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STUDY TYPE: Teratogenicity Study in Rabbits GUIDELINE: 83-3(b)

TOX.CHEM.No.: 215B MRID No.: 412505-03 PROJECT NO.: 0-0030

TEST MATERIAL: Technical Chlorothalonil

STUDY NUMBERS: Ricerca Inc Reference No. 87-0060; Bio/dynamics Project No. 87-3196

SPONSOR: Farmenta Plant Protection Company, Mentor, Ohio.

TESTING LABORATORY: Bio/dynamics, East Millstone, New Jersey.

REPORT TITLE: A Teratology Study in Rabbits with Technical Chlorothalonil. (Doc.# 1544-87-0060-TX-002)

REPORT AUTHORS: N.H Wilson and J.C. Killeen

REPORT DATE: October 4, 1988

CONCLUSION: Groups of 20 mated female New Zealand White rabbits were given oral administrations of 0, 5, 10, or 20 mg/kg/day technical chlorothalonil suspended in 0.5% aqueous methyl cellulose during days 7 through 19 of gestation; dams were sacrificed on Day 30. No maternal, embryo, or fetotoxicity or teratogenicity was observed at the 5 or 10 mg/kg dose levels. At the high-dose (20 mg/kg), maternal toxicity was manifested by decreases in body weight gain and food consumption only during the period of dosing; no other treatment-related effects were observed at this level. No treatment-related effects were observed on fetal body weights or sex ratios. Chlorothalonil did not induce any external, visceral or skeletal malformations at any of the doses tested. Under the conditions of this study, the no-observed-effect-levels (NOELs) were 10 mg/kg for maternal toxicity and 20 mg/kg/day for developmental toxicity.

CORE CLASSIFICATION: Guideline; satisfies the requirements for a developmental toxicity study in rabbits.

QUALITY ASSURANCE: A quality assurance statement was provided.

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Study Title: A Teratology Study in Rabbits with Technical Chlorothalonil.

Testing Laboratory: Bio/dynamics, East Millstone, New Jersey.

Study (Project) No. 87-3196 (bio/dynamics) 87-0060 (Ricerca Inc)

Study Performance: December 5, 1987 thru January 22, 1988

Report Date: October 4, 1988

Authors: N.H Wilson and J.C Killeen

1. OBJECTIVE

The objective of this study was to assess the teratogenic potential of technical chlorothalonil in rabbits.

2. MATERIALS AND METHODS

a. Test Material

Trade Name: Technical chlorothalonil
Chemical Name: 2,4,5,6-tetrachloroisophthalonitrile
Ricerca Compound Number: T-117-12
Farmenta Identification Number: SDS-2787-1002
Farmenta Lot Number: D-5840923
Purity: 98.1%
Description: Light gray powder
Vehicle: 0.5% (w/v) methylcellulose in water

b. Test Animals

Species/Strain: Female New Zealand White [Hra:(NZW)SPF]
Age at receipt: 4 to 5 months
Body Weight: 3 to 4 kg
Source: Hazleton Research Products, Inc, Pennsylvania
Identification: Monel self-piercing ear tag.
Acclimation: 30 days, prior to mating.
Housing: Individually in stainless steel cages.
Food: Purina Certified High Fiber Diet No.5325 ad libitum.
Water: Tap water ad libitum
Environment: Temperature- 68 ± 5°C; Humidity- 50 ± 20%;
Air Exchange- 12/hr.; Light/Dark- 12 hr.cycle

Group Assignment: 20 mated females were randomly assigned to 1 control group and 3 treatment groups.

c. Mating

Following acclimation, females were housed with sexually mature males (1:1) until mating had been observed (designated day 0 post coitum).

d. Preparation of Dosing Solutions

The test material/vehicle suspensions (w/v) were prepared fresh daily prior to dosing. The vehicle, 0.5% methylcellulose, was added to a weighed amount of the test material and the mixture was kept homogenous by constant stirring on a magnetic stirrer.

e. Analysis of the Dosing Solutions

Prior to initiation of the study, concentration, homogeneity, and stability analyses were determined for the 5 and 10 mg/kg dose levels (dosing suspensions at concentrations of 2.5 and 10.0 mg/ml,respectively). In addition, samples of dosing suspensions (all dose levels) used on days 1, 3, 7, 10, 14, 17, and 20 of dosing were also analyzed to confirm that the suspensions were prepared at their intended concentrations.

f. Administration of Test Article

The test article was administered daily orally via gavage at nominal dose levels of 5, 10, or 20 mg/kg from day 7 through 19 of gestation. The control group received the vehicle only. All groups received a dosing volume of 2 mL/kg body weight, with a daily adjustment of the individual volume to the actual body weight.

g. Observations

All animals were observed twice daily for morbidity, mortality, appearance, and clinical signs of toxicity. Dams were given a detailed physical examination on Days 0, 7, 10, 13, 16, 19, 24, and 30 of gestation. Body weights were measured on gestation days 0, 3, 7, 10, 13, 16, 19, 24 and 30. Food consumption was measured on Days 0, 3, 7, 10, 13, 16, 19, 24, and 29 post coitum. Dams aborting or delivering prematurely were sacrificed on the day such evidence was observed and given a gross postmortem evaluation.

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h. Termination

On day 30 post coitum, dams were sacrificed and post mortem examination included gross macroscopic examination of all internal organs with emphasis on uterus, uterine contents, position of fetuses in the uterus and the number of corpora lutea. The uteri (and its contents) of all females with live fetuses were weighed and the corrected body weights were calculated. Each fetus was weighed, sexed and examined for gross external abnormalities, and was prepared by fresh microdissection procedure for visceral examinations and stained with Alizarin Red S for skeletal examination.

3. RESULTS

a. Analysis of the Dosing Solutions

Homogeneity analysis indicated that the samples were accurately and homogeneously prepared; the mean values for the 2,5 and 10.0 mg/ml were 94 ± 3% and 103 ± 1%, respectively, of the nominal concentrations. Stability analyses of the two suspensions showed that the test material/methylcellulose suspensions were stable for at least a period of 24 hours at room temperature. Concentration analyses of the dosing suspensions collected during the dosing period (Days 1, 3, 7, 10, 14, 17, and 20) indicated that the suspensions were prepared accurately throughout the study. The means of the concentrations determined analytically for the 5, 10, and 20 mg/kg/day dosing suspensions were 96 ± 3%, 97 ± 5%, and 97 ± 3%, respectively, when expressed as a percentage of the expected concentrations.

b. Maternal Mortality

No mortality occurred during the study at the vehicle control, or at the low-dose (5 mg/kg) groups. The death of one female at the mid-dose (10 mg/kg) on Day 10 was considered to be non-treatment related; necropsy revealed fluid in the lungs and frothy fluid in the trachea, suggestive of an intubation error. The cause of death of one dam on Day 13 at the high-dose (20 mg/kg) was not established.

c. Maternal Body Weight Gain and Corrected Body Weight Gain.

Mean body weights for each recorded interval during gestation were comparable between the control and each treated group. Mean body weight gains were comparable for the control and each treated group during the pre-treatment period (Days 0-7 of gestation) and during the post-treatment period (Days 19-30). During the dosing period (Days 7-19 of gestation), a slight but statistically significant ($P < 0.05$) decrease in mean body weight gain was seen in dams at the 20 mg/kg/day when compared to control; dams at the 5 or 10 mg/kg/day did not show a similar effect (Table 1).

d. Maternal Food Consumption

No treatment-related effects were seen in the mean food consumption of dams between the vehicle control and the low- or mid-dose groups. At the high-dose, a slight, decrease in mean food consumption was observed during treatment days 7, 10, 13, 16, and 19; however, the difference was statistically significant ($p < 0.05$) only on Day 7 (Table 2).

e. Clinical Signs

No treatment-related clinical signs of toxicity were observed in dams at any dose groups.

f. Maternal Macroscopical Examination

No toxicologically significant macroscopical changes were observed in the dams that died or sacrificed at termination. The most frequent findings were discolored lungs (primarily red/tan/brown/ foci/areas) which were qualitatively similar in both the control and treated groups. The incidences in the control, low-dose, mid-dose, and high-dose groups were 60%, 60%, 70%, and 80%, respectively.

g. Reproduction Data

Pregnancy rates were comparable between the control and treated groups; 100% in the control, low- and mid-dose groups and 95% (19/20) in the high-dose group. There were no aborted pregnancies during the study. Premature delivery occurred in two control dams (10%), one low-dose dam (5%), two mid-dose dams (10%), and one high-dose dam (5%); the remaining 72 dams survived to scheduled sacrifice on Day 30. The mean number of corpora lutea and mean number of uterine implantations per dam were comparable between the control and treated groups. Although a slight increase in the mean pre-implantation loss index was seen at the mid-dose (0.153 ± 0.186) when compared to controls (0.077 ± 0.109), it was not considered to be treatment related since the difference was neither statistically significant nor was seen at the high-dose (0.081 ± 0.107) (Table 3).

h. Sex Ratios

The sex ratios of fetuses among the treated groups were comparable to that in the vehicle control group (Table 2).

i. Fetal Weight and Viability

Treatment altered neither fetal viability nor fetal weight.

j. External and Visceral Examinations

No treatment-induced external or visceral malformations were seen in fetuses from dams receiving 5, 10, or 20 mg/kg/day chlorothalonil. External malformations observed included a distended abdomen in one control fetus, the presence of a single external nare, and absence of upper incisors in one fetus from the low-dose group. No external malformations were seen in the 156 fetuses from the mid-dose or 158 fetuses from the high-dose Table 4).

The incidences of fetuses with visceral malformations for the control, low-, mid- and high-dose groups were 2/173 (1.2%), 1/170 (0.6%), 2/156 (1.3%), and 2/158 (1.3%). The type of malformations observed were similar to those commonly seen in rabbits of this strain (Table 5).

k. Skeletal Examination

The incidences of fetuses with skeletal malformation were: 9/173 (5.2%) in the vehicle control; 15/169 (8.9%) in the low-dose; 9/156 (5.8%) in the mid-dose; and 6/158 (3.8%) in the high-dose. The types of malformations included minor skeletal abnormalities (fused sternebrae, angulated arch of the hyoid) that are commonly seen in this strain of rabbits. No treatment-related differences in the level of skeletal ossification were seen between the vehicle control and the treated groups (Appendix 1).

4. DISCUSSION

Oral administration of chlorothalonil (0, 5, 10, or 20 mg/kg) to mated rabbits during the period of organogenesis (days 7 through 19) resulted in maternal toxicity characterized by slight decreases in mean body weight gain and mean food consumption at the high-dose (20 mg/kg) only during the dosing period; no embryotoxicity, fetotoxicity or teratogenicity was observed at this level. No maternal, embryo, fetotoxicity or teratogenicity was observed at the 5 or 10 mg/kg dose levels. Chlorothalonil did not induce external, visceral or skeletal malformations at any of the dose levels tested.

5. CONCLUSION

Based on these results, the NOELs and LOELs established are:

Maternal Toxicity NOEL: 10 mg/kg/day
LOEL: 20 mg/kg/day (HDT)
Developmental Toxicity NOEL: 20 mg/kg/day (HDT)

CORE CLASSIFICATION: Guideline; this study satisfies the requirements for a developmental toxicity study in rabbits (83-3b) and is acceptable for regulatory purposes.

Table 1. Maternal Body Weight Change In Rabbits Following Oral Administration of Chlorothalonil (grams).

Dose Level (mg/kg/day)		Gestation Days 0-7	7-10	10-13	13-16	16-19	19-30	7-19
0	Mean	106	6	24	86	21	94	136
	S.D	53	72	45	96	49	220	128
	N	20	20	20	20	20	18	20
5	Mean	107	8	3	78	22	38	111
	S.D	123	46	51	77	112	155	108
	N	20	20	20	20	20	18	20
10	Mean	89	-9	22	53	6	75	71
	S.D	59	80	66	78	84	185	183
	N	20	19	19	19	19	17	19
20	Mean	101	-26	8	21	-9	111	-8*
	S.D	81	73	59	106	104	226	206
	N	19	19	18	18	18	17	18

* Significantly ($p < 0.05$) different from controls (Dunnett's).

Table 2. Maternal Food Consumption In Rabbits Following Oral Administration of Chlorothalonil (g/kg/day).

Dose Level (mg/kg/day)		Study Days 1	3	7	10	13	16	19	24	29
0	Mean	45	49	47	46	36	40	40	25	23
	S.D	12	6	6	8	11	11	13	15	14
	N	17	19	20	19	20	19	17	20	14
5	Mean	53	50	45	47	37	36	35	25	20
	S.D	6	7	9	7	10	16	12	9	15
	N	17	19	18	18	17	18	19	18	16
10	Mean	51	51	42	46	39	36	43	27	24
	S.D	9	7	14	7	7	18	15	14	15
	N	14	20	20	17	16	17	15	16	15
20	Mean	52	52	40*	42	29	26	28	28	23
	S.D	9	10	20	13	12	15	22	13	13
	N	16	18	15	18	17	16	13	16	15

* Significantly ($p < 0.01$) different from controls (Dunnett's).

Table 3. Summary of Reproduction data in Rabbits Following Oral Administration of Chlorothalonil.

Parameters	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	20 mg/kg/day
No.of females mated	20	20	20	20
Female mortality	0	0	1	1
No. pregnant (%)	20 (100%)	20 (100%)	20 (100%)	19 (95%)
No.of pregnancies aborted	0	0	0	0
No.of premature births	2	1	2	1
No.of litters with viable fetuses	18	19	17	17
No.of litters with all resorption	0	0	0	0
No.of corpora lutea	198	192	191	184
Mean ± S.D.	11.0 ± 1.9	10.1 ± 1.9	11.2 ± 2.5	10.8 ± 2.0
No.of implantation sites	184	180	162	169
Mean ± S.D.	10.2 ± 2.4	9.5 ± 1.5	9.5 ± 2.9	9.9 ± 2.2
Preimplantation loss (M+S.D.)	0.077 ± 0.109	0.063 ± 0.091	0.153 ± 0.186	0.081 ± 0.107
No.of viable fetuses	172	170	156	158
Mean litter size ± S.D.	9.6 ± 2.2	8.9 ± 2.0	9.2 ± 2.7	9.3 ± 2.2
Mean no.of males ± S.D.	4.8 ± 1.9	3.9 ± 1.7	4.6 ± 2.1	4.5 ± 1.8
Mean no.of females ± S.D.	4.8 ± 2.1	5.1 ± 2.0	4.6 ± 2.5	4.8 ± 2.3
No.of dead fetuses	1	0	0	0
No.of resorption	11	10	6	11
Mean ± S.D.	0.6 ± 1.0	0.5 ± 1.9	0.4 ± 0.8	0.6 ± 0.6
Resorptions/implants (M±S.D.)	0.054 ± 0.001	0.049 ± 0.171	0.031 ± 0.063	0.067 ± 0.065
No.of litters with resorption (%)	7 (38.9)	2 (10.5)	4 (23.5)	10 (58.8%)
Mean fetal body weight (g)	45.68 ± 6.42	43.38 ± 5.25	43.32 ± 6.60	42.91 ± 9.37
Males	46.88 ± 6.72	43.19 ± 5.59	43.34 ± 6.94	43.89 ± 9.75
Females	44.93 ± 6.97	43.34 ± 5.28	41.99 ± 4.71	41.88 ± 8.84
Ratio of viable fetuses (Total m/f)	1.0	0.8	1.0	1.0

Table 4. Summary of External Malformations in Fetuses of Dams Given Oral Administration of Chlorothalonil.

Types of Malformations	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	20 mg/kg/day
Litters evaluated	18	19	17	17
Fetuses evaluated	173	170	156	158
Live	172	170	156	158
Dead	1	0	0	0
Distended abdomen				
Fetal incidence	1	0	0	0
Litter incidence	1	0	0	0
Single external nare				
Fetal incidence	0	1	0	0
Litter incidence	0	1	0	0
Absence of upper incisor				
Fetal incidence	0	1	0	0
Litter incidence	0	1	0	0
Ascites of the abdominal cavity				
Fetal incidence	1	0	0	0
Litter incidence	1	0	0	0
Total external malformations				
Fetal incidence	1	1	0	0
Litter incidence	1	1	0	0

Table 5. Summary of Visceral Malformations in Fetuses of Dams Given Oral Administration of Chlorothalonil.

Parameters	0 mg/kg/day	5 mg/kg/day	10 mg/kg/day	20 mg/kg/day
Litters evaluated	18	19	17	17
Fetuses evaluated	173	170	156	158
Live	172	170	156	158
Dead	1	0	0	0
Lateral ventricles distended				
Fetal incidence	0	1	0	0
Litter incidence	0	1	0	0
Persistent truncus arteriosus				
Fetal incidence	1	0	0	0
Litter incidence	1	0	0	0
Interventricular septal defect				
Fetal incidence	1	0	0	0
Litter incidence	1	0	0	0
Heart enlarged				
Fetal incidence	1	0	0	0
Litter incidence	1	0	0	0
Renal pelvis(es) distended, Papilla(ae) absent				
Fetal incidence	0	0	1	2
Litter incidence	0	0	1	1
Ectopic kidneys				
Fetal incidence	0	0	1	0
Litter incidence	0	0	1	0
Total visceral malformations				
Fetal incidence	2	1	2	2
Litter incidence	2	1	2	1

APPENDIX 1.

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