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DATA EVALUATION RECORD

STUDY TYPE: One-Generation Reproduction - Rat (Rangefinding Study)

MRID NUMBER: 412505-04

TEST MATERIAL: Chlorothalonil, Technical

SYNONYMS: 2,4,5,6-tetrachloroisophthalonitrile

STUDY NUMBER(S): 1722-87-0120-TX-001

SPONSOR: Fermenta ASC Corporation
5966 Heisley Road
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Mentor, Ohio 44061-8000

TESTING FACILITY: Ricerca, Inc.
Department of Toxicology and Animal Metabolism
7528 Auburn Road
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TITLE OF REPORT: Reproduction dose-rangefinding study in rats with technical chlorothalonil

AUTHOR(S): N. H. Wilson, J. S. Chun and J. C. Killeen

DATE REPORT ISSUED: May 18, 1989

CONCLUSIONS: Based upon the results of this study, treatment levels of 500, 1500 and 3000 ppm in diet are appropriate for inclusion in a two generation reproduction study in rats.

Systemic NOEL = ~~1500~~ 750 EAD 9/5/91 ppm for female rats and ~~750~~ 375 acX 9/5/91 ppm for male rats

Systemic LOEL = ~~750~~ 1500 EAD 9/5/91 ppm for female rats and ~~375~~ 750 acX 9/5/91 ppm for males rats on the basis of body weight gain reduction

Developmental NOEL = 1500 ppm

Developmental LOEL = 3000 ppm on the basis of reduced pup weight gain and reduced viability

CLASSIFICATION: Core - Supplementary
(This is not a guideline study.)

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I. PROTOCOL

A. Materials

1. Test Material: Technical grade chlorothalonil (stated purity of 98.1%); described as a light gray powder (Lot No. D-5840923).

2. Test species: 6-week old Charles River CD rats (Charles River Breeding Laboratory, Inc., Portage, MI.)

3. Diet preparation: Dietary mixtures containing the test material were prepared fresh weekly. Diet mixtures were stored at room temperature in the dark.

   Test diets, analyzed for homogeneity of mixtures and chemical stability in dietary mixtures for 14 days, were confirmed in test diets containing 200 and 3000 ppm. During the study, samples of each diet were collected and analyzed weekly for the first ten weeks and then during even weeks thereafter.

B. Procedures and Study Design

1. Mating: Each male was caged with one female from the same test group until a vaginal plug was observed or sperm cells were found in vaginal smears taken daily during the mating period. If sperm were not found, cohabitation was repeated each night until a positive smear was obtained or for 14 days, whichever came first.

   On day 18 of gestation, each pregnant female was individually placed into a cage with a solid bottom and Bed-O-Cobs bedding where they were kept through gestation and lactation.

2. Mating schedule: The F_0 parental animals were given test diets for ten weeks before they were mated.

3. Animal assignment: F_0 animals were randomly assigned to test groups as follows:

Test groups No.	Dose (ppm) *	Animals per group Males	Females
1	0	15	15
2	200	15	15
3	375	15	15
4	750	15	15
5	1500	15	15
6	3000	15	15

* Diets were administered from the beginning of the study until the animals were sacrificed.

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 per sex per group	Frequency
Mortality and signs of toxicity	All	Twice a day during premating and growth periods.
Detailed clinical observations	All	Once a week during growth and breeding periods.
Body weight	All	At beginning of study and weekly until termination of the study for males and the 10 week growth period for females.
	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.

2. Reproductive performance: Parental reproductive performance was assessed from breeding and parturition records of animals in the study.

The following indices were calculated:

$$\text{Male fertility index} = \frac{\text{No. females pregnant}}{\text{No. males mated}} \times 100$$

$$\text{Female fertility index} = \frac{\text{No. females pregnant}}{\text{Total no. females mated}} \times 100$$

3. Litter observations: At delivery, the date, number, weight and sex of live and stillborn pups and any external abnormalities were recorded. Daily observations for survival and overt toxic signs were conducted throughout lactation. The number and sex of live pups were recorded on days 0, 1, 4 (pre- and post-cull), 7, 14 and 21 of lactation. Total litter weights for live pups were measured on days 0, 4 (pre- and post-cull), 7 and 14. Individual body weights were measured on day 21.

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The following indices were calculated:

$$\text{Live Born Index} = \frac{\text{No. of pups born live}}{\text{Total no. of pups born}} \times 100$$

$$\text{Day 4 Viability Index} = \frac{\text{No. of pups alive at Day 4 (Precull)}}{\text{Total no. of pups born alive}} \times 100$$

$$\text{Lactation Index} = \frac{\text{No. of pups alive on Day 21}}{\text{No. of pups alive on Day 4 (Postcull)}} \times 100$$

$$\text{Litter Viability Index} = \frac{\text{No. of litters with live pups on Day 21}}{\text{No. of litters with live pups on Day 0}} \times 100$$

4. Necropsy

a. Parental animals: All parental rats were sacrificed after completion of the lactation period. All animals were subjected to post mortem examinations.

b. Offspring: The surviving F1 offspring were sacrificed at 21 days of age. These animals were subjected to gross post mortem examinations. Stillborn pups or pups that died during the study were necropsied within six hours of discovery.

c. Necropsy observations: Gross necropsy included notation of the presence or absence of implantation scars. No tissues were saved for microscopic examination.

D. Statistical Analyses: Study data were divided into four general categories for statistical analysis.

1. Interval Scale Data - This category included adult body weights (male and female), food consumption, gestation length and litter weights. Bartlett's test was used to test for normality of the data and homogeneity of variance. If Bartlett's test indicated nonsignificance, data were subjected to a one-way analysis of variance followed by mean separation using Dunnett's test. Litter weights were also analyzed by analysis of covariance with litter size as the covariate. If Bartlett's test indicated significance, data were analyzed by nonparametric procedures described in part D,3 below.

For the parametric data, regression techniques, a test for trend in dose levels and a test for lack-of-fit were performed. Where significant lack-of-fit was indicated, the trend test was discounted.

All tests were two-sided and were conducted at the 5% level of significance.

2. Count Data - Day 0 data for litter size, number of live pups and number of stillborn pups were analyzed using these procedures. Per the study report, "These parameters were assumed to be Poisson distributed, and a square root transformation was used to achieve normality. Following the transformation, the analysis of these parameters was the same as that of the interval scale data-including performing Bartlett's test on the transformed data and the use of the nonparametric tests if the Bartlett's test shows significance.

   "All tests were two-sided and were conducted at the 5% level of significance."

3. Percentage Data - These data included liveborn, stillborn, Day 4 pup viability and lactation indices and sex ratio on Day 0. "Statistical evaluation of the equality of the treatment means for these parameters (and, as needed, for parameters from the first group) was made by the Kruskal-Wallis test, a nonparametric one-way analysis of variance technique and by Dunn's summed rank test, a nonparametric technique for comparing the treatment groups to the control group. Also, Jonckheere's test was to test for a monotonic trend in the dose levels.

   "Note that for these parameters, the index (or ratio) was computed separately for each litter. These litter indices, then, are the observations that were analyzed.

   "All tests were two-sided tests and were conducted at the 5% level of significance."

4) Contingency Table Data - These data consisted of the mating and fertility indices. Per the study report, "Statistical analyses of these incidence data were performed using contingency tables. Each treatment group was compared to the control group using Fisher's Exact test for a 2x2 table. In addition, Armitage's test for a linear trend in the doses was performed.

   "The Fisher's exact tests were done at the 1% level of significance (5% divided by the number of treatment groups) in order to maintain an overall significance level of approximately 5% for each parameter. Armitage's test was performed at the 5% significance level. All tests were two-sided."

II. REPORTED RESULTS

A. Analysis of test diets: Analyses of test diets for concentration, homogeneity, and stability were conducted prior to the initiation of the study. The following recoveries were reported: 96% ± 4% (200 ppm), 98% ± 5% (400 ppm), 98% ± 4% (700 ppm), 98% ± 3% (1500 ppm) and 99% ± 4% (3000 ppm). Outliers were reported for one of 28 samples analyzed from the 400 ppm diet (89%) and one of 34 samples analyzed from the 3000 ppm diet (112%).

Homogeneity of the diets was evaluated by duplicate analyses of five samples each from the 200 and 3000 ppm diets. Concentrations were 202 ppm ± 1 ppm (101% ± 1%) and 3002 ppm ± 22 ppm (100% ± 1%), respectively.

Diet samples from the 200 and 3000 ppm diets collected after storage at room temperature for 4, 7 and 14 days. For the 200 ppm diet, concentrations were 210 ppm ± 1 ppm (104%), 181 ppm ± 1 ppm (90%) and 172 ppm ± 3 ppm (85%) for the 4, 7 and 14 day samples, respectively. For the 3000 ppm diets, comparable values were 2883 ppm ± 71 ppm (96%), 2850 ppm ± 9 ppm (95%) and 3060 ppm ± 78 ppm (102%).

Dietary concentration, homogeneity and stability with respect to the test material were acceptable.

B. Parental animals

1. Mortality and clinical signs: No deaths occurred during this study.

No treatment related clinical signs were reported.

2. Body weight and food consumption: During the premating period, body weights and body weight gains for males from the 1500 and 3000 ppm treatment groups were reduced relative to the control beginning with week 1 and persisting until mating. These observations were not paralleled by decreased food consumption. The only significant decrease noted in food consumption occurred during week 1 in the high dose group. This may have reflected an effect of the test material on palatability; food consumption increased in the subsequent weeks and no accompanying clinical signs were reported.

No treatment related effects in females on body weight, body weight gain or food consumption were reported during the premating period. Although statistical significance was indicated for food consumption in the 200 and 3000 ppm treatment groups, these increases are considered to be numerical anomalies rather than treatment effects.

Reported body weight and selected food consumption results for the premating period are summarized as follows:

Observation and study week	Dose group (ppm) 0	200	375	750	1500	3000
F_0 Generation Males - Premating						
Mean body weight (g)						
0	208.0	210.7	207.8	208.9	206.1	208.0
10	529.9	510.9	498.9	501.8	471.4**	490.2
Mean weight gain (g)						
0 - 10	321.9	300.2	291.1	292.9	265.3	282.2
Mean food consumption (g/rat/day)						
1	24.5	25.3	24.0	24.0	22.7	20.4**
2	25.9	26.5	25.1	25.7	25.1	26.7
10	27.3	28.2	26.6	27.0	24.9	27.8
F_0 Generation Females - Premating						
Mean body weight (g)						
0	147.0	148.9	145.5	146.2	146.4	149.9
10	259.8	276.4	261.3	259.7	259.5	251.2
Mean weight gain (g)						
0 - 10	112.8	127.5	115.8	113.5	113.1	101.3
Mean food consumption (g/rat/day)						
1	15.9	17.4	15.8	16.0	15.7	16.4
2	16.7	17.7	16.1	16.5	16.3	17.4
10	16.1	18.3**	17.1	16.8	16.9	18.0**

**Statistically significantly different from control, $p<0.01$.
(Data excerpted from Study Tables 1 and 2, pages 37 and 41.)

No treatment related effects were reported on gestational or lactational maternal body weights. Lactational body weight gains exhibited a dose related increase in the 750, 1500 and 3000 ppm treatment groups relative to the control.

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Selected group mean body weights for pregnant or nursing dams were summarized in the report as follows:

Observation and study week	Dose group (ppm) 0	200	375	750	1500	3000
Mean body weight (g)						
Gestation -						
Day 0	258.1	274.0	255.5	261.3	251.4	249.1
Day 20	387.1	389.8	384.2	384.3	372.5	372.2
Lactation -						
Day 0	300.7	311.9	297.0	297.9	293.5	287.9
Day 21	307.4	320.2	302.6	311.4	313.4	321.8
Mean body weight gain (g)						
Gestation -						
Days 0-20	129.0	125.8	128.7	123.0	121.1	123.1
Lactation -						
Day 0-21	6.7	8.3	5.6	13.5	19.9	33.9

(Data excerpted from Study Tables 9 and 10, pages 60 and 61.)

3. Test Substance Intake: Based on food consumption, body weight, and dietary analyses results, males consumed 14, 25, 50, 101 and 207 mg/kg/day during the peak growth periods (weeks 1 to 10) for the 200, 375, 750, 1500 and 3000 ppm treatment groups, respectively. For females, the comparable intakes of the test material were 16, 29, 57, 115 and 245 mg/kg/day.

4. Reproductive performance: No treatment related effects on reproductive performance were reported.

Results for the parental animals are summarized from the report as follows:

Observation and study week	Dose group (ppm) 0	200	375	750	1500	3000
Males						
Mated (%)	100	93	93	93	100	100
Fertile (%)	87	100	71	93	93	87
Females						
Mated (%)	100	93	93	93	100	100
Fertile (%)	87	100	71	93	93	87
Median gestation interval (days)	22.7	22.1	22.3	22.0	22.5	22.2
Number of litters	13	14	10	13	14	13
Mean litter size						
Day 0	13.5	11.1	13.2	12.2	11.4	12.1
Day 21	8.0	7.8	8.0	8.0	7.6	7.8
Mean pup weight (g)						
Day 0	6.3	6.5	6.5	6.3	6.5	6.5
Day 21	53.2	53.4	54.5	50.3	52.6	46.8**

**Statistically significantly different from control, $p<0.01$.
(Excerpted from Study Tables 11-13, pages 62-64, and Study Tables 21 and 22, pages 72-75.)

5. Necropsy results

a. Organ weights: No organ weights were measured.

b. Pathology - Macroscopic examination: The only apparent treatment related gross pathology reported was the enlargement of the kidneys (bilateral) in 5/15 male rats from the 3000 ppm treatment group, with accompanying discoloration in 2/15. Pale, mottled and enlarged kidneys were reported in one high dose female. The authors indicated that this is a common lesion seen in response to treatment of rats with chlorothalonil and thus is probably treatment related in this study.

No other treatment related gross lesions were reported.

C. Offspring

1. Viability and clinical signs: No whole litter losses were reported in any treated groups. One control female delivered a litter containing a single stillborn pup. The percentages of pups born live surviving

at day 4 (pre-cull) were 100.0, 99.5, 98.8, 98.3, 100.0 and 95.7% for groups receiving 0, 200, 375, 750, 1500 and 3000 ppm of the test material in diet. The survival in the high dose group was significantly reduced ($p<0.01$) relative to the control. No pup loss occurred after the 4-day culling.

Sex ratio was not affected by treatment.

Changes in mean litter sizes were summarized in the report as follows:

Observation and study week	Dose group (ppm) 0	200	375	750	1500	3000
Day 0	13.5	11.1	13.2	12.2	11.4	12.1
Day 4						
(Pre-Cull)	13.5	11.0	13.0	11.9	11.4	11.6
(Post-Cull)	8.0	7.8	8.0	8.0	7.6	7.8
Day 7	8.0	7.8	8.0	8.0	7.6	7.8
Day 14	8.0	7.8	8.0	8.0	7.6	7.8
Day 21	8.0	7.8	8.0	8.0	7.6	7.8

(Excerpted from Study Table 21, pages 72-73.)

No apparent treatment related clinical observations were reported in F_1 pups.

2. Body weight: Pup weights for the 3000 ppm group were decreased relative to the control at the day 14 and 21 weighings. No other treatment related effects on pup body weights were reported.

Selected group mean body weights are summarized from the report as follows:

Observation and study week	Dose group (ppm) 0	200	375	750	1500	3000
Body weight (g)						
Day 0	6.3	6.5	6.5	6.3	6.5	6.5
Day 21	53.2	53.4	54.5	50.3	52.6	46.8**
Weight gain (g)	46.9	46.9	48.0	44.0	46.1	40.3

**Statistically significantly different from control, $p<0.01$.
(Excerpted from Study Table 22, pages 74-75.)

3. Necropsy results

a. Organ weights: Organ weights were not recorded.

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b. Pathology - Macroscopic examination: No treatment related gross lesions were reported. Dilated renal pelvis was slightly more frequent in the 750 and 1500 ppm treatment group, but was similar to the control in the 3000 ppm group.

III. DISCUSSION

All treatment levels used in this study were well tolerated. No effects on reproductive parameters were reported. Males from the 1500 and 3000 ppm treatment groups exhibited slight body weight and body weight gain decrements relative to the control. Body weight gain was also slightly reduced in high dose females. Treatment related gross pathology was limited to kidney enlargement in the high dose males, an effect acknowledged by the registrant to be associated with chlorothalonil ingestion by rats.

Effects in offspring were decreased body weight gains in high dose pups and a slight reduction in viability prior to day 4 of lactation in the high dose pups.

On the basis of this study, the registrant selected 3000 ppm as a confirmed effect level, 500 ppm as a likely no effect level and 1500 ppm as an intermediate treatment level for a subsequent two generation reproduction study in rats with technical chlorothalonil. Tox Branch concurs with these selections.

CONCLUSIONS: Based upon the results of this study, treatment levels of 500, 1500 and 3000 ppm in diet are appropriate for inclusion in a two generation reproduction study in rats.

Systemic NOEL = 1500 ppm for female rats and 750 ppm for male rats
Systemic NOEL = 750 ppm for female rats and 375 ppm for males rats on the basis of body weight gain reduction
Developmental NOEL = 1500 ppm
Developmental LOEL = 3000 ppm on the basis of reduced pup weight gain and reduced viability

CLASSIFICATION: Core - Supplementary
(This is not a guideline study.)