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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Dicofol - Evaluation of Developmental Toxicity Studies in Rat and Rabbit, and a 2-Generation Reproduction Study in Rats

ToxChem No.: 093 83-3A 83-3B 83-4

Accession (MRID) Nos. 400420-46, 400420-47, and 418066-01
HED Project No.: 1-0831

FROM: for Susan L. Makris, M.S. and James N Rowe 7/17/91
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and

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Health Effects Division (H7509C) Marcia van Gemert 7/22/91

Registrant: Rohm and Haas Company
727 Norristown Road
Springhouse, Pennsylvania 19477

Action Requested: Review the following studies conducted on the chemical Dicofol:

1. Developmental Toxicity Study in Rats
2. Developmental Toxicity Study in Rabbits
3. Two-Generation Reproduction Study in Rats

Summaries and Conclusions:

1. Developmental Toxicity Study in Rats (Guideline 83-3) - Accession (MRID) No. (400420-46,) Caswell No. 093

Dicofol was administered by oral gavage to Crl:COBS^{R}CD^{R}(SD)BR females rats at doses of 0.25, 2.50 and 25.00 mg/kg/day on gestation Days 6-15. Dose- and treatment-related incidences of salivation occurred in the mid and high-dose groups during the time of dosing. At the high-dose, decrements

in maternal body weight and food consumption values were noted for the period of dosing. At necropsy, high-dose maternal absolute and relative (to body weight) liver weights were increased, and histopathological evaluation revealed a treatment-related increase in the incidence of centrilobular hepatocyte hypertrophy. The maternal NOEL = 0.25 mg/kg/day, and the maternal LOEL = 2.50 mg/kg/day. There was no evidence of developmental toxicity or teratogenicity resulting from administration of the test material; therefore, the developmental toxicity NOEL and LOEL > 25.00 mg/kg/day.

CORE Classification: Guideline

2. Developmental Toxicity Study in Rabbits (Guideline 83-3) - Accession (MRID) No. (400420-47) Caswell No. 093

Dicofol was administered to NZW rabbits by oral gavage from day 7-19 of gestation at doses of 0.4, 4.0, and 40.0 mg/kg/day. Signs of maternal toxicity in the high-dose group consisted of abnormal feces, decreased food consumption and body weight gain during dosing, a significant increase in the liver-to-terminal-body weight ratio values at necropsy, and an increase in the incidence of cytoplasmic hyalinization and diffuse vacuolation of hepatocytes at histopathological evaluation. The maternal NOEL = 4.0 mg/kg/day, and the maternal LOEL = 40.0 mg/kg/day. Although there was no evidence of fetal teratogenicity, there was an increased incidence of dams aborting in the high-dose group, and the developmental LOEL = 40.0 mg/kg/day, with a developmental NOEL = 4.0 mg/kg/day.

CORE Classification: Minimum

3. Two-Generation Reproduction Study in Rats (Guideline 83-4) - Accession No. (418066-01) Caswell No. 093, HED Project No. 1-0831

Crl:CD^{R}BR rats were exposed to Dicofol over two consecutive generations at dietary levels of 5, 25, 125, and 250 ppm. The Systemic NOEL = 5 ppm and the Systemic LOEL = 25 ppm, based upon histopathological changes in the liver and ovaries of parental animals. There were no effects on reproductive performance and/or offspring growth and development; the reproductive NOEL = 5 ppm based upon vacuolation in the ovaries of P2 females, an observation which is compatible with enhance steroidogenic activity, the reproductive toxicity LOEL = 25 ppm.

CORE Classification: Minimum

Recommendation:

Based upon the effects noted in the 2-generation reproduction study in rats, as cited above, it is recommended that the current RfD for Dicofol be reconsidered.

cc G. Ghali (H7509C)

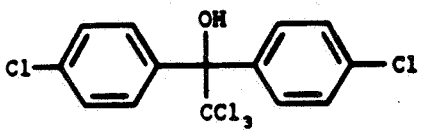
Primary Review by: Susan Lynn Makris, M.S. Susan L Makris 6-19-91
Toxicologist, Review Section III, Toxicology Branch II-HFAS/HED (H7509C)
Secondary Review by: James N. Rowe, Ph.D. James N. Rowe 6/21/91
Section Head, Review Section III, Toxicology Branch II-HFAS/HED (H7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat
Guideline: 83-3

EPA MRID (Accession) No.: 400420-46

Test Material: Dicofol (95.6% active ingredient), described as a dark brown highly viscous liquid (Lot No. MLO-0953, TD No. 84-393)



Synonym: Kelthane® Technical Miticide

Sponsor: Rohm and Haas Company
727 Norristown Road
Springhouse, Pennsylvania 19477

Study Numbers: Testing Facility Study No.: 018-010
Sponsor's Report No.: 85RC-69

Testing Facility: Argus Research Laboratories, Inc.
935 Horsham Road
Horsham, Pennsylvania 19044

Title of Report: Dicofol (Kelthane® Technical Miticide): A Developmental Toxicity Study of Dicofol Administered Via Gavage to Crl:COBS®CD®(SD)BR Presumed Pregnant Rats

Authors: A.M. Hoberman and M.S. Christian

Report Issued: July 3, 1986

Conclusions:

Dicofol was administered by oral gavage to Crl:COBS®CD®(SD)BR female rats at doses of 0.25, 2.50, and 25.00 mg/kg/day on Days 6-15 of presumed gestation. Treatment- and dose-related statistically significant incidences of salivation were noted in the mid- and high-dose groups during the period of dosing. Maternal body weight was significantly decreased on Day 16 of gestation at the high-dose level; body weight change and food consumption values were significantly decreased at this level on Days 6-16. Following the discontinuation of Dicofol administration, high-dose maternal body weight change

and food consumption values rebounded (Days 16-20); however, Day 20 body weight values remained lower than control. At necropsy, high-dose mean maternal liver weight values were slightly, but not significantly, increased, and the mean liver-to-terminal-body weight ratio was significantly increased as compared to control values. Histopathological evaluation of maternal liver tissue revealed a treatment-related increase in the incidence of centrilobular hepatocyte hypertrophy at the high-dose.

Maternal NOEL = 0.25 mg/kg/day
Maternal LOEL = 2.50 mg/kg/day

No evidence of developmental toxicity was observed. The examination of uterine contents at cesarean section revealed no treatment-related effects on numbers of corpora lutea, implantations, live and dead fetuses, or early and late resorptions. Fetal body weight, viability, and sex ratios were similar between control and treated groups. No fetal malformation or variation revealed by gross external, visceral, or skeletal evaluation was attributed to administration of Dicofol.

Developmental Toxicity NOEL = Not determined ($\geq$25.00 mg/kg/day)
Developmental Toxicity LOEL = Not determined (>25.00 mg/kg/day)

Core Classification: GUIDELINE

This study satisfies the requirements of FIFRA Guideline 83-3 for developmental toxicity studies.

A. Materials

Test Compound:
- Purity: 95.6% active ingredient
- Description: Dark brown highly viscous liquid (at room temperature)
- Lot No.: Lot No. MLO-0953, TD No. 84-393

Vehicle:
- Substance: Fisher (Kodak stripped) laboratory grade corn oil
- Manufacturer: Eastman Kodak Co., Rochester, NY 14650
- Supplier: Fisher Scientific, King of Prussia, PA 19406
- Lot No.: #D4-31

Test Animal(s):
- Species: Rat
- Strain: Crl:COBS^{R}CD^{R}(SD)BR
- Source: Charles River Breeding Laboratories, Inc. Lakeview Facility Newfield, New Jersey
- Age: 85 days at mating
- Weight: 222-300g at mating

B. Study Design

A copy of the methodology presented in report No. 018-010 is attached. This study was designed to assess the developmental toxicity potential of Dicofol when administered by oral gavage to Crl:COBS^{R}CD^{R}(SD)BR female rats on gestation Days 6 through 15, inclusive.

Mating:

Following a 2-week acclimation period, the apparently healthy virgin female rats were paired one-to-one with male breeder rats (87 days of age and 334 to 426 g) for a maximum of four days. Females were observed daily for positive evidence of copulation. Presence of spermatozoa in vaginal smears, or a copulatory plug in the vagina or cage pan, was considered to be confirmation of mating. Females with such signs were presumed to be pregnant and designated as being at Day 0 of gestation.

Group Assignment and Dosage Levels:

Group No.	Dose (mg/kg)	No. per Group	Animal Numbers
1 (Control)	0[a]	25	28,591 - 28,615
2 (Low)	0.25	25	28,616 - 28,640
3 (Mid)	2.50	25	28,641 - 28,665
4 (High)	25.00	25	28,666 - 28,690

a Vehicle (corn oil) control.

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Test Material Formulation, Administration, and Analysis:

Solutions of Dicofol in corn oil were formulated daily during the dosing period. The Dicofol was heated to 80°C, stirred to ensure homogeneity, and mixed with corn oil to concentrations providing the scheduled dosages to the rats when administered at a constant dosage volume of 5.0 ml/kg.

The test material was administered to the study animals by oral gavage on Days 6-15 of gestation. All dosage calculations were adjusted for percent active ingredient (95.6%). (Note: Analysis of the technical Kelthane® used in this study was found to contain 93.4% active ingredient - see report No. 018-101, page 171.) Dosage volumes were adjusted daily for changes in individual body weight. Solutions were stirred continuously during dosing. Control animals received the vehicle (corn oil) in the same manner.

According to the report, previous evaluations of Dicofol-corn oil solutions indicated that they were stable for the period of use required by this study; data supporting this statement was not provided. Concentration analysis was performed on control solutions and triplicate samples of dosing solutions for Days 1 (mid- and high-dose only), 5 (mid- and high-dose only), 9, and 13 of dosing. A summary of analytical results is presented in Text Table A.

Text Table A. Summary of Dosing Solution Concentration Analyses

Group	Dose (mg/kg)	Conc. (ppm)	No. Samples Analyzed	Results (ppm)			Percent of Theory
				Mean ± S.D.	Max.	Min.	
1	0	0.0	4	0.0±0.0	0.0	0.0	100
2	0.25	50.0	6	51.8±3.06	48.0	57.0	104
3	2.50	500.0	12	550.0±20.00	500.0	580.0	110
4	25.00	5000.0	12	5783.3±393.82	5200.0	6600.0	116

Note: Data were extracted from report No. 018-010, page 173.

Analysis for homogeneity of dosing solutions was not performed.

Observations:

The animals were checked for mortality twice daily during the course of the study. During the period of test material administration (Days 6-15 of gestation), observations for physical signs of test substance effect and/or viability were made three times daily (pre-dose, 30 minutes to 1 hour post-dose, and approximately 4-hours post-dose). From Days 16 through 20 of gestation, observations of general health and/or signs of abortion or natural delivery were recorded once daily.

Body weights and food consumption of the female rats were recorded on Day 0 of gestation and daily from Day 6 through 20.

Dams were sacrificed on Day 20 of gestation. Gross necropsy of each rat included examination of the thoracic, abdominal, and peritoneal cavities. Liver weights were recorded. Corpora lutea were counted, and uterine contents were examined for pregnancy, number and placement of implantations, early and late resorptions, and live and dead fetuses.

The liver and any gross lesions were preserved in 10% neutral buffered formalin. Liver specimens were evaluated histopathologically, beginning with the highest dose group and progressing to lower dosage groups until a no-observed-effect dosage level was reached.

Following removal from the uterus, the fetuses were weighed individually and examined to identify sex and gross external alterations. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations using a modification of Wilson's sectioning technique; the remainder of the fetuses were eviscerated, cleared, stained with alizarin red-S, and examined for skeletal alterations.

Historical control data were provided to allow comparison with concurrent controls.

Statistical analysis

The following statistical analysis methods were employed:

Trends in data were analyzed using the Cochran-Armitage test for linear trend. Intergroup differences were analyzed using Bartlett's test followed by either the Mann-Whitney U test or one-way ANOVA. If necessary, Dunnett's test was used following ANOVA, for comparison of individual groups with controls. Data obtained during cesarean section and data for alterations, malformations, and variations was evaluated using Jonckheere's test followed by either the Mann-Whitney U test or the Fisher's Exact Test.

Compliance

The following were provided:

- A signed Statement of No Confidentiality Claim
- A signed Statement of compliance with EPA GLPs
- A signed Quality Assurance Statement

C. Results

1. Maternal Toxicity

Mortality:

All adult females survived to scheduled sacrifice.

Clinical Observations:

Selected clinical observations noted during gestation are presented in Table 1. The incidence of salivation, which occurred in Dicofol-treated rats during the period of dosing (Days 6-15), was treatment- and dose-related. It was not clear from the data presented in the report whether or not this observation was noted immediately after dosing. Other observations noted were considered to be incidental.

Table 1. Incidence of Selected Clinical Observations [a]

Observation	0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
Salivation	0	1	5*	21*
Urine stains	0	1	0	1
Chromodacryorrhea	0	0	1	0
Alopecia	4	1	1	9
Lesion	0	0	1	2

* Significantly different from control, $p \leq 0.05$.

a Number of rats with stated observation recorded at least once during the course of the study.

Note: Data were extracted from report No. 018-010, p. 44.

Body Weight and Food Consumption:

Body weight, body weight change, and food consumption data are summarized from the report in Table 2. A significant treatment-related decrease in body weight was noted for the high-dose (25.00 mg/kg) rats at Day 16 of gestation. Body weight change and food consumption values for Days 6-16, the period of dosing, were also significantly reduced for these animals. After discontinuation of test material administration (Days 16-20 of gestation), the high-dose rats experienced a rebound effect, with a resulting statistically significant increase in Days 16-20 food consumption and biologically significant increases in Days 16-20 body weight change and Day 20 absolute body weight values.

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Table 2. Selected Mean Maternal Body Weight Change and Food Consumption Values During Gestation (g ± S.D.)

Interval	0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
Body Weight				
Day 0	247.6 ± 13.4	248.5 ± 13.7	249.0 ± 16.7	248.2 ± 14.2
Day 6	282.4 ± 15.9	280.8 ± 17.1	278.8 ± 23.0	276.4 ± 16.5
Day 16	331.9 ± 22.1	334.9 ± 24.0	328.0 ± 28.3	311.3 ± 21.9*
Day 20	390.4 ± 33.0	395.5 ± 28.7	392.5 ± 31.3	378.4 ± 26.5
Body Weight Change				
Days 0-6	34.8 ± 8.6	32.3 ± 9.2	29.8 ± 9.2	28.2 ± 11.1
Days 6-16	49.5 ± 12.1	54.1 ± 11.6	49.2 ± 9.2	34.9 ± 13.9**
Days 16-20	58.6 ± 14.9	60.6 ± 10.0	64.5 ± 10.4	67.1 ± 12.4
Days 0-20	142.8 ± 29.7	147.0 ± 21.9	143.4 ± 18.7	130.2 ± 21.2
Food Consumption				
Days 0-6	95.5 ± 8.0	92.0 ± 13.2	92.2 ± 9.5	90.9 ± 10.9
Days 6-16	74.5 ± 6.6	76.1 ± 9.3	73.1 ± 4.0	61.8 ± 8.9*
Days 16-20	75.5 ± 7.9	76.0 ± 7.2	76.4 ± 5.7	82.8 ± 7.1*
Days 0-20	77.0 ± 5.7	77.0 ± 8.0	75.8 ± 3.9	72.4 ± 5.8*

* Statistically significantly different from control value, $p \leq 0.01$.
** Statistically significantly different from control value, $p \leq 0.05$.

Note: Data were extracted from report No. 018-010, pages 46-48.

Net body weight change data (body weight change for Days 0-20, minus gravid uterine weight) were not reported.

Gross Pathology Observations:

The investigators reported the following gross pathology findings for maternal rats (Table 3):

Table 3. Incidence of Lesions Noted at Maternal Necropsy

Observation	0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
Hydronephrosis	1	3	2	4
Kidney enlarged, containing clear fluid	0	1	0	0

Note: Data compiled from report No. 018-010, Page 45.

Hydronephrosis included slight to marked dilation of the pelvis of one or both kidneys; the enlarged kidney noted at the 0.25 mg/kg/day dose level was associated with hydronephrosis in the same animal.

Due to the lack of apparent dosage-dependency and to the consideration that hydronephrosis is a common occurrence for the strain of rat used on this study (no historical data was provided to support this statement), the investigators judged the kidney lesions to be unrelated to treatment with Dicofol.

Organ Weights:

Mean absolute and relative liver weight values are presented in Table 4. Mean liver weight values were slightly, but not significantly, increased for the high-dose group (25.00 mg/kg). The mean liver-to-terminal-body weight ratio value for the high-dose group was significantly higher than control values.

Table 4. Summary of Absolute and Relative Maternal Liver Weight Values

Parameter	0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
Mean liver weight (g ± S.D.)	16.94±1.71	17.18±1.56	16.99±2.04	17.58±2.06
Mean liver/body weight ratio (% ± S.D.)	4.34±0.24	4.34±0.22	4.30±0.23	4.64±0.36*

* Statistically significantly different from control value, $p \leq 0.01$.

Note: Data were extracted from report 018-010, page 46.

Histopathology:

Result of histopathological evaluation of maternal liver tissue is presented in Table 5. There were no treatment-related changes in the livers of rats at the low- and mid-dose levels (0.25 or 2.50 mg/kg, respectively). At the high-dose level (25.00 mg/kg), treatment-related changes, consisting of minimal to slight enlargement (hypertrophy) of centrilobular hepatocytes, were noted in the liver.

Table 5. Summary of Histopathological Examination of Maternal Livers

Findings	0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
No. examined	25	25	25	25
No. normal	22	21	22	6
Centrilobular hepatocyte hypertrophy	0	0	0	17
Basophilic cell focus/foci	1	0	0	0
Multifocal extramedullary hematopoiesis	1	1	0	0
Multifocal mononuclear cellular infiltration	1	3	3	3

Note: Data were extracted from report No. 018-010, page 160.

Observations Noted at Cesarean Section:

The results of the examination of uterine contents at cesarean section are presented in Table 6. There appeared to be no treatment-related effects on the number of corpora lutea, implantation sites, live or dead fetuses, or early or late resorptions. Mean fetal weight values, fetal viability, and fetal sex ratio were similar between control and treated groups.

2. Developmental Toxicity

Observations noted at external, visceral, and skeletal evaluation of fetuses are presented in Tables 7, 8, and 9, respectively. Neither the incidence nor distribution of fetal alterations indicated a treatment-related effect. The investigators stated that the observations noted were within the range of historical control data for the testing facility (1983-1984 data was appended to the report; 1985 data for the incidence of dilation of the pelvis of one or both kidneys was also presented).

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Table 6. Summary of Cesarean Section Data

Parameter	0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
No. animals assigned (mated)	25	25	25	25
No. pregnant (%)	25(100)	25(100)	24(96)	25(100)
Maternal deaths	0	0	0	0
Total corpora lutea[a]	449	468	459	492
Corpora lutea/dam	18.0	18.7[a]	19.1	19.7
Total implantations[a]	368	370	364	385
Implantations/dam	14.7	14.8	15.2	15.4
Total live fetuses	335	352	336	365
Live fetuses/dam	13.4	14.1	14.0	14.6
Total resorptions[a]	33	18	28	20
Early	31	17	28	18
Late	2	1	0	2
Resorptions/dam[a]	1.3	0.7	1.2	0.8
Total dead fetuses	0	0	0	0
Mean fetal weight (g)	3.43	3.26	3.36	3.37[b]
Sex ratio (% males/litter)	51.4	50.4	45.8	50.9
Preimplantation loss (%)[c]	18.0	20.6	20.7	21.7
Postimplantation loss (%)[d]	9.0	4.9	7.7	5.2

a Calculated by reviewer.

b One litter (No. 28671) was excluded from the calculations because part of the litter was inadvertently not weighed.

c Calculated by reviewer:
% = (total corpora lutea - total implantation sites) / (total corpora lutea) x 100

d Calculated by reviewer:
% = (total resorptions + total dead fetuses) / (total implantations) x 100

Note: Data were extracted from report No. 018-010, pages 49-50 and 93-96.

Table 7. Summary of External Observations

Observation	0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
No. pups (litters) examined	335(25)	352(25)	336(24)	365(25)
MALFORMATIONS[a]				
HEAD/EYES/EARS				
Multiple anomalies including: small head, absent stoma and nares, anophthalmia, agenesis of the ears, and micrognathia[b]				
Litter incidence N(%)	1(4.0)	0(0.0)	0(0.0)	0(0.0)
Fetal incidence N(%)	1(0.3)	0(0.0)	0(0.0)	0(0.0)
Multiple anomalies including: small cranium, microphthalmia, bulging eyes, ectopic ears, and micrognathia[b]				
Litter incidence N(%)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
Fetal incidence N(%)	0(0.0)	0(0.0)	1(0.3)	0(0.0)
JAW				
Micrognathia[b]				
Litter incidence N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.0)
Fetal incidence N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.3)
BODY				
Umbilical hernia				
Litter incidence N(%)	1(4.0)	0(0.0)	0(0.0)	0(0.0)
Fetal incidence N(%)	1(0.3)	0(0.0)	0(0.0)	0(0.0)

Table 7. Summary of External Observations - continued

Observation		0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
TAIL					
Thread-like					
Litter incidence	N(%)	0(0.0)	2(8.0)	0(0.0)	0(0.0)
Fetal incidence	N(%)	0(0.0)	2(0.6)	0(0.0)	0(0.0)
TOTAL WITH ANY MALFORMATION					
Litter incidence	N(%)	2(8.0)	2(8.0)	1(4.2)	1(4.2)
Fetal incidence	N(%)	2(0.6)	2(0.6)	1(0.3)	1(0.3)

a No external variations were observed.
b Micrognathia occurred in conjunction with several other anomalies of the head in fetuses No. 28606-4 (control) and 28642-3 (mid-dose), but was observed as an independent anomaly in fetus No. 28679-5 (high-dose).

Note: Data were extracted from report No. 018-010, pages 52-54 and 109-124.

Table 8. Summary of Visceral Observations

Observation		0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
No. pups (litters) examined		159(23)	169(25)	161(24)	175(25)
VARIATIONS					
KIDNEY(S)					
Pelvis, slightly dilated, unilaterally					
Litter incidence	N(%)	0(0.0)	1(4.0)	1(4.2)	3(12.0)
Fetal incidence	N(%)	0(0.0)	1(0.6)	1(0.6)	3(1.7)
TOTAL WITH ANY VARIATION					
Litter incidence	N(%)	0(0.0)	1(4.0)	1(4.2)	3(12.0)
Fetal incidence	N(%)	0(0.0)	1(0.6)	1(0.6)	3(1.7)

Table 8. Summary of Visceral Observations - continued

Observation	0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
No. pups (litters) examined	159(23)	169(25)	161(24)	175(25)
MALFORMATIONS				
BRAIN Consolidated, undifferentiated tissue (lateral ventricles not present)				
Litter incidence N(%)	1(4.3)	0(0.0)	0(0.0)	0(0.0)
Fetal incidence N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
LUNG(S) Agenesis of one lobe or portion				
Litter incidence N(%)	3(13.0)	2(8.0)	0(0.0)	2(8.0)
Fetal incidence N(%)	4(2.5)	2(1.2)	0(0.0)	2(1.1)
TOTAL WITH ANY MALFORMATION				
Litter incidence N(%)	4(17.4)	2(8.0)	0(0.0)	2(8.0)
Fetal incidence N(%)	5(3.1)	2(1.2)	0(0.0)	2(1.1)

Note: Data were extracted from report No. 018-010, pages 55-56 and 109-124.

Table 9. Summary of Skeletal Observations

Observation	0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
No. pups (litters) examined	176(25)	183(25)	175(24)	190(25)
VARIATIONS				
VERTEBRAE				
Thoracic centrum, bifid				
Litter incidence N(%)	1(4.0)	0(0.0)	5(20.8)	3(12.0)
Fetal incidence N(%)	1(0.6)	0(0.0)	5(2.8)	4(2.1)
RIBS				
Incompletely ossified (hypoplastic)				
Litter incidence N(%)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
Fetal incidence N(%)	0(0.0)	0(0.0)	2(1.1)	0(0.0)
Wavy				
Litter incidence N(%)	3(12.0)	0(0.0)	1(4.2)	3(12.0)
Fetal incidence N(%)	3(1.7)	0(0.0)	2(1.1)	3(1.6)
MANUBRIUM AND STERNEBRAE				
Manubrium fused to first sternal center				
Litter incidence N(%)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
Fetal incidence N(%)	0(0.0)	0(0.0)	1(0.6)	0(0.0)
MANUBRIUM				
Incompletely ossified				
Litter incidence N(%)	1(4.0)	0(0.0)	0(0.0)	1(4.0)
Fetal incidence N(%)	1(0.6)	0(0.0)	0(0.0)	1(0.5)

Table 9. Summary of Skeletal Observations - continued

Observation		0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
No. pups (litters) examined		176(25)	183(25)	175(24)	190(25)
STERNEBRAE					
Fused					
Litter incidence	N(%)	0(0.0)	1(4.0)	1(4.2)	0(0.0)
Fetal incidence	N(%)	0(0.0)	1(0.5)	1(0.6)	0(0.0)
Not ossified					
Litter incidence	N(%)	1(4.0)	1(4.0)	0(0.0)	0(0.0)
Fetal incidence	N(%)	1(0.6)	1(0.5)	0(0.0)	0(0.0)
Incompletely ossified					
Litter incidence	N(%)	3(12.0)	7(28.0)	4(16.7)	6(24.0)
Fetal incidence	N(%)	3(1.7)	10(5.5)	5(2.8)	7(3.7)
PELVIS					
Ischia and/or pubes, incompletely ossified					
Litter incidence	N(%)	4(16.0)	2(8.0)	0(0.0)	0(0.0)
Fetal incidence	N(%)	5(2.8)	3(1.6)	0(0.0)*	0(0.0)*
TOTAL WITH ANY VARIATION					
Litter incidence	N(%)	6(24.0)	7(28.0)	8(32.0)	11(44.0)
Fetal incidence	N(%)	8(4.6)	11(6.0)	11(6.3)	14(7.4)

Table 9. Summary of Skeletal Observations - continued

Observation	0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
No. pups (litters) examined	176(25)	183(25)	175(24)	190(25)
MALFORMATIONS				
SKULL				
Multiple anomalies, including: small eye socket, fused squamosals and zygomatics, unossified mandibles, small sphenoid[a], tympanic bones fused below hyoid, and unossified palate				
Litter incidence N(%)	0(0.0)	0(0.0)	0(0.0)	1(4.2)
Fetal incidence N(%)	0(0.0)	0(0.0)	0(0.0)	1(0.6)
VERTEBRAE				
Thoracic and lumbar hemivertebra				
Litter incidence N(%)	1(4.0)	0(0.0)	0(0.0)	0(0.0)
Fetal incidence N(%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
RIBS				
Fused				
Litter incidence N(%)	1(4.0)	1(4.0)	0(0.0)	0(0.0)
Fetal incidence N(%)	1(0.6)	1(0.5)	0(0.0)	0(0.0)
Cervical rib present				
Litter incidence N(%)	0(0.0)	0(0.0)	2(8.3)	1(4,0)
Fetal incidence N(%)	0(0.0)	0(0.0)	2(1.1)	1(0.5)

Table 9. Summary of Skeletal Observations - continued

Observation	0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
No. pups (litters) examined	176(25)	183(25)	175(24)	190(25)
MANUBRIUM, STERNEBRAE, AND XIPHOID				
Duplicated				
Litter incidence N(%)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
Fetal incidence N(%)	0(0.0)	0(0.0)	1(0.6)	0(0.0)
STERNEBRAE				
One or more, asymmetric				
Litter incidence N(%)	0(0.0)	1(4.0)	0(0.0)	0(0.0)
Fetal incidence N(%)	0(0.0)	1(0.5)	0(0.0)	0(0.0)
TOTAL WITH ANY MALFORMATION				
Litter incidence N(%)	1(4.0)	1(4.0)	4(16.7)	1(4.0)
Fetal incidence N(%)	1(0.6)	1(0.5)	4(2.3)	1(0.5)

* Statistically significantly different from control value, $p \leq 0.05$.

a Small sphenoid is a variation but is included here since it was associated with multiple malformations of the skull.

Note: Data were extracted from report No. 018-010, pages 57-62 and 109-124.

Overall incidences of fetal observations are summarized in Table 10. A significant increase in the number/percent of litters with any variation observed was noted for the high-dose (25.00 mg/kg) group. These variations were comprised of several minor reversible skeletal alterations, and one visceral variation (dilation of the renal pelvis) which might be attributed to a slight delay in development. Independently, these variations did not occur at significantly high incidences, nor were they outside of historical control incidence ranges. The investigators stated that "the statistical finding was considered to be an artifact of the arbitrary classification and statistical analyses of fetal alteration in terms of malformation and variation and was not considered attributable to administration of the test material."

Table 10. Summary of Fetal Observations

Observation	0 mg/kg	0.25 mg/kg	2.50 mg/kg	25.00 mg/kg
Litters evaluated	25	25	24	25
Fetuses evaluated	335	352	336	365
Live	335	352	336	365
Dead	0	0	0	0
No. litters with fetuses with any alteration observed (%)	11(44.0)	10(40.0)	11(45.8)	16(64.0)
No. fetuses with any alteration observed (%)	14(4.2)	16(4.5)	15(4.5)	21(5.8)
Mean % fetuses with any alteration/litter	3.90	4.46	4.64	5.99
No. litters with fetuses with any malformation observed (%)	6(24.0)	5(20.0)	4(16.7)	4(16.0)
No. fetuses with any malformation observed (%)	7(2.1)	5(1.4)	4(1.2)	4(1.1)
Mean % fetuses with any malformation/litter	2.0	1.5	1.2	1.3
No. litters with fetuses with any variation observed (%)	6(24.0)	7(28.0)	9(37.5)	14(56.0)*
No. fetuses with any variation observed(%)	8(2.4)	12(3.4)	12(3.6)	17(4.6)
Mean % fetuses with any variation/litter	2.3	3.2	3.7	4.6

* Statistically significantly different from control value, $p \leq 0.05$.

Note: Data were extracted from report No. 018-010, page 51.

D. Discussion/Conclusions

a. Maternal Toxicity:

Administration of Dicofol to Crl:COBS^{R}CD^{R}(SD)BR female rats on Days 6-15 of presumed gestation by oral gavage at doses of 0.25, 2.50, and 25.00 mg/kg/day produced evidence of systemic toxicity at the 25.00 mg/kg/day (high-dose) level.

1. Treatment- and dose-related statistically significant incidences of salivation were noted in the mid- and high-dose groups during the period of test material administration.

2. Maternal body weight was significantly decreased on Day 16 of gestation at the high-dose level; body weight change and food consumption values were significantly decreased at this level on Days 6-16. Following the discontinuation of Dicofol administration, high-dose maternal body weight change and food consumption values rebounded; however, Day 20 body weight values remained lower than control.

3. At necropsy, high-dose maternal liver weights were slightly, but not significantly, increased. Due to the increased liver weights and the reduced terminal body weight of the dams, the liver-to-terminal-body weight ratio for the high-dose group was increased significantly as compared to control values. Histopathological evaluation of maternal liver tissue revealed a treatment-related increase in the incidence of centrilobular hepatocyte hypertrophy at the high-dose.

Maternal NOEL = 0.25 mg/kg/day
Maternal LOEL = 2.50 mg/kg/day

b. Developmental Toxicity:

No evidence of developmental toxicity was observed. The examination of uterine contents at cesarean section revealed no treatment-related effects on numbers of corpora lutea, implantations, live and dead fetuses, or early and late resorptions. Fetal body weight, viability, and sex ratios were similar between control and treated groups. No fetal malformation or variation revealed by gross external, visceral, or skeletal evaluation was attributed to administration of Dicofol. Although the incidence of fetuses with any variation was significantly increased in the high-dose group, this was not judged to be a sign of developmental toxicity since the types of variations observed occurred at low incidences, are common historically in this species, and are generally not comparable in etiology. In addition, some variations, e.g., wavy ribs, delayed/reduced ossification, and dilation of the renal pelves, are considered to be reversible in nature.

Developmental Toxicity NOEL = Not determined ($\geq$25.00 mg/kg/day)
Developmental Toxicity LOEL = Not determined (>25.00 mg/kg/day)

D. Study Deficiencies: None noted.

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E. Core Classification: GUIDELINE

GUIDELINE: 83-3

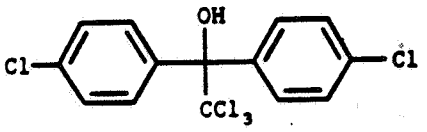
Reviewed by: Alberto Protzel, Ph.D. 5/28/91
Review Section III, Toxicology Branch II/HED (H7509C)
Secondary Review by: James N. Rowe, Ph.D. James N. Rowe 5/28/91
Section Head, Review Section III, Toxicology Branch II/HED (H7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rabbit
EPA Guideline: 83-3

EPA Identification No.s: EPA MRID (Accession) No. 400420-47
EPA ID No.
EPA Record No.
EPA Pesticide Chemical Code
Caswell No.
HED Project No.
Document No.

Test Material: Dicofol (Technical) 95.6% a.i., Lot MLO-0963, TD No. 84-393.



Synonyms: Kelthane^{R} (Technical) Miticide

Sponsor: Rohm and Haas Company. Spring House, PA.

Study Number: 86RC-15

Testing Facility: Argus Research Laboratories, Inc. Horsham, PA.

Title of Report: Dicofol (Kelthane^{R} Technical Miticide): A developmental toxicity study of dicofol administered via stomach tube to New Zealand White rabbits.

Author(s): A.M. Hoberman and M.S. Christian

Report Issued: May 28, 1986

Conclusions:

Oral administration of dicofol at 0, 0.4, 4.0, and 40.0 mg/kg/day during days 7-19 of gestation in the New Zealand White rabbit produced a significant ($p \leq 0.05$) depression in body weight gain in the HDT coupled with a significant ($p \leq 0.05$) decrease in food intake. The average weight gain for the entire gestation period in the HDT remained significantly less ($p \leq 0.05$) than controls

in spite of a rebound effect during the post-dosing period. Additional signs of toxicity in the HDT included increased incidence ($p \leq 0.05$) of abnormal feces (solid or liquid), increase ($p \leq 0.05$) in liver to body weight ratios, increase in the incidence of cytoplasmic hyalinization (8/20) and of diffuse vacuolation in hepatocytes. Although cytoplasmic hyalinization was also observed in hepatocytes from the MDT (2/19), the biological significance of this effect is unclear in the MDT in the absence of other toxic signs. The maternal NOEL is set at 4.0 mg/kg/day.

A significant ($p \leq 0.05$) incidence of irregularly shaped fontanellae was observed in the HDT (fetal frequency 4/101, litter frequency 1/14) compared to concurrent controls (0/118). This is not considered to be a developmental effect of the test material, because it was found to be within the range of historical controls. The range of observed incidences in historical control data submitted by the testing laboratory was up to: 0.9-29.5% (fetal) and 7.1-21.4% (litter).

A high frequency of abortions (4/19), in excess of concurrent (1/18) and historical controls (up to 1/14-2/15 with an outlier at 1/4), was reported for the HDT. This high incidence of abortions appears to be attributable to maternal toxicity. Direct developmental toxicity, however, cannot be definitively excluded. Thus, this study defines a developmental toxicity NOEL of 4.0 mg/kg/day and a developmental toxicity LOEL of 40.0 mg/kg/day.

Core Classification: Minimum.

A. Materials

A copy of the "Materials and Methods" section from the report is appended.

Test Compound: Purity: 95.6% a.i.
Description: Dark brown highly viscous liquid
Lot No.: MLO-0953, TD No. 84-393
Contaminant: No data

Vehicle(s): Aqueous 1.0% (w/v) methylcellulose (Sigma Chemical Co.). Hi Sil 233 (Pittsburgh Plate Glass), used as excipient. Aqueous solutions were prepared using deionized R.O. membrane processed water.

Test Animal(s): Species: rabbit
Strain: New Zealand White rabbit ([Hra:(NZW)SPF])
Source: Hazleton Research Animals, Swampbridge Road, Denver, PA
Age: approximately six months at the time of insemination
Weight: 3.01-4.41 kg on date of insemination

B. Study Design

This study was designed to assess the developmental toxicity potential of dicofol when administered by gavage to pregnant rabbits on gestation days 7 through 19, inclusive.

Insemination:

After acclimatization for 19-22 days, females were artificially inseminated. The rabbits were administered 20 USP units/kg of Human Chorionic Gonadotropin (Pregnyl^{R}, Organon, Inc.) approximately three hours prior to artificial insemination. Approximately 0.25 mL of semen that had been diluted with normal saline (Abbott) to a concentration of 6.0×10^{6} spermatozoa/0.25 mL saline was used to inseminate each rabbit. Spermatozoa were obtained from four proven male breeders. After artificial insemination, the animals were caged individually. The day of artificial insemination was designated as day 0 of the study.

Group Arrangement:

Table 1. Dosing groups for teratology study

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	20 (10001-10020)
Low Dose (LDT)	0.4	20 (10021-10040,10081)[1]
Mid Dose (MDT)	4.0	20 (10041-10060)
High Dose (HDT)	40.0	20 (10061-10080)

[1] Animal 10081 was introduced to replace rabbit 10029, which was sacrificed on day 1 of gestation.

Dosing:

All doses were administered by gavage in a volume of 5 ml/kg of body weight/day prepared daily during the dosing period. The dosing solutions were analyzed for concentration. The dosing volume (5 ml/kg/day) was adjusted daily for changes in body weight.

Observations:

The animals were checked for mortality twice each day of the entire study. General appearance observations were made at least once daily on days 0-6 of gestation. Observations for toxic signs were made three times daily during the dosing period (days 7-19 of gestation), and daily during the postdosage period (days 20-29 of gestation). Body weights were recorded on day 0 and daily on days 7-29 of gestation. The dams were sacrificed at day 29 of gestation. Examinations at sacrifice consisted of: examination of gross lesions, liver weight determination, counting of corpora lutea, determination of the number, distribution, and viability of any fetuses present, counting and examination of implantation sites to determine number of early and late resorptions.

The fetuses were examined in the following manner: the fetuses were weighed, sexed and examined for external alterations. Live fetuses were sacrificed, dissected, and examined for soft tissue alterations (including a cross-section of the brain). Skeletal alterations were evaluated after staining with alizarin red S.

Historical control data were provided to allow comparison with concurrent controls.

Statistical analysis

The following statistical analysis methods were employed:

Trends in data were analyzed using the Cochran-Armitage test for linear trend. Intergroup differences were analyzed using Bartlett's test followed by either the Mann-Whitney U test or one-way ANOVA. If necessary, Dunnett's test was used following ANOVA, for comparison of individual groups with controls. Data obtained during cesarean-section and data for alterations, malformations and variations was evaluated using Jonckheere's test followed by either the Mann-Whitney U test or Fisher's exact test.

Compliance:

A signed Statement of No Confidentiality Claim was provided.

A signed Statement of compliance with EPA GLP's was provided.

A signed Quality Assurance Statement was provided.

Results:

1. Maternal Toxicity

Mortality:

One middle dose (MDT) rabbit died as a result of intubation error. No other deaths were reported.

Clinical Observations:

Abnormal feces (dried or soft) were increased ($p \leq 0.05$) in the HDT (16 cases) compared to controls (8 cases). Increased incidence and/or duration of cases of alopecia was reported ($p \leq 0.05$) in the MDT and HDT compared to controls. Four rabbits in the HDT aborted between days 24-27 of gestation. These rabbits lost weight, had decreased feed consumption, and had intermittent episodes of abnormal feces after start of dosing. One abortion was reported for LDT (day 24), 1 for the control group (day 17), and none for the MDT. Aborted fetuses appeared normal.

Body Weight:

Body weights were recorded on day 0 of gestation, on the first day of dosing (day 7) and daily thereafter through day 29 of gestation. Body weight gains for gestation days 0-7, 7-10, 10-13, 13-16, 16-20, 20-24, and 20-29 were reported. Corrected body weights, calculated by subtracting the weight of the gravid uterus, were not reported.

As shown in Table 2, mean body weight gains were significantly depressed ($p \leq 0.01$) in the HDT with respect to controls during the dosing period. Body weight gains in the HDT rebounded significantly ($p \leq 0.01$) during the postdosing period. Overall, for the 7-29 day period, weight gains in HDT were significantly depressed with respect to controls ($p \leq 0.01$).

Table 2. Maternal mean body weight gains (mean ± S.D. in kg). Data from p. 53 of the Study Report.[1]

Group	Prior to dosing (d 0-7)	Day 7-20 of gestation[2]	Post-dosing (d 20-29)	Days 7-29 of gestation period
Control	0.16 ± 0.07	0.16 ± 0.09	0.06 ± 0.17	0.22 ± 0.22
LDT	0.19 ± 0.07	0.16 ± 0.10	0.08 ± 0.11	0.26 ± 0.13
MDT	0.18 ± 0.08	0.10 ± 0.09	0.07 ± 0.10	0.17 ± 0.12
HDT	0.20 ± 0.06	-0.29 ± 0.25**	0.22 ± 0.16**	0.01 ± 0.25**

** Significantly different from controls at $p \leq 0.01$.
[1] Corrected weight values were not available.
[2] Includes the dosing period: comprising days 7-19 of gestation.

Mean liver weights and liver to body weight ratios (as percent) are shown in Table 3. Significant elevations in liver to body weight ratios were observed at the LDT ($p \leq 0.05$) and at the HDT ($p \leq 0.01$).

Table 3. Mean liver weights (g) and liver to body ratios. Data from p. 55 of the Study Report.

Group	Liver weights (mean ± S.D., g)	Liver to body weight ratios (mean ± S.D., %)
Control	99.23 ± 19.17	2.42 ± 0.33
LDT	117.47 ± 27.64*	2.77 ± 0.55*
MDT	98.01 ± 21.42	2.41 ± 0.39
HDT	114.77 ± 24.27	2.89 ± 0.57**

* Significantly different from control at $p \leq 0.05$.
** Significantly different from controls at $p \leq 0.01$.

Food Consumption

As shown in Table 4, food consumption was significantly depressed in the HDT (-57.4% of controls, $p \leq 0.01$) during days 7-20 (which include the dosing period at days 7-19). There was an apparent rebound during days 20-29 in the HDT.

Table 4. Maternal food consumption (Data from p. 54 of the Study Report).

Group	Prior to dosing period (d. 0-7)	D. 7-20 period[1]	Post-dosing (d. 20-29)	Entire gestation (d. 0-29)
Control	45.9 ± 4.2	40.8 ± 5.1	29.9 ± 8.5	38.2 ± 3.7
LDT	45.8 ± 3.4	40.4 ± 7.0	31.5 ± 8.6	39.0 ± 4.2
MDT	46.4 ± 3.1	39.3 ± 4.4	28.9 ± 7.2	37.3 ± 3.0
HDT	46.9 ± 3.5	17.4 ± 10.9**	32.0 ± 12.3	30.8 ± 6.1**

* Significantly different from control at $p \leq 0.05$.
** Significantly different from controls at $p \leq 0.01$.
[1] Includes the dosing period plus one day: dosing was done on days 7-19 of gestation.

Gross Pathological Observations

With the exception of a lung perforation resulting from intubation error, no test substance-related gross lesions were observed.

Histopathology of the Maternal Liver

The authors reported a dose-dependent increase in the incidence of eosinophilic, hyaline material in centrilobular hepatocytes for the MDT (2/19) and HDT (8/20). In addition, the authors reported a higher incidence of diffuse cytoplasmic vacuolation (marked) for HDT (6/20).

Cesarean section observations

Pregancy rates ranged from 90% to 95% and were considered acceptable (Table 5). No treatment related effects were reported for the average numbers of corpora lutea, implantations, resorptions (early and late), mean number of dead and live fetuses, fetal weights, and sex ratio. No dose related effects were observed on pre- and post-implantation loss.

A high incidence of abortions (4/19, 21%) was noted in HDT. This incidence of abortions was higher than in concurrent controls (1/18, 5.6%), exceeded the historical control frequency, was accompanied by clinical signs and was thus considered to be treatment-related. Examination of the uterine contents and aborted fetuses revealed fetuses and late resorptions that were normal for the developmental stage at the time of abortion. Although the mean fetal body weight in the HDT (42.2 g) was smaller than that of the controls (46.3 g), the difference was not statistically significant ($p>0.05$) and was within the historical control range.

Table 5: Cesarean Section observations (From pp.55-56 and pp.103-114 of the Study Report).

Parameter	Control	LDT	MDT	HDT
#Animals Assigned	20	20	20	20
#Animals Mated/Inseminated	20	20	20	20
Pregnancy Rate (%)	18 (90%)	19 (95%)	18 (90%)	19 (95%)
Maternal Wastage				
#Died	0	0	1[1]	0
#Died/pregnant	0	0	0	0
#Non pregnant	2	1	2	1
#Aborted	1	1	0	4
#Premature Delivery	0	0	0	1
Total Corpora Lutea[2]	182	192	183	170
Corpora Lutea/dam	10.7	10.7	10.8	12.1
Total Implantations	131	111	136	108
Implantations/Dam	7.7[3]	6.2	8.0	7.7
Total Live Fetuses[2]	118	104	123	101
Live Fetuses/Dam	6.9	5.8	7.2	7.2
Total Resorptions[2]	13	7	13	7
Early	7	4	11	6
Late	6	3	2	1
Resorptions/Dam	0.8	0.4	0.8	0.5
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (gm)	46.3	50.9	46.1	42.2
Preimplantation Loss(%)[4]	28.0	42.2	25.7	36.4
Postimplantation Loss(%)[5]	10	6.3	9.6	6.5
Sex Ratio (% Males/litter)	48.5	57.9	49.4	44.5

[1] Intubation error on day 9 of gestation.
[2] Totals were computed by the reviewer.
[3] All implantations were resorbed in one control doe.
[4] Values were calculated by the reviewer:
% = (Tot. corpora lutea - Tot. implantation sites) / (Tot. corpora lutea) x 100.0
[5] Values were calculated by the reviewer:
% = (Tot. resorptions + Tot. dead fetuses) / (Total implantations) x 100.0

2. Developmental Toxicity

External examinations

As shown in Table 6, no apparent external malformations were present in the cesarean-delivered pups. One HDT dam (10074) delivered one live pup on day 29 of gestation; in addition, 6 live fetuses and 3 late resorptions were found in utero. One of the 6 fetuses from this litter (10074-9) had an open left eyelid.

Table 6. External examinations in cesarean-delivered pups. Data from p. 58 and pp. 119-134 of the Study Report.

Observations	Control	LDT	MDT	HDT
# pups (litters) examined	118(16)	104(18)	123(17)	101(14)
# pups (litters) affected	0	0	0	0

Visceral Examinations

Table 7 summarizes the visceral examination data. One case of hydrocephalus was reported in the HDT (7.1% litter incidence and 1.0% fetal incidence). The hydrocephalus was only detectable during the visceral examination. This occurrence of hydrocephalus was not considered to be treatment-related by the authors because it is relatively common in historical controls (up to 9.1% litter incidence and 1.1% fetal incidence) and it was observed only in one fetus.

Table 7. Visceral examinations in cesarean-delivered pups. Data from p.59 and pp. 119-134 of the Study Report.

Observations	Control	LDT	MDT	HDT
# pups (litters) examined	118(16)	104(18)	123(17)	101(14)
# pups (litters) affected	0	0	0	1(1)
Hydrocephalus	0	0	0	1(7.1)[1]

[1] Fetal (litter) incidence, in percent.

Skeletal Examinations

Skeletal findings are presented below in Table 8. The incidence of irregularly shaped fontanellae was significantly higher (fetal incidence = 4%, litter incidence = 7.1%, $p \leq 0.05$) in cesarean-delivered HDT fetuses (4 fetuses from the same litter, 10076-1,2,3,4) than in controls (0 fetuses affected).

These incidences in the HDT will increase slightly to 5.5% (fetal) and to 13.3% (litter) if the incidence of irregularly shaped fontanella in fetuses (10074-1 and 10074-7) from the dam that delivered a live pup is included. These incidences of irregularly shaped fontanellae, however, are still within the range of the historical control data submitted by the testing laboratory (observed incidences other than 0: fetal 0.9-29.5% and litter 7.1- 21.4%). Other skull malformations reported in this study included extra ossification centers at the parietals, frontals and nasals but their incidence did not differ significantly ($p \leq 0.05$) from controls. One HDT fetus (10061-1) presented a hemivertebra, an assymmetric vertebral centrum, unilateral fusion of two vertebral arches and two ribs, and incomplete ossification of the first sternebra (Table 8).

Table 8. Summary of skeletal observations in cesarean-delivered pups. Data from pp. 60-65 and 119-134 of the Study Report.

Observations	Control	LDT	MDT	HDT
# pups (litters) examined	118(16)	104(18)	123(17)	101(14)
MALFORMATIONS				
SKULL				
Intraparietals present				
Total litters affected	1	0	1	1
Number of fetuses (%)	2(1.7)	0	3(2.4)	1(1.0)
Intrafrontals present				
Total litters affected	3	1	0	0
Number of fetuses (%)	3(2.5)	1(1.0)	0	0
Interfrontal present				
Total litters affected	1	1	2	1
Number of fetuses (%)	2(1.7)	2(1.9)[1]	3(2.4)	1(1.0)
Internasal present				
Total litters affected	0	1	0	0
Number of fetuses (%)	0	1(1.0)	0	0
Irregularly shaped fontanelle				
Total litters affected	0	0	0	1
Number of fetuses (%)	0	0	0	4(4.0)*
HYOID				
Ala(e), angulated				
Total litters affected	0	2	2	0
Number of fetuses (%)	0	3(2.9)[1,2]	4(3.2)	0

(Continued)

Table 8. Summary of skeletal observations (Continued from previous page).

Observations	Control	LDT	MDT	HDT
# pups (litters) examined	118(16)	104(18)	123(17)	101(14)
VERTEBRAL/RIB MALFORMATIONS				
Associated vertebral and rib malformations				
Total litters affected	0	0	0	1
Number of fetuses (%)	0	0	0	1(1.0)[3,4]
VERTEBRAE				
Thoracic, hemivertebrae				
Total litters affected	1	0	0	1
Number of fetuses (%)	1(0.8)	0	0	1(1.0)[3]
Thoracic, centrum, assymetric				
Total litters affected	0	0	0	1
Number of fetuses (%)	0	0	0	1(1.0)[3]
Thoracic, arches fused				
Total litters affected	0	0	0	1
Number of fetuses (%)	0	0	0	1(1.0)[3]
Caudal, one or more, misaligned				
Total litters affected	1	2	0	0
Number of fetuses (%)	1(0.8)	2(1.9)	0	0
RIBS				
Two, fused				
Total litters affected	0	0	0	1
Number of fetuses (%)	0	0	0	1(1.0)[3]
STERNEBRAE				
One or more, assymetric				
Total litters affected	0	1	0	0
Number of fetuses (%)	0	1(1.0)	0	0
TOTAL WITH ANY MALFORMATION				
Total litters affected	6	6	5	4
Number of fetuses (%)	9(7.6)	9(8.6)	10(8.1)	7(6.9)

(Continued)

Table 8. Summary of skeletal observations (Continued from previous page).

Observations	Control	LDT	MDT	HDT
# pups (litters) examined	118(16)	104(18)	123(17)	101(14)
VARIATIONS				
SKULL				
Hole in right parietal				
Total litters affected	0	0	0	1
Number of fetuses (%)	0	0	0	1(1.0)[5]
Holes in frontal				
Total litters affected	0	0	0	1
Number of fetuses (%)	0	0	0	1(1.0)[5]
Enlarged fontanelle				
Total litters affected	0	0	0	1
Number of fetuses (%)	0	0	0	1(1.0)
RIBS				
One or more, thickened areas of ossification				
Total litters affected	4	5	3	1
Number of fetuses (%)	5(4.2)	5(4.8)	3(2.4)	1(1.0)
STERNEBRAE				
1st. Incompletely ossified				
Total litters affected	1	0	0	1
Number of fetuses (%)	1(0.8)	0	0	1(1.0)[3]
Two or more, fused				
Total litters affected	1	1	0	1
Number of fetuses (%)	1(0.8)	1(1.0)[2]	0	1(1.0)
TOTAL WITH ANY VARIATIONS				
Total litters affected	5	5	3	4
Number of fetuses (%)	7(5.9)	6(5.8)[2]	3(2.4)	5(5.0)[3,5]

* Significantly different from controls ($p \leq 0.05$).
[1] One fetus (10035-5) also had other alterations.
[2] One fetus (10035-7) also had other alterations.
[3] One fetus (10061-1) also had other alterations.
[4] The category "Associated vertebral and rib malformations", appears to have been included by the authors to highlight the simultaneous occurence of vertebrae and rib malformations listed in this table for fetus 10061-1.
[5] One fetus (10062-9) also had other alterations.

D. Discussion/Conclusions

a. Maternal Toxicity:

Oral administration of Dicofol at 0, 0.4, 4.0, or 40.0 mg/kg/day during days 7-19 of gestation in the New Zeland White rabbit produced signs of toxicity in the HDT. These signs consisted of a significant ($p \leq 0.05$) depression of body weight gain during the dosing period that was associated with a decrease in food consumption and a significant ($p \leq 0.05$) increase of abnormal feces. Gross pathological examination of the liver at sacrifice revealed a significant ($p \leq 0.05$) increase in liver to body weight ratios for LDT and HDT. Histopathological examination of the liver revealed a dose-dependent increase in the incidence of eosinophilic, hyaline material for the MDT and HDT. Although cytoplasmic hyalinization was observed in hepatocytes from the MDT (2/19), the biological significance of this effect is unclear in the MDT in the absence of other toxic signs. Thus, this study defines a maternal NOEL of 4.0 mg/kg/day and a maternal LOEL of 40.0 mg.kg/day.

b. Developmental Toxicity:

A high frequency of abortions (4/19), in excess of concurrent (1/18) and historical controls (up to 1/14-2/15 with an outlier at 1/4), was reported for the HDT. this high incidence of abortions in HDT appears to be attributable to maternal toxicity. In the absence of gross defects in the aborted fetuses and a late reabsorption rate not different from expected, this high incidence of abortions in HDT appears to be attributable to maternal toxicity. Direct developmental toxicity, however, cannot be definitively excluded. Thus, this study defines a developmental toxicity NOEL of 4.0 mg/kg/day and a developmental toxicity LOEL of 40.0 mg/kg/day.

A significant ($p \leq 0.05$) incidence of irregularly shaped fontanellae was observed in the HDT fetuses (4/101, fetal incidence; 1/14 litters, litter incidence) compared to concurrent controls (0/118). This incidence, however, was found to be within the range observed for historical controls. The range of observed incidences in historical control data submitted by the testing laboratory was up to: 0.9-29.5% (fetal) and 7.1- 21.4% (litter). Thus, it is not possible to conclude that the incidence of irregularly shaped fontanellae observed in the HDT is compound related.

D. Study Deficiencies:

No significant study deficiencies were noted.

E. Core Classification: Core minimum data.

Maternal NOEL = 4.0 mg/kg/day
Maternal LOEL = 40.0/mg/kg/day
Developmental Toxicity NOEL = 4.0 mg/kg/day
Developmental Toxicity LOEL = 40.0 mg/kg/day

F. Risk Assessment:

Development of a MOS has been determined to be unnecessary at this time.

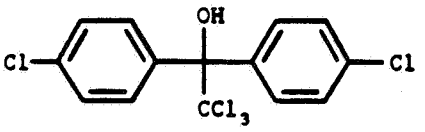
Reviewed by: Susan L. Makris, M.S. Susan L Makris 6-3-91
Toxicologist, Review Section III, Toxicology Branch II-HFAS/HED (H7059C)
Secondary Review by: James N. Rowe, Ph.D James N. Rowe 6/11/91
Review Section III, Toxicology Branch II-HFAS/HED (H7059C)

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction - Rat (Guideline 83-4)

MRID NUMBER: 418066-01

TEST MATERIAL: Dicofol (93.3% active ingredient, less than 0.1% DDT-related materials), described as a dark brown solid (Lot No. RS-4503, Toxicology Department Sample No. 85-211).



SYNONYMS: Kelthane® Technical Miticide

STUDY NUMBER(S): Protocol No. 89P-028, Report No. 89R-028

SPONSOR: Rohm and Haas Company
Toxicology Department
727 Norristown Road
Springhouse, Pennsylvania 19477

TESTING FACILITY: Rohm and Haas Company
Toxicology Department
727 Norristown Road
Springhouse, Pennsylvania 19477

TITLE OF REPORT: Dicofol: Two-Generation Reproduction Study in Rats

AUTHORS: H.M. Solomon and B.A. Kulwich

DATE REPORT ISSUED: February 18, 1991

CONCLUSIONS: Dietary administration of Dicofol, at levels of 5, 25, 125, and 250 ppm, to Crl:CD®BR rats over two generations resulted in decreased P1 premating body weight gain and/or food consumption values at 125 and/or 250 ppm. In addition, histopathological changes were observed in the liver (hypertrophy of centrilobular hepatocytes with associated vacuolation - P1 and P2 males and females at 25, 125, and 250 ppm), adrenal glands (hypertrophy/vacuolation - P1 and P2 females at 125 and 250 ppm), and ovaries (increased vacuolation - P2 females at 25, 125, and 250 ppm).

NOEL for Systemic Toxicity = 5 ppm
LOEL for Systemic Toxicity = 25 ppm

There were no treatment-related effects on reproductive performance for the P1 or P2 adult rats. Evidence of toxicity in the offspring included decreased viability of the P1F1a pups at the 250 ppm dose level and P2F2a and P2F2b pups at the 125 and 250 ppm dose levels; increased numbers of stillborn pups, pup deaths (predominantly Days 0-4 of lactation), and total litter loss were observed. A negative effect on pup growth (decreased pup weight) was noted for Days 7 and 14 of lactation for the 250 ppm dose level. Ovarian vacuolation noted in the P2 females at 25, 125, and 250 ppm was compatible with enhanced steroidogenic activity and is judged to be an effect on reproductive physiology.

NOEL for Reproductive Toxicity = 5 ppm
LOEL for Reproductive Toxicity = 25 ppm

Core Classification: Core-Minimum Data

I. PROTOCOL

A. Materials

1. Test species: 21-day old male and female Crl:CD^{R}BR rats were obtained for the first parental generation of the study from the Charles River Breeding Laboratories, Kingston Facility, Stone Ridge, New York. The rats were acclimated for a period of 2 weeks before they were placed on study.

2. Test Material Formulation and Analysis: The test material, Dicofol, was administered in the diet. Fresh diet mixtures were prepared weekly. Acetone was used as the carrier solvent. Representative samples of test diets were analyzed for concentration, homogeneity, and chemical stability of the active ingredient in dietary mixtures (study weeks 1, 2, 4, 8, 12, 16, 20, 24, 32, 36, 40, 48, 52, and 56).

B. Procedures and Study Design

1. Animal assignment: P1 animals were randomly assigned to test groups as follows:

Group No.	Test Compound	Dose (ppm)[a]	Animals per group[b]	
			Male	Female
1	Control[c]	0	25	25
2	Dicofol	5	25	25
3	Dicofol	25	25	25
4	Dicofol	125	25	25
5	Dicofol	250	25	25

a All diet concentrations are ppm of active ingredient. Diets were administered from the beginning of the study until the animals were sacrificed.
b The same number of animals were picked from the F1 litters as parents for the P2 generation.
c Solvent (acetone) control.

2. Mating: During the 21-day mating period, one male was caged with one female from the same test group. Sibling matings were avoided. Females were examined daily for positive evidence of mating (observation of a retained or exuded copulatory plug). For P2 females, mating was also confirmed by observation of sperm cells following vaginal lavage. If plug and/or sperm were not found after 10 days of cohabitation, the first male was removed and replaced by another male from the same test group that had copulated successfully during the previous 10 days. For a second (additional) breeding of the P2 animals, the number of days allowed prior to replacement of male breeders was reduced to 7, allowing a potential to pair one female with up to 3 males during the 21-day duration of mating.

Following positive evidence of copulation, each mated female was individually housed in a cage with a solid bottom and absorbent bedding where it was kept throughout the gestation and lactation periods. Females for which mating was not confirmed were presumed to be pregnant and housed similarly.

3. Mating schedule: The P1 and P2 parental animals were given test diets for 10 and 14 weeks, respectively, before they were mated. Selection of P2 parental animals was made at 22-28 days of age, and the mated animals in the study were approximately 16-17 weeks of age at the time of first mating. An additional mating of the P2 generation was conducted because, according to the investigators, some of the data from the first mating were equivocal.

C. Observation Schedule

1. Parental animals: Observations and the schedule for those observations is summarized from the report as follows:

Type of Observation	Number of Animals/Sex/Group	Frequency
Mortality and signs of toxicity	All	Twice a day during the study.
Detailed clinical observations	All	Once a week during the study.
Body weight	All	At beginning of study and weekly through premating and growth periods.
	Maternal animals	Days 0, 7, 14, and 21 of gestation; days 0, 7, 14, and 21 post partum.
Food consumption	All	Weekly during premating period.
	Maternal animals	Days 0, 7, 14, and 21 of gestation; days 0, 7, 14, and 21 of lactation.

2. Reproductive performance: Parental reproductive performance was assessed from breeding and parturition records of animals in the study. For P1 and P2 females, mating was considered successful if a sperm plug was detected in the vagina or on the absorbent paper beneath the cage. In addition, for P2 females, mating was confirmed by positive evidence of sperm in a vaginal lavage.

The following indices were calculated:

$$\text{Male mating index (\%)} = \frac{\text{No. males that mated}}{\text{No. males used for mating}} \times 100$$

$$\text{Female mating index (\%)} = \frac{\text{No. females that mated}}{\text{No. females used for mating}} \times 100$$

$$\text{Male fertility index (\%)} = \frac{\text{No. sires}}{\text{No. males mated}} \times 100$$

$$\text{Female fertility index (\%)} = \frac{\text{No. females pregnant}}{\text{No. females mated}} \times 100$$

$$\text{Gestation index (\%)} = \frac{\text{No. females producing litters with at least one live pup}}{\text{No. pregnant females}} \times 100$$

3. Litter observations: According to the report, the following litter observations were made:

Observation	Day of Observation (Lactation)				
	Day 0	Day 4	Day 7	Day 14	Day 21
Number of live pups	X	X[a]			
Number of dead pups[b]	X				
Sex of each pup	X				
Individual body weights	X	X	X	X	X
External alterations	X				
Clinical signs[c]	X	X	X	X	X

a On day 4, litters were culled randomly to 8 pups (4/sex) when possible.
b Cage-site observations to detect dead or moribund pups were conducted twice daily through lactation.
c Pups were examined for signs of ill health or reaction to treatment and for abnormal behavior or appearance.

Dead pups were examined grossly for external and internal abnormalities, and a possible cause of death was determined for pups born or found dead.

The following indices were calculated:

$$\text{Gestation index (\%)} = \frac{\text{No. females producing litters with at least one live pup}}{\text{No. pregnant females}} \times 100$$

$$\text{Viability index (\%)} = \frac{\text{No. pups/litter alive on day 4}}{\text{No. pups/litter born alive}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\text{No. pups/litter alive on day 21}}{\text{No pups/litter alive after culling (day 4)}} \times 100$$

4. Postmortem Studies

a. Sacrifice and Examination Schedules

1. Parental animals: All surviving parental males were sacrificed after the last litters in each generation were produced. Maternal

animals were sacrificed after the last litter of each generation was weaned. These animals were subjected to post mortem examinations as follows:

Animals Examined	Macroscopic	Microscopic[a]
Found dead	X	X
Unscheduled sacrifice	X	X
Scheduled sacrifice	X	X

a All tissues from control and high dose groups (250 ppm), gross lesions from low and intermediate dose groups (5, 25, and 125 ppm), livers from both sexes and adrenal glands and ovaries for females of the low and intermediate dose groups.

2. Offspring: The F1, F2a and F2b offspring were sacrificed at the end of the weaning period. These animals were subjected to post mortem examinations as follows:

Animals Examined	Macroscopic	Microscopic
Found dead	X	
Scheduled sacrifice	X	

b. Necropsy observations: Gross necropsy consisted of examination of all organs, tissues, and body cavities. The uteri of parental females that did not deliver a litter were opened at necropsy and stained with 10% ammonium sulfide to detect the presence of very early resorptions.

c. Histopathology: The following required tissues from parental animals were prepared for microscopic examination:

X Ovaries
X Uterus
X Unusual lesions
X Vagina/cervix
X Epididymides
X Prostate
X Seminal vesicles
X Testes

Additional tissues prepared for microscopic examination included liver, adrenal glands, pituitary, and coagulating gland. Tissue sections were stained with hematoxylin and eosin.

D. Data Analyses

1. General considerations: All analyses were done for each generation separately. Premating observations and fertility parameters were analyzed separately for each sex. Mean pup body weight was calculated by litter in each group before the group mean value was determined. The report stated that the litter was used as the experimental unit for the purpose of statistical evaluation. The level of significance selected was 0.05.

2. Statistical analyses: Analysis of variance was performed on the following observations: parental body weight and food consumption, offspring body weight, and length of gestation. Dunnett's t-test was used when one-way ANOVA was significant. The Fisher's Exact Test was used to assess incidence data (pregnancy, clinical signs, maternal death, litters with stillborn pups, gross necropsy, and histopathology). The Mann-Whitney U Test was used to analyze live fetuses per litter, viability and lactation indices, and sex ratio. According to the report, when more than 75% ties occurred (i.e., 75% of the litters were unaffected for a particular parameter), the Fisher's Exact test was used in place of the Mann-Whitney U test to detect significant differences between groups. The following indices were calculated using either the Fisher's Exact test or the Mann-Whitney U test: mating (male and female), fertility (male and female), gestation, viability, and lactation.

3. Compliance: The following signed statements were supplied:

- Statement of Data Confidentiality
- GLP Compliance Statement
- Flagging Statement
- Quality Assurance Statement

II. REPORTED RESULTS

A. Analysis of test diets: Analysis of sample mixtures prior to study start indicated that formulation procedures were adequate and produced homogeneous mixtures (reported range of 94-107% of target). Stability was demonstrated for formulation samples stored for 1 week at room temperature (reported range of 90-107% of target). Concentration analyses of formulations indicated actual mean concentrations of 0, 5.4, 24.4, 126.0, and 227.0 ppm which were within ±10% of nominal concentration values and were judged to be acceptable.

B. Parental animals

1. Mortality and clinical signs: Several animals were found dead or were sacrificed *in extremis* during the course of the study (Table 1). Due to the nature and distribution of these deaths, they were not considered to be related to treatment.

Table 1: Incidence of Unscheduled Deaths

Dose (ppm)	P1 Males	P1 Females	P2 Males	P2 Females
0	1	0	0	0
5	2	0	0	0
25	0	0	0	0
125	0	1a	1	1
250	1	0	1	1a

a Died during delivery.

The investigators noted sporadic statistically significant increases in the incidence of specific clinical observations as presented in Table 2.

Table 2: Selected Clinical Observations

Generation/ Sex	Observation	No. Animals Affected				
		0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
P1F1a Females	Pale in appearance (lactation Day 0)	0	0	0	0	7*
P2F2a Males	Red staining around eye	1	9*	6*	3	3
	Nodules on tail	1	4	8*	6*	4
	Malocclusion	2	6	8*	5	5

* Significantly different from control, $P<0.05$.

Due to the lack of correlation between sexes, generations, and/or groups, these clinical observations were not judged to be treatment-related.

2. Body weight and food consumption

Selected body weight and food consumption results for parental animals prior to mating and for pregnant or nursing dams are summarized below in Tables 3a, 3b, 4a, 4b, and 4c.

Premating: Premating body weight and food consumption values for P1 animals are summarized in Table 3a. The report noted a slight, nonsignificant yet apparently treatment-related decrease in body weight during the premating period for the P1 males at 250 ppm. Significant treatment-related decreases in mean body weight were observed in the P1 females at 125 and 250 ppm during premating Weeks 2-10 and 1-10, respectively. Mean body weight change values (Weeks 0-10) for both P1 males and females demonstrate a treatment-related effect at these dose levels.

Premating food consumption was marginally reduced for P1 males at 125 and 250 ppm and for P1 females at 25 ppm and significantly reduced for P1 females at 125 and 250 ppm for some intervals.

Premating body weight and food consumption data were similar between control and treated groups for the P2 animals (Table 3b).

Gestation and lactation: P1 maternal body weight and food consumption values are summarized in Table 4a. Statistically significant treatment-related decreases in mean body weight were noted for P1 females at 125 and 250 ppm during all intervals of gestation and at 250 ppm on days 0 and 7 of lactation (although overall body weight change values during gestation and lactation at all breedings were generally similar between control and treated groups). Concurrent food consumption decreases were noted during gestation, with statistical significance attained only at Days 0-7. Food consumption during lactation was similar between control and treated P1 females.

No treatment-related effect was evident in the bodyweight or food consumption data from the first or second gestation or lactation periods of the P2 females (Tables 4b and 4c).

Table 3a: Premating Body Weight and Food Consumption - P1

Premating Observations	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
P1 Males					
Mean body weight (g ± S.E.)					
Week 0	217.5 ± 3.37	215.0 ± 3.49	219.2 ± 3.24	217.4 ± 3.40	215.7 ± 3.03
Week 5	432.3 ± 9.08	430.6 ± 9.50	445.8 ± 6.74	425.8 ± 7.68	404.3 ± 6.72
Week 10	531.9 ± 13.76	537.4 ± 13.68	563.0 ± 10.39	530.9 ± 9.57	503.1 ± 9.54
Mean body weight change (g ± S.E.)[a]					
Weeks 0-10	315.5 ± 12.00	320.9 ± 11.95	343.8 ± 9.23	313.5 ± 8.31	288.1 ± 8.75
Mean food consumption (g/rat/day ± S.E.)					
Week -1	24.8 ± 0.37	25.1 ± 0.54	25.5 ± 0.36	25.4 ± 0.38	25.0 ± 0.35
Week 4	28.9 ± 0.74	28.5 ± 0.84	30.2 ± 0.67	28.3 ± 0.63	26.6 ± 0.51
Week 9	28.5 ± 0.70	28.3 ± 0.75	29.9 ± 0.63	28.1 ± 0.49	27.3 ± 0.55
P1 Females					
Mean body weight (g ± S.E.)					
Week 0	163.9 ± 2.14	161.1 ± 2.18	160.3 ± 2.17	159.6 ± 2.46	162.3 ± 1.99
Week 5	262.3 ± 4.24	251.2 ± 3.65	252.1 ± 3.92	242.0 ± 4.04*	234.6 ± 2.71*
Week 10	300.5 ± 5.08	292.4 ± 4.73	290.3 ± 5.40	279.1 ± 5.37*	267.7 ± 3.58*
Mean body weight change (g ± S.E.)[a]					
Weeks 0-10	136.6 ± 4.05	131.3 ± 4.09	130.0 ± 4.09	119.5 ± 3.80	105.4 ± 3.04
Mean food consumption (g/rat/day ± S.E.)					
Week -1	18.9 ± 0.35	19.0 ± 0.24	18.3 ± 0.36	18.6 ± 0.35	19.0 ± 0.26
Week 4	20.8 ± 0.41	19.9 ± 0.31	19.6 ± 0.45	18.9 ± 0.39*	18.2 ± 0.28*
Week 9	19.2 ± 0.37	19.0 ± 0.31	18.1 ± 0.37	18.2 ± 0.43	17.0 ± 0.26*

* Statistically significantly different from control, $p<0.05$.

a Calculated by reviewer from individual body weight data, report No. 89R-028, pages 330-339; group comparisons not performed.

Note: Data were extracted from report No. 89R-028, pages 71-74, 77-80, 85-88, and 91-94.

Table 3b: Premating Body Weight and Food Consumption - P2

Premating Observations	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
P2 Males					
Mean body weight (g ± S.E.)					
Week 0	76.7 ± 2.13	78.0 ± 2.48	81.4 ± 2.72	76.7 ± 2.53	70.2 ± 2.78
Week 5	363.7 ± 4.85	368.0 ± 5.61	379.5 ± 7.04	370.4 ± 4.81	361.6 ± 7.61
Week 10	509.9 ± 7.47	510.6 ± 10.56	542.4 ± 10.91	525.7 ± 7.54	513.6 ± 11.20
Week 15	580.9 ± 8.52	582.5 ± 13.58	614.4 ± 14.45	594.7 ± 11.17	590.3 ± 13.30
Mean body weight change (g ± S.E.)[a]					
Weeks 0-10	433.1 ± 7.68	432.6 ± 9.85	461.0 ± 9.18	449.0 ± 8.06	441.5 ± 11.27
Mean food consumption (g/rat/day ± S.E.)					
Week 0	16.4 ± 0.35	17.2 ± 0.36	17.3 ± 0.35	16.9 ± 0.39	16.3 ± 0.48
Week 4	32.5 ± 0.56	32.3 ± 0.53	32.7 ± 0.60	32.3 ± 0.44	31.8 ± 0.55
Week 9	30.6 ± 0.66	29.8 ± 0.97	32.4 ± 0.68	31.1 ± 0.51	31.2 ± 0.62
Week 14	30.3 ± 0.58	30.1 ± 0.71	30.4 ± 0.75	29.3 ± 0.77	31.1 ± 0.67
P2 Females					
Mean body weight (g ± S.E.)					
Week 0	70.6 ± 2.01	73.6 ± 1.96	76.0 ± 2.17	67.5 ± 2.57	66.2 ± 2.31
Week 5	222.9 ± 3.84	221.9 ± 2.76	227.5 ± 4.51	219.6 ± 3.79	220.9 ± 4.28
Week 10	282.6 ± 6.13	284.9 ± 5.08	293.0 ± 6.01	281.0 ± 5.97	284.4 ± 6.03
Week 15	308.7 ± 6.94	311.0 ± 6.06	320.9 ± 7.14	305.7 ± 6.85	311.9 ± 6.53
Mean body weight change (g ± S.E.)[a]					
Weeks 0-10	212.0 ± 5.82	211.3 ± 5.25	217.0 ± 5.36	202.3 ± 12.04	218.2 ± 6.23
Mean food consumption (g/rat/day ± S.E.)					
Week 0	15.3 ± 0.25	15.7 ± 0.30	15.1 ± 0.29	15.5 ± 0.31	14.4 ± 0.30
Week 4	22.2 ± 0.44	21.6 ± 0.39	21.6 ± 0.47	21.0 ± 0.44	21.2 ± 0.43
Week 9	21.3 ± 0.52	21.2 ± 0.55	20.4 ± 0.44	20.9 ± 0.57	20.9 ± 0.42
Week 14	21.0 ± 0.45	20.4 ± 0.38	20.4 ± 0.52	19.9 ± 0.42	20.2 ± 0.43

* Statistically significantly different from control, $p<0.05$.

a Calculated by reviewer from individual premating body weight data, report No. 89R-028, pages 350-369; group comparisons not performed.

Note: Data were extracted from report No. 89R-028, pages 71-74, 77-80, 85-88, and 91-94.

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Table 4a: Maternal Body Weight and Food Consumption - P1F1a Generation

Maternal Observations	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
Gestation					
Mean body weight (g ± S.E.)					
Day 0	299.9 ± 6.63	288.6 ± 5.78	281.3 ± 3.82	272.7 ± 6.16*	264.4 ± 2.57*
Day 7	330.4 ± 6.56	320.7 ± 6.02	312.1 ± 4.59	299.0 ± 5.97*	288.2 ± 3.21*
Day 14	357.5 ± 5.52	353.6 ± 6.56	343.0 ± 4.62	330.8 ± 6.54*	323.5 ± 3.92*
Day 21	436.6 ± 7.00	434.4 ± 9.32	421.2 ± 5.86	403.8 ± 10.68*	404.5 ± 5.55*
Mean body weight change (g ± S.E.)[a]					
Days 0-21	136.7 ± 5.25	145.7 ± 5.07	139.9 ± 5.13	131.1 ± 8.33	140.1 ± 4.75
Mean food consumption (g/rat/day ± S.E.)					
Days 0-7	20.3 ± 0.68	20.3 ± 0.66	20.1 ± 0.44	18.2 ± 0.44*	16.6 ± 0.45*
Days 7-14	23.5 ± 0.53	23.9 ± 0.90	23.7 ± 0.59	22.1 ± 0.60	21.6 ± 0.46
Days 14-21	24.8 ± 0.69	25.7 ± 0.75	25.1 ± 0.54	23.9 ± 0.67	24.9 ± 0.51
Lactation					
Mean body weight (g ± S.E.)					
Day 0	336.2 ± 7.07	334.0 ± 7.16	319.0 ± 7.28	315.4 ± 7.36	303.5 ± 4.68*
Day 7	346.2 ± 7.83	350.2 ± 7.53	331.1 ± 5.16	329.1 ± 7.93	310.1 ± 4.88*
Day 14	360.4 ± 6.26	354.0 ± 7.84	347.1 ± 6.80	337.0 ± 8.89	340.7 ± 4.44
Day 21	352.0 ± 4.44	352.5 ± 7.15	347.1 ± 5.59	337.7 ± 7.33	337.0 ± 3.65
Mean body weight change (g ± S.E.)[a]					
Days 0-21	15.8 ± 4.94	18.5 ± 3.77	28.2 ± 4.96	22.3 ± 3.51	33.5 ± 4.85
Mean food consumption (g/rat/day ± S.E.)					
Days 0-7	35.0 ± 1.33	38.4 ± 1.30	35.3 ± 1.50	33.9 ± 1.50	33.9 ± 1.22
Days 7-14	58.2 ± 1.42	57.8 ± 1.65	57.1 ± 1.79	52.3 ± 2.21	56.3 ± 2.25
Days 14-21	64.6 ± 2.36	64.7 ± 2.15	65.8 ± 1.68	64.0 ± 3.16	68.0 ± 2.52

* Statistically significantly different from control, $p<0.05$.

a Calculated by reviewer from individual gestation and lactation body weight data, report No. 89R-028, pages 340-349; group comparisons not performed.

Note: Data were extracted from report No. 89R-028, pages 75-76 and 89-90.

Table 4b: Maternal Body Weight and Food Consumption - P2F2a Generation

Maternal Observations	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
Gestation					
Mean body weight (g ± S.E.)					
Day 0	315.8 ± 8.95	310.4 ± 6.80	320.9 ± 8.59	307.9 ± 8.39	311.7 ± 9.92
Day 7	333.4 ± 9.01	333.0 ± 6.55	341.6 ± 8.32	331.1 ± 8.44	335.5 ± 10.01
Day 14	361.4 ± 8.73	360.8 ± 6.47	369.6 ± 7.98	362.1 ± 9.39	366.4 ± 11.13
Day 21	441.6 ± 10.60	447.3 ± 8.09	446.9 ± 8.87	443.8 ± 10.52	455.8 ± 12.32
Mean body weight change (g ± S.E.)[a]					
Days 0-21	125.9 ± 5.90	136.9 ± 5.36	126.0 ± 5.19	134.9 ± 5.19	144.1 ± 3.53
Mean food consumption (g/rat/day ± S.E.)					
Days 0-7	19.2 ± 0.75	20.1 ± 0.41	20.1 ± 0.62	20.2 ± 0.56	20.4 ± 0.61
Days 7-14	22.8 ± 1.07	22.4 ± 0.30	22.7 ± 0.66	23.0 ± 0.70	23.8 ± 0.75
Days 14-21	24.0 ± 0.46	24.4 ± 0.53	23.6 ± 0.96	25.5 ± 0.70	26.7 ± 0.85
Lactation					
Mean body weight (g ± S.E.)					
Day 0	342.7 ± 6.28	347.7 ± 5.29	347.5 ± 9.77	334.2 ± 7.58	355.3 ± 9.56
Day 7	345.1 ± 6.27	360.5 ± 4.90	352.4 ± 6.95	350.6 ± 7.01	366.7 ± 9.44
Day 14	354.1 ± 5.21	360.3 ± 7.14	352.1 ± 4.30	341.9 ± 7.38	369.2 ± 9.20
Day 21	338.2 ± 6.07	350.9 ± 5.76	337.7 ± 7.52	334.8 ± 7.51	369.4 ± 8.23*
Mean body weight change (g ± S.E.)[a]					
Days 0-21	-4.5 ± 4.52	3.2 ± 5.42	-9.8 ± 5.11	0.6 ± 3.81	14.1 ± 3.81
Mean food consumption (g/rat/day ± S.E.)					
Days 0-7	30.9 ± 1.10	33.9 ± 1.24	31.2 ± 1.32	32.9 ± 1.47	33.6 ± 1.66
Days 7-14	50.6 ± 1.91	52.4 ± 1.73	49.3 ± 2.28	51.9 ± 2.13	53.7 ± 2.29
Days 14-21	61.1 ± 1.98	61.4 ± 2.01	57.4 ± 2.51	61.6 ± 3.20	63.3 ± 2.38

* Statistically significantly different from control, $p<0.05$.

a Calculated by reviewer from individual gestation and lactation body weight data, report No. 89R-028, pages 370-379; group comparisons not performed.

Note: Data were extracted from report No. 89R-028, pages 81-82 and 95-96.

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Table 4c: Maternal Body Weight and Food Consumption - P2F2b Generation

Maternal Observations	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
Gestation					
Mean body weight (g ± S.E.)					
Day 0	344.9 ± 6.92	362.0 ± 11.24	353.6 ± 14.86	365.0 ± 13.63	349.2 ± 10.29
Day 7	371.5 ± 7.77	392.0 ± 11.92	376.7 ± 13.65	392.0 ± 13.99	376.1 ± 10.74
Day 14	400.8 ± 8.13	421.9 ± 11.99	410.3 ± 14.36	424.3 ± 16.08	406.8 ± 13.41
Day 21	481.8 ± 9.70	511.1 ± 13.21	501.3 ± 20.74	510.5 ± 16.74	485.8 ± 22.23
Mean body weight change (g ± S.E.)[a]					
Days 0-21	136.9 ± 5.38	149.1 ± 6.48	147.6 ± 12.20	145.5 ± 5.08	136.6 ± 14.65
Mean food consumption (g/rat/day ± S.E.)					
Days 0-7	21.3 ± 0.98	22.7 ± 0.77	22.0 ± 0.94	22.5 ± 0.97	21.8 ± 0.77
Days 7-14	23.3 ± 0.81	24.6 ± 0.72	26.3 ± 1.18	25.0 ± 1.28	24.5 ± 1.16
Days 14-21	23.2 ± 0.80	24.9 ± 1.16	26.4 ± 1.11	25.5 ± 0.78	26.1 ± 1.23
Lactation					
Mean body weight (g ± S.E.)					
Day 0	399.4 ± 8.85	403.0 ± 10.16	398.6 ± 11.40	388.4 ± 12.36	390.5 ± 11.48
Day 7	397.4 ± 8.34	401.4 ± 8.52	394.5 ± 9.44	396.4 ± 11.72	404.9 ± 9.70
Day 14	390.1 ± 8.20	397.2 ± 9.30	393.4 ± 11.18	378.9 ± 12.09	396.6 ± 9.30
Day 21	375.8 ± 7.20	388.2 ± 8.44	373.3 ± 9.35	374.4 ± 9.69	390.5 ± 8.77
Mean body weight change (g ± S.E.)[a]					
Days 0-21	-23.6 ± 5.25	-14.8 ± 6.53	-25.2 ± 7.23	-14.0 ± 5.77	-0.1 ± 4.14
Mean food consumption (g/rat/day ± S.E.)					
Days 0-7	31.1 ± 0.95	31.8 ± 1.19	30.8 ± 1.56	33.2 ± 1.35	34.1 ± 2.33
Days 7-14	48.5 ± 1.58	48.4 ± 1.89	45.4 ± 3.30	46.8 ± 2.03	46.9 ± 3.07
Days 14-21	59.9 ± 2.04	59.5 ± 2.73	55.2 ± 4.19	59.4 ± 2.45	58.4 ± 4.13

* Statistically significantly different from control, $p<0.05$.

a Calculated by reviewer from individual gestation and lactation body weight data, report No. 89R-028, pages 380-389; group comparisons not performed.

Note: Data were extracted from report No. 89R-028, pages 83-84 and 97-98.

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3. Test Substance Intake: Based on food consumption, body weight, and dietary analyses results, the doses expressed as mg test substance/kg body weight were as follows during the premating period:

Table 5a: Premating Test Substance Intake (mg/kg/day)

Interval	Males				Females			
	5 ppm	25 ppm	125 ppm	250 ppm	5 ppm	25 ppm	125 ppm	250 ppm
P1F1a Generation								
Week 0	0.7	3.2	15.7	29.5	0.6	3.0	14.3	25.1
Week 1	0.5	2.6	12.6	25.4	0.5	2.5	12.7	27.5
Week 2	0.4	2.2	10.9	21.5	0.5	2.3	11.5	23.1
Week 3	0.4	2.0	9.6	19.4	0.4	2.2	11.0	21.4
Week 4	0.4	1.8	8.9	17.4	0.4	2.0	10.3	20.2
Week 5	0.3	1.7	8.4	16.8	0.4	1.9	9.8	19.0
Week 6	0.3	1.6	7.9	15.7	0.4	1.8	9.3	18.1
Week 7	0.3	1.5	7.4	14.9	0.4	1.7	9.0	17.5
Week 8	0.3	1.4	7.1	14.5	0.3	1.6	8.5	16.7
Week 9	0.3	1.4	6.8	13.9	0.3	1.6	8.4	16.2
Weeks 0-9	0.4	1.9	9.5	18.9	0.4	2.1	10.5	20.5
P2F2a/b Generation								
Week 0	1.1	5.4	27.9	58.8	1.1	5.0	29.1	55.4
Week 1	0.8	4.1	21.2	44.5	0.8	3.7	20.6	41.1
Week 2	0.7	3.3	16.6	34.6	0.6	3.1	16.1	32.5
Week 3	0.6	2.8	14.2	29.2	0.6	2.7	14.2	28.5
Week 4	0.5	2.5	12.7	25.6	0.5	2.6	13.1	26.7
Week 5	0.4	2.2	11.0	22.3	0.5	2.4	12.2	24.7
Week 6	0.4	2.0	9.9	20.1	0.5	2.3	11.5	22.9
Week 7	0.3	1.7	8.7	17.4	0.4	2.0	10.3	20.9
Week 8	0.3	1.5	7.9	16.1	0.4	1.9	9.7	19.4
Week 9	0.3	1.6	7.7	15.9	0.4	1.8	9.5	18.8
Week 10	0.3	1.5	7.4	15.6	0.4	1.8	9.2	18.5
Week 11	0.3	1.4	7.3	14.9	0.4	1.7	9.1	17.8
Week 12	0.3	1.4	7.0	14.4	0.4	1.7	8.7	18.0
Week 13	0.3	1.3	6.6	14.1	0.3	1.7	8.5	17.2
Week 14	0.3	1.3	6.2	13.5	0.3	1.6	8.3	16.6
Week 15	0.3	1.2	6.3	13.1	0.3	1.6	8.1	16.6
Weeks 0-15	0.5	2.2	11.2	23.2	0.5	2.4	12.4	24.7

Note: Data were extracted from report No. 89R-028, pages 35, 99-102, and 105-108.

According to the report, calculations of test substance consumption for maternal animals, based upon food consumption, body weight, and dietary analyses results, resulted in the following values (Table 5b). The apparent increase in compound consumption for the P1 and P2 dams in all treated groups during lactation was judged to be the result of food consumption by the offspring as well as the dams.

Table 5b: Maternal Test Substance Intake (mg/kg/day)

Interval	Gestation				Lactation			
	5 ppm	25 ppm	125 ppm	250 ppm	5 ppm	25 ppm	125 ppm	250 ppm
P1F1a Generation								
Days 0 - 7	0.4	1.8	8.4	15.7	0.6	2.8	13.5	28.1
Days 7 - 14	0.4	1.9	9.2	18.7	0.8	4.3	19.9	45.7
Days 14 - 21	0.4	1.8	9.0	19.3	0.9	4.8	23.9	50.0
Days 0 - 21	0.4	1.8	8.9	17.9	0.8	4.0	19.1	41.3
P2F2a Generation								
Days 0 - 7	0.3	1.6	8.3	16.4	0.5	2.3	12.4	24.0
Days 7 - 14	0.3	1.7	8.7	17.9	0.7	3.5	18.7	37.2
Days 14 - 21	0.3	1.6	8.8	18.2	0.9	4.1	22.8	43.4
Days 0 - 21	0.3	1.6	8.6	17.5	0.7	3.3	18.0	34.8
P2F2b Generation								
Days 0 - 7	0.3	1.6	7.7	15.6	0.4	1.9	10.9	22.2
Days 7 - 14	0.3	1.8	8.0	16.2	0.6	2.9	15.0	29.5
Days 14 - 21	0.3	1.6	7.6	16.0	0.8	3.5	20.0	37.3
Days 0 - 21	0.3	1.7	7.8	15.9	0.6	2.8	15.3	29.8

Note: Data were extracted from report No. 89R-028, pages 35, 103-104, and 109-112.

4. Reproductive performance: Results for the parental animals are summarized from the report in Table 6.

There were no treatment-related effects on reproductive performance noted by the investigators for the P1 or P2 male and female rats at any interval. Significant decreases in the number of males mated at 250 ppm for the P2F2a generation and of males mated and females mated at 25 ppm for the P2F2b generation were not considered by the investigators to be related to treatment.

5. Necropsy results

a. Organ weights: No organ weights were taken at necropsy.

b. Pathology

i. Macroscopic examination: Gross necropsy observations reported for the P1 and P2 parental animals appeared to be incidental in nature and unrelated to administration of the test substance.

ii. Microscopic examination: Selected observations noted at histopathological examination of tissues from the parental animals of both generations are presented in Table 7a.

Statistically significant treatment-related findings were observed in the liver of both sexes of both generations at dose levels of 25, 125, and 250 ppm. These changes consisted of hypertrophy of centrilobular hepatocytes, with associated minimal to slight (P1) or moderate (P2) vacuolation of hepatocytes with a centrilobular to midzonal distribution in the males. Statistically significant treatment-related changes, comprised of hypertrophy and/or vacuolation, were noted in the adrenal glands of both P1 and P2 female rats at 125 and 250 ppm. In addition, statistically significant treatment-related changes (increased vacuolation) were noted in the ovaries of P1 females at the 250 ppm dose level and in the P2 females at the 25, 125, and 250 ppm dose levels. According to the investigators,

Table 6: Reproductive Performance

Observation	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
P1F1a Generation					
Mean precoital interval (days)	6.0	4.7	4.9	5.6	5.4
Males					
No. paired	25	25	25	25	25
No. mated	17	19	21	17	18
Male mating index (%)	68	76	84	68	72
No. fertile	12	17	15	14	17
Male fertility index (%)	71	89	71	82	94
Females					
No. paired	25	25	25	25	25
No. mated	25	25	25	25	25
Female mating index (%)	100	100	100	100	100
No. fertile	16	19	18	20	22
Female fertility index (%)	64	76	72	80	88
Mean gestation duration (days)	22.7	22.8	22.8	22.7	23.0
Gestation index (%)	100	100	94	85	100
P2F2a Generation					
Mean precoital interval (days)	4.6	3.2	4.6	6.3	7.9
Males					
No. paired	25	25	25	25	25
No. mated	22	22	19	19	15*
Male mating index (%)	88	88	76	76	60
No. fertile	20	19	15	17	15
Male fertility index (%)	91	86	79	89	100
Females					
No. paired	25	25	25	24	25
No. mated	25	24	22	24	20
Female mating index (%)	100	96	88	100	80
No. fertile	23	20	17	22	20
Female fertility index (%)	92	83	77	92	100
Mean gestation duration (days)	22.4	22.2	22.6	22.5	22.6
Gestation index (%)	100	100	94	96	85
P2F2b Generation					
Mean precoital interval (days)	3.3	4.8	2.0	2.8	5.2
Males					
No. paired	25	25	25	25	25
No. mated	18	15	9*	21	14
Male mating index (%)	72	60	36	84	56
No. fertile	17	15	9	19	14
Male fertility index (%)	94	100	100	90	100
Females					
No. paired	25	25	25	25	25
No. mated	21	20	13*	25	23
Female mating index (%)	84	80	52	100	92
No. fertile	20	19	13	23	20
Female fertility index (%)	95	95	100	92	87
Mean gestation duration (days)	22.2	22.4	22.6	22.8*	22.8
Gestation index (%)	100	95	92	96	85

* Statistically significantly different from control, $p<0.05$.

Note: Data were extracted from report No. 89R-028, pages 113-116, 119, and 122.

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the ovarian vacuolation, characterized by an increase in the size and/or number of vacuoles in the cytoplasm of the stromal cells, is compatible with enhanced steroidogenic activity.

Table 7a: Histopathology Findings - Parental Animals

Observation	Males					Females				
	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
P1 Generation										
Liver										
Number examined	25	25	25	25	25	25	25	25	25	25
Hypertrophy, centrilobular hepatocytes	0	0	21*	25*	24*	0	0	13*	21*	25*
Vacuolization, centrilobular to midzonal hepatocytes	2	1	10*	11*	9*	0	0	0	3	1
Bile duct hyperplasia	6	5	4	10	8	2	3	4	4	7
Focus/area of cell alteration										
a. Clear cell type	2	1	6	4	5	2	1	0	0	2
b. Eosinophilic type	1	0	0	1	3	0	0	0	1	0
Adrenal										
Number examined	25	2	1	0	25	25	25	25	25	25
Hypertrophy/vacuolization, inner cortex	0	0	0	0	0	0	0	0	7*	23*
Ovary										
Number examined						25	25	25	25	25
Increased vacuolization						1	1	3	2	20*
P2 Generation										
Liver										
Number examined	25	25	25	25	25	25	25	25	25	25
Hypertrophy, centrilobular hepatocytes	0	1	14*	24*	25*	0	0	9*	24*	24*
Vacuolization, centrilobular to midzonal hepatocytes	3	2	7	12*	16*	1	0	1	3	2
Bile duct hyperplasia	13	15	9	10	13	4	5	8	5	15*
Focus/area of cell alteration										
a. Clear cell type	9	5	10	8	11	4	3	2	1	1
b. Eosinophilic type	1	2	0	2	6*	0	0	1	6*	8*
Adrenal										
Number examined	25	2	0	1	25	25	25	25	25	25
Hypertrophy/vacuolization, inner cortex	0	0	0	0	0	0	0	0	8*	25*
Ovary										
Number examined						25	25	25	25	25
Increased vacuolization						1	1	6*	5	18*

* Statistically significantly different from control, $p<0.05$; analysis by data reviewer, Fischer's Exact Test.

Note: Data were extracted from report No. 89R-028, pages 143-154.

Observed hypertrophy of the centrilobular hepatocytes from both parental generations demonstrated a dose-related response in severity as detailed in Table 7b. The degree of change was greater in males than females and was generally similar between generations.

Table 7b: Incidence of Hypertrophy of the Centrilobular Hepatocytes

Grading	Males					Females				
	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
P1 Generation										
No. examined	25	25	25	25	25	25	25	25	25	25
Minimal	0	0	19*	12*	0	0	0	13*	14*	3
Slight	0	0	2	12*	3	0	0	0	7*	17*
Moderate	0	0	0	1	18*	0	0	0	0	5*
Mod. Severe	0	0	0	0	3	0	0	0	0	0
Total	0	0	21*	25*	24*	0	0	13*	21*	25*
P2 Generation										
No. Examined	25	25	25	25	25	25	25	25	25	25
Minimal	0	1	10*	3	1	0	0	9*	10*	6*
Slight	0	0	4	14*	8*	0	0	0	13*	15*
Moderate	0	0	0	7*	16*	0	0	0	1	3
Total	0	1	14*	24*	25*	0	0	9*	24*	24*

* Statistically significantly different from control, $p<0.05$; analysis by data reviewer, Fischer's Exact Test.

Note: Data were extracted from report No. 89R-028, pages 40-41.

C. Offspring

2. Viability: Mean litter size and viability results from pups during lactation are summarized from the report in Table 8a.

Table 8a: Litter Size and Viability

Observation	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
P1F1a Generation					
No. of litters (Day 0)	16	19	17	17	22
Mean litter size					
Day 0	12.4	12.7	11.5	11.4	11.5
Day 4 - Precull	12.1	12.5	11.9	11.3	11.4
Day 4 - Postcull	7.8	7.6	7.8	7.3	7.4
Day 7	7.8	7.5	7.8	7.3	7.4
Day 14	7.8	7.5	7.8	7.3	7.4
Day 21	7.8	7.4	7.8	7.3	7.4
Percent males (Day 0)	48	55	51	51	48
Pup mortality (No.)					
Stillborn	0	1	0	0	15a
Days 0-4	6	4	4	2	12
Days 5-21	0	2[d]	0	0	0
Total litter loss	0	0	1	0	1
Survival indices					
Viability index (mean %)	97.2	98.6	93.1	99.3	91.0
Lactation index (mean %)	100	98.5	100	100	100

(Continued)

Table 8a: Litter Size and Viability - continued

Observation	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
P2F2a Generation					
No. of litters	23	20	16	22	17
Mean litter size					
Day 0	11.5	12.9	11.3	11.9	10.4
Day 4 - Precull	11.4	12.4	10.9	10.3	11.4
Day 4 - Postcull	7.5	7.7	7.4	7.3	7.7
Day 7	7.5	7.4	7.3	6.9	7.6
Day 14	7.5	7.4	7.2	6.9	7.5
Day 21	7.5	7.4	7.2	7.2	7.5
Percent males (Day 0)	49	54	37	49	53
Pup mortality (No.)					
Stillborn	4b	8b	0	7	34c
Days 0-4	3	22	6	65	17
Days 5-21	0	6	3	9	3
Total litter loss	0	1	0	4*	3
Survival indices					
Viability index (mean %)	99.0	91.5	95.8	76.0*	78.0*
Lactation index (mean %)	100	95.4	97.7	91.7	97.5
P2F2b Generation					
No. of litters	20	18	12	22	17
Mean litter size					
Day 0	11.8	13.2	10.4	12.7	12.0
Day 4 - Precull	11.8	12.8	9.3	11.2	11.7
Day 4 - Postcull	7.7	7.8	6.9	7.2	7.6
Day 7	7.7	7.7	6.9	7.4	7.3
Day 14	7.6	7.1	6.8	6.9	7.1
Day 21	7.6	7.0	6.8	6.9	7.1
Percent males (Day 0)	53	50	55	53	47
Pup mortality (No.)					
Stillborn	2	3b	3b	1	4
Days 0-4	0	8	13	65d	17
Days 5-21	1	15	1	9d	15
Total litter loss	0	0	0	4	2
Survival indices					
Viability index (mean %)	100	97.0*	93.5*	79.9*	91.8*
Lactation index (mean %)	99.6	89.6	99.0	88.8	88.3

* Statistically significantly different from control, $p<0.05$.
a Includes 6 pups designated as "uncertain" status, taken to mean that the precise time of death (on Day 0) could not be determined accurately.
b Includes 1 pup designated as "uncertain" status.
c Includes 8 pups designated as "uncertain" status.
d Recalculated by reviewer from individual litter data, report No. 89R-028, pages 521-534.

Note: Data were extracted from report No. 89R-028, pages 116-124.

Although the number of litters available for analysis in either the P2F2a or P2F2b generations is below the Agency guideline requirement of 20 for several of the dose groups, combining the data from the first and second breeding yields a sufficient number of data points for evaluation of results from the second generation.

A decrease in offspring viability was noted at 250 ppm for the P1F1a litters and at 125 and 250 ppm for the P2F2a and P2F2b litters. This was evidenced by statistically and/or biologically significant increases in the number of stillborn pups, the number of pup deaths (predominantly Days 0-4 of lactation), and the incidence of total litter loss.

The total number of pup mortalities (stillborn, found dead, or cannibalized) per generation is listed in Table 8b.

Table 8b: Total Pup Mortality [No. of Pups (Litters)]

Generation	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
P1F1a	6(4)	7(5)	4(3)	2(1)	27(6)
P2F2a	7(6)	36(8)	9(6)	79(13)	54(12)
P2F2b	3(3)	26(7)	17(5)	75(10)	36(10)
P2F2a/P2F2b Combined	10(9)	62(15)	10(11)	154(23*)	90(22*)

* Statistically significantly different from control, $p<0.05$; analysis by reviewer, Fisher's Exact Test.

Note: Data were extracted from report No. 89R-028, pages 521-534.

Although pup mortality is significantly increased at the 5 ppm dose level, for both P2F2a and P2F2b litters, it is difficult to demonstrate a treatment-related effect due to the following factors: 1. There is no clear dose-response relationship in the mortality incidence data, whether analyzed from an individual or litter perspective. The 5 ppm mortality rate is consistantly higher than that of the 25 ppm dose group in both generations and higher than that of the 125 ppm dose level in the first generation. In addition, the number of litters containing pups that died is similar between the control group and the 5 ppm group. A statistically significant increase in the number of litters affected occurs in the 125 and 250 ppm dose groups. 2. The high mortality in the 5 ppm dose group for each breeding of the second generation can be attributed primarily to the death rates in 2 litters. Since one dam (No. 89-02217) produced two of these high-mortality litters (one at each breeding), the pup deaths could be due to factors unrelated to toxicity. 3. A significantly decreased index of viability in the 5 ppm P2F2b breeding may be a reflection of the unusually viable control group against which it was being compared.

Therefore, in the absence of clear, unequivocal evidence of toxicity, offspring mortality noted at the 5 ppm dose level is not considered to be treatment-related.

2. Pup Clinical Observations: Clinical observations recorded for pups during lactation did not indicate a treatment-related effect. The incidence of pups with malformations (litter incidence in parentheses) is summarized in Table 9.

Table 9: Selected Clinical Observations - Offspring

Malformation (Lactation Days)	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
P1F1a Generation					
Tail not developed/ anal atresia (D0-6)	0(0)	0(0)	1(1)	0(0)	0(0)
P2F2a Generation					
Vestigial tail (D0-6)	0(0)	0(0)	2(1)	0(0)	0(0)
Umbilical protrusion (D0-6)	0(0)	1(1)	0(0)	0(0)	0(0)
Microphthalmia (D14-22)	1(1)	0(0)	0(0)	0(0)	0(0)
P2F2b Generation					
Tail agenesis (D0-6)	0(0)	1(1)	0(0)	0(0)	0(0)
Microphthalmia (D14-22)	0(0)	0(0)	2(1)	0(0)	0(0)

Note: Data were extracted from report No. 89R-028, pages 62-63, 67-68, and 70.

3. Pup body weight: Statistically significant treatment-related decreases in F1a and F2a pup mean body weight values were evident at lactation Days 7 and 14 for the 250 ppm dose level. The mean body weight values for the F2b pups were also decreased at Days 7 and 14, although statistical significance was not demonstrated.

Group mean pup body weights are summarized from the report in Table 10.

Table 10: Mean Pup Weight (g)

Interval	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
P1F1 Generation					
Day 0	6.7	6.9	6.9	6.9	6.6
Day 4 - Precull	11.3	11.2	11.7	10.9	10.1
Day 4 - Postcull	11.2	11.2	11.8	10.9	10.1
Day 7	17.4	17.5	18.1	16.8	15.2*
Day 14	35.1	34.9	35.3	33.0	31.0*
Day 21	50.3	51.1	51.1	50.1	47.3
P2F2a Generation					
Day 0	6.6	6.2a	6.7	6.5	6.5a
Day 4 - Precull	11.4	9.7*	11.0	10.2a	9.7
Day 4 - Postcull	11.4	9.8	10.9	10.5	9.7
Day 7	17.7	15.6	16.9	15.4a	14.8*
Day 14	33.8	32.0	33.2	31.1a	29.4*
Day 21	50.0	47.8	49.8	49.2	45.2
P2F2b Generation					
Day 0	6.5	6.4	7.4*	6.4a	6.6a
Day 4 - Precull	11.2	9.7	12.6	10.7a	9.8
Day 4 - Postcull	11.2	9.7	12.5	10.7	9.8
Day 7	17.5	15.0	18.3	17.0	14.8
Day 14	34.3	31.3	34.9	34.5	32.5
Day 21	51.8	48.6	56.4	52.6	50.9

* Statistically significantly different from control, $p<0.05$.
a Recalculated by reviewer from individual pup weight data, report No. 89R-028, pages 580-612, due to errors in the number of litters used for calculations in the report.
Note: Data were extracted from report No. 89R-028, pages 118, 121, and 124.

3. Postmortem results: Neither the type, incidence, or distribution of gross necropsy observations indicated any treatment-related effects in the P1F1a, P2F2a, or P2F2b offspring. According to the authors, the observations, which were categorized as incidental, variations, or malformations, were typical of changes occurring in rats of this age. The malformations noted included abnormalities of the eye, palate, tail, vertebral column, limbs, and internal organs (gonads, kidney, spleen, and heart) with no apparent treatment-related incidence pattern.

The incidence of pups with malformations noted at necropsy is summarized from the report in Table 11.

Table 11: Incidence of Malformations Noted at Pup Necropsy

Observation	0 ppm	5 ppm	25 ppm	125 ppm	250 ppm
P1F1a Generation					
Litters Evaluated	16	19	16	15	22
Pups Evaluated	144	184	141	142	192
Live	144	183	141	142	192
Dead	0	1	0	0	0
No. Pups with Malformations	0	2	1	0	1
No. Affected Litters	0	2	1	0	1
P2F2a Generation					
Litters Evaluated	23	20	16	21	18
Pups Evaluated	266	246	174	225	191
Live	263	239	174	218	165
Dead	3	7	0	7	26
No. Pups with Malformations	1	1	1	1	0
No. Affected Litters	1	1	1	1	0
P2F2b Generation					
Litters Evaluated	20	19	12	21	17
Pups Evaluated	237	222	117	242	188
Live	235	220	115	241	185
Dead	2	2	2	1	3
No. Pups with Malformations	0	1	1	0	0
No. Affected Litters	0	1	1	0	0

Note: Data were extracted from report No. 89R-028, pages 126, 129, and 132.

III. DISCUSSION

A. Systemic toxicity: Dietary administration of Dicofol to rats at dose levels of 5, 25, 125, and 250 ppm over two consecutive generations produced evidence of systemic toxicity at the 25, 125, and 250 ppm levels.

1. Treatment-related decreases in premating body weight gain and/or food consumption were noted for the P1 males and females at 250 ppm and the P1 females at 125 ppm.

2. Histopathological evaluation of tissues from treated animals revealed evidence of toxicity in the liver, adrenal glands, and ovaries. Hypertrophy of centrilobular hepatocytes with associated vacuolation was noted in the livers of P1 and P2 adult rats at the 25, 125, and 250 ppm dose levels. Hypertrophy/vacuolation of the adrenal glands was observed in P1 and P2 female rats at the 125 and 250 ppm dose levels,

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and increased vacuolation of the ovaries was noted in P1 females at the 250 ppm dose level and in P2 female rats at the 25, 125 and 250 ppm dose levels.

B. Reproductive toxicity: There were no treatment-related effects on reproductive performance for the P1 or P2 adult rats; however, histopathological evaluation of ovarian tissues revealed increased vacuolation in P2 females at 25, 125, and 250 ppm. The noted changes, characterized by an increase in the size and/or number of vacuoles in the cytoplasm of the stromal cells, are compatible with enhanced steroidogenic activity and are judged to be an effect on reproductive physiology.

Evidence of toxicity to the offspring was observed at the 125 and 250 ppm dose levels.

1. Decreased viability of P1F1a pups at the 250 ppm dose level and P2F2a and P2F2b pups at the 125 and 250 ppm dose levels was evidenced by statistically and/or biologically significant increases in the number of stillborn pups, the number of pup deaths (predominantly during Days 0-4 of lactation), and the incidence of total litter loss.

2. Statistically (F1a and F2a pups) or biologically (F2b) significant treatment-related decreases in pup body weight were noted for the 250 ppm dose level at Days 7 and 14 of lactation.

C. Study deficiencies: The following deficiencies were noted:

1. There were less than 20 pregnant females (litters) per dose level for several groups at each breeding. The number of litters at each generation for Groups 1-5 respectively were as follows: P1F1a:16,19,17,17,22; P2F2a:23,20,16,22,17; and P2F2b:20,18,12,22,17. The investigators elected to add an additional breeding to the study (the P2F2b generation), thereby substantially increasing the number of available data points for the second generation. Although an additional breeding was not performed for the first generation, the validity of the study was not judged to be compromised.

2. Body weight change calculations were not provided and were calculated by the reviewer to facilitate data evaluation.

3. Calculations of several mean pup weight values (based upon litter mean values) were incorrect and were recalculated by the reviewer. Differences were minimal; statistical group comparisons were not re-run. In addition, several pup mortality counts were incorrect in the report and were corrected by the reviewer.

4. Although the methods section of the report states that statistical analysis was performed on histopathology incidence data, no statistical annotations appeared in the summary tables, nor was statistical significance discussed in the results text. Analyses were performed by the data reviewer and are discussed elsewhere in this document.

5. Neither the Compliance Statement nor the report final signature page were dated by the personnel who signed them.

D. Classification: CORE-Minimum Data

NOEL for Systemic Toxicity = 5 ppm
LOEL for Systemic Toxicity = 25 ppm
NOEL for Reproductive Toxicity = 5 ppm
LOEL for Reproductive Toxicity = 25 ppm

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