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CHLORDANE

FOR POSSIBLE CARCINOGENICITY

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CONTRIBUTORS: This report presents the results of the bioassay of chlordane for possible carcinogenicity, conducted by 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 at Gulf South Research Institute, New Iberia, Louisiana, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Incorporated, prime contractor for the NCI carcinogen bioassay program.

The experimental design was determined by Drs. J. H. Weisburger[1,2] and R. R. Bates[1]. The doses were selected by Drs. T. E. Shellenburger[3,4], J. H. Weisburger and R. R. Bates. Animal treatment and observations were supervised by Drs. T. E. Shellenburger and H. P. Burchfield[3], with the technical assistance of Ms. D. H. Monceaux[3] and Mr. D. Broussard[3].

Necropsies were performed under the supervision of Drs. E. Bernal[3] and B. Buratto[3]. The histopathologic evaluation was conducted at Experimental Pathology Laboratories by Dr. R. A. Renne[5,9] and Dr. J. F. Ferrell[5], and the diagnoses included in this report represent the interpretation of these pathologists. Pathologists from Tracor Jitco and NCI reviewed selected slides and concurred with the overall pathologic evaluation of the study.

Compilation of individual animal survival and summary tables was performed by EG&G Mason Research Institute[7]; pathology tables

were prepared by Dr. R. A. Renne; and statistical analyses were performed by Mr. J. Nam[8] and Dr. J. J. Gart[8].

This report was prepared at Tracor Jitco under the direction of NCI. Those responsible for the report at Tracor Jitco were the toxicologists, Drs. W. E. MacDonald and J. F. Robens; the chemist, Dr. S. S. Olin; the technical editor, Dr. E. W. Gunberg; and the technical writers, Dr. G. L. Miller, Mr. W. E. Reichardt, and Ms. Y. E. Presley. The final report was reviewed by members of the participating organizations[1,3,5,6].

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SUMMARY

A bioassay of analytical-grade chlordane 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 chlordane for 80 weeks, then observed for 29 weeks. Because of toxic effects, doses were reduced for both male and female rats during the course of the tests. Time-weighted average doses used for the male rats were 203.5 and 407.0 ppm; for the females, 120.8 and 241.5 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 109 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 each of the treated groups was tested along with a concurrent control. Because of toxic effects, doses were reduced for female mice during the course of the tests; however, it was possible to increase the doses for the male mice. The time-weighted average doses used for the male mice were 29.9 and 56.2 ppm; for the females, 30.1 and 63.8 ppm. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched-control groups combined with 70 untreated male and 80 untreated female mice from similar bioassays of five other compounds. All surviving mice were killed at 90-91 weeks.

The effects of chlordane 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 the average body weights of the high-dose male and female rats were consistently lower than those of the untreated controls, while differences between the low-dose and control rats were negligible. Body weights of mice given either low or high doses showed little or no effect of the chlordane; however, other adverse clinical signs were seen with greater frequency in treated than in control mice.

The effects of chlordane on survival rates indicated that mortality was dose-related for female rats and for male mice. However, a substantial proportion of most groups of animals survived to an age at which tumors could be expected to appear; male control rats, for unknown reasons, showed an abnormally low survival rate.

Hepatocellular carcinoma showed a highly significant dose-related trend for mice, using either matched controls (for males, controls 2/18, low dose 16/48, high dose 43/49, $P < 0.0001$; for females, controls 0/19, low dose 3/47, high dose 34/49, $P < 0.0001$) or pooled controls (for males, controls 17/92, $P < 0.0001$; for females, controls 3/78, $P < 0.0001$). These high levels of significance were maintained when hepatocellular carcinoma was combined with nodular hyperplasia or when the data were subjected to life-table adjustment. No other tumors were found in mice in sufficient numbers to justify analysis.

In contrast to findings with mice, hepatocellular carcinoma failed to appear at a significant rate of incidence in rats administered chlordane. Further, the number of lesions of the liver in rats did not become significant with the addition of nodular neoplasia or with the application of life-table adjustment to the data.

There was significant statistical evidence for the induction in treated male rats of proliferative lesions of follicular cells of the thyroid and of malignant fibrous histiocytoma, but these findings were discounted because the rates of incidence were comparatively low and/or are known to be variable in control rat populations.

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

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I. INTRODUCTION

Chlordane is a member of the cyclodiene group of chlorinated insecticides, which includes aldrin, dieldrin, endrin, heptachlor, and endosulfan. It was introduced in 1945 and was the first chlorinated cyclodiene developed for insect control (Chlordane Formulation Guide, 1962). It is effective on a wide variety of insects of agricultural, industrial, and domestic importance. The compound was registered for use on more than 40 vegetable and 27 fruit crops. About a third of the amount used in the United States is applied to pests of the home, garden lawn, and turf (EPA, 1976).

On November 18, 1974, the EPA issued an order of intent to cancel the registration and use of pesticides containing chlordane, 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 chlordane 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 were the use of chlordane to control root weevils infecting Florida citrus crops, and certain other uses.

Chlordane persists in the environment, lasting 10 years or more in treated soils (EPA, 1974). Translocation to untreated areas occurs through soil erosion and dusting. Plants grown in treated soil, particularly root crops, also may absorb chlordane residues and thus enter the food chain.

Estimates of human exposure through the diet, based on Food and Drug Administration (FDA) market basket surveys conducted from June 1968 through April 1970, showed only trace amounts of chlordane in a few root crops, such as potatoes, which contributed less than 0.001 mg chlordane per person per day (Duggan and Corneliussen, 1972). The FAO/WHO (1968) tentative acceptable daily intake of chlordane in man is 0.001 mg/kg/day, a value considerably higher than the actual human exposure reported by the FDA. Exposure may also occur with persons applying chlordane and with workers producing the compound and its formulations.

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 (Secretary's Commission on Pesticides, 1969) pointed out the need for an evaluation of the carcinogenicity of several pesticides, including certain members

of the cyclodiene group. Because of known biological effects of low doses of chlordane over extended periods of time (Ingle, 1965), 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 analytical-grade chlordane obtained from Velsicol Chemical Corporation. The Velsicol preparation is a developmental product and is considerably purer than the commercial technical grade. According to the manufacturer's analysis, the batch used for the bioassay contained 94.8% chlordane (71.7% alpha- or cis-chlordane and 23.1% gamma- or trans-chlordane), 0.3% heptachlor, 0.6% nonachlor, 1.1% hexachlorocyclopentadiene, 0.25% chlordene isomers, and other chlorinated compounds. Chemically, chlordane is the product of the addition of chlorine to chlordene; chlordene is the Diels-Alder product of hexachlorocyclopentadienol and cyclopentadiene.

Under the conditions employed for gas chromatography at Gulf South Research Institute, two major peaks were observed, probably corresponding to alpha- and gamma-chlordane. The analyses did not, however, detect the minor components, such as heptachlor and nonachlor. From the mass spectrum, the group of parent ions around m/e 410 was consistent with the molecular formula of chlordane. The elemental analysis was correct for chlordane. Other physical, spectral, and chromatographic data were consistent with the manufacturer's analysis.

The chlordane used for the tests was stored in a refrigerator.

B. Dietary Preparation

All diets were formulated using Wayne® Lab Blox Meal (Allied Mills, Inc., Chicago, Ill.) to which was added the required amount of chlordane for each dietary concentration. The test compound was first dissolved in a small amount of acetone (Mallinckrodt Chemical Works, St. Louis, Mo.), which was added to the feed. Corn oil equal to 2% of the final feed weight 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 chlordane. The diets were mixed mechanically to assure homogeneity and to allow the evaporation of the acetone. The corn oil (Louana®) was produced by Opelousas Refinery Co., Opelousas, Louisiana. The prepared diets were analyzed regularly throughout the test to assure uniformity. Water and the formulated diets were made available ad libitum to the experimental animals and were replaced three times per week.

The stability of chlordane in feed was checked by analyzing formulated diets for the concentration of chlordane at intervals over a 7-day period. Diets containing 30 and 100 ppm chlordane showed no change on standing at ambient temperature for this period.

Theoretical chlordane 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 level from 30 to 800 ppm, the mean of the analytical concentrations for the samples checked was within 5% of the theoretical concentration, and the coefficient of variation was never more than 5.6%. 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 obtained 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 then assigned to treatment and control groups.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. Incoming air was filtered through fiberglass air conditioner 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 suspended wire cages; the mice, in plastic cages equipped with filter caps. Female mice were housed five per cage and males two or three per cage. 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 chlordane, along with their matched controls, were housed in a room by themselves. Mice being treated with chlordane were maintained in a room housing other mice being treated with toxaphene, heptachlor, 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 maximum tolerated doses of chlordane, 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 the high doses. In the subchronic studies, chlordane 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 50 to 1,600 ppm were used for male rats; 100 to 1,600 ppm, for females. Concentrations from 20 to 640 ppm were used for both male and female mice. Weights of animals and consumption of feed were measured weekly, and deaths were noted.

All male rats fed 1,600 ppm died during the second week of the study, while those fed 800 ppm showed no deaths and their gain in weight and consumption of feed were low only for the first week of treatment. Four of five female rats died, however, when given 800 ppm. When given 400 ppm or less, the gain in weight and consumption of feed in the treated females were generally similar to those of the controls. Based on these findings, low and high doses of 400 and 800 ppm, respectively, were selected for the chronic studies in the male rats; 200 and 400 ppm, for the females.

With mice fed 320 ppm, two of five males and all females died; at 160 ppm again two of five males died, but no females; at 80 ppm, no males or females died. Gain in weight and consumption of

feed, however, were unaffected in either male or female mice by any of the doses tested. Based on the mortality data, concentrations of 40 and 80 ppm were selected as low and high doses for the chronic studies in male mice; 80 and 160 ppm, for the females.

F. Design of Chronic Studies

Designs of the chronic studies with rats and mice, including test and matched control groups, are presented in tables 1 and 2. When tests with the rats were initiated at doses indicated by the subchronic studies, toxic effects were observed; doses were, therefore, reduced during the course of the tests. When tests with the mice were initiated at doses indicated by the subchronic studies, high mortality resulted, and the original high-dose groups were discarded at 14 weeks. The 40-ppm males and 80-ppm females then became the high-dose groups, and the study was continued using the original control mice (10 males, 10 females). A new low-dose group was started at 20 ppm for males and 40 ppm for females, along with additional control mice (10 males, 10 females). After 1 week, the doses for the males could be increased, while those for the females had to be reduced, due to toxic effects.

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

Table 1. Design of Chlordane Chronic Feeding Studies in Rats

Sex and Treatment Group	Initial No. of Animals[a]	Chlordane in Diet (ppm)	Time on Study: Treated (weeks)	Time on Study: Untreated[b] (weeks)	Time-Weighted Average Doses[c] (ppm)
MALE					
Matched-Control	10	0	0	109	
Low-Dose					
	50	400	33		203.5
		100	14		
		50	33		
		0	0	29	
High-Dose	50	800	33		407.0
		200	14		
		100	33		
		0	0	29	
FEMALE					
Matched-Control	10	0	0	109	
Low-Dose	50	200	33		120.8
		100	14		
		50	33		
		0	0	29	
High-Dose	50	400	33		241.5
		200	14		
		100	33		
		0	0	29	

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

[b]When diets containing chlordane were discontinued, treated rats and their matched controls were fed plain feed diets (without corn oil) for 12 weeks, then control diets (2% corn oil added) for an additional 17 weeks.

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

Table 2. Design of Chlordane Chronic Feeding Studies in Mice

Sex and Treatment Group	Initial No. of Animals[a]	Chlordane 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	91	
Low-Dose	50	20	1		29.9
		30	79		
		0	0	10	
High-Dose	50	40	15		56.2
		60	65		
		0	0	10	
FEMALE					
Matched-Control	20[d]	0	0	91	
Low-Dose	50	40	1		30.1
		30	79		
		0	0	10	
High-Dose	50	80	15		63.8
		60	65		
		0	0	10	

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

[b]When diets containing chlordane were discontinued, mice received the control diet (2% corn oil added) until termination.

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

[d]Initially 10 animals of each sex were placed on test as matched controls; however, when the study was restarted, 10 additional animals of each sex were placed on test as matched controls.

similar bioassays of other compounds with the matched-controls for chlordane. 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 dieldrin, chlordane, heptachlor, dichlorvos, and dimethoate; this resulted in pooled-control groups containing 60 males and 60 females. 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 resulted in pooled-control groups containing 100 males and 80 females. 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 the following slight differences in the dichlorvos matched-control rats that were pooled for use as controls in the chlordane 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 Insitute, 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, diaphragm, 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 uncensored 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 the 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, exact (or conditional) tests for proportions were used as given by Cox (1970). For the comparison of two groups, this is simply the Fisher exact test. When three groups are compared, this is 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

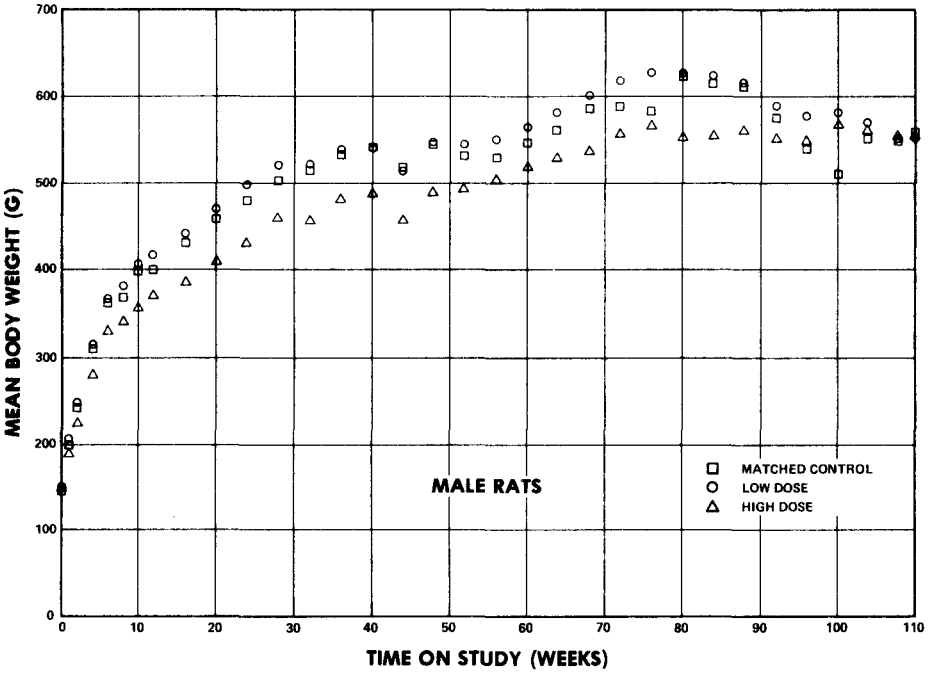
A. Body Weights and Clinical Signs (Rats)

The average body weights of high-dose male and female rats were consistently lower than those of the untreated controls, while differences between low-dose rats and the controls were slight or absent (figure 1).

During the first year of study, the appearance and behavior of the treated rats were generally comparable to those of the control rats. At week 44 a majority of the high-dose females had generalized tremors along with loss of weight, but the tremors were not noted again. Adverse clinical signs in all treated groups occurred frequently during the first year of study and gradually increased in frequency during the second year. These signs included loss of weight, rough and discolored hair coats, palpable masses, and in some cases multiple tumors. Surviving rats at the termination of the study (109 weeks) were generally in a poor physical condition.

B. Survival - (Rats)

Curves showing the probability of survival of treated and control rats are shown in figure 2. For male rats there was no indication of a significant difference in survivorship among the



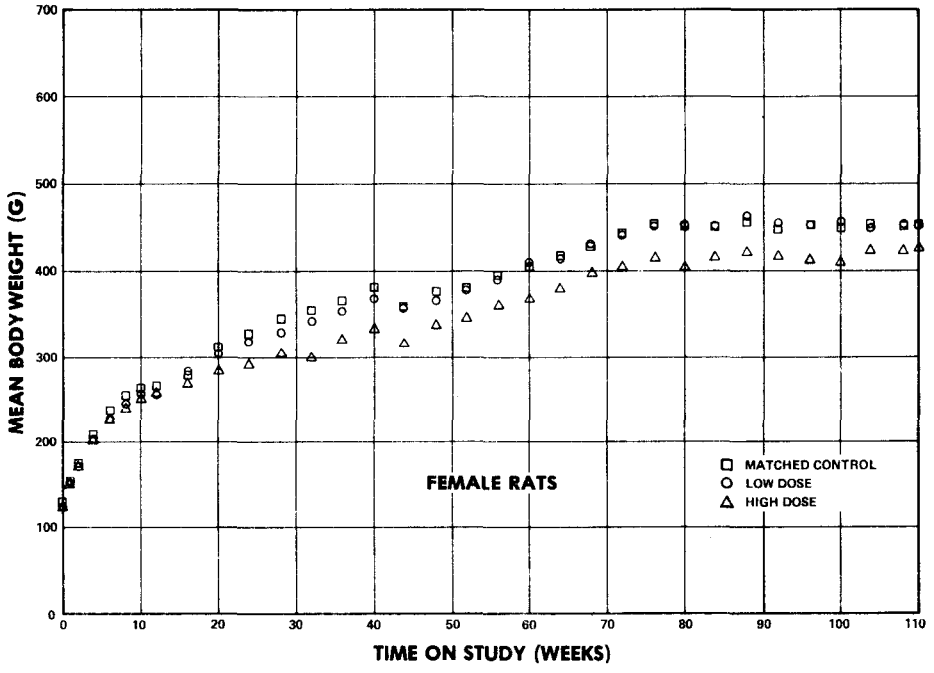


Figure 1. Growth Curves for Rats Fed Chlordane in the Diet

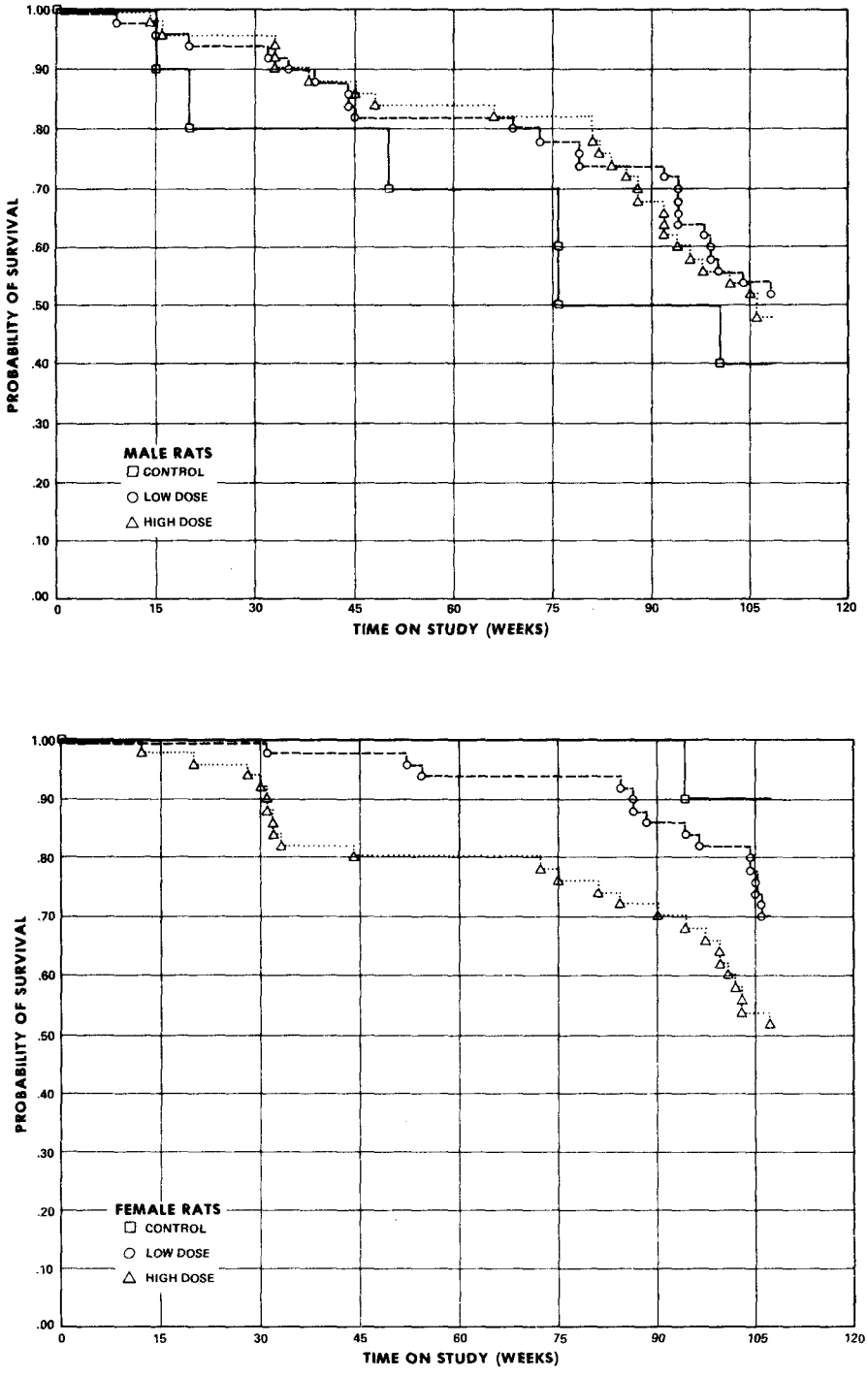


Figure 2. Survival Curves for Rats Fed Chlordane in the Diet

three groups. For the female rats there was a highly significant dose-related trend (P = 0.003) in mortality from other than accidental kills or scheduled sacrifice.

C. Pathology (Rats)

Histopathologic findings are summarized 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 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 gland. 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 larger and had more basophilic cytoplasm than the surrounding normal thyroid 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 heterogenous 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, the follicular-cell adenomas of the thyroid (Bloodworth, 1968; Meissner and Warren, 1968) characteristically were more distinctly circumscribed, had some evidence of encapsulation by connective tissue, 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, and hyperchromatic nuclei and mitotic figures

were numerous. Evidence of connective tissue and/or inflammatory response and necrosis was often present. Although some carcinomas were large enough to be noted at necropsy, others were noted only on microscopic examination. Metastasis was not observed.

Proliferative C-cell lesions presented a spectrum of histologic characteristics ranging from very minimal increases in the number of C-cells interspersed among normal thyroid follicles, with no compression or distortion of follicular architecture, to grossly visible tumors that invaded adjacent tissue. 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:

Hyperplasia

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

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

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 occur.

(3) There may be an increase in basophilia of C-cells.

(4) Invasion of thyroid capsule, adjacent tissue, or lymphatics is lacking.

(5) Encapsulation is rare.

Carcinoma

(1) There is invasion of thyroid capsule, adjacent tissue, or vessels.

(2) There may be metastasis.

(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.

Although no metastasis of C-cell carcinoma was observed in rats from this study, metastatic C-cell carcinomas have been found in rats of the same strain (Osborne-Mendel) from other similar studies.

The incidence of other endocrine neoplasms is summarized in Table A3. Pituitary adenomas occurred frequently in all groups, whereas neoplasms of the adrenal gland and pancreatic islets occurred infrequently.

Table A4 summarizes the incidence of primary hepatic neoplasms. These included a number of lesions, classified as "neoplastic nodules," which consisted of nodules of enlarged hepatocytes

compressing adjacent tissue, and which were 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.

The incidence of primary renal tumors is summarized in table A5. Those renal neoplasms classified as "malignant mixed tumors" contained neoplastic tissue components having the microscopic appearance of renal mesenchymal stroma, adipose tissue, and primitive renal epithelium, in various proportions.

Table A6 summarizes the incidence of neoplasms of the reproductive system. Endometrial stromal polyps represented the most frequently occurring neoplasm of the reproductive tract. Numerous mammary fibroadenomas, some of which were multiple, were observed in both test and control females (table A7).

Various other types of neoplasms occurred infrequently in both test and control rats. The incidence of these neoplasms is summarized in table A8. Several tumors occurred in the skin, subcutis, and/or skeletal muscles. With the exception of two obvious carcinomas, one osteosarcoma, and two lipomas, all of these tumors had a somewhat similar microscopic appearance. The proliferating cells in these tumors were apparently histiocytes,

in some cases appearing as a pleomorphic population of round or polyhedral cells having large vesicular nuclei with prominent nucleoli, forming numerous multinucleated giant cells surrounded by varying amounts of stroma. Other tumors in the spectrum appeared as interlacing bundles of oval cells having the appearance of fibroblasts. In some areas, the histologic appearance was similar to a granulomatous inflammatory response within fascia and subcutis. Similar lesions occurred in and around the mesentery, pancreas, spleen, and, less frequently, the pleura. Staining representative sections of all morphologic variants of these lesions with Gomori's method for reticulum demonstrated the presence of numerous reticulin fibrils intertwined among the proliferating cells in all lesions except those resembling fibrosarcomas, in which there was little intercellular reticulin. There appeared to be a histologic spectrum of lesions ranging from a proliferation of plump histiocytes, some forming multinucleated giant cells, with a mixture of granulocytic and mononuclear inflammatory cells and foci of acute necrosis, to an obviously malignant neoplastic process with pleomorphic cells, numerous mitoses, and extensive invasion of adjacent normal muscle and parenchymatous organs. The relatively high incidence of involvement of tissues around the pancreas and mesenteric vessels and the presence of degenerating arteries in some nodules of proliferating tissue suggest the possible relationship of

these lesions to periarteritis nodosa, a common lesion in aging rats often involving pancreatic and mesenteric vessels. However, these proliferative lesions occurred in many cases in this study in the absence of histologically evident arteritis. Another possibility is the presence of some infectious agent or foreign body in tissues of these rats, which elicited a granulomatous response leading to a neoplastic proliferation of histiocytic cells. However, no organism or foreign body was observed microscopically in acid-fast, PAS-, GMS-, or Gram-stained tissue sections from representative lesions. Examinations of representative tissues by electron microscopy supported the conclusion that the basic proliferating cell in all these lesions was the histiocyte, regardless of the variability in cellular architecture, the degree of inflammatory reactions, or the tissues involved. Recent reports in the literature have described neoplasms of histiocytes in humans, (Fu et al., 1975; Stout and Lattes, 1967), including the extreme variability in the histologic picture they may present with light microscopy, (Vilanova and Flint, 1974), and involvement of visceral organs (Chawla et al., 1975; Okayasu et al., 1975). The proliferative lesions described above in this study fit the light microscopic and ultrastructural criteria for "fibrous histiocytomas" as given in the cited literature and are classified as such in this report.

Tables A1-A8 summarize the incidence of all neoplasms observed in

this study. There was an increased incidence of total follicular-cell neoplasms (adenomas and carcinomas) in high-dose rats of both sexes when compared with the incidence in controls of the same sex (6/31 [19%] in high-dose males versus 4/51 [8%] in pooled controls and 0/6 in matched controls; 6/32 [19%] in high-dose females versus 3/59 [5%] in pooled controls and 0/10 in matched controls). 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 chlordane were mentioned in the studies cited. A factor which complicates interpretation is the large number of test rats from which no section of thyroid was available for microscopic examination. 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 chlordane 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 chlordane in rats.

D. Statistical Analyses of Results (Rats)

Statistical analyses of neoplasms in rats fed chlordane are given in Appendix E, tables E1 - E14.

Only one hepatocellular carcinoma was found among the 170 chlordane-treated animals of both sexes 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. Large numbers of female rats were found with neoplastic nodules in the low-dose group (23%) compared with the controls (10%). This difference, however, was not significant by either statistical test. Comparisons using the pooled controls reinforced most of these conclusions (table E2). However, the incidence of neoplastic nodules in low-dose females (23%) was significantly higher than in the pooled controls (8%) by either test, with $P < 0.05$.

There were several follicular-cell carcinomas of the thyroid found among males and females of the treated groups, but there were not enough to reach statistical significance (table E3). When carcinomas were combined with adenomas and comparisons were made with matched controls, significant linear trend was found in the males but not in the females. When comparisons were made with pooled controls, however, the combination of carcinomas and

adenomas yielded a significant linear trend in the females but not in the males (figure E4).

Several C-cell carcinomas of the thyroid were found, but three were in the female control group (table E5). No statistically significant results were found here. When C-cell adenomas were added to the carcinomas, a significant result was found in the life-table-adjusted test for the female rats. This finding was vitiated by the fact that there was a significant departure from the linear trend. The control group and the high-dose group had similar results, and the "significant" linear trend was mainly a comparison of the low dose with the high dose. In the males a significantly negative trend was found for the life-table-adjusted test ($P = 0.02$). Analyses using the pooled controls yielded similar results (table E6), since the category of carcinoma yielded no significant differences of interest. Among females the combination of carcinoma and adenoma yielded results of an erratic character: the response to the high dose was significantly higher than that to the pooled controls, while the response to the low dose was significantly lower.

Thus, the thyroid results were ambiguous for the follicular-cell and C-cell tumors of the thyroid. Although dose-response relationships were found to be consistently positive for follicular-cell tumors of both male and female rats, the negative

relationship among males for C-cell tumors was somewhat offset by an indication of a positive relationship among females.

There was a highly significant finding of dose-related trend of incidence of malignant fibrous histiocytoma in male rats ($P = 0.0007$ by the life-table-adjusted test), but there was no indication of such tumor induction in females (table E7). Comparisons using pooled controls reinforced these findings (table E8).

An appreciable number of animals in both the matched control and treated groups had pituitary tumors, but with no significant dose-related trends in either sex by either statistical test (table E9). 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.

Comparisons using pooled controls (table E10) generally agreed with those using matched controls. The only exception was in the category in which adenoma was added, where the female low-dose group showed a significantly lower incidence than the pooled controls by both tests ($P < 0.01$).

When different categories of mammary tumors were considered together, a large number of such tumors were found in female

rats. No significant differences were found, however, between treated animals and matched controls (table E11). The incidence in the low-dose group significantly exceeded that in the pooled controls ($P < 0.05$) in the category which included adenomas, fibroadenomas, and fibromas (table E12).

The high-dose female group had two animals with sarcomas of the uterus, but this was not a large enough response to achieve significance (tables E13 and E14).

IV. RESULTS - MICE

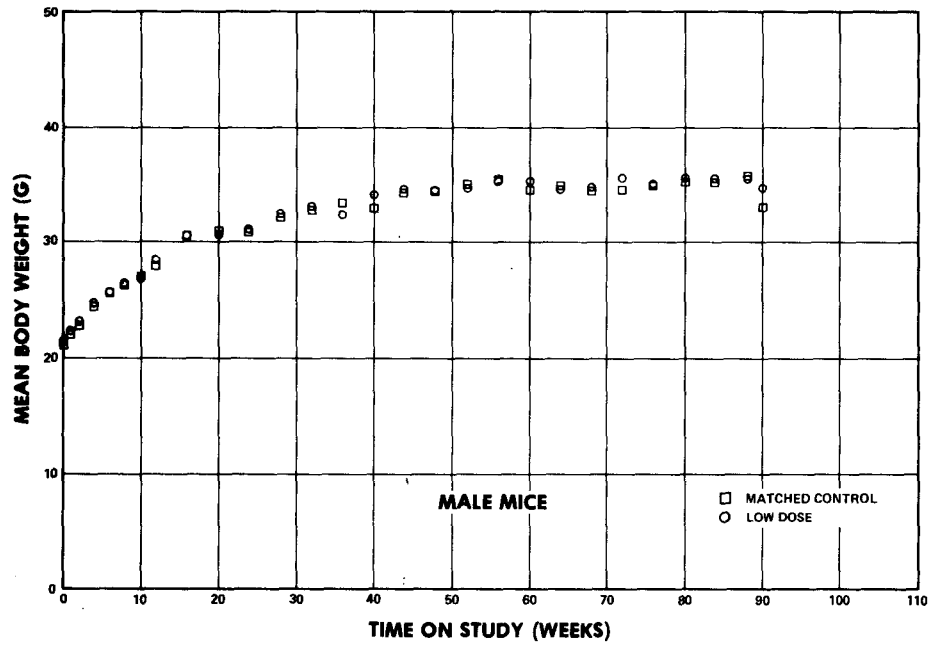
A. Body Weights and Clinical Signs (Mice)

The average body weights of mouse groups receiving low and high doses did not differ appreciably from those of the untreated groups during the course of the tests (figures 3 and 4).

During the first year of the study the appearances and behaviors of the treated and control mice were generally comparable. At week 20, generalized tremors were observed in the high-dose male and female mice even though, as noted above, their weights were hardly affected. After 50 weeks of treatment, alopecia (general and/or local) and a hunched appearance were noted in a few animals. Abdominal distention was observed in all groups but was predominant in the females.

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 significant differences between the low-dose and control groups ($P = 0.02$) and high-dose and control groups ($P = 0.01+$) in mortality from other than accidental death or scheduled sacrifice. This may reflect toxicity of the treatment in the male mice. On the other hand, there was no such indication at



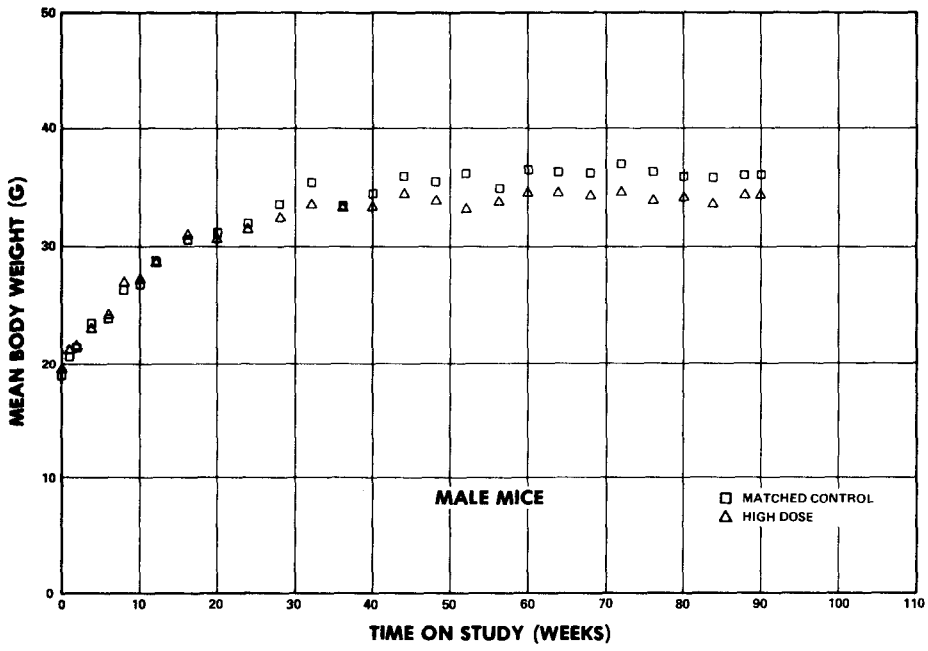


Figure 3. Growth Curves for Male Mice Fed Chlordane in the Diet

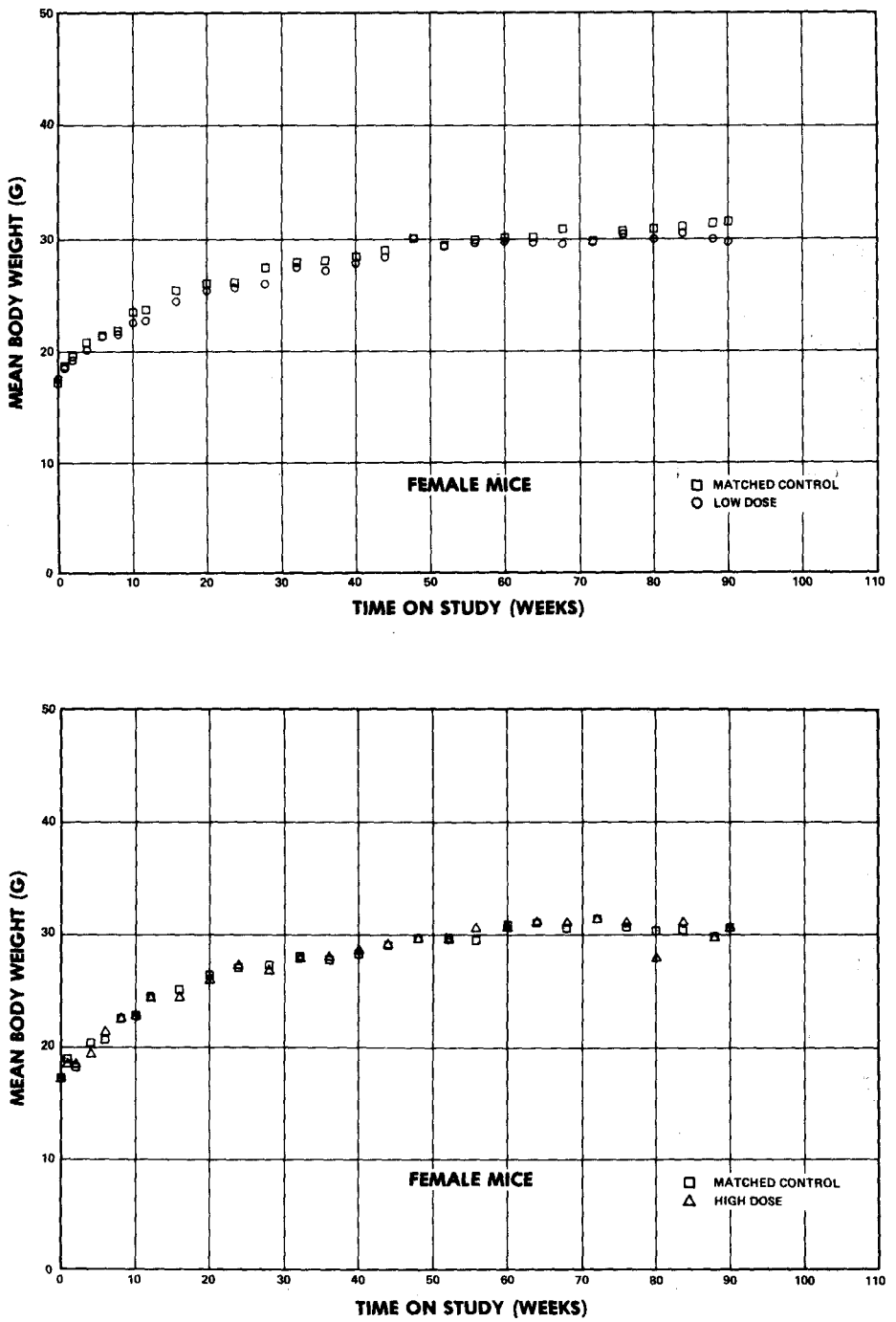


Figure 4. Growth Curves for Female Mice Fed Chlordane in the Diet

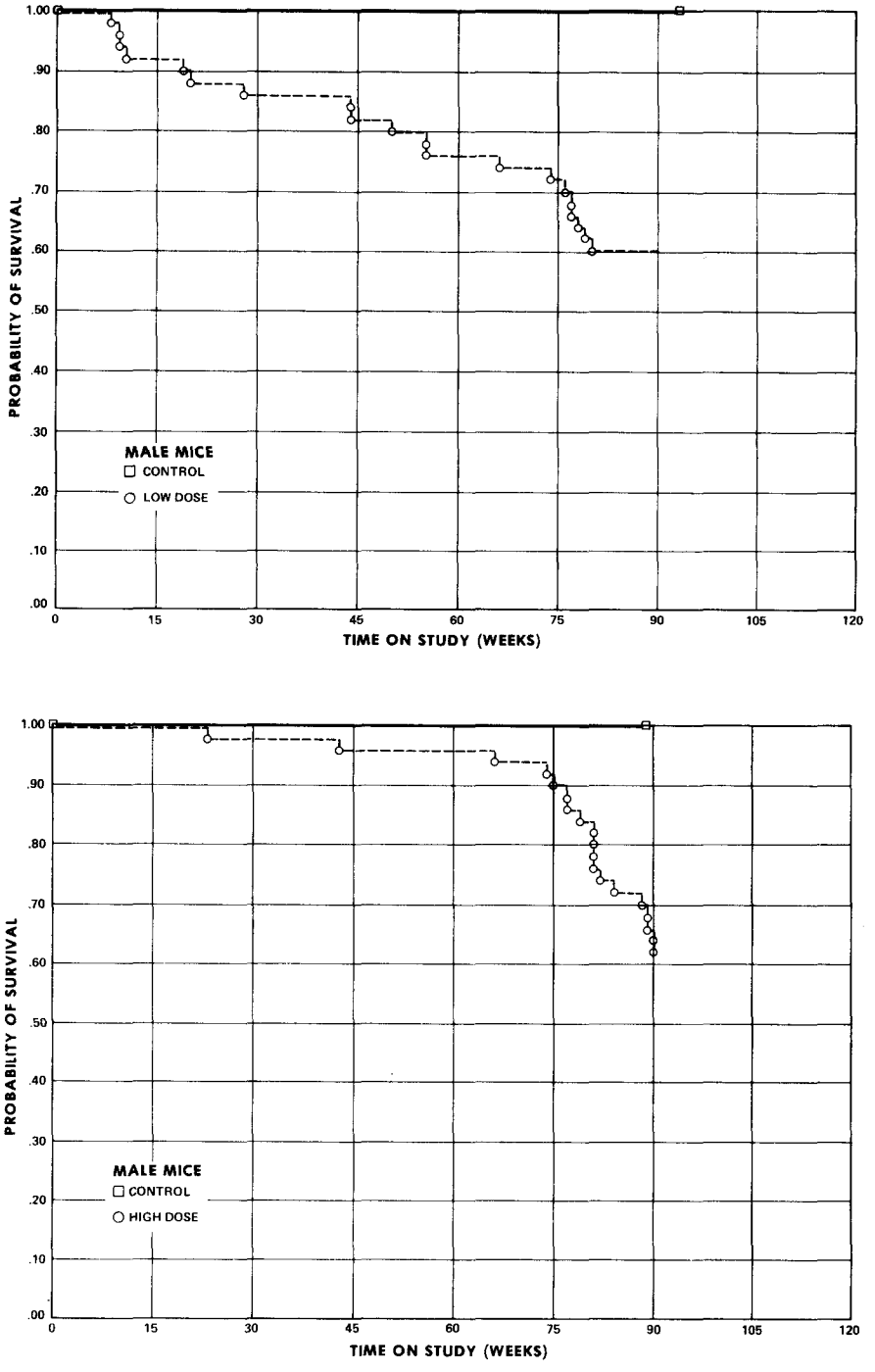


Figure 5. Survival Curves for Male Mice Fed Chlordane in the Diet

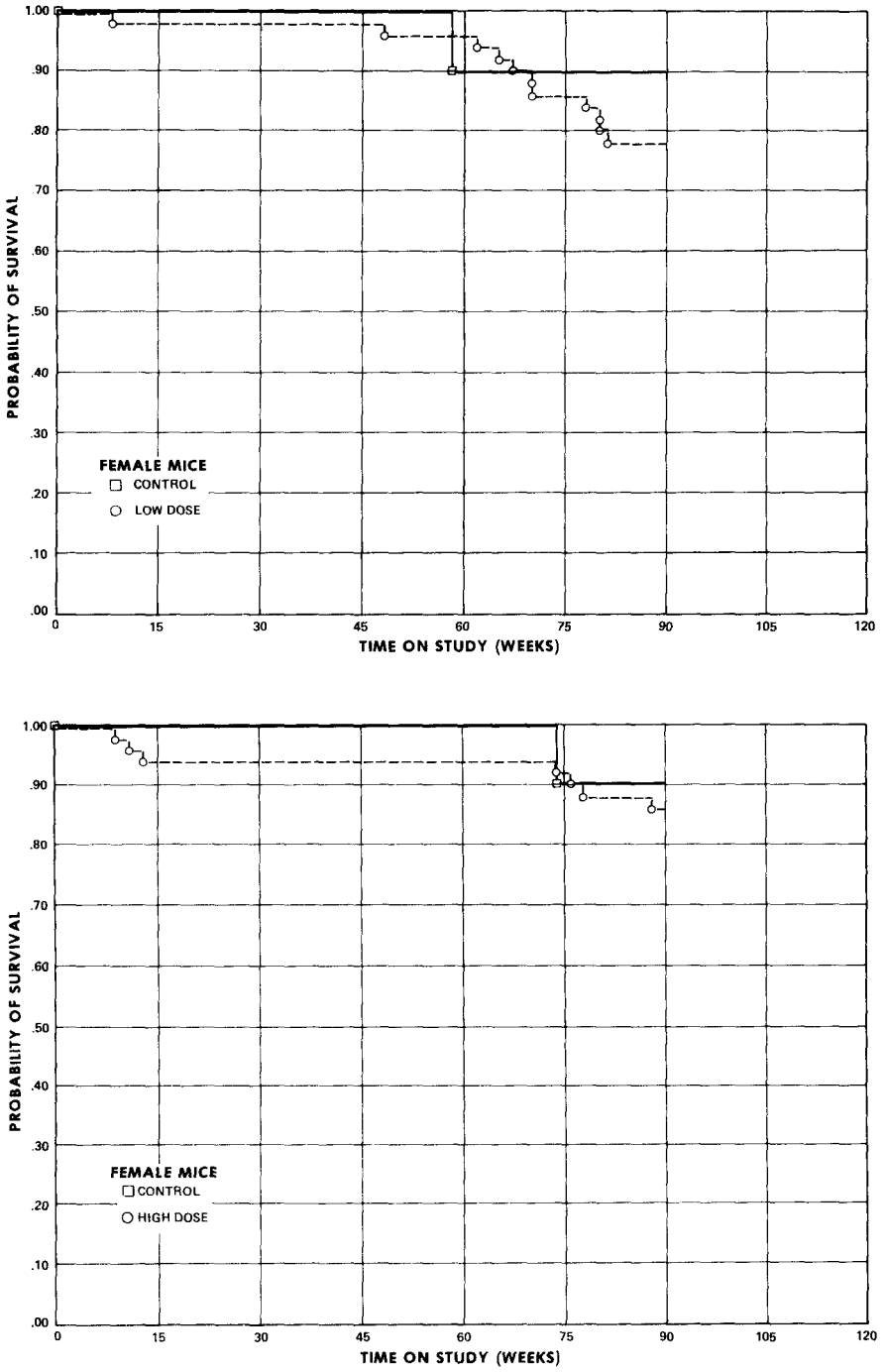


Figure 6. Survival Curves for Female Mice Fed Chlordane in the Diet

all in female mice, where the survivals of the treated groups and their respective control groups were virtually identical.

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.

A variety of inflammatory, degenerative, and proliferative lesions occurred with approximately equal frequency in chlordane-treated and control mice. These included purulent oophoritis, cystic ovaries, cystic endometrial hyperplasia and/or suppurative endometritis, lymphoid hyperplasia of the spleen, chronic nephritis, and amyloidosis of the kidney.

With the exception of primary hepatic neoplasms (table B2), a low overall incidence of neoplasms occurred in both control and test groups of mice in this study. Hepatocellular carcinomas occurred in 17/92(18%) of pooled-control males, 2/18(11%) of matched-control males, 16/48(33.3%) of low-dose males, 43/49(88%) of high-dose males; 3/78(4%) of pooled-control females, 0/19 of matched-control females, 3/47(6%) of low-dose females, and 34/49(69%) of high-dose females. The incidence of hepatocellular carcinomas, therefore, was clearly increased in both males and females at the higher dosage level; a less distinct increase in

incidence was present in the low-dose male group. The incidence in low-dose females (6%) was slightly higher than in the control females, but lower than in the control males.

There was a wide range of histologic variation from normal liver among the hepatocellular carcinomas (Lemon, 1967). Some of these lesions consisted of nodules of hepatocytes with only moderate variation from adjacent normal hepatic parenchyma in staining characteristics, size and shape of cells, and lobular architecture. Although these tumors clearly compressed adjacent normal parenchyma, they usually did not appear to be growing by invasion. At the other end of the spectrum were tumors with clearly anaplastic cytologic characteristics, hepatocytes in solid sheets or haphazardly arranged in papillary or pseudoacinar patterns, with no resemblance to normal lobular architecture within the tumor. Metastasis of hepatocellular carcinoma to the lung occurred in two high-dose males and three high-dose females.

Hepatic lesions that were classified as nodular hyperplasia (table B2) 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.

The incidence of primary pulmonary neoplasms was slightly increased in male mice (table B3), but the amount of increase was

insufficient to indicate clearly a carcinogenic effect on the lung.

Alveolar-cell adenomas (Baillif and Jones, 1973) consisted of discrete, densely cellular nodules of small, round epithelial cells arranged in rows or nests, compressing adjacent pulmonary parenchyma. The neoplastic cells had spherical, central nuclei and eosinophilic cytoplasm. Carcinomas of alveolar cells were larger and less discrete; nuclei of tumor cells were much more pleomorphic and hyperchromatic, and the nuclear:cytoplasmic ratio was much greater in the carcinomas. The neoplastic cells in the carcinomas were larger than in the adenomas: their cytoplasm was indistinct and sometimes contained clear vacuoles.

Several other types of neoplasms involving various tissues occurred infrequently (table B3), with no distinct differences in incidence between test and control groups.

D. Statistical Analyses of Results (Mice)

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

The small size of the control group notwithstanding, a highly significant difference in hepatocellular carcinoma was found between the high-dose male group and its control (table F1) by the simple proportion analysis ($P < 0.0001$) and by the life-table-

adjusted analysis ($P < 0.0001$). The low-dose group was marginally significantly different ($P = 0.05+$) from its control with the life-table-adjusted test. Comparison with the pooled controls (table F2) reinforced all of these results. In particular, the low-dose group had significantly more hepatocellular carcinoma than the pooled controls ($P = 0.04$ and 0.001) by each of the two tests.

In female mice, the difference between the high-dose and control groups with respect to the incidence of hepatocellular carcinoma (table F3) was highly significant ($P = 0.0001$ and 0.0002). The difference between the low-dose and control groups was not significant, although it was always in the direction of a positive response. The results were similar for comparisons using pooled controls (table F4).

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

V. DISCUSSION

Chlordane is a member of the organochlorine group of pesticides that can be classed as neurotoxins (Brooks, 1975). Acute exposure to chlordane stimulates the control nervous system, but the compound is less toxic than other cyclodiene pesticides. Tremors, a manifestation of the neurotoxicity that is characteristic of such compounds, developed in female high-dose rats at week 44 of treatment with chlordane and in both male and female high-dose mice at week 20.

The effects of chlordane 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 the average body weights of the high-dose male and female rats were consistently lower than those of the untreated controls, while differences between the low-dose and control rats were negligible. Adverse clinical signs in treated rats included rough and discolored hair coats and the occurrence of palpable masses. For mice given either low or high doses, body weights showed little or no effect of the chlordane; however, adverse clinical signs of alopecia, hunched appearance, and, predominantly in females, bloating or abdominal distention were observed with greater frequency in treated than in untreated mice.

The effects of chlordane on survival rates showed a dose-related decrease only in the females; in the males, the survival of the controls, for unknown reasons, was abnormally low. For mice, mortality rates were increased in low- and high-dose males but not in females. Except for the male control rats, a substantial proportion of all groups survived 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 chlordane (Appendix F, tables F1-F4), particularly at the high dose, using either matched controls or pooled controls. At the low dose, the incidence of the tumor was significant only for male mice, using pooled controls. The significance became greater when animals dying prior to appearance of the first tumor in any of the groups were eliminated from the denominators in the calculations. Finally, dose-related trends for hepatocellular carcinoma were consistently significant, using either matched or pooled controls for both males and females.

In contrast to findings with mice, the incidence of hepatocellular carcinoma in rats (Appendix E, tables E1 and E2) was extremely low in both treated and control animals. Lesions of the liver in rats did not become statistically significant with

the addition of nodular neoplasia to carcinoma or with the application of life-table adjustment to the data.

A significant dose-related trend in follicular-cell lesions of the thyroid was found, however, in the rats (tables E3 and E4). Although the trend for follicular-cell carcinoma alone was not significant, that for follicular-cell carcinoma combined with adenoma was significant or nearly significant. The trend for thyroid follicular-cell lesions was significant for females, but not for males, when pooled controls were used for comparison instead of the matched controls. A significant dose-related trend in malignant fibrous histiocytoma in male rats also was found, with the use of either matched or pooled controls (tables E7 and E8). There was, however, a large number of test rats from which no sections of thyroid were available for microscopic examination, and, in the judgment of the pathologist, the nature, incidence, and severity of neither the thyroid lesions nor the histiocytomas were sufficient to indicate clearly a carcinogenic effect of chlordane in rats.

Chlordane is metabolized primarily to the epoxide, oxychlordane, which is the principal form in which the insecticide is stored in body fat (Barnett and Dorough, 1974). Minor quanitites of other metabolites are excreted in the urine and feces.

Livers of rats fed diets containing 10 to 320 ppm (Ambrose et al., 1953), 2.5 to 25 ppm (Ortega et al., 1957), or 5 to 300 ppm (Ingle, 1965) chlordane for periods up to 2 years were reported to show abnormalities of hepatocytes, such as the appearance of intracytoplasmic bodies, enlargement of nuclei and nucleoli, and hypertrophy; however, no hepatocytic neoplasms were described. Brooks (1975) has questioned whether such lesions represent damage to the liver or simply adaptational response to challenge by foreign compounds.

In the present bioassay, chlordane was hepatocarcinogenic in mice but not in rats. In rats, chlordane induced statistically significant increases in proliferative follicular-cell lesions of the thyroid in both males and females and in malignant fibrous histiocytomas in the males. The relationship of these lesions to administration of the compound is not clear, however, 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 CHLORDANE 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	50	50
Animals Necropsied	58	8	44	43	60	10	49	44
Animals Examined Histopathologically	58	8	44	43	60	10	49	42

TABLE A2

PROLIFERATIVE LESIONS OF THE THYROID[a]

	MALE RATS				FEMALE RATS			
	Pooled Control (51)	Matched Control (6)	Low Dose (34)	High Dose (31)	Pooled Control (58)	Matched Control (10)	Low Dose (43)	High Dose (32)
Follicular-cell Carcinoma	1	0	1	2	1	0	2	2
Follicular-cell Adenoma	3	0	0	4	2	0	2	4
C-cell Carcinoma	1	0	1	2	5	3	0	3
C-cell Adenoma	3	0	4	3	7	0	3	7
Follicular-cell Hyperplasia	0	0	1	4	4	1	4	5
C-cell Hyperplasia	29	4	15	6	30	4	22	10

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

TABLE A3

OTHER ENDOCRINE NEOPLASMS[a]

	MALE RATS				FEMALE RATS			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
ADRENAL	(55)	(7)	(39)	(36)	(56)	(9)	(48)	(36)
Cortical Carcinoma	1	0	0	0	0	0	1	1
Cortical Adenoma	2	0	1	0	0	0	0	0
PANCREATIC ISLETS	(52)	(7)	(42)	(36)	(60)	(10)	(45)	(40)
Adenoma	1	0	0	0	1	0	0	1
PITUITARY	(48)	(6)	(34)	(34)	(52)	(10)	(46)	(37)
Carcinoma	2	0	1	1	0	0	1	0
Adenoma	14	0	9	7	23	6	7	11

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

TABLE A4

PRIMARY HEPATIC NEOPLASMS[a]

	MALE RATS				FEMALE RATS			
	Pooled Control (58)	Matched Control (8)	Low Dose (44)	High Dose (40)	Pooled Control (59)	Matched Control (10)	Low Dose (47)	High Dose (39)
Hepatocellular Carcinoma	1	0	1	0	0	0	0	0
Neoplastic Nodule	2	0	1	0	5	1	11	6
Hemangiosarcoma	3	0	0	0	0	0	0	1

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

TABLE A5

PRIMARY RENAL NEOPLASMS[a]

	MALE RATS				FEMALE RATS			
	Pooled Control (57)	Matched Control (8)	Low Dose (43)	High Dose (42)	Pooled Control (58)	Matched Control (10)	Low Dose (49)	High Dose (42)
Malignant Mixed Tumor	0	0	4	0	1	0	0	0
Tubular Adenoma	3	1	0	0	0	0	0	0

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

TABLE A6

NEOPLASMS OF THE REPRODUCTIVE SYSTEM[a]

	MALE RATS				FEMALE RATS			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
TESTIS	(58)	(8)	(43)	(42)	---	---	---	---
Interstitial-cell Tumor	0	0	1	1				
OVARY	---	---	---	---	(58)	(9)	(48)	(36)
Granulosa-cell Tumor					1	0	0	1
UTERUS	---	---	---	---	(56)	(9)	(47)	(33)
Sarcoma, N.O.S.					0	0	0	2
Hemangiosarcoma					0	0	1	0
Adenocarcinoma					0	0	1	0
Endometrial Stromal Polyp					6	0	3	1

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

TABLE A7

NEOPLASMS OF THE MAMMARY GLAND[a]

	MALE RATS				FEMALE RATS			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
MAMMARY GLAND	(58)	(8)	(44)	(43)	(60)	(10)	(49)	(44)
Carcinoma	0	0	0	0	2	1	2	3
Adenoma	0	0	0	0	1	0	10	4
Fibroadenoma	0	0	0	0	8	1	6	3
Fibroma	1	0	0	0	1	0	1	0
Lipoma	0	0	0	1	0	0	0	0

[a]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
Malignant Lymphoma	(58)[b]	(8)[b]	(44)[b]	(44)[b]	(60)[b]	(10)[b]	(49)[b]	(44)[b]
	1	1	0	1	2	0	0	0
Malignant Fibrous Histiocytoma (any site)	2	0	1	7	1	1	1	1
SKIN/SUBCUTIS	(58)[b]	(8)[b]	(44)[b]	(43)[b]	(60)[b]	(10)[b]	(49)[b]	(44)[b]
Squamous-cell Carcinoma	0	0	0	1	0	0	0	0
Carcinoma, N.O.S.	0	0	1	0	0	0	0	0
Lipoma	0	0	1	0	1	0	0	0
LUNG	(58)	(8)	(43)	(39)	(58)	(10)	(48)	(48)
Alveolar-cell Adenoma	0	0	1	0	0	0	0	0
BRAIN	(57)	(8)	(44)	(42)	(59)	(10)	(49)	(41)
Glioma	1	1	0	0	0	0	1	0

TABLE A8

MISCELLANEOUS NEOPLASMS[a]

(continued)

	MALE RATS				FEMALE RATS			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
DIAPHRAGM	(58)[b]	(8)[b]	(44)[b]	(43)[b]	(60)[b]	(10)[b]	(49)[b]	(44)[b]
Osteosarcoma	0	0	0	1	0	0	0	0
SPLEEN	(56)	(8)	(44)	(38)	(55)	(10)	(49)	(38)
Hemangioma	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 CHLORDANE 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	18	50	50	80	20	50	50
Animals Necropsied	92	18	49	49	79	20	50	50
Animals Examined Histopathologically	92	18	48	49	79	20	50	50

TABLE B2

PROLIFERATIVE HEPATIC LESIONS[a]

	MALE MICE				FEMALE MICE			
	Pooled Control	Matched Control	Low Dose	High Dose	Pooled Control	Matched Control	Low Dose	High Dose
LIVER	(92)	(18)	(48)	(49)	(78)	(19)	(47)	(49)
Hepatocellular Carcinoma	17	2	16	43	3	0	3	34
Nodular Hyperplasia	3	2	7	0	2	1	3	3
Diffuse Hyperplasia	3	3	3	0	1	0	3	3
Hepatocytomegaly	0	0	1	1	1	0	0	0

[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
LUNG	(91)	(17)	(46)	(46)	(79)	(20)	(47)	(47)
Alveolar-cell Carcinoma	0	0	1	1	0	0	0	0
Alveolar-cell Adenoma	6	1	5	2	0	0	0	1
HEMATOPOIETIC SYSTEM	(92)[b]	(18)[b]	(49)[b]	(49)[b]	(79)[b]	(20)[b]	(50)[b]	(50)[b]
Malignant Lymphoma[c]	5	1	1	0	8	1	1	0
THYROID	(79)	(17)	(36)	(35)	(72)	(16)	(43)	(35)
Follicular-cell Adenoma	1	0	0	1	1	0	0	0
KIDNEY	(92)	(18)	(48)	(47)	(78)	(20)	(46)	(48)
Tubular Adenoma	0	0	0	1	0	0	0	0
MAMMARY GLAND	(92)[b]	(18)[b]	(49)[b]	(49)[b]	(79)[b]	(20)[b]	(50)[b]	(50)[b]
Fibroadenoma	0	0	0	0	0	0	1	0
Neurofibroma	0	0	0	0	0	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
LACRIMAL GLAND	(92)[b]	(18)[b]	(49)[b]	(49)[b]	(79)[b]	(20)[b]	(50)[b]	(50)[b]
Adenoma	1	0	0	0	1	1	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.

[c]For purposes of this summary, the following lesions are grouped under malignant lymphoma: lymphocytic lymphosarcoma, lymphosarcoma, and reticulum-cell sarcoma (any site).

APPENDIX C

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

TABLE C1

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	8 (100%)	44 (100%)	47 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	8	44	47
ANIMALS WITH NON-TUMOR PATHOLOGY	8 (100%)	43 (98%)	38 (81%)
INTEGUMENTARY SYSTEM *	1 (13%)	3 (7%)	
SUBCUT TISSUE	1	3	
GRANULOMA		1	
GRANULATION TISSUE	1	2	
RESPIRATORY SYSTEM	3 (38%)	25 (57%)	15 (32%)
TRACHEA	1	3	
INFLAMMATION CHRONIC	1	3	
LUNG	3	23	15
EMPHYSEMA		2	
ATELECTASIS		2	
INFLAMMATION INTERSTITIAL		1	
PNEUMONIA ASPIRATION		1	
CHOLESTEROL DEPOSITION		3	1
ALVEOLAR MACROPHAGES	2	21	15
LYMPHOID HYPERPLASIA	1	1	
CIRCULATORY SYSTEM		21 (48%)	15 (32%)
MYOCARDIUM		21	14
INFLAMMATION		3	
FIBROSIS		21	14
ENDOCARDIUM			1
FIBROSIS			1
AORTA		1	2
ARTERIOSCLEROSIS		1	2
CORONARY ARTERY		1	
CALCIFICATION		1	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C1 MALE RATS: NONNEOPLASTIC LESIONS (CONT.)

	CONTROL	LOW DOSE	HIGH DOSE
PULMONARY ARTERY		1	1
INFLAMMATION		1	
INFLAMMATION ACUTE			1
MESENTERIC ARTERY			1
PERIARTERITIS			1
DIGESTIVE SYSTEM *	6 (75%)	40 (91%)	31 (66%)
SALIVARY GLAND			1
FIBROSIS			1
LIVER	5	28	16
CONGESTION		1	
METAMORPHOSIS FATTY		1	
CYTOPLASMIC VACUOLIZATION	3	19	3
HEPATOCYTOMEGALY	4	25	13
HYPERPLASIA DIFFUSE			2
ANGIECTASIS	1	4	5
BILE DUCT	4	35	25
DILATATION		1	
INFLAMMATION	2	18	20
FIBROSIS		7	3
HYPERPLASIA	4	32	19
PANCREAS		8	7
ECTOPIA		1	
INFLAMMATION ACUTE		1	
FIBROSIS		5	6
PERIARTERITIS		2	3
PANCREATIC ACINUS	1	4	4
ATROPHY	1	4	4
STOMACH		2	2
EROSIVE INFLAMMATION		1	2
CALCIFICATION DYSTROPHIC		1	
GASTRIC MUCOSA		1	3
CALCIFICATION		1	3

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C1 MALE RATS: NONNEOPLASTIC LESIONS (CONT.)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM *	3 (38%)	38 (86%)	32 (68%)
KIDNEY	3	38	32
INFLAMMATION INTERSTITIAL		1	
INFLAMMATION CHRONIC	3	35	31
PYELONEPHRITIS CHRONIC		2	1
HEMATOPOIESIS		1	
KIDNEY/PELVIS		2	5
INFLAMMATION CHRONIC			1
HYPERPLASIA EPITHELIAL		2	4
URINARY BLADDER		5	5
INFLAMMATION CHRONIC		2	
HYPERPLASIA EPITHELIAL		5	5
ENDOCRINE SYSTEM	5 (63%)	31 (70%)	19 (40%)
PITUITARY	1	6	3
CYST		2	1
HEMORRHAGE		1	
ATROPHY			1
HYPERPLASIA CHROMOPHOBE-CELL	1	3	1
ANGIECTASIS		1	
ADRENAL CORTEX	1	17	8
CYTOMEGALY	1	16	6
PHAGOCYTIC CELL			1
HYPERPLASIA FOCAL		1	1
THYROID	4	21	13
ULTIMOBRANCHIAL CYST		2	3
CYSTIC FOLLICLES		5	2
ATROPHY			1
HYPERPLASIA C-CELL	4	15	6
HYPERPLASIA FOLLICULAR-CELL		1	4
PARATHYROID	1	4	3
HYPERPLASIA	1	3	3
HYPERPLASIA DIFFUSE		1	
PANCREATIC ISLETS		2	
HYPERPLASIA		2	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C1 MALE RATS: NONNEOPLASTIC LESIONS (CONT.)

	CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM *		8 (18%)	9 (19%)
SPLEEN		7	9
HEMOSIDEROSIS		3	2
HYPERPLASIA RETICULUM-CELL		2	
HEMATOPOIESIS		4	7
LYMPH NODE		1	1
GRANULOMA			1
LYMPHOID HYPERPLASIA		1	
CERVICAL LYMPH NODE		1	
INFLAMMATION		1	
REPRODUCTIVE SYSTEM	3 (38%)	33 (75%)	26 (55%)
PROSTATE	1	3	5
INFLAMMATION SUPPURATIVE	1	3	5
INFLAMMATION ACTIVE CHRONIC	1		
METAPLASIA SQUAMOUS		1	3
TESTIS	3	32	24
EDEMA	1	1	
PERIARTERITIS		1	6
ATROPHY	3	32	24
NERVOUS SYSTEM			
NONE			
MUSCULOSKELETAL SYSTEM			1 (2%)
FASCIA			1
INFLAMMATION			1
SPECIAL SENSE ORGANS			1 (2%)
EYE			1
CATARACT			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 *	2 (25%)	10 (23%)	15 (32%)
FOOT			1
GRANULATION TISSUE			1
NO ASSOCIATED ORGAN	2	7	11
NO LESION REPORTED		1	4
ACCIDENTAL DEATH			4
AUTOLYSIS/NO NECROPSY PERFORMED	2	6	3
ADIPOSE TISSUE		1	
INFLAMMATION		1	
MESENTERY		2	3
HEMORRHAGE		1	
PERIARTERITIS		1	1
ARTERIOSCLEROSIS			1
NECROSIS FAT			1

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C2

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10 (100%)	49 (100%)	44 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	42
ANIMALS WITH NON-TUMOR PATHOLOGY	10 (100%)	48 (98%)	41 (93%)
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM *	2 (20%)	18 (37%)	20 (45%)
TRACHEA		1	
INFLAMMATION CHRONIC		1	
LUNG	2	16	20
EMPHYSEMA		4	
ATELECTASIS		4	
CONGESTION		1	
INFLAMMATION INTERSTITIAL			1
CHOLESTEROL DEPOSITION		2	2
ALVEOLAR MACROPHAGES	2	14	20
HYPERPLASIA ALVEOLAR-CELL			1
LYMPHOID HYPERPLASIA			1
LUNG/ALVEOLI		5	2
INFLAMMATION SUPPURATIVE		5	2
CIRCULATORY SYSTEM	3 (30%)	9 (18%)	5 (11%)
HEART		3	
FIBROSIS		3	
MYOCARDIUM	3	6	5
FIBROSIS	3	5	5
DEGENERATION	1		
CALCIFICATION		1	
ENDOCARDIUM		1	
FIBROSIS		1	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONT.)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM *	9 (90%)	42 (86%)	38 (86%)
LIVER	6	28	28
CONGESTION			1
FIBROSIS FOCAL		1	
DEGENERATION			1
NECROSIS		1	1
NECROSIS FOCAL		6	4
METAMORPHOSIS FATTY			1
CYTOPLASMIC VACUOLIZATION	6	8	17
HEPATOCYTOMEGALY	6	20	22
HYPERPLASIA DIFFUSE		1	
ANGIECTASIS		2	16
LIVER/HEPATOCYTES			1
CYTOPLASMIC VACUOLIZATION			1
BILE DUCT	5	38	30
DILATATION		3	1
INFLAMMATION	3	25	19
FIBROSIS	1	13	9
HYPERPLASIA	4	31	24
PANCREAS	1	6	1
INFLAMMATION CHRONIC		1	
GRANULOMA			1
FIBROSIS	1	5	
PANCREATIC ACINUS		3	
ATROPHY		3	
URINARY SYSTEM	9 (90%)	27 (55%)	30 (68%)
KIDNEY	6	21	28
CYST			1
INFLAMMATION INTERSTITIAL	1	1	
INFLAMMATION CHRONIC	5	20	26
FIBROSIS FOCAL			1
KIDNEY/PELVIS	3	7	3
HYPERPLASIA EPITHELIAL	3	7	3
URINARY BLADDER	3	3	1
HYPERPLASIA EPITHELIAL	3	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 *	8 (80%)	33 (67%)	25 (57%)
PITUITARY	3	7	6
CONGESTION	1		
HEMORRHAGE		1	
HEMOSIDEROSIS		1	
HYPERPLASIA FOCAL			1
HYPERPLASIA CHROMOPHOBE-CELL	2	5	5
ADRENAL	2	5	
DEGENERATION	1		
ANGIECTASIS	1	5	
ADRENAL CORTEX	3	12	12
METAMORPHOSIS FATTY			1
CYTOMEGALY	2	11	8
HYPERPLASIA	1		1
HYPERPLASIA FOCAL			2
ANGIECTASIS		1	
THYROID	5	25	15
ULTIMOBRANCHIAL CYST		1	1
CYSTIC FOLLICLES		4	
SCAR	1		
ATROPHY FOCAL	1		
HYPERPLASIA C-CELL	4	22	11
HYPERPLASIA FOLLICULAR-CELL	1	4	5
PARATHYROID		1	
HYPERPLASIA		1	
PANCREATIC ISLETS	1		
HYPERPLASIA	1		
HEMATOPOIETIC SYSTEM	4 (40%)	13 (27%)	12 (27%)
SPLEEN	4	8	11
INFLAMMATION CYSTIC		1	
HEMOSIDEROSIS	3	5	6
HYPERPLASIA RETICULUM-CELL			1
HEMATOPOIESIS	2	2	5
CERVICAL LYMPH NODE		1	
DILATATION/SINUS		1	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE C2 FEMALE RATS: NONNEOPLASTIC LESIONS (CONT.)

	CONTROL	LOW DOSE	HIGH DOSE
THYMUS		4	1
CYST		4	
ATROPHY			1
REPRODUCTIVE SYSTEM *	1 (10%)	3 (6%)	6 (14%)
MAMMARY GLAND	1		
HYPERPLASIA	1		
UTERUS/ENDOMETRIUM		3	5
INFLAMMATION SUPPURATIVE		1	1
HYPERPLASIA		1	1
HYPERPLASIA CYSTIC		2	4
OVARY			1
GRANULATION TISSUE			1
NERVOUS SYSTEM			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
ALL OTHER SYSTEMS		1 (2%)	10 (23%)
ABDOMINAL CAVITY			1
GRANULOMA			1
NO ASSOCIATED ORGAN		1	9
NO LESION REPORTED			1
AUTOLYSIS/NECROPSY PERF/NO HISTO			2
AUTOLYSIS/NO NECROPSY PERFORMED		1	6

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

APPENDIX D

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

TABLE D1

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

	HIGH DOSE CONTROL	LOW DOSE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	10	50	50
ANIMALS NECROPSIED	10 (100%)	8 (100%)	49 (100%)	49 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	8	48	49
ANIMALS WITH NON-TUMOR PATHOLOGY	2 (20%)	3 (38%)	14 (29%)	6 (12%)
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM *				1 (2%)
LUNG				1
CONGESTION				1
CIRCULATORY SYSTEM			2 (4%)	
HEART			1	
DEGENERATION PARENCHYMATOUS			1	
MYOCARDIUM			1	
INFLAMMATION CHRONIC			1	
DIGESTIVE SYSTEM	2 (20%)	2 (25%)	10 (20%)	2 (4%)
LIVER	2	1	10	1
HEPATOCYTOMEGALY			1	1
HYPERPLASIA NODULAR	1	1	7	
HYPERPLASIA DIFFUSE	2	1	3	
STOMACH				1
ULCER CHRONIC				1
SMALL INTESTINE		1		
POLYP		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
URINARY SYSTEM *			2 (4%)	1 (2%)
KIDNEY			2	1
INFLAMMATION INTERSTITIAL			2	1
ENDOCRINE SYSTEM			1 (2%)	1 (2%)
THYROID			1	1
ULTIMOBRANCHIAL CYST			1	
HYPERPLASIA FOLLICULAR-CELL				1
HEMATOPOIETIC SYSTEM	1 (10%)	1 (13%)	2 (4%)	
SPLEEN			2	
LYMPHOID HYPERPLASIA			2	
LYMPH NODE	1			
INFLAMMATION	1			
MESENTERIC LYMPHNODE		1		
EDEMA		1		
REPRODUCTIVE SYSTEM			1 (2%)	1 (2%)
TESTIS			1	1
ATROPHY			1	1
NERVOUS SYSTEM				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
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
ALL OTHER SYSTEMS *	7 (70%)	5 (63%)	20 (41%)	5 (10%)
PERITONEUM		1		
INFLAMMATION		1		
NO ASSOCIATED ORGAN	7	4	20	5
NO LESION REPORTED	7	4	18	4
AUTOLYSIS/NECROPSY PERF/NO HISTO			1	
AUTOLYSIS/NO NECROPSY PERFORMED			1	1

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE D2

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

	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	10	50	50
ANIMALS NECROPSIED	10 (100%)	10 (100%)	50 (100%)	50 (100%)
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	10	50	50
ANIMALS WITH NON-TUMOR PATHOLOGY	7 (70%)	6 (60%)	28 (56%)	16 (32%)
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM *	2 (20%)		1 (2%)	1 (2%)
LUNG	2		1	1
CONGESTION PASSIVE			1	
EDEMA			1	
INFLAMMATION FOCAL				1
HYPERPLASIA ALVEOLAR-CELL	2			
CIRCULATORY SYSTEM			1 (2%)	
RENAL ARTERY			1	
INFLAMMATION			1	
DIGESTIVE SYSTEM	1 (10%)	1 (10%)	6 (12%)	9 (18%)
LIVER	1	1	5	6
INFLAMMATION	1			1
INFLAMMATION FOCAL GRANULOMATOUS			1	
NECROSIS FOCAL				1
HYPERPLASIA NODULAR		1	3	3
HYPERPLASIA DIFFUSE			3	3
HEMATOPOIESIS			1	
PANCREAS			1	2
ATROPHY			1	2
STOMACH				1
INFLAMMATION ACUTE				1

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE D2 FEMALE MICE: NONNEOPLASTIC LESIONS (CONT.)

	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM *	1 (10%)	1 (10%)	3 (6%)	2 (4%)
KIDNEY	1	1	3	2
LYMPHOCYTIC INFLAM INFILTRATE				1
INFLAMMATION INTERSTITIAL			2	1
INFLAMMATION GRANULOMATOUS			1	
AMYLOIDOSIS	1	1		
ENDOCRINE SYSTEM			1 (2%)	1 (2%)
THYROID			1	1
HYPERPLASIA FOLLICULAR-CELL			1	1
HEMATOPOIETIC SYSTEM	2 (20%)	1 (10%)	3 (6%)	1 (2%)
SPLEEN	2		3	1
INFLAMMATION GRANULOMATOUS			1	
LYMPHOID HYPERPLASIA	2			1
HEMATOPOIESIS			2	
LYMPH NODE		1	1	1
CONGESTION CHRONIC PASSIVE		1		
INFLAMMATION				1
INFLAMMATION GRANULOMATOUS			1	
RENAL LYMPH NODE	1			
INFLAMMATION	1			
REPRODUCTIVE SYSTEM	3 (30%)	5 (50%)	21 (42%)	3 (6%)
UTERUS			2	1
INFLAMMATION				1
INFLAMMATION SUPPURATIVE			2	
UTERUS/ENDOMETRIUM	1	1	5	1
INFLAMMATION SUPPURATIVE	1			
HYPERPLASIA CYSTIC		1	5	1
OVARY/OVIDUCT		1	1	
INFLAMMATION		1		
INFLAMMATION ACUTE SUPPURATIVE			1	

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

TABLE D2 FEMALE MICE: NONNEOPLASTIC LESIONS (CONT.)

	LOW DOSE CONTROL	HIGH DOSE CONTROL	LOW DOSE	HIGH DOSE
OVARY	3	4	16	1
CYST		1		
FOLLICULAR CYST		1	3	1
CORPUS LUTEUM CYST		1		
INFLAMMATION SUPPURATIVE			4	
INFLAMMATION ACUTE SUPPURATIVE	2		6	
INFLAMMATION SUBACUTE	1		1	
INFLAMMATION CHRONIC		1	2	
NERVOUS SYSTEM				
NONE				
MUSCULOSKELETAL SYSTEM *			1 (2%)	
SKELETAL MUSCLE			1	
DEGENERATION			1	
SPECIAL SENSE ORGANS				
NONE				
ALL OTHER SYSTEMS	3 (30%)	4 (40%)	17 (34%)	9 (18%)
PERITONEUM	1			
INFLAMMATION ACUTE	1			
NO ASSOCIATED ORGAN	2	4	17	9
NO LESION REPORTED	2	4	17	9

*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

APPENDIX E

STATISTICAL ANALYSES OF NEOPLASMS IN RATS FED CHLORDANE IN THE DIET

Table E1. Statistical Analysis of Neoplasms of the Liver in Rats Fed Chlordane 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	0/8 0%	1/44 2%	0/40 0%	0.57[c]	--[d]
Female	0/10 0%	0/47 0%	0/39 0%	--	
Hepatocellular Carcinoma and/or Neoplastic Nodule					
Male	0/8 0%	2/44 5%	0/40 0%	0.39[c]	--[d]
Female	1/10 10%	11/47 23%	6/39 15%	0.51[c]	0.43

[a] Males: 203.5 ppm; females: 120.8 ppm.
[b] Males: 407.0 ppm; females: 241.5 ppm.
[c] P value given in the direction of a negative linear trend.
[d] Validity of test is dubious because of the small number of animals with tumors.

Table E2. Statistical Analysis of Neoplasms of the Liver in Rats Fed Chlordane 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%	1/44 2%	0/40 0%	0.42[c]	--[e]
Female	0/59 0%	0/47 0%	0/39 0%	--	--
Hepatocellular Carcinoma and/or Neoplastic Nodule					
Male	3/58 5%	2/44 5%	0/40 0%	0.15[c]	0.08[c]
Female	5/59 8%	11/47[d] 23%	6/39 15%	0.10	0.08

[a]Males: 203.5 ppm; females 120.8 ppm.
[b]Males: 407.0 ppm; females: 241.5 ppm.
[c]P value given in the direction of a negative linear trend.
[d]Significantly higher than its pooled control in both test ($P < 0.05$).
[e]Validity of test is dubious because of the small number of animals with tumors.

Table E3. Statistical Analysis of Neoplasms of Thyroid Follicular Cells in Rats Fed Chlordane 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	0/6 0%	1/34 3%	2/31 6%	0.36	--[d]
Female	0/10(0/9) 0% (0%)	2/43(2/38) 5% (5%)	2/32(2/27) 6% (7%)	0.37(0.35)	--[d]
Carcinoma and/or Adenoma					
Male	0/6 0%	1/34 3%	6/31 19%	0.02	0.01+
Female	0/10 0%	4/43 9%	6/32 19%	0.06	0.02

[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; 203.5 ppm; females: 120.8 ppm.
[c]Males: 407.0 ppm; females: 241.5 ppm.
[d]Validity of test is dubious because of the small number of animals with tumors.

Table E4. Statistical Analysis of Neoplasms of Thyroid Follicular Cells in Rats Fed Chlordane 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%	1/34 3%	2/31 6%	0.23	--[e]
Female	1/58 2%	2/43 5%	2/32 6%	0.18	0.11
Carcinoma and/or Adenoma					
Male	4/51 8%	1/34 3%	6/31 19%	0.10	0.09
Female	3/58 5%	4/43 9%	6/32[d] 19%	0.03	0.01

[a]Males: 203.5 ppm; females: 120.8 ppm.
[b]Males: 407.0 ppm; females: 241.5 ppm.
[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 animals with tumors.

Table E5. Statistical Analysis of Neoplasms of Thyroid C-cells in Rats Fed Chlordane in the Diet (using matched 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	0/6 0%	1/34 3%	2/31 6%	0.36	--[e]
Female	3/10 30%	0/43 0%	3/32 9%	0.25[c,d]	0.23[c,d]
Carcinoma and/or Adenoma					
Male	0/6 0%	5/34 15%	5/31 16%	0.30	0.21
Female	3/10 30%	3/43 7%	10/32 31%	0.16[d]	0.046[d]

[a]Males: 203.5 ppm; females: 120.8 ppm.
[b]Males: 407.0 ppm; females: 241.5 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]Validity of test is dubious because of the small number of animals with tumors.

Table E6. Statistical Analysis of Neoplasms of Thyroid C-cells in Rats Fed Chlordane 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%	1/34 3%	2/31 6%	0.23	--[h]
Female	5/58 9%	0/43[f] 0%	3/32 9%	0.36[c,d]	0.48[c,d]
Carcinoma and/or Adenoma					
Male	4/51 8%	5/34 15%	5/31 16%	0.16	0.16
Female	11/58 19%	3/43[f] 7%	10/32[g] 31%	0.18[d]	0.08[e]

[a]Males: 203.5 ppm; females: 120.8 ppm.
[b]Males: 407.0 ppm; females: 241.5 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]Data depart significantly from a linear trend ($P < 0.01$).
[f]Significantly lower than its pooled control in life-table adjusted test ($P < 0.05$).
[g]Significantly higher than its pooled control in life-table adjusted test ($P < 0.05$).
[h]Validity of test is dubious because of the small number of animals with tumors.

Table E7. Statistical Analysis of Malignant Fibrous Histiocytoma in Rats Fed Chlordane in the Diet (using matched control)

Sex	No. Rats with Lesion/ No. Rats Necropsied[a] Control	Low Dose[b]	High Dose[c]	Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Male	0/8(0/5) 0% (0%)	1/44(1/37) 2% (3%)	7/44(7/37) 16% (19%)	0.014(0.016)	0.007
Female	1/10(1/10) 10% (10%)	1/49(1/46) 2% (2%)	1/44(1/36) 2% (3%)	0.32[d](0.37)[d]	--[e]

[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: 203.5 ppm; females: 120.8 ppm.
[c]Males: 407.0 ppm; females: 241.5 ppm.
[d]P value given in the direction of a negative linear trend.
[e]Validity of test is dubious because of the small number of animals with tumors.

Table E8. Statistical Analysis of Malignant Fibrous Histiocytoma in Rats Fed Chlordane in the Diet (using pooled control)

Sex	No. Rats with Lesion/ No. Rats Necropsied			Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
	Control	Low Dose[a]	High Dose[b]		
Male	2/58	1/44	7/44[c]		
	3%	2%	16%	0.02	0.01
Female	1/60	1/49	1/44		
	2%	2%	2%	0.54	--[d]

[a]Males: 203.5 ppm; females: 120.8 ppm.
[b]Males: 407.0 ppm; females: 241.5 ppm.
[c]Significantly higher than its pooled control in both tests ($P < 0.05$).
[d]Validity of test is dubious because of the small number of animals with tumors.

Table E9. Statistical Analysis of Neoplasms of the Pituitary Gland in Rats Fed Chlordane in the Diet (using matched control)

Sex	No. Rats with Lesion/ No. Rats with Pituitary Examined[a] Control	Low Dose[b]	High Dose[c]	Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Chromophobe Adenocarcinoma or Carcinoma					
Male	0/6(0/5) 0% (0%)	1/34(1/32) 3% (3%)	1/34(1/31) 3% (3%)	0.64(0.64)	--[g]
Female	0/10(0/9) 0% (0%)	1/46(1/41) 2% (2%)	0/37(0/32) 0% (0%)	0.60[d](0.61)[d]	--[g]
Chromophobe Adenocarcinoma or Carcinoma and/or Chromophobe Adenoma					
Male	0/6 0%	10/34 29%	8/34 24%	0.39	0.23
Female	6/10 60%	8/46 17%	11/37 30%	0.26[d,e]	0.38[d,f]

[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: 203.5 ppm; females: 120.8 ppm.
[c]Males: 407.0 ppm; females: 241.5 ppm.
[d]P value given in the direction of a negative linear trend.
[e]Data depart significantly from a linear trend ($P < 0.01$).
[f]Data depart significantly from a linear trend ($P < 0.05$).
[g]Validity of test is dubious because of the small number of animals with tumors.

Table E10. Statistical Analysis of Neoplasms of the Pituitary Gland in Rats Fed Chlordane in the Diet (using pooled 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 and/or Adenoma					
Male	16/48 33%	10/34 29%	8/34 24%	0.20	0.18[c]
Female	23/52 44%	8/46[e] 17%	11/37 30%	0.04[c,d]	0.13[c,d]

[a]Males: 203.5 ppm; females: 120.8 ppm.
[b]Males: 407.0 ppm; females: 241.5 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 both tests ($P < 0.01$).

Table E11. Statistical Analysis of Neoplasms of the Mammary Gland in Female Rats Fed Chlordane in the Diet (using matched control)

No. Rats with Lesion/ No. Rats Necropsied[a]			Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Control (0 ppm)	Low Dose (120.8 ppm)	High Dose (241.5 ppm)		
Carcinoma or Adenocarcinoma				
1/10(1/10)	2/49(2/49)	3/44(3/40)		
10% (10%)	4% (4%)	7% (8%)	0.63(0.59)	0.41
Carcinoma or Adenocarcinoma and/or Adenoma, Fibroadenoma or Fibroma				
2/10	18/49	8/44		
20%	37%	18%	0.17[b]	0.34[b]

[a]The early deaths are eliminated in those figures in parentheses, i.e., deaths before the first tumor was found in any of those groups being compared to one another.

[b]P value is given in the direction of a negative linear trend.

Table E12. Statistical Analysis of Neoplasms of the Mammary Gland in Female Rats Fed Chlordane in the Diet (using pooled control)

No. Rats with Lesion/ No. Rats Necropsied			Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Control (0 ppm)	Low Dose (120.8 ppm)	High Dose (241.5 ppm)		
Carcinoma or Adenocarcinoma				
2/60 3%	2/49 4%	3/44 7%	0.27	0.17
Carcinoma or Adenocarcinoma and/or Adenoma, Fibroadenoma or Fibroma				
10/60 17%	18/49[c] 37%	8/44 18%	0.30[a]	0.16[b]

[a]Data depart significantly from a linear trend ($P < 0.01$).
[b]Data depart significantly from a linear trend ($P < 0.05$).
[c]Significantly higher than its pooled control in both tests ($P < 0.05$).

Table E13. Statistical Analysis of Neoplasms of the Uterus in Female Rats Fed Chlordane in the Diet (using matched control)

No. Rats with Lesion/ No. Rats with Uterus Examined[a]			Exact Test for Dose-related Trend (P)	Tests After Life-table Adjustment (P)
Control (0 ppm)	Low Dose (120.8 ppm)	High Dose (241.5 ppm)		
Sarcoma				
0/9(0/8) 0% (0%)	0/47(0/35) 0% (0%)	2/33(2/22) 6% (9%)	0.13(0.11)	--[c]
Sarcoma and/or Endometrial Stromal Polyp				
0/9 0%	3/47 6%	3/33 9%	0.28	0.12
Adenocarcinoma				
0/9(0/8) 0% (0%)	1/47(1/41) 2% (2%)	0/33(0/23) 0% (0%)	0.63[b](0.68)[b]	--[c]
Sarcoma and/or Adenocarcinoma				
0/9 0%	1/47 2%	2/33 6%	0.27	--[c]
Sarcoma and/or Adenocarcinoma and/or Endometrial Stromal Polyp				
0/9 0%	4/47 9%	3/33 9%	0.36	0.17

[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 is given in the direction of a negative linear trend.
[c]Validity of test is dubious because of the small number of animals with tumors.

Table E14. Statistical Analysis of Neoplasms of the Uterus in Female Rats Fed Chlordane in the Diet (using pooled control)

No. Rats with Lesion/ No. Rats with Uterus Examined			Exact Test for Dose-related Trend	Tests After Life-table Adjustment
Control (0 ppm)	Low Dose (120.8 ppm)	High Dose (241.5 ppm)	(P)	(P)
Sarcoma				
0/56 0%	0/47 0%	2/33[b] 6%	0.06	--[c]
Sarcoma and/or Endometrial Stromal Polyp				
6/56 11%	3/47 6%	3/33 9%	0.36[a]	0.44[a]
Sarcoma and/or Adenocarcinoma				
0/56 0%	1/47 2%	2/33[b] 6%	0.07	--[c]
Sarcoma and/or Adenocarcinoma and/or Endometrial Stromal Polyp				
6/56 11%	4/47 9%	3/33 9%	0.41[a]	0.47[a]

[a]P value is given in the direction of a negative trend.
[b]Significantly higher than its pooled control in life-table adjusted test ($P < 0.05$).
[c]Validity of test is dubious because of the small number of animals with tumors.

APPENDIX F

STATISTICAL ANALYSES OF NEOPLASMS IN MICE FED CHLORDANE IN THE DIET

Table F1. Statistical Analysis of Neoplasms of the Liver in Male Mice Fed Chlordane 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 (29.9 ppm)	High Dose (56.2 ppm)			
Hepatocellular Carcinoma					
1/10(1/10)	--	43/49(43/47)			
10% (10%)	--	88% (91%)	< 0.0001(< 0.0001)		< 0.0001
1/8(1/8)	16/48(16/36)	--			
12%(12%)	33%(44%)	--	0.23(0.10)		0.05+
Combined				< 0.0001(< 0.0001)	< 0.0001

[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.

Table F2. Statistical Analysis of Neoplasms of the Liver in Male Mice Fed Chlordane 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 (29.9 ppm)	High Dose (56.2 ppm)			
Hepatocellular Carcinoma					
17/92	--	43/49			
18%	--	88%	< 0.0001		< 0.0001
17/92	16/48	--			
18%	33%	--	0.04		0.001
17/92	16/48	43/49			
18%	33%	88%		< 0.0001[a]	< 0.0001

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

Table F3. Statistical Analysis of Neoplasms of the Liver in Female Mice Fed Chlordane 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 (30.1 ppm)	High Dose (63.8 ppm)			
Hepatocellular Carcinoma					
0/9(0/9)	--	34/49(34/47)			
0% (0%)	--	69% (72%)	0.0001(0.0001)		0.0002
0/10(0/9)	3/47(3/46)	--			
0% (0%)	6% (7%)	--	0.58(0.55)		--[b]
Combined				0.0001(0.0001)	0.0002

[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]Validity of test is dubious because of the small number of animals with tumors.

Table F4. Statistical Analysis of Neoplasms of the Liver in Female Mice Fed Chlordane 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 (30.1 ppm)	High Dose (63.8 ppm)			
Hepatocellular Carcinoma					
3/78	--	34/49			
4%	--	69%	< 0.0001		< 0.0001
3/78	3/47	--			
4%	6%	--	0.41		0.26
3/78	3/47	34/49			
4%	6%	69%		< 0.0001[a]	< 0.0001[a]

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

APPENDIX G

ANALYSIS OF FORMULATED DIETS FOR CONCENTRATION OF CHLORDANE

APPENDIX G

Analysis of Formulated Diets for

Concentration of Chlordane

A 100 g sample of the diet mixture was shaken with 12 ml acetone at room temperature for 16 hrs., then filtered through Celite with acetone washes, and reduced in volume to 10 ml. After appropriate dilutions, the solution was quantitatively analyzed for chlordane 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)
30	21	30.1	5.6	27.6-34.6
40	4	39.4	3.5	38.1-40.6
50	10	49.2	3.1	46.0-51.8
60	12	59.8	3.2	55.0-62.5
80	4	78.7	3.9	74.2-80.6
100	12	100.0	4.4	93.5-111.5
160	3	154.0	3.8	150-161
200	8	198.0	1.8	195-204
400	9	391.0	3.6	374-412
800	5	802.0	2.9	770-833

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