A 5/23/95

DATA EVALUATION RECORD
§ 71-4 -- AVIAN REPRODUCTION TEST

1. **CHEMICAL:** Acetochlor **PC Code No.:** 121601

2. **TEST MATERIAL:** Acetochlor. Batch no. QUE-9001-1482T
Purity: 92.07%

3. **CITATION**

Authors: Vanessa A. Redgrave
Title: Acetochlor: Reproduction in the Bobwhite Quail.
Study Completion Date: August 5, 1993
Laboratory: Huntingdon Research Centre Ltd.
Sponsor: Monsanto Agricultural Company
Laboratory Report ID: MTO 25/921670
MRID No.: 433831-02
DP Barcode: D208318

4. **REVIEWED BY:** F. Nicholas Mastrota, Biologist, EEB, EFED

Signature: F. Nicholas Mastrota **Date:** 5/8/95

5. **APPROVED BY:** Daniel D. Rieder, Head of Section 3, EEB, EFED

Signature: Daniel Rieder **Date:** 5-23-95

6. **STUDY PARAMETERS**

Scientific Name of Test Organism: Colinus virginianus
Age of Test Organisms at Test Initiation: 24 weeks
Definitive Study Duration: 23 weeks

7. **CONCLUSIONS:** This study is scientifically sound, but because of numerous guideline deviations, it does not fulfill the data requirement for an avian reproduction test for the bobwhite quail (guideline no. 71-4a). Based on this test, a chronic dietary exposure to acetochlor at 750 ppm ai impairs reproductive success of the bobwhite by reducing the weight and survival of the young produced. A dietary concentration of 150 ppm ai does not cause observable effects.

Results Synopsis
Most sensitive endpoints: Number of 14-day survivors, hatchling weight, 14-day survivor weight.
NOEC: 150 ppm ai **LOEC:** 750 ppm ai

8. **ADEQUACY OF THE STUDY**

A. Classification: Supplemental.

B. Rationale: There were numerous deviations from the EPA guidelines for this test. The most important deviation was

that the photoperiod was increased at the beginning of week 7 rather than the beginning of week 9.

C. Repairability: The guideline deviations 1, 2, 4, and 6 could be rectified be submitting additional information that was absent from the study report. However, guideline deviations 3, 5, and 7 cannot be repaired. As deviations 5 is critical, this study cannot be upgraded to core.

9. GUIDELINE DEVIATIONS

1. The study report if the birds used were phenotypically indistinguishable from wild bobwhites.

2. The Standard Evaluation Document states that the health of birds should be observed for 2 to 6 weeks prior to selecting birds for treatments. No such observation period was mentioned in the study report. Also, the report gives no description of the health and mortality of birds prior to the initiation of the test.

3. The relative humidity in the room where the adult birds were housed was 73%, whereas the guidelines state that they should be approximately 55%.

4. The study report did not describe the ventilation provided in the room where the adult birds were housed.

5. The photoperiod was increased from a 7-h day to a 16-h day at the beginning of Week 7, whereas the guidelines state that this increase should occur at the beginning of Week 9.

6. The study report did not describe how the premix was stored.

7. Body weights of adult birds were not measured for biweekly intervals up to the onset of egg laying, as the guidelines require. Instead, they were only measured for test initiation, Day 71, and test termination (Day 162).

10. SUBMISSION PURPOSE: In support of registration of the chemical.

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11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species A wild waterfowl species, preferably the mallard (Anas platyrhynchos), or an upland game species, preferably the northern bobwhite (Colinus virginianus)	Northern bobwhite (Colinus virginianus)
Age at beginning of test Birds should be approaching their first breeding season.	8 months. Birds were approaching their first breeding season.
Supplier All birds should be from the same source.	Mr. Potter, Rosedean, Woodhurst, Huntington, Cambridgeshire, England.
Were birds pen-reared?	Yes
Were birds phenotypically indistinguishable from wild birds?	Not reported
Health observation period 2 to 6 weeks.	None stated.
Were birds healthy and without excessive mortality prior to the test?	Not reported

B. Test System

Guideline Criteria	Reported Information
Were pens for adult birds of adequate size and designed to conform to good husbandry practices?	Yes

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Guideline Criteria	Reported Information
Were pens for chicks of adequate size and designed to conform to good husbandry practices?	**Yes. (Housing used for chicks was only described as "wooden box floor pens," but the small control mortality of chicks suggests that these facilities were adequate.)**
Where pens constructed of a nonbinding material such as galvanized or stainless steel?	**Yes for adult pens; no for chick pens.**
Was adequate ventilation provided?	**Not reported**
Temperature Approx. 21°C (70°F)	Range: 19-22°C
Relative humidity Approx. 55%	Range: 73%
Lighting First 8 weeks: 7 h per day. Thereafter: 16-17 h per day. At least 6 footcandles at bird level.	First 6 weeks: 7 h per day. Thereafter: 16 h per day.
Diet A commercial breeder feed (or its equivalent) that is appropriate for the test species.	Quail layer diet, manufactured by Special Diets Services, Witham, Essex, England.
Preparation of test diet A premixed containing the test substance should be mechanically mixed with basal diet. If an evaporative vehicle is used, it must be completely evaporated prior to feeding.	A premix was prepared by mixing test material with basal diet without use of a vehicle. The premix was then diluted with basal diet to achieve the final test diets. Test diet were prepared weekly.
Was the premix stored under conditions which maintain stability?	**Not reported**
Was the diet analyzed to verify homogeneity and stability of the test substance?	**Yes**

Guideline Criteria	Reported Information
Replenishment of feed	Feed was provided ad libitum throughout the study.

C. Test Design

Guideline Criteria	Reported Information
Nominal concentrations At least two concentrations other than the control are required; three or more are strongly recommended. The highest test concentrations should show a significant effect or be at or above the maximum field residue level.	Nominal concentrations: 30, 150 and 750 ppm ai Max. residue level: 728 ppm ai
Control Vehicle control.	Untreated feed was used in the control since no vehicle was used.
Vehicle Corn oil or other appropriate vehicle.	None
Vehicle amount (% of diet by weight) Not more than 2%.	N/A
Number of birds per pen One male and 1 female per pen is strongly recommended. For quail, 1 male and 2 females may be acceptable. For ducks, 2 males and 5 females may be acceptable.	1 males and 1 females per pen.
Number of pens per group At least 5 replicate pens are required for mallards housed in groups of 7. For other arrangements, at least 12 pens are required, but considerably more may be needed if birds are kept in pairs. At least 16 pens are strongly recommended.	24 pens per group.

Guideline Criteria	Reported Information
Pre-laying exposure duration At least 10 weeks prior to the onset of egg-laying.	11 weeks
Exposure duration with egg-laying At least 10 weeks.	12 weeks
Withdrawal period If reduced reproduction is evident, a withdrawal period of up to 3 weeks may be added to the test phase.	N/A

D. Egg Collection and Incubation

Guideline Criteria	Reported Information
Were eggs collected daily?	Yes
Egg storage temperature Approximately 16°C (61°F)	16°C
Egg storage humidity Approximately 65%	Not reported.
Were eggs set weekly?	Yes
Were eggs candled for cracks prior to being set for incubation on Day 0?	Yes
Candling for fertility Quail: approx. Day 11 Ducks: approx. Day 14	Eggs were candled twice, on Day 11 and Day 18.
Transfer of eggs for hatching Bobwhite: Day 21 Mallard: Day 23	Eggs were transferred on Day 21.
Hatching temperature 39°C (102°F) is recommended	39°C
Hatching humidity 70% is recommended	70%

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Guideline Criteria	Reported Information
Day after egg set that chicks were removed and counted Bobwhite: Day 24 Mallard: Day 27	Chicks were removed and counted on Day 24.

E. Eggshell Thickness Measurement

Guideline Criteria	Reported Information
Collection Schedule At least once every two weeks (Week 1, 3, 5, 7 and 9).	Eggs were collected on the first day of Week 2, 4, 6, 8, 10 and 12 of egg laying.
Were shells opened, washed, and air dry for at least 48 hours before measuring?	Yes
Measurement 3-4 measurements per eggs to the nearest 0.01 mm.	Each egg shells was measured in four places to the nearest 0.01 mm.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Did diet analysis verify the concentrations of test material?	Yes
Did diet analysis show that the test substance was stable and homogeneous?	Yes
Were body weights of adults reported for test initiation and biweekly up to week 8 or the onset of egg laying?	No. Body weights were reported only for Days -7, 1, 71 and 162.
Was average food consumption of adults reported at least biweekly?	Yes

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Guideline Criteria	Reported Information
Reproductive Endpoints The following endpoints should be reported: • Eggs laid • Eggs cracked • Eggs set • Viable embryos • Live 3-week embryos • Normal hatchlings • 14-day-old survivors • Weights of 14-day-old survivors • Egg shell thickness • Total food consumption • Initial and final body weights, by sex	The following endpoints were measured: • Eggs laid • Eggs cracked • Eggs set • Viable embryos • Live 3-week embryos • Number dead in shell - not pipped. • Number dead in shell - pipped. • Normal hatchlings • 14-day-old survivors • Weights of 14-day-old survivors • Egg shell thickness • Total food consumption • Initial and final body weights, by sex
Were data reported by pen for all endpoints?	Yes

Significant Results: The proportion of normal hatch which survived for 14 days was significantly lower in the 750 ppm dose group than in the control group ($P<0.01$). Also, the body weights of both hatchlings and 14-day-old survivors were significantly less in 750 ppm group than in the control ($P<0.01$). No other significant differences were observed between the treatment and control groups. No notable behavioral or gross physiological effects were observed. The no observed effect level (NOEL) for reproductive effects on the bobwhite was 750 ppm ai.

13. VERIFIED STATISTICAL RESULTS

Means and Standard Deviations of Endpoints

Endpoint	Control	30 ppm	150 ppm	750 ppm
Eggs laid (EL)	54.6 (20.8)	53.1 (22.6)	55.3 (18.8)	53.5 (19.7)
Eggs cracked (EC)	2.00 (2.3)	2.08 (2.14)	0.70 (0.97)	2.22 (4.08)
Eggs set (ES)	49.1 (18.5)	47.4 (20.5)	50.5 (17.1)	47.8 (17.7)

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Endpoint	Control	30 ppm	150 ppm	750 ppm
Viable embryos (VE)	44.5 (19.8)	39.1 (24.1)	47.8 (17.1)	37.7 (21.0)
Live 3-wk embryos (LE)	43.3 (19.4)	38.1 (23.7)	46.9 (16.4)	36.8 (21.0)
Normal hatchlings (NH)	39.2 (18.5)	34.3 (21.9)	43.3 (15.0)	32.6 (19.5)
14-day-old survivors (HS)	36.0 (17.7)	30.9 (20.4)	38.8 (14.2)	26.8 (17.2)
Egg shell thickness	0.20 (0.01)	0.20 (0.01)	0.20 (0.01)	0.21 (0.01)
Hatchling weight	6.7 (0.5)	6.7 (0.6)	6.6 (0.5)	6.3 (0.5)
14-day-old survivor weight	24.4 (2.1)	24.5 (2.4)	23.8 (1.6)	22.8 (2.3)
Mean food consumption	17.5 (1.5)	17.5 (1.7)	17.1 (2.0)	17.4 (1.5)
Final weight of males	205.8 (18.7)	204.2 (18.5)	196.6 (17.0)	196.6 (15.4)
Final weight of females	214.6 (21.2)	217.7 (17.0)	221.9 (18.4)	219.0 (22.9)

Statistically Significant Endpoints

Endpoint	Statistical Method	Levels at which Effect Was Observed
14-day hatchling survivors	Williams	750 ppm
Hatchling survivors per eggs laid	Williams	750 ppm
Hatchling weight	Dunnett's Test	750 ppm
14-day survivor wt.	Dunnett's Test	750 ppm

14. REVIEWER'S COMMENTS

Acetochlor impairs the reproduction of the northern bobwhite at relatively high dietary concentrations (750 ppm ai). It does not appear to effect the number of eggs laid or the hachability of

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the eggs. However, the chicks that are hatched from eggs from hens exposed to high concentrations of acetochlor are small and have reduced survival.