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FINAL

DATA EVALUATION RECORD

TETRAMETHRIN

Study Type: Developmental Toxicity in Rabbits

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Pia Lindstrom Date Jan 22, 1992

Independent Reviewer Sanju Diwan Date 1/22/92

QA/QC Manager Sharon Segal Date 1/22/92

Contract Number: 68D10075
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Project Officer: James Scott

EPA Reviewer: William Dykstra, Ph.D.
Toxicologist, Toxicology Branch I/HED
EPA Section Head: Roger Gardner, Ph.D.
Toxicology Branch I/HED

Signature: William Dykstra 11/28/92
Date: 11/28/92
Signature: Roger Gardner
Date: 4/28/93

DATA EVALUATION RECORD

STUDY TYPE: Developmental toxicity study in rabbits

EPA IDENTIFICATION NUMBERS

TOX CHEM. NUMBER:

MRID NUMBER: 419950-05

TEST MATERIAL: Tetramethrin 95.1% pure

SYNONYMS: Neo-pynamin, phthaltrin

SPONSOR: Sumitomo Chemical Company Ltd., Osaka, Japan

STUDY NUMBER: 95088

TESTING FACILITY: Bioresearch Laboratories Ltd., Montreal, Canada

TITLE OF REPORT: An Oral Teratology Study of Neo-Pynamin in the Rabbit

AUTHORS: K. Robinson, G. Washer, and J. Noveroske

REPORT ISSUED: July 18, 1991

CONCLUSIONS: A developmental toxicity study was conducted in which New Zealand White rabbits were administered tetramethrin via gavage at 0, 30, 100, 300, or 500 mg/kg/day during gestational days (GD) 7 through 19. Analytical chemistry results, however, indicate that the highest dose level was 420 mg/kg/day (approximately 15% less than the nominal concentration). Maternal toxicity, observed at 300 and 420 mg/kg/day, was manifested as a nonsignificantly decreased corrected body weight gain as well as decreased body weight gain during the dosing and gestational periods. Consequently, the maternal NOEL and LOEL were 100 and 300 mg/kg/day, respectively.

Developmental toxicity was not observed in this study. Consequently, the developmental NOEL was 420 mg/kg/day; the LOEL was not determined.

CLASSIFICATION: Core Minimum. This study meets the minimum requirements set forth under EPA Guideline 83-3 for a developmental toxicity study in rabbits. Although the affected parameter was a nonsignificant decrease in body weight gain, the decrease was substantial (10-20%), and a similar decrease in weight loss had been observed in a range-finding study at 500 mg/kg.

A. MATERIALS

Test Compound

Purity:	95.1%
Description:	White/pale yellow powder
Lot number:	90304
Receipt date:	January 16, 1990
Contaminants:	Not reported

Vehicle: 0.5% w/v carboxymethylcellulose (source: Sigma Chemical Company, St. Louis, Missouri)

Test Animals

Species:	Rabbit
Strain:	New Zealand White
Source:	Hazleton Research Products Inc., Denver, PA
Age:	5 months on GD 0
Weight:	2.6-3.7 kg on GD 0
Males used:	Proven bucks of same strain and source

B. STUDY DESIGN

This study was designed to assess the potential of tetramethrin to cause developmental toxicity in rabbits when administered daily via gavage from GD 7 through 19, inclusive.

Mating: Following 26 days of acclimation, females were artificially inseminated using a diluted pool of semen (at least 5×10^7 spermatozoa/mL) from proven bucks of the same strain and supplier. A volume of 0.70-0.90 mL of the pooled semen was used to inseminate each doe. To ensure ovulation, each doe was given an intravenous injection of 50 I.U. of human chorionic gonadotropin (source not reported) 19 days and again 2-4 hours prior to insemination. The day of insemination was designated day 0 of gestation.

Animal Husbandry: Food (Certified Purina Rabbit Laboratory Chow # 5322) was given in the amount of 180 g/day, while water (tap water, treated by reverse osmosis and ultraviolet sterilization) was freely available. A 12-hour light/dark cycle was maintained. Temperature and humidity were maintained at 17°C ± 3° and 50% ± 20%, respectively, with 15 ± 1.5 air changes per hour.

Group Arrangement: Animals were randomly assigned to dose groups (using a computer-based randomization procedure) as follows:

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	20
2	30	20
3	100	20
4	300	21[a]
5	500	21[a]

[a]One animal died on GD 7 and was replaced.

Dose Administered: Doses were administered daily via gavage from GD 7 through 19 in a volume of 10 mL/kg. The individual body weights recorded on GD 7 were used to provide the correct mg/kg/day dose. Dose suspensions were prepared daily in 0.5% aqueous carboxymethylcellulose using a Brinkman homogenizer; the vehicle was prepared weekly. During the dosing, the suspensions were kept on a stir plate. Analyses for stability and homogeneity were performed prior to study initiation. Analyses for concentration were performed on suspensions taken on three occasions during the dosing period.

The selection of dose levels was based on the results of a range-finding study. Five pregnant rabbits per group were administered tetramethrin at dose levels of 0, 250, 500, 1000, or 1500 mg/kg/day from GD 7 through 19. Maternal toxicity, observed at 500, 1000, and 1500 mg/kg/day, was manifested as increased rates of mortality (1, 4, and 1 does at 500, 1000, and 1500 mg/kg/day, respectively) and abortions (2, 4, and 5 at 500, 1000, and 1500 mg/kg/day, respectively) and significantly decreased body weight. Developmental toxicity, observed at 1000 and 1500 mg/kg/day, was manifested as increased rates of resorptions.

Observations: Animals were observed twice daily for mortality, moribundity, and general appearance and behavior during pre- and postdosing periods, in addition to overt signs of toxicity immediately following dosing from GD 7 to 19. Females that aborted and/or died were necropsied, and gross findings and fetuses from these animals were preserved in 10% neutral buffered formalin for possible microscopic examination. Body weight was recorded on GD 0, 3, 5, 7, 10, 13, 16, 19, 22, 25, 27, and 29. Food consumption was recorded daily during the entire gestation. Females were sacrificed on GD 29 by an intravenous injection of pentobarbital followed by exsanguination. Litters were delivered by cesarean section. Examination of the does at sacrifice included the following:

- Gross pathological observations (lesions preserved in 10% neutral buffered formalin);

- Number of corpora lutea;
- Gravid uterine weight;
- Number of implantation sites; and
- Number and location of resorptions (early and late) and live and dead fetuses.

Uteri from apparently nonpregnant animals were stained with 10% ammonium sulfide to detect early embryonic death.

All fetuses (after being killed by a subcutaneous injection of T61 euthanasia solution) were examined in the following manner:

- Fetuses were weighed;
- External anomalies were recorded;
- Visceral anomalies were recorded using a dissection microscope;
- Heads from one-third of the fetuses were removed and placed in Bouin's solution and later examined using Wilson's technique (1965); and
- Skeletal anomalies were recorded following fetal evisceration and staining with Alizarin Red S using a modification of Dawson's technique (1926).

Statistical Analysis: The following methods were used.

- Maternal body weight, maternal body weight gain, maternal food consumption, and fetal body weight--ANOVA and Dunnett's test;
- Numbers of corpora lutea, implantation sites, resorptions, and live fetuses, percentage of pre- and postimplantation losses, sex ratio (% males), and % skeletal variations per litter--Kruskal Wallis test and Mann-Whitney U-test; and
- Fetal and litter incidences of malformations and variations--Chi Square test (with Yate's correction) or Fisher's Exact test.

Compliance

- A signed Statement of No Data Confidentiality Claim, dated August 7, 1991, was provided;
- A signed Statement of Compliance with EPA GLPs, dated July 18 and 25, and August 16, 1991, was provided; and
- A signed Quality Assurance Statement, dated July 18, 1991, was provided.

C. RESULTS

1. Test Material Analyses

Analyses for homogeneity of the test material in the vehicle revealed a potential mixing problem at the highest dose level. While the range for the 30-, 100-, and 300-mg/kg groups was 93-103% of nominal values, it was only 84-89% for the 500-mg/kg group. Recovery of the test material in the vehicle on the same day as preparation revealed values between 90% and 109% of nominal concentrations. Analyses for concentration of the dosing suspensions included values outside the normally accepted ±10% range on several occasions. For the 30-mg/kg/group, the concentrations were 94%, 78%, 68%, 90%, 92%, and 97% of target; for the 100-mg/kg group, the concentrations were 98%, 101%, 78%, 85%, and 88% of target; and for the 500-mg/kg group, the concentrations were 86%, 86%, 94%, 98%, 85%, and 77% of target.

2. Maternal Toxicity

Mortality: No compound-related mortalities were observed. From the 100-mg/kg group, one animal died on GD 17; from the 300-mg/kg group, one animal died on GD 7 and one on GD 18; and from the 500-mg/kg group, one animal died on GD 7 and and one on GD 18. For all these animals, the cause of death was considered to be a gavage error based upon clinical behavior, time of death, and/or pathological findings at necropsy.

Abortion: No compound-related abortions were observed. One doe aborted on GD 23 in the 30-mg/kg group. Uterine examination revealed seven implantation sites and one early and five late resorptions. Necropsy revealed no gross findings.

Clinical Observations: No compound-related clinical findings were observed. Incidental findings in all dose groups included alopecia, scabs, reflux/liquid from nares, convulsive and/or decreased movements, gasping, and blood around muzzle.

Body Weight: A summary of maternal body weight gain for selected time intervals is presented in Table 1. Body weight (data not shown) was not significantly affected by the test compound. However, body weight gain (Table 1), recalculated by the reviewers and analyzed by ANOVA for the predosing (GD 0-7), dosing (GD 7-19), and gestational (GD 0-29) periods (Table 1), decreased by 20% during dosing and 10% during gestation in the 300- and 500-mg/kg groups. In addition, as shown by the authors, there were substantial decreases in corrected body weight gain at these same dose levels.

Food Consumption: A summary of food consumption for selected time intervals is presented in Table 2. No compound-related effects were observed. The slight decreases noted during dosing in the 300- and 500-mg/kg groups were <5% and were not considered to be important.

Gross Pathological Observations: No compound-related gross findings were observed. Incidental findings in all dose groups included small gall bladder, ovarian and/or oviduct cyst(s), red stomach, and thickened urinary bladder.

Cesarean Section Observations: A summary of cesarean section data is presented in Table 3. No compound-related effects were observed for any parameter.

3. Developmental Toxicity

Summaries of malformations and variations are presented in Tables 4, 5, and 6. No compound-related anomalies were observed.

External Examinations: The following external malformations were observed (Table 4): anencephaly (one fetus in the 100-mg/kg group) and intestines protruding at umbilicus (one fetus in the 300-mg/kg group). No variations were noted.

Visceral Examinations: The following visceral malformations were observed (Table 5): absent accessory lobe of lungs (one fetus in the 100-mg/kg group); common truncus (one fetus in the 30-mg/kg group); interventricular septal defect (one fetus in the 30-mg/kg group); and retinal folding (one fetus in the 30-mg/kg group). Variations included the eye one fetus each in the control, 30-, and 100-mg/kg groups), gallbladder (all dose groups), liver (one fetus in the 100-mg/kg group), and thymus (one fetus in the 300-mg/kg group).

Skeletal Examinations: No skeletal malformations were observed (Table 6). Variations were observed in all dose groups and included variations in skull bones (reduced/irregular ossification, additional sutures, absent, and/or bipartite), vertebrae (extra presacral or reduced number of caudal vertebrae), sternebrae (misaligned, fused, and/or bifurcated), ribs (notched), and pelvic girdle (reduced ossification).

D. DISCUSSION/CONCLUSIONS

1. Acceptance Criteria

The reviewers have completed an Acceptance Criteria checklist (Attachment I) that is included with this evaluation. All criteria were satisfied.

2. Test Material Analyses

The variability in the homogeneity data indicates a potential mixing problem. While the deviations in the 30-mg/kg group do not affect the study results since no effects were observed at doses up to and including 300 mg/kg/day, it appears that the concentrations at the highest dose level were mostly in the range of 84-89% of target when analyzed for homogeneity and concentration. Therefore, the reviewers

suggest that the highest dose level be recalculated to 84% of 500 mg (i.e., 420 mg).

3. Maternal Toxicity

Maternal toxicity, observed at 300 and 500 mg/kg/day, was evidenced by decreased corrected body weight gain as well as decreased body weight gain during the dosing and gestational periods. These decreases, 10-20%, were considered by the reviewers to be compound-related and biologically adverse, although they were not statistically significant. This conclusion is further supported by the results from the range-finding study in which significant body weight loss was reported at doses of 500 mg/kg/day and above.

Based on these results, the maternal NOEL and LOEL values were 100 and 300 mg/kg/day, respectively.

4. Developmental Toxicity

- Deaths/Resorptions: No compound-related effects were observed.
- Altered Growth: No compound-related effects were observed.
- Developmental Anomalies: No compound-related effects were observed.

No developmental toxicity was observed in this study. Consequently, the NOEL was 420 mg/kg/day and a LOEL was not determined.

5. Study Deficiency

The protocol for and results of the range-finding study were not submitted. These data would further support the conclusion in the present study, and it is therefore, recommended that they be submitted.

Historical control data on body weight gain during gestation may also strengthen the conclusion that the decreased body weight gain (observed during dosing and gestation in the present study) is a true effect of the test compound as opposed to normal variation. Therefore, it is recommended that these data as well be submitted.

E. CLASSIFICATION: Core Supplementary Data

Maternal NOEL = 100 mg/kg/day
Maternal LOEL = 300 mg/kg/day based on decreased body weight gain and corrected body weight gain
Developmental toxicity NOEL = 420 mg/kg/day
Developmental toxicity LOEL = not determined

F. RISK ASSESSMENT: Not applicable

TABLE 1. Mean Body Weight Gain (g ± S.D.)[a]

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 0-7)[b]	Dosing Period (GD 7-19)	Post Dosing Period (GD 19-29)[b]	Entire Gestation Period (GD 0-29)[b]	Corrected Weight Gain (GD 7-29)
0	140 ± 65	226 ± 49	238 ± 44	610 ± 75	40 ± 98
30	147 ± 55	230 ± 63	203 ± 99	579 ± 132	10 ± 92
100	126 ± 59	226 ± 94	241 ± 87	587 ± 106	50 ± 98
300	115 ± 59	181 ± 125	240 ± 85	551 ± 122	-40 ± 115
500	141 ± 67	182 ± 102	242 ± 99	565 ± 144	-10 ± 115

[a]Data were extracted from Study No. 95088, Table 3 and 4 and Appendix 2.

[b]Calculated by the reviewers and analyzed by ANOVA.

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TABLE 2. Mean Food Consumption (g/animal/day ± S.D.)[a,b]

Dose Group (mg/kg/day)	Prior to Dosing Period (GD 0-6)	Dosing Period (GD 7-19)	Post Dosing Period (GD 20-28)	Entire Gestation Period (GD 0-28)
0	175 ± 12.6	176 ± 9.9	165 ± 20.3	172 ± 11.7
30	180 ± 1.1	178 ± 4.2	167 ± 19.7	174 ± 7.8
100	178 ± 5.6	177 ± 7.5	174 ± 9.4	177 ± 6.6
300	178 ± 7.1	168 ± 24.0	172 ± 11.6	173 ± 10.5
500	178 ± 7.2	167 ± 32.8	164 ± 21.4	169 ± 17.7

[a]Data were extracted from Study No. 95088, Appendix 5.

[b]Calculated by the reviewers and analyzed by ANOVA.

TABLE 3. Cesarean Section Observations[a]

Parameter	Dose Level (mg/kg/day) 0	30	100	300	500
No. animals assigned	20	20	20	21	21
No. animals pregnant	19	20	19	19	19
Pregnancy rate (%)	95	100	95	90	90
Maternal wastage					
No. died or killed/pregnant	0	0	1	1	2
No. died/nonpregnant	0	0	0	1	0
No. nonpregnant	1	0	1	1	2
No. aborted	0	1	0	0	0
Total no. corpora lutea[b]	163	175	163	160	146
Corpora lutea/doe	8.6 ± 1.74[c]	9.2 ± 2.35	9.1 ± 1.39	8.9 ± 1.57	8.6 ± 1.91
Total no. implantations[b]	129	141	126	144	128
Implantations/doe	6.8 ± 1.75	7.4 ± 2.29	7.0 ± 2.38	8.0 ± 1.94	7.5 ± 1.46
Total no. live fetuses[b]	121	131	108	134	114
Live fetuses/doe	6.4 ± 1.80	6.9 ± 2.16	6.0 ± 2.74	7.4 ± 2.20	6.7 ± 1.93
Total no. resorptions[b]	8	10	18	10	14
Early	6	7	15	7	9
Middle	0	2	1	1	0
Late	2	1	2	2	5
Resorptions/doe	0.4 ± 0.69	0.5 ± 0.96	1.0 ± 2.03	0.6 ± 0.70	0.8 ± 1.33
Gravid uterine weight (g)	420 ± 95.6	436 ± 98.7	407.8 ± 163.6	464.8 ± 88.8	435.5 ± 111.9
Total no. dead fetuses	0	0	0	0	0
Fetal weight/litter (g)	47.2 ± 5.52	45.7 ± 5.69	48.6 ± 5.04	45.2 ± 5.35	45.2 ± 3.92
Preimplantation loss (%)	19.6	17.6	21.2	9.7	9.8
Postimplantation loss (%)	6.1	6.2	12.8	7.7	11.5
Sex ratio (% male)	53.0	47.9	50.7	55.3	51.3

[a]Data were extracted from Study No. 95088, Table 8 and 9 and Appendices 1 and 8.

[b]Calculated by the reviewers.

[c]Mean ± S.D.

TABLE 4. Summary of Fetal External Anomalies[a]

Finding[b]	Dose Level (mg/kg/day) 0	30	100	300	500
No. fetuses (litters) examined	121 (19)	131 (19)	108 (17)[c]	134 (18)	114 (17)
MALFORMATIONS					
Anencephaly	0	0	1	0	0
Intestines protruding at umbilicus	0	0	0	1	0
Total no. fetuses (litters) with external malformations	0	0	1	1	0
VARIATIONS					
None	0	0	0	0	0
Total no. fetuses (litters) with external variations	0	0	0	0	0

[a]Data were extracted from Study No. 95088, Table 10 and Appendix 13.

[b]More than one finding may be observed in one fetus.

[c]Complete resorption in one doe.

B

TABLE 5. Summary of Fetal Visceral Anomalies[a]

Finding[b]	Dose Level (mg/kg/day) 0	30	100	300	500
No. of fetuses (litters)	121 (19)	131 (19)	108 (17)[c]	134 (18)	114 (17)
MALFORMATIONS					
Accessory lobe of lungs absent	0	0	1	0	0
Common truncus, pulmonary trunk absent	0	1	0	0	0
Interventricular septal defect	0	1	0	0	0
Retinal folding with intraocular hemorrhage	0	1	0	0	0
Total no. fetuses (litters) with visceral malformations	0	2 (2)	1	1	0
VARIATIONS					
Eye					
Lenses oval	1	1	1	0	0
Bitreous body reduced	0	1	0	0	0
Gallbladder					
Absent	1	1	1	2 (2)	2 (1)
Reduced	3 (2)	4 (3)	2 (2)	0	4 (3)
Liver, additional lobe	0	0	1	0	0
Thymus, undescended	0	0	0	1	0
Total no. fetuses (litters) with visceral variations	5 (3)	7 (6)	5 (4)	2 (2)	6 (3)

[a]Data were extracted from Study No. 95088, Table 10 and Appendix 13.

[b]More than one finding may be observed in one fetus.

[c]Complete resorption in one doe.

TABLE 6. Summary of Fetal Skeletal Anomalies[a]

Finding[b]	Dose Level (mg/kg/day) 0	30	100	300	500
No. fetuses (litters)	121 (19)	131 (19)	108 (17)[c]	134 (18)	107 (16)[d]
MALFORMATIONS:					
None	0	0	0	0	0
Total no. fetuses (litters) with skeletal malformations	0	0	0	0	0
VARIATIONS					
Skull, hyoid bone					
Reduced ossification	5 (5)	5 (3)	7 (5)	9 (2)	4 (3)
Bipartite	0	2 (2)	0	0	0
Semibipartite	0	1	0	0	1
Irregular ossification	1	0	0	0	0
Absent	0	1	0	0	0
Skull, frontal bones					
Reduced ossification	2 (2)	0	0	1	0
Skull, frontal/parietal bones					
Additional sutures	0	0	1	1	0
Skull, parietal bones					
Irregular ossification	1	0	0	0	0
Vertebral column					
Extra presacral vertebrae	0	0	1	0	0
Reduced no. caudal vertebrae	0	0	1	0	0
Sternebrae, misaligned	2 (2)	0	1	1	1
Fused	1	0	0	1	1
Bifurcate	0	0	0	1	0
Ribs, notched rib	1	0	1	0	0
Pelvic girdle, pubic bones					
Reduced ossification	0	1	0	0	0
Total no. fetuses (litters) with skeletal variations	11 (8)	9 (7)	12 (8)	9 (5)	7 (6)

[a]Data were extracted from Study No. 95088, Table 10 and Appendix 13.

[b]More than one finding may be observed in one fetus.

[c]Complete resorption in one doe.

[d]One litter excluded due to extensive mechanical damage during staining.

ATTACHMENT I

83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1. YES Technical form of the active ingredient tested.

2. YES At least 20 pregnant animals/dose group for mice, rats, or hamsters are available. At least 12 pregnant animals/dose group for rabbits are available (three test groups and control group).

3. YES At the high dose, overt maternal effects such as slight weight loss are reported (or a limit dose is given, 1,000 mg/kg).

4.* YES At the low dose, no developmental toxicity is reported.

5. YES Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.

6.* YES Analysis for test material stability, homogeneity, and concentration in dosing medium.

7. YES Individual daily observations.

8. YES Individual body weights.

9. YES Individual food consumption.

10. YES Necropsy on all animals.

11. YES Individual uterine examination, including numbers of fetal deaths, early and late resorptions, and viable fetuses per sex.

12. YES All ovaries examined to determine number of corpora lutea.

13. YES Individual litter weights and/or individual fetal weights/sex/litter.

14. YES Individual fetal external examination.

15. YES Individual fetal skeletal examination for 1/3 to 1/2 of each litter for rodents and all for rabbits.

16. YES Individual fetal soft tissue examination.

Criteria marked with a * are supplemental, may not be required for every study.