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Public Health Service
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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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

CONTRIBUTORS: This bioassay of toxaphene was conducted by Gulf South Research Institute, New Iberia, Louisiana, initially under direct contract to NCI (1) and currently under a subcontract to Tracor Jitco, Inc., Rockville, Maryland, prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by Drs. J. H. Weisburger (1,2) and R. R. Bates (1,3); the doses were selected by Drs. T. E. Shellenberger (4,5), J. H. Weisburger, and R. R. Bates. Administration of the test chemical and observation of the animals were supervised by Drs. T. E. Shellenberger and H. P. Burchfield (4), with the technical assistance of Ms. D. H. Monceaux (4) 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) and Ms. P. L. Yong (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).

This report was prepared at Tracor Jitco (7) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg, Director of the Bioassay Program; Dr. L. A. Campbell, Deputy Director for Science; Dr. J. F. Robens, toxicologist; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Mr. W. D. Reichardt, and Ms. L. A. Waitz, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley and Ms. P. J. Graboske.

The following scientists at NCI were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr. Dr. J. Fielding Douglas, Dr. Dawn G. Goodman (9), Dr. Richard A. Griesemer, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Robert A. Squire (10), Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

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

Groups of 50 rats of each sex were administered toxaphene at one of two doses for 80 weeks, then observed for 28 or 30 weeks. Time-weighted average doses for males were 556 or 1,112 ppm; for females they were 540 or 1,080 ppm. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls consisted of the matched-control groups for toxaphene combined with 45 untreated male and 45 untreated female rats from similar bioassays of five other test chemicals. All surviving rats were killed at 108-110 weeks.

Groups of 50 mice of each sex were administered toxaphene at one of two doses for 80 weeks, then observed for 10 or 11 weeks. Time-weighted average doses were 99 or 198 ppm for both males and females. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched-control groups for toxaphene 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.

Mean body weights attained by low- and high-dose female rats and high-dose male mice were lower than those of matched controls, but weights of other dosed groups were essentially unaffected by the toxaphene. Other clinical signs of toxicity in rats included generalized body tremors at week 53 in high-dose male and female animals, and later, leg paralysis, ataxia, epistaxis, hematuria, and vaginal bleeding, predominantly in the dosed groups of rats of each sex. Abdominal distention, diarrhea, dyspnea, and rough hair coats were common to both dosed rats and dosed mice. There were dose-related decreases in survival rates in mice but not in rats. Sufficient numbers of both rats and mice were at risk for the development of late-appearing tumors.

In the male rats, the incidence of follicular-cell carcinomas or adenomas of the thyroid was dose related ($P = 0.007$) using the pooled controls (matched controls 1/7, pooled controls 2/44, low-dose 7/41, high-dose 9/35). In the females, the incidence of follicular-cell adenomas of the thyroid was dose related using either the matched ($P = 0.022$) or pooled ($P = 0.008$) controls (matched controls 0/6, pooled controls 1/46, low-dose 1/43,

high-dose 7/42). Direct comparisons of dosed and pooled-control groups but not matched controls showed significantly increased incidences of follicular-cell carcinomas or adenomas in the high-dose males (P = 0.008) and of follicular-cell adenomas in the high-dose females (P = 0.021). Two follicular-cell tumors in the high-dose males were carcinomas; all other follicular-cell tumors in the rats were adenomas.

In the mice, the incidence of hepatocellular carcinomas was dose related (P less than 0.001) for both males (matched controls 0/10, pooled controls 4/48, low-dose 34/49, high-dose 45/46) and females (matched controls 0/9, pooled controls 0/48, low-dose 5/49, high-dose 34/49), using either matched or pooled controls. Direct comparisons showed that the incidences of hepatocellular carcinomas in low- and high-dose male mice and high-dose female mice were all significantly higher (P less than 0.001) than those in the respective matched or pooled controls. Statistical significance was maintained when the incidence of hepatocellular carcinomas was combined with that of neoplastic nodules of the liver.

It is concluded that under the conditions of this bioassay, toxaphene was carcinogenic in male and female B6C3F1 mice, causing increased incidences of hepatocellular carcinomas. The test results also suggest carcinogenicity of toxaphene for the thyroid of male and female Osborne-Mendel rats.

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I. INTRODUCTION

Toxaphene (CAS 8001-35-2; NCI C00259) is an organochlorine insecticide that belongs to the class of compounds known as polychlorinated bicyclic terpenes with chlorinated camphenes predominating (Brooks, 1975); an insecticide marketed as Strobane-T® (Tenneco Chemical Co., Piscataway, N. J.) is identical with toxaphene. Toxaphene is obtained from camphene by photochemical chlorination, which produces a heterogeneous mixture of chemicals containing 67-69% chlorine. Although the exact composition of toxaphene has not been determined, it is similar to that of Strobane®, an insecticide differing from Strobane-T® and now discontinued. Strobane® has been shown to cause hepatomas in mice (Innes et al., 1969). Persons involved in the manufacture or handling of toxaphene, as well as human volunteers exposed twice a week at 3-week intervals to 500 mg/m^3 aerosol of toxaphene 30 min/day for 10 days, showed no toxic manifestations (Deichmann, 1973).

Toxaphene is registered for use on a wide range of fruits, vegetables, nuts, field crops, animals, and agricultural premises (EPA Compendium of Registered Pesticides, 1974). Tolerances for residues of toxaphene have been established for many of the

various agricultural commodities. Toxaphene was selected for study in the Carcinogenesis Testing Program because it is known to be related to Strobane®, a compound inducing hepatomas, because it has been used extensively in agriculture, and because its persistence in the ecosystem (Brooks, 1975) may lead to chronic human exposure through residues in food and water.

II. MATERIALS AND METHODS

A. Chemical

The technical-grade toxaphene used in the chronic phase of the bioassay was obtained in a single batch from Hercules, Inc., Wilmington, Delaware (Lot No. X-16189-49). Analyses performed at Gulf South Research Institute (melting range, elemental analysis, gas-liquid chromatography, infrared spectrophotometry) confirmed the expected heterogeneity of this product. The chemical was stored at approximately 0°C.

B. Dietary Preparation

All diets containing toxaphene were formulated using Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) to which was added the required amount of toxaphene 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. The diets were stored at room temperature until used, but no longer than 1 week.

As a quality control test on the accuracy of preparation of the diets, the concentration of toxaphene 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 value obtained for the checked samples was within 3.2% of the theoretical, and the coefficient of variation was never more than 6.9%.

C. Animals

Rats and mice of each sex, obtained through contracts of the Division of Cancer Treatment, National Cancer Institute, were used in these bioassays. The rats were of the Osborne-Mendel strain obtained from 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 (rats for

7 days, mice for 13 days) and then assigned to control or dosed groups.

D. Animal Maintenance

All animals were housed in rooms in which the temperature ranged from 22 to 24°C, and the relative humidity from 40 to 70%. The air entering each room was filtered (Air Maze Incom International, Cleveland, Ohio), and room air was changed 10 to 12 times per hour. Fluorescent lighting provided illumination 10 hours per day. Food and water were provided ad libitum. Fresh feed was provided daily, and excess remaining feed was discarded.

The rats were housed individually in hanging galvanized steel mesh cages (Hoeltge, Inc., Cincinnati, Ohio), and the mice were housed in polypropylene cages (Lab Products, Inc., Garfield, N.J.), five females per cage or two or three males per cage. Mouse cages were covered with polyester filter bonnets (Lab Products, Inc.). The rat racks and cages were sanitized every 2 weeks. The mouse cages were sanitized each week. These cages and racks were washed in an industrial washer at 82°C with Acclaim® detergent (Economics Laboratory, Inc., St. Paul, Minn.) and then rinsed. Absorbent Kimpak® cage liners

(Kimberly Clark Corp., Neenah, Wis.) were placed under the rat cages and were changed three times per week. Absorb-dri® hardwood chip bedding (Lab Products, Inc.), used in the mouse cages, was provided two times per week for males and three times per week for females. Filter bonnets were sanitized each week. Feed jars and water bottles were changed and sanitized three times per week. Sipper tubes and stoppers were sanitized two times per week.

The filter bonnets, feed jars, water bottles, sipper tubes, and stoppers were washed in a Vulcan Autosan washer (Louisville, Ky.).

Cage racks for each species were rotated to a new position in the room once per week; at the same time, each cage was moved to a different row within the same column of a rack. Rats receiving toxaphene, along with their matched controls, were housed in a room by themselves. Mice were maintained in a room housing mice from the following studies:

Feed Studies

(CAS 57-74-9) chlordane
(CAS 143-50-0) chlordecone

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses of toxaphene, on the basis of which two concentrations (hereinafter referred to as "low" and "high" doses) were determined for administration in the chronic studies. In these subchronic studies, toxaphene 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. The test chemical was provided in feed to dosed groups of five male and five female animals of each species for 6 weeks, followed by observation for 2 weeks. Untreated-control groups consisted of five animals of each species and each sex. A second study was performed on male and female rats at doses ranging from 1,280 to 5,120 ppm to confirm the results and to extend the concentration range of the first study.

At 1,280 ppm in the first and second studies, there were no deaths among the rats, and mean weight gains of both males and females were comparable to those of corresponding controls. At 2,560 ppm, two female rats died in the first study; however, the mean weights of the survivors were not adversely affected. During the second study, one male and one female died at 2,560 ppm. On the basis of these results, the low and high doses for

the chronic studies using rats were set at 1,280 and 2,560 ppm for males, and 640 and 1,280 ppm for females.

Four male and two female mice died at 640 ppm, and one male and one female mouse given 320 ppm died. Mean weight gains of mice given 320 ppm were comparable to those of controls. On the basis of these results, the low and high doses for the chronic studies using mice were set at 160 and 320 ppm for males and females.

F. Chronic Studies

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

Since the numbers of animals in the matched-control groups were small, pooled-control groups also were used for statistical evaluation. For the rats, matched controls from the current bioassay on toxaphene were combined with matched controls from studies performed on captan (CAS 133-06-02), chloramben (CAS 133-90-4), lindane (CAS 58-89-9), malathion (CAS 121-75-5), and picloram (CAS 1918-02-1) to give pooled-control groups consisting of 55 males and 55 females. For the mice, matched controls from the current bioassay were combined with matched controls from

Table 1. Toxaphene Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals(a)	Toxaphene in Diet(b) (ppm)	Time on Study Dosed(c) (weeks)	Time on Study Observed(d) (weeks)	Time-Weighted Average Dose(e) (ppm)
Male					
Matched-Control	10	0		108-109	
Low-Dose	50	1,280	2		556
		640	53		
		320	25		
		0		28	
High-Dose	50	2,560	2		1,112
		1,280	53		
		640	25		
		0		28	
Female					
Matched-Control	10	0		108-109	
Low-Dose	50	640	55		540
		320	25		
		0		30	
High-Dose	50	1,280	55		1,080
		640	25		
		0		30	

(a) All animals were 5 weeks of age when placed on study.

(b) Initial doses shown were toxic; therefore, doses were lowered after 2 weeks and again at 53 or 55 weeks, as shown.

(c) All animals were started on study on the same day.

(d) When diets containing toxaphene were discontinued, dosed rats and their matched controls were fed control diets without corn oil for 20 weeks, then control diets (2% corn oil added) for an additional 8 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. Toxaphene Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals(a)	Toxaphene in Diet(b) (ppm)	Time on Study Dosed(c) (weeks)	Time on Study Observed(d) (weeks)	Time-Weighted Average Dose(e) (ppm)
Male					
Matched-Control	10	0		90-91	
Low-Dose	50	160	19		99
		80	61		
		0		11	
High-Dose	50	320	19		198
		160	61		
		0		10	
Female					
Matched-Control	10	0		90-91	
Low-Dose	50	160	19		99
		80	61		
		0		11	
High-Dose	50	320	19		198
		160	61		
		0		10	

(a) All animals were 5 weeks of age when placed on study.

(b) Initial doses shown were toxic; therefore, doses were lowered at 19 weeks, as shown.

(c) All animals were started on study on the same day.

(d) When diets containing toxaphene were discontinued, dosed mice and their matched controls were fed control diets without corn oil for 7 weeks, then control diets (2% corn oil added) for an additional 3 to 4 weeks.

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

studies performed on lindane, malathion, phosphamidon (CAS 13171-21-6), and tetrachlorvinphos (CAS 961-11-5) to give pooled-control groups consisting of 50 males and 50 females. These studies on chemicals other than toxaphene were also conducted at Gulf South Research Institute and were started less than 5 months apart. In the studies on captan and malathion, the control groups of the rats were started at different times, but only those started within 5 months of the time of starting the matched controls for toxaphene were used in the pooled-control groups. The matched-control groups for the different test chemicals were of the same strains and from the same suppliers, and they were all examined by the same pathologists.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, weighed every 2 weeks for the first 12 weeks and monthly thereafter, and palpated for masses at each weighing. Sick, tumor-bearing, and moribund animals were observed daily. Moribund animals and animals that survived to the end of the bioassay were killed using ether and necropsied. Necropsies were also performed on all animals found dead, unless precluded by autolyses or severe cannibalization.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions. 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 may have died early, or been in advanced states of cannibalization or autolysis. 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 appropriate statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for

a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each

dose level. When results for a number of dosed groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

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

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When

such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

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

The approximate 95 percent confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true

binomial probability of the incidence of a specific type of tumor in a dosed group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a dosed group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the dosed group than in the control.

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

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

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

The mean body weights of the low- and high-dose female rats were lower than those of the matched controls throughout most of the bioassay, suggesting a dose-related effect of toxaphene on body weights (figure 1). Weights of low- and high-dose male rats were essentially unaffected by the toxaphene. Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation.

During the first 16 weeks of the bioassay, the dosed animals were generally comparable to the controls in appearance and behavior, with the exception of the high-dose males, which appeared hyperactive during week 2; doses for male rats were lowered at that time. At week 53, the concentration of toxaphene in the feed was reduced because a majority of the high-dose males and females developed generalized body tremors. From week 52 to week 80, clinical signs including alopecia, diarrhea, dyspnea, pale mucous membranes, rough hair coats, dermatitis, ataxia, leg paralysis, epistaxis, hematuria, abdominal distention, and vaginal bleeding were noted predominantly in the dosed groups.

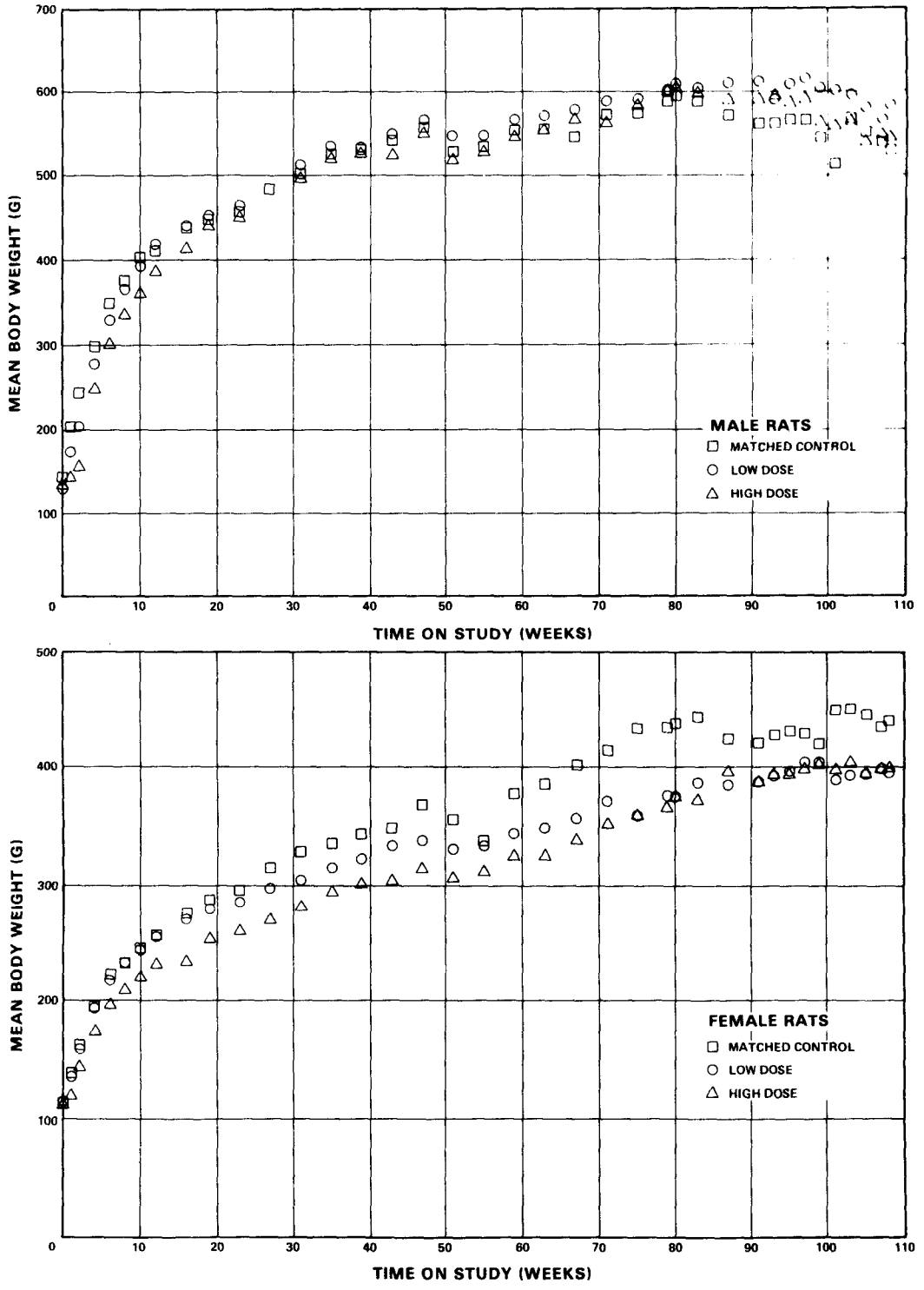


Figure 1. Growth Curves for Rats Fed Toxaphene in the Diet

During the second half of the year, these adverse signs were noted with increasing frequency in both control and dosed groups. Two females, one high-dose and one low-dose, had impaired equilibrium. A purulent vaginal discharge was noted in one high-dose female.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats fed toxaphene in the diet at the doses of this bioassay, together with those of the controls, are shown in figure 2. The result of the Tarone test for dose-related trend in mortality is not significant in either sex.

In male rats, 45/50 (90%) of the high-dose group, 47/50 (94%) of the low-dose group, and all 10 animals of the control group lived at least as long as week 52 on study. In females, 48/50 (96%) of the high-dose group, 46/50 (92%) of the low-dose group, and all 10 animals of the control group lived beyond week 52 on study. Sufficient numbers of dosed rats of each sex were at risk for the development of tumors.

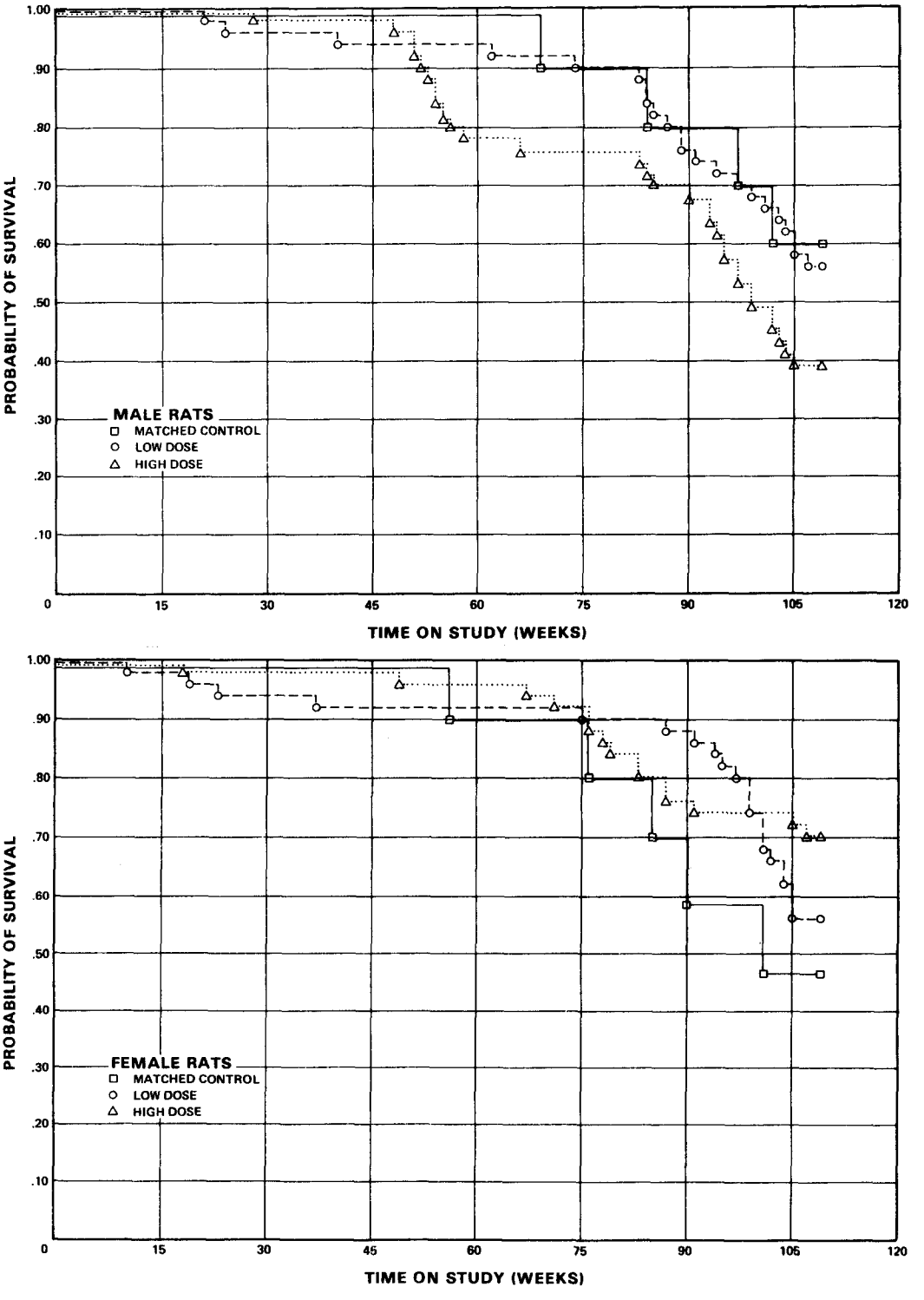


Figure 2. Survival Curves for Rats Fed Toxaphene in the Diet

C. Pathology (Rats)

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

The majority of neoplastic and nonneoplastic lesions observed in the rats were not unusual findings, and the incidences of lesions in animals of the dosed groups were generally similar to those of the matched-control groups. Certain lesions (follicular-cell tumors of the thyroid; follicular and C-cell hyperplasias of the thyroid; endometrial stromal polyps of the uterus; chromophobe adenomas of the pituitary gland; tumors of the mammary gland; cyst of the bile duct; and testicular atrophy) appeared with a higher incidence in the dosed rats; however, they are frequently observed in this strain of rat independent of any chemical administration. The follicular-cell neoplasms, primarily adenomas, were seen in 1/7 control males, 7/41 low-dose males, 9/35 high-dose males; 0/6 control females, 1/43 low-dose females, and 7/42 high-dose females. Thyroid follicular-cell hyperplasias were observed only in dosed rats (males: low-dose 3/41, high-dose 3/35; females: low-dose 5/43, high-dose 3/42). These tumors and lesions occurred at relatively low incidences, and, in view of the low numbers of animals used in the matched controls,

it could not be concluded from comparisons of incidences in dosed and matched-control groups that the increased incidences of neoplasms or proliferative lesions were a result of the administration of toxaphene.

Based on the histopathologic examination, there was no conclusive evidence that toxaphene was carcinogenic in Osborne-Mendel rats under the conditions of this bioassay, although thyroid follicular-cell neoplasms may have been associated with administration of the test chemical.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In male rats, the Fisher exact test shows that the incidence of follicular-cell carcinomas or adenomas of the thyroid was significantly higher ($P = 0.008$) in the high-dose group than in the pooled-control group, and the Cochran-Armitage test for linear trend, using the pooled controls, had a probability level

of P = 0.007. All of these tumors were adenomas except for two carcinomas in the high-dose group.

In the female rats, follicular-cell adenomas of the thyroid appeared in the high-dose group in significant proportions (P = 0.021), when comparisons were made with the pooled controls. When compared with the historical controls (data obtained to date on 20 studies at this laboratory in this bioassay program), the incidence in the high-dose group (7/42 or 17%) was higher than that reported for follicular-cell adenomas in the female historical controls (4/184 or 2.2%). Furthermore, the incidences in neither the matched nor the pooled controls were different from that in the historical controls. The Cochran-Armitage test for significant linear trend applied to the proportions in pooled-control (1/46), low-dose (1/43), and high-dose (7/42) females showed a probability level of P = 0.008; the Cochran-Armitage test applied to the data using the matched controls (P = 0.022) supported the findings using the pooled controls.

Also in the female rats, the Cochran-Armitage test results on the incidence of animals with chromophobe adenomas, chromophobe carcinomas, or adenomas of the pituitary are significant (P = 0.012 using the pooled controls and P = 0.046 using the matched

controls). The Fisher exact test shows that the incidence in the high-dose group is significantly higher than that in the pooled-control group (P = 0.013). The historical-control data obtained to date on 20 similar studies at this laboratory show an incidence of 58/185 (31.4%), although there are incidences as high as 6/10 (60%), 5/10 (50%), 3/6 (50%), and 4/9 (44%). Considering these high spontaneous incidences seen in control groups, the conclusion cannot be made that there is an association between administration of the chemical and the increased incidence of tumors of the pituitary in female Osborne-Mendel rats under the conditions of this bioassay.

In male rats, the Fisher exact test on the incidence of neoplastic nodules of the liver between the low-dose and pooled-control groups indicates a P value of 0.034, which is above the 0.025 level required for significance when the Bonferroni inequality criterion is used for multiple comparison. The high-dose group does not show a significantly higher incidence than that in either control group. The results of the Cochran-Armitage test are not significant.

Significant results in the negative direction are seen in the incidence of adrenal tumors in male rats.

In summary, the statistical results suggest that the incidence of thyroid tumors in each sex of rats is associated with the administration of toxaphene.

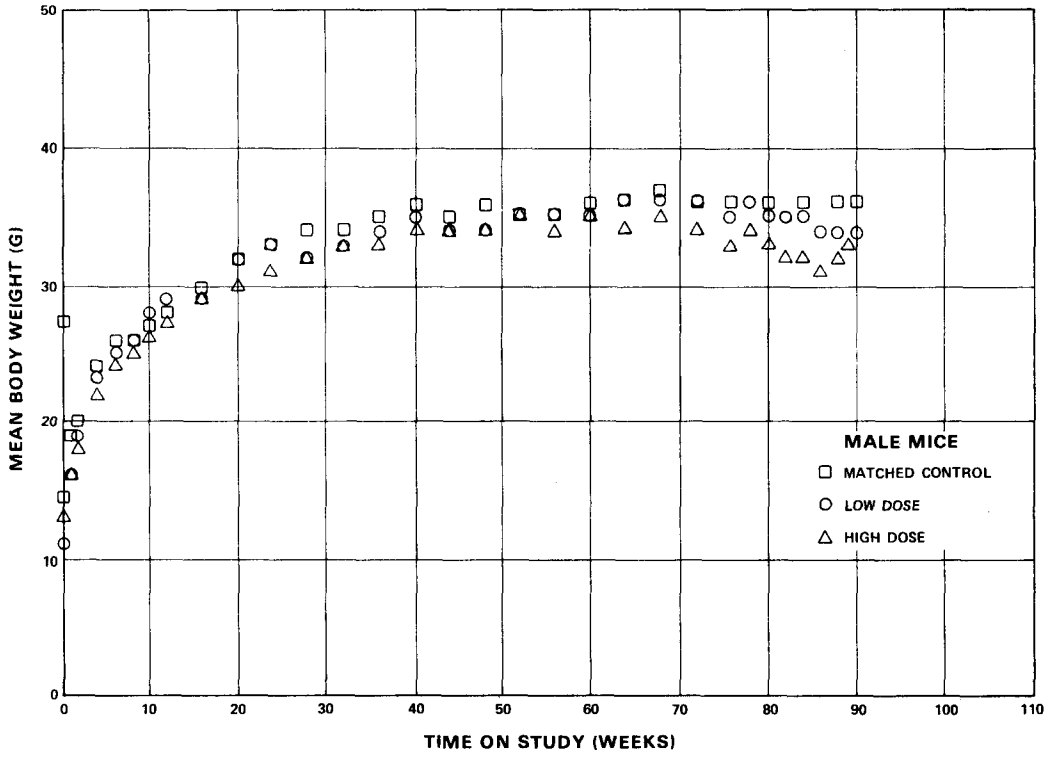
IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

The mean body weights attained by the high-dose male mice were generally lower than those of the matched controls, while weights of low-dose males and both low- and high-dose females were essentially unaffected by the toxaphene (figure 3). Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation.

Several animals died before week 19 of the bioassay, and doses were lowered at that time. Following this, the dosed mice were generally comparable to the controls in appearance and behavior during the first year of the study. A few animals, both control and dosed, had alopecia.

During the second year of the bioassay, abdominal distention was noted in all dosed groups, but predominantly in the high-dose males. Other clinical signs included alopecia, diarrhea, rough hair coats, and dyspnea. From weeks 60 to 76, the low-dose males appeared hyperexcitable.



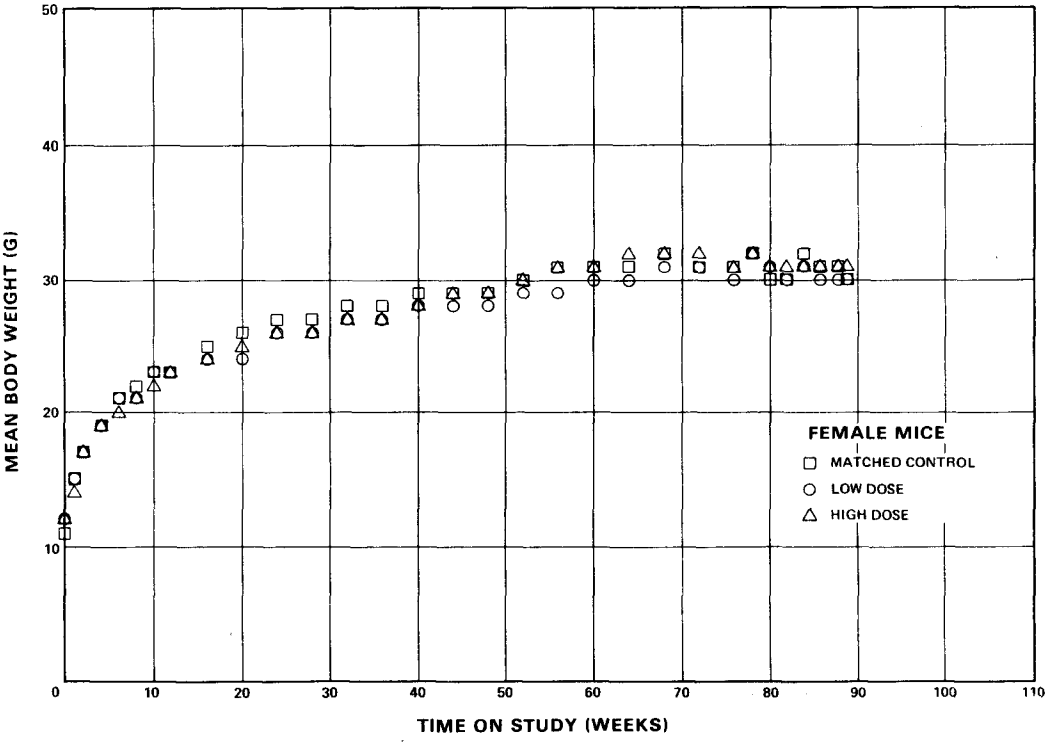


Figure 3. Growth Curves for Mice Fed Toxaphene in the Diet

B. Survival (Mice)

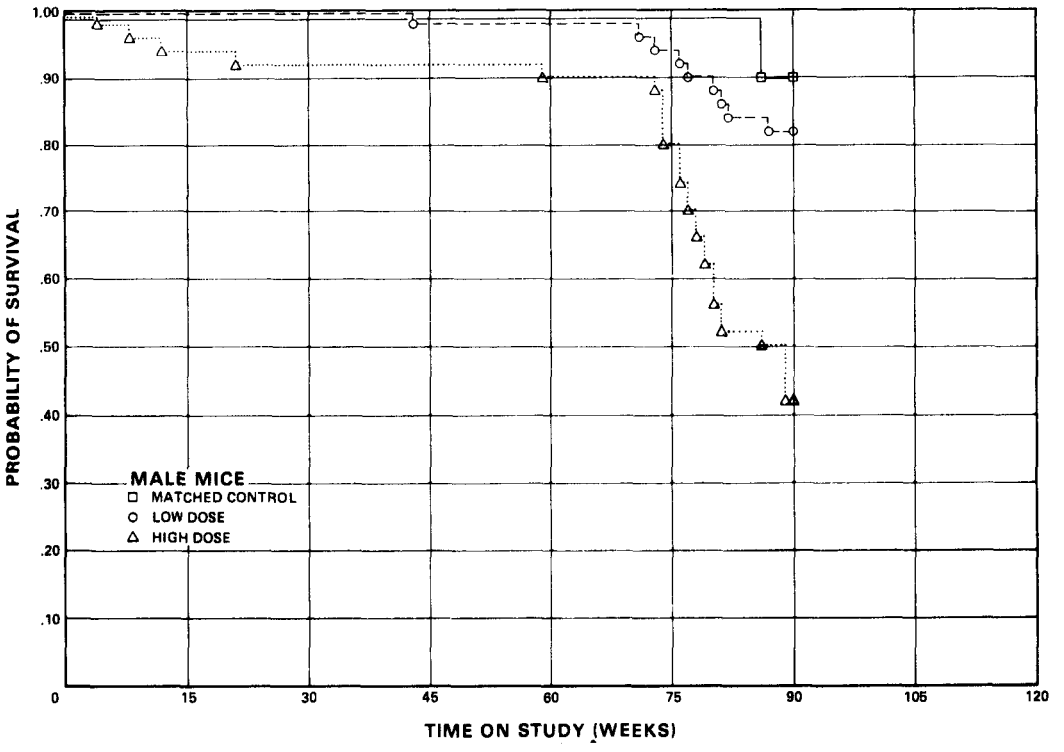
The Kaplan and Meier curves estimating the probabilities of survival for male and female mice fed toxaphene in the diet at the doses of this bioassay, together with those of the controls, are shown in figure 4. In each sex, the result of the Tarone test for dose-related trend in mortality is significant (P less than 0.001 in males and P = 0.017 in females).

In male mice, 46/50 (92%) of the high-dose group, 49/50 (98%) of the low-dose group, and all 10 animals of the control group lived beyond week 52 on study. In females, 46/50 (92%) of the high-dose group, 46/50 (92%) of the low-dose group, and 9/10 (90%) of the control group lived beyond week 52 on study.

Sufficient numbers of dosed mice of each sex were at risk for the development of tumors.

C. Pathology (Mice)

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



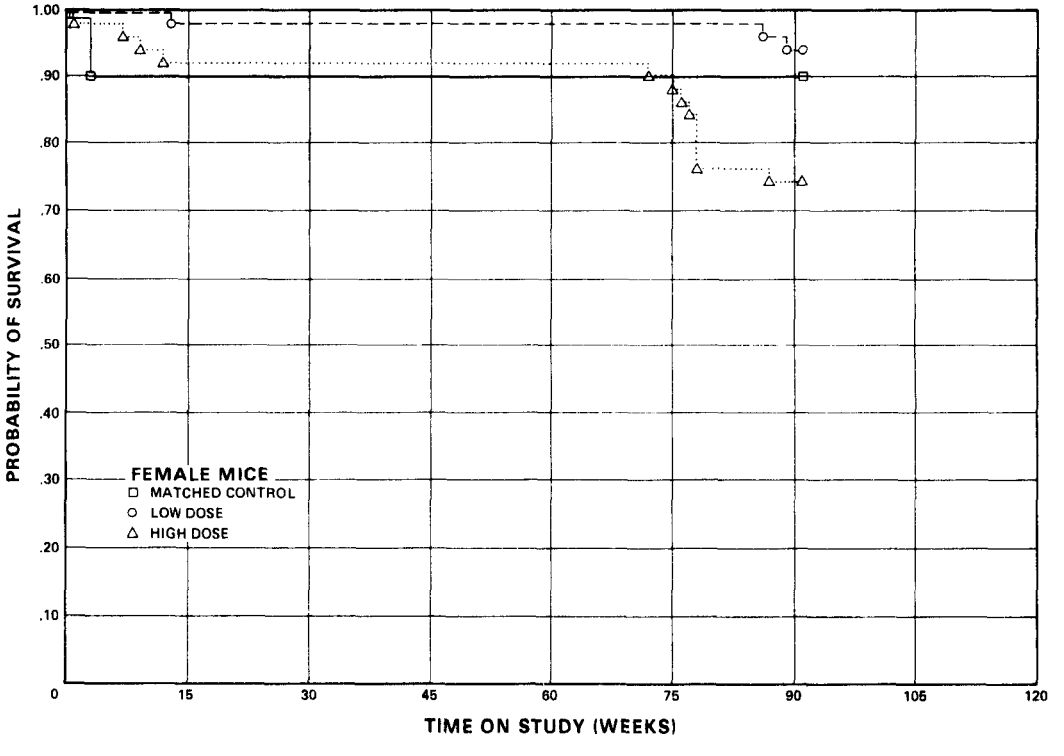


Figure 4. Survival Curves for Mice Fed Toxaphene in the Diet

With the exception of the incidences of hepatocellular carcinomas and neoplastic nodules of the liver, the incidences of lesions in the control mice were comparable to those in the dosed animals.

Hepatocellular carcinomas occurred only in the dosed mice — males: low-dose 34/49 (69%), high-dose 45/46 (98%); females: low-dose 5/49 (10%), high-dose 34/49 (69%). In male mice without hepatocellular carcinoma, neoplastic nodules of the liver occurred in 2/10 (20%) matched-control males, 6/49 (12%) low-dose males, 0/9 (0%) matched-control females, 13/49 (27%) low-dose females, and 6/49 (12%) high-dose females.

The lesions diagnosed as hepatocellular carcinomas varied in gross appearance from small, single growths to large, multinodular growths, randomly positioned throughout the liver. These growths were generally tan to dark brown in color and variegated in pattern, with tiny scattered areas of hemorrhage and necrosis. Histologically they were quite variable. They appeared as circumscribed masses of obviously malignant cells encroaching on the periphery. Liver cell plates were thickened and branched haphazardly; occasionally nests were formed, as were pseudorosettes. For the most part the cells were more basophilic than normal, and the cytoplasmic and nuclear borders were commonly enlarged and/or irregular in outline. Markedly enlarged

hyperchromatic nuclei were frequently seen. Areas of infraction and hemorrhage were not uncommon. Mitoses were also seen with some abnormal forms. Metastases were not found to be associated with the hepatocellular carcinomas.

The diagnosis "neoplastic nodule" is used to describe one or more than one clearly delineated nodular growth within the liver parenchyma. Grossly, these nodules varied in size up to 0.5 cm in diameter. Most commonly they were pale tan and homogeneous on the cut surface. Histologically they were expansive growths compressing the surrounding tissue. The sinusoidal pattern was mostly haphazard, the cords being of variable thickness. A tinctorial change was commonly evident between the proliferative mass and the surrounding liver. The cells were regular and the nuclei were essentially within normal limits. Vascular structures within these lesions were not remarkable. Bile ducts were not evident. The diagnosis of neoplastic nodules vs. small carcinomas was based on the more anaplastic cellular features of the latter. Occasionally, cellular atypia was relatively marked, suggesting malignant transformation.

Most of the other neoplastic and nonneoplastic lesions observed in the mice were not unusual findings, and the incidences of lesions in animals of the control groups were similar to those of

the dosed groups. These lesions are, therefore, considered to have occurred spontaneously.

Based on the histopathologic examination, toxaphene was carcinogenic in B6C3F1 mice, causing increased incidences of hepatocellular carcinomas and neoplastic nodules under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group. Only tumors of the liver occurred at this incidence; however, in this report, the incidence of neoplastic nodules is not listed separately but is combined with the incidence of hepatocellular carcinomas.

The proportions of hepatocellular carcinomas in the low- and high-dose groups of male mice and in the high-dose group of female mice were significant (P less than 0.001) when compared with either the matched- or pooled-control groups by the Fisher

exact test. Also, the results of the Cochran-Armitage test for dose-related trend, using either of the control groups, had values of P less than 0.001 in both male and female groups, but with significant departures from linearity due to the steep rise in incidence in the dosed groups compared with that in the controls. Although the matched controls of the present studies show no hepatocellular carcinomas, the historical records taken from 20 similar studies at this laboratory showed 45/245 (18.4%) hepatocellular carcinomas in male B6C3F1 mice and 6/224 (2.7%) in females.

When the incidence of hepatocellular carcinomas was combined with that of neoplastic nodules, the results of the Fisher exact test for comparisons of incidences for low- and high-dose males and high-dose females with those of the respective matched and pooled controls indicated that in each of these tests P was less than 0.001.

V. DISCUSSION

Mean body weights attained by low- and high-dose female rats and high-dose male mice were lower than those of matched controls, but weights of other dosed groups were essentially unaffected by the toxaphene. Other clinical signs of toxicity included generalized body tremors in high-dose male and female rats at week 53, after which the concentrations of toxaphene were reduced. Later, leg paralysis, ataxia, epistaxis, hematuria, and vaginal bleeding were observed in a few animals, predominantly in the dosed groups. Abdominal distention, diarrhea, dyspnea, and rough hair coats were observed predominantly in the dosed groups of both rats and mice; the abdominal distention was noted particularly among the high-dose male mice. Several high-dose male mice died during later weeks of the study, and the survival rates showed a significant dose-related trend in male mice. High-dose female mice had a significant decrease in survival. Sufficient numbers of both rats and mice were at risk for the development of late-appearing tumors.

In the male rats, the incidence of follicular-cell carcinomas or adenomas of the thyroid was dose related ($P = 0.007$) using the pooled controls (matched controls 1/7, pooled controls 2/44,

low-dose 7/41, high-dose 9/35). In the females, the incidence of follicular-cell adenomas of the thyroid was dose-related using either the matched (P = 0.022) or pooled (P = 0.008) controls (matched controls 0/6, pooled controls 1/46, low-dose 1/43, high-dose 7/42). Direct comparisons of dosed and pooled-control groups showed significantly increased incidences of the follicular-cell carcinomas or adenomas in the high-dose males (P = 0.008) and of the follicular-cell adenomas in the high-dose females (P = 0.021). Two follicular-cell tumors in the high-dose males were carcinomas; all other follicular-cell tumors in the rats were adenomas.

In the female rats, the incidence of tumors of the pituitary (adenomas, chromophobe adenomas, and chromophobe carcinomas) was dose related using either matched (P = 0.046) or pooled (P = 0.012) controls, and, in a direct comparison, the incidence of pituitary tumors in the high-dose group was significantly higher (P = 0.013) than that in the pooled-control group (matched controls 3/8, pooled controls 17/51, low-dose 15/41, high-dose 23/39). One pituitary tumor, in a high-dose female, was a carcinoma; all other pituitary tumors in the rats were adenomas. The historical-control data obtained to date on 20 similar studies at this laboratory show an incidence of pituitary tumors of 58/185 (31.4 %), although there are incidences as high as 6/10

(60%), 5/10 (50%), 3/6 (50%), and 4/9 (44%). Considering these high spontaneous incidences observed in control groups, the conclusion cannot be made that the tumors in this study are associated with the administration of the test chemical.

In the mice, the incidence of hepatocellular carcinomas was dose related (P less than 0.001) for both males (matched controls 0/10, pooled controls 4/48, low-dose 34/49, high-dose 45/46) and females (matched controls 0/9, pooled controls 0/48, low-dose 5/49, high-dose 34/49), using either matched or pooled controls. Direct comparisons showed that the incidences of hepatocellular carcinomas in low- and high-dose male mice and high-dose female mice were all significantly higher (P less than 0.001) than those in the respective matched or pooled controls. Statistical significance was maintained when the incidence of hepatocellular carcinomas was combined with that of neoplastic nodules of the liver.

Both the FDA in 1949 and Kettering Laboratories in 1952 (Lehman, 1965) conducted 2-year feeding studies of toxaphene in rats (strain not specified). Concentrations of toxaphene used in the two investigations were 25, 100, 400, and 1,600 ppm and 10, 100, 1,000, and 1,500 ppm, respectively. Histologic changes in the liver were noted in rats given more than 25 ppm in the FDA study

and more than 100 ppm in the Kettering study, but no increase in the incidence of tumors was noted. Doses used in the present study fall within these ranges. Sherman rats fed 50 or 200 ppm toxaphene in the diet for 9 months did not show clinical signs, but on histopathologic examination, mild liver changes were found in some of the dosed animals (Ortega et al., 1957). In other studies, hybrid mice were administered the related chemical, Strobane®, at 4.64 mg/kg by stomach tube for 3 weeks, then at a concentration of 11 ppm in the diet for 75 weeks (Innes et al., 1969). A significantly elevated incidence of hepatomas was reported in male C57BL/6 x C3H/Anf hybrid mice and of lymphomas in male C57BL/6 x AKR hybrid mice.

It is concluded that under the conditions of this bioassay, toxaphene was carcinogenic in male and female B6C3F1 mice, causing increased incidences of hepatocellular carcinomas. The test results also suggest carcinogenicity of toxaphene for the thyroid of male and female Osborne-Mendel rats.

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APPENDIX A

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

TABLE A1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	10	50	45
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	47	45
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(50)	(45)
SQUAMOUS CELL PAPILLOMA			1 (2%)
*SUBCUT TISSUE	(10)	(50)	(45)
FIBROMA		1 (2%)	
FIBROUS HISTIOCYTOMA		1 (2%)	
FIBROUS HISTIOCYTOMA, MALIGNANT		2 (4%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(9)	(45)	(43)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(50)	(45)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#SPLEEN	(9)	(45)	(42)
HEMANGIOMA		3 (7%)	3 (7%)
#THYMUS			(1)
CARCINOMA,NOS			1 (100%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(9)	(44)	(45)
NEOPLASTIC NODULE	1 (11%)	6 (14%)	4 (9%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FIBROUS HISTIOCYTOMA, METASTATIC		1 (2%)	
#BILE DUCT	(9)	(44)	(45)
PAPILLARY ADENOMA			1 (2%)
HAMARTOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY	(9)	(45)	(45)
TUBULAR-CELL ADENOMA		2 (4%)	1 (2%)
MIXED TUMOR, MALIGNANT		1 (2%)	1 (2%)
† HAMARTOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(7)	(42)	(31)
CARCINOMA,NOS			1 (3%)
ADENOMA, NOS	1 (14%)	1 (2%)	
CHROMOPHOBE ADENOMA	2 (29%)	12 (29%)	4 (13%)
#ADRENAL	(9)	(41)	(37)
ADENOMA, NOS	2 (22%)		
CORTICAL ADENOMA	2 (22%)	4 (10%)	3 (8%)
CORTICAL CARCINOMA		1 (2%)	
PHEOCHROMOCYTOMA	1 (11%)		1 (3%)
#THYROID	(7)	(41)	(35)
FOLLICULAR-CELL ADENOMA	1 (14%)	7 (17%)	7 (20%)
FOLLICULAR-CELL CARCINOMA			2 (6%)
C-CELL ADENOMA		1 (2%)	
C-CELL CARCINOMA		1 (2%)	
#PARATHYROID	(5)	(26)	(21)
ADENOMA, NOS		1 (4%)	
#PANCREATIC ISLETS	(9)	(42)	(30)
ISLET-CELL ADENOMA		1 (2%)	1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(45)
CARCINOMA,NOS		1 (2%)	

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

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*PROSTATE	(9)	(37)	(35)
SARCOMA, NOS			1 (3%)
NERVOUS SYSTEM			
*BRAIN	(9)	(44)	(43)
ASTROCYTOMA		1 (2%)	
MENINGIOMA			1 (2%)
*CRANIAL NERVE	(10)	(50)	(45)
NEURILEMOMA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKULL	(10)	(50)	(45)
OSTEOBLASTOMA			1 (2%)
*SKELETAL MUSCLE	(10)	(50)	(45)
SARCOMA, NOS			1 (2%)
BODY CAVITIES			
*BODY CAVITIES	(10)	(50)	(45)
MESOTHELIOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(10)	(50)	(45)
UNDIFFERENTIATED CARCINOMA		1 (2%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@	2	9	14
MORIBUND SACRIFICE	2	13	15
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	6	28	19
ANIMAL MISSING			1
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	33	24
TOTAL PRIMARY TUMORS	10	49	42
TOTAL ANIMALS WITH BENIGN TUMORS	7	27	20
TOTAL BENIGN TUMORS	9	35	26
TOTAL ANIMALS WITH MALIGNANT TUMORS		7	10
TOTAL MALIGNANT TUMORS		8	11
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	1	6	5
TOTAL UNCERTAIN TUMORS	1	6	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

@ INCLUDES AUTOLYZED ANIMALS

* 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 TOXAPHENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(10)	(50)	(49)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	3 (6%)
LIPOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(9)	(46)	(48)
TERATOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(7)	(47)	(48)
HEMANGIOMA		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(42)	(40)
NEOPLASTIC NODULE	1 (10%)	4 (10%)	4 (10%)
HEPATOCELLULAR CARCINOMA		1 (2%)	
#BILE DUCT	(10)	(42)	(40)
HAMARTOMA			1 (3%)
#PANCREAS	(9)	(46)	(45)
LIPOMA		1 (2%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(8)	(49)	(48)
TUBULAR-CELL ADENOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(8)	(41)	(39)
ADENOMA, NOS	1 (13%)		4 (10%)
CHROMOPHOBE ADENOMA	2 (25%)	15 (37%)	18 (46%)
CHROMOPHOBE CARCINOMA			1 (3%)
#ADRENAL	(8)	(44)	(43)
CORTICAL ADENOMA		3 (7%)	4 (9%)
CORTICAL CARCINOMA			2 (5%)
#THYROID	(6)	(43)	(42)
FOLLICULAR-CELL ADENOMA		1 (2%)	7 (17%)
C-CELL CARCINOMA	1 (17%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(49)
ADENOMA, NOS		2 (4%)	1 (2%)
ADENOCARCINOMA, NOS		1 (2%)	1 (2%)
PAPILLARY ADENOCARCINOMA			1 (2%)
FIBROMA		1 (2%)	2 (4%)
FIBROADENOMA	1 (10%)	10 (20%)	10 (20%)
TERATOMA, MALIGNANT			1 (2%)
#UTERUS	(9)	(41)	(45)
CARCINOMA,NOS		1 (2%)	
PAPILLARY ADENOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP		9 (22%)	5 (11%)
#OVARY	(8)	(40)	(36)
CARCINOMA,NOS			1 (3%)
GRANULOSA-CELL TUMOR		1 (3%)	
NERVOUS SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@	2	4	4
MORIBUND SACRIFICE	4	18	11
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	4	28	35
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	31	40
TOTAL PRIMARY TUMORS	6	52	70
TOTAL ANIMALS WITH BENIGN TUMORS	4	28	36
TOTAL BENIGN TUMORS	4	43	56
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	4	10
TOTAL MALIGNANT TUMORS	1	4	10
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	5	4
TOTAL UNCERTAIN TUMORS	1	5	4
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 TOXAPHENE IN THE DIET

TABLE B1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	50	46
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(46)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (10%)	1 (2%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(50)	(50)
GRANULOCYTIC LEUKEMIA		2 (4%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(49)	(46)
NEOPLASTIC NODULE	2 (20%)	6 (12%)	
HEPATOCELLULAR CARCINOMA		34 (69%)	45 (98%)
ANGIOSARCOMA		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(10)	(50)	(50)
PAPILLARY CYSTADENOMA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@		3	8
MORIBUND SACRIFICE	1	6	21
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	9	41	21
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	44	45
TOTAL PRIMARY TUMORS	3	45	47
TOTAL ANIMALS WITH BENIGN TUMORS	1	2	2
TOTAL BENIGN TUMORS	1	2	2
TOTAL ANIMALS WITH MALIGNANT TUMORS		37	45
TOTAL MALIGNANT TUMORS		37	45
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	2	6	
TOTAL UNCERTAIN TUMORS	2	6	
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 TOXAPHENE IN THE DIET

	MATCHED 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			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (10%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(8)	(46)	(47)
ADENOMA, NOS		1 (2%)	
#LIVER	(9)	(49)	(49)
NEOPLASTIC NODULE		13 (27%)	6 (12%)
HEPATOCELLULAR CARCINOMA		5 (10%)	34 (69%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(50)
ADENOMA, NOS		1 (2%)	
#UTERUS	(6)	(48)	(44)
LEIOMYOMA		1 (2%)	
ENDOMETRIAL STROMAL POLYP		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(10)	(50)	(50)
PAPILLARY CYSTADENOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@	1	1	6
MORIBUND SACRIFICE		2	7
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	9	47	37
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	1	22	40
TOTAL PRIMARY TUMORS	1	23	41
TOTAL ANIMALS WITH BENIGN TUMORS	1	4	1
TOTAL BENIGN TUMORS	1	4	1
TOTAL ANIMALS WITH MALIGNANT TUMORS		6	34
TOTAL MALIGNANT TUMORS		6	34
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		13	6
TOTAL UNCERTAIN TUMORS		13	6
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 TOXAPHENE IN THE DIET

TABLE C1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	10	50	45
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	47	45
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(50)	(45)
EPIDERMAL INCLUSION CYST		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(9)	(45)	(43)
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(9)	(45)	(42)
HEMORRHAGE		1 (2%)	
FIBROSIS, FOCAL		1 (2%)	
PERIARTERITIS		1 (2%)	
HYPERPLASIA, RETICULUM CELL			1 (2%)
#LYMPH NODE	(8)	(43)	(35)
INFLAMMATION, FOCAL			1 (3%)
CIRCULATORY SYSTEM			
#HEART	(9)	(47)	(42)
FIBROSIS, DIFFUSE			1 (2%)
#MYOCARDIUM	(9)	(47)	(42)
INFLAMMATION, CHRONIC	1 (11%)		
FIBROSIS, FOCAL	1 (11%)		
#ENDOCARDIUM	(9)	(47)	(42)
FIBROSIS		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*AORTA	(10)	(50)	(45)
ARTERIOSCLEROSIS, NOS	1 (10%)		
MEDIAL CALCIFICATION		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(9)	(44)	(45)
DEGENERATION, BALLOONING		1 (2%)	1 (2%)
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY		2 (5%)	4 (9%)
FOCAL CELLULAR CHANGE	1 (11%)		
ANGIECTASIS		1 (2%)	
#LIVER/CENTRILOBULAR	(9)	(44)	(45)
METAMORPHOSIS FATTY	1 (11%)		
#BILE DUCT	(9)	(44)	(45)
HYPERPLASIA, NOS	1 (11%)	3 (7%)	1 (2%)
HYPERPLASIA, FOCAL	1 (11%)		
#PANCREATIC ACINUS	(9)	(42)	(30)
ATROPHY, NOS			1 (3%)
#STOMACH	(9)	(43)	(40)
ULCER, NOS		1 (2%)	
ULCER, CHRONIC	1 (11%)		
CALCIFICATION, METASTATIC	1 (11%)		
ACANTHOSIS			1 (3%)
URINARY SYSTEM			
#KIDNEY	(9)	(45)	(45)
CYST, NOS	1 (11%)		
MULTIPLE CYSTS		1 (2%)	
INFLAMMATION, CHRONIC	4 (44%)	9 (20%)	20 (44%)
DEGENERATION, CYSTIC		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(7)	(42)	(31)
DEGENERATION, CYSTIC		2 (5%)	2 (6%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NOS	1 (14%)		
ANGIECTASIS		1 (2%)	
#ADRENAL	(9)	(41)	(37)
METAMORPHOSIS FATTY	1 (11%)		
ANGIECTASIS			2 (5%)
#ADRENAL CORTEX	(9)	(41)	(37)
HYPERPLASIA, NOS	1 (11%)		
#THYROID	(7)	(41)	(35)
FOLLICULAR CYST, NOS		2 (5%)	1 (3%)
HYPERPLASIA, C-CELL		2 (5%)	1 (3%)
HYPERPLASIA, FOLLICULAR-CELL		3 (7%)	3 (9%)
#PARATHYROID	(5)	(26)	(21)
HYPERPLASIA, NOS	1 (20%)		
REPRODUCTIVE SYSTEM			
#PROSTATE	(9)	(37)	(35)
INFLAMMATION, NOS			2 (6%)
INFLAMMATION, ACUTE		1 (3%)	1 (3%)
INFLAMMATION, CHRONIC			1 (3%)
#TESTIS	(9)	(46)	(43)
ATROPHY, NOS	2 (22%)	11 (24%)	10 (23%)
ATROPHY, FOCAL		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*FEMUR	(10)	(50)	(45)
FIBROUS OSTEODYSTROPHY	1 (10%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(10)	(50)	(45)
NECROSIS, FAT	1 (10%)		
*MESENTERY	(10)	(50)	(45)
THROMBOSIS, NOS	1 (10%)		
PERIARTERITIS		1 (2%)	2 (4%)
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		6	5
ACCIDENTAL DEATH			1
NECROPSY PERF/NO HISTO PERFORMED		1	1
AUTO/NECROPSY/HISTO PERF			1
AUTO/NECROPSY/NO HISTO	1	2	
AUTOLYSIS/NO NECROPSY			3

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

TABLE C2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(9)	(46)	(48)
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(7)	(47)	(48)
HEMORRHAGE		1 (2%)	
CIRCULATORY SYSTEM			
#ENDOCARDIUM	(9)	(49)	(47)
FIBROSIS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(10)	(42)	(40)
METAMORPHOSIS FATTY	1 (10%)	2 (5%)	2 (5%)
BASOPHILIC CYTO CHANGE			1 (3%)
CLEAR-CELL CHANGE		1 (2%)	
#BILE DUCT	(10)	(42)	(40)
HYPERPLASIA, NOS	2 (20%)	1 (2%)	1 (3%)
HYPERPLASIA, CYSTIC			3 (8%)
#STOMACH	(9)	(47)	(49)
ULCER, NOS		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ULCER, CHRONIC	1 (11%)		
URINARY SYSTEM			
#KIDNEY	(8)	(49)	(48)
CAST, NOS	1 (13%)		
INFLAMMATION, CHRONIC	1 (13%)	8 (16%)	2 (4%)
#KIDNEY/TUBULE	(8)	(49)	(48)
CAST, NOS	1 (13%)		
ENDOCRINE SYSTEM			
#PITUITARY	(8)	(41)	(39)
CYTOPLASMIC VACUOLIZATION	1 (13%)		
HYPERPLASIA, FOCAL	1 (13%)		
#ADRENAL	(8)	(44)	(43)
ACCESSORY STRUCTURE	1 (13%)		
ANGIECTASIS	2 (25%)	3 (7%)	1 (2%)
#ADRENAL CORTEX	(8)	(44)	(43)
HYPERPLASIA, FOCAL			1 (2%)
#THYROID	(6)	(43)	(42)
FOLLICULAR CYST, NOS			2 (5%)
HYPERPLASIA, C-CELL		4 (9%)	2 (5%)
HYPERPLASIA, FOLLICULAR-CELL		5 (12%)	3 (7%)
REPRODUCTIVE SYSTEM			
*VAGINA	(10)	(50)	(49)
INFLAMMATION, SUPPURATIVE	1 (10%)		
POLYP, INFLAMMATORY	1 (10%)		
#UTERUS/ENDOMETRIUM	(9)	(41)	(45)
CYST, NOS	1 (11%)	2 (5%)	
INFLAMMATION, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*PHALANGES	(10)	(50)	(49)
GRANULOMA, NOS	1 (10%)		
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		11	7
AUTO/NECROPSY/HISTO PERF	1		
AUTO/NECROPSY/NO HISTO		1	
AUTOLYSIS/NO NECROPSY			1

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

APPENDIX D

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

TABLE D1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	50	46
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(50)	(50)
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#LYMPH NODE	(7)	(36)	(39)
INFLAMMATION, CHRONIC		1 (3%)	
#MESENTERIC L. NODE	(7)	(36)	(39)
HYPERPLASIA, NOS		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(49)	(46)
FOCAL CELLULAR CHANGE	1 (10%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
#TESTIS	(10)	(49)	(45)
ATROPHY, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	7	6	1
AUTO/NECROPSY/NO HISTO			4

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

TABLE D2.

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

	MATCHED 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			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(49)
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(10)	(48)	(44)
HYPERPLASIA, LYMPHOID		2 (4%)	1 (2%)
#MESENTERIC L. NODE	(7)	(44)	(40)
INFLAMMATION, NOS			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(9)	(49)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
METAMORPHOSIS FATTY			1 (2%)
#SMALL INTESTINE	(9)	(49)	(45)
HYPERPLASIA, LYMPHOID			1 (2%)
URINARY SYSTEM			
#KIDNEY/TUBULE	(10)	(47)	(48)
CAST, NOS			1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#THYROID	(7)	(45)	(38)
HYPERPLASIA, FOLLICULAR-CELL			1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
#UTERUS	(6)	(48)	(44)
INFLAMMATION, CHRONIC		1 (2%)	
DEGENERATION, CYSTIC		1 (2%)	
#UTERUS/ENDOMETRIUM	(6)	(48)	(44)
DILATATION, NOS		1 (2%)	
INFLAMMATION, FOCAL			1 (2%)
DEGENERATION, CYSTIC		4 (8%)	
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, CYSTIC	1 (17%)	1 (2%)	1 (2%)
#OVARY/OVIDUCT	(6)	(48)	(44)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, CHRONIC	1 (17%)		
#OVARY	(7)	(48)	(45)
DISTENTION		1 (2%)	
CYST, NOS		1 (2%)	
FOLLICULAR CYST, NOS		2 (4%)	
INFLAMMATION, SUPPURATIVE	1 (14%)	5 (10%)	
INFLAMMATION ACUTE AND CHRONIC			1 (2%)
INFLAMMATION, CHRONIC	1 (14%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*MESENTERY	(10)	(50)	(50)
NECROSIS, FAT			1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	6	17	6
AUTO/NECROPSY/HISTO PERF			2
AUTO/NECROPSY/NO HISTO		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 TOXAPHENE IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Toxaphene in the Diet (a)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Neoplastic Nodule (b)	1/9 (11)	1/52 (2)	6/44 (14)	4/45 (9)
P Values (c,d)	N.S.	N.S.	P = 0.034**	N.S.
Relative Risk (Matched Control) (f)			1.227	0.800
Lower Limit			0.190	0.099
Upper Limit			55.074	38.517
Relative Risk (Pooled Control) (f)			7.091	4.622
Lower Limit			0.908	0.479
Upper Limit			317.788	222.281
Weeks to First Observed Tumor	109	--	108	94
Pituitary: Chromophobe Adenoma, Carcinoma, NOS, or Adenoma, NOS (b)	3/7 (43)	8/46 (17)	13/42 (31)	5/31 (16)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			0.722	0.376
Lower Limit			0.315	0.118
Upper Limit			3.371	2.069
Relative Risk (Pooled Control) (f)			1.780	0.927
Lower Limit			0.763	0.260
Upper Limit			4.429	2.878
Weeks to First Observed Tumor	102	--	85	95

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Toxaphene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Adrenal: Adenoma, NOS, Cortical Adenoma, or Carcinoma (b)	4/9 (44)	5/52 (10)	5/41 (12)	3/37 (8)
P Values (c,d)	P = 0.019 (N)	N.S.	P = 0.043 (N)*	P = 0.020 (N)*
Relative Risk (Matched Control) (f)			0.274	0.182
Lower Limit			0.088	0.039
Upper Limit			1.189	0.931
Relative Risk (Pooled Control) (f)			1.268	0.843
Lower Limit			0.311	0.138
Upper Limit			5.126	4.032
Weeks to First Observed Tumor			85	85
Spleen: Hemangioma (b)	0/9 (0)	0/49 (0)	3/45 (7)	3/42 (7)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			Infinite	Infinite
Lower Limit			0.136	0.145
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control) (f)			Infinite	Infinite
Lower Limit			0.656	0.703
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	83	85

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Toxaphene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Thyroid: Follicular-cell Carcinoma or Adenoma (b)	1/7 (14)	2/44 (5)	7/41 (17)	9/35 (26)
P Values (c,d)	N.S.	P = 0.007	N.S.	P = 0.008**
Relative Risk (Matched Control) (f)			1.195	1.800
Lower Limit			0.211	0.346
Upper Limit			53.423	76.080
Relative Risk (Pooled Control) (f)			3.756	5.657
Lower Limit			0.769	1.272
Upper Limit			35.292	50.584
Weeks to First Observed Tumor	109	--	104	56

(a) Dosed groups received time-weighted average doses of 556 or 1,112 ppm.

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

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

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats
Fed Toxaphene in the Diet (a)

(continued)

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

(e) The probability level for departure from linear trend is given when P less than 0.05 for any comparison.

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

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Toxaphene in the Diet (a)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Integumentary System: Malignant Fibrous Histiocytoma of the Subcutaneous Tissue (b)	0/10 (0)	0/55 (0)	1/50 (2)	3/49 (6)
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			0.059	0.674
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		105	83
Mammary Gland: Fibroadenoma (b)	1/10 (10)	6/55 (11)	10/50 (20)	10/49 (20)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			2.000	2.041
Lower Limit			0.357	0.363
Upper Limit			84.786	86.461
Relative Risk (Pooled Control) (f)			1.833	1.871
Lower Limit			0.652	0.667
Upper Limit			5.693	5.801
Weeks to First Observed Tumor	87	--	19	67

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Toxaphene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Neoplastic Nodule (b)	1/10 (10)	1/55 (2)	5/42 (12)	4/40 (10)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			1.190	1.000
Lower Limit			0.165	0.121
Upper Limit			54.892	46.976
Relative Risk (Pooled Control) (f)			6.548	5.500
Lower Limit			0.770	0.570
Upper Limit			301.508	263.516
Weeks to First Observed Tumor	109	--	108	109
Pituitary: Chromphobe Adenoma, Carcinoma, or Adenoma, NOS (b)	3/8 (38)	17/51 (33)	15/41 (37)	23/39 (59)
P Values (c,d)	P = 0.046	P = 0.012	N.S.	P = 0.013**
Relative Risk (Matched Control) (f)			0.976	1.573
Lower Limit			0.410	0.706
Upper Limit			4.521	6.767
Relative Risk (Pooled Control) (f)			1.098	1.769
Lower Limit			0.583	1.062
Upper Limit			2.021	2.897
Weeks to First Observed Tumor	85	--	75	79

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Toxaphene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Thyroid: Follicular-cell Adenoma (b)	0/6 (0)	1/46 (2)	1/43 (2)	7/42 (17)
P Values (c,d)	P = 0.022	P = 0.008	N.S.	P = 0.021**
Relative Risk (Matched Control) (f)			Infinite	Infinite
Lower Limit			0.009	0.340
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control) (f)			1.070	7.667
Lower Limit			0.014	1.049
Upper Limit			82.048	335.959
Weeks to First Observed Tumor	--	--	102	105
Adrenal: Cortical Adenoma or Carcinoma (b)	0/8 (0)	3/50 (6)	3/44 (7)	6/43 (14)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			Infinite	Infinite
Lower Limit			0.126	0.344
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control) (f)			1.136	2.326
Lower Limit			0.160	0.530
Upper Limit			8.065	13.580
Weeks to First Observed Tumor	--	--	104	87

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Toxaphene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp (b)	0/9 (0)	5/53 (9)	9/41 (22)	5/45 (11)
P Values (c,d)	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control) (f)			Infinite	Infinite
Lower Limit			0.657	0.286
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control) (f)			2.327	1.178
Lower Limit			0.760	0.288
Upper Limit			8.142	4.787
Weeks to First Observed Tumor	--	--	87	109

(a) Dosed groups received time-weighted average doses of 540 or 1,080 ppm.

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

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

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Toxaphene in the Diet (a)

(continued)

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

(e) The probability level for departure from linear trend is given when P less than 0.05 for any comparison.

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

APPENDIX F

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

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Toxaphene in the Diet (a)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	0/10 (0)	4/48 (8)	34/49 (69)	45/46 (98)
P Values (c,d)	P less than 0.001	P less than 0.001	P less than 0.001*	P less than 0.001*
			P less than 0.001**	P less than 0.001**
Departure from Linear Trend (e)	P = 0.028	P = 0.044		
Relative Risk (Matched Control) (f)			Infinite	Infinite
Lower Limit			2.610	4.108
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control) (f)			8.327	11.739
Lower Limit			3.379	5.764
Upper Limit			27.877	15.812
Weeks to First Observed Tumor	--	--	73	59

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Toxaphene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Neoplastic Nodule (b)	2/10 (20)	7/48 (15)	40/49 (82)	45/46 (98)
P Values (c,d)	P less than 0.001	P less than 0.001	P less than 0.001*	P less than 0.001*
			P less than 0.001**	P less than 0.001**
Departure from Linear Trend (e)	P = 0.007	P = 0.002		
Relative Risk (Matched Control) (f)			4.082	4.891
Lower Limit			1.430	1.883
Upper Limit			28.580	9.530
Relative Risk (Pooled Control) (f)			5.598	6.708
Lower Limit			2.949	4.049
Upper Limit			11.332	7.956
Weeks to First Observed Tumor	90	--	73	59

(a) Dosed groups received time-weighted average doses of 99 or 198 ppm.

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

(c) Beneath the incidence of tumors in a control group is the probability level for the Cochran-Armitage test when P less than 0.05; otherwise not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group (*) or with the pooled-control group (**) when P less than 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 Toxaphene in the Diet (a)

(continued)

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

(e) The probability level for departure from linear trend is given when P less than 0.05 for any comparison.

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

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Toxaphene in the Diet (a)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	0/9 (0)	0/48 (0)	5/49 (10)	34/49 (69)
P Values (c,d)	P less than 0.001	P less than 0.001	P = 0.030**	P less than 0.001*
				P less than 0.001**
Departure from Linear Trend (e)	P = 0.022	P = 0.002		
Relative Risk (Matched Control) (f)			Infinite	Infinite
Lower Limit			0.262	2.384
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control) (f)			Infinite	Infinite
Lower Limit			1.237	11.267
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	89	72

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Toxaphene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Neoplastic Nodule (b)	0/9 (0)	0/48 (0)	18/49 (37)	40/49 (82)
P Values (c,d)	P less than 0.001	P less than 0.001	P = 0.026*	P less than 0.001*
			P less than 0.001**	P less than 0.001**
Relative Risk (Matched Control) (f)			Infinite	Infinite
Lower Limit			1.200	2.854
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control) (f)			Infinite	Infinite
Lower Limit			5.649	13.554
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	89	72

(a) Dosed groups received time-weighted average doses of 99 or 198 ppm.

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

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

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Toxaphene in the Diet (a)

(continued)

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

(e) The probability level for departure from linear trend is given when P less than 0.05 for any comparison.

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

APPENDIX G

ANALYSIS OF FORMULATED DIETS FOR CONCENTRATIONS OF TOXAPHENE

APPENDIX G

Analysis of Formulated Diets for Concentrations of Toxaphene

A 2 g sample of formulated diet was shaken at ambient temperature with 50 ml hexane for 2 hours, then filtered through Celite with hexane washes, and reduced in volume to 10 ml.

The toxaphene then was dehydrohalogenated by the following procedure. A 1 ml aliquot was added to 0.5 ml of alcoholic KOH (2.5 g KOH/10 ml 95% ethanol), and the mixture was heated at 75-80°C for 15 minutes. The mixture was allowed to cool, 1 ml hexane and 8 ml saturated aqueous Na_2SO_4 were added, and the mixture was shaken for 2 minutes. The hexane layer was drawn off, another 1 ml of hexane was added, and the mixture was again shaken and the hexane layer removed. This extraction was repeated one more time, and the combined hexane extracts were brought up to 10 ml volume with hexane and dried over anhydrous Na_2SO_4.

The resultant solution was analyzed quantitatively by gas-liquid chromatography (electron capture detector, 5% QF-1 on Chromosorb

W column). Recoveries were checked with spiked samples carried through the entire procedure.

Theoretical Concentrations in Diet (ppm)	No. of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
80	4	81.8	1.8%	81-84
160	8	155.0	3.3%	150-164
320	3	330.0	1.0%	327-333
640	11	640.0	6.9%	592-714
1,280	12	1,286.0	2.9%	1,200-1,340

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

August 31, 1978

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

A toxicologist with Hercules presented a public statement regarding the bioassay of Toxaphene. He noted that Hercules is the sole U.S. manufacturer of Toxaphene, the largest selling insecticide in the world. He said that Hercules disagreed with the conclusion in the report that "The test results also suggest carcinogenicity of Toxaphene for the thyroid of male and female Osborne-Mendel rats." Based on an evaluation of the histopathology and statistics by consultants to Hercules, the representative said that the follicular-cell thyroid tumor incidence in treated and pooled control male rats was virtually identical. Furthermore, the incidence of follicular-cell thyroid tumors in females was not statistically significant. He said that classic thyroid carcinogens induce mainly bilateral tumors, whereas those observed in control and treated animals were essentially unilateral. He objected to the use of the term "carcinogen" in reference to benign tumors. He noted a discrepancy in the historical control tissue count (for thyroids) between that given in the report and the number found in the Hercules evaluation. The Hercules representative recommended that the report include the range of thyroid tumors observed in each control group.

The primary reviewer indicated that his remarks were predicated solely on the information in the report and not from other sources. He concluded that the bioassay demonstrated that the test compound induced a neoplastic response in the rat thyroid and the mouse liver. He also pointed out an increased number of liver carcinomas observed in low dose treated male rats. He suggested that this finding should receive greater emphasis. He further noted that the test compound came from a single batch of Toxaphene. Since Toxaphene is a mixture, he wondered if the carcinogenic component(s) would be common to all batches. Although he agreed with the conclusion in the report, the primary reviewer cautioned against estimating the potential hazard of Toxaphene for man, particularly since no evidence exists that polychlorinated alkanes or terpenes are human carcinogens. He recommended that the report on the bioassay of Toxaphene be accepted as written. He also recommended that the test batch of Toxaphene be analyzed to determine its quantitative similarity to other batches of Toxaphene in general use.

Although the staff viewed the thyroid tumors as "suspicious," a Program pathologist said that the histopathology was confirmed at several review levels. Despite the question about the thyroid tumors, he noted that the liver tumors in mice were clearly treatment-related. He acknowledged that any necessary modification to the report would be made after examining the concerns expressed by Hercules.

A motion was approved unanimously that the report on the bioassay of Toxaphene be accepted as written.

Members present were:

Arnold L. Brown (Chairman), University of Wisconsin School of Medicine
Joseph Highland, Environmental Defense Fund
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center

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

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