UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR#: 0053453

MEMORANDUM

Date: June 22, 2006

Subject: **Dicofol:** Review of Special One-generation Reproduction Study - Rat (MRIDs 44253801, 44559901 and 44624301)

PC Code.: 010501
DP Barcode No: D325924

From: Guruva B. Reddy, Veterinary Medical Officer
Registration Action Branch 1
Health Effects Division (HED) (7509C)

To: Daniel Rosenblatt, RM 05
Registration Division (7505C)

Through: P.V. Shah, Ph.D., Branch Senior Scientist
Registration Action Branch 1
Health Effects Division (HED) (7509C)

1 CONCLUSIONS

The Health Effects Division has evaluated the special one-generation rat reproduction study (MRIDs 44253801, 44559901 and 44624301) for dicofol and provided the Data Evaluation Record (DER). The study is classified as **acceptable/non-guideline** and does satisfy the purpose for which it was conducted; to determine the effects of dicofol on reproductive performance in parental animals and determine the potential endocrine effects of dicofol in F_0 and F_1 rats. Exposure to dicofol and/or its metabolites did not effect reproductive function or performance and/or endocrine organs. The animals were adequately exposed to the test material during all phases study as shown by quantifiable levels of dicofol and metabolites in adult serum,

milk, prenursing neonate tissues and weanling serum. Further, administration of dicofol did not affect the follicle counts and/or the estrus cycle length.

II ACTION REQUESTED

The Registration Division has requested that the Health Effects Division (HED) review the special one-generation rat reproduction study for Dicofol, MRIDs 44253801, 44559901 and 44624301 in support of registration.

CITATION: Rowe, J.N. (1997) Reproduction and postnatal study of potential endocrine effects of dicofol administered orally (diet) to rats. Argus Research Laboratories, Inc., Horsham, PA. Laboratory project ID 018-021, March 5, 1997. MRID 44253801. Unpublished.

Hoberman, A.L. (1998)Supplement to Reproduction and postnatal study of potential endocrine effects of dicofol administered orally (diet) to rats. Argus Research Laboratories, Inc., Horsham, PA. Laboratory project ID 018-021, March 5, 1997 (MRID 44253801). Supplemental information. April 2, 1998. MRID 44559901. Unpublished.

Lomax, L (1998) Ovary Follicle Counts - Supplement to Reproduction and postnatal study of potential endocrine effects of dicofol administered orally (diet) to rats. Argus Research Laboratories, Inc., Horsham, PA. Laboratory project ID 018-021, March 5, 1997 (MRID 44253801). July 20, 1998. MRID 44624301. Unpublished.

EXECUTIVE SUMMARY: In a single-generation reproduction study (MRIDs 44253801, 44559901 and 44624301) Dicofol (96.4% a.i., Lot/Batch #687) was administered to Crl:CD®Br VAF/PLUS Sprague-Dawley derived rats (30 males and 30 females per dose). Dicofol was administered in the diet at concentrations of 0, 5, 25, and 125 ppm (0, 0.3, 1.7, and 8.7 mg/kg/day for males and 0, 0.4, 2.0, and 9.8 mg/kg/day for females during premating). F_0 females were administered the control or test diet for 10 weeks before cohabitation, during cohabitation, gestation, lactation, and for 1 to 2 weeks after the last F_1 litter was weaned. F_0 males were administered the control or test diet continuously for 10 weeks before cohabitation, during cohabitation, and until sacrifice when all F_1 litters were weaned. F_1 rats (23 to 29 per sex per dose) selected for continued evaluation were weaned onto the same diets as their parents. F_1 males were sacrificed at about 90 to 100 days of age, and F_1 females were sacrificed at about 70 days of age. A satellite group consisting of 10 F_0 females per dose were administered test or control diet as described for females in the main study; these animals, which were mated with F_0 males from the main study to produce F_1 offspring, were used for evaluation of test material and metabolite residues in serum during the premating phase of the study, in milk on day 2 and 12 postpartum, in prenursing neonate tissue, and in serum from weanling pups.

No treatment-related effects were observed on mortality or clinical signs of toxicity at any dose in adult F_0 or F_1 animals. One F_0 control male rat was sacrificed moribund due to causes unrelated to treatment with the test material; all other animals survived to study termination. Localized alopecia was observed in six F_0 male rats at 125 ppm compared with only one control ($p<0.05$). In 125-ppm group F_0 males, body weights were slightly decreased by 4 to 5% ($p<0.05$ compared with controls) from days 22 to 50. Body weights or body weight gain in other male and female F_0 and F_1 groups were not affected by

treatment with the test material at any time during the study. Food consumption values were similar in treated and controls groups.

In F_0 males at 125 ppm relative liver weights to body increased (7%) significantly ($p<0.05$) compared to controls. In the F_1 male and female weanlings at 125 ppm, significant ($p<0.05$) increases were observed in the absolute liver weight (19 and 14%), liver to body weight ratio (20 and 16%), and liver to brain weight ratio (19 and 14%), compared to controls, respectively. Treatment-related microscopic findings in the liver of 125-ppm group animals included hypertrophy of centrilobular hepatocytes with increased cytoplasmic eosinophilia in almost all F_0 and adult F_1 male and female rats, vacuolization of centrilobular hepatocytes in almost all F_1 weanlings examined, and hypertrophy of centrilobular hepatocytes with or without increased cytoplasmic eosinophilia in three F_1 female weanlings. These lesions were not observed in controls or treated animals administered 5 or 25 ppm of the test material. At 125 ppm, absolute spleen weight and spleen to brain weight ratio were significantly decreased in F_0 males; spleen to body weight and spleen to brain weight ratios were significantly decreased in F_1 male and female weanlings. The transient nature of the effect in males, the small difference between treated and control animals (-11 to -17%), and the lack of associated histopathological lesions suggest that the changes in spleen weight were equivocal.

The LOAEL for general systemic toxicity is 125 ppm (8.7/9.8 mg/kg/day)based on the histopathologic findings in the liver of adult F_0 and F_1 male and female rats (centrilobular hypertrophy of hepatocytes with increased cytoplasmic eosinophilia), F_1 male and female weanlings (vacuolization of centrilobular hepatocytes), and F_1 female weanlings (hypertrophy of centrilobular hepatocytes with or without increased cytoplasmic eosinophilia). The corresponding NOAEL is 25 ppm (1.7/2.0 mg/kg/day).

No treatment-related effects were observed on parameters of reproductive function or performance: length and periodicity of estrous cycle; epididymal sperm count, concentration, motility, and morphology; testicular spermatid count and concentration; sexual maturation as evidenced by age of vaginal opening in females and preputial separation in males; mean precoital interval; mating and fertility indices; and median gestation length. No treatment-related effects were observed on viability, clinical signs, body weight, or body weight gain of offspring. The viability and lactation indices were similar in treated and control groups. No treatment-related effects were observed on the number of stillborns, mean litter sizes at birth, or the sex ratio.

The weight of the testes and male accessory organs were similar in treated and control groups. Microscopic examination of the testes showed normal spermatogenic cycles in the seminiferous tubules of treated animals. Although an increase in the percent of nonmotile sperm was observed at 5 and 125 ppm in F_1 males, the lack of a corresponding decrease in the percent of motile sperm suggest that this effect is not treatment related.

Small, transient increases in mean absolute ovarian weight (23%, $p<0.05$) and the ovarian to body weight ratio (18%, $p<0.05$) at 125 ppm compared to controls in F_1 female weanling were not considered to be a treatment-related. No clear dose-response was observed as the mean ovarian weight at 125 ppm was 0.016 g compared with 0.015 g at 5 ppm, and the mean weight at 25 ppm (0.013 g) was identical to that of controls. No increase in ovarian weight was observed in adult F_0 or F_1 females at 125 ppm. No histopathologic lesions were observed in the ovaries of either generation. Ovarian follicle counts (primordial, growing, and antral) in F_0 and F_1 females in the control and 125-ppm groups showed a significantly increased number of antral follicles in treated F_1 females (33%, $p<0.05$) compared with the

control value. However, a recount of the follicles in F_1 females did not confirm the statistically significant differences noted for the original counts. Supplemental data (MRID 44624301)confirms the original findings that there is no treatment-related effects on the numbers of primordial and growing follicles in the adult F_0 or F_1 females Therefore, the results of the original antral follicle count should not be considered evidence of a treatment-related effect on the ovary.

Organ weight data showed statistically significant ($p<0.05$, compared with controls) changes in adrenal gland, thymus, and pituitary gland weights; however, these changes were considered to be unrelated (adrenals, thymus, and pituitary) to treatment with Dicofol at the doses used in this study. Absolute, organ to body weight ratios, and organ to brain weight ratios were increased by 31 to 50% for adrenal glands, 16 to 28% for thymus, and 66 to 76% for pituitary, compared to controls, at all doses tested. However, the lack of clear dose-response relationships, corresponding histopathological lesions, and consistency of results between generations or the sexes suggest that these organ weight changes are not related to treatment.

Exposure to Dicofol did not affect reproductive parameters or the on viability, clinical signs, body weight, or body weight gain of offspring at the doses used in this study. **Therefore, the reproductive and/or offspring toxicity NOAEL is >125 ppm (> 8.7/9.8 mg/kg/day).** Exposure to Dicofol also did not affect endocrine organs at the doses used in this study. The animals were adequately exposed to the test material during all phases of the study as shown by quantifiable levels of the Dicofol and metabolites in adult serum, milk, prenursing neonate tissue, and weanling serum.

This special reproduction study in the rat is classified **acceptable (nonguideline)** and does satisfy the purpose for which it was conducted: to determine the effects of Dicofol on reproductive performance in F_0 rats and determine the potential endocrine effects of Dicofol in F_0 and F_1 rats.

EPA Reviewer: Guruva B. Reddy, D.V.M., Ph.D.
Registration Action Branch 1 (7509C)
Work Assignment Manager: P.V. Shah, Ph.D.
Registration Action Branch 1 (7509C)

Date 7/13/06
Date 7/18/06

TXR# 0053453

DATA EVALUATION RECORD

STUDY TYPE: One-Generation Reproduction Study - Rat

DP BARCODE: D325924
P.C. CODE: 010501

SUBMISSION CODE:
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): Dicofol (96.4% a.i.; 82.2% p,p'- and 14.2% o,p'-isomer)

SYNONYMS: Kelthane®

CITATION: Rowe, J.N. (1997) Reproduction and postnatal study of potential endocrine effects of dicofol administered orally (diet) to rats. Argus Research Laboratories, Inc., Horsham, PA. Laboratory project ID 018-021, March 5, 1997. MRID 44253801. Unpublished.

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SPONSOR: Rohm and Haas Co. Box 904, 727 Norristown Rd., Spring House, PA 19477-0904.

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This special reproduction study in the rat is classified **acceptable (nonguideline)** and does satisfy the purpose for which it was conducted: to determine the effects of Dicofol on reproductive performance in F_0 rats and determine the potential endocrine effects of Dicofol in F_0 and F_1 rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. A Flagging statement was not provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Dicofol

Description: red-brown semi-solid
Lot/Batch #: 687 (TD No. 95-060)
Purity: 96.4% a.i.
CAS #: 115-32-5-2 (*p,p'*-Dicofol)

2. Vehicle

The test material was administered in feed (Purina Certified Rodent Diet® #5002)

3. Test animals

Species: rat
Strain: Crl:CD®BR VAF/Plus® (Sprague-Dawley derived)
Age at start of dosing: (F_0): 45 to 46 days (F_1): 21 days
Weight at start of dosing:
(F_0) Males: 160 - 186 g; Females: 138 - 164 g
(F_1) Males: 40 - 66 g; Females: 35 - 60 g
Source: Charles River Laboratories, Inc., Portage, MI
Housing: The rats were individually housed in stainless steel cages except during cohabitation and postpartum periods. One male and one female were housed together during cohabitation. Females were individually housed in nesting boxes at least one day before parturition and housed with pups during lactation.
Diet: Purina Certified Rodent Diet® No. 5002 *ad libitum*
Water: Municipal water *ad libitum*
Environmental conditions:
Temperature: 70 - 78°F
Humidity: 40 - 70%
Air changes: 10/hr
Photoperiod: 12 hrs dark/12 hrs light
Acclimation period (F_0): 7 days

B. PROCEDURES AND STUDY DESIGN

1. Mating procedure

One male and one female from the same test group (main study group) were allowed to cohabit for a maximum of 2 weeks. The females in the satellite group were allowed to cohabit with a male in the same test group that had already mated. No male was allowed to mate with more than two females. Females with sperm-positive vaginal smears or evidence of a copulatory plug were considered to be at day 0 of pregnancy.

2. Study schedule

F_0 males were administered the test or control diets continuously for 70 days before cohabitation, during cohabitation, and after cohabitation for a total of about 126 to 130 days of treatment. F_1 males selected for continued evaluation were given the same diets as their parents for 90 to 100 days of age. F_0 females were administered the test or control diets continuously for 70 days before cohabitation, during cohabitation, gestation, lactation, and for about 1 to 2 weeks after their litters were weaned. F_1 females selected for continued evaluation were administered the same diets as their parents until they were about 70 days of age. One F_1 male and one F_1 female pup were randomly selected from each litter for gross examination at 21 days postpartum

3. Animal assignment

F_0 animals were randomly assigned to the main study groups using a computer-generated randomization procedure based on animal weight. A computer-generated randomization was also used to assign ten females per group to the satellite study. The animals were assigned to the dietary concentrations as noted in Table 1. One male and one female F_1 weanling were randomly selected from each litter for further evaluation; they were assigned to the same test group as their parents.

TABLE 1. Animal assignment

Test group	Conc. in diet[a] (ppm)	Animals/group			
		F_0 Males	F_0 Females[b]	F_1 Males	F_1 Females
Control	0	30	40	25	25
Low (LDT)	5	30	40	24	23
Mid (MDT)	25	30	40	26	26
High (HDT)	125	30	40	29	28

Data taken from page 43, MRID 44253801.
[a]Dietary concentrations were based on purity of 98.9% a.i. reported initially.
[b]Included 10 rats/group for satellite study.

4. Dose selection rationale

Doses were selected based on previous two-generation studies conducted by the sponsor (Rohm and Haas Report No. 98R-028). Details were not presented by the study author.

5. Dosage preparation and analysis

Test diets were prepared every 7 to 16 days by the testing facility; no additional details regarding preparation were reported by the study author. Prepared diets were stored in a freezer (<-15°C) until used. Prior to the start of the study, homogeneity of the test substance was determined on duplicate samples taken from the top, middle, and bottom levels. Stability of the test substance in feed was evaluated on three sets of duplicate samples taken from the middle level. One sample each was stored in the light for 9 or 23 days at room temperature and then frozen. The third sample was stored frozen in the dark for 30 days. During the study, samples of treated food from ten different preparation dates were analyzed to verify concentration.

Results -
Homogeneity analysis: Each individual sample concentration was within ±10% of each target concentration. The ranges were as follows: 5 ppm: 5.05 to 5.20 ppm; 25 ppm: 23.9 to 24.8 ppm; 125 ppm: 126 to 128 ppm.

Stability analysis: All samples were within ±10% of the concentration on day 0 for all concentrations and storage conditions.

Concentration analysis: The average analytical concentrations were within ±10% of the target concentrations except for one 25-ppm sample (+14%). The ranges were as follows: 5 ppm: 4.87 to 5.47 ppm; 25 ppm: 24.0 to 28.5 ppm; 125 ppm: 118 to 135 ppm

The analytical data indicated that the mixing procedure was adequate, the test material was stable at ambient temperature for 23 days or frozen for 30 days, and the variance between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. Parental animals

Observations and the schedule for observations are summarized in Table 2. Food consumption was measured during cohabitation but not tabulated. The number of days of cohabitation and maternal behavior of the dams were recorded. Animals assigned to the satellite group were observed for mortality, clinical signs, body weight changes, and food consumption; data were recorded but not tabulated. Blood samples were collected from the lateral tail vein of all satellite animals during weeks 5 and 10 and milk was collected from up to nine satellite animals

per group on days 2 and 12 postpartum for quantification of dicofol (*o,p-* and *p,p-* isomers) and the metabolite (FW152) of each isomer.

Post weaning observations of F_1 animals included the age of vaginal patency (opening) in females, the age of preputial separation in males, and estrous cycling in females starting at 50 days of age.

Table 2. Observation schedule for F_0 and F_1 parental animals

Type of observation	No. Animals per sex per Group	Time of observation	Frequency of observation
Mortality	All	Throughout study	Twice daily
Clinical observations	All	Throughout study	Daily
Estrous cycle	All	Premating/mating	Daily for 21 days before mating, during mating until sperm positive
Maternal behavior	Dams that delivered litters naturally	Lactation	Days 1, 5, 8, 15, and 21
Body weight			
Females	All	Premating period	Once a week[a]
	All	Presumed gestation	Days 0, 7, 14, 20, 25
	All	Lactation	Days 1, 5, 8, 15, and 21
Males	All	Throughout study	Twice during acclimation, once a week during treatment, and at sacrifice
Food consumption			
Females	All	Premating period	Once a week
	All	Presumed gestation	Days 1, 7, 14, 20, and 25
	All	Lactation	Days 1, 5, 8, and 15
Males	All	Throughout study	Once a week except during mating

Data taken from pages 44-45, MRID 44253801.
[a]F_0 adults were weighed until cohabitation; F_1 animals were weighed until sacrificed.

2 Litter observations

According to the report, the following litter observations (X) were made (see Table 3). On day 5 postpartum, litters were standardized to a maximum of 8 pups/litter (4 per sex/litter, as nearly as possible). Physical observations included gross external physical anomalies. Litters of females assigned to the satellite group were counted each day, the results were recorded but not tabulated.

Blood samples or tissue from pups in each satellite dose group were collected for analysis of Dicofol and metabolites. The first three pups born to litters delivered by dams (selected for milk samples) between 0700 and 1900 EST or three pups that had not nursed (also from satellite dams) were collected for analysis of whole neonate tissue. Blood samples were collected from three decapitated day 5 culls from each litter in the satellite groups (not analyzed). Blood was also collected from the vena cava of one male and one female 21-day old pup from five randomly selected litters in each treatment group.

TABLE 3. F_1 Litter observations

Observation	Time of observation (lactation day)					
	Day 0	Day 5[a]	Day 5[b]	Day 8	Day 15	Day 21
Number of live pups	Daily					
Pup weight	X	X	X	X	X	X
Physical observations and external alterations	Daily					
Number of dead pups	Daily					
Sex of each pup (M/F)	X	X	X	X	X	X

Data taken from page 45, MRID 44253801.
[a]Before standardization (culling).
[b]After standardization (culling).

3. Sacrifice and postmortem observation

a. Parental animals

Gross and microscopic observations: Maternal animals in the satellite groups were sacrificed on day 12 postpartum without further evaluation. All surviving parental males were sacrificed immediately after all F_1 pups were weaned. Maternal animals in the main study were sacrificed 1 to 2 weeks after all F_1 pups were weaned. Maternal animals that did not deliver a litter were sacrificed and necropsied on day 25 of presumed gestation. F_1 males were sacrificed at 90 to 100 days of age and F_1 females were sacrificed about 70 days of age and subjected to postmortem and microscopic examination as described for F_0 rats. The animals were anesthetized by pentobarbital injection

(i.v.) followed by exsanguination and subjected to postmortem examinations.

Gross necropsy consisted of examination of the thoracic, abdominal, and pelvic viscera of all rats. The number of implantation sites was recorded for F_0 dams. The CHECKED (X) tissues were collected for microscopic examination and the (XX) organs were weighed. The pituitary gland, thymus, and adrenal glands were weighed after fixation; other organs were weighed fresh. Microscopic examination was performed on all tissues in the control and high dose group except for brain, and the left testes and left epididymis (used for sperm evaluation). Tissues from low- and mid-dose groups were examined microscopically if treatment-related effects were observed at the high-dose. Reproductive organs were examined from rats at the low- and mid-doses if reduced fertility was suspected. The right ovary was serially sectioned and 10 sections were selected randomly for oocyte count, and a section from the middle of the ovary was examined for pathologic changes. The uteri of apparently nonpregnant rats (sacrificed on day 25 of presumed pregnancy) were examined for implantation sites. The right epididymis was sectioned longitudinally so that the caput, corpus, and cauda could be examined.

XX	Ovaries/oviduct	XX	Epididymides
XX	Uterus/cervix	XX	Prostate
X	Vagina	XX	Seminal vesicles & coagulating gland
X	Mammary gland	XX	Testis
XX	Pituitary gland	XX	Adrenal gland
XX	Brain	XX	Kidney
XX	Liver	XX	Thymus
XX	Spleen	XX	Gross lesions

Data taken from page 49, MRID 44253801.

Sperm Evaluation: Sperm from the left epididymis and left testis of F_0 males sacrificed at study termination (about 126 to 130 days) were evaluated. Sperm were collected from the left distal cauda epididymis for evaluation of sperm motility and morphology. Sperm morphology and motility was evaluated in fresh specimens, and sperm morphology was also evaluated in fixed specimens. The percentage of normal sperm per 200 sperm was determined, and the number of abnormal sperm was determined qualitatively. The total number of sperm in the entire cauda epididymis (cauda reserves) were counted. In addition, spermatids were counted in the left testis. F_1 males were sacrificed at about 90 days of age and evaluated as described for F_0 males.

b. Offspring

Culled F_1 pups were sacrificed by carbon dioxide asphyxiation on day 5. Gross lesions from 1- to 4-day-old and 5- to 21-day-old pups were retained in fixative. All satellite pups not used for evaluation of test material and metabolite concentrations were discarded after day 12. On day 21 postpartum, postmortem examinations were conducted and organ weights were determination on one male and one female F_1 pup randomly selected from each litter. The 21-day-old pups were discarded if not selected for continued evaluation or postmortem examination. The CHECKED (X) tissues from the 21-day old pups were collected for microscopic examination and the (XX) organs were weighed. The thymus and adrenal glands were weighed after fixation, and the other organs were weighed fresh.

XX	Ovaries/oviduct	XX	Testis
XX	Adrenal gland	XX	Kidney
XX	Liver	XX	Thymus
XX	Brain	XX	Gross lesions
XX	Spleen		

Data taken from page 51, MRID 44253801.

D. DATA ANALYSIS

1. Statistical analyses

The individual rat was the unit of analysis for adult animals, and the litter was the unit for analysis for offspring. Statistical analyses were conducted as follows:

Paternal, maternal, and pup incidence data: Variance Test for Homogeneity of the Binomial Distribution.

Body weights, organ weights, food consumption values, pup body weight, sex ratio, mortality of pups: Bartlett's test for homogeneity of variance and analysis of variance (ANOVA) when Bartlett's test was not significant followed by Dunnett's test for determining statistical significance ($p \leq 0.05$) if ANOVA was significant. If Bartlett's test was significant, nonparametric test were applied (Kruskal-Wallis test when there were ≤75% ties or Fisher's exact test when there were >75% ties). If Kruskal-Wallis test was significant, Dunn's method of multiple comparison was used to identify statistical significance.

Natural delivery data: Kruskal-Wallis test

Sperm motility data (expressed as percentages): subjected to arcsine transformation followed by a parametric method.

2. Indices

a. Reproductive indices

The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Mating index: percentage of pairings that resulted in matings

Fertility index: (No. of pregnancies/No. of rats mated)×100

Gestation index (%): (No. dams with liveborn pups/No. pregnant rats) × 100

b. Offspring viability indices

The following indices were calculated from lactation records of litters in the study:

Viability index: (No. live pups at day 5 (precull)/No. live pups on day 1 postpartum) × 100

Lactation index: (No. live pups at day 21/No. live pups on day 5 (postcull)) × 100

3. Historical control data
Historical control data were provided for thymus, pituitary, and adrenal gland weights.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

Alopecia was observed on the limbs of six F_0 male rats in the 125-ppm group ($p<0.05$) compared with only one control. No statistically significant increases in the incidence of alopecia was observed in F_1 males, F_0 females, or F_1 females. There were no treatment related deaths during the study. One male rat in the F_0 control group was sacrificed moribund on day 65 because of effects resulting from a broken palate.

2. Body weight and food consumption

a. Premating/postweaning periods

Body weights, body weight gain, and food consumption data are summarized in Tables 4a (F_0 animals) and 4b (F_1 animals). Slight, but statistically significant decreases (4 to 5%) in body weight were seen during days 22 to 50 in F_0 male rats administered 125 ppm of the test material. Body weight measurement at other time points and dose levels in F_0 males and body weights at all time points and dose levels in F_1

males, F_0 females, and F_1 females were similar to the weights of corresponding controls. Body weight gain was similar for all time intervals in all treated and corresponding control groups except for statistically significant increases in 25- (42%) and 125-ppm (33%) group F_0 males between days 70 and 77 and in 5- (29%) and 25-ppm (25%) F_0 females between days 50 and 57. Overall weight gain in treated groups was similar to that observed for corresponding controls. Food consumption was similar in all treated and corresponding control groups except for a few sporadic time points.

b. Gestation and lactation periods

Mean absolute body weights of F_0 females administered all dose levels of the test material were similar (generally within ±2%) to controls during the gestational, lactational, and post lactational periods. Body weight gain in low- and high-dose groups were significantly lower (14 or 15%, $p<0.05$) than control weight gain for the first week of pregnancy, and weight gain over the entire gestation period for the low-dose group was significantly lower (10%, $p<0.05$) than that of the control group. Because mean body weights were generally within 2% of control weights and a clear dose-response relationship was not observed for body weight gain, these changes are not considered to be treatment related. Weight gain during the lactational and postlactational periods was similar in treated groups and controls. Food consumption was similar in treated groups and controls during gestation, lactation, and after lactation except for some sporadic time intervals.

Table 4a. Body weight, body weight gain, and food consumption during premating and postmating (males only) periods in F_0 rats fed Dicofol

Observations/study day	Concentration (ppm) 0	5	25	125
F_0 Generation Males - Premating/Postmating				
Mean body weight (g)				
day 1	175.9	173.6	174.2	174.4
day 22	331.5	329.0	326.9	319.2* (3.7)
day 50	447.3	451.4	445.8	428.2*(4.3)
day 70	495.9	504.0	496.6	475.9
day 126	578.6	584.0	582.6	556.0
Mean weight gain (g)				
day 1-70 (premating)	320.4	330.3	322.4	301.6
day 84-126 (postmating)	62.9	58.9	63.6	55.7
Mean food consumption - day 1-70				
(g/animal/day)	26.1	26.4	26.0	25.2
(g/kg b.w./day)	69.8	70.0	69.6	69.8
F_0 Generation Females - Premating				
Mean body weight (g)				
day 1	149.1	149.1	149.1	149.2
day 22	215.3	215.0	213.3	212.2
day 50	261.5	263.7	260.6	256.5
day 70	275.8	280.9	277.5	271.4
Mean weight gain (g) day 1-70	126.6	131.9	128.4	122.3
Mean food consumption - day 1-70				
(g/animal/day)	18.7	18.8	18.3	18.0
(g/kg b.w./day)	81.2	80.9	79.1	78.8

Data taken from Tables B3, B4, B5, B6 (pp. 87-94), C3, C4 (pp. 187-188), C11, and C12 (pp. 195-196), MRID 44253801.
*$p<0.05$, statistically significant compared with controls

Table 4b. Body weight, body weight gain, and food consumption during the postweaning period in F_1 rats fed Dicofol

Observations/study day	Concentration (ppm) 0	5	25	125
F_1 Generation Males				
Mean body weight (g)				
day 1	53.7	50.7	50.1	49.9
day 22	223.0	219.3	214.1	217.5
day 50	425.1	418.0	418.8	421.0
day 78	522.3	525.6	523.8	522.5
Mean weight gain (g) day 1-78	468.6	474.9	473.7	472.6
Mean food consumption - day 1-78				
(g/animal/day)	26.6	26.4	26.7	26.5
(g/kg b.w./day)	81.0	81.1	82.7	81.9
F_1 Generation Females				
Mean body weight (g)				
day 1	50.1	48.8	47.6	49.6
day 22	159.8	160.6	159.9	162.6
day 43	224.4	225.0	223.5	224.4
Mean weight gain (g) day 1-43	174.4	176.1	176.0	174.7
Mean food consumption - day 1-43				
(g/animal/day)	17.7	17.5	17.7	17.6
(g/kg b.w./day)	119.3	118.1	120.2	118.5

Data taken from Tables D3, D4, D5, D6 (pp. 347-350), E3, E4, E5, and E6 (431-434), MRID 44253801.

3. Test Substance Intake

Compound consumption was based on food consumption, body weight, and analytical purity of 98.9% a.i. The doses expressed as average daily intake in mg of Dicofol/kg body weight for the various treatment periods are presented in Table 5.

Table 5. Average test substance intake (mg/kg body weight/day)

Treatment period	Male			Female		
	5 ppm	25 ppm	125 ppm	5 ppm	25 ppm	125 ppm
F_0 Generation						
Premating	0.3	1.7	8.7	0.4	2.0	9.8
Gestation	-	-	-	0.4	1.8	9.0
Lactation	-	-	-	0.6	3.3	17.0
F_1 Generation						
Postweaning	0.4	2.1	10.2	0.6	3.0	14.8

Data extracted from pages 56, 57, and 65, MRID 44253801.

4. Reproductive function

a. Estrous cycle length and periodicity

Table 6 presents the summary of estrus cycling in F_0 and F_1 females on Dicofol. Vaginal smears were evaluated to determine the number of estrous cycles attained within a 21-day period. No treatment-related effect was observed. The mean number of estrous cycles in F_0 female rats ranged from 5.2 in the control group to 5.4 in the 25-ppm group. In F_1 females the mean number of estrous cycles in 21 days ranged from 4.6 for controls to 5.3 for the 25-ppm group. In the F_0 females, only one animal (5-ppm group) remained in diestrus for ≥6 days and no animals in any group remained in estrus for •6 days. Supplemental data reveal that 2 control and 2 low dose (5ppm) F_1 females were in diestrus for •6 days (MRID 44559901). The data also indicate that none of the animals remained in estrus for more than 6 days.

Table 6. *Estrous Cycle Length in F_0 and F_1 Females on Dicofol*

Observation	(0) Conrol	5 ppm	25 ppm	125 ppm
F_0 Females				
Estrous stages/21 Days[a]	5.2 ± 0.6	5.3 ± 1.0	5.4 ±0.7	5.3 ±0.7
3 or more consecutive Days of estrus	6	1	2	2
4 or more consecutive Days of diestrus	1	1	0	1
6 or more consecutive Days of estrus	0	0	0	0
6 or more consecutive Days of diestrus	0	1	0	0
F_1 Females				
Estrous stages/21 Days[a]	4.1 ± 1.0	4.8 ± 1.0	5.3 ± 0.5	5.0 ± 0.7
3 or more consecutive Days of estrus	0	1	1	2
4 or more consecutive Days of diestrus	4	4	1	3
6 or more consecutive Days of estrus	0	0	0	0
6 or more consecutive Days of diestrus	2	2	0	0

Data extracted from pages 7 and 8, MRID 44559901.
[a] Mean ± S.D.

b. Sperm measures

In F_0 males, the total number of motile, nonmotile, and motile plus nonmotile sperm, the average sperm count and concentration, the percent abnormal sperm, and sperm morphology in the cauda epididymis did not differ significantly between treatment and control groups. Evaluation of fixed specimens showed that the average number of sperm with no heads was significantly elevated at the 5-ppm level (3.3 vs 2.2 per rat in controls, $p<0.05$). Because no increase was observed at the 25- or 125-ppm levels, this effect is not considered to be treatment related. Testicular spermatid counts and concentrations in F_0 males were not significantly different in treated groups compared with controls.

In F_1 males, the total number of nonmotile sperm was slightly, but significantly increased ($p \leq 0.05$) in rats administered 5 (17%) and 125 ppm (16%), compared to the controls. The percent nonmotile sperm in males administered the 25-ppm dose showed a greater increase (37%), but did not achieve statistical significance compared with that of controls; the increase was probably due to the large number of abnormal sperm observed in one 25-ppm group animal. No significant decrease was observed in the percent of motile sperm suggesting that the small increase in nonmotile sperm is unlikely to be treatment-related. There were no significant increases in the average count, concentration, or percent abnormal caudal epididymal sperm in F_1 rats. In addition, the morphology of caudal epididymal sperm in 125-ppm group F_1 males was not different from that of controls. The testicular spermatid count and concentration were also unaffected by treatment with the test material.

c. Sexual maturation (F_1)

Sexual maturation was unaffected by treatment with the test material as measured by the age of preputial separation in F_1 male rats or the age of vaginal patency in F_1 female rats.

5. Reproductive performance

Results for the parental animals are summarized in Table 7. No treatment-related effects were observed on reproductive performance of F_0 male and female rats. The fertility index was low for 5-ppm male and female rats compared with the other groups, but sufficient numbers of litters were produced by all groups for F_1 evaluations. The precoital and gestation intervals were similar for all groups.

Table 7. Reproductive performance in F_0 male and female rats fed Dicofol

Observation	Concentration (ppm)			
	0	5	25	125
Mean precoital interval (days)	2.8 ±1.6	3.4±2.8	2.6±1.2	3.1±1.4
Males				
No. paired	29	30	30	30
No. that mated	28	30	28	30
No. fertile[a]	25	23	26	29
Intercurrent deaths	1	0	0	0
Females				
No. paired	30	30	30	30
No. that mated	29	30	28	30
No. fertile[a]	25	23	26	29
Intercurrent deaths	0	0	0	0
No. of litters	25	23	26	29
Indices (%)				
Mating index - males	96.6	100.0	93.3	100.0
Mating index - females	96.7	100.0	93.3	100.0
Fertility index - males	89.3	76.7	92.8	96.7
Fertility index - females	86.2	76.7	92.8	96.7
Gestation index	100.0	100.0	100.0	100.0
Median gestation interval (days)	23.0±0.3	23.0±0.4	23.0±0.4	22.9±0.4

Data taken from Tables B7 (p. 95), C17, and C18 (pp. 202-203), MRID 44253801.
[a]Determined by the number of confirmed pregnancies of females.

6. Parental postmortem results

a. Organ weights

Mean organ weights showing statistically significant differences between the treated and control groups are summarized in Table 8. No treatment related effects of organ weights were observed in F_0 or F_1 adult female rats. Absolute liver weights and the liver to brain weight ratio in F_0 males were not significantly elevated at 125 ppm compared with control, but the liver to body weight ratio was significantly elevated (7%). Liver weights were slightly elevated in F_0 females and F_1 males and females at 125 ppm; however, statistical significance

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was not achieved. Absolute spleen weight (11%) and the spleen to brain weight ratio (11%) were significantly decreased in 125 ppm F_0 males compared with controls; the spleen to body weight ratio (8%) was not significantly decreased. In F_1 males, absolute weights, organ to body weight ratios, and organ to brain weight ratios of adrenal glands (29 to 50%), thymus (16 to 28%), and pituitary weights (66 to 76%) were significantly increased ($p<0.05$) at all dose levels. However, no clear dose-response relationships were observed for any of these organ weight changes.

Table 8. Organ weights (g) and organ weight ratios (%) in adult male rats fed Dicofol

Organ	Concentration (ppm)			
	0	5	25	125
F_0 generation				
Body weight[a]	582.1 ± 51.0	588.8 ± 41.6	587.4 ± 58.6	561.8 ± 47.8
Liver	19.22 ± 2.50[b] 3.296 ± 0.269 927.50 ± 123.30	19.73 ± 2.99 3.344 ± 0.386 963.42 ± 151.78	19.82 ± 2.68 3.370 ± 0.266 956.33 ± 136.42	19.82 ± 2.54 3.525 ± 0.294* 957.75 ± 129.62
Spleen	0.84 ± 0.17 0.144 ± 0.025 40.48 ± 7.91	0.82 ± 0.12 0.140 ± 0.021 40.23 ± 5.93	0.84 ± 0.14 0.144 ± 0.029 40.73 ± 6.79	0.75 ± 0.12* 0.133 ± 0.021 36.13 ± 4.86*
F_1 generation				
Body weight[a]	551.7 ± 41.2	553.9 ± 46.1	554.4 ± 45.1	551.8 ±53.3
Adrenals	0.0498 ± 0.0116 9.047 ± 2.013 2.41 ±0.58	0.0745 ±0.0093* 13.378 ±2.142* 3.62 ±0.46*	0.0681 ± 0.0104* 12.446 ± 1.983* 3.37 ± 0.60*	0.0654 ± 0.0065* 11.969 ± 1.346* 3.20 ± 0.33*
Thymus	0.4121 ± 0.1124 74.776 ±20.003 19.88 ± 5.78	0.5241 ± 0.1031* 94.792 ± 18.213* 25.49 ± 5.25*	0.5139 ± 0.0814* 92.996 ± 14.596* 25.18 ± 3.91*	0.4767 ± 0.1401 86.756 ± 24.730* 23.29 ± 6.64*
Pituitary	0.0104 ± 0.0029 1.893 ± 0.558 0.50 ±0.14	0.0179 ± 0.0019* 3.254 ± 0.402* 0.87 ± 0.09*	0.0180 ± 0.0029* 3.250 ± 0.470* 0.88 ± 0.15*	0.0173 ± 0.0021* 3.146 ± 0.375* 0.84 ± 0.10*

Data taken from Tables B8-B10 (pp. 96-101) D9-D11 (pp. 353-358, MRID 44253801.
[a]Terminal body weight in g ± standard deviation.
[b]Mean ± standard deviation; first row, absolute organ weights; second row, (organ weight/terminal body weight) × 100; third row. (organ weight/brain weight) × 100

b. Pathology

Macroscopic examination: There was no statistically significant increase in gross lesions at any site in male or female rats of either generation compared with corresponding controls. However, an increase was observed in the incidence of dilation of the kidney pelvis in 125-ppm group F_1 females (5/28 vs 1/25 in controls, N.S.).

Microscopic examination: Table 9 summarize the microscopic findings in F_0 and F_1 male and female rats administered the test material. The only treatment-

related findings were hypertrophy and increased cytoplasmic eosinophilia of centrilobular hepatocytes in almost all male and female rats of both generations administered 125 ppm of the test material. The histologic findings were distributed diffusely in all lobes in F_0 animals and multifocally to diffusely in F_1 animals. These findings were not seen in control or the lower dose groups.

The spermatogenic cycle in seminiferous tubules was examined in control and 125-ppm group males. The stages appeared normal in all F_0 males in the 125-ppm and control groups; abnormal spermatogenic stages were seen in one F_1 male in the control group. Unilateral atrophy of the testes was observed in one F_0 male and two F_1 males in the 125-ppm group, no F_0 controls, and in four F_1 controls.

No treatment-related histopathological changes were observed on the testes, ovaries, other reproductive organs, or endocrine organs in treated animals of either generation. A section through the middle of the ovary was examined for pathological variations; no abnormalities were observed in the ovaries of 125-ppm group females of either generation. In addition, the stage of the estrous cycle was determined for 125-ppm and control group animals at the time of sacrifice; no differences were observed in the distribution of animals in the four stages of the estrous cycle as compared with controls.

Follicle counts are summarized in Table 10a. Follicle counts were conducted on every tenth serial section of the right ovaries of control and 125-ppm F_0 and F_1 group females. Primordial, growing, and antral follicles were counted separately. The number of antral follicles per animal was significantly elevated at the 125-ppm level for F_1 females (33%, $p<0.05$ compared with controls). Because of the lack of histomorphological changes accompanying the increased follicle count and the interindividual variations in follicle counts by the technicians, follicles in F_1 control and 125-ppm females were recounted in a blind study. The statistical difference in the antral follicle count was not confirmed by the recount. In fact, the mean values for the recount were statistically significantly different ($p<0.01$ Student's t-test calculated by the reviewer) from the original counts for primordial, growing, and antral follicles in controls and for growing and antral follicles in 125-ppm group animals. In later supplemental submission (MRID 44624301) the follicle counts were conducted on the slices of right ovary yielded similar conclusions as described above (Table 10b).

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Table 9. Microscopic findings in male and female F_0 and F_1 generation rats fed Dicofol

Organ/lesion	Concentration (ppm)			
	0	5	25	125
F_0 Generation				
Males				
Liver, centrilobular hepatocytes				
Hypertrophy	0/30[a]	0/30	0/30	30/30**
Increased cytoplasmic eosinophilia	0/30	0/30	0/30	30/30**
Females				
Liver, centrilobular hepatocytes				
Hypertrophy	0/30	0/30	0/30	28/30**
Increased cytoplasmic eosinophilia	0/30	0/30	0/30	28/30**
F_1 Generation				
Males				
Liver, centrilobular hepatocytes				
Hypertrophy	0/25	0/23	0/26	29/29**
Increased cytoplasmic eosinophilia	0/25	0/23	0/26	29/29**
Females				
Liver, centrilobular hepatocytes				
Hypertrophy	0/25	0/23	0/26	21/28**
Increased cytoplasmic eosinophilia	0/25	0/23	0/26	21/28**

Data taken from Pathology Report Tables 5 (pp. 829-831) and 9 (pp. 889-892), MRID 44253801.
[a]Number of animals with a lesion/number of animals examined.
*p<0.05, **p<0.01 compared with controls calculated by the reviewer using the Fisher exact test.

Table 10a. Follicle counts in F_0 and F_1 generation females fed Dicofol

Treatment group	No. examined	Follicle stage		
		Primordial	Growing	Antral
F_0 Generation				
Control	30	270 ± 115.5[a]	33 ± 19.9	22 ± 10.1
125 ppm	30	271 ± 124.5	38 ± 22.6	18 ± 8.1
F_1 Generation				
Control	25	257 ± 71.7	47 ± 25.4	27 ± 10.5
125 ppm	27	260 ±76.1	53 ± 30.2	36 ± 18.6*
F_1 Generation - Recount				
Control	25	299 ± 68.9	80 ± 22.1	45 ± 13.3
125 ppm	27	307 ± 117.2	85 ± 32.5	54 ± 27.8

Data taken from Ovarian Histopathology and Follicle Count, Tables 2 and 3 (pp. 971-973).
[a]Mean ± standard deviation
*p<0.05, compared with the control group.

Table 10b. Follicle re-counts in F_0 and F_1 generation females fed Dicofol		
Treatment group	**No. examined**	**Follicle stage**
		Primordial and Growing
F_0 Generation		
Control	30	126 ± 47.7[a]
125 ppm	30	126 ± 45.7
F_1 Generation		
Control	25	101 ± 27.6
125 ppm	27	116 ± 42.6

Data taken from Ovarian Histopathology and Follicle Count, Tables 1 (pp. 9), MRID 44624301.
[a]Mean ± standard deviation
*$p<0.05$, compared with the control group.

B. OFFSPRING

1. Viability and clinical signs

Mean litter size and viability results from pups during lactation are summarized in Table 11. There were no statistically significant differences between treated groups and controls for any of the parameters examined. No treatment-related clinical signs were observed in the pups.

Table 11. Mean litter size and viability of F_1 generation pups				
Observation/study time	**Concentration (ppm)**			
	0	**5**	**25**	**125**
Total no. of litters	25	23	26	29
Total no. pups born	328	301	333	379
Total. no. liveborn	328	301	330	377
Total no. stillborn	0	0	3	2
Mean litter size - day 1	13.1 ±1.9	13.1 ± 2.1	12.8 ± 2.7	13.1 ± 3.1
Mean no. live pups/litter ± standard deviation				
Day 1	13.1 ± 1.9	13.1 ± 2.1	12.7 ± 2.7	13.0 ± 3.0
Day 5 (precull)	12.9 ±1.9	12.9 ±2.0	12.3 ± 2.9	12.8 ± 3.0
Day 5 (postcull)	8.0 ± 0.0	8.0 ± 0.0	7.7 ± 1.2	7.8 ± 1.1
Day 21	8.0 ± 0.0	8.0 ± 0.0	7.7 ± 1.2	7.8 ± 1.1
Survival indices				
Viability index (%)	98.5	98.7	97.0	98.7
Lactation index (%)	100.0	100.0	100.0	100.0
Sex ratio (% males) - day 1	50.3	48.2	50.0	50.6
Sex ratio (% males) - day 5 (precull)	50.1	48.6	50.7	50.5

Data taken from Table C19, pp. 204-206, MRID 44253801.

2. Body weight

Mean body weights and weight gain of pups in treated groups were not statistically significantly different from those of the control group. Selected mean pup body weight data are presented in Table 12.

Table 12. Mean litter weights in F_1 generation pups				
Day of lactation	Concentration (ppm)			
	0	5	25	125
Mean pup weight per litter (g) ± standard deviation				
Day 1	6.3 ± 0.6	6.5 ± 0.6	6.3 ± 0.6	6.3 ± 0.6
Day 5 (precull)	10.3 ± 1.0	10.4 ± 1.1	10.4 ± 1.4	10.2 ± 1.3
Day 5 (postcull)	10.5 ± 1.0	10.5 ± 1.1	10.6 ± 1.3	10.4 ± 1.2
Day 15	34.5 ± 2.8	33.6 ± 2.7	33.7 ± 3.5	33.1 ± 3.1
Day 21	47.0 ± 3.8	46.1 ± 4.4	45.6 ± 6.4	45.9 ± 4.9
Weight gain (g)[a]				
Day 1-5 (precull)	4.0	3.9	4.1	3.9
Day 5 (postcull)-15	24.0	23.1	23.1	22.7
Day 15-21	12.5	12.5	11.9	12.8
Day 1-21	40.7	39.6	39.3	39.6

Data taken from Table C19, p. 206, MRID 44253801.
[a] Body weight gain calculated by the reviewer.

3. Offspring postmortem results

a. Organ weights

Organ weights in 21-day old pups are summarized in Table 13. At 125 ppm, liver, kidney, and spleen weights in male pups and liver, spleen, and ovarian weights in female pups showed statistically significant ($p<0.05$) changes relative to controls. Absolute liver weights were significantly ($p<0.05$) elevated by 19 and 14%, liver to body weight ratios by 20 and 16%, and liver to brain weight ratios by 19 and 14% at 125 ppm in male and female pups, compared to the controls, respectively. Spleen to body weight ratios were significantly reduced in both sexes (15 to 16%) at 125 ppm and in females at 5 ppm (15%). The spleen to brain weight ratio was significantly reduced only in female pups (17%) at 125 ppm. Absolute spleen weights (15 to 17%) in both sexes at 125 ppm and spleen to brain weight ratio (16%, $p<0.05$) for male pups were reduced. Kidney to body weight (8%) and kidney to brain weight (9%) ratios in 125-ppm group male pups were reduced relative to controls; the 9% reduction for absolute kidney weight did not achieve statistical significance. Absolute ovarian weight (23%) and the ovarian to body weight ratio (18%) were significantly elevated in female pups at 125 ppm; the ovarian to brain weight ratio (15%) was elevated, but not significantly.

Table 13. Organ weights in F_1 male and female weanlings fed Dicofol

Organ	Concentration (ppm)			
	0	5	25	125
Males				
Liver	2.10 ± 0.28[a] 4.428 ± 0.330 137.48 ± 17.65	2.04 ± 0.28 4.370 ± 0.357 134.65 ± 19.14	2.16 ± 0.37 4.618 ± 0.419 141.16 ± 20.68	2.49 ± 0.35* 5.319 ± 0.344* 163.64 ± 20.01*
Spleen	0.24 ± 0.07 0.495 ± 0.110 15.49 ± 4.24	0.23 ± 0.05 0.484 ± 0.075 14.95 ± 2.89	0.22 ± 0.06 0.474 ± 0.106 14.56 ± 3.59	0.20 ± 0.06 0.421 ± 0.104* 13.02 ± 3.72
Kidneys	0.66 ± 0.07 1.397 ± 0.080 43.36 ± 4.65	0.63 ± 0.08 1.364 ± 0.121 41.93 ± 5.54	0.65 ± 0.09 1.401 ± 0.235 42.43 ± 5.50	0.60 ± 0.08 1.288 ± 0.112* 39.49 ± 4.91*
Females				
Liver	2.09 ± 0.29 4.530 ± 0.446 142.37 ± 20.54	1.95 ± 0.34 4.305 ± 0.420 132.15 ± 25.91	2.08 ± 0.38 4.665 ± 0.497 143.80 ± 25.74	2.39 ± 0.36* 5.235 ± 0.475* 162.49 ± 21.92*
Spleen	0.26 ± 0.07 0.562 ± 0.144 17.69 ± 4.85	0.22 ± 0.06 0.475 ± 0.087* 14.69 ± 3.88*	0.23 ± 0.06 0.502 ± 0.096 15.61 ± 3.70	0.22 ± 0.06 0.473 ± 0.120* 14.70 ± 3.97*
Ovaries	0.013 ± 0.004 29.200 ± 7.25 0.92 ± 0.25	0.015 ± 0.004 33.454 ± 8.552 1.03 ± 0.34	0.013 ± 0.003 29.444 ± 5.559 0.90 ± 0.18	0.016 ± 0.004* 34.329 ± 7.838* 1.06 ± 0.25

Data taken from Tables C25-C30 (213-218), MRID 44253801.
[a]Mean ± standard deviation; first row, absolute organ weights; second row, organ weight/terminal body weight) × 100; third row, (organ weight/brain weight) × 100
*$p<0.05$ compared with controls.

b. Pathology

Macroscopic examination: Gross findings in the treated groups were similar in type and incidence to those seen in controls.

Microscopic examination: Microscopic findings in F_1 weanlings are summarized in Table 14. No lesions were observed at 5 and 25 ppm. Treatment-related lesions were observed in the liver of male and female weanlings at the 125 ppm dose level. Vacuolization of the centrilobular hepatocytes occurred in 93% of the male weanlings examined and in 96% of the female weanlings. In addition, hypertrophy of the centrilobular hepatocytes was seen in three female weanlings; hypertrophy was accompanied by increased cytoplasmic eosinophilia in two weanlings.

Table 14. Microscopic findings in F_1 generation weanlings

Organ/lesion	Concentration (ppm)			
	0	5	25	125
Males				
Liver, centrilobular hepatocytes				
Vacuolization	0/25	0/23	0/25	27/29**
Hypertrophy	0/25	0/23	0/25	0/29
Increased cytoplasmic eosinophilia	0/25	0/23	0/25	0/29
Females				
Liver, centrilobular hepatocytes				
Vacuolization	0/25	0/23	0/25	27/28**
Hypertrophy	0/25	0/23	0/25	3/28
Increased cytoplasmic eosinophilia	0/25	0/23	0/25	2/28

Data taken from the Pathology Report, Table 13, p. 941, MRID 44253801.
**p<0.01, compared with the control group.

C. DICOFOL AND METABOLITES IN SERUM, MILK, AND NEONATE TISSUE

1. Analysis of serum and milk from adult satellite animals

Serum was collected from five adult satellite F_0 females after 5 and 10 weeks on study and milk was collected from five satellite F_0 dams on day 2 and day 12 postpartum for analysis of the two isomers of Dicofol (*p,p'*-Dicofol and *o,p'*-Dicofol) and their metabolites (*p,p'*-FW152 and *o,p'*-FW152). The limit of quantitation (LOQ) was 0.02 ppm for serum and milk. Samples with reported concentrations <LOQ were given the value of 0.02 ppm, and samples reported as not detectable levels (not detectable = ND) were given values ½ LOQ (0.01 ppm). Generally, mean values ≤0.02 ppm were comprised of individual values that were not quantifiable (<LOQ or ND). The results are summarized in Table 15.

No residues were quantifiable (<LOQ or ND) in control serum; *p,p'*-Dicofol was the only residue quantifiable in control milk. Exposure to the adult rats was confirmed by quantifiable levels of Dicofol residues in adult serum. *p,p'*-Dicofol was quantifiable in serum at all doses and showed a clear dose-related increases at 5 and 10 weeks. *p,p'*-FW152 was quantifiable in serum only at the 125-ppm dose level. The concentrations of the Dicofol in adult serum remained constant with continued treatment between 5 and 10 weeks. The *o,p'*-isomer and metabolite were not quantifiable in serum. Exposure of the pups during lactation was confirmed by quantifiable concentrations of Dicofol in milk. The concentrations of *p,p'*-Dicofol and the

metabolite showed clear dose-related increases in milk. The concentrations in milk decreased considerably between day 2 and 12 of lactation; *p,p'*-Dicofol decreased by 70 to 75% and its metabolite decreased by 76 to 85%, excluding the 5 ppm concentration that was below the LOQ. Where quantifiable concentrations of *o,p'*-Dicofol were found in milk, the concentrations were very low (<1%) compared with that of *p,p'*-Dicofol, although *o,p'*-Dicofol constituted about 14% of the technical formulation.

Table 15. Dicofol and metabolite concentrations in serum and milk of adult F_0 female rats

Sample/time	Dose group	Residue recovered (ppm)[a]			
		***p,p'*-Dicofol**	***o,p'*-Dicofol**	***p,p'*-FW152**	***o,p'*-FW152**
Serum/5 weeks	Control	0.0120	0.0100	0.0160	0.0120
	5 ppm	0.0665	0.0100	0.0100	0.0140
	25 ppm	0.269	0.0100	0.0200	0.0160
	125 ppm	1.19	0.0140	0.0224	0.0200
Serum/10 weeks	Control	0.0120	0.0100	0.0100	0.0200
	5 ppm	0.0827	0.0100	0.0100	0.0200
	25 ppm	0.315	0.0100	0.0200	0.0200
	125 ppm	1.20	0.0160	0.0247	0.0200
Milk/day 2 p.p.	Control	0.0234	0.0120	0.0140	0.0180
	5 ppm	4.58	0.0289	0.0217	0.0221
	25 ppm	18.7	0.0604	0.229	0.0796
	125 ppm	72.3	0.103	1.97	0.179
Milk/day 12 p.p.	Control	0.0621	0.0100	0.0180	0.0200
	5 ppm	1.38	0.0199	0.0180	0.0200
	25 ppm	4.59	0.0337	0.0539	0.0348
	125 ppm	18.0	0.100	0.297	0.0773

Data taken from Appendix I, Tables I (pp. 674-676) and III (679-681), MRID 44253801.
[a]Residues reported as less than the limit of quantitation (<LOQ) were assigned a value of 0.02 ppm, which is equal the LOQ; residues reported as not detectable (ND) were assigned values of ½ LOQ (0.01 ppm).
p.p. = postpartum

2. Analysis of neonate tissue and serum from weanling rats

Whole tissue from neonates that had not nursed and serum from 21-day old weanlings were analyzed for the presence of Dicofol and metabolites. The results are summarized in Table 16. Exposure of the fetus to Dicofol was confirmed by detectable residue levels in the whole tissue of prenursing neonates. The concentrations of the *p,p'*-isomer and its metabolite, *p,p'*-FW152, showed dose-related increases in neonate tissue and weanling serum. The concentrations of *o,p'*-isomer and its metabolite were very low, such that quantifiable levels were found in neonate tissue only at the 125-ppm dose level; therefore, *p,p'*-Dicofol also comprised almost all the residue recovered from neonate tissue and weanling serum.

Table 16. Dicofol and metabolite concentrations in F_1 neonate tissue and weanling serum

Sample/time	Dose group	Residue recovered (ppm)[a]			
		p,p'-Dicofol	*o,p'*-Dicofol	*p,p'*-FW152	*o,p'*-FW152
Neonate tissue	Control	0.0050	0.0050	0.0050	0.0050
	5 ppm	0.0962	0.0050	0.0101	0.0050
	25 ppm	0.345	0.0050	0.0537	0.0100
	125 ppm	1.43	0.0128	0.284	0.0100
Serum/weanlings	Control	0.0200	0.0120	0.0160	0.0200
	5 ppm	0.241	0.0100	0.0229	0.0200
	25 ppm	0.492	0.0100	0.0360	0.0200
	125 ppm	1.25	0.0180	0.107	0.0180

Data taken from Appendix I, Tables II (pp. 677-678) and IV (682-683), MRID 44253801.
[a]Residues reported as less than the limit of quantitation (<LOQ) were assigned a value of 0.02 ppm (weanling serum) or 0.01 ppm (neonate tissue), which is equal the LOQ; residues reported as not detectable (ND) were assigned values of ½ LOQ (0.01 or 0.0050 ppm for weanling serum and neonate tissue, respectively).

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study author concluded that Dicofol at concentrations as high as 125 ppm did not cause adverse reproductive or endocrine effects in parental animals or offspring. *In utero* and lactational exposure was confirmed by detectable levels of Dicofol in the prenursing neonate, weanling serum,

and dam milk. The no-observed-effect level (NOAEL) for general toxicity was 25 ppm (1.7 and 2.0 mg/kg/day premating dose for F_0 male and female) based on the following effects at 125 ppm: transient decrease in body weight and organ weight changes in F_0 males, histopathologic alterations in the liver of F_0 and adult F_1 males and females, organ weight changes (liver, spleen, and kidneys) and histopathologic alterations in the liver of F_1 weanlings. The NOAEL for reproductive and developmental effects was also 25 ppm based on equivocal ovarian weight changes in F_1 weanlings at 125 ppm.

B. REVIEWER'S DISCUSSION

Groups of 30 male and 30 female rats were administered Dicofol in the diet at concentrations of 0, 5, 25, or 125 ppm for 70 days before mating, during mating, gestation, lactation, and after lactation. F_1 male and female rats produced by mating the F_0 parents were weaned onto the same diets as their respective parents. F_1 males were administered the diets until about 90 days of age and F_1 females until about 70 days of age. In addition, a satellite study using groups of 10 F_0 females treated the same as females in the main study was conducted to investigate residues of Dicofol and metabolite concentrations in serum during premating, milk during lactation, whole tissue of prenursing pups, and serum from 21-day old pups.

1. *Systemic toxicity*

Except for one F_0 control male that was sacrificed moribund because of effects unrelated to treatment with Dicofol, no animals died during the study. The only clinical sign showing a statistically significant increased incidence was alopecia in F_0 males. Alopecia is a common finding as indicated by similar incidences in treated F_1 males, F_0 females, and F_1 females and their corresponding controls. Therefore, alopecia is not considered to be a treatment-related effect in F_0 males. Body weights in 125-ppm group F_0 male rats showed statistically significant, transient decreases that did not exceed 5% compared with the control values; body weight gain in F_0 males was not affected by treatment. In the absence of an effect on body weight gain, the small decrease in body weight is not considered to be toxicologically significant. Dicofol had no effect on body weights or body weight gain in adult F_1 males, F_0 females, or F_1 females. In addition, treatment-related effects were not observed on body weight, body weight gain, or food consumption in F_0 dams during gestation, lactation, or the postlactational period.

Absolute liver weights in adult F_0 males were not affected by treatment, but the liver to terminal body weight ratio was slightly but statistically significantly elevated in F_0 male rats at 125 ppm. Absolute liver weights, liver to body weights, and

liver to brain weights of male and female weanlings also were significantly elevated at 125 ppm. Increases in liver weights were observed in adult F_1 males, adult F_0 females, and F_1 females at 125 ppm, but statistical significance was not achieved. Hypertrophy of centrilobular hepatocytes with increased cytoplasmic eosinophilia was observed in almost all adult male and female rats administered 125 ppm of the test material, and vacuolization of centrilobular hepatocytes was observed in the liver of almost all male and female weanlings. In addition, hypertrophy with or without increased cytoplasmic eosinophilia was observed in a few female weanlings. The increases in liver weights and microscopic findings in the liver are treatment-related findings; the liver is a target for Dicofol.

Absolute and relative (to brain weight) spleen weights were significantly decreased at 125 ppm in F_0 males and appeared to be dose related. Spleen weights (organ to body weight and brain weight ratios) were significantly reduced in male and female weanlings. The reduced relative spleen weight ratios of the weanlings did not carry over to the spleen weights of adult F_1 males and females, which were not affected by treatment with Dicofol. No histopathologic effects were observed in the spleen of adult or weanling rats. The evidence for the relationship between treatment and reduced spleen weights is equivocal, because the different effect on spleen weights between adult F_0 and F_1 males cannot be explained, decrease in weanlings was transient, and histopathologic lesions were not observed in the spleen.

The kidney weight in male weanlings showed a slight but statistically significant decrease at 125 ppm; there was no associated microscopic findings. In addition, no effect on kidney weight was observed in adult F_0 or F_1 males. Therefore, the decreased kidney weights are not considered to be treatment related.

In conclusion, the lowest-observed-effect level (LOAEL) for general systemic toxicity is 125 ppm (8.7/9.8 mg/kg/day) based on the histopathologic findings in the liver of adult F_0 and F_1 male and female rats (centrilobular hypertrophy of hepatocytes with increased cytoplasmic eosinophilia), F_1 male and female weanlings (vacuolization of centrilobular hepatocytes), and F_1 female weanlings hypertrophy of centrilobular hepatocytes with or without increased cytoplasmic eosinophilia). The corresponding NOAEL is 25 ppm (1.7/2.0 mg/kg/day).

2. Reproductive toxicity and endocrine effects

No treatment-related effects were observed on parameters of reproductive function or performance: length and periodicity of estrous cycle; epididymal sperm count, concentration, motility, and morphology;

testicular spermatid count and concentration; sexual maturation as evidenced by age of vaginal opening in females and preputial separation in males; mean precoital interval; mating and fertility indices; and median gestation length. No treatment-related effects were observed on viability, clinical signs, body weight, or body weight gain of offspring. The viability and lactation indices were similar in treated and control groups. There was no increase in the number of stillborns, no decrease in mean litter sizes at birth, and no effect on the sex ratio in the treated groups compared with controls. Reduced viability, increased numbers of stillborn pups, total litter loss, and reduced pup body weights were observed at 125 and 250 ppm in a two-generation reproduction study (MRID 41806601).

The number of sperm with no heads was significantly increased at 5 ppm in F_0 males. However, the increase was not considered to be treatment-related, because no effect was observed at the higher doses. The percent of nonmotile sperm was increase at all doses in F_1 males; statistical significance was achieved at the 5- and 125 ppm doses but not at the 25-ppm dose level. The increase in the number of nonmotile sperm is unlikely to be treatment-related, because no clear dose-response was observed and the percent of motile sperm was not decreased. Weight measurements and microscopic examination of the testes and accessory organs showed no treatment-related effects. The individual stages of the spermatogenic cycles in the seminiferous tubules also appeared normal in male rats at 125 ppm.

Microscopic examination of the ovaries showed no changes relative to controls. The ovarian follicle (primordial, growing, and antral) counts in F_0 and F_1 females administered 125 ppm of the test material were similar to those of the corresponding control groups, except for the statistically significant increase in the antral follicle count for F_1 females administered 125 ppm of the test material. However, a recount of the follicles by a different technician did not confirm these results, i.e., no significant increase was observed in the number of antral follicles. Further, the difference between the original counts and the recount was greater than the differences between the original counts for treated and control female rats, suggesting that the results of the original counts should not be considered as evidence of a treatment-related effect on the ovary. The mean ovarian weight of female weanlings was significantly increased at 125 ppm compared with that of controls. The weight increase relative to controls, however, was small (23% for absolute weight), and no clear dose-response was observed as the weight at 125 ppm (0.016 g, $p<0.05$) was similar to that at 5 ppm (0.015 g, N.S.). In addition, the ovarian weights of 70-day old F_1 females at sacrifice were slightly decreased at 125

ppm compared with controls rather than increased as observed for 21-day old weanlings. Therefore, the transient increase in ovarian weights in 125-ppm female weanlings is probably not related to treatment with the test material. Vacuolation of the ovaries in P2 (F_1) females at ≥25 ppm was reported in a two-generation reproduction study (MRID 41806601) but not in the current study.

Adrenal gland, thymus, and pituitary gland weights were statistically significantly elevated in all treatment groups of F_1 male rats. The increased weights did not become progressively more severe as the dose increased (no dose-response relationship), and no microscopic findings were associated with the increased weights. Further, the adrenal glands, thymus, and pituitary were weighed after fixation. The study authors did not state whether the organs were dissected free of adhering tissues before or after fixation, so trimming errors may have been a factor. Historical control data were provided, but the organs were weighed fresh rather than after fixation. Further, historical control animals and those used in the current study were not the same age at the time of sacrifice. Therefore, the historical control data cannot be used to compare with the results from the current study. Because of no clear dose-response relationship, no associated microscopic findings, and no consistency between generations or the sexes, the changes in weight of the thymus, adrenal glands, and pituitary are not considered to be related to treatment with Dicofol at the doses used in this study. Adrenal gland effects (vacuolation and/or hypertrophy of cortical cells) have been reported in 90-day subchronic studies in male and female rats (TRID 470158014) (dose not reported), in females administered 125 and 250 ppm of Dicofol in a two-generation reproduction study (MRID 41806601), and in male and female rats administered 250 ppm in a chronic/carcinogenicity study (MRID 41150001). The current study showed no morphological effects on the adrenal gland at doses up to 125 ppm. The previous studies did not include adrenal gland weights.

The results from this study showed a dose-related increase in Dicofol (particularly the *p,p'*-isomer and its metabolite) in all adult serum, milk, neonate tissue, and weanling serum. Serum levels for Dicofol or metabolites did not increase between 5 and 10 weeks of treatment. Exposure of F_1 animals to the test material during all stages of development was confirmed by the presence of residues in prenursing pups (*in utero* exposure), milk (lactational exposure), and weanling serum (lactational and food consumption). Concentrations of residues in 21-day-old pup serum were comparable to concentrations measured in adult female serum during the premating period. These results indicate that adults and offspring at all

stages of development were adequately exposed to the test material.

Exposure to Dicofol did not affect reproductive organs or the viability, body weights and body weight gains of offspring at the doses used in this study. **Therefore, the reproductive and/or offspring NOAEL is >125 ppm (8.7/9.8 mg/kg/day).** Exposure to Dicofol also did not affect endocrine organs at the doses used in this study. The evidence showed that the animals were exposed to the test material at all phases of the study.

C. STUDY DEFICIENCIES

No details were given for test diet preparation.

The adrenal glands, thymus, and pituitary glands were weighed after fixation rather than fresh.

This study was very difficult to review; the report was poorly organized, and pertinent information was not easily located in the report.

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R124612

Chemical: Carbendazim

PC Code: 128872
HED File Code: 61200 SRRD CDC
Memo Date: 3/20/2001
File ID: 00000000
Accession #: 412-06-0197

HED Records Reference Center
7/25/2006