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ACEPHATE TECHNICAL **RAT TERATOLOGY (OPPTS §870.3700; OPP §83-3A)**

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DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat Guideline: 83-3 a

EPA ID No.s: EPA MRID No. 41081602
EPA Pesticide Chemical Code 103301
Toxicology Chemical Code 002A

Test Material: ORTHENE™ INSECTICIDE

Synonyms: Chevron Acephate Technical

Citation: E.A. Lochry (1989). ORAL TERATOGENICITY AND DEVELOPMENTAL TOXICITY WITH CHEVRON ACEPHATE TECHNICAL, Argus Research Laboratories, Inc., for Chevron Chemical Company, Ortho Agricultural Chemical Division, Laboratory Project Identification S-3200, Study No. 303-008, CEHC Reference NO. 87-245, February 13, 1989 (Unpublished); EPA MRID No. 41081602.

Executive Summary: In a developmental (teratology) study (MRID# 41081602), virgin female rats (Strain: Crl:CD®(SD)BR from Source: Charles River Breeding Laboratories, Inc., Raleigh, North Carolina) received either 0, 5, 20, or 75 mg/kg/day Acephate Technical (Purity: 99.7% a.i.; Lot No.: SX-1725) in reverse osmosis membrane processed deionized water (R.O. Deionized water) by oral gavage from gestation days 6 through 15.

The maternal animals of the high dose group presented with statistically significantly ($p \leq 0.01$) increased incidence of rats with tremors and decreased motor activity. The mid and high dose had reduced body weights and body weight gains during the dosing period (gestation days 6-16; 47.1-84.2% of control for body weight gains) the period including the post dosing period (gestation days 6-20; 80.4-90.3% for body weight gains and when corrected for gravid uterine weights; 37.3-71.0%) and the entire gestation period (gestation days 0-20; 86.3-92.0% for body weight gains and when corrected for gravid uterine weights; 71.2-84.2%). There was a rebound in body weights in the high dose group during the period following dosing (gestation days 16-20) and a decrease in body weight gain during the same period when corrected for gravid uterine weight. There was also reduced food consumption and food efficiency in the mid and high dose groups during the dosing period (73.1-92.4%; statistically significant reduced food consumption for both doses, statistics were not performed on food

efficiency), for the dosing plus post dosing period (81.4-93.7%; statistically significant reduced food consumption for both doses) and for the entire gestation period (87.3-95.9%; statistically significant reduced food consumption for the high dose only). **The Maternal Toxicity NOEL was 5 mg/kg/day and the Maternal Toxicity LOEL was 20 mg/kg/day based reduced body weights and body weight gain, reduced food consumption and reduced food efficiency.**

Developmental Toxicity was noted in the high dose group as slight decreases in the mean number of ossified caudal vertebrae, sternal centers, metacarpals and the fore- and hindlimb phalanges with the hindlimb phalanges statistically significantly reduced. **The Developmental Toxicity NOEL was 20 mg/kg/day and the Developmental Toxicity LOEL was 75 mg/kg/day based on decreases in mean numbers of ossification centers per litter.**

This study is classified as Acceptable-Guideline and satisfies the guideline requirements (§83-3a) for a teratology study in rats.

Compliance: A signed and dated CONFIDENTIALITY STATEMENT (no claim of confidentiality was made), GOOD LABORATORY PRACTICE STATEMENT, FLAGGING statement (according to the investigators the study neither meed nor exceeds any of the applicable criteria), CERTIFICATE OF COMPLIANCE WITH EPA FIFRA AND TSCA GOOD LABORATORY PRACTICE STANDARDS and QUALITY ASSURANCE UNIT FINAL REPORT CERTIFICATE was provided.

THIS REVIEW CONTAINS TEXT INFORMATION SCANNED BY THE REVIEWER INTO ELECTRONIC FORMAT (USED IN MATERIALS AND METHODS, STUDY DESIGN AND CONCLUSIONS-INVESTIGATORS SUMMARY SECTIONS).

A. Materials and Methods

Test Compound: CHEVRON Acephate Technical
Purity: 99.7% a.i.
Specific Gravity: 1.35
Description: a white powder with a strong cabbage-like odor
Lot No.: SX-1725
Receipt Date: April 11, 1988
Other provided information: Stored at room temperature

Vehicle(s): Reverse osmosis membrane processed deionized water (R.O. Deionized water); was analyzed for possible bacterial and chemical contamination.

Test Animal(s): Species: Virgin female rats
Strain: Crl:CD®(SD)BR
Source: Charles River Breeding Laboratories, Inc., Raleigh, North Carolina
Age: Females: 71 days; Males: 73 days
Body Weight: Females: 179-233 g; Males: 223-327 g
Males: same strain and vender but from the Portage, MI. Facility.
Acclimation Period: 2 weeks

B. Study Design (from page 11 of the study report)

The purpose of this study was to evaluate CHEVRON Acephate Technical for any r embryo-fetal toxicity or teratogenic effect in Crl:CD®(SD)BR pregnant rats. The standards of the U.S. Environmental Protection Agency[1,2,3], the U.S. Food and Drug Administration[4,5], the Organization for Economic Cooperation and Development[6], and the Japanese Society of Agricultural Chemical Industry[7] were used as guidelines.

The test substance, CHEVRON Acephate Technical, was administered via gavage once daily to female rats on days 6 through 15 of presumed gestation.

Mating Procedure: (from pages 18-19 of the study report)

Following the acclimation period (two weeks) 150 virgin female rats that appeared to be in good health were placed in cohabitation with the male rats (one female rat was paired with one male rat; no male rat mated more than one female rat assigned to this study). Female rats with spermatozoa observed in a vaginal lavage or a copulatory plug observed in situ were considered to be at day 0 of presumed gestation and assigned to individual housing. The male rats were used only for the purpose of breeding the female rats were not administered the test substance, and therefore, were not considered to be part of the Test System.

Day 0 of presumed gestation occurred between June 21 and June 24, 1988 for the 100 female rats that were assigned to the study. These female rats appeared to be disease-free and weighed from 237 to 302 g each on day 0 of presumed gestation. The rats were assigned to the four dosage groups using a computer generated (weight-ordered) randomization procedure based on their body weights on day 0 of presumed gestation.

Animal Husbandry: (from pages 20-21 of the study report)

The female rats were housed individually in wire-bottomed stainless-steel cages suspended above absorbent paper. During cohabitation, male and female rats were placed together one female per male in wire-bottomed cages for a minimum of four days. Upon observation of spermatozoa in a vaginal lavage or a copulatory plug in situ the female rat was removed from cohabitation and individually housed. Because the dams were terminated prior to the expected day of natural delivery nesting materials were not supplied.

The female rats were housed in Room 2 (166 square feet of floorspace) throughout the study The study room was independently supplied with a minimum of 12 changes per hour of 100% fresh air that bad been passed through 99.97% HEPA filters. The temperature in the study room was maintained at 18°C to 26°C throughout the study. Humidity was monitored weekly throughout the acclimation period and daily for the remainder of the study (beginning the first day of the cohabitation period) and was documented to range from approximately 42% to 56% in the study room. No deviations in these environmental parameters occurred that adversely affected the results of the study. An automatically-controlled fluorescent light cycle was maintained at 12 hours light:12 hours dark with each dark cycle beginning at 1900 hours EST.

The rats were given Certified Rodent Chow® #5002 (Ralston Purina, meal form) that was available ad libitum throughout the study.

Analyses were routinely performed by the feed supplier. No contaminants in the feed or deviations from expected nutritional requirements were detected by these analyses.

Local water that had been deionized by passage through a reverse osmosis membrane (R.O. deionized water) was available to the rats ad libitum from a stainless steel automatic watering system. The processed water is analyzed annually for possible chemical contamination (Lancaster Laboratories. Inc, Lancaster, Pennsylvania) and monthly for possible bacterial contamination. (Purity-Standard Laboratories, Chalfont, Pennsylvania). Chlorine was added to the processed water as a bacteriostat. The monthly water samples contained from 0.7 to 1.4 ppm of chlorine at the time of analysis: the water was determined to be suitable for consumption.

Group Arrangement: (from page 19 of the study report)

Following completion of the four-day cohabitation period, a computer generated (weight-ordered) randomization procedure was used to assign 100 mated female rats that appeared to be in good health to one of the four dosage groups (25 rats/group). Each selected female rat was individually identified with a Monel® self-piercing ear tag (Gey Band and Tag Co., Inc . No. MSPT 20101) inscribed with the rat's designated unique permanent number.

Test Group	Dose Level (mg/kg)	Number Assigned
Control	Vehicle	25
Low Dose	5	25
Mid Dose	20	25
High Dose	75	25

From page 22 of the study report:

Dosages of CHEVRON Acephate Technical selected for use in this study were 0(Vehicle), 5, 20 and 75 mg/kg/day. These dosages were selected by the Sponsor on the basis of a dosage-range study (Argus Research Laboratories Inc Study Number 303-008P). [Not provided]

Dose Administration: (from page 11 and 15 of the study report)

The test substance, CHEVRON Acephate Technical, was administered via gavage once daily to female rats on days 6 through 15 of presumed gestation. Dosages of 0 (Vehicle), 5, 20 and 75 mg/kg/day were given to the rats (25/group) using reverse osmosis membrane processed deionized water (R.O. deionized water) as the vehicle. The dosages were prepared at concentrations of 0(Vehicle Control), 0.5, 2 and 7.5 mg/mL respectively. All dosages were given to the rats via gavage at a dosage volume of 10 mL/kg/day that was adjusted daily on the basis of body weight.

From page 23 of the study report:

Solutions of the formulated test substance (800 mL per concentration) in R.O. deionized water were prepared three times during the study.

Duplicate or triplicate 10 g samples of each concentration were reserved from each batch of CHEVRON Acephate Technical solutions prepared for use in the study. These duplicate or triplicate sets of 10 g samples were reserved on the day following preparation of each batch and on the last day that each batch was used. Both 10 g samples of the duplicate set and two 10 g samples of each triplicate set were sent to the Sponsor for concentration and stability verification. The third 10 g sample of each triplicate set was retained at the Test Facility. All samples were within acceptable limits; the results of these analyses are available in the Sponsor's records.

On July 1, 1988, three samples of approximately 10 g each were taken from the

top, middle and bottom of each concentration. Each of these samples were sent to the Sponsor for homogeneity determinations. Seven days later (on July 8, 1988), three samples of approximately 10 g each were taken from each concentration. Two 10 g samples of each triplicate set were sent to the Sponsor for stability determinations. The other 10 g sample was retained at the Test Facility.

Two 5 g samples of CHEVRON Acephate Technical (bulk test substance) were received during the study. The first sample was taken immediately after preparation of the first batch of test substance solutions; the second sample was taken immediately after preparation of the third batch of test substance solutions. These samples were sent to the Sponsor for possible analysis.

One 20 g sample of CHEVRON Acephate Technical (bulk test substance) was reserved on the first day that the test substance solutions were prepared for use in the study and was retained at the Test Facility.

CHEVRON Acephate Technical was administered orally via gavage to female rats on days 6 through 15 of presumed gestation Dosage volumes (10 mL/kg) were adjusted daily for changes in body weights.

Daily dosage of the rats occurred at approximately the same time each day (between 0950 and 1120 hours EST).

Observations: (from pages 23-25 of the study report)

The female rats were observed for clinical signs and/or general appearance several times during the acclimation period (adverse observations were recorded by exception) and on day 0 of presumed gestation. Viability in the rats was noted a minimum of twice daily throughout the study. Observations for clinical signs of test substance effect, abortion and/or viability were also made several times each day during the dosage period (days 6 through 15 of presumed gestation). These observations were made once daily during the postdosage period (days 16 through 20 of presumed gestation).

Body weights and feed consumption values for the rats were recorded at least once weekly prior to mating and on days 0, 3 and 6 through 20 of presumed gestation.

All rats were sacrificed by carbon dioxide asphyxiation on day 20 of presumed gestation. The abdomen of each rat was opened, and the intact uterus was extracted and weighed. Uteri which had no observable implantation sites were stained with ammonium sulfide to confirm pregnancy status[13]. The thoracic and abdominal cavities were examined for gross lesions. Gross lesions observed in ... dams were preserved in neutral buffered 10% formalin for possible ... evaluation. All other maternal tissues were discarded.

Corpora lutea in each ovary were counted. The number and placement of implantations, early and late resorptions and live and dead fetuses were

noted. An early resorption was defined as one in which organogenesis was not evident. A late resorption was defined as one in which the occurrence of organogenesis was evident. A live fetus was defined as a term fetus that responded to mechanical stimuli. Non-responding term fetuses were considered to be dead. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption.

Each fetus was removed from the uterus, placed in an individual container and individually identified with a tag. Each fetus was subsequently weighed and examined to identify sex and gross external alterations. Live fetuses were then sacrificed by carbon dioxide asphyxiation.

Approximately one-half of the fetuses in each litter were examined for soft tissue alterations using an adaptation of the microdissection technique of Staples[8]. Fetuses selected for visceral examination were decapitated, and the heads fixed in Bouin's solution. The decapitated body was examined by microdissection and the viscera were removed and discarded. The decapitated specimens were preserved in ethanol for possible evaluation for skeletal alterations. The fetal heads were sectioned with a razor blade and evaluated for soft tissue alterations. The remaining fetuses in each litter were eviscerated, cleared, stained with alizarin red S[9] and examined for skeletal alterations.

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

Statistical analysis: (from page 27 of the study report)

The investigators provided a "flow-chart" for the statistical analysis of the data (on page 26 of the study report) and the following statistical analysis methods were employed:

Maternal and fetal incidence data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution[14].

Maternal body weight, gravid uterine weight and feed consumption averages (g/day and g/kg/day) as well as litter averages for percent male fetuses, fetal body weight, fetal ossification sites and percent fetal alterations were analyzed using Bartlett's Test of Homogeneity of Variances[15] and the Analysis of Variance[16], when appropriate [i.e. when Bartlett's Test was not significant ($P>0.05$)]. If the analysis of Variance was significant ($P\leq0.05$) then Dunnett's Test[17] was used to identify the statistical significance of individual groups. If the Analysis of Variance was not appropriate [i.e., when Bartlett's Test was significant ($P\leq0.05$)], the Kruskal-Wallis Test[18] was used when less than or equal to 75% ties were present; when more than 75% ties were present, the Fisher's Exact Test[19] was used. In cases in which the Kruskal-Wallis Test was statistically significant ($P\leq0.05$), Dunn's Method of Multiple Comparisons[20] was used to identify the statistical significance

of individual groups.

The Analysis of Covariance[21] was used to evaluate average maternal body weight changes during days 0 to 6 of gestation and days 0 to 20[a] of gestation. This test was also used to evaluate changes in average maternal body weight during days 6 to 7, 9, 12, 16 and 20[a] of gestation. The methods previously described for Bartlett's Test[15] and the Analysis of Variance[16], were used to evaluate maternal body weight change data on days 7 to 8, 8 to 9, 9 to 12, and 12 to 16 of gestation (intervals which began during the dosage period), and for evaluation of maternal body weight change data during the postdosage period because there were significant ($P \leq 0.01$) differences among the groups for average maternal body weight on day 16 of gestation.

All other Caesarean-sectioning data were evaluated using the procedures previously described for the Kruskal-Wallis Test[18].

a. Includes statistical analyses of maternal body weight change intervals involving the corrected day 20 of gestation body weight values.

REFERENCES (from pages 41-43 of the study report)

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NOTE FROM THE REVIEWER: THE PROTOCOL DESCRIBED ABOVE IN THE MATERIALS AND METHODS SECTION IS ACCEPTABLE TO FULFILL THE GUIDELINE §83-3A.

C. Results

Maternal Toxicity:

Mortality

No animals were reported to have died during the study.

Clinical Observations

The high dose group presented with statistically significantly ($p \leq 0.01$) increased incidence of rats with tremors and decreased motor activity, see Table I below (from Table 12, pages 70-74 of the study report):

Table I: Clinical Signs of Toxicity

Dose:	Control	5	20	75
Tremors				
# animals days	0	0	0	191
# animals	0	0	0	25
Decreased motor activity				
# animals days	0	0	0	6
# animals	0	0	0	4

Body Weight

The investigators supplied group summary and individual animal data. The following tables present mean body weights and body weight gains (from Table 3, pages 51-54 of the study report), tables exclude 2 control litters, one with 2 fetuses and 1 with a fetus and an early resorption:

Table II: Body Weights (grams)

Day:	0	6	15	20	20C[1]
Dose (mg/kg/day):					
Control	264.5±11.2	304.6±17.1	373.7±22.5	435.4±26.2	345.6±19.4
5	265.3±9.0	304.6±12.6	371.8±15.2	438.6±20.0	343.6±15.4
20	265.8±9.5	304.9±11.4	363.0±16.2	423.0±19.6	334.0±17.6*
75	266.6±11.6	309.0±17.2	341.5±19.5**	414.1±20.6**	324.3±18.0**

[1] = corrected for gravid uterine weight; * = $p \leq 0.05$; ** $p \leq 0.01$.

Table III: Body Weight Gains (grams)

Days:	0-6	6-16	16-20	6-20	0-20
Dose (mg/kg/day):					
Control	40.1±12.1	69.0±9.4	61.7±6.9	130.8±14.1	170.9±22.8
5	39.4±7.7	67.1±10.0 (97.2)[1]	66.8±9.6 (108.3)	133.9±15.9 (102.4)	173.3±19.1 (101.4)
20	39.1±8.5	58.1±11.1** (84.2)	60.0±9.0 (97.2)	118.1±13.8** (90.3)	157.2±17.7* (92.0)
75	42.4±10.7	32.5±11.6** (47.1)	72.6±10.7** (117.7)	105.2±13.9** (80.4)	147.5±17.2** (86.3)
CORRECTED FOR GRAVID UTERINE WEIGHT					
Control			-28.1±8.7	41.0±8.9	81.1±15.5
5			-28.2±8.3	38.9±8.4 (94.9)[1]	78.3±12.5 (96.6)
20			-28.9±7.6	29.1±11.5** (71.0)	68.3±15.7** (84.2)
75			-17.2±9.3** (61.2)	15.3±10.6** (37.3)	57.7±14.0** (71.2)

[1] = percent of control; * = $p \leq 0.05$; ** = $p \leq 0.01$.

The mid and high dose had reduced body weights and body weight gains during the dosing period (gestation days 6-16) the period including the post dosing period (gestation days 6-20 and when corrected for gravid uterine weights) and the entire gestation period (gestation days 0-20 and when corrected for gravid uterine weights). There was a rebound in body weights in the high dose group during the period following dosing (gestation days 16-20) and a decrease in body weight gain during the same period when corrected for gravid uterine weight.

Food Consumption

The investigators supplied group summary and individual animal data. The following table presents food consumption data for periods similar to body weight gains above in grams/day (from Table 4, pages 55-58 of the study report), tables exclude 2 control litters, one with 2 fetuses and 1 with a fetus and an early resorption:

Table IV: Food Consumption Data (grams/day)

Days:	0-6	6-16	16-20	6-20	0-20
Dose (mg/kg/day):					
Control	22.6±2.3	24.9±2.3	26.1±1.8	25.2±2.0	24.4±2.0
5	22.4±1.8	24.6±1.7 (98.8)	26.3±1.6 (100.7)	25.1±1.6 (99.6)	24.3±1.5 (99.6)
20	22.6±2.1	23.0±2.4** (92.4)	25.3±2.2 (96.9)	23.6±2.2** (93.7)	23.4±2.0 (95.9)
75	23.0±2.5	18.2±2.2** (73.1)	26.5±2.2 (101.5)	20.5±1.6** (81.4)	21.3±1.6** (87.3)

* = $p \leq 0.05$; ** $p \leq 0.01$.

Table V: Food Efficiency Data (%)

Days:	0-6	6-16	16-20	6-20	0-20
Dose (mg/kg/day):					
Control	29.6	27.7	47.3	37.1	35.0
5	29.3	27.3	50.8	38.1	35.7
20	28.8	25.3	47.4	35.7	33.6
75	30.7	17.9	54.8	36.7	34.6

There was reduced food consumption and food efficiency in the mid and high dose groups during the dosing period (statistically significant reduced food consumption for both doses, statistics were not performed on food efficiency), for the dosing plus post dosing period (statistically significant reduced food consumption for both doses) and for the entire gestation period (statistically significant reduced food consumption for the high dose only).

Gross Pathological Observations

The investigators observed urinary tract lesions at post mortem. There was moderate dilation of the renal pelvis in the left kidney and marked dilation of the renal pelvis of the right kidney in one high dose animal and another high dose animal had two white stones in the urinary bladder. These observations are not considered to be related to treatment and are within historical control data for this strain of animals (provided on page 29 of the study report).

Cesarean Section Observations

The investigators supplied group summary and individual animal data. The following table presents the cesarean section observations (from Tables 5 and 15, pages 59-62 and 91-94 of the study report):

Table VI: Cesarean Section Observations

Dose:	Control	5	20	75
#Animals Assigned	25	25	25	25
#Animals Mated/Inseminated	25	25	25	25
#Animals Pregnant	24	23	24	24
Pregnancy Rate (%)	96	92	96	96
Maternal Wastage				
#Died	0	0	0	0
#Died/pregnant	0	0	0	0
#Non pregnant	1	2	1	1
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
#excluded[1]	2	0	0	0
Total Litters Examined	21	23	24	24
Total Corpora Lutea[2]	425(466)[3]	455	484	459
Corpora Lutea/dam	20.2±2.1 (19.4±3.2)[3]	20.7±2.3[4]	20.2±1.8	20.0±1.9[4]
Total Implantations[2]	361(367)[3]	410	406	416
Implantations/Dam	17.2±2.0 (15.3±5.4)[3]	17.8±1.9	16.9±2.1	16.5±2.4
Total Live Fetuses	346(351)[3]	394	386	397
Live Fetuses/Dam	16.5±2.3 (14.6±5.4)[3]	17.1±2.4	16.1±2.3	16.5±2.4
Total Resorptions	15(16)[3]	16	20	19
Early	15(16)[3]	16	19	18
Late	0	0	1	1
Resorptions/Dam	4.4±5.6 (5.9±10.8)[3]	4.2±6.3	4.9±5.9	4.6±5.1
Total Dead Fetuses	0	0	0	0
Mean Fetal Weight (gm)	3.45±0.18 (3.62±0.58)[3]	3.52±0.23	3.50±0.15	3.38±0.25
Preimplantation Loss(%)[2]	15.1	9.9	16.1	9.4
Postimplantation Loss(%)[2]	4.2	3.9	4.9	4.6
Sex Ratio (% Male)	47.4±12.8 (43.5±18.0)[3]	47.6±14.8	54.1±15.3	51.5±10.8

[1] = excludes 3 control litters, 1 with 2 fetuses, 1 with 2 fetuses and 1 with 1 fetus and an early resorption; [2] = calculated by the reviewer; [3] = included excluded litters; [4] = excluded with an incorrect corpora lutea count.

No treatment related effects were noted in the above data.

2. Developmental Toxicity

External Examinations

The investigators provided group summary and individual animal data. The following table presented the external observation data (from Table 8, page 64 of the study report):

Table VII: External Examinations

Dose (mg/kg/day)	Control	5	20	75
#pups/litters examined	351/24	394/23	386/24	397/24
Observations				
Meningocele	0/0[1]	0/0	0/0	1/1[2]
Eyes:				
Bulges, depressed, bilateral	0/0	0/0	0/0	1/1[2]
Nose:				
Cleft	0/0	0/0	0/0	1/1[2]
Body:				
Edematous	0/0	0/0	0/0	1/1[2]
Umbilical hernia	0/0	0/0	0/0	1/1[2]
Tail:				
Constricted at tip	0/0	0/0	1/1	0/0

[1] = fetal incidence/litter incidence; [2] = observations in same fetus.

No treatment related effects were noted in the above data.

Visceral Examinations

The investigators provided group summary and individual animal data. No soft tissue alterations were noted.

Table VIII: Visceral Examinations

Dose (mg/kg/day)	Control	5	20	75
#pups/litters examined	168/23	193/23	187/24	191/24
#pups/litters affected	0/0	0/0	0/0	0/0

No treatment related effects were noted in the above data.

Skeletal Examinations

The investigators provided group summary and individual animal data. The following table presented the external observation data (from Table 8, page 64 of the study report):

Table IX: Skeletal Examinations

Dose (mg/kg/day)	Control	5	20	75
#pups/litters examined	168/23	193/23	187/24	191/24
Observations				
Skull:				
Multiple skull bones altered	0/0	0/0	0/0	1/1[2]
Vertebrae:				
Cervical:				
arches present	0/0	0/0	0/0	1/1[2]
cervical rib	1/1	0/0	2/5	2/2
Thoracic:				
centrum, bifid	0/0	2/2	2/2	1/1
centrum, not ossif.	0/0	0/0	0/0	1/1[2]
Rib(s):				
1, split & 1 short	0/0	1/1[3]	0/0	0/0
Manubrium:				
incompletely ossif.	0/0	0/0	2/2[4]	0/0
Sternebrae:				
one or more, incompletely or not ossified	3/3	2/2[3]	3/3[4]	0/0
Pelvis:				
Ischium and/or pubes, incompletely or not ossified	1/1	2/3	2/2[4]	3/5
Ischium:				
incompletely ossif.	0/0	0/0	2/2[4]	0/0
not ossified	0/0	0/0	0/0	1/1[2]
Pubes:				
incompletely ossif.	0/0	2/3	1/1[4]	2/4
not ossified	1/1	0/0	0/0	0/0
Scapulae:				
Irregularly shaped	0/0	0/0	0/0	1/1[2]

[1] = fetal incidence/litter incidence; [2], [3], [4] = observations in a single fetus.

No treatment related effects were noted in the above data.

Fetal Ossification Sites

The investigators provided group summary data only, no individual animal data. The following table presents the fetal ossification site data (from Table 11, page 69 of the study report):

Table X: Fetal Ossification Sites

Dose (mg/kg/day)	Control	5	20	75
#pups/litters examined	182/24	201/23	199/24	204/24
Ossification sites/litter				
Hyoid:	0.81±0.22	0.81±0.25	0.76±0.24	0.89±0.17
Vertebrae:				
Cervical	7.00±0.00	7.00±0.00	7.00±0.00	7.00±0.00
Thoracic	13.04±0.06	13.05±0.07	13.03±0.05	13.02±0.07
Lumbar	5.96±0.07	5.95±0.07	5.96±0.07	5.98±0.07
Sacral	3.00±0.00	3.00±0.00	3.00±0.00	2.99±0.06
Caudal	4.84±0.77	4.88±0.40	4.84±0.33	4.60±0.40
Ribs:	13.03±0.06	13.02±0.04	13.02±0.05	13.02±0.05
Sternebrae:				
Manubrium	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
Sternal	3.72±0.34	3.74±0.27	3.72±0.22	3.62±0.28
Xiphoid	1.00±0.02	0.99±0.04	1.00±0.00	0.98±0.05
Forepaws:				
Carpals	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Metacarpals	3.57±0.31	3.58±0.31	3.49±0.37	3.35±0.31
Phalanges	5.14±0.90	5.09±0.40	4.93±0.36	4.75±0.58
Hindpaws:				
Tarsals	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Metatarsals	4.04±0.21	4.00±0.00	4.00±0.00	3.98±0.09
Phalanges	4.74±0.65	4.66±0.69	4.63±0.54	4.12±1.10*

* = P < 0.05.

The high dose group had slight decreases in the mean number of ossified caudal vertebrae, sternal centers, metacarpals and the fore- and hindlimb phalanges with the hindlimb phalanges statistically significantly reduced.

D. Discussion/Conclusions

i. Investigators Conclusions: (from pages 12-14 of the study report)

No female rat died during the study.

Administration of the 75 mg/kg/day dosage of CHEVRON Acephate Technical, as compared with the vehicle, significantly increased ($P \leq 0.01$) the number of rats with tremors and decreased motor activity. No other clinical observation was noted during this study.

Administration of the 20 and 75 mg/kg/day dosages of the test article to the dams, as compared with the vehicle, resulted in significant inhibitory effects ($P \leq 0.05$ to $P \leq 0.01$) on average maternal body weight gain and feed consumption relative to body weight values during the dosage period. A significant decrease ($P \leq 0.01$) in average maternal body weight gain occurred for the 5 mg/kg/day dosage group between days 6 and 9 of gestation, the first three days the test substance was given. This observation was followed by a significant increase ($P \leq 0.05$) in average maternal body weight gain between days 9 and 12 of gestation, the three days of dosage. Between days 12 and 16 of gestation, the last four days of dosage, the average body weight gain for the 5 mg/kg/day dosage group dams was essentially identical to the control group value. These transient changes in maternal body weight gain at the 5 mg/kg/day dosage were interrelated with minimal (not significant; $P > 0.05$) transient decreases in average maternal feed consumption values. All of which were probable pharmacotoxic responses of the pregnant animal to administration of the test substance. These observations were not sufficiently persistent or severe to preclude this dosage from identification as a No-Observable-Adverse-Effect Level (NOAEL).

During the postdosage period (days 16 to 20 of gestation), average maternal body weight gain and feed consumption values relative to body weight values (g/kg/day) were significantly increased ($P \leq 0.01$) for the 75 mg/kg/day dosage, as compared with the control values, a rebound phenomenon.

Pregnancy incidences (23 or 24 pregnant rats per group) were comparable in all dosage groups. No Caesarean-delivery parameter was affected by administration of the test substance to the dams. The average numbers of corpora lutea, implantations, resorptions, and fetuses per litter were equivalent for the four dosage groups. Similarly, the incidences of dams with viable fetuses did not differ among the groups. Differences in the incidences of these parameters were neither biologically remarkable nor significant ($P > 0.05$).

Administration of the maternally toxic 75 mg/kg/day dosage of CHEVRON Acephate Technical to the dams, as compared with the vehicle. decreased average fetal body weight per litter (not significant; $P > 0.05$). This decrease was significant ($P < 0.05$) for the high dosage group female fetuses, as compared with the control group value. Fetal body weights for the 0(Vehicle), 5 and 20 mg/kg/day dosage group litters were comparable and not significantly ($P > 0.05$) different.

Administration of the maternally toxic 75 mg/kg/day dosage of CHEVRON Acephate Technical to the dams, as compared with the vehicle, resulted, in small reversible delays in skeletal ossification, expected observations in fetuses s with decreased body weights. As compared with the control group values, high dosage group litters had slight decreases in the average numbers of ossified caudal vertebrae, sternal centers, metacarpals and fore- and hindpaw phalanges. Of these decreases, only the average number of hindpaw phalanges was significantly decreased ($P \leq 0.05$), as compared with the control group value, and all average values for fetal ossification sites were within the ranges observed historically.

No malformation or other variation revealed by gross external, soft tissue or skeletal examination of the fetuses was attributed to the test substance. The fetal and litter incidences of all other observed alterations were not dosage-dependent, not significantly ($P>0.05$) increased from control values and/or are common fetal alterations in this strain of rat.

On the basis of these data, the maternal no-observable adverse effect level (NOAEL) was 5 mg/kg/day, and the developmental NOEL for CHEVRON Acephate Technical was 20 mg/kg/day. Adverse effects of CHEVRON Acephate Technical on embryo-fetal development are expected to occur only at dosages that are also toxic to the dams.

ii. Reviewers' Conclusions

a. Maternal Toxicity:

The high dose group presented with statistically significantly ($p \leq 0.01$) increased incidence of rats with tremors and decreased motor activity. The mid and high dose had reduced body weights and body weight gains during the dosing period (gestation days 6-16) the period including the post dosing period (gestation days 6-20 and when corrected for gravid uterine weights) and the entire gestation period (gestation days 0-20 and when corrected for gravid uterine weights). There was a rebound in body weights in the high dose group during the period following dosing (gestation days 16-20) and a decrease in body weight gain during the same period when corrected for gravid uterine weight. There was also reduced food consumption and food efficiency in the mid and high dose groups during the dosing period (statistically significant reduced food consumption for both doses, statistics were not performed on food efficiency), for the dosing plus post dosing period (statistically significant reduced food consumption for both doses) and for the entire gestation period (statistically significant reduced food consumption for the high dose only).

b. Developmental Toxicity:

i. Deaths/Resorptions:

No treatment related effects were noted.

ii. Altered Growth:

No treatment related effects were noted.

iii. Developmental Anomalies:

The high dose group had slight decreases in the mean number of ossified caudal vertebrae, sternal centers, metacarpals and the fore- and hindlimb phalanges with the hindlimb phalanges statistically significantly reduced.

iv. Malformations:

No treatment related effects were noted.

E. Study Deficiencies:

The investigators provided only group summary data, no individual animal data for fetal ossification sites.

F. This study is classified as Acceptable-Guideline and satisfies the guideline requirements (§83-3a) for a teratology study in rats.

Maternal Toxicity NOEL = 5 mg/kg/day
Maternal Toxicity LOEL = 20 mg/kg/day

Developmental Toxicity NOEL = 20 mg/kg/day
Developmental Toxicity LOEL = 75 mg/kg/day