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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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REPORT ON THE BIOASSAY OF DICOFOL
FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of dicofol 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 chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because 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 a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of dicofol was conducted by Hazleton Laboratories America, Inc., Vienna, Virginia, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. M. B. Powers (3), Dr. R. W. Voelker (3), Dr. W. A. Olson (3,4) and Dr. W. M. Weatherholtz (3). Chemical analysis was performed by Dr. C. L. Guyton (3, 5) and the analytical results were reviewed by Dr. N. Zimmerman (6); the technical supervisor of animal treatment and observation was Ms. K. J. Petrovics (3).

Histopathologic examinations were performed by Dr. R. H. Habermann (3) and reviewed by Dr. R. W. Voelker (3) at the Hazleton Laboratories America, Inc., and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (7).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (8); the statistical analysis was performed by Mr. W. W. Belew (6) and Dr. J. R. Joiner (7), using methods selected for the Carcinogenesis Bioassay Program by Dr. J. J. Gart (9).

This report was prepared at METREK, a Division of The MITRE Corporation (6) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (6), task leader Dr. M. R. Kornreich (6), senior biologist Ms. P. Walker (6), biochemist Mr. S. C. Drill (6), and technical editor Ms. P. A. Miller (6). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1), Dr. R. A. Griesemer (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,10), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay of technical-grade dicofol for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F1 mice. Dicofol was administered in the feed, at either of two concentrations, to groups of 50 males and 50 females of each species. The high and low time-weighted average concentrations of dicofol were, respectively, 942 and 471 ppm for male rats, 760 and 380 ppm for female rats, 528 and 264 ppm for male mice, and 243 and 122 ppm for female mice. For each species, 20 animals of each sex were placed on test as controls. The period of compound administration was 78 weeks, followed by 34 weeks of observation in rats and 14 or 15 weeks in mice.

There was no statistically significant positive association between dietary concentration and mortality in either sex or species.

Hepatocellular carcinomas in dosed male mice were the only neoplasms that occurred in any dosed group of either species in statistically significant increased incidences when compared to controls. The Cochran-Armitage test as well as the Fisher exact test for both the high and low dose groups supported the association between compound administration and increased incidences of this tumor in the male mice. No increase in hepatocellular carcinomas was observed in dosed female mice.

Under the conditions of this bioassay, technical-grade dicofol was carcinogenic in male B6C3F1 mice, causing hepatocellular carcinomas. No evidence for carcinogenicity was obtained for this compound in Osborne-Mendel rats of either sex or in female B6C3F1 mice.

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I. INTRODUCTION

Dicofol (NCI No. C00486), a synthetic organochlorine acaricide, was selected for bioassay by the National Cancer Institute because it is an alcohol analog of the known tumorigen DDT (Innes et al., 1969). Its widespread use on edible crops was also an important factor in its selection for testing.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 4-chloro-alpha-(4-chlorophenyl)-alpha-(trichloromethyl)benzenemethanol.* It is also called 1,1-bis (p-chlorophenyl)-2,2,2-trichloroethanol; 4,4'-dichloro-alpha-(trichloromethyl)benzhydrol; and 2,2,2-trichloro-1,1-di-(4-chlorophenyl) ethanol.

Dicofol is a nonsystemic acaricide that is used to control mites on cotton, corn, and other field crops; vegetables; citrus and non-citrus fruits; and nursery and greenhouse crops (Martin and Worthing, 1977). In 1971, 447 thousand pounds of dicofol were used to treat 474 thousand acres of crops in the United States. Cotton was the major single crop treated, accounting for 189 thousand pounds or 42 percent of total dicofol usage (Andrilenas, 1974). The vast majority of dicofol usage (409 thousand pounds on 428,000 acres) took place in the Pacific States (California, Oregon, and Washington).

*The CAS registry number is 115-32-2.

Specific production statistics for dicofol are not available; however, the inclusion of the compound in Synthetic Organic Chemicals, U.S. Production and Sales, 1975 (U.S. International Trade Commission, 1977) implies an annual commercial production in excess of 1000 pounds or $1000 in value. Approximately 4 million pounds of dicofol were produced in 1971 (Ouellette and King, 1977). No imports of the pesticide were reported in the period 1970-1974 inclusive (U.S. Department of Agriculture, 1975).

Agricultural workers have the greatest potential for exposure to dicofol, although the production and storage of the compound may also present a significant risk to workers in the pesticide manufacturing industry. The general population may be exposed to dicofol in house and garden pesticides for evergreens, shrubs, and flower and vegetable gardens (Gosselin et al., 1975); to airborne dicofol after commercial agricultural spraying; to residues in rivers and streams as a result of industrial discharge; and to dicofol residues in crops and soils. Dicofol has been found in concentrations of up to 0.066 ppm in "ready-to-eat" fruits (Manske and Corneliussen, 1974). Residues in soil decrease rapidly, but traces may persist for a year or longer (Martin and Worthing, 1977). Industrial wastewater in the Soviet Union has been found to contain as much as 0.397 mg/l of dicofol (Diatlovitskaia and Botvinova, 1971).

The effects of dicofol poisoning presumably resemble those of DDT, although the latter is somewhat more toxic. The primary sites

of action appear to be the cerebellum and higher motor cortex (Gosselin et al., 1976).

Leukopenia, neutropenia, and a decrease in the hemoglobin level were found in humans working with dicofol-chlorophos-copper oxychloride mixtures 8 to 10 hours daily for several months (Stuneeva, 1973).

Although it is stored to a certain extent throughout the body, dicofol, like DDT, appears to be preferentially stored in fat; however, some dicofol is apparently converted into DDE, a DDT metabolite, in rats (Brown, 1972).

Workers using a number of pesticides, including dicofol, were found to have an abnormally large amount of lymphocyte chromosomal damage (Yoder et al., 1973); however, the compound showed no mutagenic activity in Escherichia coli, failing to induce reversions to prototype in a tryptophan-dependent mutant (WP2 Try-) (Ashwood-Smith et al., 1972).

No indications of teratogenicity were found in mice fed dicofol in their diet in amounts of up to 500 ppm over five generations (Brown, 1972).

II. MATERIALS AND METHODS

A. Chemicals

Technical-grade dicofol (Figure 1) [1,1-bis(p-chlorophenyl)-2,2,2-trichloroethanol] was purchased from Rohm and Haas Chemical Company and chemical analysis was performed by Hazleton Laboratories America, Inc., Vienna, Virginia. The wide range observed for the melting point (45° to 60°C) and the difference from the literature value (77° to 78°C) suggested a compound of low purity. Although the effectiveness of gas-liquid chromatography is limited due to thermal decomposition of the compound, analysis using this technique suggested a purity between 40 and 60 percent. Analyses performed twelve months later revealed similar results. Within the next twelve-month period a significant amount of the stored material liquified, suggestive of substantial decomposition.

Throughout this report the term dicofol is used to represent this technical-grade material.

B. Dietary Preparation

The basal laboratory diet for both treated and control animals consisted of 2 percent Duke's® corn oil (S. F. Sauer Company, Richmond, Virginia) by weight added to Wayne Lab-Blox® meal (Allied Mills, Inc., Chicago, Illinois). Fresh mixtures of dicofol in corn oil were prepared each week and stored in the dark. The mixtures of dicofol in corn oil were incorporated into the appropriate amount of basal laboratory diet in a twin-shell blender fitted with an accelerator bar.

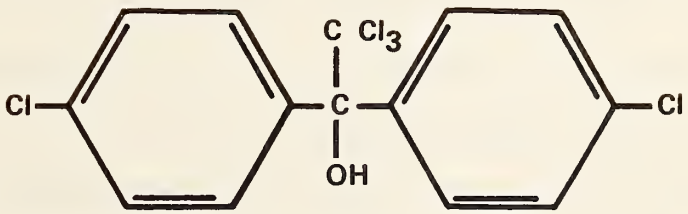


FIGURE 1
CHEMICAL STRUCTURE OF DICOFOL

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. The Osborne-Mendel rat was selected on the basis of a comparative study of the tumorigenic responsiveness to carbon tetrachloride of five different strains of rats (Reuber and Glover, 1970). The B6C3F1 mouse was selected because it has been used by the NCI for carcinogenesis bioassays and has proved satisfactory in this capacity.

Rats and mice of both sexes were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. The Osborne-Mendel rats were procured from the Battelle Memorial Institute, Columbus, Ohio, and the B6C3F1 mice were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Upon receipt, animals were quarantined for at least 10 days, observed for visible signs of disease or parasites, and assigned to the various dosed and control groups.

D. Animal Maintenance

All animals were housed by species in temperature- and humidity-controlled rooms. The temperature range was 20° to 24°C, and the relative humidity was maintained between 45 and 55 percent. The air conditioning system in the laboratory provided filtered air at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

The rats were individually housed in suspended galvanized-steel wire-mesh cages with perforated floors. Mice were housed by sex in

groups of ten in solid-bottom, polypropylene cages equipped with filter tops. Sanitized cages with fresh bedding (Sanichips®, Pinewood Sawdust Company, Moonachie, New Jersey) were provided once each week for mice. Rats received sanitized cages with no bedding with the same frequency. Food hoppers were changed and heat-sterilized once a week for the first 10 weeks and once a month thereafter. Fresh heat-sterilized glass water bottles and sipper tubes were provided three times a week. Food and water were available ad libitum.

The dicofol-treated and control rats were housed in the same room with rats receiving diets containing* dioxathion (78-34-2); mexacarbate (315-18-4); nitrofen (1836-75-5); endosulfan (115-29-7); and trifluralin (1582-09-8).

All mice used in the dicofol study, including controls, were housed in the same room as other mice receiving diets containing trifluralin (1582-09-8); p,p'-DDE (72-55-9); dioxathion (78-34-2); methoxychlor (72-43-5); p,p'-TDE (72-54-8); chlorobenzilate (510-15-6); safrole (94-59-7); acetylaminofluorene (53-96-3); clonitralid (1420-04-8); sulfallate (95-06-7); p,p'-DDT (50-29-3); amitrole (61-82-5); pentachloronitrobenzene (82-68-8); nitrofen (1836-75-5); endosulfan (115-29-7); and mexacarbate (315-18-4).

E. Selection of Initial Concentration

In order to establish the maximum tolerated concentrations of dicofol for administration to treated animals in the chronic studies,

*CAS registry numbers are given in parentheses.

subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among six groups, each consisting of five males and five females. Dicofol was premixed with a small amount of laboratory diet. The mixture was then incorporated into the basal laboratory diet and fed ad libitum to five of the six rat groups in concentrations of 178, 316, 562, 1000, and 1780 ppm and to five of the six mouse groups in concentrations of 100, 178, 316, 562, and 1000 ppm. The sixth group of each species served as a control group, receiving only the basal laboratory diet. The dosed dietary preparations were administered for a period of 6 weeks, followed by a 2-week observation period during which all animals were fed the basal laboratory diet.

A dosage inducing no mortality and resulting in a depression in mean group body weight of approximately 20 percent relative to controls was selected as the initial high concentration. When weight gain criteria were not applicable, mortality data alone were utilized.

Mean body weight depression was observed at all dosage levels in both male and female rats. At a concentration of 562 ppm, the depression in mean group body weight was 3 percent in male rats and 2 percent in females. At 1000 ppm the depression in mean body weight was 20 percent in males and 11 percent in females. No deaths occurred in groups receiving concentrations of 1000 ppm or less. The initial concentration used in the chronic bioassay for high dose male and female rats was 760 ppm. This was later increased for male rats to 1000 ppm.

Mean body weight depression in mice was observed in all treated groups. For males the mean group body weight depressions were 20 percent in the group receiving 178 ppm and 28 percent in those receiving 316 ppm. One male receiving 178 ppm died. Among females mean body weight depression was 19 percent at 100 ppm and 27 percent at 178 ppm. No deaths were reported for any of the female groups. The initial high concentrations used for male and female mice in the chronic study were 300 and 110 ppm, respectively. This was later increased, as shown in Table 2.

F. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, actual concentrations administered, duration of treated and untreated observation periods, and the time-weighted average concentrations) are summarized in Tables 1 and 2.

All rats were approximately 6 weeks old at the time they were placed on test. The initial dietary concentrations of dicofol administered to rats were 760 and 380 ppm. Throughout this report those rat groups initially receiving the former concentration are referred to as the high dose groups and those initially receiving the latter concentration are referred to as the low dose groups. In week 20, the high and low concentrations for males were increased to 1000 and 500 ppm, respectively, as the treated males had apparently tolerated the previous levels. These levels were maintained throughout the remainder of the dosing period. The high and low dose rats

TABLE 1

DESIGN SUMMARY FOR OSBORNE-MENDEL RATS
DICOFOL FEEDING EXPERIMENT

	INITIAL GROUP SIZE	DICOFOL CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION[b]
MALE					
CONTROL	20	0		110	0
LOW DOSE	50	380	19		
		500	59		471
		0		34	
HIGH DOSE	50	760	19		
		1000	59		942
		0		34	
FEMALE					
CONTROL	20	0		111	0
LOW DOSE	50	380	78		380
		0		34	
HIGH DOSE	50	760	78		760
		0		34	

[a] Concentrations given in parts per million.

[b] Time-weighted average concentration = $\frac{\Sigma(\text{concentration X weeks received})}{\Sigma(\text{weeks receiving chemical})}$

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
DICOFOL FEEDING EXPERIMENT

	INITIAL GROUP SIZE	DICOFOL CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION[b]
MALE					
CONTROL	20	0		91	0
LOW DOSE	50	150	4		264
		200	15		
		250	14		
		300	45		
		0		14	
HIGH DOSE	50	300	4		528
		400	15		
		500	14		
		600	45		
		0		14	
FEMALE					
CONTROL	20	0		91	0
LOW DOSE	50	55	9		122
		85	10		
		100	14		
		150	45	15	
HIGH DOSE	50	110	9		243
		170	10		
		200	14		
		300	45		
		0		15	

[a] Concentrations in parts per million.

[b] Time-weighted average concentration = $\frac{\Sigma(\text{concentration X weeks received})}{\Sigma(\text{weeks receiving chemical})}$

were treated for 78 weeks followed by 34 weeks of observation during which they received the basal laboratory diet.

All mice were approximately 6 weeks old at the time the experiment began. The initial dietary concentrations administered to male mice were 300 and 150 ppm. Female mice received initial concentrations of 110 and 55 ppm. Throughout this report males initially receiving 300 ppm and females initially receiving 110 ppm are referred t as the high dose groups while males initially receiving 150 ppm and females initially receiving 55 ppm are referred to as the low dose groups. Dosage levels were increased on three separate occasions for both male and female mice, as apparent tolerance of previous concentrations was observed. The high and low concentrations administered to male mice were increased to 400 and 200 ppm, respectively, during week 5. In week 10, the high and low concentrations administered to the female mice were increased to 170 and 85 ppm, respectively. During week 20 the high and low concentrations administered to treated mice were increased, respectively, to 500 and 250 ppm for the males and to 200 and 100 ppm for the females. Final increases in concentrations were made during week 34, when high and low concentrations were increased to 600 and 300 ppm for males, and to 300 and 150 ppm for females. These dosage levels were maintained for the remainder of the 78-week period of chemical administration. A 14- to 15-week observation period followed, during which the animals received the basal laboratory diet.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. Body weights, food consumption, and data concerning appearance, behavior, signs of toxic effects, and incidence, size, and location of tissue masses were recorded at weekly intervals for the first 10 weeks and at monthly intervals thereafter. From the first day, all animals were inspected daily for mortality. The presence of tissue masses was determined by observation and palpation of each animal.

During the course of this bioassay several pathology protocols were in effect, each for different periods of time. The minimum protocol required that, if possible, certain tissues were to be taken and examined histopathologically from all control animals, from any animal in which a tumor was observed during gross examination, and from at least 10 grossly normal males and 10 grossly normal females from each treated group. In addition, any tissues showing gross abnormalities were to be taken and examined histopathologically. Under later protocols, some tissues were taken from additional dosed animals. The number of animals in each group from which a tissue was examined is indicated in Appendices A through D.

A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized by exsanguination under sodium pentobarbital anesthesia, and were immediately necropsied.

The histopathologic examination consisted of gross and microscopic examination of major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in 10 percent buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination. An occasional section was subjected to special staining techniques for more definitive diagnosis.

Slides were prepared from the following tissues from selected animals: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, muscle, uterus, mammary gland, and ovary.

H. Data Recording and Statistical Analyses

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

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing 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 was 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, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) 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, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test determined 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 was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

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

The approximate 95 percent confidence interval for the relative risk of each 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 treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

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

III. CHRONIC TESTING RESULTS: RATS

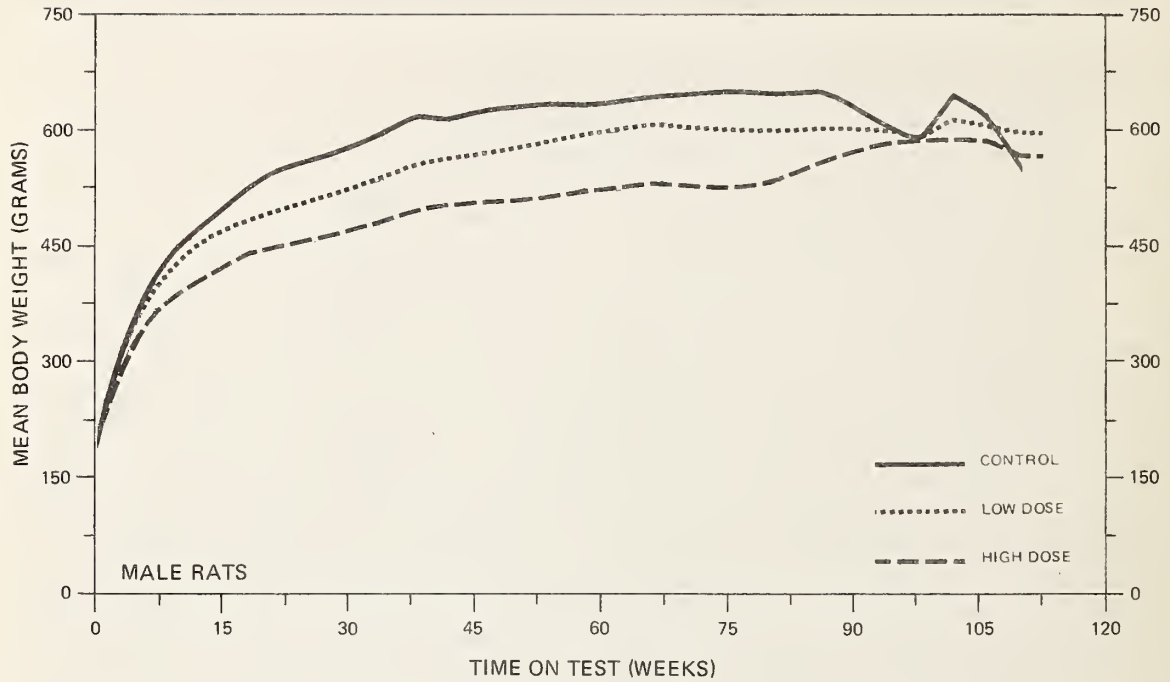
A. Body Weights and Clinical Observations

Dose-related mean body weight depression was apparent in both male and female rats throughout the bioassay (Figure 2).

During the first 30 weeks of the study, appearance and behavior of the treated rats were generally comparable with those of the untreated controls. As the study progressed (from week 30 until cessation of chemical administration in week 78), a hunched appearance was observed in a slightly greater number of treated rats than untreated controls. During the subsequent observation period, this characteristic was noted in comparable numbers of treated and control animals.

Respiratory signs involving labored respiration, wheezing, and/or nasal discharge were noted at a low incidence in all groups during the study.

Clinical signs often associated with aging in laboratory rats were observed at a comparable rate in control and treated animals during the second year of the study. These included sores on the body, abdominal urine stains, rough fur, localized alopecia, eyes reddened or discharging, swollen areas of the body or bloating, and tissue masses or palpable nodules. Isolated observations noted in one to three rats during the study included circling, salivation, undersized gonads, red vaginal discharge, and ataxia.



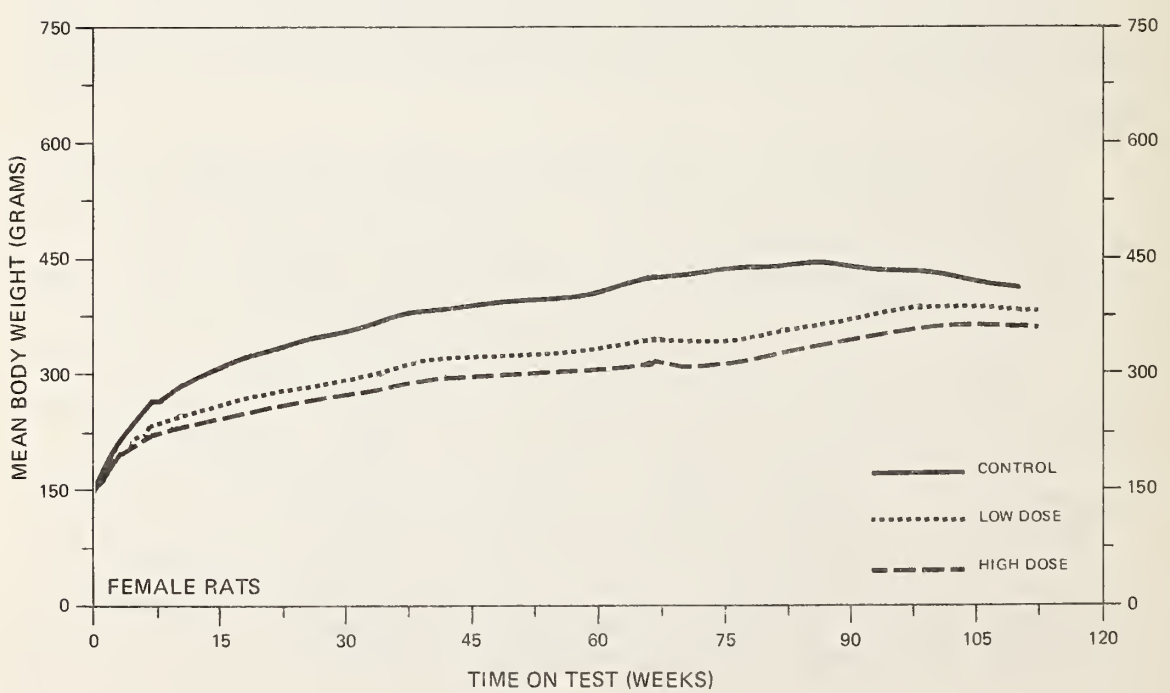


FIGURE 2
GROWTH CURVES FOR DICOFOL CHRONIC STUDY RATS

B. Survival

The estimated probabilities of survival for male and female rats in the control and dicofol-dosed groups are shown in Figure 3. For both male and female rats there was no significant positive association between dosage and mortality.

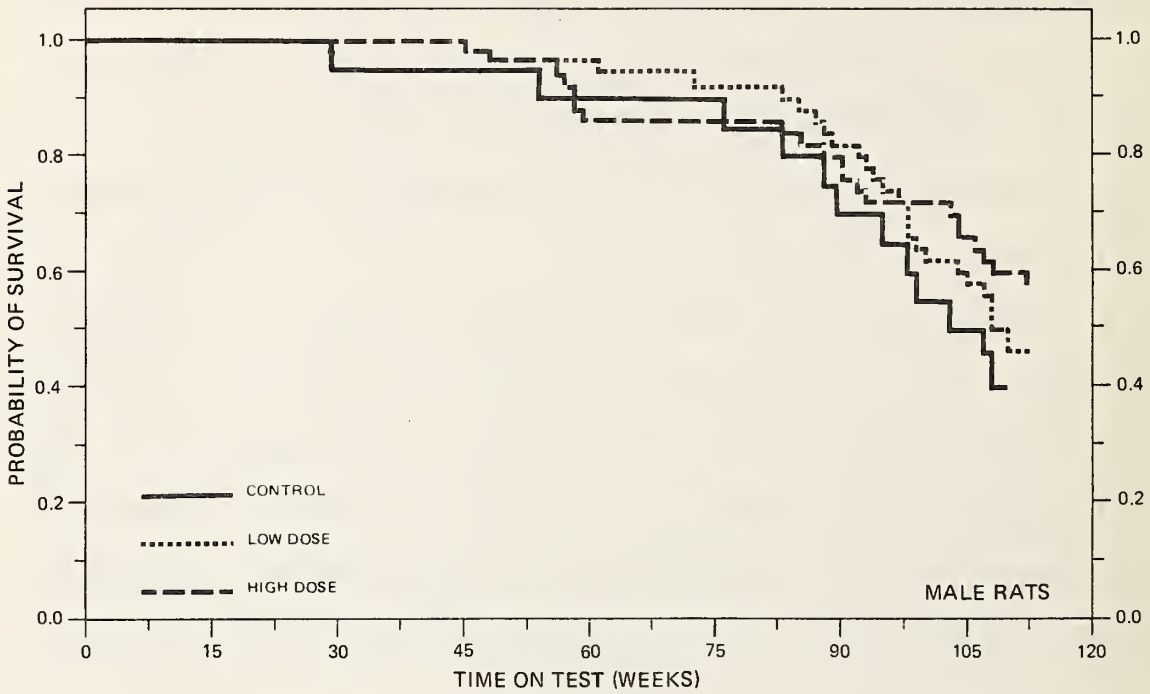
Adequate numbers of male rats were at risk from late-developing tumors as 72 percent (36/50) of the high dose, 64 percent (32/50) of the low dose, and 55 percent (11/20) of the control group survived at least 100 weeks. For female rats the survival was also adequate as 88 percent (44/50) of the high dose, 92 percent (46/50) of the low dose, and 80 percent (16/20) of the control group survived at least 100 weeks.

C. Pathology

Histopathologic findings on neoplasms in rats are tabulated in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are tabulated in Appendix C (Tables C1 and C2).

The types of tumors represented have been encountered previously as naturally occurring lesions in the Osborne-Mendel rat and were without apparent relationship to the administration of the chemical.

The incidences of inflammatory, degenerative, and proliferative lesions were similar in treated and control animals and were consistent with spontaneous lesions found in untreated aged Osborne-Mendel rats.



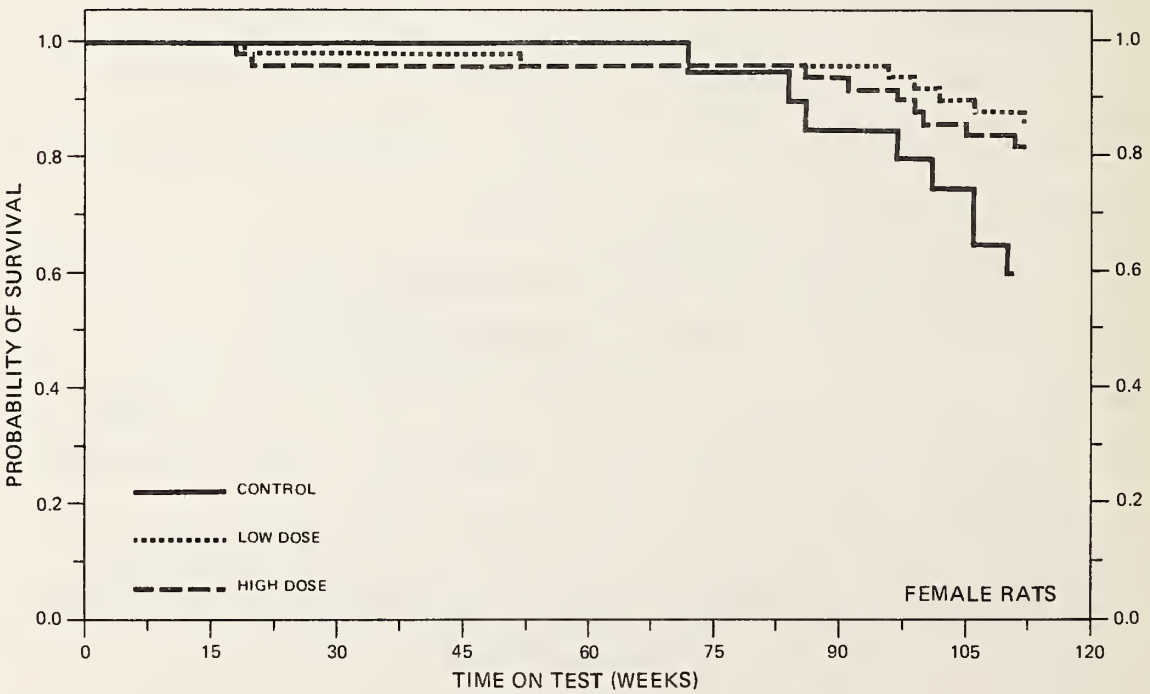


FIGURE 3
SURVIVAL COMPARISONS OF DICOFOL CHRONIC STUDY RATS

This histopathlogic examination provided no evidence for the carcinogenicity of dicofol in Osborne-Mendel rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or dicofol-dosed groups and where such tumors were observed in at least 5 percent of the group.

For rats of both sexes none of the statistical tests indicated a significant positive association between the administration of dicofol and the incidence of any tumor. Thus, at the dose levels used in this experiment there was no convincing statistical evidence that dicofol was a carcinogen in Osborne-Mendel rats.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In all of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should also be noted that all of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by dicofol that could not be established under the conditions of this test.

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE RATS TREATED WITH DICOFOL[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic: Malignant Lymphoma[b]	0/20(0.00)	4/50(0.08)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.386	0.255
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	72	93
Pituitary: Chromophobe Adenoma[b]	6/18(0.33)	5/28(0.18)	3/17(0.18)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.536	0.529
Lower Limit	---	0.157	0.102
Upper Limit	---	1.815	2.049
Weeks to First Observed Tumor	95	95	104
Thyroid: Follicular-Cell Carcinoma[b]	1/19(0.05)	3/31(0.10)	3/27(0.11)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.839	2.111
Lower Limit	---	0.164	0.188
Upper Limit	---	93.219	106.380
Weeks to First Observed Tumor	110	83	83

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: Follicular-Cell Adenoma or Follicular-Cell Carcinoma[b]	1/19(0.05)	4/31(0.13)	3/27(0.11)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.452	2.111
Lower Limit	---	0.272	0.188
Upper Limit	---	116.423	106.380
Weeks to First Observed Tumor	110	83	83
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	0/19(0.00)	3/31(0.10)	1/27(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.386	0.039
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	112

[a]Treated groups received time-weighted average doses of 471 and 942 ppm in feed.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE RATS TREATED WITH DICOFOL[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	9/20(0.45)	14/32(0.44)	15/30(0.50)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.972	1.111
Lower Limit	---	0.503	0.588
Upper Limit	---	2.077	2.303
Weeks to First Observed Tumor	84	112	100
Thyroid: C-Cell Carcinoma[b]	1/19(0.05)	2/25(0.08)	0/22(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.520	0.000
Lower Limit	---	0.085	0.000
Upper Limit	---	85.947	15.847
Weeks to First Observed Tumor	111	112	---
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	3/19(0.16)	2/25(0.08)	0/22(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.507	0.000
Lower Limit	---	0.047	0.000
Upper Limit	---	4.010	1.378
Weeks to First Observed Tumor	111	112	---

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma[b]	5/20(0.25)	6/50(0.12)	5/50(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	4.800	0.400
Lower Limit	---	0.143	0.107
Upper Limit	---	1.807	1.583
Weeks to First Observed Tumor	86	102	86
Uterus: Endometrial Stromal Polyp[b]	2/20(0.10)	1/32(0.03)	4/31(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.313	1.290
Lower Limit	---	0.006	0.208
Upper Limit	---	5.661	13.323
Weeks to First Observed Tumor	111	112	112

[a]Treated groups received doses of 380 and 760 ppm in feed.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

IV. CHRONIC TESTING RESULTS: MICE

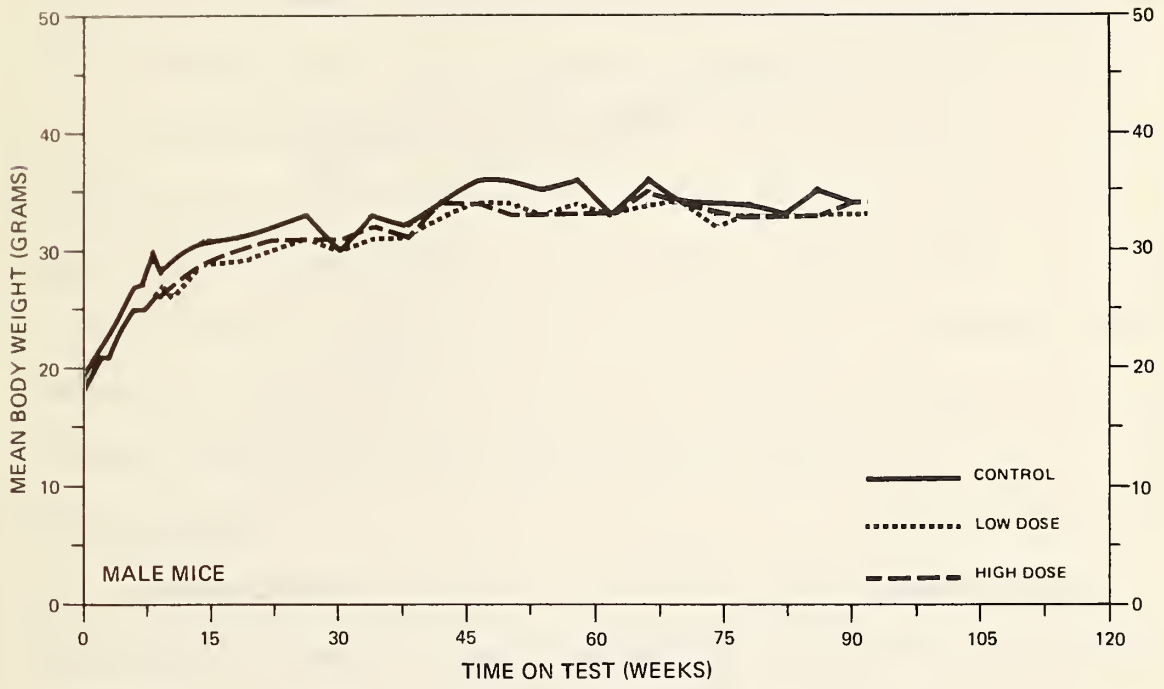
A. Body Weights and Clinical Observations

Dose-related mean body weight depression was apparent in female mice from approximately week 40 until the bioassay was terminated (Figure 4). No dose-related mean body weight depression was apparent in males.

Both the physical appearance and behavior of the treated and control mice were comparable during the first 18 weeks of the study. Following the dosage increases in weeks 20 and 34 of the study, a hunched appearance was observed in approximately 75 percent of the treated male mice. Only a few treated females and untreated control males and females exhibited this sign during the first 78 weeks; however, during the last 14 to 15 weeks of the study, most of the surviving control and treated mice appeared hunched. Signs often observed in laboratory mice, particularly in group-housed animals, were noted at a comparable rate in control and treated animals with the incidences increasing as the animals aged. These included sores and/or desquamation on parts of the body (more prevalent in males due to fighting), localized alopecia, stains on fur, genital irritation, palpable nodules or tissues masses, and bloated appearance.

B. Survival

The estimated probabilities of survival for male and female mice in the control and dicofol-dosed groups are shown in Figure 5. For



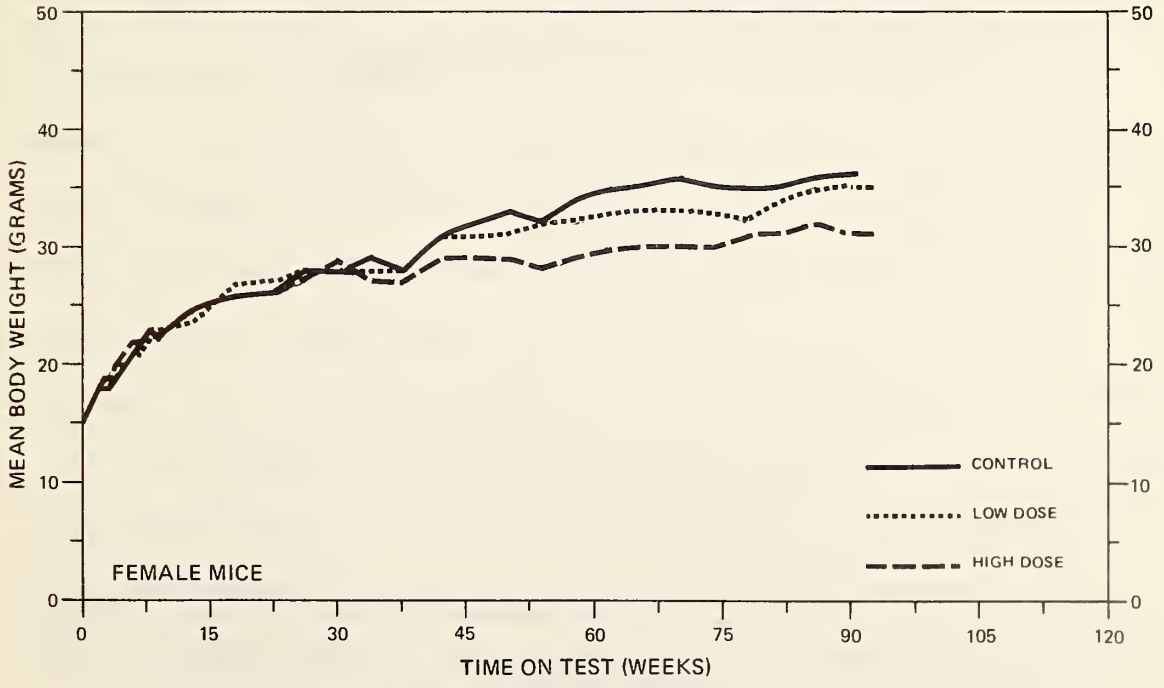


FIGURE 4
GROWTH CURVES FOR DICOFOL CHRONIC STUDY MICE

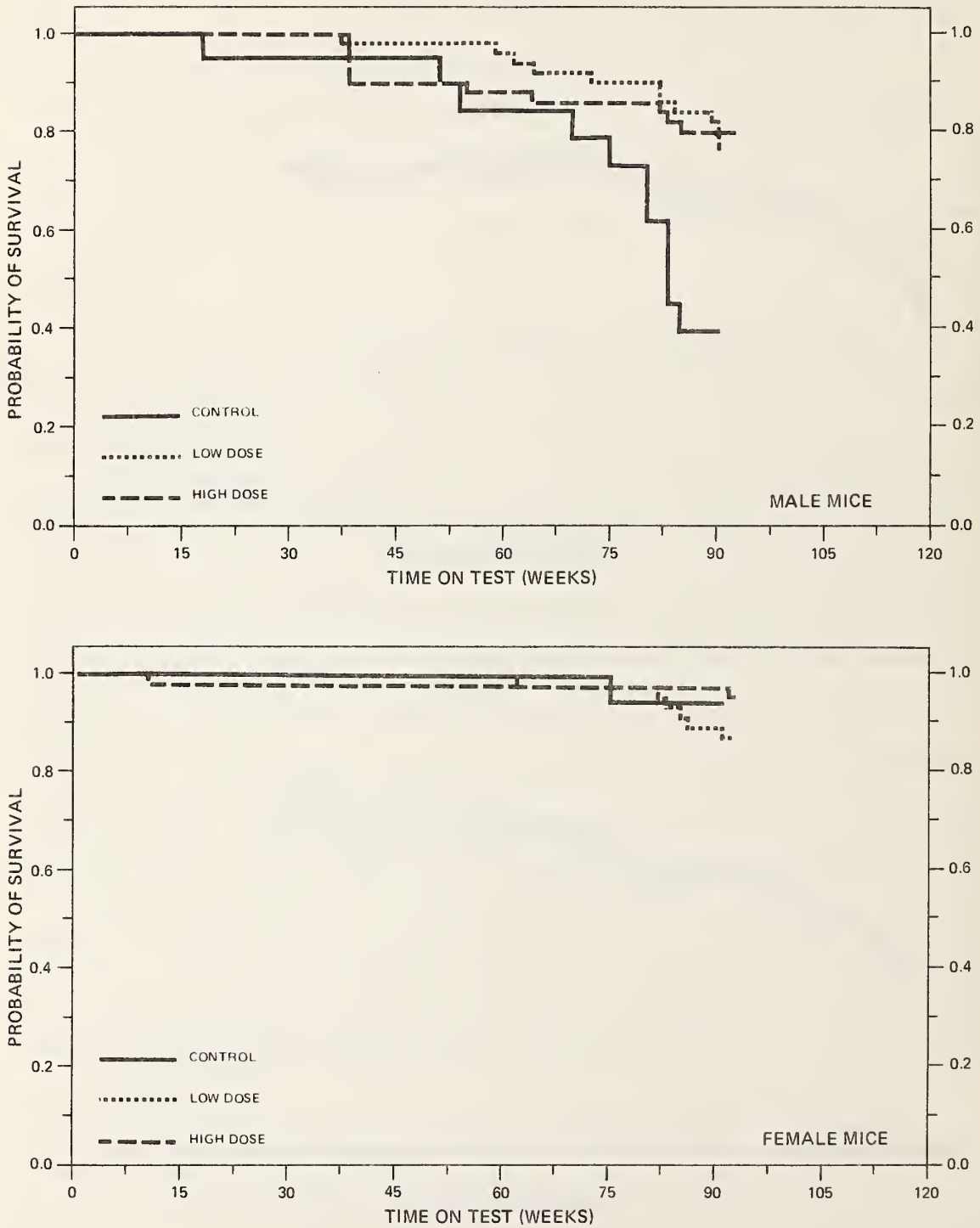


FIGURE 5
SURVIVAL COMPARISONS OF DICOFOL CHRONIC STUDY MICE

both male and female mice there was no significant positive association between dosage and mortality.

For male mice 76 percent (38/50) of the high dose and 76 percent (38/50) of the low dose but only 35 percent (7/20) of the control group survived until the end of the study. For the females the survival was relatively good as 96 percent (48/50) of the high dose, 84 percent (42/50) of the low dose, and 95 percent (19/20) of the control mice survived until the end of the study.

C. Pathology

Histopathologic findings on neoplasms in mice are tabulated in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are tabulated in Appendix D (Tables D1 and D2).

Hepatocellular carcinomas occurred in 3/18 (17 percent) control males, 22/50 (44 percent) low dose males, 35/47 (74 percent) high dose males, 1/20 (5 percent) control females, 0/44 low dose females, and 0/50 high dose females. Microscopically, the hepatocellular carcinomas varied greatly in appearance. Some lesions contained well-differentiated hepatic cells that had a relatively uniform arrangement of the cords, and others had very anaplastic liver cells with large hyperchromatic nuclei, often with pseudo-inclusion bodies, and with vacuolated, pale cytoplasm. Mitotic figures were often present. Some of the tumors were characterized by discrete areas of highly anaplastic cells. The hepatic neoplasms occurring in the control mice were not different in appearance from those noted in the treated mice.

Other neoplasms that occurred in this study were considered to be lesions that occur naturally in untreated B6C3F1 mice. There were no appreciable differences in frequency between the control and treated groups.

Incidences of other inflammatory, degenerative, and proliferative lesions that occurred were without appreciable difference in the control and treated mice.

Based upon this histopathologic examination dicofol was carcinogenic in male mice, as it was associated with an increased incidence of hepatocellular carcinomas. There was no evidence of compound-related neoplasia in the female mice.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or dicofol-dosed groups and where such tumors were observed in at least 5 percent of the group.

Significant numbers of liver tumors were observed in the treated male mice. The Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dosage and the incidence of hepatocellular carcinomas. The Fisher exact test supported this result with a significant ($P < 0.001$) comparison of the high dose group to the control group; the comparison of the low dose group to the

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH DICOFOL[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	0/18(0.00)	6/50(0.12)	0/48(0.00)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.006	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.605	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	91	---
Subcutaneous Tissue: Fibrosarcoma[b]	2/18(0.11)	7/50(0.14)	2/48(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.260	0.375
Lower Limit	---	0.276	0.030
Upper Limit	---	11.800	4.932
Weeks to First Observed Tumor	91	82	92
Lung: Alveolar/Bronchiolar Adenoma[b]	1/18(0.06)	2/36(0.06)	5/39(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.000	2.308
Lower Limit	---	0.057	0.292
Upper Limit	---	57.355	106.089
Weeks to First Observed Tumor	83	91	92

TABLE 5 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic: Malignant Lymphoma[b]	0/18(0.00)	1/50(0.02)	3/48(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.020	0.236
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	90
Liver: Hepatocellular Carcinoma[b]	3/18(0.17)	22/50(0.44)	35/47(0.74)
P Values[c]	P < 0.001	P = 0.035	P < 0.001
Relative Risk (Control)[d]	---	2.640	4.468
Lower Limit	---	0.950	1.737
Upper Limit	---	12.504	18.987
Weeks to First Observed Tumor	83	61	64
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	3/18(0.17)	23/50(0.46)	36/47(0.77)
P Values[c]	P < 0.001	P = 0.025	P < 0.001
Relative Risk (Control)[d]	---	2.760	4.596
Lower Limit	---	1.000	1.797
Upper Limit	---	13.003	19.249
Weeks to First Observed Tumor	83	61	64

TABLE 5 (CONCLUDED)

[a]Treated groups received time-weighted average doses of 264 and 528 ppm in feed.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH DICOFOL[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic: Malignant Lymphoma[b]	2/20(0.10)	4/44(0.09)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.909	0.600
Lower Limit	---	0.146	0.076
Upper Limit	---	9.544	6.861
Weeks to First Observed Tumor	91	62	93

[a]Treated groups received time-weighted average doses of 122 and 243 ppm in feed.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

control group had a probability level of P = 0.035, a marginal result which was not significant using the Bonferroni criterion. To further examine these results an additional, life-table analysis was performed. Figure 6 shows the probability of survival without a known hepatocellular carcinoma for male mice. The Tarone test indicated a significant (P = 0.010) positive association between dosage and tumor incidence.

Based upon these results the administration of dicofol was associated with the elevated incidence of hepatocellular carcinomas in male B6C3F1 mice.

No statistically significant positive association between dosage and tumor incidence was observed at any other site in either male or female mice.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by dicofol that could not be established under the conditions of this test.

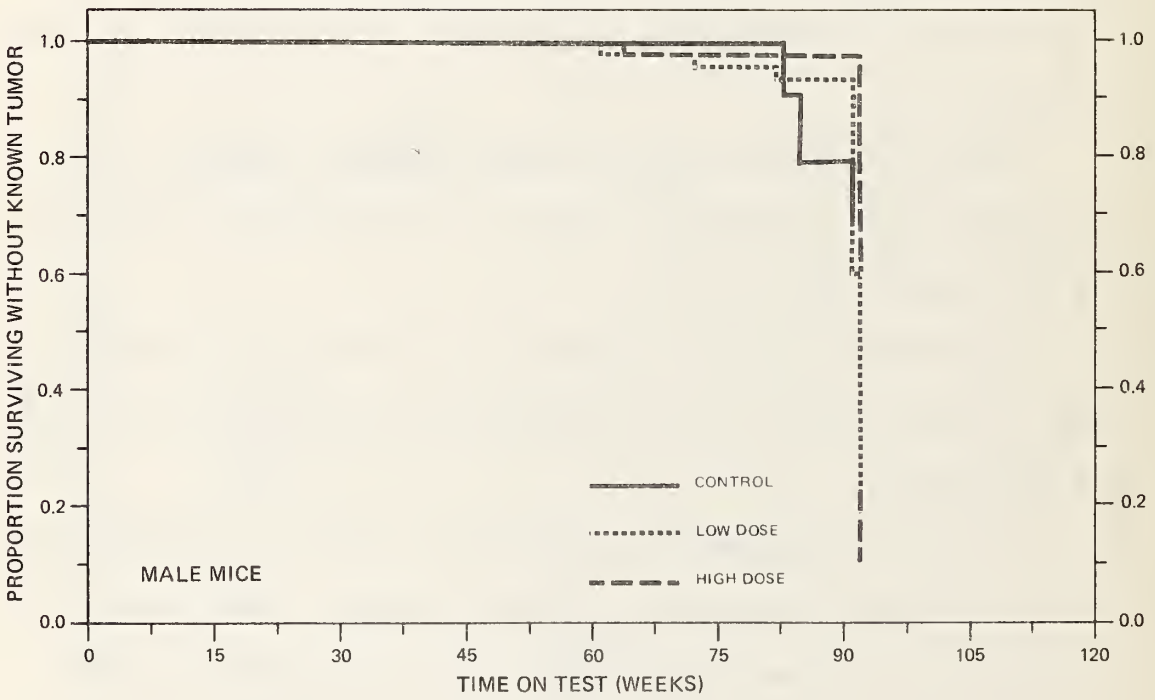


FIGURE 6
COMPARISONS OF DICOFOL CHRONIC STUDY MALE MICE SURVIVING WITHOUT OBSERVED HEPATOCELLULAR CARCINOMAS

V. DISCUSSION

Dietary administration of dicofol was not associated with a significant accelerated mortality in either sex of either species although some mice died early from liver cancer. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

In rats the only apparent effect of dicofol administration was distinct, dose-related mean body weight depression in males and females. No consistent unusual clinical observations were reported, no unusual or rare neoplasms or nonneoplastic lesions were observed, and none of the neoplasms that did occur were present in statistically significant increased incidences when compared to controls.

In mice dose-related mean body weight depresion was evident in treated females but not in males. There were no unusual clinical observations recorded for either sex. Hepatocellular carcinomas were observed in 3/18 (17 percent), 22/50 (44 percent), and 35/47 (74 percent) of the control, low dose, and high dose males, respectively, and 1/20 (5 percent), 0/44, and 0/50 of the control, low dose, and high dose females, respectively. Statistical analysis of the incidences of this neoplasm, using the Cochran-Armitage test and life-table analysis, indicated a significant positive association between dosage and incidence in the treated males. This finding was supported by the Fisher exact comparison of high dose to control. No unusual tumors were observed during the histopathologic examination, and

statistical significance was not attributed to the incidences of other tumors.

Long-term ingestion of p,p'-DDE, p,p'-DDT or technical-grade DDT (compounds with similar chemical structures to dicofol) has been found to induce liver tumors in both sexes of several strains of mice (International Agency for Research on Cancer, 1974). However, in this bioassay of dicofol, these lesions were induced only in male B6C3F1 mice.

Under the conditions of this bioassay, technical-grade dicofol was carcinogenic in male B6C3F1 mice, causing hepatocellular carcinomas. No evidence for carcinogenicity was obtained for this compound in Osborne-Mendel rats of either sex or in female B6C3F1 mice.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH DICOFOL

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH DICOFOL

	CONTROL (VEH) 01-M038	LOW DOSE 01-M039	HIGH DOSE 01-M040
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	19	49	47
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(49)
FIBROMA	1 (5%)	1 (2%)	
FIBROSARCOMA	1 (5%)	1 (2%)	
LIPOMA		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(40)	(38)
CORTICAL CARCINOMA, METASTATIC			1 (3%)
OSTEOSARCOMA, METASTATIC	1 (5%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(49)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		4 (8%)	1 (2%)
#SPLEEN	(19)	(25)	(19)
HEMANGIOSARCOMA		2 (8%)	
#LIVER	(19)	(42)	(40)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(8)	(16)	(14)
MIXED TUMOR, MALIGNANT		1 (6%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	CONTROL (VEH) 01-M038	LOW DOSE 01-M039	HIGH DOSE 01-M040
#LIVER	(19)	(42)	(40)
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (3%)
CORTICAL CARCINOMA, METASTATIC			1 (3%)
URINARY SYSTEM			
#KIDNEY	(19)	(40)	(18)
MIXED TUMOR, MALIGNANT		1 (3%)	
#URINARY BLADDER	(18)	(20)	(14)
PAPILLOMA, NOS			1 (7%)
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(28)	(17)
CHROMOPHOBE ADENOMA	6 (33%)	5 (18%)	3 (18%)
#ADRENAL	(19)	(25)	(20)
CORTICAL CARCINOMA			1 (5%)
#THYROID	(19)	(31)	(27)
FOLLICULAR-CELL ADENOMA		1 (3%)	
FOLLICULAR-CELL CARCINOMA	1 (5%)	3 (10%)	3 (11%)
C-CELL ADENOMA		2 (6%)	
C-CELL CARCINOMA		1 (3%)	1 (4%)
#PANCREATIC ISLETS	(19)	(25)	(14)
ISLET-CELL ADENOMA	1 (5%)	1 (4%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
ADENOCARCINOMA, NOS		2 (4%)	1 (2%)
#PROSTATE	(14)		(1)
FIBROMA	1 (7%)		
NERVOUS SYSTEM			
#BRAIN	(19)	(23)	(16)
EPENDYMOMA		1 (4%)	

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

TABLE A1 (CONTINUED)

	CONTROL (VEH) 01-M038	LOW DOSE 01-M039	HIGH DOSE 01-M040
ASTROCYTOMA	1 (5%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*VERTEBRAL COLUMN	(20)	(50)	(49)
OSTEOSARCOMA	1 (5%)		
*MUSCLE OF HEAD	(20)	(50)	(49)
SQUAMOUS CELL CARCINOMA		1 (2%)	
*MUSCLE OF NECK	(20)	(50)	(49)
FIBROSARCOMA			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(49)
LIPOMA			1 (2%)
MIXED TUMOR, MALIGNANT		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	12	27	21
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	8	23	29
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	CONTROL (VEH) 01-M038	LOW DOSE 01-M039	HIGH DOSE 01-M040
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	10	23	16
TOTAL PRIMARY TUMORS	13	30	17
TOTAL ANIMALS WITH BENIGN TUMORS	8	9	6
TOTAL BENIGN TUMORS	9	11	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	16	10
TOTAL MALIGNANT TUMORS	4	19	11
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		1
TOTAL SECONDARY TUMORS	1		2
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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH DICOFOL

	CONTROL (VEH) 01-F038	LOW DOSE 01-F075	HIGH DOSE 01-F076
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(50)
CYSTADENOCARCINOMA, NOS			1 (2%)
FIBROMA	1 (5%)		
HEMANGIOSARCOMA	1 (5%)	1 (2%)	
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (4%)	1 (2%)
#OVARY	(20)	(23)	(23)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (4%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#PANCREAS	(20)	(23)	(19)
CYSTADENOCARCINOMA, METASTATIC		1 (4%)	
URINARY SYSTEM			
#KIDNEY	(20)	(23)	(20)
HAMARTOMA+	1 (5%)	1 (4%)	1 (5%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

+ THIS IS CONSIDERED TO BE A BENIGN FORM OF THE MALIGNANT MIXED TUMOR OF THE KIDNEY AND CONSISTS OF PROLIFERATIVE LIPOCYTES, TUBULAR STRUCTURES, FIBROBLASTS, AND VASCULAR SPACES IN VARYING PROPORTIONS.

TABLE A2 (CONTINUED)

	CONTROL (VEH) 01-F038	LOW DOSE 01-F075	HIGH DOSE 01-F076
#URINARY BLADDER	(20)	(22)	(20)
LEIOMYOSARCOMA			1 (5%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(32)	(30)
CHROMOPHOBE ADENOMA	9 (45%)	14 (44%)	15 (50%)
#ADRENAL	(20)	(23)	(22)
CORTICAL ADENOMA		1 (4%)	
CORTICAL CARCINOMA		1 (4%)	1 (5%)
#THYROID	(19)	(25)	(22)
FOLLICULAR-CELL CARCINOMA			1 (5%)
C-CELL ADENOMA	2 (11%)		
C-CELL CARCINOMA	1 (5%)	2 (8%)	
#PANCREATIC ISLETS	(20)	(23)	(19)
ISLET-CELL ADENOMA		1 (4%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOMA, NOS		1 (2%)	1 (2%)
ADENOCARCINOMA, NOS	1 (5%)		
FIBROADENOMA	5 (25%)	6 (12%)	5 (10%)
*VAGINA	(20)	(50)	(50)
LEIOMYOSARCOMA			1 (2%)
#UTERUS	(20)	(32)	(31)
ADENOMA, NOS			1 (3%)
ENDOMETRIAL STROMAL POLYP	2 (10%)	1 (3%)	4 (13%)
#OVARY	(20)	(23)	(23)
CYSTADENOCARCINOMA, NOS		1 (4%)	
GRANULOSA-CELL TUMOR			1 (4%)
NERVOUS SYSTEM			
#BRAIN	(20)	(23)	(19)
EPENDYMOMA			1 (5%)
SPECIAL SENSE ORGANS			
NONE			

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

TABLE A2 (CONTINUED

	CONTROL (VEH) 01-F038	LOW DOSE 01-F075	HIGH DOSE 01-F076
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	8	7	9
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	12	43	41
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL(VEH) 01-F038	LOW DOSE 01-F075	HIGH DOSE 01-F076
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	16	28	28
TOTAL PRIMARY TUMORS	23	33	36
TOTAL ANIMALS WITH BENIGN TUMORS	15	22	22
TOTAL BENIGN TUMORS	20	25	27
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	8	7
TOTAL MALIGNANT TUMORS	3	8	8
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS
IN MICE TREATED WITH DICOFOL

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH DICOFOL

	CONTROL (VEH) 02-M032	LOW DOSE 02-M033	HIGH DOSE 02-M034
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	2		1
ANIMALS NECROPSIED	18	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	18	48	47
INTEGUMENTARY SYSTEM			
*SKIN	(18)	(50)	(48)
SQUAMOUS CELL CARCINOMA		1 (2%)	
SEBACEOUS ADENOMA	1 (6%)		
*SUBCUT TISSUE	(18)	(50)	(48)
FIBROMA		6 (12%)	
FIBROSARCOMA	2 (11%)	7 (14%)	2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(18)	(36)	(39)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (6%)	2 (6%)	5 (13%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(18)	(50)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	3 (6%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(18)	(50)	(47)
HEPATOCELLULAR ADENOMA		1 (2%)	1 (2%)
HEPATOCELLULAR CARCINOMA	3 (17%)	22 (44%)	35 (74%)
#STOMACH	(18)	(31)	(39)
SQUAMOUS CELL CARCINOMA			1 (3%)
URINARY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL (VEH) 02-M032	LOW DOSE 02-M033	HIGH DOSE 02-M034
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(18)	(50)	(48)
LIPOMA		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	11	12	11
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	7	38	38
ANIMAL MISSING	2		1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1 (CONCLUDED)

	CONTROL (VEH) 02-M032	LOW DOSE 02-M033	HIGH DOSE 02-M034
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	5	34	38
TOTAL PRIMARY TUMORS	7	41	47
TOTAL ANIMALS WITH BENIGN TUMORS	2	9	6
TOTAL BENIGN TUMORS	2	10	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	28	37
TOTAL MALIGNANT TUMORS	5	31	41
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

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH DICOFOL

	CONTROL (VEH) 02-F032	LOW DOSE 02-F035	HIGH DOSE 02-F036
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		2	
ANIMALS NECROPSIED	20	44	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	44	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(44)	(50)
FIBROSARCOMA		1 (2%)	
LIPOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(19)	(19)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(44)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)		1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (5%)	1 (2%)
#SPLEEN	(20)	(16)	(18)
HEMANGIOSARCOMA			1 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		
#PANCREATIC L.NODE	(20)	(15)	(19)
SQUAMOUS CELL CARCINOMA, METASTA			1 (5%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (7%)	
#MESENTERIC L. NODE	(20)	(15)	(19)
SQUAMOUS CELL CARCINOMA, METASTA			1 (5%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (5%)
#LIVER	(20)	(44)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL (VEH) 02-F032	LOW DOSE 02-F035	HIGH DOSE 02-F036
DIGESTIVE SYSTEM			
#LIVER	(20)	(44)	(50)
HEPATOCELLULAR CARCINOMA	1 (5%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(14)	(15)
CHROMOPHOBE ADENOMA			1 (7%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(44)	(50)
ADENOCARCINOMA, NOS	1 (5%)		
#UTERUS	(20)	(26)	(23)
SQUAMOUS CELL CARCINOMA			1 (4%)
HEMANGIOMA			1 (4%)
#OVARY	(20)	(17)	(25)
CYSTADENOMA, NOS			1 (4%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE B2 (CONCLUDED)

	CONTROL (VEH) 02-F032	LOW DOSE 02-F035	HIGH DOSE 02-F036
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	6	2
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	19	42	48
ANIMAL MISSING		2	
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	5	6	8
TOTAL PRIMARY TUMORS	5	6	8
TOTAL ANIMALS WITH BENIGN TUMORS			3
TOTAL BENIGN TUMORS			3
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	6	5
TOTAL MALIGNANT TUMORS	5	6	5
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			2
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 TREATED WITH DICOFOL

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH DICOFOL

	CONTROL (VEH) 01-M038	LOW DOSE 01-M039	HIGH DOSE 01-M040
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	19	49	47
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(49)
HYPERKERATOSIS	1 (5%)		
ACANTHOSIS	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(49)
ABSCESS, NOS	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(19)	(40)	(38)
INFLAMMATION, NOS			1 (3%)
PNEUMONIA, CHRONIC MURINE	6 (32%)	12 (30%)	13 (34%)
CALCIUM DEPOSIT			1 (3%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(21)	(14)
METAMORPHOSIS FATTY	1 (5%)		1 (7%)
#SPLEEN	(19)	(25)	(19)
INFLAMMATION, NOS			1 (5%)
HYPERTROPHY, NOS			1 (5%)
HEMATOPOIESIS	3 (16%)	1 (4%)	
CIRCULATORY SYSTEM			
#HEART	(19)	(23)	(18)
CALCIUM DEPOSIT		1 (4%)	1 (6%)
#MYOCARDIUM	(19)	(23)	(18)
DEGENERATION, NOS	1 (5%)	2 (9%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	CONTROL (VEH) 01-M038	LOW DOSE 01-M039	HIGH DOSE 01-M040
#ENDOCARDIUM	(19)	(23)	(18)
HYPERPLASIA, NOS		1 (4%)	3 (17%)
*AORTA	(20)	(50)	(49)
ARTERIOSCLEROSIS, NOS		3 (6%)	2 (4%)
DIGESTIVE SYSTEM			
#LIVER	(19)	(42)	(40)
METAMORPHOSIS FATTY	3 (16%)	6 (14%)	8 (20%)
HYPERPLASIA, NOS	1 (5%)		
#PANCREAS	(19)	(25)	(14)
PERIARTERITIS		1 (4%)	1 (7%)
#STOMACH	(19)	(28)	(30)
INFLAMMATION, NOS	1 (5%)		
ULCER, FOCAL	1 (5%)		
CALCIUM DEPOSIT		3 (11%)	3 (10%)
#COLON	(19)	(21)	(15)
PARASITISM	1 (5%)		
URINARY SYSTEM			
#KIDNEY	(19)	(40)	(18)
PYELONEPHRITIS, NOS		1 (3%)	1 (6%)
INFLAMMATION, CHRONIC	11 (58%)	18 (45%)	8 (44%)
CALCIUM DEPOSIT		3 (8%)	
#URINARY BLADDER	(18)	(20)	(14)
CALCULUS, NOS			1 (7%)
INFLAMMATION, NOS	1 (6%)		1 (7%)
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(28)	(17)
CYST, NOS		2 (7%)	
HYPERPLASIA, NOS		1 (4%)	
#ADRENAL	(19)	(25)	(20)
ANGIECTASIS		1 (4%)	

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

TABLE C1 (CONTINUED)

	CONTROL (VEH) 01-M038	LOW DOSE 01-M039	HIGH DOSE 01-M040
#THYROID	(19)	(31)	(27)
CYST, NOS		7 (23%)	7 (26%)
HYPERPLASIA, C-CELL		1 (3%)	
#PARATHYROID	(19)	(24)	(17)
HYPERPLASIA, NOS		4 (17%)	4 (24%)
REPRODUCTIVE SYSTEM			
#TESTIS	(19)	(30)	(17)
ATROPHY, NOS	7 (37%)	14 (47%)	4 (24%)
*EPIDIDYMIS	(20)	(50)	(49)
ATROPHY, NOS	1 (5%)		
NERVOUS SYSTEM			
#BRAIN	(19)	(23)	(16)
GLIOSIS			1 (6%)
SPECIAL SENSE ORGANS			
*EYE	(20)	(50)	(49)
CATARACT		1 (2%)	2 (4%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	3	3

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

TABLE C1 (CONCLUDED)

	CONTROL (VEH) 01-M038	LOW DOSE 01-M039	HIGH DOSE 01-M040
NECROPSY PERF/NO HISTO PERFORMED	1		
AUTC/NECROPSY/NO HISTO		1	2
AUTOLYSIS/NO NECROPSY			1

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH DICOFOL

	CONTROL (VEH) 01-F038	LOW DOSE 01-F075	HIGH DOSE 01-F076
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(34)	(24)
INFLAMMATION, NOS	1 (5%)		
PNEUMONIA, CHRONIC MURINE	9 (45%)	5 (15%)	10 (42%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(23)	(19)
METAMORPHOSIS FATTY	2 (10%)		1 (5%)
#SPLEEN	(20)	(26)	(20)
HEMATOPOIESIS	1 (5%)	3 (12%)	1 (5%)
#MESENTERIC L. NODE	(19)	(22)	(19)
CYST, NOS			1 (5%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(24)	(21)
DEGENERATION, NOS		1 (4%)	2 (10%)
#ENDOCARDIUM	(20)	(24)	(21)
HYPERPLASIA, NOS			1 (5%)
*CORONARY ARTERY	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(41)	(40)
INFLAMMATION, NOS	1 (5%)	1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL (VEH) 01-F038	LOW DOSE 01-F075	HIGH DOSE 01-F076
METAMORPHOSIS FATTY	2 (10%)	2 (5%)	
HYPERPLASIA, NOS			1 (3%)
#STOMACH	(20)	(24)	(25)
ULCER, FOCAL	1 (5%)	1 (4%)	
URINARY SYSTEM			
#KIDNEY	(20)	(23)	(20)
PYELONEPHRITIS, NOS	2 (10%)		1 (5%)
INFLAMMATION, CHRONIC	1 (5%)	5 (22%)	2 (10%)
#URINARY BLADDER	(20)	(22)	(20)
INFLAMMATION, NOS	1 (5%)		
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(32)	(30)
CYST, NOS	1 (5%)		
#ADRENAL	(20)	(23)	(22)
ANGIECTASIS		2 (9%)	2 (9%)
#THYROID	(19)	(25)	(22)
CYST, NOS		2 (8%)	1 (5%)
REPRODUCTIVE SYSTEM			
*VAGINA	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)		
POLYP		2 (4%)	
#UTERUS	(20)	(32)	(31)
HYDROMETRA	1 (5%)	6 (19%)	6 (19%)
INFLAMMATION, NOS		2 (6%)	
#UTERUS/ENDOMETRIUM	(20)	(32)	(31)
INFLAMMATION, NOS	1 (5%)		1 (3%)
HYPERPLASIA, CYSTIC	3 (15%)	3 (9%)	
#OVARY	(20)	(23)	(23)
CYST, NOS	1 (5%)	1 (4%)	4 (17%)
NERVOUS SYSTEM			
NONE			

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

TABLE C2 (CONCLUDED)

	CONTROL (VEH) 01-F038	LOW DOSE 01-F075	HIGH DOSE 01-F076
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
ABSCESS, NOS	1 (5%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		13	8
NECROPSY PERF/NO HISTO PERFORMED		1	1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH DICOFOL

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH DICOFOL

	CONTROL (VEH) 02-M032	LOW DOSE 02-M033	HIGH DOSE 02-M034
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	2		1
ANIMALS NECROPSIED	18	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	18	48	47
INTEGUMENTARY SYSTEM			
*SKIN	(18)	(50)	(48)
EPIDERMAL INCLUSION CYST	2 (11%)		
INFLAMMATION, NOS	1 (6%)		
RESPIRATORY SYSTEM			
#LUNG	(18)	(36)	(39)
PNEUMONIA, CHRONIC MURINE			1 (3%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(16)	(36)	(40)
AMYLOIDOSIS	5 (31%)	3 (8%)	
HEMATOPOIESIS			2 (5%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(18)	(31)	(36)
INFLAMMATION, NOS	1 (6%)		
#ENDOCARDIUM	(18)	(31)	(36)
INFLAMMATION, NOS	1 (6%)		
*AORTA	(18)	(50)	(48)
PERIARTERITIS	1 (6%)		
DIGESTIVE SYSTEM			
#LIVER	(18)	(50)	(47)
THROMBOSIS, NOS	1 (6%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL (VEH) 02-M032	LOW DOSE 02-M033	HIGH DOSE 02-M034
INFLAMMATION, NOS		1 (2%)	2 (4%)
HYPERPLASIA, NODULAR		1 (2%)	2 (4%)
*PANCREAS	(18)	(31)	(36)
CYSTIC DUCTS			1 (3%)
ATROPHY, NOS			1 (3%)
*RECTUM	(18)	(50)	(48)
PROLAPSE		2 (4%)	
URINARY SYSTEM			
*KIDNEY	(18)	(36)	(41)
HYDRONEPHROSIS	1 (6%)	1 (3%)	
PYELONEPHRITIS, NOS	1 (6%)		
INFLAMMATION, CHRONIC	8 (44%)	6 (17%)	
AMYLOIDOSIS	5 (28%)	1 (3%)	
#URINARY BLADDER	(17)	(30)	(38)
INFLAMMATION, NOS		1 (3%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(18)	(50)	(48)
CYST, NOS	1 (6%)		
*PREPUTIAL GLAND	(18)	(50)	(48)
ABSCESS, NOS			1 (2%)
#PROSTATE	(18)	(30)	(36)
INFLAMMATION, NOS	1 (6%)		
*SEMINAL VESICLE	(18)	(50)	(48)
INFLAMMATION, NOS	1 (6%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE D1 (CONCLUDED)

	CONTROL (VEH) 02-M032	LOW DOSE 02-M033	HIGH DOSE 02-M034
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	10	5
ANIMAL MISSING/NO NECROPSY	2		1
NECROPSY PERF/NO HISTO PERFORMED		1	
AUTO/NECROPSY/HISTO PERF			1
AUTO/NECROPSY/NO HISTO		1	1
AUTOLYSIS/NO NECROPSY			1

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH DICOFOL

	CONTROL (VEH) 02-F032	LOW DOSE 02-F035	HIGH DOSE 02-F036
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		2	
ANIMALS NECROPSIED	20	44	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	44	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(44)	(50)
VERRUCA			1 (2%)
*SUBCUT TISSUE	(20)	(44)	(50)
NECROSIS, FAT		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(19)	(19)
PNEUMONIA, CHRONIC MURINE		1 (5%)	
HYPERPLASIA, ADENOMATOUS		1 (5%)	
METAPLASIA, SQUAMOUS		1 (5%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(16)	(18)
HEMATOPOIESIS		2 (13%)	1 (6%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(44)	(50)
CYST, NOS		1 (2%)	
THROMBOSIS, NOS		1 (2%)	
INFLAMMATION, NOS		1 (2%)	
#PANCREAS	(20)	(14)	(17)
INFLAMMATION, NOS		1 (7%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (VEH) 02-F032	LOW DOSE 02-F035	HIGH DOSE 02-F036
URINARY SYSTEM			
#KIDNEY	(20)	(18)	(18)
HYDRONEPHROSIS			1 (6%)
INFLAMMATION, CHRONIC		1 (6%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(26)	(23)
HYDROMETRA	2 (10%)	2 (8%)	6 (26%)
INFLAMMATION, NOS	2 (10%)	2 (8%)	3 (13%)
#UTERUS/ENDOMETRIUM	(20)	(26)	(23)
HYPERPLASIA, CYSTIC	6 (30%)	13 (50%)	
#OVARY	(20)	(17)	(25)
CYST, NOS	2 (10%)	4 (24%)	9 (36%)
INFLAMMATION, NOS			1 (4%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE D2 (CONCLUDED)

	CONTROL (VEH) 02-F032	LOW DOSE 02-F035	HIGH DOSE 02-F036
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	20	26
ANIMAL MISSING/NO NECROPSY		2	
AUTOLYSIS/NO NECROPSY		4	

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

April 26, 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 Dicofol for carcinogenicity.

The primary reviewer thought that the conduct and the design of the bioassay were adequate, although he disagreed with the presentation of the conclusion on the carcinogenicity of Dicofol. He said that the conclusion should focus on the negative response found in three of the treatment groups rather than on the positive in only a single sex of one species. He concluded that: 1) Dicofol should not be classified as a carcinogen without replication of the study and 2) Dicofol would not appear to pose a carcinogenic risk for man on the basis of this bioassay.

The secondary reviewer said that the inadequacies of the bioassay made it difficult to interpret the significance of the increased incidence of liver tumors found in treated male mice, particularly in the absence of other positive data. He was especially critical of: the lack of analytical data on the stability of Dicofol during storage and in the treatment diet (it was noted that a significant amount of

the stored Dicofol liquified); the inadequate subchronic data for selecting chronic dose levels; the change in dose levels during the chronic phase; and the poor survival among control male mice. He concluded that the test was poorly conducted and recommended that it be repeated.

A Program staff member commented that the survival among control male mice was fairly high until about 78 weeks. He said that the incidence of hepatocellular carcinomas among treated male mice was 74% in the high dose group and 44% in the low dose one, as compared to a high of 25% in historic controls at the testing laboratory. He opined that the induction of a statistically significant incidence of hepatocellular carcinomas in one sex and species was sufficient to base a conclusion of carcinogenicity. The primary reviewer repeated that such a limited positive response was overemphasized in the report. He suggested that Dicofol be termed no more than a hepatocarcinogen in male mice. The secondary reviewer contended that the bioassay was too inadequate to draw any conclusion.

A subgroup member offered an amendment to a motion put forth earlier by the primary reviewer. The amended motion read: "Under the conditions of this bioassay, technical grade Dicofol produced no evidence of carcinogenicity in Osborne-Mendel rats of either sex or in female B6C3F1 mice; the failure to determine the stability of Dicofol throughout the study prohibits drawing any conclusion concerning its carcinogenicity." A vote on the amended motion passed unanimously.

In further discussion, it was recommended that a sample of the original Dicofol be analyzed to determine its composition. Based on the results of the analysis, a decision could be made as to whether the compound should be considered for retest.

Members present were:

Michael Shimkin (Acting Chairman), University of California at San Diego
Joseph Highland, Environmental Defense Fund
George Roush, Jr., Monsanto Company
Louise Strong, University of Texas Health Sciences Center
John Weisburger, American Health Foundation

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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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