

UNDATED

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

1. CHEMICAL: PIRATE PC Code No.: 129093
2. TEST MATERIAL: AC 303,630 technical Purity: 94.5 %
3. CITATION : Reproduction study with AC 303,630 Technical in the Northern Bobwhite (*Colinus virginianus*)

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Title: see citation
Study Completion Date: 10/28/94
Laboratory: Bio-Life Associates, Ltd.
Sponsor: American Cyanamid Company
Laboratory Report ID: 105-024-07
MRID No.: 434928-11
DP Barcode: D210808

4. REVIEWED BY: N.E. Federoff, Wildlife Biologist, EEB, EFED
Signature: Date: 8/4/95
5. APPROVED BY: Ann Stavola, Head of Section (#), EEB, EFED
Signature: Date:
6. STUDY PARAMETERS

Scientific Name of Test Organism: (given in citation)
Age of Test Organisms at Test Initiation: 21 weeks of age
Definitive Study Duration: 168 consecutive treatment days
206 days (adult, incubation, and survival phases)
69-76 days (duration of adult exposure prior to egg laying)

7. CONCLUSIONS: This study is scientifically sound, although some irregularities did occur. Therefore it does not meet EPA guideline requirements for an avian reproduction study. With an NOEL of 0.5 ppm and a LOEC of 1.5 ppm, the primary effects are a significant difference in 14 day survivors at 4.5 ppm and a significant difference in hatchling weight in the 1.5 and 4.5 ppm treatment groups when compared to the control group. No clinical signs of toxicity were noted in either the adults or hatchlings during the study period.

Results Synopsis

Most sensitive endpoints:

NOEC: 0.5 ppm ai

LOEC: 1.5 ppm ai

8. ADEQUACY OF THE STUDY

A. **Classification:** Supplemental

B. **Rationale:** Irregularities during duration of study.

C. **Repairability:** The NOEC is low at 0.5 ppm and it is doubtful that a new study would provide significantly different results. Therefore the requirement for a new study is waived.

9. GUIDELINE DEVIATIONS

1. Adult birds (n=10) died during the study, eight of which suffered from excoriation and two from impacted intestines. One bird from the control, two birds from the 0.5 ppm group, one bird from the 1.5 ppm group, and four birds from the 4.5 ppm group were reported to have suffered and died from excoriation. A two hour power outage was reported while chicks from week 19 were in the brooders, and a fire, which killed numerous hatchlings, was reported while chicks from week 21 were in the brooders. Data from these 2 weeks was removed from the reported statistical analysis and may or may not have been significant in the final results.

10. SUBMISSION PURPOSE: To support registration for use on cotton.

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> A wild waterfowl species, preferably the mallard (<i>Anas platyrhynchos</i>), or an upland game species, preferably the northern bobwhite (<i>Colinus virginianus</i>)	Northern Bobwhite (<i>Colinus virginianus</i>)
<u>Age at beginning of test</u> Birds should be approaching their first breeding season.	21 weeks old
<u>Supplier</u> All birds should be from the same source.	Oak Ridge Game Farm Gravette, AR. 72736
Were birds pen-reared?	Not reported
Were birds phenotypically indistinguishable from wild birds?	Yes
<u>Health observation period</u> 2 to 6 weeks.	3 weeks
Were birds healthy and without excessive mortality prior to the test?	Yes with the exception of excoriation. No mortality was recorded during the quarantine period.

B. Test System

Guideline Criteria	Reported Information
Were pens for adult birds of adequate size and designed to conform to good husbandry practices?	One adult male and female per pen were housed in 30.5cm square pens constructed of 1" wire mesh.
Were pens for chicks of adequate size and designed to conform to good husbandry practices?	Chicks were divided by treatment group and housed in 91x71x28cm battery brooders (up to 30 chicks per brooder) in a room separate from the adults.
Where pens constructed of a nonbinding material such as galvanized or stainless steel?	Yes
Was adequate ventilation provided?	Yes
<u>Temperature</u> Approx. 21°C (70°F)	Min ave:20C Max ave:22C SD: not reported
<u>Relative humidity</u> Approx. 55%	Average relative humidity: 48% Range: 32-68%
<u>Lighting</u> First 8 weeks: 7 h per day. Thereafter: 16-17 h per day. At least 6 footcandles at bird level.	First 8 weeks: 7 h per day. Thereafter: 17 h per day. 6 footcandles at bird level.
<u>Diet</u> A commercial breeder feed (or its equivalent) that is appropriate for the test species.	Adults:Purina custom game bird Layena 28% was fed during duration of testing. Hatchlings:Purina game bird Startena was used during the 14-day post-hatch observation period.

Guideline Criteria	Reported Information
<p>Preparation of test diet A premix 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.</p>	<p>A 10 kg premix was prepared by blending the entire amount of test substance dissolved in acetone with the diet for 15 minutes. The remainder of the diet was added to the premix and blended for an additional 15 minutes. Thereafter, diets were stored at room temp. All diets were prepared weekly 24 hrs prior to being presented to adult birds.</p>
<p>Was the premix stored under conditions which maintain stability?</p>	<p>Yes</p>
<p>Was the diet analyzed to verify homogeneity and stability of the test substance?</p>	<p>Yes</p>
<p>Replenishment of feed</p>	<p>Not reported</p>

C. Test Design

Guideline Criteria	Reported Information
<p>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.</p>	<p>Nominal concentrations: Control, 0.5, 1.5, and 4.5ppm Max. residue level: 76.8ppm for short grass at a single maximum application rate of 0.32 lbs ai/A.</p>
<p>Control Vehicle control.</p>	<p>Test diet treated with Acetone</p>
<p>Vehicle Corn oil or other appropriate vehicle.</p>	<p>Acetone</p>

Guideline Criteria	Reported Information
<p><u>Vehicle amount (% of diet by weight)</u> Not more than 2%.</p>	1%
<p><u>Number of birds per pen</u> 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.</p>	1 male and 1 female per pen.
<p><u>Number of pens per group</u> 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.</p>	16 pens per group: 1 control group, 0.5ppm group, 1.5ppm group, and 4.5ppm group. 128 birds total.
<p><u>Pre-laying exposure duration</u> At least 10 weeks prior to the onset of egg-laying.</p>	10-11 weeks
<p><u>Exposure duration with egg-laying</u> At least 10 weeks.</p>	13-14 weeks
<p><u>Withdrawal period</u> If reduced reproduction is evident, a withdrawal period of up to 3 weeks may be added to the test phase.</p>	N/A

D. Egg Collection and Incubation

Guideline Criteria	Reported Information
Were eggs collected daily?	Yes
Egg storage temperature Approximately 16°C (61°F)	15.5°C average (60.5F average)
Egg storage humidity Approximately 65%	58% average relative humidity
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 on Day 10.
Transfer of eggs to hatcher Bobwhite: Day 21 Mallard: Day 23	Eggs were transferred on Day 21.
Hatching temperature 39°C (102°F) is recommended	37.2-37.8°C (ave:37.5 C)
Hatching humidity 70% is recommended	62%-77% (ave:69.5%)
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
<p>Collection Schedule At least once every two weeks (Week 1, 3, 5, 7 and 9).</p>	<p>Eggs were collected on the first day of eight separate intervals: weeks 11,13,15,17, 19,21,23,and 25.</p>
<p>Were shells opened, washed, and air dry for at least 48 hours before measuring?</p>	<p>Yes</p>
<p>Measurement 3-4 measurements per eggs to the nearest 0.01 mm.</p>	<p>3 measurements per egg to the nearest 0.01mm using an Ames Pocket Thickness Measure.</p>

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 test 0 .5 1.5 4.5 diet 0 .46 1.42 4.12 concentrations listed as ppm
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?	Yes, biweekly through week 10 and at termination of study.
Was average food consumption of adults reported at least biweekly?	Yes, calculated weekly.
<p>Reproductive Endpoints The following endpoints should be reported:</p> <ul style="list-style-type: none"> • 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 	<p>The following endpoints were measured:</p> <ul style="list-style-type: none"> *All eggs laid were reported. *All eggs cracked were noted. *All eggs set were reported. *All viable embryos reported. *Live 17-day embryos reported. *Normal hatchlings reported. *All 14-day survivors noted. *All weights of 14-day survivors were recorded. *All egg shell thickness noted *TFC reported for adults. **All day 1 and day 14 body weights were recorded but not recorded according to sex. Adult weights recorded by sex.
Were data reported by pen for all endpoints?	Yes, for those that were reported.

Significant Results: Reported statistical analysis, consisting of Levene's test and/or Dunnett's test, conducted by Bio-life Associates found these significant differences: Hatchling day 1 body weights at 4.5 and 1.5 ppm were significantly different than the control; the number of viable embryos/eggs set, live 17-day embryos/eggs set, and normal hatchlings/eggs set at 4.5 ppm were significantly different than the control; and the number of 14-day survivors/eggs set and 14-day survivors/chicks hatched at 0.5, 1.5, and 4.5 ppm were significantly different than the control.

13. VERIFIED STATISTICAL RESULTS

Means of Endpoints and SD (# below the mean)

Endpoint	Control	0.5ppm	1.5ppm	4.5ppm
Eggs laid (EL)	66.44 23.63	62.25 25.165	63.75 18.884	56.813 26.77
Eggs cracked (EC)	1.81 1.91	0.937 0.929	1.75 1.807	1.062 1.57
Eggs set (ES)	59.7 21.98	56.50 23.36	56.813 18.049	51.188 23.583
Viable embryos (VE)	57.5 21.45	53.063 22.101	53.813 18.741	43.625 25.303
Live 3-wk embryos (LE)	57.13 21.13	53.813 22.358	53.50 18.779	43.125 24.899
Normal hatchlings (NH)	53.31 20.35	49.313 21.291	49.250 19.696	38.563 22.491
14-day-old survivors (HS)	49.25 18.93	41.375 19.873	41.813 17.463	26.437 14.688
Egg shell thickness (THICK)	0.23 0.013	0.230 0.015	0.229 0.018	0.235 0.012
Hatchling weight (HATWT)	7.14 0.473	7.073 0.556	6.781 0.390	6.621 0.356
14-day-old survivor weight (SURVWT)	30.156 4.374	30.187 2.97	29.819 2.640	29.914 2.381
Mean food consumption (FOOD)	7429.75 621.616	6976.125 896.445	7260.50 730.577	6992.438 879.386
Final weight of males (POSTM)	218.533 20.33	223.187 17.482	222.312 16.688	219.786 19.974

Endpoint	Control	0.5ppm	1.5ppm	4.5ppm
Final weight of females (POSTF)	253.88 25.393	256.00 29.611	248.467 33.827	251.077 29.245

Statistically Significant Endpoints

Endpoint	Statistical Method	Levels at which Effect Was Observed
14 day survivors	Tukey's/Dunnett's	4.5 ppm
Hatchling weight	Tukey's Dunnett's	4.5 ppm 1.5 & 4.5 ppm

14. **REVIEWER'S COMMENTS:** Statistically significant endpoints other than those already mentioned above were also found using the Least Squared Means test (LSM) but were not reported due to the inflated experimentwise error caused by (LSM) comparisons.