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LINDANE

FOR POSSIBLE CARCINOGENICITY

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CONTRIBUTORS: This report presents the results of the bioassay of lindane for possible carcinogenicity, conducted for the Carcinogen Bioassay and Program Resources Branch, Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), Bethesda, Maryland. The bioassay was conducted by Gulf South Research Institute, New Iberia, Louisiana, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI carcinogenesis bioassay program.

The experimental design was determined by Drs. J. H. Weisburger[1,2] and R. R. Bates[1,3]; the doses were selected by Drs. T. E. Shellenberger[4,5], J. H. Weisburger, and R. R. Bates. Animal treatment and observation were supervised by Drs. T. E. Shellenberger and H. P. Burchfield[4], with the technical assistance of Ms. D. H. Monceaux[4] and Mr. D. Broussard[4]. Histopathology was performed by Drs. E. Bernal[4] and B. Buratto[4] at Gulf South Research Institute, and the diagnoses included in this report represent the interpretation of these pathologists.

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

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SUMMARY

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

Groups of 50 rats of each sex were administered lindane at one of two doses for 80 weeks, then observed for 29-30 weeks. Time-weighted average doses for males were 236 or 472 ppm; those for females were 135 or 270 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 45 untreated male and 45 untreated female rats from similar bioassays of four other test chemicals. All surviving rats were killed at 108-110 weeks.

Groups of 50 mice of each sex were administered lindane at one of two doses, either 80 or 160 ppm, for 80 weeks, then observed for an additional 10-11 weeks. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 40 untreated male and 40 untreated female mice from similar bioassays of four other test chemicals. All surviving mice were killed at 90-91 weeks.

Neither the mean body weights of rats nor those of mice showed consistent effects from the administration of lindane. The physical condition of the surviving treated mice deteriorated during the final 6 weeks on study. Except for the female matched-control group of rats, survival of all groups of rats and mice was adequate for meaningful statistical analyses of the incidence of tumors.

In rats, no tumor occurred at a statistically significant incidence in the treated groups of either sex.

In mice, the incidence of hepatocellular carcinoma in low-dose males was significant when compared with that in the pooled controls (controls 5/49, low-dose 19/49, P = 0.001). This finding, by itself, is insufficient to establish the carcinogenicity of lindane. The incidence of hepatocellular carcinoma in high-dose male mice (9/46) was not significantly different from that in the matched (2/10) or pooled controls.

It is concluded that under the conditions of this bioassay, lindane was not carcinogenic for Osborne-Mendel rats or B6C3F1 mice.

TABLE OF CONTENTS

APPENDIXES

TABLES

FIGURES

I. INTRODUCTION

Lindane (CAS 58-89-9; NCI C00204) is an organochlorine pesticide that is registered for use in soil, foliar, and seed treatment for a large variety of fruit and vegetable crops, and for use on livestock, pets, and agricultural premises (EPA, 1971, 1973). Residues of lindane may be persistent in soil and foods (Hayes, 1975). There may also be direct human exposure to lindane through its use in pharmaceutical preparations or in public health pest control.

Lindane was selected for testing because data regarding its carcinogenicity were considered inadequate, and because there was a potential for long-term human exposure due to its extensive use and its persistence in the environment.

II. MATERIALS AND METHODS

A. Chemical

Lindane is the gamma isomer of 1,2,3,4,5,6-hexachlorocyclohexane. It was obtained in two batches for the chronic study, the first batch from City Chemical Co., New York, N. Y., and the second batch from Diamond Shamrock Co., Agricultural Chemicals Division, Cleveland, Ohio. Both suppliers reported the chemical to be essentially 100% pure.

The identity and purity of these batches were confirmed at Gulf South Research Institute using melting point; elemental analysis (C, H, Cl) for $C_6H_6Cl_6$; infrared, nuclear magnetic resonance, and mass spectroscopy; and thin-layer and gas-liquid chromatography. The chemical was stored at approximately 4^oC.

B. Dietary Preparation

All diets were formulated using finely ground Wayne® Lab Blox meal (Allied Mills, Inc., Chicago, Ill.) to which was added the required amount of lindane for each dietary concentration. The test chemical was first dissolved in a small amount of acetone (Mallinckrodt, Inc., St. Louis, Mo.), which was then added to the feed. Corn oil (Louana®, Opelousas Refinery Co., Opelousas, La.) was also added to the feed, primarily as a dust suppressant, and the diets were mixed mechanically to assure homogeneity of the

mixtures and evaporation of the acetone. Final diets, including those for the control groups of animals, contained corn oil equal to 2% of the final weight of feed. Formulated diets were stored at approximately 17°C until used, but no longer than 1 week.

The stability of lindane in feed was tested by determining the concentration of the chemical in formulated diets at intervals over a 7-day period. Diets containing 80 or 320 ppm lindane showed no change in concentration on standing at ambient temperature for this period.

As a quality control test on the accuracy of preparation of the diets, the concentration of lindane was determined in different batches of formulated diets during the chronic study. The results are summarized in Appendix G. At each dietary concentration, the mean of the analytical concentrations for the checked samples was within 2.4% of the theoretical concentration, and the coefficient of variation was never more than 5.1%. Thus, the evidence indicates that the formulated diets were prepared accurately.

C. Animals

Rats and mice of both sexes, obtained through contracts of the Division of Cancer Treatment, National Cancer Institute, were used in these bioassays. The rats were of the Osborne-Mendel

strain obtained from Battelle Memorial Institute, Columbus, Ohio, and the mice were B6C3F1 hybrids obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. On arrival at the laboratory, all animals were quarantined for an acclimation period (rats for 10 days, mice for 12 days) and were then assigned to control and treated groups.

D. Animal Maintenance

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

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

Cages for control and treated mice were placed on separate racks in the same room. Animal racks for both species were rotated laterally once per week; at the same time each cage was changed to a different position in the row within the same column. Rats receiving lindane, along with their matched controls, were housed in a room by themselves. Mice receiving lindane were maintained in a room housing mice administered safrole (CAS 94-59-7) or N-2-fluorenylacetamide (CAS 53-96-3), together with their respective matched controls.

E. Subchronic Studies

Subchronic studies were conducted to estimate the maximum tolerated doses of lindane, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the chronic studies. In these subchronic studies, lindane was added to the animal feed in twofold increasing concentrations, ranging from 160 to 2,560 ppm for rats and from 40 to 1,280 ppm for mice. Treated and control groups each consisted of five male and five female animals. The chemical was provided in feed to the treated groups for 6 weeks, followed by a period of observation for 2 weeks.

In male or female rats receiving 320 or 640 ppm, weight gains decreased during the first 3 weeks. Later, weight gains of

treated male rats approached those of the controls. There were no deaths at these doses; however, one male rat and two female rats treated at 1,280 ppm died during week 2. The low and high doses for the chronic studies using rats were set at 320 and 640 ppm.

In the mice, there was no effect on weight gains at 80 and 160 ppm, and no deaths occurred. At 320 ppm, one male died during week 1 and two females died during week 5. By week 5, all mice receiving 640 ppm died. The low and high doses for the chronic studies using mice were set at 80 and 160 ppm.

F. Designs of Chronic Studies

The designs of the chronic studies are shown in tables 1 and 2.

Since the numbers of animals in the matched-control groups were small, pooled-control groups also were used for statistical comparisons. Matched controls from the current studies on lindane were combined with matched controls from studies performed on tetrachlorvinphos (CAS 961-11-5), toxaphene (CAS 8001-35-2), endrin (CAS 72-20-8), and malathion (CAS 121-75-5). The pooled controls for statistical tests using rats consisted of 55 males and 55 females; using mice, 50 males and 50 females. The studies on chemicals other than lindane were also conducted at Gulf South Research Institute and overlapped the lindane study

Table 1. Design of Lindane Chronic Feeding Studies in Rats

Sex and Treatment Group	Initial No. of Animals[a]	Lindane in Diet[b] (ppm)	Time on Study: Treated[c] (weeks)	Time on Study: Untreated[d] (weeks)	Time-Weighted Average Dose[e] (ppm)
Male					
Matched-Control	10	0		109	
Low-Dose	50	320	38		236
		160	42		
		0		30	
High-Dose	50	640	38		472
		320	42		
		0		30	
Female					
Matched-Control	10	0		108-109	
Low-Dose	50	320	2		135
		160	49		
		80	29		
		0		29-30	
High-Dose	50	640	2		270
		320	49		
		160	29		
		0		30	

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

[b]Doses of lindane were lowered during the study, as indicated, due to deaths among the treated animals.

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

[d]When diets containing lindane were discontinued, treated rats and their matched controls were fed control diets without corn oil for 15 weeks, then control diets (2% corn oil added) for an additional 15 weeks.

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

Table 2. Design of Lindane Chronic Feeding Studies in Mice

Sex and Treatment Group	Initial No. of Animals[a]	Lindane in Diet (ppm)	Time on Study: Treated[b] (weeks)	Time on Study: Untreated[c] (weeks)
MALE				
Matched-Control	10	0		90
Low-Dose	50	80	80	
		0		10
High-Dose	50	160	80	
		0		10
FEMALE				
Matched-Control	10	0		90
Low-Dose	50	80	80	
		0		10
High-Dose	50	160	80	
		0		10-11

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

[b]All animals were started on study on the same day.

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

by at least 1 year. The matched-control groups for the different test chemicals were of the same strain and from the same supplier, and they were examined by the same pathologists.

G. Clinical and Pathologic Examinations

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

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, gallbladder (mice), pancreas, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, and brain. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

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

H. Data Recording and Statistical Analyses

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

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

Probabilities of survival were estimated by the product-limit

procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a

significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of treated animals at each dose level. When results for a number of treated groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

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

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the

first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

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

The approximate 95 percent confidence interval for the relative risk of each treated group compared to its control was calculated

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

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

induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of the male rats were unaffected by lindane (figure 1). The weights of the low-dose females were consistently higher than those of the matched-control and high-dose females, and the weights of the high-dose females were higher than those of the matched controls near the end of the study. Except for occasional weight loss by individual animals, the treated animals were generally comparable to the controls in appearance and behavior during the first year of the study.

Clinical signs in all treated groups were noted at a low or moderate incidence during the first half of the second year, and with gradually increasing frequency during the remainder of the study. These signs included rough and discolored hair coats (primarily among the male animals), pale mucous membranes, dermatitis, and vaginal bleeding.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats receiving lindane at the doses used in this experiment, together with those of the matched controls, are shown in figure 2.

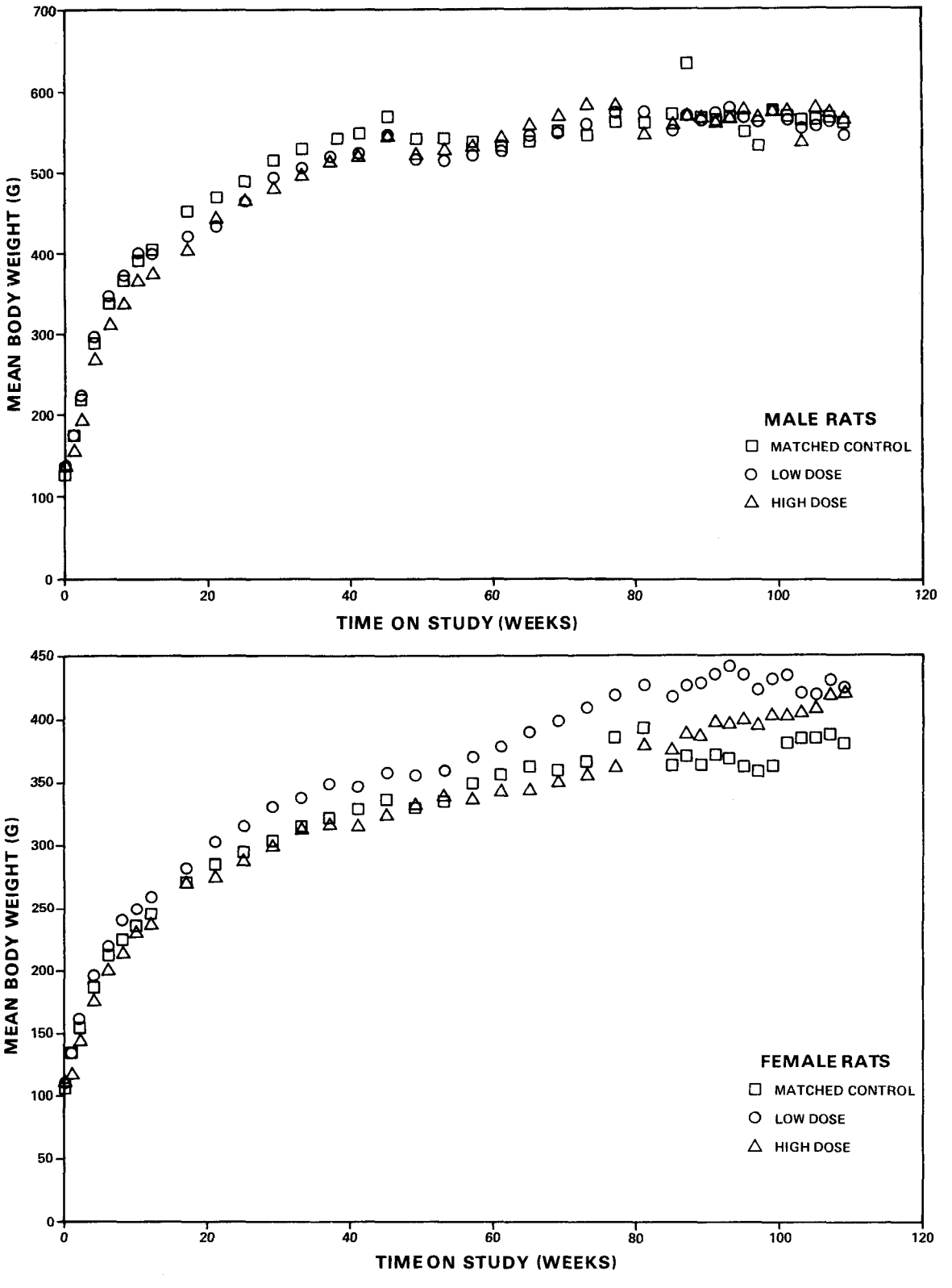

Figure 1. Growth Curves For Rats Fed Lindane In The Diet

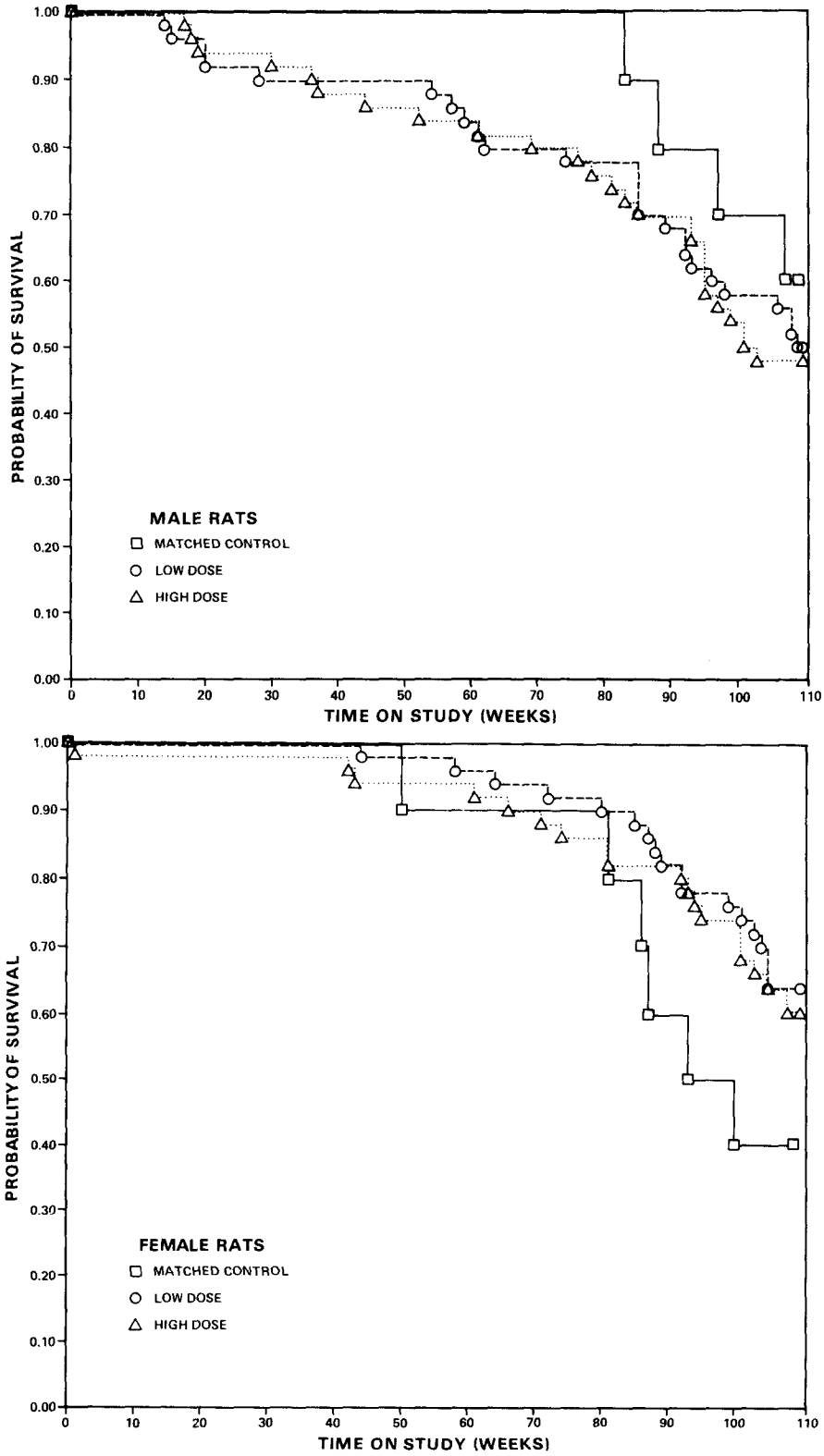

Figure 2. Survival Curves For Rats Fed Lindane In The Diet

In neither sex was the Tarone test result significant at the 0.05 level for positive dose-related trend in mortality over the period. In male rats, 48% of the high-dose group, 50% of the low-dose group, and 60% of the controls lived to the end of the study. In females, only 40% of the controls survived to the end of the study, while at least 60% of the low- and high-dose groups survived. Early deaths of these rats were not tumor associated. Over 80% of these animals lived at least as long as 52 weeks on study, providing adequate numbers of rats of both sexes for meaningful statistical analyses of the incidences of late-developing tumors.

C. Pathology (Rats)

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

A variety of neoplastic, proliferative, degenerative, and inflammatory lesions occurred in a random manner among rats of the control and lindane-treated groups. The majority of these lesions, commonly observed in this strain of rat, occurred with a low incidence within a given group.

The incidences of most of these lesions in the treated groups were comparable to those in the controls. Occasionally, however,

these lesions occurred only in animals of the treated groups. This may reflect the small number of control animals rather than any harmful effect caused by the chemical.

In the judgment of the pathologists, there was no evidence of carcinogenicity induced in Osborne-Mendel rats by the administration of lindane under the conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

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

In male rats, the Cochran-Armitage test for positive dose-related trend in proportions for hemangioma of the spleen has a probability level of 0.030 using the pooled controls; however, this is based on only a minimal incidence in the high-dose group (3/44, 7%), and under this condition, the analysis based on a hypothesis of linear trend is questionable. Female rats do not show any incidence of this tumor. There is inadequate statistical evidence to conclude that the occurrence of this tumor is related to treatment, since the results of the Fisher exact test are not significant.

In the analyses of the incidence of chromophobe adenoma of the pituitary in female rats, although the result of the Cochran-Armitage test for positive dose-related trend in proportions is not significant at the 0.05 level, the departure from linearity has a probability level of 0.048 using the pooled controls, due to the higher incidence in the low-dose group than in the high-dose group. The Fisher exact test shows a P value of 0.033 when the incidence in the low-dose females is compared with that in the pooled controls, but this probability level is above the 0.025 level required by the Bonferroni criterion for multiple comparisons. In male rats, the statistical test results for the incidence of this tumor are not significant in the positive direction. No direct relationship between the incidence of this tumor and the administration of lindane can be concluded.

In female rats, the Fischer exact tests shows a P value of 0.049 when the incidence of C-cell adenoma of the thyroid in the low-dose group is compared with that in the pooled-control group; however, this probability level is above the 0.025 level required by the Bonferroni criterion for multiple comparisons.

There are no other specific incidences of tumors for which the Cochran-Armitage test or the Fisher exact test shows significance in the positive direction. A negative Cochran-Armitage test result is observed in the incidence of follicular-cell adenoma of

the thyroid in male rats when the incidence in the pooled controls exceeds the incidences in the treated groups. In each of the 95% confidence intervals for relative risk, shown in the tables, the value of one is included; this indicates the negative aspects of the results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by this chemical, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of the male mice were unaffected by lindane (figure 3). The weights of the low-dose females were consistently lower than those of the matched-control and high-dose females.

During the first year of the study, the treated animals were generally comparable to the controls in appearance and behavior. A few treated animals had alopecia. During the second year, all of the treated females appeared excitable when handled, and many of the treated males were observed fighting. Clinical signs including rough hair coats, alopecia, and abdominal distention (predominantly in treated males) were noted with increasing frequency during the remainder of the study. Treated mice were generally in poor physical condition during the last 6 weeks of the study.

B. Survival (Mice)

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

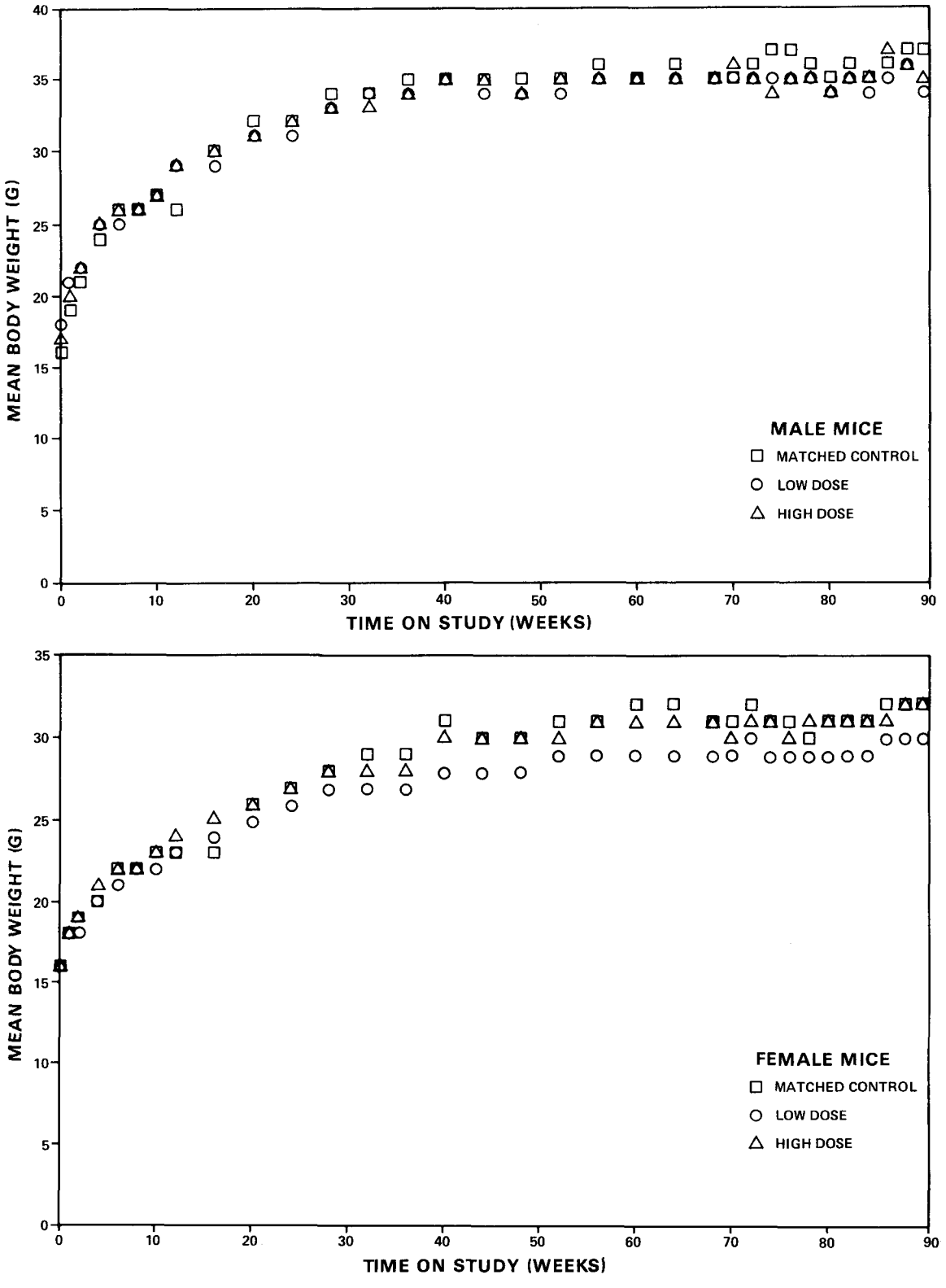

Figure 3. Growth Curves For Mice Fed Lindane In The Diet

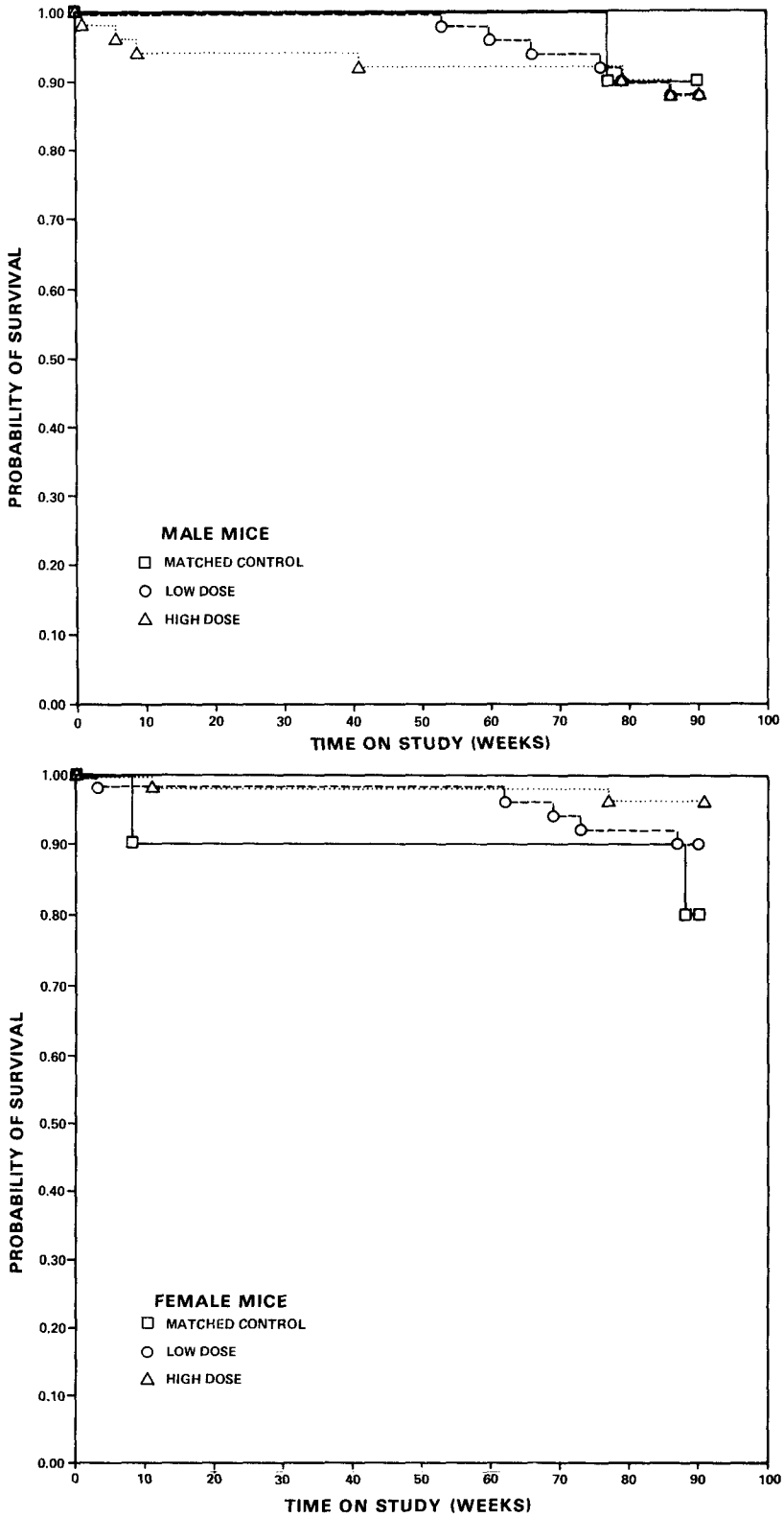

Figure 4. Survival Curves For Mice Fed Lindane In The Diet

In neither sex was the Tarone test result significant for positive dose-related trend in mortality over the period.

The survivals for treated and control groups within each sex are comparable. At least 88% of the males and 80% of the females lived to the end of the study, providing sufficient animals for meaningful statistical analyses of the incidence of tumors.

C. Pathology (Mice)

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

A variety of neoplastic, proliferative, degenerative, and inflammatory lesions occurred in a random manner among mice of the control and lindane-treated groups. An exception was the relatively high incidence of neoplastic lesions in the liver.

Hepatocellular carcinoma and neoplastic nodules of the liver, as described by Squire and Levitt (1975), were the most numerous lesions, occurring with approximately equal frequency among control and high-dose mice. Hepatocellular carcinomas occurred in 2/10 (20%) control males, 0/10 (0%) control females, 9/46 (20%) high-dose males, and 3/46 (7%) high-dose females. These lesions were most frequent in the low-dose males (19/49 [39%]).

The hepatocellular carcinomas were quite variable in morphology. Grossly, the lesions were large single masses (1 to 2 cm. in diameter), or multiple discrete, confluent nodules varying in size from microscopic to several millimeters in diameter. The consistency also varied, but in most cases the tumors were soft, particularly when there were extensive areas of necrosis. They were well circumscribed and readily distinguishable from the surrounding normal tissue. The major lobes were most frequently affected with lesions within the parenchyma or bulging just beneath the capsule. Microscopically, the most salient features of the lesions were tinctorial and architectural. These features varied not only from tumor to tumor, but also among different areas of the same tumor. It was not uncommon to see nodular structures within other nodules. The cytoplasm of the affected cells was most commonly basophilic. The arrangement of the liver plates was either nearly normal or so disorganized as to destroy the trabecular pattern completely. Dilation of the sinuses also contributed to the focal or diffuse distortion of the neoplastic tissue. Because of these changes and the compression of the adjacent parenchyma, the outline of the lesion was usually well defined. The cytology was more or less that of well-differentiated hepatocytes with some variation of size and shape. Intracytoplasmic eosinophilic bodies were present in a large number of cells. The nuclei were smaller, of the same size, or

larger than the normal ones. Abnormal mitoses were rare and normal mitoses were relatively few. Occasionally, neoplastic changes were associated with degenerative features such as lipid deposition, focal or massive necrosis, hemorrhage, or with inflammatory changes. Metastases to the lung (two animals), diaphragm (one animal), or epididymis (one animal) were observed in male mice only.

The nonneoplastic lesions were degenerative or inflammatory in nature. The distribution of these nonneoplastic lesions among the animals of the different groups suggests that they are not attributable to the chemical tested; rather, they are coincidental lesions.

In the judgment of the pathologists, there was insufficient evidence to conclude that carcinogenicity in B6C3F1 mice was induced by the administration of lindane under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

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

In male mice, although the Cochran-Armitage test result for positive dose-related trend in proportions for hepatocellular carcinoma is not significant at the 0.05 level, the departure from linearity has a probability level of 0.002 using the pooled controls, due to the higher incidence in the low-dose group than in the high-dose group. The Fisher exact test shows a significantly higher proportion (P = 0.001) of this tumor in the low-dose group than in the pooled controls, and the lower and upper limits of the 95% confidence interval of the relative risk have values greater than one. The incidence of hepatocellular carcinoma in female mice is not statistically significant. When hepatocellular carcinoma and neoplastic nodule of the liver in females are considered together, the statistical test results of the combined incidence are still not significant. In males, after similar grouping, the result of the Cochran-Armitage test of proportions remains not significant, with a probability level of 0.011 for the departure from linearity, due to a higher proportion in the low-dose group than in the high-dose group. The Fisher exact test shows that the combined incidence in the low-dose males is significantly higher than that in the pooled controls (P = 0.010). The incidence in the high-dose group is not significant, and this conclusion could not be attributed to low survival, since the survival in the high-dose group was comparable to that in the low-dose group. The results of the

historical controls compiled to date at this laboratory indicate that hepatocellular carcinomas and neoplastic nodules of the liver occurred in 75/360 (20.8%) of B6C3F1 male mice. Due to this high spontaneous incidence, and because the incidence among high-dose males in this study is not significant, these tumors cannot be related conclusively to treatment.

There are no other specific incidences of tumors in mice for which the statistical tests show significance. In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one is included; this indicates the negative aspects of the results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by lindane, which could not be detected under the conditions of this test.

V. DISCUSSION

Lindane was toxic to both rats and mice, as shown by clinical signs and effects on weight and/or mortality. During the first year of the study, the treated rats were generally comparable to the controls in appearance and behavior. Clinical signs, including rough and discolored hair coats, pale mucous membranes, dermatitis, and vaginal bleeding were noted with increasing frequency in the treated animals during the second year of the study.

Mean body weight gains in rats were not adversely affected by lindane; however, individual treated animals lost weight at various times during the study. Survival of the treated rats was not statistically different from that of the matched controls; however, there were several early deaths among the controls. Survival to termination of the study was from 48% to 64% for each of the treated groups.

In mice, the mean body weights were unaffected by treatment, with the exception of the low-dose females. During the second year, the female mice were excitable; among the males, increased fighting, rough hair coats, alopecia, and abdominal distention were noted. Treated mice were generally in poor physical condition during the last 6 months on study; however, treatment

did not affect survival, and there were adequate numbers of treated animals for meaningful statistical analyses of the incidence of tumors.

In rats, a variety of neoplastic lesions occurred in a random manner among both control and treated animals, but the incidences in the treated groups were not significantly different from those in either the matched- or pooled-control groups.

In mice, a relatively high incidence of neoplastic lesions in the liver was observed in both control and treated animals. Hepatocellular carcinomas in the males occurred in 2/10 matched controls, 5/49 pooled controls, 19/49 low-dose, and 9/46 high-dose mice. The incidence in the low-dose males was significantly higher than that in the pooled controls ($P = 0.001$). However, neither the incidence in the high-dose males nor the dose-related trend was significant, and the significance of the findings was not increased when hepatocellular carcinomas were combined with neoplastic nodules. No hepatic hyperplasia was observed in treated mice of either sex. In control animals, hepatocellular carcinomas and neoplastic nodules occurred in 23% of all B6C3F1 male mice on study at the laboratory. Thus, the incidence of hepatocellular carcinoma in male mice cannot clearly be related to treatment. The incidence of hepatocellular carcinoma among female mice was not significant.

The chronic toxicity of lindane has been studied previously. Fitzhugh et al. (1950) fed lindane to groups of Wistar rats at doses of 5 to 1,600 ppm for their life spans. At doses > 100 ppm, the chemical produced low incidences of liver enlargement and small foci of necrosis, as seen by gross examination, and hepatic-cell enlargement, hepatic-cell atrophy, fatty degeneration, and focal necrosis, as seen by microscopic examination. The mortality was high in this study, and the mean age at death for rats fed 100 ppm was 64 weeks.

Thorpe and Walker (1973) fed 400 ppm lindane (purity > 99.5%) to groups of 30 male and 30 female CF1 mice for 2 years. Among males, hyperplastic nodules of the liver occurred in 38% of the treated animals and in 20% of the controls, while hepatic neoplasms (types not delineated) occurred in 55% of the treated animals and in only 4% of the controls. Among females, hyperplastic nodules of the liver occurred in 34% of the treated animals and in 23% of the controls, while hepatic neoplasms (types not delineated) occurred in 34% of the treated animals and in none of the controls. Mortality was high in both sexes of treated animals, and the calculations were based on the incidence of tumors subsequent to appearance of the first liver tumor in the group being analyzed. The concentration of lindane used in

this study was 2-1/2 times the high dose fed to the mice in the present bioassay.

Herbst et al. (1975) fed NMRI mice diets containing 12.5, 25, and 50 ppm lindane for 80 weeks. No tumor-inducing effect was observed in the livers of these animals. The highest dose used in the Herbst study was less than the low dose used in the present bioassay.

It is concluded that under the conditions of this bioassay, lindane was not carcinogenic for Osborne-Mendel rats or B6C3F1 mice.

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APPENDIX A

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

TABLE A1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	48	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	47	46
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(48)	(49)
PAPILLOMA, NOS			1 (2%)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
*SUBCUT TISSUE	(10)	(48)	(49)
FIBROMA		1 (2%)	3 (6%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(8)	(44)	(44)
HEMANGIOMA			3 (7%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(8)	(39)	(44)
SARCOMA, NOS		1 (3%)	
#LIVER	(10)	(45)	(45)
NEOPLASTIC NODULE		3 (7%)	2 (4%)
URINARY SYSTEM			
#KIDNEY	(10)	(46)	(46)
TUBULAR-CELL ADENOMA			1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MIXED TUMOR, MALIGNANT			1 (2%)
†HAMARTOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(32)	(35)
CARCINOMA,NOS		1 (3%)	
ADENOMA, NOS			2 (6%)
CHROMOPHOBE ADENOMA		3 (9%)	1 (3%)
#ADRENAL	(10)	(37)	(38)
CORTICAL ADENOMA			1 (3%)
#THYROID	(6)	(37)	(37)
FOLLICULAR-CELL ADENOMA	1 (17%)	5 (14%)	
FOLLICULAR-CELL CARCINOMA		1 (3%)	4 (11%)
C-CELL ADENOMA	1 (17%)	3 (8%)	1 (3%)
#PARATHYROID	(3)	(23)	(28)
ADENOMA, NOS		1 (4%)	
#PANCREATIC ISLETS	(9)	(41)	(39)
ISLET-CELL ADENOMA	2 (22%)	1 (2%)	1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(48)	(49)
CARCINOMA,NOS		1 (2%)	
ADENOMA, NOS			2 (4%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
† This is considered to be a benign form of the malignant mixed tumor of the kidney and consists of lipocytes, tubular structures, and fibroblasts in varying proportions.

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*PLEURA	(10)	(48)	(49)
MESOTHELIOMA, NOS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@		14	13
MORIBUND SACRIFICE	4	11	13
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	6	25	24
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	3	19	17
TOTAL PRIMARY TUMORS	4	24	23
TOTAL ANIMALS WITH BENIGN TUMORS	3	13	13
TOTAL BENIGN TUMORS	4	15	16
TOTAL ANIMALS WITH MALIGNANT TUMORS		5	4
TOTAL MALIGNANT TUMORS		5	5
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		4	2
TOTAL UNCERTAIN TUMORS		4	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(10)	(50)	(50)
SARCOMA, NOS	1 (10%)	1 (2%)	
FIBROMA		1 (2%)	
LIPOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(10)	(48)	(47)
SARCOMA, NOS, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(48)	(45)
BILE DUCT ADENOMA		1 (2%)	
NEOPLASTIC NODULE		4 (8%)	2 (4%)
*BILE DUCT	(10)	(50)	(50)
BILE DUCT ADENOMA		2 (4%)	
HAMARTOMA		1 (2%)	
#STOMACH	(9)	(48)	(47)
LEIOMYOMA		1 (2%)	
URINARY SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(7)	(45)	(41)
CARCINOMA,NOS		1 (2%)	
ADENOMA, NOS			2 (5%)
CHROMOPHOBE ADENOMA	3 (43%)	14 (31%)	8 (20%)
#ADRENAL	(9)	(42)	(44)
CORTICAL ADENOMA		3 (7%)	2 (5%)
#THYROID	(8)	(44)	(42)
FOLLICULAR-CELL ADENOMA		1 (2%)	1 (2%)
FOLLICULAR-CELL CARCINOMA		1 (2%)	
C-CELL ADENOMA		4 (9%)	3 (7%)
#PANCREATIC ISLETS	(10)	(48)	(47)
ISLET-CELL ADENOMA		2 (4%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(50)
CARCINOMA,NOS	1 (10%)	1 (2%)	
ADENOMA, NOS		3 (6%)	1 (2%)
ADENOCARCINOMA, NOS	1 (10%)		
FIBROMA		2 (4%)	1 (2%)
FIBROADENOMA	3 (30%)	12 (24%)	9 (18%)
#UTERUS	(9)	(47)	(44)
ADENOCARCINOMA, NOS		1 (2%)	
LEIOMYOSARCOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP	1 (11%)	6 (13%)	7 (16%)
#UTERUS/ENDOMETRIUM	(9)	(47)	(44)
CARCINOMA,NOS			1 (2%)
#OVARY	(8)	(46)	(44)
SERTOLI-CELL TUMOR			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(9)	(49)	(49)
OLIGODENDROGLIOMA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(10)	(50)	(50)
LIPOMA			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(10)	(50)	(50)
SARCOMA, NOS		1 (2%)	
FIBROUS HISTIOCYTOMA, MALIGNANT			1 (2%)
SITE UNKNOWN			
CARCINOMA,NOS			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@		2	4
MORIBUND SACRIFICE	6	16	16
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	4	32	30
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	40	31
TOTAL PRIMARY TUMORS	10	65	42
TOTAL ANIMALS WITH BENIGN TUMORS	6	37	29
TOTAL BENIGN TUMORS	7	54	36
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	6	4
TOTAL MALIGNANT TUMORS	3	7	4
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		4	2
TOTAL UNCERTAIN TUMORS		4	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

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

TABLE B1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	50	47
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(50)	(47)
FIBROUS HISTIOCYTOMA			1 (2%)
*SUBCUT TISSUE	(10)	(50)	(47)
HEMANGIOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(10)	(50)	(47)
HEPATOCELLULAR CARCINOMA, METAST		2 (4%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (20%)	2 (4%)	3 (6%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(50)	(47)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(49)	(46)
NEOPLASTIC NODULE	1 (10%)		1 (2%)
HEPATOCELLULAR CARCINOMA	2 (20%)	19 (39%)	9 (20%)
URINARY SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*EPIDIDYMIS	(10)	(50)	(47)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
DIAPHRAGM			
HEPATOCELLULAR CARCINOMA, METAST		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@		1	4
MORIBUND SACRIFICE	1	5	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	9	44	44
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	21	15
TOTAL PRIMARY TUMORS	5	21	16
TOTAL ANIMALS WITH BENIGN TUMORS	2	2	5
TOTAL BENIGN TUMORS	2	2	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	19	10
TOTAL MALIGNANT TUMORS	2	19	10
TOTAL ANIMALS WITH SECONDARY TUMORS#		3	
TOTAL SECONDARY TUMORS		4	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1		1
TOTAL UNCERTAIN TUMORS	1		1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(10)	(48)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (10%)		
OSTEOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(8)	(49)	(48)
FIBROUS HISTIOCYTOMA	1 (13%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(47)	(46)
NEOPLASTIC NODULE	1 (10%)	2 (4%)	
HEPATOCELLULAR CARCINOMA		2 (4%)	3 (7%)
#CECUM			(1)
SARCOMA, NOS			1 (100%)
URINARY SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#ADRENAL	(7)	(46)	(44)
CORTICAL CARCINOMA		1 (2%)	
#THYROID	(7)	(43)	(41)
FOLLICULAR-CELL ADENOMA			2 (5%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(49)	(49)
ADENOMA, NOS	1 (10%)		
#UTERUS	(7)	(44)	(43)
SARCOMA, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SACRUM	(10)	(49)	(49)
OSTEOSARCOMA		1 (2%)	
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@	1	2	2
MORIBUND SACRIFICE	1	3	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	8	45	48
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	7	8
TOTAL PRIMARY TUMORS	4	7	9
TOTAL ANIMALS WITH BENIGN TUMORS	2	1	4
TOTAL BENIGN TUMORS	2	1	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	4	5
TOTAL MALIGNANT TUMORS	1	4	5
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	2	
TOTAL UNCERTAIN TUMORS	1	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN RATS FED LINDANE IN THE DIET

TABLE C1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	48	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	47	46
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(48)	(49)
HYPERKERATOSIS	1 (10%)		
ACANTHOSIS	1 (10%)		
RESPIRATORY SYSTEM			
#LUNG	(9)	(46)	(46)
ATELECTASIS		1 (2%)	
PNEUMONIA, CHRONIC MURINE		1 (2%)	
NECROSIS, FOCAL			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(8)	(44)	(44)
HEMORRHAGE		2 (5%)	
INFLAMMATION, GRANULOMATOUS		1 (2%)	
SCLEROSIS		1 (2%)	1 (2%)
HEMATOPOIESIS			1 (2%)
HYPOPLASIA, LYMPHOID		1 (2%)	
#MESENTERIC L. NODE	(10)	(31)	(34)
INFLAMMATION, NOS	1 (10%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(9)	(46)	(46)
INFLAMMATION, CHRONIC FOCAL	1 (11%)		
#ENDOCARDIUM	(9)	(46)	(46)
SCLEROSIS			1 (2%)
FIBROSIS, FOCAL		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(10)	(45)	(45)
CIRRHOSIS, NOS			1 (2%)
DEGENERATION, BALLOONING			1 (2%)
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY		3 (7%)	5 (11%)
FOCAL CELLULAR CHANGE	1 (10%)	1 (2%)	1 (2%)
ANGIECTASIS		1 (2%)	2 (4%)
*BILE DUCT	(10)	(48)	(49)
DILATATION, NOS			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL	1 (10%)	2 (4%)	
HYPERPLASIA, NOS	2 (20%)		3 (6%)
HYPERPLASIA, FOCAL			2 (4%)
#PANCREAS	(9)	(41)	(39)
INFLAMMATION, CHRONIC	1 (11%)		
#STOMACH	(9)	(43)	(38)
EROSION	1 (11%)		1 (3%)
CALCIFICATION, DYSTROPHIC			1 (3%)
#GASTRIC MUCOSA	(9)	(43)	(38)
CALCIFICATION, DYSTROPHIC		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(10)	(46)	(46)
INFLAMMATION, DIFFUSE		1 (2%)	
INFLAMMATION, CHRONIC	5 (50%)	22 (48%)	28 (61%)
PYELONEPHRITIS, CHRONIC		1 (2%)	
#RENAL PAPILLA	(10)	(46)	(46)
CALCIUM DEPOSIT			1 (2%)
#URINARY BLADDER	(9)	(34)	(37)
INFLAMMATION, ACUTE	1 (11%)		
*URETHRA	(10)	(48)	(49)
INFLAMMATION, SUPPURATIVE	1 (10%)		
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(32)	(35)
CYST, NOS	2 (20%)	2 (6%)	4 (11%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DEGENERATION, CYSTIC		1 (3%)	1 (3%)
ANGIECTASIS			1 (3%)
#ADRENAL MEDULLA	(10)	(37)	(38)
HYPERPLASIA, NOS			1 (3%)
#THYROID	(6)	(37)	(37)
HYPERPLASIA, C-CELL		3 (8%)	1 (3%)
HYPERPLASIA, FOLLICULAR-CELL	1 (17%)	4 (11%)	3 (8%)
#PARATHYROID	(3)	(23)	(28)
HYPERPLASIA, NOS		1 (4%)	2 (7%)
REPRODUCTIVE SYSTEM			
#PROSTATE	(9)	(38)	(37)
INFLAMMATION, SUPPURATIVE	1 (11%)		
INFLAMMATION, ACUTE			3 (8%)
INFLAMMATION, CHRONIC		1 (3%)	
#TESTIS	(10)	(43)	(44)
PERIARTERITIS		1 (2%)	1 (2%)
ATROPHY, NOS	4 (40%)	1 (2%)	8 (18%)
ATROPHY, FOCAL		1 (2%)	
ATROPHY, DIFFUSE		1 (2%)	
#TESTIS/TUBULE	(10)	(43)	(44)
DEGENERATION, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(10)	(48)	(49)
PERIARTERITIS		3 (6%)	4 (8%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	10	6
AUTO/NECROPSY/HISTO PERF		4	2
AUTO/NECROPSY/NO HISTO		1	3
AUTOLYSIS/NO NECROPSY		2	1

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

TABLE C2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	49
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(50)	(50)
GRANULOMA, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(10)	(48)	(47)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, FOCAL			1 (2%)
ABSCESS, NOS			1 (2%)
INFLAMMATION, CHRONIC			2 (4%)
HEMATOPOIETIC SYSTEM			
#ABDOMINAL LYMPH NODE	(9)	(37)	(39)
INFLAMMATION, CHRONIC		1 (3%)	
#MESENTERIC L. NODE	(9)	(37)	(39)
INFLAMMATION, NOS	1 (11%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(9)	(45)	(43)
DEGENERATION, CYSTIC		1 (2%)	
#LIVER	(10)	(48)	(45)
METAMORPHOSIS FATTY		2 (4%)	2 (4%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BASOPHILIC CYTO CHANGE			2 (4%)
FOCAL CELLULAR CHANGE		5 (10%)	4 (9%)
ANGIECTASIS		5 (10%)	2 (4%)
*BILE DUCT	(10)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
HYPERPLASIA, NOS			1 (2%)
#ESOPHAGUS	(1)	(15)	(12)
HYPERKERATOSIS		1 (7%)	1 (8%)
URINARY SYSTEM			
#KIDNEY	(10)	(46)	(48)
CYST, NOS			1 (2%)
INFLAMMATION, CHRONIC		3 (7%)	5 (10%)
#KIDNEY/PELVIS	(10)	(46)	(48)
METAPLASIA, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(7)	(45)	(41)
CYST, NOS			1 (2%)
#ADRENAL	(9)	(42)	(44)
HEMORRHAGE			3 (7%)
DEGENERATION, CYSTIC		1 (2%)	2 (5%)
ANGIECTASIS	2 (22%)	3 (7%)	
#ADRENAL MEDULLA	(9)	(42)	(44)
HYPERPLASIA, NOS	1 (11%)		
#THYROID	(8)	(44)	(42)
HYPERPLASIA, C-CELL	1 (13%)		
HYPERPLASIA, FOLLICULAR-CELL		2 (5%)	3 (7%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(50)
HYPERPLASIA, NOS	1 (10%)		
ADENOSIS	1 (10%)		
#UTERUS	(9)	(47)	(44)
HYDROMETRA		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		5	7
AUTO/NECROPSY/HISTO PERF			2
AUTO/NECROPSY/NO HISTO		1	1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS

IN MICE FED LINDANE IN THE DIET

TABLE D1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	47
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	50	47
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(10)	(50)	(47)
CONGESTION, PASSIVE			1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	
#CERVICAL LYMPH NODE	(9)	(42)	(37)
INFLAMMATION, FOCAL			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(49)	(46)
INFLAMMATION, CHRONIC			1 (2%)
CLOUDY SWELLING			3 (7%)
FOCAL CELLULAR CHANGE			1 (2%)
*BILE DUCT	(10)	(50)	(47)
DILATATION, NOS	1 (10%)		
URINARY SYSTEM			
#KIDNEY	(10)	(50)	(47)
HYDRONEPHROSIS		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC FOCAL			1 (2%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	6	26	25
AUTO/NECROPSY/HISTO PERF		1	1
AUTOLYSIS/NO NECROPSY			3

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

TABLE D2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(8)	(49)	(48)
HYPERPLASIA, NODULAR			1 (2%)
HYPERPLASIA, LYMPHOID		2 (4%)	3 (6%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(47)	(46)
METAMORPHOSIS FATTY	1 (10%)		
*BILE DUCT	(10)	(49)	(49)
CYST, NOS			1 (2%)
#PANCREAS	(9)	(46)	(48)
DILATATION/DUCTS			1 (2%)
#PEYERS PATCH	(9)	(48)	(48)
HYPERPLASIA, LYMPHOID		2 (4%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(10)	(49)	(47)
INFLAMMATION, INTERSTITIAL	2 (20%)		
INFLAMMATION, CHRONIC	1 (10%)		1 (2%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#UTERUS	(7)	(44)	(43)
HYDROMETRA		1 (2%)	
#UTERUS/ENDOMETRIUM	(7)	(44)	(43)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL			1 (2%)
HYPERPLASIA, CYSTIC		3 (7%)	2 (5%)
#OVARY	(7)	(42)	(46)
FOLLICULAR CYST, NOS		2 (5%)	2 (4%)
INFLAMMATION, NOS	2 (29%)	2 (5%)	5 (11%)
INFLAMMATION, SUPPURATIVE	1 (14%)	10 (24%)	2 (4%)
INFLAMMATION, CHRONIC			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	26	27
AUTO/NECROPSY/HISTO PERF	1		
AUTOLYSIS/NO NECROPSY		1	1

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

APPENDIX E

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

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

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Subcutaneous Tissue: Fibroma[b]	0/10 (0.00)	0/49 (0.00)	1/48 (0.02)	3/49 (0.06)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.012	0.136
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.055	0.602
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		108	69
Liver: Neoplastic Nodule[b]	0/10 (0.00)	0/49 (0.00)	3/45 (0.07)	2/45 (0.04)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.149	0.073
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.656	0.322
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		110	110

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	0/10 (0.00)	6/47 (0.13)	3/32 (0.09)	1/35 (0.03)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.209	0.017
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			0.734	0.224
Lower Limit			0.125	0.005
Upper Limit			3.148	1.720
Weeks to First Observed Tumor	--		110	110
Thyroid: Follicular-cell Adenoma[b]	1/6 (0.17)	3/42 (0.07)	5/37 (0.14)	0/37 (0.00)
P Values[c,d]	P = 0.028 (N)	N.S.	N.S.	N.S.
Departure from Linear Trend[e]		P = 0.048		
Relative Risk (Matched Control)[f]			0.811	0.000
Lower Limit			0.131	0.000
Upper Limit			37.268	3.028
Relative Risk (Pooled Control)[f]			1.892	0.000
Lower Limit			0.397	0.000
Upper Limit			11.410	1.869
Weeks to First Observed Tumor	109		110	--

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Thyroid: Follicular-cell Carcinoma[b]	0/6 (0.00)	1/42 (0.02)	1/37 (0.03)	4/37 (0.11)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.010	0.185
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			1.135	4.541
Lower Limit			0.015	0.479
Upper Limit			86.730	217.008
Weeks to First Observed Tumor	--		85	93
Thyroid: Follicular-cell Adenoma or Carcinoma[b]	1/6 (0.17)	3/42 (0.07)	6/37 (0.16)	4/37 (0.11)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.973	0.649
Lower Limit			0.171	0.093
Upper Limit			43.453	31.077
Relative Risk (Pooled Control)[f]			0.270	1.514
Lower Limit			0.524	0.274
Upper Limit			13.112	9.698
Weeks to First Observed Tumor	109		85	93

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Thyroid: C-cell Adenoma[b]	1/6 (0.17)	2/42 (0.05)	3/37 (0.08)	1/37 (0.03)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.486	0.162
Lower Limit			0.056	0.003
Upper Limit			24.871	12.423
Relative Risk (Pooled Control)[f]			1.703	0.568
Lower Limit			0.206	0.010
Upper Limit			19.427	10.429
Weeks to First Observed Tumor	109		110	110
Adrenal: Cortical Adenoma[b]	0/10 (0.00)	2/54 (0.04)	0/37 (0.00)	1/38 (0.03)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]				Infinite
Lower Limit				0.015
Upper Limit				Infinite
Relative Risk (Pooled Control)[f]			0.000	0.711
Lower Limit			0.000	0.012
Upper Limit			4.894	13.091
Weeks to First Observed Tumor	--		--	103

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Spleen: Hemangioma[b]	0/8 (0.00)	0/52 (0.00)	0/44 (0.00)	3/44 (0.07)
P Values[c,d]	N.S.	P = 0.030	N.S.	N.S.
Relative Risk (Matched Control)[f]				Infinite
Lower Limit				0.126
Upper Limit				Infinite
Relative Risk (Pooled Control)[f]				Infinite
Lower Limit				0.711
Upper Limit				Infinite
Weeks to First Observed Tumor	--		--	97

[a]Treated groups received time-weighted average doses of 236 or 472 ppm in feed.

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

[c]Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; otherwise, not significant (N.S.) is indicated.

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

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

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

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

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Subcutaneous Tissue: Fibroma[b]	0/10 (0.00)	0/54 (0.00)	1/50 (0.02)	0/50 (0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	
Lower Limit			0.012	
Upper Limit			Infinite	
Relative Risk (Pooled Control)[f]			Infinite	
Lower Limit			0.058	
Upper Limit			Infinite	
Weeks to First Observed Tumor	--		110	--
Liver: Neoplastic Nodule[b]	0/10 (0.00)	1/49 (0.02)	4/48 (0.09)	2/45 (0.04)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.215	0.073
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			4.083	2.178
Lower Limit			0.424	0.117
Upper Limit			196.654	125.581
Weeks to First Observed Tumor	--		104	110

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	3/7 (0.43)	6/46 (0.13)	14/45 (0.31)	8/41 (0.20)
P Values[c,d]	N.S.	N.S.	P = 0.033**	N.S.
Departure from Linear Trend[e]		P = 0.048		
Relative Risk (Matched Control)[f]			0.726	0.455
Lower Limit			0.321	0.175
Upper Limit			3.371	2.294
Relative Risk (Pooled Control)[f]			2.385	1.496
Lower Limit			0.954	0.498
Upper Limit			6.877	4.786
Weeks to First Observed Tumor	109		87	95
Thyroid: Follicular-cell Adenoma[b]	0/8 (0.00)	0/48 (0.00)	1/44 (0.02)	1/42 (0.02)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.011	0.011
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.059	0.061
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		110	110

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Thyroid: Follicular-cell Carcinoma[b]	0/8 (0.00)	0/48 (0.00)	1/44 (0.02)	0/42 (0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	
Lower Limit			0.011	
Upper Limit			Infinite	
Relative Risk (Pooled Control)[f]			Infinite	
Lower Limit			0.059	
Upper Limit			Infinite	
Weeks to First Observed Tumor	--		110	--
Thyroid: Follicular-cell Adenoma or Carcinoma[b]	0/8 (0.00)	0/48 (0.00)	2/44 (0.05)	1/42 (0.02)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.061	0.011
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.323	0.061
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		110	110

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Thyroid: C-cell Adenoma[b]	0/8 (0.00)	0/48 (0.00)	4/44 (0.09)	3/42 (0.07)
P Values[c,d]	N.S.	N.S.	P = 0.049**	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.194	0.132
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			1.013	0.689
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		101	103
Adrenal: Cortical Adenoma[b]	0/9 (0.00)	0/51 (0.00)	3/42 (0.07)	2/44 (0.05)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.145	0.068
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.731	0.343
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		105	103

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Mammary Gland: Adenoma, NOS[b]	0/10 (0.00)	0/47 (0.00)	3/50 (0.06)	1/50 (0.02)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.134	0.012
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.566	0.050
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		103	101
Mammary Gland: Adenoma or Carcinoma, NOS[b]	1/10 (0.10)	1/47 (0.02)	4/50 (0.08)	1/50 (0.02)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.800	0.200
Lower Limit			0.097	0.003
Upper Limit			38.616	15.415
Relative Risk (Pooled Control)[f]			3.760	0.940
Lower Limit			0.390	0.012
Upper Limit			181.269	72.331
Weeks to First Observed Tumor	100		103	101

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	3/10 (0.30)	8/42 (0.19)	12/50 (0.24)	9/50 (0.18)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.800	0.600
Lower Limit			0.293	0.202
Upper Limit			3.948	3.093
Relative Risk (Pooled Control)[f]			1.260	0.945
Lower Limit			0.528	0.358
Upper Limit			3.227	2.573
Weeks to First Observed Tumor	50		44	61
Uterus: Endometrial Stromal Polyp[b]	1/9 (0.11)	4/52 (0.08)	6/47 (0.13)	7/44 (0.16)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			1.149	1.432
Lower Limit			0.177	0.235
Upper Limit			51.671	62.922
Relative Risk (Pooled Control)[f]			1.660	2.068
Lower Limit			0.421	0.566
Upper Limit			7.527	9.021
Weeks to First Observed Tumor	109		87	93

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

(continued)

[a]Treated groups received time-weighted average doses of 135 or 270 ppm in feed.

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

[c]Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; otherwise, not significant (N.S.) is indicated.

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

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

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

APPENDIX F

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

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

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	2/10 (0.20)	3/48 (0.06)	2/50 (0.04)	3/47 (0.06)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.200	0.319
Lower Limit			0.018	0.046
Upper Limit			2.592	3.576
Relative Risk (Pooled Control)[f]			0.640	1.021
Lower Limit			0.056	0.143
Upper Limit			5.345	7.264
Weeks to First Observed Tumor	77		90	90
Liver: Hepatocellular Carcinoma[b]	2/10 (0.20)	5/49 (0.10)	19/49 (0.39)	9/46 (0.20)
P Values[c,d]	N.S.	N.S.	P = 0.001**	N.S.
Departure from Linear Trend[e]		P = 0.002		
Relative Risk (Matched Control)[f]			1.939	0.978
Lower Limit			0.615	0.263
Upper Limit			15.778	8.635
Relative Risk (Pooled Control)[f]			3.800	1.917
Lower Limit			1.516	0.626
Upper Limit			11.891	6.755
Weeks to First Observed Tumor	77		60	79

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	3/10 (0.30)	8/49 (0.16)	19/49 (0.39)	10/46 (0.22)
P Values[c,d]	N.S.	N.S.	P = 0.010**	N.S.
Departure from Linear Trend[e]		P = 0.011		
Relative Risk (Matched Control)[f]			1.293	0.725
Lower Limit			0.518	0.253
Upper Limit			6.021	3.657
Relative Risk (Pooled Control)[f]			2.375	1.332
Lower Limit			1.109	0.519
Upper Limit			5.616	3.536
Weeks to First Observed Tumor	77		60	79

[a]Treated groups received doses of 80 or 160 ppm in feed.

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

[c]Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; otherwise, not significant (N.S.) is indicated.

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

(continued)

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

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

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

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

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	0/10 (0.00)	1/48 (0.02)	1/48 (0.02)	2/48 (0.04)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.012	0.068
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			1.000	2.000
Lower Limit			0.013	0.108
Upper Limit			76.886	115.535
Weeks to First Observed Tumor	--		90	91
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	1/10 (0.10)	2/48 (0.04)	1/48 (0.02)	2/48 (0.04)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.208	0.417
Lower Limit			0.003	0.026
Upper Limit			16.043	24.099
Relative Risk (Pooled Control)[f]			0.500	1.000
Lower Limit			0.009	0.075
Upper Limit			9.277	13.306
Weeks to First Observed Tumor				

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	0/10 (0.00)	2/47 (0.04)	2/47 (0.04)	3/46 (0.07)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.069	0.145
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			1.000	1.533
Lower Limit			0.075	0.184
Upper Limit			13.295	17.650
Weeks to First Observed Tumor	--		90	91
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	1/10 (0.10)	3/47 (0.06)	4/47 (0.09)	3/46 (0.07)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.851	0.652
Lower Limit			0.104	0.064
Upper Limit			41.020	33.512
Relative Risk (Pooled Control)			1.333	1.022
Lower Limit			0.237	0.143
Upper Limit			8.665	7.260
Weeks to First Observed Tumor	90		90	91

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

(continued)

[a]Treated groups receiving doses of 80 or 160 ppm in feed.

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

[c]Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when $P < 0.05$; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; otherwise, not significant (N.S.) is indicated.

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

[e]The probability level for departure from linear trend is given when $P < 0.05$ for any comparison.

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

APPENDIX G

ANALYSIS OF FORMULATED DIETS FOR CONCENTRATIONS OF LINDANE

APPENDIX G

Analysis of Formulated Diets for Concentrations of Lindane

A 100-g sample of the diet was shaken with 125 ml hexane for 16 hrs., then filtered through Celite with hexane washes and reduced in volume to 10 ml. After appropriate dilutions, the solution was quantitatively analyzed for lindane 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 Concentration in Diet (ppm)	No. of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
80	26	79.2	4.0%	74.2-85.6
160	33	160.2	4.0%	144-174
320	16	317.8	3.2%	296-337
640	11	655.5	5.1%	600-708

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