7-6-88

DATA EVALUATION RECORD PAGE 1 OF

CASE: GS0103 PHORATE FRSTR

CONT-CAT: 01 GUIDELINES: 71-4

MRID: 158334

Beavers, J. (1986) Phorate Technical: A One-generation Reproduction Study with the Mallard (Anas platyrhynchos):Final Report: Project No.130-142. Unpublished study prepared by Wildlife International Ltd. 104 p.

REVIEW RESULTS:

VALID ✓ INVALID _____ INCOMPLETE _____

GUIDELINE: SATISFIED ___ PARTIALLY SATISFIED ✓ NOT SATISFIED ___

DIRECT RVW TIME = 40 hr START DATE: 6/27/88 END DATE: 7/1/88

REVIEWED BY: Ann Stavola

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1/16

1. Chemical: Phorate

2. Test Material: Technical, 92.1% ai

3. Study/Action Type: Avian Reproduction
Mallard (Anas platyrhynchos)

4. Study ID: MRID 158334. Beavers, J. (1986) Phorate Technical: A one-generation reproduction study with the mallard (Anas platyrhynchos). Final Report: Project No. 130-142. Unpublished study prepared by Wildlife International, Ltd. MRID 158334

5. Reviewed By: Ann Stavola
Aquatic Biologist
HED/EEB
Signature: Ann Stavola
Date: 7/25/88

6. Approved By: Douglas Urban
Supervisory Biologist
HED/EEB
Signature: Douglas J Urban
Date: 10/11/88

7. Conclusions:

The study is scientifically sound and meets EPA guideline requirements for an avian reproduction study with a waterfowl species. The study indicated that a diet of 60 ppm phorate significantly inhibited the ability of mallard ducks to reproduce normally. The NOEL was 5 ppm.

8. Recommendations: N/A

9. Background:

Avian reproduction studies were required in the Phorate Registration Standard (1983).

10. Materials and Methods:

a. Test Animals - Mallard ducks (Anas platyrhynchos). Pen-reared, phenotypically indistinguishable from wild birds. Purchased from Whistling Wings, Hanover, IL. All were from the same hatch and approximately 44 weeks old and approaching their first breeding season at the start of the study.

b. Test System - Adults - There were one drake and one hen in each pen, 75 x 90 x 45 cm high, and there were 16 pens per treatment group. Every week, a 7-day supply of food was placed in each pen. If the birds wasted an excessive amount of food, they were given more food. Water was provided ad libitum. The average temperature and humidity in the study room were, respectively, 63 ± 12 °F and 79 percent. The air system in the room constantly replaced the room's air with fresh air. For the first 8 weeks of the study, the photoperiod was 8L:16D. During week 9, the photoperiod was adjusted to 17L:7D, and it stayed at this photoperiod until the adult birds were sacrificed. The strength of the light was 12 footcandles.

The test diets were prepared by mixing phorate (92.1% ai), corn oil and acetone with the basal diet. The final concentrations of phorate fed to the adult mallards were 5, 20, and 60 ppm in addition to control pens. The adults were not fed any medication in their food.

c. Egg Collection and Incubation - Eggs were collected daily, washed with a chlorine-based detergent to prevent pathogen contamination and stored in a cold room at 55 ± 2 °F, 76 percent RH until they were placed in incubators. Incubation was done on a weekly basis. The incubator had a temperature of 99.2 ± 0.2 °F and RH of 55 percent. The incubator rotated the eggs every hour in a 100 °arc for 24 days to prevent adhesion of the embryos.

d. Hatching - On day 24, the eggs were transferred to hatchers where the temperature and RH were, respectively 98.9 ± 0.5 °F and 77 percent. All hatchlings, unhatched eggs and egg shells were removed on day 26 or 27.

e. Ducklings - The ducklings were fed untreated diet and received no medications. They were housed in pens, 72 x 90 x 24 cm high. Temperature was 100 °F from the time of hatching until they were 5 to 7 days old; then it was reduced to ambient temperature. The photoperiod was 17L:7D.

f. Study Design - The phases of the study were:

1) Acclimation - Approximately 7 weeks.

2) Pre-photostimulation - Approximately 9 weeks.

3) Pre-egg laying (with photostimulation) - Approximately 2 weeks.

4) Egg-laying - Approximately 9 weeks.

5) Post-adult sacrifice (final incubation, hatching, 14-day offspring rearing period) - 6 weeks.

The treatment groups were: Control, 5 ppm, 20 ppm, and 60 ppm. The test material was phorate technical grade, 92.1% ai.

g. Observations - Adult Bird Observations - Daily for signs of toxicity or abnormal behavior; weekly for food consumption for each pen; body weights at initiation, weeks 2, 4, 6, 8 and terminal sacrifice; post-mortem necropsy at death or at end of adult phase of study.

Eggs

- Eggs laid.

- Eggshell thickness: weekly throughout egg-laying period, 1 egg was collected from each of the odd-numbered pens in odd weeks and from each of the even-number pens in even weeks. The eggs were opened at the mid-section, washed out and air-dried for a week at ambient temperature. The shell plus membrane were measured at 5 points with a micrometer to the nearest 0.005 mm.

- Eggs cracked: determined by candling eggs before placing into incubator.

- Eggs set: the numbers laid minus the numbers cracked for each pen and studied for shell thickness.

- Viable embryos: determined by candling on day 14 of incubation.

- Live 3-week embryos determined by candling on day 21 of incubation.

- Hatchlings: the number that hatched per pen and the average body weight of the hatchlings by pen was determined.

- Ducklings: on day 14 after hatching, the average body weight by parental pen was determined.

11. Statistical Analyses:

Dunnett's method was used to determine statistically significant differences between the control group and each

treatment group. Sample units were the individual pens for each group. If an adult duck died during the study, the pen was not used in the statistical analyses. In the case where a drake died at the end of the study, that pen was included in the analyses. The following parameters were analyzed with statistics:

Adult body weight
Adult feed consumption
Eggs laid of maximum laid (59 eggs was the maximum laid by one hen)
Eggs cracked of eggs laid
Viable embryos of eggs set
Live 3-week embryos of viable embryos
Hatchlings of 3-week embryos
14-Day old survivors of hatchlings
14-Day old survivors of eggs set
Hatchlings of maximum set (53 eggs was the maximum set by 1 hen)
14-Day old survivors of maximum set
Offspring's body weight
Eggshell thickness

12. Reported Results:

Three ducks eating the 60 ppm diet died during the study--a drake during week 8, a drake during week 16, and a drake at the end of the study. Necropsies indicated a number of abnormalities such as loss of muscle mass and body fat, atrophy of reproductive organs and hemorrhaging.

Other adult ducks exhibited lesions or abnormal behavior associated with pen wear and tear or aggression. A few ducks at 20 and 60 ppm exhibited clinical signs of exposure to an OP pesticide such as limb weakness and loss of coordination.

When gross necropsies were done on all ducks still alive at the end of the study, many birds fed the 60 ppm diet had atrophying reproductive organs.

A summary of adult body weight data is given in Table 1 (attached). There were treatment-related effects at 60 ppm in males throughout the study and females at the ends of weeks 2, 4, and 8.

A summary of food consumption is given in Table 2 (attached). Food consumption decreased significantly in birds fed 60 ppm during weeks 1, 2, 3, and 12.

The 5 ppm treatment group had a statistically significant difference in the number of live 3-week embryos as a percent of viable embryos, the number of hatchlings as a percent of live 3-week embryos and the number of 14-day old survivors as a percent of the number of hatchlings. The authors did not

consider these differences to be biologically important.

The 20 ppm treatment group was not statistically different from the controls in any reproductive parameter.

The 60 ppm treatment group was statistically different from the controls with regard to eggs laid, viable embryos as a percent of eggs set, number of hatchlings and 14-day old survivors as a percent of the maximum number of eggs set.

There were no differences between controls and treatment groups regarding egg shell thickness and body weight of hatchlings. Only the 60 ppm treatment group showed a significant increase in body weight of the 14-day old ducklings. This effect may have been caused by the small number of hatchlings reared together for this group.

13. Author's Conclusions/QA Statement:

"Dietary concentrations of up to 20 ppm of technical phorate did not produce treatment-related mortality in adult mallards during thn 19-week exposure period. There were no apparent treatment-related effects on body weight of adults ai 5 ppm or feed consumption among adults at 5 ppm and 20 ppm. A slight effect on adult body weight occurred at 20 ppm, and a marked effect on adult body weight and feed consumption was observed at 60 ppm. At 5 ppm there was no treatment-related effect upon any of the reproduction parameters. At 20 ppm, there appeared to be a slight effect on the number of viable embryos as a percent of eggs set. There was a marked effect upon reproductive performance at 60 ppm. The no-observed-effect concentration was 5 ppm."

QA Statement: "This study was conducted so as to conform with Goox Laboratory Practices as published by U.S. Environmental Protection Agency, OPP . . . ."

14. Reviewer's Evaluation and Interpretation of the Study:

a. Test Procedures - The test procedures used on this study follow those in EPA's Pesticide Assessment Guidelines Subdivision E, EPA-540/9-82-024, October 1982.

b. Statistical Analyses - The data were analyzed with ANOVA with the SAS procedure (Big Bird program). The variables were converted via arcsine transformation prior to the ANOVA analyses. Duncan's Multiple Range Test was used to compare significant differences between the means of the treatment groups for each parameter. In addition, a Power Test was done for each variable to measure the statistical strength of the study.

c. Results and Discussion - The parameters analyzed by the "Big Bird" program were eggs laid, eggs cracked, eggs set, viable embryos, live embryos and number of hatchlings. Our analyses indicated that for all parameters except eggs cracked, the means for the 60 ppm treatment group were significantly lower than those for the control and lower treatment groups. The power test indicated that the study is statistically sound.

The following data summary table indicates that, in addition to number of eggs cracked, there were no differences between control and treatment groups regarding 14-day old survivors as a percent of normal hatchlings, weight of hatchlings and egg shell thickness. With regard to this last parameter, the mean thickness of eggs from the 60 ppm treatment group is slightly lower than those of the other groups, but the difference is not statistically significant.

The data indicate that:

- Long term exposure of 60 ppm technical phorate, 92.1%, can produce some mortalities (9%) in adult mallards.

- This dietary exposure produces morphological changes in reproductive organs such as regressed gonads and egg yolk peritonitis.

- Ducks at 20 ppm and 60 ppm gained significantly less weight than controls by the end of the study. Ducks at 60 ppm actually lost weight.

- Food consumption was significantly reduced during several weeks for the birds fed 60 ppm phorate. However, overall differences in food consumption between controls and treated birds were not significant.

- Exposure to 60 ppm phorate caused significant reductions in eggs laid, eggs set, viable embryos, live embryos, and number of hatchlings.

- The inability of ducks fed 60 ppm phorate in their diet to reproduce normally was likely the result of physiological changes phorate caused in the reproductive organs.

d. Conclusions

1) Category - Core.

2) Rationale - The study followed recommended EPA procedures and is scientifically sound.

Summary of Phorate Effects on Mallard Duck Reproduction Parameters

Parameter	Nominal Concentration of Phorate 0	5	20	60
Eggs laid				
Total number	700	630	668	119
Number/hen	44	39	42	9
Eggs cracked				
Total number	37	23	24	13
Number/hen	2.3	1.4	1.5	0.9
% of eggs laid	5.3	3.7	3.6	10.9
Eggs set				
Total number	599	540	580	88
% of eggs laid	85.6	85.7	86.8	73.9
Viable embryos (14-day)				
Total number	555	496	456	60
% of eggs laid	79.3	78.7	68.3	50.4
% of eggs set	92.7	91.9	78.6	68.2
Live embryos (21-day)				
Total number	545	473	450	60
% of viable embryos	98.2	95.4	98.7	100
Hatchlings				
Total number	484	358	399	57
% of eggs laid	69.1	56.8	59.7	47.9
% of eggs set	80.8	66.3	68.8	64.8
% of viable embryos	87.2	72.2	87.5	95
% of live embryos	88.8	75.7	88.7	95
14-Day Old Survivors				
Total number	482	349	394	57
Number/hen	30.1	21.8	24.6	4.1
% of normal hatchlings	99.6	97.5	98.7	100
Average hatchling weight (g)	40	40	40	36
Average 14-Day Old duckling weight (g)	222	225	231	259
Mean Adult Weight At study termination				
Females (g/bird)	1299	1314	1210	1028
Males (g/bird)	1228	1263	1240	1075

Summary of Phorate Effects on Mallard Duck
Reproduction Parameters (Cont'd)

Parameter	Nominal Concentration of Phorate			
	0	5	20	60
Mean change from study initiation				
Females (g/bird)	+233	+216	+123	-78
Males (g/bird)	+37	+62	+8	-79
Mean eggshell thickness (mm)	0.382 ± 0.022	0.381 ± 0.030	0.374 ± 0.035	0.357 ± 0.049
Average feed Consumption (g/bird/day)				
Pre-egg production	137	147	135	122
Egg production	212	236	230	230
Mean total	349	383	365	352

Please see EEB review

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