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EXCERPT
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DATA EVALUATION RECORD

CYHALOTHRIN

Teratogenicity Study in Rabbits

STUDY IDENTIFICATION: Killick, M. E. Cyhalothrin: Oral (gavage) teratology study in the New Zealand white rabbit. (Unpublished study No. R8 0169 and report No. 2700-72/211 by Hazleton Laboratories Europe Ltd., Harrogate, England, for Imperial Chemical Industries Limited, Cheshire, England; dated June 1981.) Accession No. 073206.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
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Signature: I. Cecil Felkner

Date: 11-26-85

1. CHEMICAL: Cyhalothrin; [(R,S)α-cyano-3-phenoxybenzyl-(±)-cis-3,3 (Z-2-chloro-3,3,3-trifluoroprop-1-en)-2,2-dimethylcyclopropane carboxylate].

2. TEST MATERIAL: Cyhalothrin, from batch No. 005, was a brown viscous liquid (at room temperature) described as a technical grade pyrethroid mixture containing 89.25 percent cyhalothrin.

3. STUDY/ACTION TYPE: Teratogenicity study in rabbits.

4. STUDY IDENTIFICATION: Killick, M. E. Cyhalothrin: Oral (gavage) teratology study in the New Zealand white rabbit. (Unpublished study No. RB 0169 and report No. 2700-72/211 by Hazleton Laboratories Europe Ltd., Harrogate, England, for Imperial Chemical Industries Limited, Cheshire, England; dated June 1981.) Accession No. 073206.

5. REVIEWED BY:

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7. CONCLUSIONS:

A. We could not assess the NOEL and LOEL for maternal and fetal toxicity of cyhalothrin in this study due to the high incidence of illness-related maternal deaths and to deficiencies in the design and conduct of fetal examinations.

B. This study is classified Core Supplemental; it did not provide adequate information for assessing the potential teratogenicity of the test material.

8. RECOMMENDATIONS:

To upgrade the classification of this study, we recommend that:

1. Healthy animals be used, and that their reproductive history be reported.

2. Pregnancies be terminated on day 29 or 30 of gestation, and not on day 28.

3. Fetuses be sacrificed by carbon dioxide inhalation or intraperitoneal injection, and not by intracardiac injection.

4. A more thorough method for craniofacial examination be implemented. If brain tissues were fixed and saved, they should be sectioned and examined by the methods described by Wilson and the data should be submitted. The methods used for visceral examination should be cited or described.

5. The above recommendations, if implemented, would yield more meaningful results in future studies and would permit the determination of maternal and fetal NOELs and LOELs for cyhalothrin in rabbits.

9. BACKGROUND:

A range-finding study in pregnant rabbits was conducted at Hazleton Laboratories Europe, Ltd. (report No. 2603-72/210) to determine dose levels for the teratogenicity study. The author did not include details or results from this range-finding study.

Item 10--see footnote 1.

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[1]Only items appropriate to this DER have been included.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Cyhalothrin was described as a brown, viscous liquid consisting of 89.25 percent active ingredient. The test material was supplied by Imperial Chemical Industries, Ltd. under the code No. Y00102/010/005. Corn oil was used as the vehicle and control substance. Dosage formulations were prepared once (3 days before the initiation of dosing), divided into daily aliquots, and stored at room temperature until used. The dosage levels of 0, 3, 10, and 30 mg/kg/day were achieved by mixtures containing 0.0, 1.7, 5.6, and 16.8 mg of test material (adjusted for purity) per mL of corn oil. The doses were administered by gavage. Treatment volumes were adjusted to 2 mL/kg of body weight and were based on maternal body weights recorded on gestation day 6. These volumes were reduced for animals whose body weights decreased below their respective reference level of gestation day 6, but were not increased to compensate for body weight gains above their reference level.

2. Test Animals and Test System: New Zealand white rabbits were obtained from Morton Commercial Rabbits, Essex, England. Prior to mating, females were examined by a veterinarian to assure their suitability for the study. Following an acclimatization period of 20 days, 72 sexually mature females (3.14-4.09 kg) were mated to 3 different males; the day of mating was designated gestation day 0. An additional 10 females from a later shipment were mated after an acclimatization period of 8 days, and 6 of these were used as replacements. After mating, each female was injected intravenously with chorionic gonadotropin to stimulate ovulation. A total of 18 females were initially assigned to each group. However, 1, 1, and 4 animals were subsequently assigned to the 0, 3, and 10 mg/kg/day dosage groups, respectively, to replace animals that died early in the study. All surviving females were dosed from gestation day 6 through 18 and sacrificed on gestation day 28.

3. Parameters Measured: Chemical analyses were conducted on samples of dose formulations obtained on the day of preparation and 28 days later when dosing was completed.

All animals were observed at least once daily to determine their health status and to record clinical signs of toxicity. Mortality checks were performed twice daily. Maternal body weights were recorded on gestation days 0, 6 through 19, 24, and 28. Maternal food consumption was recorded on gestation days 0, 3, 6, 9, 12, 15, 18, 21, 24, and 28. Necropsies were conducted on mated females on gestation day 28; at this time, gross maternal findings, gravid uterine weight, and number of corpora lutea were recorded. In addition, the number, type, and location of implantations within uteri were recorded.

Fetal weight, crown-to-rump length, and sex were determined after sacrificing the fetuses with intracardiac injections of Euthatal. Subsequently, all fetuses were examined for gross external abnormalities, skinned, dissected, and examined for visceral abnormalities. Eviscerated fetuses were fixed, and their cranial cavities were examined through single slices at the level of the fronto-parietal suture. Skeletal structures were stained with Alizarin Red and examined for abnormalities. Data were processed where appropriate to give mean values, group mean values and standard deviations. All statistical tests were carried out at 1% significance levels.

12. REPORTED RESULTS:

A. Test Material: Results from gas chromatographic analyses performed at the time of preparation of dose formulations, and at the end of the dosing period, indicate that all formulations ranged from 92-110 percent of intended concentrations and that the test material was stable during the entire dosing period.

B. Maternal Effects: Several mated animals died prior to their scheduled sacrifice date. The mortality incidence was 1/19, 2/19, 6/22, and 2/18 animals in the 0, 3, 10, and 30 mg/kg/day groups, respectively (Table 1). The study author indicated that most of these deaths appeared to be related to pulmonary disorders and not to the test material.

No compound-related clinical observations were noted during gestation. Also, macroscopic examinations of maternal organs conducted during necropsies revealed no abnormalities associated with the test material.

Maternal body weights were slightly reduced in the high-dose group from the initiation of dosing until sacrifice. The resulting reduction in group mean body weight gain from gestation days 6 through 9 was statistically significant for this group of animals when compared with controls. No other notable effects on maternal body weight were reported (Tables 2a and 2b). Statistically significant reductions in food intake were recorded for the 30 mg/kg/day dosage group between gestation days 6 and 15 (Table 3).

According to the study author, the percentage of pregnant animals in this study was within the normal range of historical controls in their laboratory, and no compound-related effects on fertility indices were evident (Table 4). No statistically significant effects related to the test article were noted in gravid uterine weights or in corrected body weight gains (Table 5). The mean numbers of corpora lutea per female were comparable for all groups (Table 6).

TABLE 1. Mated Females Found Dead or Sacrificed Prior to Gestation Day 28

Dosage Group (mg/kg/day)	Animal No.	Died/Sacrificed on Gestation Day	Pregnancy Status	Respiratory/ Pulmonary Involvement
0	4082	6	pregnant	yes
3	4106	6	pregnant	yes
3	4111	21	pregnant	yes
10	4117	12	pregnant	yes
10	4126	9	pregnant	yes
10	4127	23	pregnant	no
10	4128	6	not pregnant	yes
10	4130	22	not pregnant	no
10	4154	25	pregnant	no
30	4135	18	pregnant	yes
30	4138	25	pregnant	no

TABLE 2a. Effects of Cyhalothrin on Mean Maternal Body Weight (kg) During Gestation in Rabbits

Gestation Day	Dosage (mg/kg/day) 0	3	10	30
0	3.54	3.54	3.59	3.58
6	3.73	3.66	3.73	3.76
9	3.74	3.71	3.77	3.66
12	3.83	3.79	3.82	3.71
15	3.91	3.90	3.91	3.80
18	3.96	3.93	3.96	3.87
28	4.19	4.13	4.23	4.15

TABLE 2b. Effects of Cyhalothrin on Mean Maternal Body Weight Gain (kg) During Gestation in Rabbits

Gestation Days	Dosage (mg/kg/day) 0	3	10	30
0 - 6 (predosing)	0.21	0.12	0.14	0.18
6-18 (dosing)	0.23 [6.2%]	0.27 [7.4%]	0.23 [6.2%]	0.11 [2.9%]
18-28 (postdosing)	0.23	0.20	0.27	0.28
0-28 (gestation)	0.65 [18.4%]	0.59 [16.7%]	0.64 [17.8%]	0.57 [15.9%]

TABLE 3. Effects of Cyhalothrin on Mean Maternal Food Consumption (g/day)During Gestation in Rabbits

Gestation Days	Dosage (mg/kg/day)			
	0	3	10	30
0- 3	197	190	196	201
3- 6	223	215	216	229
6- 9	154	164	161	111*
9-12	183	184	188	130**
12-15	185	188	158	143*
15-18	158	159	164	146
18-21	223	202	193	227
21-24	200	181	221	229
24-28	179	157	172	185

*Statistically different from control value ($p < 0.05$).

**Statistically different from control value ($p < 0.01$).

TABLE 4. Effects of Cyhalothrin on Fertility Indices in Rabbits

Parameter	Dosage (mg/kg/day)			
	0	3	10	30
No. mated	19	19	22	18
No. pregnant	17	15	18	14
% pregnant	90	79	82	78
No. examined on gestation day 28	18	17	16	16
No. pregnant on gestation day 28[a]	16	13	14	12
% pregnant on gestation day 28[a]	89	77	88	75

[a] Based on females surviving until gestation day 28.

TABLE 5. Effects of Cyhalothrin on Adjusted Maternal Body Weight[a] and Gravid Uterine Weight in Rabbits

Parameter	Dosage (mg/kg/day) 0	3	10	30
Group mean body weight (kg) on gestation day 28	4.19	4.13	4.23	4.15
Group mean gravid uterine weight (kg)	0.383	0.364	0.400	0.414
Group mean adjusted body weight (kg) on gestation day 28	3.81	3.77	3.83	3.74
% adjusted gestational body weight gain	7.6	6.5	6.7	4.5

[a] Calculated by subtracting gravid uterine weight from maternal body weight on gestation day 28.

TABLE 6. Effects of Cyhalothrin on Reproductive Indices in Rabbits

Parameter	Dosage (mg/kg/day)			
	0	3	10	30
No. corpora lutea/female	9.4	9.2	9.3	10.3
No. implantations/litter	7.6	7.5	8.1	8.4
% preimplantation loss	19.9	19.2	12.3	18.5
No. resorptions/litter	0.63	0.69	0.79	0.83
% postimplantation loss	8.3	9.3	9.6	9.9
Live fetuses/litter	6.9	6.8	7.4	7.6
Mean fetal weight (g)	38.8	40.0	38.0	37.6
Fetal male/female ratio	1.22	1.10	0.91	1.17

C. Embryonic/Fetal Effects: No compound-related effects were reported in preimplantation losses. Postimplantation losses were slightly increased in the dosage groups; however, this effect was not statistically significant and was not considered compound related. The group mean number of fetuses, crown-to-rump lengths, and fetal sex ratios were considered to be similar for all groups. Very slight decreases in group mean fetal weight were reported for the mid- and high-dose groups, but these decreases were not statistically significant (Table 6).

No compound-related effects were reported for the type or incidences of malformations or variations.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study author concluded that the only maternal effects associated with cyhalothrin were body weight losses and reductions in food consumption in the high-dose animals. These effects indicated that 30 mg/kg/day elicited maternal toxicity in rabbits. However, no conclusive compound-related effects were noted in any aspect of fetal development, even at the highest dose tested.

B. A quality assurance statement was signed and dated on July 1, 1981.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. 1. Maternal Effects: Very high incidences of maternal mortality were seen for all study groups (Table 7). Data from clinical observations conducted during the in-life portion of the study and from macroscopic observations made during necropsies indicate that most of these deaths resulted from respiratory/pulmonary disease (Table 1). No conclusive compound-related association could be established for these deaths; however, the mortality incidences among dosage groups were at least twice as high as that reported for the control group. Slight reductions in the mean maternal body weight gain, mean adjusted body weight gain, and food consumption in the 30 mg/kg/day dosage group suggested that cyhalothrin elicited mild maternal effects at this dosage level. However, we could not assess the biological significance of these mild effects due to the presence of ongoing maternal illness during gestation.

2. Embryonic/Fetal Effects: The percentage of pregnant females was 90, 79, 82, and 78 percent for the 0, 3, 10, and 30 mg/kg/day dosage groups, respectively; these data suggest a slight increase in the incidence of females with no embryonic implantations or with implantations completely resorbed very early in gestation. However, this could not be verified by the reviewers since no method for confirmation of pregnancy

TABLE 7. Group Incidences of Mortality Among Pregnant Animals

Parameter	Dosage (mg/kg/day)			
	0	3	10	30
No. pregnant	17	15	18	14
No. dead/sacrificed	1	2	4	2
% dead/sacrificed	6	13	22	14

status (such as immersion of uterine tissues in ammonium sulfide) was presented by the study author. In addition, the mean number of resorptions per litter increased in a dose-related pattern (0.63, 0.69, 0.79, and 0.83 in the 0, 3, 10, and 30 mg/kg/day dosage groups, respectively). These increases resulted in slight dose-related elevations in the percentage of postimplantation losses (8.3, 9.3, 9.6, and 9.9 for the 0, 3, 10, and 30 mg/kg/day dosage groups, respectively); however, these changes were not statistically significant. Mild decreases in fetal body weights were reported for the 30 mg/kg/day dosage group; these body weight reductions may be associated with slight increases in the mean number of live fetuses per litter in this group. The male to female fetal ratios were comparable for all groups.

No compound-related increases in the incidences of malformations or variations were noted except for a slight increase in the incidence of a single extra rib (9, 13, 13, and 15 percent for the 0, 3, 10, and 30 mg/kg/day dosage groups, respectively). This variation is often considered an indication of mild fetotoxicity.

B. The following are differences between the reviewers' and study author's conclusions:

1. The study author reported that animals were examined by a veterinarian and confirmed as being suitable for this study. However, considering the extremely high incidence of female mortalities, which the study author indicated were attributable to pulmonary disorders (and not to the test material), we conclude that the respiratory illness was associated with an unacceptably high incidence of maternal death. Therefore, we assess that the health status of these animals was unacceptable. Furthermore, because the author did not provide the reproductive history for the females, we could not confirm if these animals were acceptable (i.e., nulligravid) for a teratogenicity study.

2. We conclude that the mean number of resorptions increased with increasing dosages but that these increases were not statistically significant. Differing from the study author's conclusion, we do not rule out a biologically significant association between the test material and the increases in embryolethality.

3. We conclude that the deficiencies in methods implemented in fetal examinations (see Section 14C, below) precluded a definitive assessment of the teratogenic potential of the test material. Therefore, we do not agree with the study author's conclusion that cyhalothrin was not teratogenic in this study; instead we consider their assessment to be based on inconclusive data.

C. The following deficiencies in study design and conduct have negatively affected the scientific validity of the study:

1. The high incidence of maternal mortality associated with pulmonary illness is considered unacceptable. A definitive assessment of maternal and fetal toxic effects cannot be made on the basis of animals with such high incidences of illness related deaths. In addition, the data obtained from surviving animals are questionable since it is possible that their health may have also been affected.

2. The following deficiencies in fetal examinations precluded a definitive assessment of teratogenic potential of the test material.

   a. Scheduled Laparotomies: It would have been more acceptable if pregnancies were terminated on gestation day 29 or 30. The sacrifice of study females on gestation day 28 is considered too early and may have contributed to the presence of small pups with reductions in skeletal ossification and an apparent increase in skeletal and visceral variants.

   b. Fetal Euthanasia: The procedure of intracardiac injection is considered unacceptable due to the physical perforation of cardiac structures and the possible distortion of cardiac and major vessel anatomy produced by the volume of fluid injected into the cardiac chambers. The anatomic disruptions resulting from these procedures may have negatively affected the accuracy of cardiovascular examinations by masking the visualization of cardiac septal defects, valve malformations, pericardial hemorrhages, and various other malformations or lesions in the mediastinum of the fetuses.

   c. Fetal Visceral Examinations: The methods used for examination of the thoracic and abdominal cavities were not indicated or described in the study report, nor was it stated whether these examinations were conducted with the aid of a dissecting microscope. This is of particular concern since cardiac structures were perforated during fetal sacrifices prior to examination for intracardiac abnormalities. In addition, the method of intracranial examination, as described in the study report, was precarious. The author stated that fixed heads were sliced through the line of the fronto-parietal suture to examine the fetal brains for "visible abnormalities." It would have been more acceptable to examine the intracranial structures through serial coronal planes to provide sectional views of the nasal cavities and septum, olfactory lobes of the brain, eyes, lateral, third and fourth ventricles, vestibulocochlear apparatus, and

cerebellum. The inherent deficiencies of the single coronal section method described by the author would not permit the visualization of a number of malformations and variations. Therefore, we conclude that the methods used in this study precluded an adequate assessment of the potential teratogenic effects of the test material.

Item 15--see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods, CBI pp. A4-A23.