National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 96
1979

BIOASSAY OF COUMAPHOS FOR POSSIBLE CARCINOGENICITY

CAS No. 56-72-4

NCI-CG-TR-96

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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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DHEW Publication No. (NIH) 79-1346

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FOREWORD: This report presents the results of the bioassay of coumaphos 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 animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of coumaphos 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., Rockville, Maryland, prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design for this bioassay is based on guidelines for carcinogen bioassays in small animals that have been established by NCI (1). The doses for the chronic studies were selected by Drs. E. E. Storrs (2) and O. G. Fitzhugh (3,4), and the principal investigator was Mr. R. J. Wheeler (2). Chemicals were analyzed during the bioassay by Mr. Wheeler and dosed feed mixtures by Mr. S. M. Billedeau (2). Reanalysis of the test chemical after completion of the bioassay was performed at Midwest Research Institute under the supervision of Dr. E.

Murrill (5). The results of these analyses were reviewed by Dr. C. W. Jameson (3). Histologic examination of animal tissues was performed by Drs. R. A. Ball (2) and E. Bernal (2), 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 (3) and Ms. P. L. Yong (3), using methods selected for the bioassay program by Dr. J. J. Gart (7).

This report was prepared at Tracor Jitco (3) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. L. A. Campbell, Director of the Bioassay Program; Dr. S. S. Olin, 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 (1) 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 (8), Dr. Richard A. Griesemer, Dr. Morton H. Levitt, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Robert A. Squire (9), Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

A bioassay of coumaphos for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F1 mice.

Groups of 50 rats of each sex and 50 mice of each sex were administered the coumaphos in the diet at one of two doses, either 10 or 20 ppm, for 103 weeks and then observed for 0-1 additional weeks. Matched controls consisted of groups of 25 untreated animals of each species and sex. All surviving animals were killed at 103-105 weeks.

Mean body weights of the dosed female rats were lower than those of corresponding controls, while mean body weights of dosed male rats and of dosed male and female mice were essentially unaffected. No clinical signs that are typical of organophosphorus poisoning were reported in either rats or mice. Survival of the rats and mice was not affected by administration of the test chemical. The test animals may have been able to tolerate higher doses. Sufficient numbers of animals in all groups of the rats and mice were at risk for the development of late-appearing tumors.

In both rats and mice, no tumors occurred in the dosed groups of either sex at incidences that were significantly higher than those in corresponding control groups.

It is concluded that under the conditions of this bioassay, coumaphos was not carcinogenic for either F344 rats or B6C3F1 mice.

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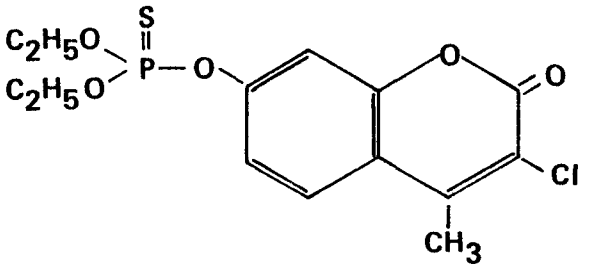
I. INTRODUCTION



COUMAPHOS

Coumaphos (CAS 56-72-4; NCI C08662), the O-ester of 3-chloro-7-hydroxy-4-methylcoumarin and 0,0-diethylphosphorothioate, is an organophosphorus pesticide that was developed in Germany by G. Schrader, who was responsible for much of the early work on the organophosphates, and to whom is attributed the synthesis of parathion (Martin, 1973; Murphy, 1975). Organophosphates inhibit cholinesterase, which leads to an accumulation of acetylcholine in the nervous system and produces symptoms of excessive nervous stimulation and eventual respiratory failure from broncho-constriction (Murphy, 1975). Coumaphos, which has a relatively low mammalian toxicity in relation to the other organophosphates (Martin, 1973), is used principally on livestock and poultry to

control ectoparasites. Most of the chemical is used on beef cattle to help prevent weight losses caused by irritation of the animals by such insects as hornflies, face flies, and stable flies (Ayers and Johnson, 1976). Applications of the insecticide are normally topical, although coumaphos is also administered orally in feed to eliminate intestinal parasites (Eto, 1974; Brown and Maniscalco, 1974; Meister, 1977) and larvae that are deposited in manure (Miller et al., 1970).

With the cancellation of the registration of many organochlorine pesticides, the organophosphates have become the main choice as substitutes (USITC, 1977). In 1975, the production of the organophosphates superseded that of the organochlorines for the first time (USITC, 1977). Approximately 400,000 pounds of coumaphos were used by the agricultural industry in 1974, for the control of livestock pests (Ayers and Johnson, 1976).

Coumaphos was selected for study in the Carcinogenesis Testing Program as a part of the effort to assess the carcinogenic potential of pesticides which have become distributed in the environment as a result of extensive use.

II. MATERIALS AND METHODS

A. Chemical

Coumaphos was obtained as the technical-grade material in a single batch (Lot No. 4153048) for the chronic phase of the study from the Chemagro division of Mobay Chemical Corporation, Kansas City, Missouri. The melting point range for this batch of coumaphos was 88-90° (literature: 95°C) (Eto, 1974). Vapor-phase chromatography (vpc) indicated a 5% impurity, which was not identified. Elemental analyses (C, H, P, S, Cl) were consistent with $C_{14}H_{16}O_5PSCl$, the molecular formula of coumaphos. Nuclear magnetic resonance, infrared (ir), and ultraviolet spectra also were consistent with the structure. The bulk chemical was stored at 4°C. Reanalysis of this lot of coumaphos at Midwest Research Institute after completion of the bioassay gave vpc and ir results which were similar to those obtained previously at Gulf South Research Institute, indicating that the chemical was stable under these conditions of storage.

The term coumaphos is used in the remainder of this report to designate the technical-grade material.

B. Dietary Preparation

All diets were formulated weekly using Wayne® Lab-Blox animal meal (Allied Mills Inc., Chicago, Ill.) to which was added the required amount of coumaphos for each dietary concentration. The test compound 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, Opelousas, La.) was also added to the feed, primarily as a dust suppressant. The diets were mixed mechanically for 25 minutes to assure the homogeneity of the mix and to allow for the evaporation of the acetone. Final diets, including those for the control groups, contained 2% corn oil by weight. Formulated diets were stored at room temperature until used, but no longer than 1 week.

The stability of coumaphos in feed was tested by determining the concentration of the compound in formulated diets at intervals over a 7-day period. Diets containing 40 or 320 ppm coumaphos showed no significant change in concentration on standing at ambient temperatures for this period.

As a quality control check on the accuracy of preparation of the diets, the concentration of coumaphos was determined in randomly selected samples from formulated diets at 8-week intervals during

the chronic study. The results of these analyses are reported in Appendix G. At each dietary concentration, the mean of the analytical concentrations for the checked samples was within 2% of the theoretical concentration, and the coefficient of variation was less than 0.06.

C. Animals

F344 (Fischer) rats and B6C3F1 mice of each sex, obtained through contracts of the Division of Cancer Treatment, National Cancer Institute, were used in these bioassays. The rats and mice were bred and supplied by the NCI Frederick Cancer Research Center, Frederick, Maryland. On arrival at the laboratory, all animals were quarantined for 14 days and then assigned to control or dosed groups.

D. Animal Maintenance

All animals were housed in rooms having a temperature range of 22-24°C and a relative humidity of 40-70%. The air in each room was filtered through permanent air maze filters (Air Maze Incom International, Cleveland, Ohio) and was changed 10-12 times

per hour. Fluorescent lighting provided illumination 10 hours per day. Food and tap water were provided ad libitum. Fresh feed was provided twice per week, and uneaten 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.), containing five females 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 (Industrial Washing Machine Corp., Matawan, N.J.) 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 sanitized in a Vulcan Autosan washer (Vulcan Autosan, 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 and mice receiving coumaphos were housed in separate rooms. Control and dosed rats were housed on the same rack, whereas cages for control and dosed mice were placed on separate racks in the same room. Coumaphos was the only compound on study in each room.

E. Subchronic Studies

Feeding studies were conducted to estimate the maximum tolerated doses of coumaphos, on the basis of which two concentrations (referred to in this report as "high" and "low" doses) were determined for administration in the chronic studies. In the subchronic studies, coumaphos was added to the animal feed at twofold increasing doses over the range of 10 to 640 ppm for both rats and mice. Control groups of rats and of mice received only a basal diet. Each dosed and control group consisted of 10 male and 10 female animals. The chemical was provided in feed to dosed groups for 13 weeks, after which the animals were killed and necropsied. Body weights were measured weekly.

In male rats administered 640 ppm, the mortality was 20%, and the weight gain of the survivors by the end of the study was 59% of that of the controls; in the females at 640 ppm, the mortality was 90%. At 320 ppm, there was no mortality in either males or females, the weight gain in the males was 93% of that of the controls, and the weight gain in the females was 67% of that of the controls. Weight gains of dosed animals were comparable to those of controls at all lower doses. Gross and microscopic pathologic examinations showed no evidence of abnormalities.

Tissue analyses from a male and a female rat fed 640 ppm or 320 ppm coumaphos, respectively, showed 0.7 ppm and 0.5 of the compound, respectively, in fat. A trace of the compound was detected in the liver of the male. Other organs of both sexes showed no detectable level of the compound. Fecal analysis indicated increasing quantities of the compound were excreted with increases in the dose administered.

There was no mortality in the mice at any dose. By the end of the study, the cumulative weight gains in the male and female mice at 640 ppm were 71 to 72% of those of corresponding control animals. Weight gain was also affected at doses as low as 10 ppm in the males and 20 ppm in the females (mean weight gain was 80% of controls in either sex). Gross and histopathologic

examinations of tissues indicated no abnormalities. Analysis of pooled tissues from a female mouse fed 640 ppm of coumaphos showed only a trace amount of the compound at the end of the study.

Previous work (FAO/WHO, 1969) showed that administration of coumaphos to rats at doses of 25 and 100 ppm in 2-year chronic feeding studies shortened the average life spans of the animals by 10% and 25%, respectively. In the same study, doses of 10 ppm and higher produced a dose-related inhibition of erythrocyte and serum cholinesterase. On the basis of both the previous and present findings, 10 and 20 ppm were selected as the low and high doses, respectively, for use in the chronic studies. It was believed that any higher level would increase mortality in rats, and probably in mice.

F. Chronic Studies

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

Table 1. Coumaphos Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals (a)	Coumaphos in Diet (b) (ppm)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Matched-Control	25	0		104
Low-Dose	50	10	103	0-1
High-Dose	50	20	103	0-1
Female				
Matched-Control	25	0		104-105
Low-Dose	50	10	103	0-1
High-Dose	50	20	103	0-1

(a) All animals were 9 weeks of age when placed on study.

(b) Diets were provided ad libitum.

Table 2. Coumaphos Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals (a)	Coumaphos in Diet (b) (ppm)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male				
Matched-Control	25	0		103-104
Low-Dose	50	10	103	0-1
High-Dose	50	20	103	0-1
Female				
Matched-Control	25	0		103-104
Low-Dose	50	10	103	0-1
High-Dose	50	20	103	0-1

(a) All animals were 8 weeks of age when placed on study.

(b) Diets were provided ad libitum.

G. Clinical and Pathologic Examinations

All animals were observed twice per day for signs of toxicity, weighed every 2 weeks, and palpated for masses at each weighing. Animals that were moribund at the time of clinical examination and those that survived to the end of the bioassay were killed using pentobarbitol 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 may have been 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 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 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 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 high-dose group of male rats were slightly lower than those of the corresponding control group throughout the bioassay, while the mean body weights of the low-dose group were not consistently affected (figure 1). The mean body weights of both the low- and high-dose groups of female rats were consistently lower than those of the corresponding control group throughout the bioassay, and the depressions in weight were about the same for both dosed groups.

During the first year of study, the appearance and behavior of the dosed rats were comparable to those of the controls. During the second year, clinical signs such as rough and discolored hair coats, dark urine, tachypnea, pale mucous membranes, vaginal bleeding in the females, and loose stools were more evident in the dosed groups than in the control groups. No clinical signs of central nervous system toxicity were reported. At the termination of the study, poor physical condition was observed in surviving animals in all groups.

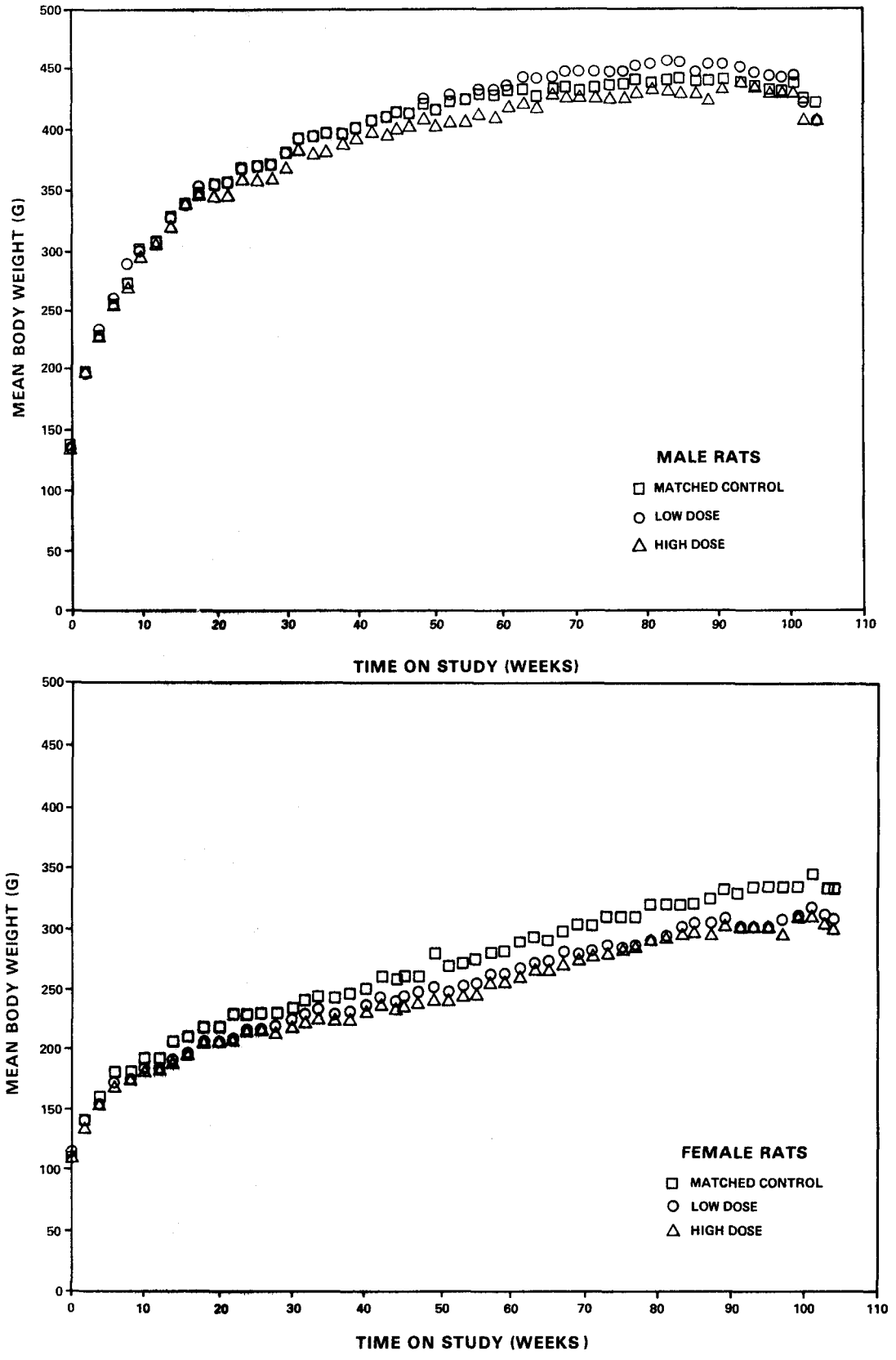


Figure 1. Growth Curves for Rats Administered Coumaphos in the Diet

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered coumaphos in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2. In neither sex were the results of the Tarone test for dose-related trend in mortality significant.

In male rats, 30/50 (60%) of the high-dose group, 36/50 (72%) of the low-dose group, and 17/25 (68%) of the matched controls lived to the end of the bioassay. In females, 35/50 (70%) of the high-dose group, 35/50 (70%) of the low-dose group, and 16/25 (64%) of the matched controls survived to the end of the bioassay. Sufficient numbers of rats of each sex were at risk for the development of late-appearing 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.

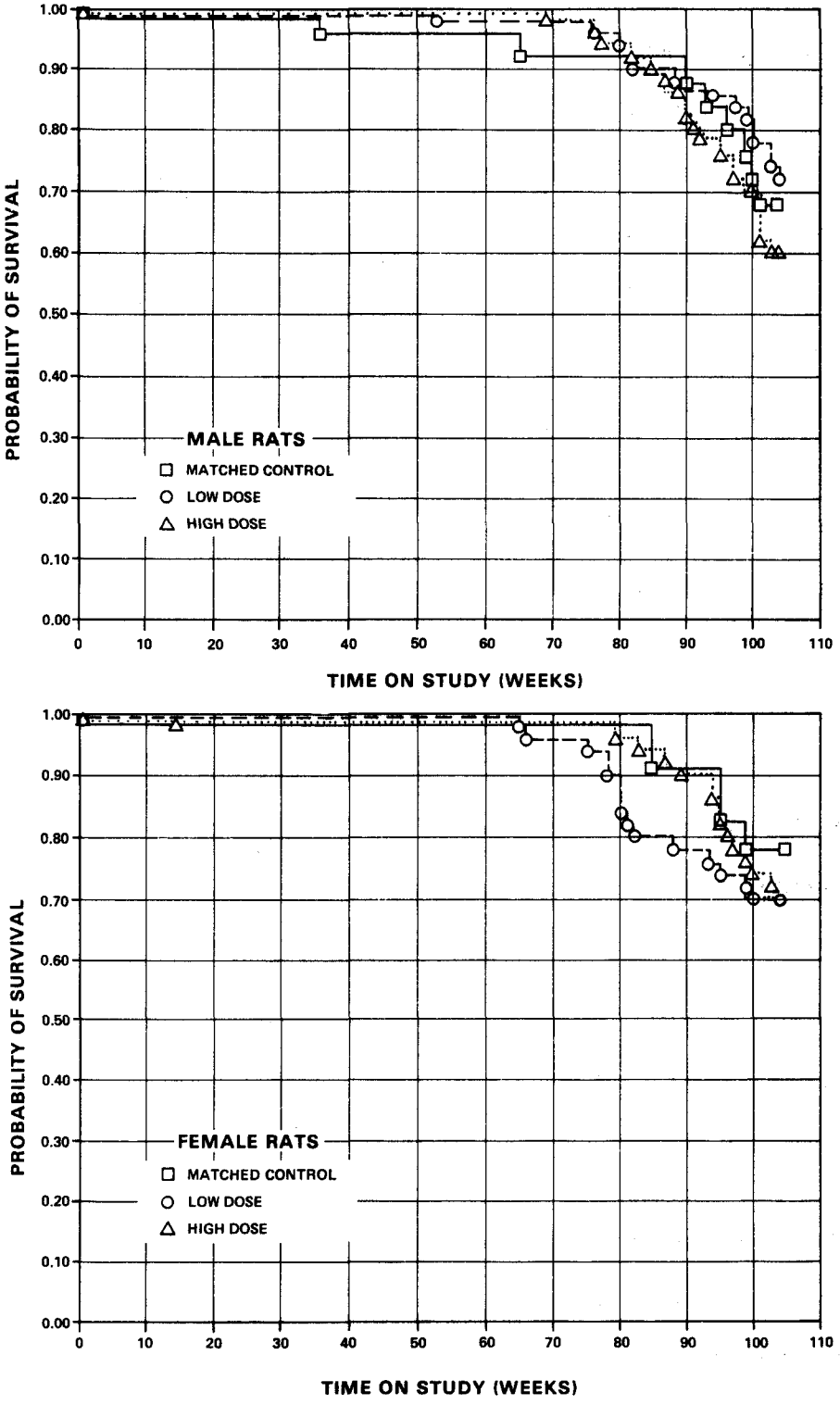


Figure 2. Survival Curves for Rats Administered Coumaphos in the Diet

For the most part, neoplasms occurred with a comparable incidence among dosed and control animals. An exception to this was seen in C-cell adenomas of the thyroid. Although these lesions were seen primarily in dosed rats, there was a greater incidence in the low-dose group than in the high-dose group (for both males and females). However, these neoplasms are often seen in aged F344 rats.

A common variety of nonneoplastic lesions were encountered. For the most part, the numbers of specific lesions were small and similar incidences were observed in control and dosed animals.

The results of the histopathologic examination indicate that coumaphos was not carcinogenic in F344 rats of either sex under the conditions of this bioassay.

D. Statistical Analysis 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 in one group and with an incidence of at least 5% in one or more than one group.

In each sex, the results of the Cochran-Armitage test for dose-associated trend are not significant in any of the incidences of tumors. The Fisher exact comparison of the combined incidence of C-cell adenomas and carcinomas of the thyroid in low-dose male rats with the incidence of those lesions in male matched controls indicates a P value of 0.028, which is above the 0.025 level required for significance when the Bonferroni inequality criterion is used for multiple comparison. There is no other incidence of tumors with results that are significant using the Fisher exact test.

In each of the 95% confidence intervals of relative risk shown in the tables (except for the incidence of C-cell adenomas or carcinomas of the thyroid in low-dose male rats), the value of one is included; this indicates the absence of significant positive 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 coumaphos, which could not be detected under the conditions of this test.

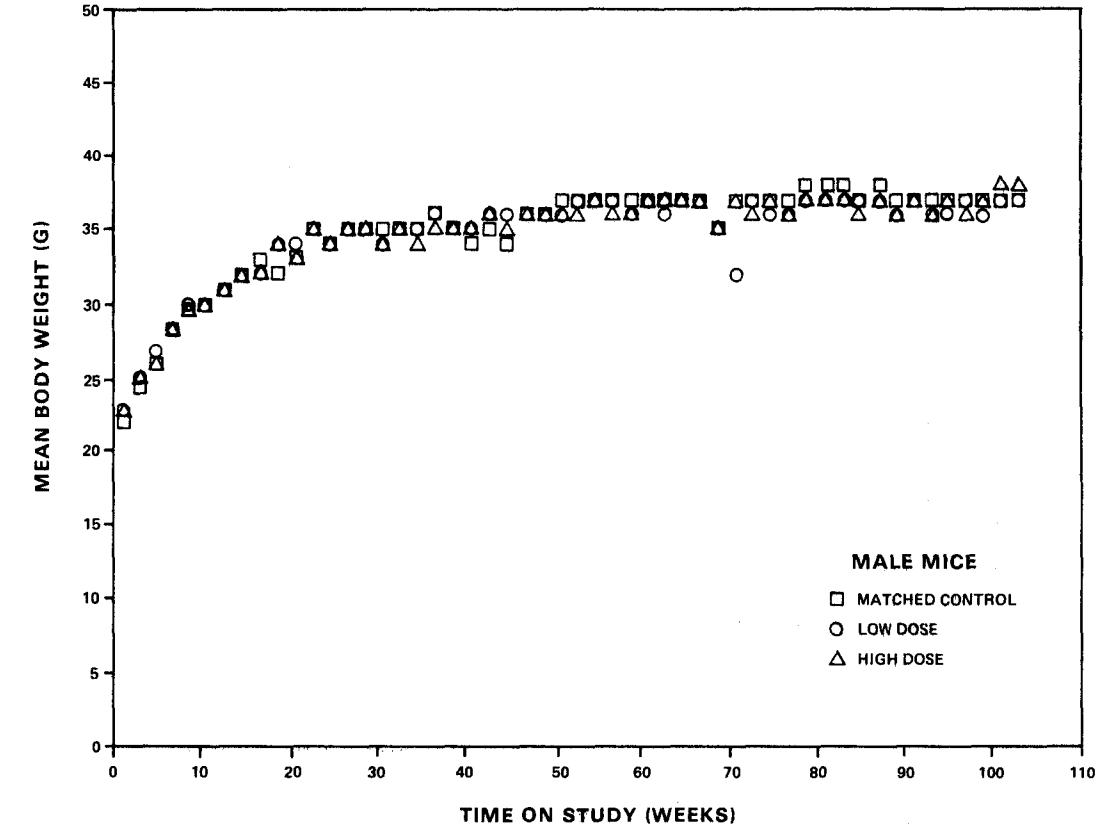
IV. RESULTS-MICE

A. Body Weights and Clinical Signs (Mice)

The mean body weights of the male and female mice were not affected by the administration of coumaphos (figure 3). Clinical signs such as rough and discolored hair coats, alopecia, pale mucous membranes, abdominal distention, and hyperactivity were noted in the second year of the bioassay, but were common to dosed and control groups of both the males and the females. However, several animals in all dosed groups had mucous in their feces, and several animals, primarily in the high-dose group of females, were hunched and lethargic. No clinical signs of toxicity to the respiratory system or central nervous system were reported.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered coumaphos in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 4. In neither sex were the



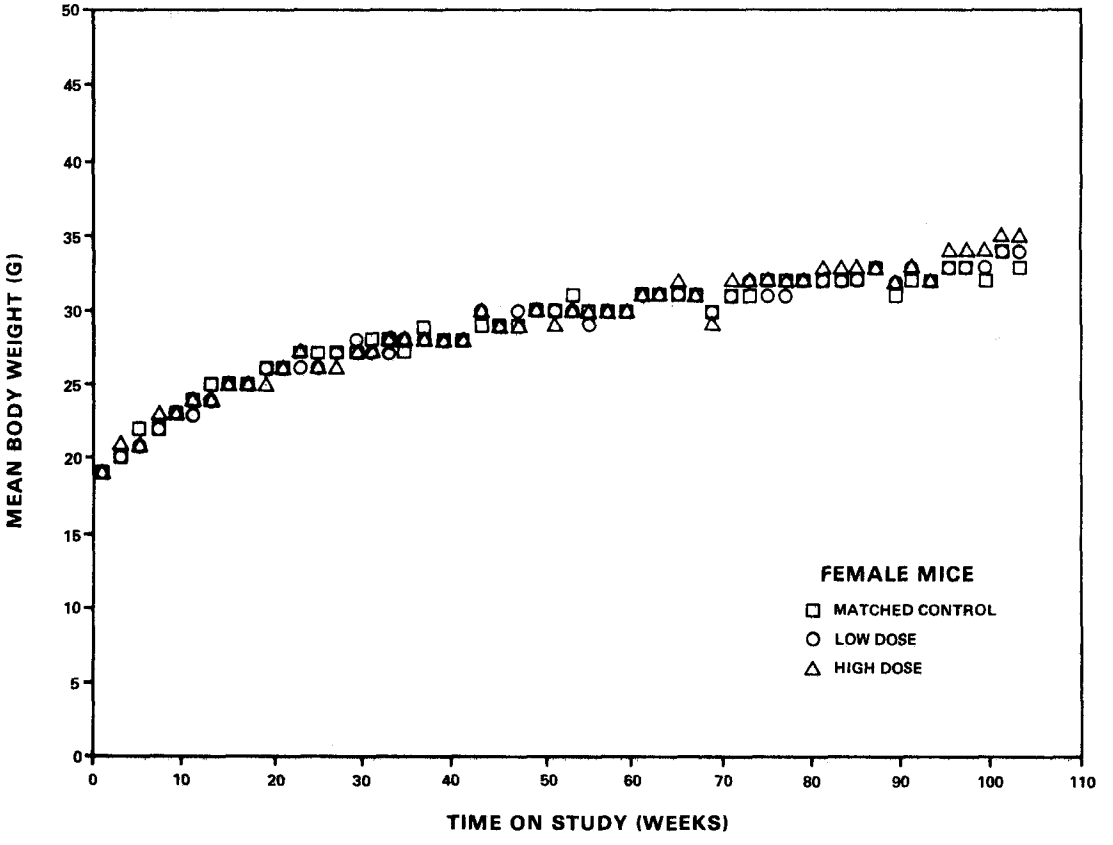


Figure 3. Growth Curves for Mice Administered Coumaphos in the Diet

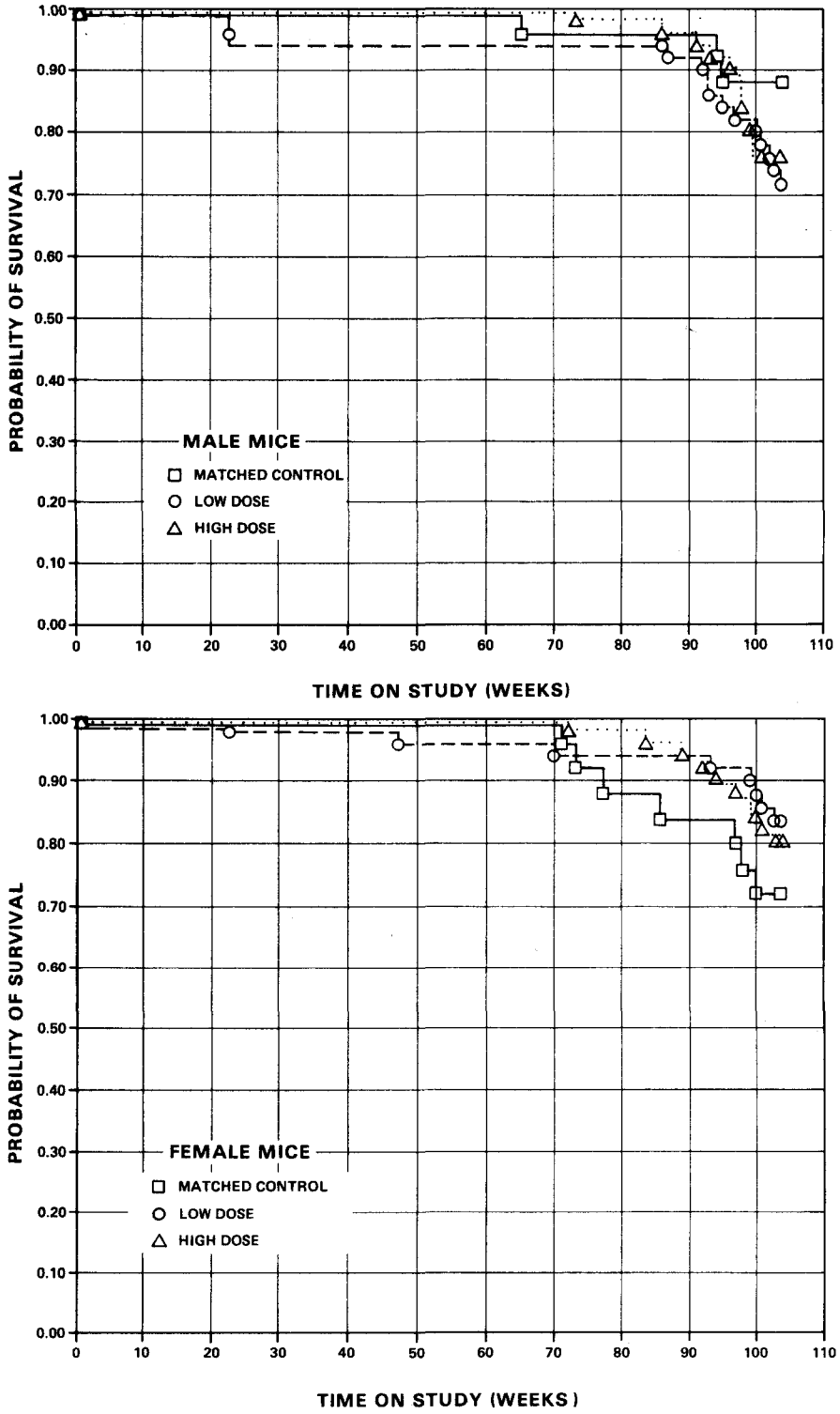


Figure 4. Survival Curves for Mice Administered Coumaphos in the Diet

results of the Tarone test for dose-related trend in mortality significant.

In male mice, 38/50 (76%) of the high-dose group, 36/50 (72%) of the low-dose group, and 22/25 (88%) of the matched controls lived to the end of the bioassay. In females, 40/50 (80%) of the high-dose group, 41/50 (82%) of the low-dose group, and 18/25 (72%) of the controls lived to the end of the bioassay. Sufficient numbers of mice of each sex were at risk for the development of late-appearing 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.

Hepatocellular carcinomas were observed in 4/48 (8%) of the low-dose and 5/50 (10%) of the high-dose female mice, but not in the matched controls. In the males, the incidence of hepatocellular carcinomas in the low-dose group (14/48, or 29%) was similar to that of the control group (7/24, or 29%). The incidence of this tumor, however, was lower in the high-dose male

mice (9/49, or 18%) than in the controls (7/24, or 29%). The majority of other neoplasms in males and females occurred with approximately equal frequency in dosed and control mice.

An occasional nonneoplastic lesion was observed in dosed or control mice; however, these lesions are not considered to be related to the administration of the test compounds.

The results of the histopathologic examination indicate that coumaphos was not carcinogenic in B6C3F1 mice of either sex 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 in one group and with an incidence of at least 5% in one or more than one group.

In each sex, the results of the Cochran-Armitage test for positive dose-related trend and those of the Fisher exact test comparing the incidence in the matched-control group with that in each of the dosed groups in the positive direction are not

significant. Significant results in the negative direction are observed in the incidence of subcutaneous tissue tumors in male mice, where the incidence in the control group exceeds that in the high-dose group.

In summary, there were no statistically significant increases in incidences of any tumors in the dosed groups compared with the controls. In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one is included; this indicates the absence of significant positive 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 coumaphos, which could not be detected under the conditions of this test.

V. DISCUSSION

In rats and mice, the mean body weights of the dosed groups were lower than those of the control group only in the female rats. In the rats, clinical signs such as rough and discolored hair coats, dark urine, tachypnea, pale mucous membranes, vaginal bleeding, and loose stools were observed more often in the dosed groups than in the controls during the second year of the bioassay. In the mice, several animals in all dosed groups had mucous in their feces, and, primarily in the high-dose females, several animals were hunched and lethargic. No signs of central nervous system toxicity that are typical of organophosphorus toxicity were reported. Survival of the rats and mice was not affected by administration of the test chemical. Sufficient numbers of animals in all groups of the rats and mice were at risk for the development of late-appearing tumors. Male rats and male and female mice may have been able to tolerate higher doses, because their mean body weights and survival were only marginally affected and they showed no signs typical of organophosphorus poisoning.

In the male rats, C-cell carcinomas or C-cell adenomas of the thyroid occurred in the low-dose group at an incidence that was

significant (P = 0.028); this value, however, was above P = 0.025, which is required for significance by the Bonferroni criterion. Furthermore, neither the incidence in the high-dose group nor the dose-related trend was significant. The occurrence of these tumors in the male rats cannot, therefore, be clearly related to administration of the coumaphos.

In the female rats and in both the male and female mice, no tumors occurred in dosed groups at incidences that were significantly higher than incidences in corresponding control groups.

The oral LD_{50} of coumaphos has been reported as 41 mg/kg for male Sherman strain rats and 16 mg/kg for the females (Gaines, 1969). It has also been reported as 38.5 mg/kg for albino rats and as 28.0 mg/kg for white mice (Kutakov, 1968). The sex was not specified for these animals. When coumaphos was administered to rats at doses of 25 and 100 ppm in 2-year chronic feeding studies, it shortened the average life spans of the animals by 10 and 25%, respectively; at doses of 10 ppm and higher, a dose-related inhibition of erythrocyte and serum cholinesterase was observed. No pathologic changes in tissues were observed that could be attributed to the test chemical, and no increase in

the incidence of tumors was reported (FAO/WHO, 1969; Lehman, 1965).

Coumaphos contains the moiety coumarin, a carcinogen shown to induce carcinomas of the bile duct in rats (IARC, 1976). Aflatoxins also contain the moiety coumarin and are known to induce tumors of the liver in both rats and man (IARC, 1972).

It is concluded that under the conditions of this bioassay, coumaphos was not carcinogenic for either Fischer 344 rats or B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED COUMAPHOS IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED COUMAPHOS IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	25	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(25)	(50)	(50)
PAPILLOMA, NOS		1 (2%)	
BASAL-CELL TUMOR	1 (4%)		1 (2%)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(25)	(50)	(50)
CARCINOMA, NOS, METASTATIC	1 (4%)		1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
C-CELL CARCINOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(25)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
LEUKEMIA,NOS	1 (4%)	1 (2%)	3 (6%)
GRANULOCYTIC LEUKEMIA	1 (4%)	1 (2%)	
MONOCYTIC LEUKEMIA	4 (16%)	5 (10%)	10 (20%)
#SPLEEN	(24)	(49)	(47)
FIBROMA	1 (4%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(25)	(49)	(49)
FIBROMA		1 (2%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(25)	(50)	(50)
NEOPLASTIC NODULE		1 (2%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(25)	(49)	(49)
TUBULAR-CELL ADENOMA	1 (4%)		
ENDOCRINE SYSTEM			
#PITUITARY	(23)	(48)	(47)
CARCINOMA,NOS		1 (2%)	2 (4%)
ADENOMA, NOS	1 (4%)	1 (2%)	1 (2%)
CHROMOPHOBE ADENOMA	4 (17%)	10 (21%)	9 (19%)
#ADRENAL	(25)	(49)	(50)
CARCINOMA,NOS	1 (4%)		1 (2%)
PHEOCHROMOCYTOMA			3 (6%)
#THYROID	(24)	(46)	(44)
C-CELL ADENOMA		7 (15%)	4 (9%)
C-CELL CARCINOMA		1 (2%)	1 (2%)
#PANCREATIC ISLETS	(23)	(47)	(49)
ISLET-CELL ADENOMA	1 (4%)	7 (15%)	5 (10%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(25)	(50)	(50)
FIBROMA		1 (2%)	
#TESTIS	(25)	(50)	(50)
INTERSTITIAL-CELL TUMOR	20 (80%)	43 (86%)	43 (86%)
NERVOUS SYSTEM			
#BRAIN	(25)	(50)	(50)
EPENDYMOMA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(25)	(50)	(50)
PARAGANGLIOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	25	50	50
NATURAL DEATH@	2	2	4
MORIBUND SACRIFICE	6	12	16
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	36	30
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	24	48	49
TOTAL PRIMARY TUMORS	36	84	86
TOTAL ANIMALS WITH BENIGN TUMORS	23	46	48
TOTAL BENIGN TUMORS	29	71	66
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	12	16
TOTAL MALIGNANT TUMORS	7	12	18
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		2
TOTAL SECONDARY TUMORS	1		2
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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED COUMAPHOS IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	23	48	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	23	48	50
INTEGUMENTARY SYSTEM			
*SKIN	(23)	(48)	(50)
FIBROMA	1 (4%)		
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(23)	(48)	(50)
LEUKEMIA,NOS			1 (2%)
MONOCYTIC LEUKEMIA	2 (9%)	7 (15%)	10 (20%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(23)	(47)	(50)
NEOPLASTIC NODULE		2 (4%)	2 (4%)
HEPATOCELLULAR CARCINOMA			1 (2%)
HEMANGIOMA			1 (2%)
URINARY SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(23)	(45)	(50)
CARCINOMA,NOS			1 (2%)
ADENOMA, NOS		5 (11%)	
CHROMOPHOBE ADENOMA	11 (48%)	18 (40%)	20 (40%)
#ADRENAL	(23)	(46)	(50)
CORTICAL ADENOMA			1 (2%)
PHEOCHROMOCYTOMA			2 (4%)
#THYROID	(19)	(46)	(45)
FOLLICULAR-CELL ADENOMA		1 (2%)	1 (2%)
C-CELL ADENOMA	1 (5%)	5 (11%)	2 (4%)
#PANCREATIC ISLETS	(22)	(47)	(47)
ISLET-CELL ADENOMA		2 (4%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(23)	(48)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
FIBROMA			1 (2%)
FIBROADENOMA	1 (4%)	3 (6%)	4 (8%)
#UTERUS	(22)	(45)	(47)
LEIOMYOMA		1 (2%)	
ENDOMETRIAL STROMAL POLYP	5 (23%)	10 (22%)	10 (21%)
HEMANGIOMA			1 (2%)
#OVARY	(23)	(47)	(50)
GRANULOSA-CELL TUMOR		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EAR CANAL	(23)	(48)	(50)
CARCINOMA,NOS		2 (4%)	
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(23)	(48)	(50)
FIBROSARCOMA	1 (4%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	25	50	50
NATURAL DEATH@	1	6	1
MORIBUND SACRIFICE	4	9	14
SCHEDULED SACRIFICE**	2		
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	35	35
ANIMAL MISSING			
ANIMAL DELETED (WRONG SEX)	2		
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	17	41	38
TOTAL PRIMARY TUMORS	22	58	58
TOTAL ANIMALS WITH BENIGN TUMORS	15	32	31
TOTAL BENIGN TUMORS	19	45	43
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	10	13
TOTAL MALIGNANT TUMORS	3	10	13
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		2	2
TOTAL UNCERTAIN TUMORS		3	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

** Animals are in fact early terminal sacrifices, but appear as scheduled sacrifices due to system interpretation.

\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED COUMAPHOS IN THE DIET

TABLE B1.
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED COUMAPHOS IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	25	49	49
INTEGUMENTARY SYSTEM			
*SKIN	(25)	(49)	(49)
FIBROMA			1 (2%)
*SUBCUT TISSUE	(25)	(49)	(49)
SARCOMA, NOS	4 (16%)	7 (14%)	1 (2%)
FIBROSARCOMA		2 (4%)	
RESPIRATORY SYSTEM			
#LUNG	(25)	(47)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	4 (16%)	4 (9%)	4 (8%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(25)	(49)	(49)
MALIGNANT LYMPHOMA, NOS	2 (8%)	4 (8%)	2 (4%)
#MESENTERIC L. NODE	(13)	(41)	(42)
MALIGNANT LYMPHOMA, NOS			2 (5%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(24)	(48)	(49)
HEPATOCELLULAR CARCINOMA	7 (29%)	14 (29%)	9 (18%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMANGIOMA			2 (4%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(20)	(42)	(45)
FOLLICULAR-CELL ADENOMA			1 (2%)
FOLLICULAR-CELL CARCINOMA	1 (5%)		
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(25)	(49)	(49)
ADENOMA, NOS	1 (4%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(25)	(49)	(49)
LIPOMA		1 (2%)	
ALL OTHER SYSTEMS			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	25	50	50
NATURAL DEATH@	1	4	2
MORIBUND SACRIFICE	2	10	10
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	22	36	38
ANIMAL MISSING			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	15	26	22
TOTAL PRIMARY TUMORS	19	32	24
TOTAL ANIMALS WITH BENIGN TUMORS	5	5	8
TOTAL BENIGN TUMORS	5	5	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	12	23	16
TOTAL MALIGNANT TUMORS	14	27	16
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
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 B2.
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED COUMAPHOS IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	25	49	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(25)	(48)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		4 (8%)	4 (8%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (4%)		
OSTEOSARCOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(25)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	3 (12%)	5 (10%)	8 (16%)
#SPLEEN	(24)	(48)	(49)
FIBROUS HISTIOCYTOMA			1 (2%)
HEMANGIOMA		1 (2%)	
#MESENTERIC L. NODE	(20)	(40)	(45)
MALIGNANT LYMPHOMA, NOS		1 (3%)	1 (2%)
#LIVER	(25)	(48)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(24)	(47)	(48)
ADENOMA, NOS		1 (2%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(25)	(48)	(50)
HEPATOCELLULAR CARCINOMA		4 (8%)	5 (10%)
#STOMACH	(24)	(46)	(49)
SQUAMOUS CELL PAPILLOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(41)	(36)
ADENOMA, NOS	3 (19%)	2 (5%)	2 (6%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(25)	(49)	(50)
CARCINOMA,NOS			1 (2%)
ADENOCARCINOMA, NOS		1 (2%)	1 (2%)
#UTERUS	(20)	(48)	(28)
ADENOCARCINOMA, NOS			1 (4%)
SARCOMA, NOS	1 (5%)		
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
ENDOMETRIAL STROMAL POLYP	1 (5%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(25)	(49)	(50)
ADENOMA, NOS			1 (2%)
*HARDERIAN GLAND	(25)	(49)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
MUSCULOSKELETAL 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
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	25	50	50
NATURAL DEATH@	1	2	2
MORIBUND SACRIFICE	6	6	8
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	18	41	40
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	20	24
TOTAL PRIMARY TUMORS	9	22	27
TOTAL ANIMALS WITH BENIGN TUMORS	4	8	9
TOTAL BENIGN TUMORS	4	8	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	13	18
TOTAL MALIGNANT TUMORS	5	14	18
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
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 ADMINISTERED COUMAPHOS IN THE DIET

TABLE C1.
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED COUMAPHOS IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	25	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(25)	(50)	(50)
CYST, NOS	1 (4%)		
EPIDERMAL INCLUSION CYST		2 (4%)	3 (6%)
*SUBCUT TISSUE	(25)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (4%)		
RESPIRATORY SYSTEM			
#LUNG	(25)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
BRONCHOPNEUMONIA, ACUTE		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(24)	(49)	(47)
FIBROSIS, FOCAL		1 (2%)	
#LYMPH NODE	(24)	(38)	(40)
CYST, NOS		1 (3%)	
#MANDIBULAR L. NODE	(24)	(38)	(40)
HYPERPLASIA, NOS			1 (3%)
#MESENTERIC L. NODE	(24)	(38)	(40)
INFLAMMATION, ACUTE/CHRONIC		1 (3%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(25)	(50)	(50)
FIBROSIS, FOCAL		1 (2%)	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
FIBROSIS, MULTIFOCAL		1 (2%)	
DEGENERATION, NOS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(25)	(50)	(50)
DEGENERATION, HYDROPIC			1 (2%)
METAMORPHOSIS FATTY	1 (4%)	2 (4%)	
FOCAL CELLULAR CHANGE		1 (2%)	1 (2%)
HEPATOCYTOMEGALY	1 (4%)		
*BILE DUCT	(25)	(50)	(50)
INFLAMMATION, CHRONIC	3 (12%)	3 (6%)	3 (6%)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, DIFFUSE	1 (4%)		
#PANCREAS	(23)	(47)	(49)
PERIARTERITIS		1 (2%)	
ATROPHY, NOS		1 (2%)	
#PANCREATIC ACINUS	(23)	(47)	(49)
ATROPHY, NOS		1 (2%)	
#STOMACH	(23)	(48)	(47)
ULCER, ACUTE		1 (2%)	1 (2%)
ULCER, CHRONIC	1 (4%)	1 (2%)	1 (2%)
#SMALL INTESTINE	(21)	(49)	(49)
INFLAMMATION, ACUTE NECROTIZING		1 (2%)	
#ILEUM	(21)	(49)	(49)
NECROSIS, FOCAL		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(25)	(49)	(49)
INFLAMMATION, CHRONIC	17 (68%)	37 (76%)	40 (82%)
INFARCT, NOS	1 (4%)		
HYPERPLASIA, FOCAL			1 (2%)
#KIDNEY/CORTEX	(25)	(49)	(49)
INFARCT, NOS			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
ENDOCRINE SYSTEM			
#PITUITARY	(23)	(48)	(47)
CYST, NOS		2 (4%)	1 (2%)
HEMORRHAGE	2 (9%)	2 (4%)	2 (4%)
HYPERPLASIA, FOCAL	1 (4%)	2 (4%)	4 (9%)
#PARATHYROID	(19)	(34)	(34)
HYPERPLASIA, DIFFUSE			1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(25)	(50)	(50)
DYSPLASIA, NOS	1 (4%)	1 (2%)	2 (4%)
*MAMMARY LOBULE	(25)	(50)	(50)
HYPERPLASIA, NOS	1 (4%)	1 (2%)	
#TESTIS	(25)	(50)	(50)
ATROPHY, NOS		1 (2%)	2 (4%)
*EPIDIDYMIS	(25)	(50)	(50)
NECROSIS, FAT		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EAR	(25)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(25)	(50)	(50)
NECROSIS, FAT			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
*MESENTERY	(25)	(50)	(50)
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

TABLE C2.
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED COUMAPHOS IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	23	48	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	23	48	50
INTEGUMENTARY SYSTEM			
*SKIN	(23)	(48)	(50)
EPIDERMAL INCLUSION CYST	1 (4%)		
RESPIRATORY SYSTEM			
#LUNG	(22)	(47)	(50)
GRANULOMA, FOREIGN BODY		1 (2%)	
HEMATOPOIETIC SYSTEM			
#MESENTERIC L. NODE	(19)	(42)	(44)
CYST, NOS		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(23)	(48)	(50)
CALCIFICATION, NOS			1 (2%)
#MYOCARDIUM	(23)	(48)	(50)
FIBROSIS, FOCAL			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(23)	(47)	(50)
INFLAMMATION, GRANULOMATOUS			1 (2%)
METAMORPHOSIS FATTY		1 (2%)	3 (6%)
FOCAL CELLULAR CHANGE	1 (4%)	1 (2%)	3 (6%)
#PANCREAS	(22)	(47)	(47)
FIBROSIS		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
ATROPHY, NOS		1 (2%)	
#STOMACH	(22)	(47)	(50)
ULCER, NOS			1 (2%)
ULCER, ACUTE		1 (2%)	
ULCER, CHRONIC		1 (2%)	
CALCIFICATION, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(23)	(47)	(50)
INFLAMMATION, CHRONIC	7 (30%)	17 (36%)	15 (30%)
CALCIFICATION, NOS			1 (2%)
ATROPHY, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(23)	(45)	(50)
CYST, NOS	1 (4%)	4 (9%)	5 (10%)
CONGESTION, NOS			1 (2%)
HEMORRHAGE	2 (9%)	4 (9%)	3 (6%)
HYPERPLASIA, NOS	1 (4%)	1 (2%)	4 (8%)
HYPERPLASIA, FOCAL	2 (9%)	3 (7%)	6 (12%)
#ADRENAL	(23)	(46)	(50)
HEMORRHAGE			1 (2%)
#ADRENAL CORTEX	(23)	(46)	(50)
METAMORPHOSIS FATTY			1 (2%)
#ADRENAL MEDULLA	(23)	(46)	(50)
HYPERPLASIA, NODULAR			1 (2%)
#THYROID	(19)	(46)	(45)
HYPERPLASIA, C-CELL	2 (11%)	1 (2%)	2 (4%)
#PARATHYROID	(17)	(35)	(35)
HYPERPLASIA, NOS			1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(23)	(48)	(50)
DYSPLASIA, NOS	5 (22%)	6 (13%)	5 (10%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ADENOSIS			1 (2%)
*MAMMARY LOBULE	(23)	(48)	(50)
HYPERPLASIA, NOS	2 (9%)		7 (14%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EAR CANAL	(23)	(48)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(23)	(48)	(50)
PERIARTERITIS		1 (2%)	
ALL OTHER SYSTEMS			
OMENTUM			
LIPOGRANULOMA			1
NECROSIS, FAT			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	2	2
AUTOLYSIS/NO NECROPSY		2	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED COUMAPHOS IN THE DIET

TABLE D1.
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED COUMAPHOS IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	25	49	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(25)	(47)	(49)
ATELECTASIS		1 (2%)	
BRONCHOPNEUMONIA, NOS			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(25)	(48)	(47)
HEMOSIDEROSIS	1 (4%)		
HYPERPLASIA, LYMPHOID			1 (2%)
#MESENTERIC L. NODE	(13)	(41)	(42)
CONGESTION, NOS			2 (5%)
INFLAMMATION, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(24)	(48)	(49)
INFLAMMATION, MULTIFOCAL		1 (2%)	
NECROSIS, NOS	1 (4%)	1 (2%)	
#LIVER/CENTRILOBULAR	(24)	(48)	(49)
NECROSIS, NOS	1 (4%)		

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#URINARY BLADDER	(21)	(46)	(46)
EDEMA, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#THYROID	(20)	(42)	(45)
CYSTIC FOLLICLES		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(25)	(49)	(49)
EDEMA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	9	20	26

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
AUTOLYSIS/NO NECROPSY		1	1

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

TABLE D2.
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED COUMAPHOS IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	25	50	50
ANIMALS NECROPSIED	25	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	25	49	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(25)	(48)	(50)
INFLAMMATION, CHRONIC		2 (4%)	
#LUNG	(25)	(48)	(50)
ATELECTASIS	1 (4%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(24)	(48)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(25)	(48)	(50)
INFLAMMATION, MULTIFOCAL		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, NODULAR		1 (2%)	
#PANCREAS	(23)	(46)	(50)
ATROPHY, NOS	1 (4%)		
#SMALL INTESTINE	(23)	(45)	(47)
HYPERPLASIA, LYMPHOID			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
URINARY SYSTEM			
#KIDNEY	(25)	(49)	(50)
HYDRONEPHROSIS	1 (4%)		1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(41)	(36)
ANGIECTASIS	1 (6%)		
#ADRENAL CORTEX	(22)	(44)	(46)
HYPERPLASIA, NOS	1 (5%)		
#THYROID	(23)	(41)	(41)
CYST, NOS			1 (2%)
CYSTIC FOLLICLES	1 (4%)		1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS/ENDOMETRIUM	(20)	(48)	(28)
HYPERPLASIA, CYSTIC			1 (4%)
#OVARY	(24)	(45)	(48)
CYST, NOS	1 (4%)	1 (2%)	1 (2%)
FOLLICULAR CYST, NOS		1 (2%)	
INFLAMMATION, NOS		1 (2%)	
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			
*PERITONEUM	(25)	(49)	(50)
INFLAMMATION, CHRONIC	1 (4%)	1 (2%)	
INFLAMMATION, GRANULOMATOUS			1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	12	24	21
AUTOLYSIS/NO NECROPSY		1	

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED COUMAPHOS IN THE DIET

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Leukemia (b)	6/25 (24)	7/50 (14)	13/50 (26)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.583	1.083
Lower Limit		0.192	0.448
Upper Limit		1.909	3.121
Weeks to First Observed Tumor	36	99	82
Hematopoietic System: Leukemia or Lymphoma (b)	6/25 (24)	8/50 (16)	13/50 (26)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.667	1.083
Lower Limit		0.233	0.448
Upper Limit		2.114	3.121
Weeks to First Observed Tumor	36	99	82

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma (b)	4/23 (17)	10/48 (21)	9/47 (19)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.198	1.101
Lower Limit		0.398	0.353
Upper Limit		4.798	4.489
Weeks to First Observed Tumor	96	94	76
Pituitary: Adenoma, NOS (not otherwise specified), Carcinoma, NOS, or Chromophobe Adenoma (b)	5/23 (22)	12/48 (25)	12/47 (26)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.150	1.174
Lower Limit		0.441	0.450
Upper Limit		3.760	3.835
Weeks to First Observed Tumor	65	82	85

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Pheochromocytoma (b)	0/25 (0)	0/49 (0)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		--	Infinite
Lower Limit		--	0.309
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	97
Thyroid: C-cell Adenoma or Carcinoma (b)	0/24 (0)	8/46 (17)	5/44 (11)
P Values (c,d)	N.S.	P = 0.028	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		1.229	0.709
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	88	92

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pancreatic Islets: Islet-cell Adenoma (b)	1/23 (4)	7/47 (15)	5/49 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		3.426	2.347
Lower Limit		0.487	0.289
Upper Limit		150.675	108.596
Weeks to First Observed Tumor	104	100	69
Testis: Interstitial-cell Tumor (b)	20/25 (80)	43/50 (86)	43/50 (86)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.075	1.075
Lower Limit		0.867	0.867
Upper Limit		1.393	1.393
Weeks to First Observed Tumor	90	76	69

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

(continued)

(a) Dosed groups received 10 or 20 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is 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 when P is less than 0.05; otherwise not significant (N.S.) is indicated.

(d) A negative (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 is less than 0.05 for any comparison.

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Leukemia (b)	2/23 (9)	7/48 (15)	11/50 (22)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.677	2.530
Lower Limit		0.358	0.624
Upper Limit		15.752	22.312
Weeks to First Observed Tumor	99	93	14
Liver: Neoplastic Nodule or Hepatocellular Carcinoma (b)	0/23 (0)	2/47 (4)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.149	0.285
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	104	103

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma (b)	1/19 (5)	5/46 (11)	2/45 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.065	0.844
Lower Limit		0.259	0.048
Upper Limit		95.429	48.728
Weeks to First Observed Tumor	105	104	104
Mammary Gland: Fibroadenoma (b)	1/23 (4)	3/48 (6)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.438	1.840
Lower Limit		0.125	0.199
Upper Limit		73.860	88.746
Weeks to First Observed Tumor	105	103	87

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma (b)	11/23 (48)	18/45 (40)	20/50 (40)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.836	0.836
Lower Limit		0.472	0.481
Upper Limit		1.645	1.632
Weeks to First Observed Tumor	85	65	89
Pituitary: Adenoma, NOS, Carcinoma, NOS, or Chromophobe Adenoma (b)	11/23 (48)	23/45 (51)	21/50 (42)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.069	0.878
Lower Limit		0.634	0.510
Upper Limit		2.003	1.698
Weeks to First Observed Tumor	85	65	89

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp (b)	5/22 (23)	10/45 (22)	10/47 (21)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.978	0.936
Lower Limit		0.357	0.342
Upper Limit		3.282	3.151
Weeks to First Observed Tumor	104	78	83

(a) Dosed groups received 10 or 20 ppm.

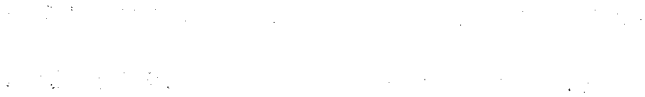
(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is 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 when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) A negative (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 is less than 0.05 for any comparison.

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



APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED COUMAPHOS IN THE DIET

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Sarcoma, NOS, or Fibrosarcoma of the Subcutaneous Tissue (b)	4/25 (16)	9/49 (18)	1/49 (2)
P Values (c,d)	P = 0.024(N)	N.S.	P = 0.042(N)
Relative Risk (f)		1.148	0.128
Lower Limit		0.364	0.003
Upper Limit		4.705	1.213
Weeks to First Observed Tumor	65	87	101
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	4/25 (16)	4/47 (9)	6/49 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.532	0.765
Lower Limit		0.110	0.204
Upper Limit		2.653	3.418
Weeks to First Observed Tumor	103	87	101

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma (b)	2/25 (8)	4/49 (8)	4/49 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.020	1.020
Lower Limit		0.160	0.160
Upper Limit		10.792	10.792
Weeks to First Observed Tumor	94	93	86
Liver: Hepatocellular Carcinoma (b)	7/24 (29)	14/48 (29)	9/49 (18)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.000	0.630
Lower Limit		0.449	0.245
Upper Limit		2.585	1.779
Weeks to First Observed Tumor	103	100	91

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

(continued)

(a) Dosed groups received 10 or 20 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is 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 when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) A negative (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 is less than 0.05 for any comparison.

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveoloar/Bronchioloar Adenoma or Carcinoma (b)	1/25 (4)	4/48 (8)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.083	2.000
Lower Limit		0.224	0.215
Upper Limit		100.372	96.452
Weeks to First Observed Tumor	104	104	103
Hematopoietic System: Lymphoma (b)	3/25 (12)	7/49 (14)	9/50 (18)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.190	1.500
Lower Limit		0.304	0.422
Upper Limit		6.690	8.064
Weeks to First Observed Tumor	73	70	72

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	0/25 (0)	4/48 (8)	5/50 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.496	0.648
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	103	100
Pituitary: Adenoma, NOS (b)	3/16 (19)	2/41 (5)	2/36 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.260	0.296
Lower Limit		0.024	0.028
Upper Limit		2.105	2.383
Weeks to First Observed Tumor	104	104	97

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

(continued)

(a) Dosed groups received 10 or 20 ppm.

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

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is 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 when P is less than 0.05; otherwise not significant (N.S.) is indicated.

(d) A negative (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 is less than 0.05 for any comparison.

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

APPENDIX G

ANALYSIS OF FORMULATED DIETS FOR CONCENTRATIONS OF COUMAPHOS

APPENDIX G

Analysis of Formulated Diets for

Concentrations of Coumaphos

Ten-gram dosed feed samples were extracted in 250 ml benzene and mechanically agitated for 3 hours. Aliquots of the supernatant were diluted to appropriate concentrations and analyzed by gas chromatography using a flame photometric detector in the phosphorus mode. Spiked samples were worked up simultaneously with the dosed feed samples, and used to correct the recoveries from the dosed feed samples for losses due to the method.

Theoretical Concentrations (ppm)	No. of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
10	11	9.8	5.55	9.0-10.9
20	11	20.2	4.89	18.6-21.6

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

October 25, 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, and State health officials. 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 Coumaphos for carcinogenicity.

The primary reviewer for the report on the bioassay of Coumaphos said that, under the conditions of test, Coumaphos was not carcinogenic in treated rats or mice. After commenting on the experimental design, he noted that the study appeared to have been adequately conducted. He did question, however, whether maximum tolerated doses were tested and he pointed out the increased incidence of C-cell adenomas of the thyroid among low dose treated male rats. The primary reviewer concluded that, based on the results of the bioassay, Coumaphos would not appear to pose a carcinogenic risk to humans.

The secondary reviewer agreed that the compound was not carcinogenic in rats or mice, under the conditions of test. He opined that there was no carcinogenic risk for man in so far as it was possible to extrapolate from the bioassay.

There was no objection to a recommendation that the report on the bioassay of Coumaphos be accepted as written.

Clearinghouse Members present:

Arnold L. Brown (Chairman) University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center

Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
(Michael B. Shimkin, University of California at San Diego, submitted a written review)
Kenneth Wilcox, Michigan State Health Department

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

☆U.S. GOVERNMENT PRINTING OFFICE: 1978-281-217/3301