(7-19-91)

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

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: 004005. Esbiothrin. Review of Developmental Toxicity Studies in the Rat and Rabbit

Tox. Chem. No. 025
Project No. 1-0772

TO: Richard King, PM Team # 74
Special Review and Reregistration Division
(H7508W)

FROM: Pamela M. Hurley, Toxicologist Pamela M. Hurley 7/11/91
Section I, Toxicology Branch I
Health Effects Division (H7509C)

THRU: Roger L. Gardner, Section Head
Section I, Toxicology Branch I
Health Effects Division (H7509C)
Roger L. Gardner
7-16-91

Record No(s). S391832

KB 7/19/91

Background and Request:

Two developmental toxicity studies have been submitted by Roussel Bio Corporation in response to a data call-in. The Toxicology Branch has been requested to review and comment on the studies.

Toxicology Branch Response:

The Toxicology Branch (TB-I) has reviewed the two developmental toxicity studies in the rat and rabbit on Esbiothrin and have found them both to be acceptable. The regulatory requirements for developmental toxicity studies in two species are now satisfied.

In the rabbit, Esbiothrin was tested at the following dose levels: 0, 30, 100 and 300 mg/kg/day by gavage. The NOEL for maternal toxicity is 100 mg/kg/day and the the LOEL is 300 mg/kg/day (HDT) based on deaths and clinical signs of toxicity (tremors, decreased motor activity and ataxia). The NOEL for developmental toxicity is 300 mg/kg/day (HDT).

In the rat, Esbiothrin was tested at the following dose levels: 0, 5, 25, and 125 mg/kg/day by gavage. The maternal NOEL

Reviewed By: Pamela Hurley, Ph.D. Pamela M Hurley 6/19/91
Section I, Tox. Branch (H7509C)
Secondary Reviewer: Roger L. Gardner, Head Roger L. Gardner 7-16-91
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DATA EVALUATION REPORT

STUDY TYPE: Teratology - Developmental Toxicity (83-3)

SPECIES: Rabbit

TOX. CHEM. NO./CASWELL NO.: 025A

ACCESSION NUMBER/MRID NO.: 416322-02

TEST MATERIAL: Esbiothrin

STUDY NUMBER(S): 718-002

REPORT NUMBER: Not given

SPONSOR: Roussel Bio Corporation, Lincoln Park, New Jersey

TESTING FACILITY: Argus Research Laboratories, Inc., Horsham, PA

TITLE OF REPORT: Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of Esbiothrin Technical Administered Orally Via Stomach Tube to New Zealand White Rabbits

AUTHOR(S): A. M. Hoberman

REPORT ISSUED: 08/31/90

CONCLUSION: Esbiothrin was tested in a developmental toxicity study in the rabbit at the following dose levels: 0, 30, 100 and 300 mg/kg/day. The NOEL for maternal toxicity is 100 mg/kg/day and the the LOEL is 300 mg/kg/day (HDT) based on deaths and clinical signs of toxicity (tremors, decreased motor activity and ataxia). The NOEL for developmental toxicity is 300 mg/kg/day (HDT).

Classification: Core Guideline

Testing Guideline Satisfied: 83-3

A. MATERIALS AND METHODS:

1. Test Compound(s):

Chemical Name: d-trans chrysanthemic acid of d-allethrolone (72%) and d-trans chrysanthemic acid of l-allethrolone (21%)

Description: thick amber liquid
Batch #(s), Other #(s): Lot 9N0317B3 and Lot 9N0947B3
Purity: 95.2% and 94.6%
Source: Roussel Bio Corporation
Vehicle (if applicable): aqueous 0.5% methyocellulose
Contaminant: list in CBI appendix - none listed

2. Test Animals:

Species and Strain (sexes): Hra:(NZW)SPF rabbits, male and female
Age: 5 months (F), age for males not given.
Weight(s): 2.36-3.82 (F), weights for males not given.
Source(s): Hazleton Research Products, Inc., Denver, PA

3. Study Design:

This study was designed to assess the developmental toxicity potential of Esbiothrin when administered by stomach tube to rabbits on gestation days 6 through 18, inclusive.

a. Mating:

Natural or artificial insemination? artificial
Describe technique used: Approximately 3 hours prior to insemination, the selected female rabbits were intravenously administered 20 USP Units of Human Chorionic Gonadotropin (HCG) per kg body weight. An estimated 0.25 ml of semen that had been diluted with normal saline to a concentration of 6.0×10^6 spermatozoa/0.25 ml saline was used to artificially inseminate each rabbit. That day was designated as day 0 of presumed gestation.

b. Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0 (vehicle)	20
Low Dose	30	20
Mid Dose	100	20
High Dose	300	20

c. Dosing:

All doses were in a volume of 5 ml/kg of body weight/day. Dosing was based on daily gestation day body weights.

1) Basis For Selection of Dose Levels: The dosages were selected on the basis of a pilot evaluation conducted by Argus Research Labs. In the pilot study, four rabbits/group were tested at the following dose levels: 0 (three rabbits), 25, 50, 100, 200 and 400 mg/kg/day. Two rabbits died in the 400 mg/kg/day group. One of the 2 animals had clinical signs of toxicity (tremors, impaired or lost righting reflex and biting). This doe had an eroded area in the stomach mucusa. Both does lost weight and had decreased food consumption. No other does in any of the treated groups had clinical signs related to exposure to the test substance. Does in the 400 mg/kg/day group had weight loss and reduced food consumption during the dosage period. There were no apparent effects on fetal development in any of the treated groups. Based on this study, the dose levels for the main study were set at 30, 100 and 300 mg/kg/day.

2) Preparation: Suspensions in aqueous 0.5% (w/w) methylcellulose were prepared daily during the dosing period.

3) Frequency of Preparation: Daily.

4) Storage Conditions: The bulk test substance was stored at room temperature. The prepared vehicle was stored refrigerated for up to 7 days.

5) Stability Analyses: Only done on bulk test material (see concentration analyses below).

6) Homogeneity Analyses: Not done

7) Concentration Analyses: Two samples of the bulk test substance were taken, one at the beginning of the dosage period and the other at the end of the dosage period. These samples were sent to the Sponsor for analysis. Triplicate 10 ml samples from each of the first and last dosage suspensions prepared for administration were retained and frozen. Two of the 3 samples from each concentration were sent for analysis. The third samples were retained.

d. Maternal Examinations:

1) Clinical Observations and Mortality: The does were examined twice daily for mortality throughout the study. They were observed several times for clinical signs during the acclimation period and on day 0 of presumed gestation. During the treatment period, observations for clinical signs of toxicityl, abortions, premature deliveries and deaths were conducted immediatedly prior to each daily administration, approximately 1/2 hour and 1 hour after each administration and more frequently for those animals with clinical signs of toxicity. These additional observations were made once daily during the postdosage period (days 19-29 of presumed gestation).

2) Body Weight Determinations: Recorded at least once weekly prior to insemination, and then on day 0 and days 6-29 of presumed gestation.

3) Food Consumption: Recorded daily during the acclimation and study periods.

4) Gross Necropsy:

Animals which died or were sacrificed in moribund condition prior to end of exposure period and were subjected to complete gross pathological examinations: all animals were necropsied on the day the event occurred. Uterine weight, pregnancy status and uterine contents were recorded.

Animals sacrificed at the end of the treatment/observation period which were subjected

to complete gross pathological examinations: All sacrificed by i.v. injection of T-61 euthanasia solution on day 29 of presumed gestation. Thoracic and abdominal cavities examined for gross lesions.

5) Uterine Examinations: Intact uteri excised and weighed. Uteri from apparent nonpregnant does were stained with 10% ammonium sulfide to ascertain the presence or absence of very early resorptions. Tissues with gross lesions were preserved in neutral buffered 10% formalin. All remaining tissues discarded. The following were recorded:

Number of corpora lutea
Number of live fetuses
Number of dead fetuses
Early and late resorptions
Number and placement of implantations
Individual fetal weights

e. Fetal Examinations:

The fetuses were examined in the following manner: Each was weighed and examined for gross external alterations. Live fetuses were killed with an injection of phenobarbitol sodium. All were examined to identify sex and visceral alterations; the brain was free-hand cross-sectioned (a single cross-section was made between the parietal and the frontal bones) and examined for hydrocephaly. The fetuses were then eviscerated, stained with alizarin red S and evaluated for skeletal alterations. All skeletal preparations were stored in 80% glycerin with thymol crystals added to retard fungal growth.

f. Historical Control Data:

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

g. Statistical analysis:

The following statistical analysis methods were employed (excerpted from the summary statement of the report): Maternal body weight, body weight change, feed consumption and organ weight data, and litter averages for percent male fetuses, fetal body weight, fetal ossification sites and percent fetal alterations were analyzed using

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Bartlett's Test of Homogeneity of Variances and the Analysis of Variance, when appropriate. If the Analysis of Variance was significant, Dunnett's Test was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate, the Kruskal-Wallis Test was used, when less than or equal to 75% ties were present; when more than 75% ties were present, the Fisher's Exact Test was used. In cases in which the Kruskal-Wallis Test was statistically significant, Dunn's Method of Multiple Comparisons was used to identify the statistical significance of the individual groups. All other Caesarean-delivery data were evaluated using the precedures previously described for the Kruskall-Wallis Test.

h. Compliance:

A signed Statement of No Data Confidentiality Claim was provided.

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

B. RESULTS:

1. Dosage Preparation: The results of the analyses of the bulk test material before and after the study were 94.6%, 94.91% and 95.04% pure test material. Therefore, the sample remained stable over the testing period. The results from the testing of the samples taken were as follows: "Concentrations of individual diet samples show the analytically determined mean concentrations for the 5 mg/g test substance level to range 20% to 32% from the target concentration; 6 mg/g level to range 3% to 23%; 20 mg/g level 11% to 24%; 40 mg/g level to be 11%; 60 mg/g level 15% to 18% and the 80 mg/g level to range from 2% to 8% from the target level." Some of these ranges appear to be somewhat wide, particularly for the lower concentrations, however, it may be difficult to accurately measure suspensions.

2. Maternal Toxicity:

a. Clinical Observations and Mortality: In the 300 mg/kg/day dose group, four does died during the treatment period (3 on day 9 and 1 on day 10 of the gestation period). All except one displayed clinical signs of toxicity (tremors, decreased motor activity and ataxia). One of the three does

also displayed impaired righting reflex and another of the 3 displayed excess salivation. No other deaths were observed in any of the other dose groups. The authors stated that "surviving does [in this dose group] did not have clinical observations attributable to the test substance after day 12 of gestation, indicating that the effects of this test substance were most severe during the first seven days of the 13-day dosage period." Does in the other treated groups did not have clinical signs of toxicity related to treatment.

b. Body Weight Determinations: All of the does that died had reduced body weight. In the highest dose group, weight loss was observed on the first 2 days after dosing and during days 6-9 of gestation. These were generally influenced by the weight losses of those animals that died in this dose group. If these animals are excluded (see table below for body weight changes), there are no significant differences in body weights or body weight gains between the high dose group and the controls at any time period. With the exception of the mid-dose group during the dosing period, there were no significant differences in body weights or body weight gains between any of the treated and control groups throughout any of the time periods. During the dosing period, the mid-dose group gained statistically significantly less than the control group. Without the effect of the 4 animals that died in the high dose group, this change in body weight gain is not dose related and is thus not likely to be an effect of treatment.

There were no significant differences in gravid uterine weights when treated animals were compared to controls.

The investigators supplied the following data:

Table I: Body Weight Gains (kilograms)[a]

Group:	Prior to Dosing Period	Dosing Period	Post Dosing Period	Entire Gestation Period	Corrected Body Weight Gains Dosing P.[1]	Entire[2]
Control	0.15	0.22	0.22	0.59	Not given	0.16
LDT	0.15	0.17	0.20	0.51	"	0.12
MDT	0.18	0.11*	0.26	0.55	"	0.17
HDT	0.18	0.18[b]	0.23[b]	0.60[b]	"	0.18[b]

[1] = corrected body weight gain for dosing period = body weight gain for dosing period minus gravid uterus weight.
[2] = corrected body weight gain for entire gestation period = body weight gain for entire gestation period minus gravid uterus weight.
a = Data extracted from (study Argus 718-002, table 5)
b = Excludes body weights for rabbits that were found dead.
* = Statistically significant ($p<0.05$)

c. Food Consumption: All of the does that died had reduced food consumption. Reduced food consumption was generally seen in the high dose group during the dosing period. None of the other treated groups had any significant reduction in food consumption when compared to controls. Absolute food consumption was reduced (but not statistically significantly so) in the high dose group on days 6-9 of the gestation period (the first 3 days of treatment) after exclusion of does which resorbed their litters. Relative food consumption was reduced in the high dose group on days 8 and 9 of gestation, with and without exclusion of does which resorbed their litters. Overall, when one examines the table below, it does not appear that food consumption was much affected beyond the first 3 days of treatment.

The investigators supplied the following data:

Table II: Food Consumption Data (G/Day)[a]

Group:	Prior to Dosing Period	Days 6-9	Dosing Period	Post-Dosing Period	Entire Gestation Period
Control	177.0	176.1	175.2	152.3	167.7
LDT	171.2	162.3	166.6[b]	149.1	160.9
MDT	176.2	169.0	160.8	149.5	160.1
HDT	176.0	145.2	169.5[c]	160.6[c]	168.6[c]

[a] = Data extracted from (study Argus 718-002, table 6)
[b] = Excludes feed consumption values that were associated with spillage.
[c] = Excludes feed consumption values for rabbits that were found dead.

d. Gross Pathology:

The investigators supplied the following data: No gross lesions attributable to the test substance were observed in any of the does, including those that died. Two does, one each in the low and in the mid-dose groups were observed to have fluid-filled abdominal cavities. One doe in the low-dose group had a degenerated corpora lutea. Parovarian cysts were found in all groups in the following frequendies: 8/20, 6/20, 8/20 and 6/20 in the control, low dose, mid-dose and high dose groups, respectively.

e. Cesarean Section Observations: The authors stated that there were no instances in which does aborted or prematurely delivered a litter during this study. One low dose group doe had a litter which consisted entirely of only one early resorption. Two middle dose group does had litters which consisted entirely of 5 and 9 early resorptions, respectively. All the control and high dose does had litters which contained at least one live conceptus, including those does that died. The resorbed litters in the low and middle dose groups were considered unrelated to the test substance because: 1) the events were not dose-dependent; and 2) the incidences were within the historical control range.

There were no statistically significant differences between the treated and control groups in pregnancy indices, averages for corpora lutea, implantations, live litter sizes, resorptions, fetal sex ratios, fetal body weights, percent resorbed conceptuses, or the numbers of does with any resorptions or with viable fetuses. All values were within the ranges observed historically. Based on calculations from the table below, it appears that the number of early resorptions/dam in the mid- and high dose groups are fairly high (although not statistically significant) when compared to controls (0.39, 0.28, 0.95 and 1.2 for the controls, low, mid and high dose groups, respectively). The range of the historical control data is from 0 - 1.3 with a mean of 0.4. The values for the mid- and high dose groups are within the range of the historical control data, but are on the high side of the range. It is possible that there may be a slight effect on early resorptions, but it should be noted that the % preimplantation loss and the number of fetal deaths/dam are higher in the control and low-dose groups when compared to the mid- and high dose groups.

Table III: Cesarean Section observations[a]

	Control	LDT	MDT	HDT
Dose: mg/kg/day	0	30	100	300
#Animals Assigned	20	20	20	20
#Animals Mated/Inseminated	20	20	20	20
Pregnancy Rate (%)	18 (90)	18 (90)	19 (95)	17 (85)
Maternal Wastage				
#Died	0	0	0	4(23.5)*
#Died/pregnant	0	0	0	4
#Non pregnant	2	2	1	3
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea	173	171	192	127
Corpora Lutea/dam	9.6	9.5	10.1	9.8
Total Implantations	130	119	141	104
Implantations/Dam	7.2	6.6	7.4	8.0
Total Live Fetuses	118	110	120	89
Live Fetuses/Dam	6.6	6.1	6.3	6.8
Total Resorptions	9	7	20	15
Early	7	5	18	15
Late	2	2	2	0
Resorptions/Dam	0.5	0.4	1.0	1.2
Total Dead Fetuses	2	1	0	0
Dead Fetuses/Dam	0.1	0.05	0.0	0.0
Mean Fetal Weight (gm/litter)	45.81	45.58	43.56	44.65
Preimplantation Loss(%)	24.9	30.4	26.6	18.1
Postimplantation Loss(%)	9.2	7.6	14.9	14.4
Sex Ratio (% Male)	41.0	48.8	49.1	45.8

[a] = Data extracted from (study Argus 718-002, tables 8 and 9)
* = Statistically significant $p<0.01$

3. Developmental Toxicity: The authors stated that there were no fetal gross, external, soft tissue or skeletal alterations that were considered to be related to treatment. The following were given as reasons why the authors believed that the alterations that were observed were not considered to be biologically significant: some were single events; some values were not statistically significant; some were decreases from controls when increases would have been biologically

significant; many were not dose-related; many were fetal incidences that occurred in the absence of significant changes in the litter incidences, the parameter generally considered the more appropriate for analysis; and all were common and within the historical control range. The following statements from the report summarize those observations that were statistically significantly different from the controls.

"Significant increases (P<=0.01) in the fetal incidences of smaller than normal gallbladder occurred for the 100 and 300 mg/kg/day dosage groups. A significant increase (P<=0.01) occurred in the fetal incidence of irregular nasal-frontal suture for the 100 mg/kg/day dosage group. Significant decreases (P<=0.05 to P<=0.01) in the litter and fetal incidences of irregular ossification of the frontal bones, irregular frontal suture and irregularly shaped scapular alae occurred for the low, middle and high dosage groups.

There were no statistically significant or biologically remarkable differences in average ossification sites among the groups.

Administration of the test substance to the does at dosages as high as 300 mg/kg/day did not affect the number of litters with fetuses with alterations, the number of fetuses with any alterations or the percentage of fetuses per litter with any variation. There were no dosage-dependent or significant differences among the four groups."

In examining the data, the Toxicology Branch agrees with the authors' statement that the incidences of small gallbladders were not biologically significant. The incidences were 0 (0), 0 (0), 4 (2) and 4 (1) for the control, low dose, mid-dose and high dose groups, respectively, [fetal (litter) incidences] for this effect. The historical control range is 0-1 for both litters (0-8.3%) and fetuses (0-1.2%); 2/32 studies had the alteration; 2/454 litters (0.44%) and 2/3320 fetuses (0.06%). The litter incidences are generally within the historical control range. In addition, there was no dose-response with the litter incidences. Although the incidences were statistically significant with fetal incidences, it is not likely that this is a treatment-related response.

Table IV: External Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined[b]	120 (18)	111 (17)	120 (17)	89 (13)
Cleft palate, medial	1 (1)[a]	0 (0)	0 (0)	0 (0)
Umbilical hernia	1 (1)	0 (0)	0 (0)	0 (0)

([+]) some observations may be grouped together
([a]) fetal [litter] incidence
([b]) All values for dead conceptuses were excluded from statistical analyses.

Table V: Visceral Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined[b]	120 (18)	111 (17)	120 (17)	89 (13)
Aneurysm in ascending aorta	1 (1)[a]	0 (0)	0 (0)	0 (0)
Persistent truncus arteriosis	0 (0)	0 (0)	0 (0)	1 (1)
Agenesis of intermediate lobe of lung	2 (2)	3 (3)	3 (2)	4 (4)
Nodule attached at junction of liver and bile duct	0 (0)	1 (1)	0 (0)	0 (0)
Small gall bladder	0 (0)	0 (0)	4[c](2)	4[c](1)
Round gallbladder	0 (0)	1 (1)	0 (0)	0 (0)

([+]) some observations may be grouped together
([a]) fetal [litter] incidence
([b]) All values for dead conceptuses were excluded from statistical analyses.
([c]) Significantly different from vehicle control group ($p<=0.01$)

Skeletal Examinations - table summaries of the skeletal examination results are on the following tables xeroxed directly from the report.

C. DISCUSSION:

1. Maternal Toxicity: One animal died in the high dose group. The toxic effects seen in the dams were those seen generally with synthetic pyrethroids: excess salivation, urine-staining of the abdominal fur, tremors, body jerks and hypersensitivity to sound. These clinical signs were only seen in the high dose group. Chromorrhinorrhea was also observed in one dam in the high dose group. No other clinical signs of toxicity, including body weight changes, decreases in food consumption or abortions were observed in any of the treated animals.

2. Developmental Toxicity: There were no indications of developmental toxicity of any kind in any of the treated groups.

   a. Deaths/Resorptions: There were no fetal deaths. The total number of resorptions for each dose group were as follows: 24, 23, 26 and 17 for the control (24 litters), low dose (25 litters), mid-dose (24 litters) and high dose (24 litters) groups.

   b. Altered Growth: There was no indication of altered growth in any of the treated groups.

   c. Developmental Anomalies: There were no treatment-related developmental anomalies in any of the treated groups.

   d. Malformations: There were no treatment-related malformations in any of the treated groups.

D. Study Deficiencies: This study meets all the requirements for an adequate study.

E. Core Classification: Core Guideline Data.

Maternal NOEL = 25 mg/kg/day
Maternal LOEL = 125 mg/kg/day
Developmental Toxicity NOEL = 125 mg/kg/day (HDT)
Developmental Toxicity LOEL = > 125 mg/kg/day

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C. DISCUSSION:

1. Maternal Toxicity: The NOEL for maternal toxicity is 100 mg/kg/day and the the LOEL is 300 mg/kg/day (HDT) based on deaths and clinical signs of toxicity (tremors, decreased motor activity and ataxia). One of the high dose does also displayed impaired righting reflex and another displayed excess salivation.

2. Developmental Toxicity:

   a. Deaths/Resorptions: There were no treatment-related increases in either deaths or resorptions in any of the treated groups when compared to controls.

   b. Altered Growth: There were no treatment-related increases in altered growth in any of the treated groups when compared to controls.

   c. Developmental Anomalies: There were no treatment-related increases in developmental anomalities in any of the treated groups when compared to controls.

   d. Malformations: There were no treatment-related increases in malformations in any of the treated groups when compared to controls.

D. Study Deficiencies: There are no major study deficiencies.

E. Core Classification: Core Guideline Data.

Maternal NOEL = 100 mg/kg/day
Maternal LOEL = 300 mg/kg/day
Developmental Toxicity NOEL = 300 mg/kg/day (HDT)

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Section I, Tox. Branch (H7509C)
Secondary Reviewer: Roger L. Gardner, Head Roger L. Gardner
Section I, Tox. Branch (H7509C) 7-16-91

DATA EVALUATION REPORT

STUDY TYPE: Teratology - Developmental Toxicity (83-3)

SPECIES: Rat

TOX. CHEM. NO./CASWELL NO.: 025

ACCESSION NUMBER/MRID NO.: 416322-01

TEST MATERIAL: Esbiothrin

SYNONYMS: Member of allethrin family

STUDY NUMBER(S): Argus 718-001

SPONSOR: Roussel Bio Corporation, Lincoln Park, New Jersey

TESTING FACILITY: Argus Research Laboratories, Inc., Horsham PA

TITLE OF REPORT: Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of Esbiothrin Technical Administered Orally via Gavage to Crl:CD^{R}BR VAF/Plus Presumed Pregnant Rats

AUTHOR(S): E. Lochry

REPORT ISSUED: 8/31/90

CONCLUSION: Esbiothrin was tested in a developmental toxicity study in rats at the following dose levels: 0, 5, 25, and 125 mg/kg/day by gavage. The maternal NOEL is 25 mg/kg/day and the maternal LOEL is 125 mg/kg/day (death, clinical signs of toxicity). The developmental toxicity NOEL is 125 mg/kg/day (HDT).

Classification: Core Guideline

Testing Guideline Satisfied: 83-3

A. MATERIALS AND METHODS:

1. Test Compound

Chemical Name: d-trans chrysanthemic acid of d-allethrolone (72%) and d-trans chrysanthemic acid of l-allethrolone (21%)

Description: Yellow, viscous liquid
Batch #(s), Other #(s): Lot 9N0317B3
Purity: 95.2%
Source: Roussel
Vehicle (if applicable): Mazola corn oil

2. Test Animals)

Species and Strain (sexes): Male and female Crl:CD^{R}BR VAF/Plus rats
Age: 72 days (F), 64 days (M) at receipt; were cohabited one week later.
Weight(s): 193-239 g (F on day after arrival); 224-306 g (M on day after arrival); 388-778 g (M at initiation of cohabitation period).
Source(s): Charles River Laboratories (Raleigh, NC - Females; Portage, MI - Males)

3. Study Design:

This study was designed to assess the developmental toxicity potential of esbiothrin when administered by gavage to rats on gestation days 6 through 15, inclusive.

a. Mating:

Natural or artificial insemination? Natural
Describe technique used: Female rats were placed in cohabitation with male rats (1:1). When a copulatory plug was observed in any female, she was considered to be at day 0 of presumed gestation and assigned to individual housing.

b. Group Arrangement:

Test Group	Dose Level (mg/kg/day)	Number Assigned
Control	0	25
Low Dose	5	25
Mid Dose	25	25
High Dose	125	25

c. Dosing:

All doses were in a volume of 5 ml/kg of body weight/day. Dosing was based on daily gestation day body weight.

1) Basis For Selection of Dose Levels: Selected on the basis of a range-finding study in which test substance-related maternal deaths occurred in 2/8 rats given 200 mg/kg/day and in all of the 8 rats which were given a dosage of 400 mg/kg/day.

2) Preparation: Prepared as a solution in corn oil.

3) Frequency of Preparation: Daily.

4) Storage Conditions: Test substance and vehicle were stored at room temperature.

5) Stability Analyses: Not done.

6) Homogeneity Analyses: Not done.

7) Concentration Analyses: Triplicate samples from each concentration (0, 1, 5, and 25 mg/ml) were taken on the first and last days of the dosing period. These were analyzed.

d. Maternal Examinations:

1) Clinical Observations and Mortality: The dams were examined for clinical signs and/or general appearance several times during the acclimation period and on day 0 of gestation. They were observed several times per day for clinical signs of toxicity, abortions, premature deliveries and mortality during the dosing period and once daily during the postdosage period. In addition, they were observed for viability at least twice per day throughout the entire study.

2) Body Weight Determinations: Body weights were recorded at least once weekly prior to mating. They were then recorded on day 0 and on days 6 through 20 of presumed gestation. thereafter.

3) Food Consumption: Food consumption was recorded on day 0 and on days 6 through 20 of presumed gestation.

4) Gross Necropsy: The abdomen of each rat was opened and the intact uterus was excised and weighed. Uteri from dams that appeared nonpregnant were pressed between two glass plates to confirm pregnancy status. The thoracic and abdominal cavities were examined for gross lesions.

Animals which died or were sacrificed in moribund condition prior to end of exposure period and were subjected to complete gross pathological examinations: All animals.

Animals sacrificed at the end of the treatment/observation period which were subjected to complete gross pathological examinations: All animals.

5) Uterine Examinations: The following parameters were examined:

Number of corpora lutea
Number of live fetuses
Number of dead fetuses
Early and late resorptions
Total implantations and placement
Individual fetal weights

e. Fetal Examinations:

The fetuses were examined in the following manner: Each fetus was removed, weighed, sexed and the uterine placement was noted. In addition, each one was examined for gross external alterations. Live fetuses were sacrificed. Approximately 1/2 of each litter were fixed in Bouin's solution and examined for soft tissue alterations by using a variation of Wilson's sectioning technique. The remaining fetuses were eviscerated, cleared, stained with alizarin red S and examined for skeletal alterations. Late resorptions were examined to the extent possible.

f. Historical Control Data:

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

g. Statistical analysis:

The following statistical analysis methods were employed (quoted directly from the document): "Maternal and fetal incidence data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution.

Maternal body weight, body weight change, gravid uterine weight and feed consumption data, and litter averages for percent male fetuses, fetal ossification sites and percent fetal alterations were analyzed using Bartlett's Test of Homogeneity of Variances and the Analysis of Variance, when appropriate [i.e., when Bartlett's Test was not significant ($p>0.05$)]. If the Analysis of Variance was significant ($P<=0.05$), Dunnett's Test was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate [i.e., when Bartlett's Test was significant ($p<=0.05$)], the Kruskal-Wallis Test was used, when less than or equal to 75% ties were present; when more than 75% ties were present, the Fisher's Exact Test was used. In cases in which the Kruskal-Wallis Test was statistically significant ($p<=0.05$), Dunn's Method of Multiple Comparisons was used to identify the statistical significance of the individual groups. Data for sternal centers were also analyzed using the Analysis of Covariance with fetal body weight as the covariate.

All other Cesarian-sectioning data were evaluated using the procedures previously described for the Kruskal-Wallis Test."

h. Compliance:

A signed Statement of Confidentiality Claim was provided.

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

B. RESULTS:

1. Dosage Preparation: The concentration analyses gave the following mean ranges (2 replicates per test per sample) of concentrations for each dose level tested: 5 mg/ml: -10% to 18%; 10 mg/ml: 7% to 15%; 20 mg/ml: 3% to 5%; 25 mg/ml: 4% to 38%; 40 mg/ml: 8% to 10%; 80 mg/ml: 11% to 13% from the target level concentration.

It should be noted that the concentrations listed here are not the same as those listed in the main report as having been analyzed.

2. Maternal Toxicity:

a. Clinical Observations and Mortality: One rat died at 125 mg/kg/day (HDT, day 10 of gestation). The following clinical signs of toxicity were observed in the high dose group: excess salivation, urine-staining of the abdominal fur, tremors, body jerks and hypersensitivity to sound. The report stated that these observations generally occurred for approximately 4 hours after intubation on days 10 through 15 of gestation and did not persist overnight. Chromorrhinorrhea was also observed in one dam. No treatment-related clinical signs toxicity were observed in any of the other groups.

b. Body Weight Determinations: No treatment-related effects in either body weights or body weight gains were observed in any of the treated groups at any time during the study.

The investigators supplied the following data:

Table I: Body Weight Gains (grams)[a]

Group:	Prior to Dosing Period	Dosing Period	Post Dosing Period	Entire Gestation Period	Corrected Body Weight Gains Dosing P.[1]	Entire[2]
Control	35.3	67.0	72.1	174.4	Not given	86.3
LDT	36.4	67.2	71.5	175.1	Not given	85.6
MDT	35.2	69.8	71.3	176.3	Not given	90.4
HDT	35.0	69.4[3]	71.2[3]	175.8	Not given	89.3[3]

[1] = corrected body weight gain for dosing period = body weight gain for dosing period minus gravid uterus weight.
[2] = corrected body weight gain for entire gestation period = body weight gain for entire gestation period minus gravid uterus weight.
[3] = Excludes value for 1 dam which died on day 10 of gestation.
a = Data extracted from (Argus 718-001 table 4)

c. Food Consumption: No treatment-related differences in food consumption were observed in any group.

The investigators supplied the following data:

Table II: Food Consumption Data (g/kg/day)[a]

Group:	Prior to Dosing Period	Dosing Period	Post-Dosing Period	Entire Gestation Period
Control	21.9	22.6	29.8	23.8
LDT	22.4	22.4	29.1	23.8
MDT	22.2	22.8	29.4	24.0
HDT	22.0	22.5[1]	30.1[1]	23.9[1]

[a] = Data extracted from (Argus 718-001 table 5)
[1] = Excludes values for 1 dam which died on day 10 of gestation.

d. Gross Pathology: The vagina and right uterine horn of one low dose group rat was moderately distended with clear, thin, yellow fluid that contained white particulate material. No other gross lesions were found in the dams.

e. Cesarean section Observations: Pregnancy incidences; the average number of corpora lutea, implantations, resorptions and fetuses per litter; the number of dams with viable fetuses; and the litter averages for fetal sex ratios, body weights and percent resorbed conceptuses were comparable with the control group for all treated groups. The following table summarizes the cesarian section observations.

Table III: Cesarean Section observations[a]

	Control	LDT	MDT	HDT
Dose (mg/kg/day)	0	5	25	125
#Animals Assigned	25	25	25	25
#Animals Mated/Inseminated	25	25	25	25
Pregnancy Rate (%)	24 (96)	25 (100)	24 (96)	25 (100)
Maternal Wastage				
#Died	0	0	0	1
#Died/pregnant	0	0	0	1
#Non pregnant	1	0	1	0
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea	422	449	435	414
Corpora Lutea/dam	17.6	18.0	18.1	17.2
Total Implantation	393	417	395	378
Implantations/Dam	16.4	16.7	16.4	15.8
Total Live Fetuses	369	394	369	361
Live Fetuses/Dam	15.4	15.8	15.4	15.0
Total Resorptions	24	23	26	17
Early	24	22	25	16
Late	0	1	1	1
Resorptions/Dam	1.0	0.9	1.1	0.7
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (gm)	3.54	3.44	3.49	3.52
Preimplantation Loss(%)	29 (7)	32 (7)	40 (9)	36 (9)
Postimplantation Loss(%)	24 (6)	23 (6)	26 (7)	17 (4)
Sex Ratio (% Male)	50.7	49.7	48.4	52.8

[a] = Data extracted from (Argus 718-001 table 7)

3. Developmental Toxicity: No treatment-related gross external, soft tissue or skeletal alterations were observed in any dose group. The following tables summarize the results.

Table IV: External Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	369 (24)	394 (25)	369 (24)	361 (24)
#pups(litters) affected	0 (0)	0 (0)	0 (0)	3 (3)
Spina Bifida (thoracic-lumbar area	0 (0)[a]	0 (0)	0 (0)	1 (1)
Thread-like tail	0 (0)	0 (0)	0 (0)	2 (2)

(+) some observations may be grouped together
(a) fetal [litter] incidence

Table V: Visceral Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
#pups(litters) examined	180 (24)	191 (25)	180 (24)	175 (24)[1]
#pups(litters) affected	0 (0)	1 (1)	1 (1)	0 (0)
Pelvis of kidney, moderate dilation	0 (0)[a]	1 (1)	0 (0)	0 (0)
Close-set kidneys	0 (0)	0 (0)	1 (1)	0 (0)
Moderate dilation of ureters	0 (0)	0 (0)	1 (1)	0 (0)

(+) some observations may be grouped together
(a) fetal [litter] incidence
(1) Includes 14 fetuses from 2 high dose group litters that were inadvertently eviscerated after gross external evaluation. No soft tissue abnormalities were identified at evisceration. The heads (palate, tongue, eyes and brain) and spinal cord (anterior to forelimbs) of these fetuses were sectioned and evaluated for alterations.

Skeletal Examinations: These are summarized in the following tables copied directly from the report.

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is 25 mg/kg/day and the maternal LOEL is 125 mg/kg/day (death, clinical signs of toxicity). The developmental toxicity NOEL is 125 mg/kg/day (HDT).

Both studies were classified as Core Guideline.