)
ABDOMINAL CAVITY		1	
NECROSIS FAT		1	
PERITONEUM		1	
INFLAMMATION		1	
NO ASSOCIATED ORGAN		7	6
NO LESION REPORTED		2	5
AUTO/NECROPSY PERF/HISTO PERF			1
AUTOLYSIS/NO NECROPSY PERFORMED		5	
ADIPOSE TISSUE			1
INFLAMMATION GRANULOMATOUS			1
MESENTERY		1	3
PERIARTERITIS		1	3

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONT.)

	CONTROL	LOW DOSE	HIGH DOSE
LIVER (CONT.)			
INFLAMMATION SUPPURATIVE			1
FIBROSIS		1	
NECROSIS			1
METAMORPHOSIS FATTY		2	4
HEPATOCYTOMEGALY	2	16	15
ATROPHY			1
HYPERPLASIA FOCAL			1
ANGIECTASIS	1	23	21
BILE DUCT	6	21	34
DILATATION	1	1	2
INFLAMMATION	5	13	21
FIBROSIS	1	6	6
HYPERPLASIA	4	17	24
PANCREAS		1	6
FIBROSIS		1	4
PERIARTERITIS			2
PANCREATIC ACINUS		3	5
ATROPHY		3	5
STOMACH		1	
ULCER FOCAL		1	
CALCIFICATION		1	
URINARY SYSTEM *	8 (80%)	34 (69%)	31 (65%)
KIDNEY	8	30	31
INFLAMMATION CHRONIC	8	31	31
METAMORPHOSIS FATTY			1
KIDNEY/PELVIS	1	7	5
HYPERPLASIA EPITHELIAL	1	7	5
URINARY BLADDER	1	3	1
INFLAMMATION CHRONIC		1	
HYPERPLASIA EPITHELIAL	1	3	1

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONT.)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM *	5 (50%)	29 (59%)	27 (56%)
PITUITARY	1	3	8
HYPERPLASIA		1	1
HYPERPLASIA CHROMOPHOBE-CELL			2
ANGIECTASIS	1	3	6
ADRENAL		1	3
METAMORPHOSIS FATTY			2
CYTOMEGALY		1	1
ADRENAL CORTEX	1	9	8
CYTOMEGALY	1	9	8
THYROID	4	23	15
ULTIMOBRANCHIAL CYST		2	3
CYSTIC FOLLICLES	1	1	3
INFLAMMATION ACUTE			1
HYPERPLASIA C-CELL	4	21	14
HYPERPLASIA FOLLICULAR-CELL	1	4	4
HEMATOPOIETIC SYSTEM	2 (20%)	7 (14%)	8 (17%)
SPLEEN	2	7	7
INFLAMMATION FOCAL GRANULOMATOUS		1	
HEMOSIDEROSIS	1	3	1
ANGIECTASIS		1	
HEMATOPOIESIS	1	2	6
CERVICAL LYMPH NODE			1
LYMPHOID HYPERPLASIA			1
PELVIC LYMPH NODE			1
INFLAMMATION CHRONIC			1
ANGIECTASIS			1

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONT.)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM *		2 (4%)	4 (8%)
UTERUS		1	2
HEMORRHAGE			1
ULCER		1	1
INFLAMMATION FOCAL		1	
INFLAMMATION SUPPURATIVE			1
UTERUS/ENDOMETRIUM		1	2
CYST			1
HYPERPLASIA CYSTIC		1	1
NERVOUS SYSTEM	1 (10%)		
BRAIN	1		
HEMORRHAGE	1		
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS		3 (6%)	8 (17%)
NO ASSOCIATED ORGAN		2	8
NO LESION REPORTED		1	6
AUTOLYSIS/NO NECROPSY PERFORMED		1	2
MESENTERY		1	
PERIARTERITIS		1	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

APPENDIX D

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

TABLE D1

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

	HIGH DOSE CONTROL	LOW DOSE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	54	48
ANIMALS NECROPSIED	10 (100%)	10 (100%)	47 (100%)	47 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	10	46	47
ANIMALS WITH NON-TUMOR PATHOLOGY	3 (30%)	3 (30%)	21 (45%)	10 (21%)
INTEGUMENTARY SYSTEM *	1 (10%)		1 (2%)	
SKIN	1		1	
ULCER	1			
INFLAMMATION GRANULOMATOUS			1	
RESPIRATORY SYSTEM	1 (10%)	1 (10%)	4 (9%)	1 (2%)
LUNG/BRONCHUS			1	
INFLAMMATION FOCAL			1	
LUNG/BRONCHIOLE			1	
INFLAMMATION SUPPURATIVE			1	
LUNG	1	1	2	1
INFLAMMATION FOCAL	1			
ALVEOLAR MACROPHAGES			2	
HYPERPLASIA ALVEOLAR-CELL		1		1
LUNG/ALVEOLI	1		1	
INFLAMMATION	1			
INFLAMMATION SUPPURATIVE			1	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM	1 (10%)	1 (10%)	13 (28%)	8 (17%)
LIVER	1	1	13	8
INFLAMMATION FOCAL			1	
DEGENERATION			1	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE D1 MALE MICE: NONNEOPLASTIC LESIONS (CONT.)

	HIGH DOSE CONTROL	LOW DOSE CONTROL	LOW DOSE	HIGH DOSE
LIVER (CONT.)				
METAMORPHOSIS FATTY	1			
HEPATOCYTOMEGALY			1	
HYPERPLASIA NODULAR		1	9	6
HYPERPLASIA DIFFUSE			1	3
URINARY SYSTEM *			6 (13%)	
KIDNEY			6	
LYMPHOCYTIC INFLAM INFILTRATE			4	
INFLAMMATION CHRONIC			2	
ENDOCRINE SYSTEM			1 (2%)	2 (4%)
THYROID			1	2
HYPERPLASIA C-CELL			1	
HYPERPLASIA FOLLICULAR-CELL				2
HEMATOPOIETIC SYSTEM			1 (2%)	
MESENTERIC LYMPHNODE			1	
CONGESTION			1	
EDEMA			1	
HYPERPLASIA RETICULUM-CELL			1	
REPRODUCTIVE SYSTEM			1 (2%)	
TESTIS			1	
ATROPHY			1	
NERVOUS SYSTEM		1 (10%)		
BRAIN		1		
CORPORA AMYLACEA		1		
MUSCULOSKELETAL SYSTEM				
NONE				

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE D1 MALE MICE: NONNEOPLASTIC LESIONS (CONT.)

	HIGH DOSE CONTROL	LOW DOSE CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS				
NONE				
ALL OTHER SYSTEMS *	5 (50%)	3 (30%)	20 (43%)	6 (13%)
NO ASSOCIATED ORGAN	5	3	20	6
NO LESION REPORTED	4	3	16	5
AUTOLYSIS/NECROPSY PERF/NO HISTO	1		1	
AUTOLYSIS/NO NECROPSY PERFORMED			3	1

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE D2

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED HEPTACHLOR IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10 (100%)	48 (100%)	45 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	48	43
ANIMALS WITH NON-TUMOR PATHOLOGY	6 (60%)	25 (52%)	12 (27%)
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM *			1 (2%)
LUNG			1
INFLAMMATION CHRONIC			1
CIRCULATORY SYSTEM	1 (10%)		
ARTERY	1		
INFLAMMATION	1		
DIGESTIVE SYSTEM	1 (10%)	6 (13%)	4 (9%)
LIVER	1	3	2
HEMORRHAGE	1		
NECROSIS	1		
HEPATOCYTOMEGALY			1
HYPERPLASIA NODULAR		3	
NODULAR REGENERATION			1
LIVER/CAUDATE LOBE			1
NECROSIS			1
BILE DUCT			1
HYPERPLASIA			1
PANCREAS		3	
INFLAMMATION CHRONIC		1	
INFLAMMATION GRANULOMATOUS		2	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE D2 FEMALE MICE: NONNEOPLASTIC LESIONS (CONT.)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM *	1 (10%)	7 (15%)	2 (4%)
KIDNEY	1	7	2
INFLAMMATION			1
LYMPHOCYTIC INFLAM INFILTRATE		6	
INFLAMMATION CHRONIC	1	1	1
ENDOCRINE SYSTEM		3 (6%)	2 (4%)
THYROID		3	2
INFLAMMATION CHRONIC		1	
HYPERPLASIA C-CELL		1	
HYPERPLASIA FOLLICULAR-CELL		1	2
HEMATOPOIETIC SYSTEM			2 (4%)
SPLEEN			2
LYMPHOID HYPERPLASIA			2
LYMPH NODE			1
HYPERPLASIA RETICULUM-CELL			1
REPRODUCTIVE SYSTEM	3 (30%)	18 (38%)	6 (13%)
UTERUS			2
INFLAMMATION SUPPURATIVE			1
INFLAMMATION ACUTE			1
UTERUS/ENDOMETRIUM	2	10	2
INFLAMMATION SUPPURATIVE		5	1
ABSCESS		1	
HYPERPLASIA CYSTIC	2	4	1
DECIDUA		1	
OVARY/OVIDUCT			1
INFLAMMATION SUBACUTE			1
OVARY	1	13	3
FOLLICULAR CYST		1	
INFLAMMATION		2	
INFLAMMATION SUPPURATIVE	1	9	2
INFLAMMATION ACUTE SUPPURATIVE			1
INFLAMMATION SUBACUTE		1	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE D2 FEMALE MICE: NONNEOPLASTIC LESIONS (CONT.)

	CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM *	2 (20%)	1 (2%)	
BRAIN/MENINGES		1	
INFLAMMATION		1	
BRAIN	2		
CORPORA AMYLACEA	2		
MUSCULOSKELETAL SYSTEM			2 (4%)
BONE			1
FIBROSIS			1
SKELETAL MUSCLE			1
INFLAMMATION ACUTE			1
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS	2 (20%)	19 (40%)	14 (31%)
NO ASSOCIATED ORGAN	2	19	14
NO LESION REPORTED	2	17	7
AUTOLYSIS/NECROPSY PERF/NO HISTO			2
AUTOLYSIS/NO NECROPSY PERFORMED		2	5

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

APPENDIX E

STATISTICAL ANALYSES OF NEOPLASMS IN RATS FED HEPTACHLOR IN THE DIET

Table E1. Statistical Analysis of Neoplasms of the Liver in Rats Fed Heptachlor in the Diet (using matched control)

Sex	No. Rats with Lesion/ No. Rats with Liver Tissue Examined: Control	Low Dose[a]	High Dose[b]	Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Hepatocellular Carcinoma					
Male	0/10 0%	0/44 0%	0/49 0%	--	
Female	0/10 0%	0/48 0%	0/46 0%	--	
Hepatocellular Carcinoma and/or Neoplastic Nodule					
Male	1/10 10%	3/44 7%	6/49 12%	0.37	0.20
Female	1/10 10%	9/48 19%	5/46 11%	0.38[c]	0.41[c]

[a]Males: 38.9 ppm; females: 25.7 ppm.
[b]Males: 77.9 ppm; females: 51.3 ppm.
[c]P value given in the direction of a negative linear trend.

Table E2. Statistical Analysis of Neoplasms of the Liver in Rats Fed Heptachlor in the Diet (using pooled control)

Sex	No. Rats with Lesion/ No. Rats with Liver Tissue Examined: Control	Low Dose[a]	High Dose[b]	Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Hepatocellular Carcinoma					
Male	1/58 2%	0/44 0%	0/49 0%	--	
Female	0/59 0%	0/48 0%	0/46 0%	--	
Hepatocellular Carcinoma and/or Neoplastic Nodule					
Male	3/58 5%	3/44 7%	6/49 12%	0.13	0.14
Female	5/59 8%	9/48 19%	5/46 11%	0.37	0.20

[a]Males: 38.9 ppm; females: 25.7 ppm.
[b]Males: 77.9 ppm; females: 51.3 ppm.

Table E3. Statistical Analysis of Neoplasms of Thyroid Follicular Cells in Rats Fed Heptachlor in the Diet (using matched control)

Sex	No. Rats with Lesion/ No. Rats with Thyroid Examined[a] Control	Low Dose[b]	High Dose[c]	Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Carcinoma					
Male	0/8(0/8) 0% (0%)	4/38(4/37) 11% (11%)	0/38(0/33) 0% (0%)	0.23[d,e](0.26)[d]	--[f]
Female	1/9(1/7) 11%(14%)	2/43(2/38) 5% (5%)	5/38(5/29) 13% (17%)	0.31(0.26)	0.19
Carcinoma and/or Adenoma					
Male	1/8 13%	9/38 24%	3/38 8%	0.16[d]	0.15[d]
Female	1/9 11%	3/43 7%	14/38 37%	0.002	0.001

[a]The early deaths are eliminated in those figures in parentheses, i.e., deaths before the first tumor was found in any of the groups being compared to one another.
[b]Males: 38.0 ppm; females: 25.7 ppm.
[c]Males: 77.0 ppm; females: 51.3 ppm.
[d]P value given in the direction of a negative linear trend.
[e]Data depart significantly from a linear trend ($P < 0.05$).
[f]Validity of test is dubious because of the small number of tumorous animals observed.

Table E4. Statistical Analysis of Neoplasms of Thyroid Follicular Cells in Rats Fed Heptachlor in the Diet (using pooled control)

Sex	No. Rats with Lesion/ No. Rats with Thyroid Examined: Control	Low Dose[a]	High Dose[b]	Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Carcinoma					
Male	1/51 2%	4/38 11%	0/38 0%	0.51[c,d]	0.30[c,d]
Female	1/58 2%	2/43 5%	5/38[f] 13%	0.02	0.001
Carcinoma and/or Adenoma					
Male	4/51 8%	9/38[g] 24%	3/38 8%	0.48[e]	0.36
Female	3/58 5%	3/43 7%	14/38[h] 37%	< 0.01[e]	< 0.01[e]

[a]Males: 38.9 ppm; females: 25.7 ppm
[b]Males: 77.9 ppm; females: 51.3 ppm
[c]P value given in the direction of a negative linear trend.
[d]Data depart significantly from a linear trend ($P < 0.01$).
[e]Data depart significantly from a linear trend ($P < 0.05$).
[f]Significantly higher than its pooled control in both tests ($P < 0.05$).
[g]Significantly higher than its pooled control in unadjusted exact test ($P < 0.05$).
[h]Significantly higher than its pooled control in both tests ($P < 0.01$).

Table E5. Statistical Analysis of Neoplasms of Thyroid C-cells in Rats Fed Heptachlor in the Diet (using matched control)

Sex	No. Rats with Lesion/ No. Rats with Thyroid Examined[a] Control	Low Dose[b]	High Dose[c]	Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Carcinoma					
Male	0/8(0/8) 0% (0%)	3/38(3/36) 8% (8%)	1/38(1/32) 3% (3%)	0.37[d] (0.56)[d]	--[f]
Female	1/9 11%	0/43 0%	0/38 0%	0.10[d,e]	--[f]
Carcinoma and/or Adenoma					
Male	0/8 0%	7/38 18%	2/38 5%	0.34[d,e]	0.34[d,e]
Female	3/9 33%	7/43 16%	3/38 8%	0.046[d]	0.03[d]

[a]The early deaths are eliminated in those figures in parentheses, i.e., deaths before first tumor was found in any of the groups being compared to one another.
[b]Males: 38.9 ppm; females: 25.7 ppm.
[c]Males: 77.9 ppm; females: 51.3 ppm.
[d]P value given in the direction of negative linear trend.
[e]Data depart significantly from a linear trend ($P < 0.05$)
[f]Validity of test is dubious because of the small number of tumorous animals observed.

Table E6. Statistical Analysis of Neoplasms of Thyroid C-cells in Rats Fed Heptachlor in the Diet (using pooled control)

Sex	No. Rats with Lesion/ No. Rats with Thyroid Examined: Control	Low Dose[a]	High Dose[b]	Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Carcinoma					
Male	1/51 2%	3/38 8%	1/38 3%	0.49	0.48
Female	5/58 9%	0/43[e] 0%	0/38[e] 0%	0.11[c]	0.01[c]
Carcinoma and/or Adenoma					
Male	4/51 8%	7/38 18%	2/38 5%	0.48[c,d]	0.28[c]
Female	11/58 19%	7/43 16%	3/38 8%	0.10[c]	0.07[c]

[a]Males: 38.9 ppm; females: 25.7 ppm.
[b]Males: 77.9 ppm; females: 51.3 ppm.
[c]P value given in the direction of a negative linear trend.
[d]Data depart significantly from a linear trend ($P < 0.05$).
[e]Significantly lower than its pooled control in life-table adjusted test ($P < 0.05$).

Table E7. Statistical Analysis of Neoplasms of the Pituitary Gland in Rats Fed Heptachlor in the Diet (using matched control)

Sex	No. Rats with Lesion / No. Rats with Pituitary Examined: Control	Low Dose[a]	High Dose[b]	Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Chromophobe Adenocarcinoma or Carcinoma					
Male	0/7 0%	0/42 0%	0/34 0%	--	--
Female	0/6 0%	0/39 0%	0/34 0%	--	--
Chromophobe Adenocarcinoma or Carcinoma and/or Chromophobe Adenoma					
Male	4/7 57%	8/42 19%	8/34 24%	0.21[c]	0.14[c]
Female	3/6 50%	9/39 23%	8/34 24%	0.25[c]	0.16[c]

[a]Males: 38.0 ppm; females: 25.7 ppm.
[b]Males: 77.0 ppm; females: 51.3 ppm.
[c]P Value given in the direction of a negative linear trend.

Table E8. Statistical Analysis of Neoplasms of the Pituitary Gland in Rats Fed Heptachlor in the Diet (using pooled control)

	No. Rats with Lesion/ No. Rats with Pituitary Examined			Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Sex	Control	Low Dose[a]	High Dose[b]		
Chromophobe Adenocarcinoma or Carcinoma and/or Adenoma					
Male	16/48 33%	8/42[e] 19%	8/34[e] 24%	0.16[c]	0.001[c,d]
Female	23/52 44%	9/39[f] 23%	8/34[f] 24%	0.02[c]	0.02[c]

[a]Males: 38.9 ppm; females: 25.7 ppm.
[b]Males: 77.9 ppm; females: 51.3 ppm.
[c]P value given in the direction of a negative linear trend.
[d]Data depart significantly from a linear trend (P = 0.01).
[e]Significantly lower than its pooled control in life table adjusted test (P < 0.05).
[f]Significantly lower than its pooled control in both tests (P < 0.05).

Table E9. Statistical Analysis of Neoplasms of the Mammary Gland in Female Rats Fed Heptachlor in the Diet (using matched control)

No. Rats with Lesion/ No. Rats Necropsied[a]			Exact Test for Dose-related Trend	Tests After Life-table Adjustment
Control (0 ppm)	Low Dose (25.7 ppm)	High Dose (51.3 ppm)	(P)	(P)
Adenocarcinoma or Papillary Adenocarcinoma				
1/10(1/10) 10% (10%)	1/48(1/46) 2% (2%)	1/48(1/39) 2% (3%)	0.17[b] (0.34)[b]	--[c]
Sarcoma or Fibrosarcoma				
0/10 0%	1/48 2%	0/48 0%	0.30[b]	--[c]
Adenocarcinoma or Papillary Adenocarcinoma and/or Sarcoma or Fibrosarcoma				
1/10 10%	2/48 4%	1/48 2%	0.23[b]	--[c]
Adenocarcinoma, or Papillary Adenocarcinoma and/or Sarcoma or Fibrosarcoma and/or Fibroma or Adenoma or Fibroadenoma				
2/10 20%	18/48 38%	8/48 17%	0.09[b]	0.22[b]

[a]The early deaths are eliminated in those figures in parentheses, i.e., deaths before first tumor was found in any of the groups being compared to one another.
[b]P value given in the direction of a negative linear trend.
[c]Validity of test is dubious because of the small number of tumorous animals observed.

Table E10. Statistical Analysis of Neoplasms of the Mammary Gland in Female Rats Fed Heptachlor in the Diet (using pooled control)

No. Rats with Lesion/ No. Rats Necropsied			Exact Test for Dose-related Trend	Tests After Life-table Adjustment
Control (0 ppm)	Low Dose (25.7 ppm)	High Dose (51.3 ppm)	(P)	(P)
Adenocarcinoma or Papillary Adenocarcinoma				
2/60 3%	1/48 2%	1/48 2%	0.46[a]	--[e]
Sarcoma or Fibrosarcoma				
0/60 0%	1/48 2%	0/48 0%	0.62	--[e]
Adenocarcinoma or Papillary Adenocarcinoma and/or Sarcoma or Fibrosarcoma				
2/60 3%	2/48 4%	1/48 2%	0.48[a]	0.44[a]
Adenocarcinoma or Papillary Adenocarcinoma and/or Sarcoma or Fibrosarcoma and or Fibroma				
10/60 17%	18/48[d] 38%	8/48 17%	0.47[b]	0.22[c]

[a]P value given in the direction of a negative linear trend.
[b]Data depart significantly from a linear trend ($P < 0.01$).
[c]Data depart significantly from a linear trend ($P < 0.05$).
[d]Significantly higher than its pooled control in both tests ($P < 0.05$).
[e]Validity of test is dubious because of the small number of tumorous animals observed.

Table E11. Statistical Analysis of Neoplasms of the Uterus in Female Rats Fed Heptachlor in the Diet (using matched control)

No. Rats with Lesion/ No. Rats with Uterus Examined			Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Control (0 ppm)	Low Dose (25.7 ppm)	High Dose (51.3 ppm)		
Endometrial Stromal Sarcoma				
0/10 0%	0/43 0%	2/41 5%	0.19	--[a]
Endometrial Stromal Sarcoma and/or Endometrial Stromal Polyp				
2/10 20%	7/43 16%	8/41 20%	0.52	0.39

[a]Validity of test is dubious because of the small number of tumorous animals observed.

Table E12. Statistical Analysis of Neoplasms of the Uterus Fed Heptachlor in the Diet (using pooled control)

No. Rats with Lesion/ No. Rats with Uterus Examined			Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Control (0 ppm)	Low Dose (25.7 ppm)	High Dose (51.3 ppm)		
Endometrial Stromal Sarcoma				
0/56 0%	0/43 0%	2/41[a] 5%	0.08	--[b]
Endometrial Stromal Sarcoma and/or Endometrial Stromal Polyp				
6/56 11%	7/43 16%	8/41 20%	0.14	0.09

[a]Significantly higher than its pooled control in life table adjusted test ($P < 0.05$).
[b]Validity of test is dubious because of the small number of tumorous animals observed.

APPENDIX F

STATISTICAL ANALYSES OF NEOPLASMS IN MICE FED HEPTACHLOR IN THE DIET

Table F1. Statistical Analysis of Neoplasms of the Liver in Male Mice Fed Heptachlor in the Diet (using matched control)

No. Mice with Lesion/ No. Mice with Liver Tissue Examined[a]			Fisher Exact Test (P)	Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Control (0 ppm)	Low Dose (6.1 ppm)	High Dose (13.8 ppm)			
Hepatocellular Carcinoma					
1/9(1/9)	--	34/47(34/46)			
11%(11%)	--	72% (74%)	0.001(0.0007)		0.002
4/10(4/8)	11/46(11/43)	--			
40% (50%)	24% (26%)	--	0.25[b](0.17)[b]		0.05+[b]
Combined				0.001[c](0.0025)[b]	0.003

[a]The early deaths are eliminated in those figures in parentheses, i.e., deaths before the first tumor was found in any of the groups being compared to one another.
[b]P value given in the direction of a negative linear trend.
[c]Two slopes are significantly different ($P < 0.05$).

Table F2. Statistical Analysis of Neoplasms of the Liver in Male Mice Fed Heptachlor in the Diet (using pooled control)

No. Mice with Lesion/ No. Mice with Liver Tissue Examined			Fisher Exact Test (P)	Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Control (0 ppm)	Low Dose (6.1 ppm)	High Dose (13.8 ppm)			
Hepatocellular Carcinoma					
17/92	--	34/47			
18%	--	72%	< 0.0001		< 0.0001
17/92	11/46	--			
18%	24%	--	0.30		0.26
17/92	11/46	34/47			
18%	24%	72%		< 0.0001[a]	< 0.0001[b]

[a]Data depart significantly from a linear trend ($P < 0.05$).
[b]Data depart significantly from a linear trend ($P < 0.01$).

Table F3. Statistical Analysis of Neoplasms of the Liver in Female Mice Fed Heptachlor in the Diet (using matched control)

No. Mice with Lesion / No. Mice with Liver Tissue Examined[a]			Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Control (0 ppm)	Low Dose (9 ppm)	High Dose (18 ppm)		
Hepatocellular Carcinoma				
2/10(2/10)	3/47(3/43)	30/42[b](30/38)[b]		
20% (20%)	6% (7%)	71% (79%)	< 0.0001(< 0.0001)	< 0.0001[c]

[a]The early deaths are eliminated in those figures in parentheses, i.e., deaths before the first tumor was found in any of the groups being compared to one another.
[b]Significantly higher than matched control (P < 0.01).
[c]Data depart significantly from linear trend (P < 0.01).

Table F4. Statistical Analysis of Neoplasms of the Liver in Female Mice Fed Heptachlor in the Diet (using pooled control)

No. Mice with Lesion/ No. Mice with Liver Tissue Examined			Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Control (0 ppm)	Low Dose (9 ppm)	High Dose (18 ppm)		
Hepatocellular Carcinoma				
3/78 4%	3/47 6%	30/42[b] 71%	< 0.0001[a]	< 0.0001[a]

[a]Data depart significantly from a linear trend (P < 0.01).
[b]Significantly higher than pooled controls (P < 0.01).

APPENDIX G

ANALYSIS OF FORMULATED DIETS

FOR CONCENTRATION OF HEPTACHLOR

APPENDIX G

Analysis of Formulated Diets for Concentration of Heptchlor

A 100 g sample of the diet mixture was shaken with 125 mls hexane at room temperature for 16 hrs., then filtered through Celite with hexane washes, and reduced in volume to 10 mls. After appropriate dilutions, the solution was quantitatively analyzed for heptachlor by gas-liquid chromatography (electron-capture detector, 10% DC-200 on Gas-Chrom Q column). Recoveries were checked with spiked samples, and external standards were used for calibration.

Theoretical Dietary Level (ppm)	No. of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
5	17	4.92	7.5	4.0-5.4
10	18	9.83	3.7	9.2-10.6
20	20	20.2	5.9	17.5-23.4
40	12	39.2	5.7	35.2-42.1
80	10	80.6	6.4	71.0-90.7
120	2	110.0	0	110.0
160	5	151.2	6.9	137.0-162.0

*U.S. GOVERNMENT PRINTING OFFICE: 1977 241-161/3051 1-3

DHEW Publication No. (NIH) 77-809