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 Mallard Duck.
Study Completion Date: August 9, 1993
Laboratory: Huntingdon Research Centre Ltd.
Sponsor: Monsanto Agricultural Company
Laboratory Report ID: MTO 24/921662
MRID No.: 433831-01
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: *Anas platyrhynchos*
Age of Test Organisms at Test Initiation: 24 weeks
Definitive Study Duration: 22 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 mallard duck (guideline no. 71-4a). Based on this test, dietary concentrations of acetochlor of 150 ppm and greater impair reproduction of the mallard, whereas a dietary concentration of 30 ppm ai causes no observable effects.

Results Synopsis
Most sensitive endpoints: Live 3-week embryos, normal hatchlings, and 14-day-old survivors.
NOEC: 30 ppm ai LOEC: 150 ppm ai

8. **ADEQUACY OF THE STUDY**

A. Classification: Supplemental.

B. Rationale: There were numerous deviations from the EPA guidelines for this test (see below). The most significant deviations were that the temperature of the rooms housing

the birds was too low and that the increase in photoperiod to stimulate reproduction took place at the improper time.

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

9. GUIDELINE DEVIATIONS

1. The study did not report if the birds used were phenotypically indistinguishable from wild mallards.

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 environmental conditions of the rooms in which the birds were housed deviated significantly from the guidelines for this study. The guidelines state that the temperature should be approximately 21°C (70°F) and 55% relative humidity. In the two rooms used for this study, the temperature ranged from 15-17 °C and the relative humidity ranged from 70-71 %.

4. 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.

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

6. 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 155).

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

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*)	Mallard (*Anas platyrhynchos*)
Age at beginning of test Birds should be approaching their first breeding season.	24 weeks old. Birds were approaching their first breeding season.
Supplier All birds should be from the same source.	The County Game Farms, Hothfield, Ashford, Kent, 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
Were pens for chicks of adequate size and designed to conform to good husbandry practices?	Yes

Guideline Criteria	Reported Information
Where pens constructed of a nonbinding material such as galvanized or stainless steel?	Yes
Was adequate ventilation provided?	Yes
Temperature Approx. 21°C (70°F)	Range: 15-18°C
Relative humidity Approx. 55%	Range: 70-71%
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
Replenishment of feed	Feed was provided ad libitum throughout the study.

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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 Max. residue level: 728 ppm Effects were shown at the two highest levels.
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.	2 males and 5 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.	7 pens per group.
Pre-laying exposure duration At least 10 weeks prior to the onset of egg-laying.	10 weeks

Guideline Criteria	Reported Information
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 14 and Day 21.
Transfer of eggs for hatching Bobwhite: Day 21 Mallard: Day 23	Eggs were transferred on Day 24.
Hatching temperature 39°C (102°F) is recommended	37.5°C
Hatching humidity 70% is recommended	Not reported.
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 155.
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 number of eggs laid per pen and per female was lower in the 750 ppm group than in the controls ($P<0.05$). The proportions of eggs set that produced viable embryos, live 3-week embryos and normal hatchlings were significantly reduced at 750 ppm ($P<0.01$). The proportion of eggs laid that produced 14-day survivors was also significantly reduced at 750 ppm ($P<0.01$). The mean body weight of hatchlings and 14-day survivors were significantly reduced at 750 ppm ($P<0.01$). The number of 14-day survivors per adult female was significantly reduced at both 150 ppm ($P<0.05$) and 750 ppm ($P<0.01$). The no observed effect level (NOEL) was determined to be 30 ppm.

13. VERIFIED STATISTICAL RESULTS

Means and Standard Deviations of Endpoints

Endpoint	Control	30 ppm	150 ppm	750 ppm
Eggs laid (EL)	174 (27)	158 (48)	142 (40)	113 (62)
Eggs cracked (EC)	6.86 (3.44)	6.00 (3.16)	4.14 (2.34)	4.00 (2.45)

Endpoint	Control	30 ppm	150 ppm	750 ppm
Eggs set (ES)	155 (26)	142 (45)	128 (33)	100 (56)
Viable embryos (VE)	146 (22)	131 (36)	112 (18)	64.4 (32.2)
Live 3-wk embryos (LE)	138 (21)	122 (33)	103 (16)	48.4 (23.9)
Normal hatchlings (NH)	100.6 (25.0)	88.1 (22.7)	68.7 (13.0)	25.3 (13.1)
14-day-old survivors (HS)	99.0 (25.0)	86.7 (21.9)	68.0 (12.6)	23.9 (12.6)
Egg shell thickness	0.343 (0.011)	0.337 (0.011)	0.349 (0.011)	0.347 (0.015)
Hatchling weight	34.0 (4.5)	34.7 (1.4)	35.3 (1.0)	31.8 (1.3)
14-day-old survivor weight	254 (9)	263 (24)	264 (8)	218 (17)
Mean food consumption	190 (15)	194 (24)	178 (19)	181 (33)
Final weight of males	1124 (51)	1112 (52)	1161 (67)	1172 (62)
Final weight of females	1084 (45)	1049 (26)	1076 (67)	1049 (77)

Statistically Significant Endpoints

Endpoint	Statistical Method	Levels at which Effect Was Observed
Live 3-week embryos	Dunnett's Test	150 and 750 ppm
Normal hatchlings	Dunnett's Test	150 and 750 ppm
14-day-old survivors	Dunnett's Test	150 and 750 ppm
Viable embryos	Dunnett's Test	750 ppm
14-day survivor wt.	Dunnett's Test	750 ppm

14. REVIEWER'S COMMENTS

Statistical analysis by the reviewer found that, in addition to the number 14-day-old survivors, the number of live 3-

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week embryos and normal hatchlings were significantly reduced at both 150 and 750 ppm.