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

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JUN 24 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg No.: 239-2471. Acephate: Review of a multi-generation reproduction study with rats conducted by the Argus Research Laboratories and dated April 3, 1987.

TOX CHEM No.: 2A
TOX PROJECT No.: 7-1084
Record No.: 203929

FROM: John Doherty
Toxicology Branch
Hazard Evaluation Division (TS-769)

TO: William H. Miller
Product Manager #16
Registration Division (TS-767)

THROUGH: Edwin Budd
Section Head
Toxicology Branch
Hazard Evaluation Division (TS-769)

The Chevron Chemical Co. has submitted a multi-generation reproduction study with acephate in rats in response to a request from the Agency to provide a study which clearly demonstrates a NOEL (refer to Dr. K. Locke's review dated November 18, 1983).

Toxicology Branch (TB) has reviewed this study and the following comments apply.

Toxicology Branch Comments.

1. The study was reviewed and determined to be CORE GUIDELINES.

2. The study data support a NOEL of 50 ppm. Refer to the Data Evaluation Record attached.

1/B

3. The effects of concern which were reported in the low dose group (50 ppm) in the 1983 study (refer to Dr. K. Locke review dated November 18, 1983):

i. decreased fertility (in the F1 group)

ii. total litter loss

iii. number of implantation sites related to total young at birth

iv. decreases in the size and weight of the total litters

v. weight loss in females during gestation and lactation

were not evident in the low dose group (receiving 25 ppm) or in the mid dose group (receiving 50 ppm) in this 1987 study. Refer to the Data Evaluation Record for the responses noted in the group receiving 500 ppm.

[Note: No data on implantation sites were presented in the 1987 study although they were reported to be looked for and they were mentioned in the necropsy description and report for the 1987 study. Thus, no direct comparison between the two studies could be made for item iii above.]

Study Reviewed

Study	Result	Classification
83-4. Multi-generation reproduction study with rats. Argus Research Laboratories Study # 303005 (and S-2497 Chevron Chem. Co.) April 3, 1987.	NOEL = 50 ppm. LEL = 500 ppm. At this level there were decreases in body weight gain for adults and pups; increases in food consumption relative to body weight in adults; clinical signs in males (alopecia and loose stools); decreases in litter size (25-30%) for 4 of the 5 litter groups; and decreases in pup survival to day 4.	GUIDELINES

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Reviewed by: J.D. Doherty
Section II, Tox. Branch (TS-769C)
Secondary reviewer: E.R. Budd
Section II, Tox. Branch (TS-769C)

6/22/88

DATA EVALUATION REPORT

STUDY TYPE: 83-4. Multi-generation reproduction-rats.

TOX. CHEM. NO.: 2A

MRID
~~ACCESSION NUMBER~~: 403234-01 (3 volumes) and 406057-01 containing corrected pages.

TEST MATERIAL: Acephate Technical, Lot SX-1102 of 98.7% purity.

SYNONYMS: Orthene

STUDY NUMBER(S): 303005 (Argus Research Lab), S-2497 (Chevron Co.)

SPONSOR: Chevron Chem. Co.

TESTING FACILITY: Argus Research Laboratories, Horsham. Pa.

TITLE OF REPORT: "Two-generation (two litter) Reproduction Study in Rats with Chevron Acephate Technical"

AUTHOR(S): Alan M. Hoberman, Ph.D.

REPORT ISSUED: April 3, 1987

CONCLUSIONS: NOEL = 50 ppm
LEL = 500 ppm. At 500 ppm there were decreases in body weight gain for adults and in some litters of the pups; increases in food consumption relative to body weight; clinical signs in males (alopecia and loose stools); decreases in litter size (25-30%) for 4 of the five litter groups; and decreases in pup survival to day 4.

Classification: core-GUIDELINES

Special Review Criteria (40 CFR 154.7): N/A

Quality Assurance Statement.

A quality assurance statement signed by Jane E. Goeke, James A. Hills and Cynthia A. Dutt was provided attesting that thirty six inspections of this study were made.

Review

A. Basic Design.

In this study, four groups of 30 rats of each sex (Crl: COBS*CD(SD)BR, obtained from the Charles River Breeding Laboratories) were dosed with either 0, 25, 50 or 500 ppm of acephate technical (Lot No.: SX-1102 and stated to be of 98.7% purity). The F_o groups were dosed with this diet for 75 days before being bred to produce the F1a and later the F1b litters. Pups from the F1b litters were selected and bred to produce F2a and F2b litters. Because of low fertility in all groups including the control for the F1b and F2b litters, a third generation (F3a) was produced from the F2b litters. The cohabitation (mating period) was scheduled to be for three weeks and the rats were mated in pairs. All rats were continuously exposed to acephate or the control diets either directly in their feed or through the mothers milk during lactation.

The test diets were prepared weekly by combining the test material with Certified Rodent Chow Meal #5002 (Ralston Purina) to provide the nominal concentrations of 0, 25, 50 and 500 ppm. During the first 12 weeks of the study the diets were replaced weekly with freshly prepared samples. After 12 weeks, each freshly prepared batch was divided in halves with one half being placed in a freezer and the other being offered to the rats without freezing. During the week the frozen diet (after thawing) replaced the diet offered to the rats. Samples of each diet preparation and samples from the feed hoppers were taken for chemical analysis for both accuracy of the diet preparation and stability of the test material in the hoppers.

The analysis for acephate was performed separately by the Chevron Chemical Co.. The report authored by A.A. Spiros is in Volume III (EPA Acc. No.: 403234-01). Analysis revealed that the test material was of 98.5% purity based on the results of several trials (this is slightly lower than 98.7% purity originally reported). The homogeneity of the diets were found to be within 85.4% to 99.4% of the uniform target concentrations. The stability tests for acephate in the diet indicated that 79.7. 87.0 and 76.0% of the acephate was present after seven days in the hoppers for the 25, 50 and 500 ppm diet groups. The analytical data also indicated that the levels of acephate were 94.3 +/-7.6%, 95.6 +/-9.5% and 91.7 +/-4.3% of the target concentrations for the low, mid and high dose test groups respectively for the freshly prepared diets.

It should be noted that on weeks 14, 15, 46, 50, 66 and 70, acephate at up to about 6 ppm was found in some control diet samples. No explanation for this was provided. The contamination

might have been at either the diet preparation or in the analytical laboratory.

B. Results.

1. Mortality.

No mature rats died as a result of exposure to acephate.

2. Body weight.

i. Adults

The study report presents an extensive write up and analysis of body weight data. Statistically significant body weight decreases were evident in the high dose group males for each generation. For example, the Fo generation male rats were 6% lower at termination than the controls. Some decreases in female body weight were also evident particularly during gestation and lactation. Although the F3a mid dose group was statistically significantly lower during gestation, this was reasonably attributed to the small litter size which was delivered for this group. Other examples of body weight changes were similarly related to litter size.

ii. Pups

Pup body weights (as average weight per litter) for all dosage groups for all litters were comparable with the possible exception of growth during days 14-21 of lactation when some decreases were noted. Pup weights (at day 21 as average weight per litter) were also comparable among all groups except that the F2a high dose group (-8%) and F3a (-7%) being statistically significantly decreased.

The overall conclusion by TB is that no consistent effects on body weight were evident for the 25 and 50 ppm groups. The effects at 500 ppm were of a small magnitude.

NOEL = 50 ppm, LEL = 500 ppm for overall body weight effects (adults and pups).

3. Food Consumption.

Increases in food consumption were noted for the males starting at weeks 2-4 and continuing to week 10 for the first and second generations for the high dose group. Increases were also

noted for females during the premating period but decreases were noted during the gestation period for the high dose group.

Food consumption varied widely with the age and stage of development of the rats and for females its condition regarding pregnancy. As the food consumption varied so did the intake of acephate. The study report investigated this aspect of the data intensively and prepared numerous data tables for the various times in the life cycles of the rats such as growth, pre-mating period, gestation, lactation, and weaning etc for each of the parental groups and litters available. Much data is presented to show the mg acephate/kg/day ingested at various stages of development. The report states that the symptoms of acephate toxicity (weight loss, some clinical signs) were most obvious when the rats were consuming the most food and hence the most acephate.

NOEL = 50 ppm, LEL = 500 ppm for increased food consumption relative to body weight.

4. Clinical signs.

The only clinical responses to acephate treatment were reported as being in the high dose group males. No effects were reported in females. These were increased incidences of alopecia in the first generation and increased incidences of soft or liquid stools in the second and third generations.

NOEL = 50 ppm, LEL = 500 ppm for clinical signs.

5. Mating performance.

No <u>dose related</u> effects on mating performances were evident for any of the parental groups which produced the F1a, F1b, F2a or F2b litters. A statistically significant decrease ($p < 0.01$) in the mating performance for the high dose parental group (F2b) which produced the F3a litters was evident (refer to Table 1 below). This resulted in less females being pregnant in this group (60% in the high dose group versus 90% in the control) and only 162 pups delivered versus 372 pups in the control group. The study report did not relate this decrease in mating performance to the presence of acephate in the diet. According to the study report this decrease in performance was said to be related to "heavier body weight" of the rats assigned to cohabitation and the "inbreeding" that had occurred as a result of random selection from the limited population of rats for three generations.

Inspection of the data related to <u>matings not resulting in pregnancy</u> (fertility index) revealed comparable incidences among all groups except for the F3a high dose test group which had 7 incidences versus only 2 incidences in the controls

indicating a possible effect for this group.

There were no obvious consistent test chemical effects on the days spent in cohabitation except that the high dose group for the F3a litter appeared to average a day longer than the other three groups in this generation (i.e. 2 days vs 3 days).

The gestation index (percentage of pregnancies resulting in litters) was not affected by acephate in the diet.

TB believes that the decrease in mating for the high dose group for the F3a generation may be related to the test material and the explanation provided by the study report, although plausible, is not readily acceptable.

Note: The mating performance for the rats in this study varied among the five mating groups as shown in Table 1 below, In particular, the rats in the second matings (to produce the F1b and F2b litters) showed decreases in the mating performance relative to the same rats in these groups which produced the F1a and F2a litters. This is best illustrated by examining the data for the F1b litters which had mating frequencies of 30.0 to 43.3 % which were much lower than the 73.3 to 83.3 % obtained for the F1a matings. The reduced mating performance was also evident but to a somewhat lesser degree in the F2b (53.3 to 70 %) when compared to the F2a (66.7 to 83.3 %). Although the mating performance was decreased in the F1b and F2b litters, there was no evidence of this being related to acephate in the diet (i.e. the control groups were similarly affected). The testing laboratory made an elaborate effort to attempt to determine the cause of this decrease in mating performance (for example, refer to letters between Mildred S. Christian and E. Marshall Johnson dated Jan 14, 1986 and Jan 17, 1986 discussing this matter). The explanation for the reduced mating performance was not precisely determined but was attributed to natural variations in the cycle of the rats and was considered to be independent of the presence of the test material.

T B recognizes that the mating performance in the F1b and F2b groups was decreased but does not consider that this was related to the test material or significantly compromised the acceptability of the study.

Table 1. Pregnancy rate (pregnant rats relative to rats co-habitated, expressed as a percentage).

Dose Level	Generation/Litter				
	F1a[1]	F1b[1]	F2a[2]	F2b[2]	F3a[3]
Control	83.3	43.3	83.3	53.3	90.0
25 ppm	73.3	33.3	73.3	63.3	80.0
50 ppm	76.7	30.0	70.0	70.0	96.7
500 ppm	76.7	36.7	66.7	53.3	60.0**

1. Appendix A Tables 37 and 38
2. Appendix B Tables 35 and 36
3. Appendix C Table 25

** Statistically significant $p<0.01$

6. Duration of gestation and delivery of litters.

These parameters were not significantly affected by the presence of acephate in the diet.

7. Litter size and stillborn pups.

The average litter size at birth was statistically significantly decreased (25-30%) by the presence of acephate in the diet for 4 of the five litters produced . The Table 2 illustrates the average litter size (number of pups per litter) at birth.

Table 2. Litter size (mean number of pups per litter +/- standard deviation).

Dose Level	Generation/Litter				
	F1a	F1b	F2a	F2b	F3a
Control	12.6(3.1)	13.1(1.8)	13.6(3.2)	13.2(2.2)	13.8(2.6)
25 ppm	12.2(2.9)	10.4(4.7)	13.5(1.6)	13.8(2.6)	13.5(1.7)
50 ppm	11.3(3.8)	11.1(2.1)	12.6(3.9)	14.3(3.0)	12.3(2.8)*
500 ppm	11.3(2.5)	9.7(3.0)*	10.2(3.4)**	9.9(3.4)**	9.7(2.8)**

*statistically significant $p < 0.05$
**statistically significant $p < 0.01$

Although the F3a mid dose group reaches statistical significance, the testing laboratory does not attribute this decrease to acephate in the diet. The control group for the F3a generation (13.8) is high and the mid dose group (12.3) is within the range for the other non affected groups.

NOEL = 50 ppm, LEL = 500 ppm for decrease in litter size.

The effect of acephate on litter size may have occurred early in pregnancy since there was no increase in the number of stillborn pups due to acephate in the diet.

8. Pup survival.

The number of pups surviving to day 4 (as a percentage of live pups delivered) was slightly decreased for the mid (-3.2%) and high (-3.5%) dose groups for the F1a generation and for the high dose group for the F2a (-6.9%) generation. Refer to the Table 3 below.

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Table 3. Pup survival (pups surviving 4 days relative to total liveborn pups, expressed as a percentage).

Dose level	Generation/Litter				
	F1a	F1b	F2a	F2b	F3a
Control	99.7	98.8	99.4	99.0	98.1
25 ppm	98.1	99.0	99.0	97.7	96.8
50 ppm	96.8**	100	98.8	99.3	98.6
500 ppm	96.5**	98.1	93.1**	99.4	95.1

** statistically significant $p<0.01$

The inconsistency of the decreases in the 4 day survival for all litter groups and the small percentages involved do not convincingly indicate a test material related effect. The study report, however, considers these decreases to be "biologically remarkable only for the high dose group". No effect of acephate was noted on the 4-21 day survival.

NOEL = 50 ppm, LEL = 500 ppm for pup survival.

9. Sex ratio.

There were no effects evident on the ratio of males to females.

10. Gross Necropsy and Histopathology.

Complete necropsies were performed on all parental rats in the Fo, F1b and F2b generations. The females were reported to have also been evaluated for the number of implantation sites at necropsy.

No test chemical related increases in lesions were noted at gross necropsy for the rats dosed with acephate. Information on the total number of implantation sites could not be found in the study report.

The histopathology report is presented in Appendix H and was prepared by W. Ray Brown, D.V.M., Ph.D., Veterinary Pathologist (report dated April 24, 1987).

Microscopic evaluations of the control and high dose rats in the Fo, F1b and F2b parental groups were made. The tissues specified for evaluation were testes, epididymides, prostate, seminal vesicles, uteri, cervix, and tissues with gross lesions. Preparation consisted of embedding the tissue in paraffin, sectioning at 5 microns, and staining with hematoxylin and eosin.

No test chemical related microscopic lesions were noted in any of the parental rats for the Fo, F1b or F2b generations.

NOEL = 500 ppm.

CONCLUSION.

This study is classified as CORE GUIDELINES. Sufficient data were generated to define a NOEL of 50 ppm. At 500 ppm there were decreases in body weight gain for adults and pups; increases in food consumption relative to body weight for adults; clinical signs in male adults (alopecia and loose stools); decrease in litter size (25-30%) in 4 of the 5 litter groups; and decreases in pup survival to day 4.

It should be noted that this study did not assess for inhibition of AChE or ChE.

Summary Tables

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meter	Dosage (ppm)	F0-F1a	F0-F1b	F1b-F2a	F1b-F2b	F2b-F3a
ng dex (%)	0	28/30(93.3)	20/30(66.7)	29/30(96.7)	28/30(93.3)	29/30(96.7)
	25	27/30(90.0)	19/30(63.3)	30/30(100.0)	29/30(96.7)	29/29(100.0)
	50	28/30(93.3)	20/30(66.7)	28/30(93.3)	30/30(100.0)	29/30(96.7)
	500	29/30(96.7)	20/30(66.7)	28/30(93.3)	28/30(93.3)	25/30(83.3)**
	0a	30/30(100.0)	27/30(90.0)	25/25(100.0)	25/25(100.0)	—
ility dex (%)	0	25/28(89.3)	13/20(65.0)	25/29(86.2)	16/28(57.1)	27/29(93.1)
	25	22/27(81.5)	10/19(52.6)	22/30(73.3)	19/29(65.5)	24/29(82.8)
	50	23/28(82.1)	9/20(45.0)	21/28(75.0)	21/30(70.0)	29/29(100.0)
	500	23/29(79.3)	11/20(55.0)	20/28(71.4)	16/28(57.1)	18/25(72.0)**
	0a	26/30(86.7)	22/27(81.5)	20/25(80.0)	18/25(72.0)	—
natal rviral dex (%) ys 0-4)	0	307/308(99.7)	166/168(98.8)	332/334(99.4)	210/212(99.0)	365/372(98.1)
	25	259/264(98.1)	103/104(99.0)	291/294(99.0)	251/257(97.7)	300/310(96.8)
	50	241/249(96.8)**	100/100(100.0)	260/263(98.8)	297/299(99.3)	344/349(98.6)
	500	248/257(96.5)**	105/107(98.1)	188/202(93.1)**	157/158(99.4)	154/162(95.1)
	0a	344/346(99.4)	255/263(97.0)	260/260(100.0)	238/242(98.3)	—
ation dex (%) ys 4-21)	0	194/195(99.5)	104/104(100.0)	194/194(100.0)	128/128(100.0)	211/213(99.1)
	25	171/172(99.4)	69/70(98.6)	178/178(100.0)	149/150(99.3)	182/184(98.9)
	50	159/163(97.5)	70/70(100.0)	159/160(99.4)	164/164(100.0)	228/228(100.0)
	500	173/178(97.2)	83/84(98.8)	136/137(99.3)	115/117(98.3)	125/125(100.0)
ter Size (Total)	0	12.6±3.1	13.1±1.8	13.6±3.2	13.2±2.2	13.8±2.6
	25	12.2±2.9	10.4±4.7	13.5±1.6	13.8±2.6	13.5±1.7
	50	11.3±3.8	11.1±2.1	12.6±3.9	14.3±3.0	12.3±2.8*
	500	11.3±2.5	9.7±3.0*	10.2±3.4**	9.9±3.4**	9.7±2.8**
	0a	13.5±3.4	12.0±4.6	13.0±2.4	13.5±3.1	—
y Weight (Grams)	0					
	Day 0	6.3±0.6	6.5±0.6	6.0±0.8	6.3±0.5	6.0±0.5
	0a	6.2±0.4	6.4±1.0	6.2±0.6	6.2±0.6	—
	Day 21	55.8±4.1	58.0±4.4	48.8±5.3	55.0±4.6	53.5±5.5
	25					
	Day 0	6.3±0.9	6.8±1.1	6.1±0.5	6.0±0.5	6.1±0.4
	Day 21	55.2±5.8	57.1±5.7	49.7±3.4	51.6±4.4	49.9±4.7*
	50					
	Day 0	6.5±0.6	6.6±0.8	6.2±0.7	6.0±0.7	6.0±0.6
	Day 21	56.0±3.3	56.9±6.0	47.5±6.3	53.3±6.5	50.8±4.4
	500					
	Day 0	6.4±0.5	6.7±0.7	6.2±0.7	6.4±0.7	6.2±0.6
	Day 21	53.5±3.6	55.9±4.9	44.8±6.0*	53.9±6.3	51.4±3.9

* = Significantly different from vehicle control ($P<0.05$)
** = Significantly different from vehicle control ($P<0.01$)
0a. Values for another two generation study (two litters per generation) simultaneously conducted in an adjacent ro
Mating Index (%) can be found in the Mating and Fertility Tables for each generation as "Rats Which Mated".
Fertility Index (%) can be found in the Mating and Fertility Tables for each generation as "Pregnant Rats/Mated".
Postnatal Survival Index (%) (days 0-4) can be found in the Natural Delivery and Litter Data Tables for each generation as "Pups Surviving 4 Days/Total Liveborn Pups (Preculling)".
Lactation Index (%) (days 4-21) can be found in Natural Delivery and Litter Data Tables for each generation as "Pups Surviving 21 Days/Pups Selected on Day 4".