5-17-89

Accession No. 407009-09

DATA EVALUATION RECORD

1. CHEMICAL: Ethyltrianol ~~Ethyltrianol~~ [Tebuconazole]

2. TEST MATERIAL: Technical HWG 1608 (FOLICUR^{TM}); 97.4% a.i., Batch No. PT16012/86.

3. STUDY TYPE: Avian Reproduction on the Mallard Duck. Species Tested: Anas platyrhynchos.

4. CITATION: Toll, P. A. 1988. Effects of HWG 1608 (FOLICUR^{TM}) on Mallard Duck Reproduction. Study No. 87-675-01. Prepared by Mobay Corp., Biochemistry/Wildlife Effects Group. Research and Development Dept. Stilwell, KA. Submitted by MOBAY Corp., Agricultural Chemicals Division, Kansas City, MO. EPA Accession No. 407009-09.

5. REVIEWED BY:

Jeffrey L. Lincer, Ph.D.,
Eco-Analysts, Inc.
Sarasota, FLorida

Signature:
Date: 11/13/88

6. APPROVED BY:

James R. Newman, Ph.D.,
Proj. Mgr., KBN Engineering and Applied Sciences, Inc.

Signature:
Date:

Henry T. Craven
Chief EEB/HED
USEPA

Signature:
Date: 5/17/89

7. CONCLUSIONS The submitted study is scientifically sound and concluded that feeding Ethyltianol (97.4% a.i.) up to 75.8 ppm did not produce any treatment-related effects in mallard ducks (Anas platyrhynchos). The study fulfills data requirements for an avian reproductive study.

8. RECOMMENDATIONS: N/A.

9. BACKGROUND: N/A.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES: N/A.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Test Animals: Pen-reared mallard ducks (Anas platyrhynchos) that were apparently healthy and phenotypically indistinguishable from wild birds were obtained from Whistling Wings Inc., Hanover, Il. All birds were from the same hatch and were approximately 18 weeks of age at test initiation. The birds were approaching their first breeding season and had not been used in previous testing. Test birds were acclimated to the laboratory facilities for 14 days prior to the initiation of the test. At test initiation, prior to final assignment to exposure groups, all birds were examined for physical injuries and general health. Birds that were injured or did not appear healthy were discarded.

Adult birds were identified by leg bands containing an individual animal number and were housed in cages containing a color-coded, numbered cage card. All eggs laid during the study were marked using a pencil with the cage number and study number for identification. Hatchlings were identified by individual wing bands as to parental cage. Brooders were identified by cage card containing parental dose group and hatch date.

B. Dosage and Design: The primary phases of the study and their approximate durations were:

1. Acclimation - two weeks.
2. Pre-photostimulation - eight weeks.
3. Pre-egg laying (with photostimulation) - two weeks.
4. Egg laying - ten weeks
5. Final incubation, hatching, and 14-day offspring rearing period - six weeks.

Treatment levels were based on known toxicity data from acute studies, a range finding study and similar triazole compounds. One hundred and twenty (60 drakes and 60 hens) were randomly distributed into four treatment groups as shown below.

Nominal Dose Concentration	Number of Pens	Birds per Pen Drakes	Hens
Control	15	1	1
5 ppm	15	1	1
20 ppm	15	1	1
80 ppm	15	1	1

The test compound was dissolved in corn oil and acetone then placed in a separatory funnel and slowly added to the feed while mixing in a 30-quart bowl of a Hobart Mixer. Acetone, which was also used as a rinsing agent for the glassware used in the preparation of the diets evaporated from the feed.

Each group contained 15 pairs of birds with one male and one female per pen. Three of the groups were fed a diet containing nominal concentrations of 5, 20 and 80 mg of technical HWG-1608 as active ingredient per kg of diet. The fourth group was fed control diet containing an amount of carrier (corn oil) equivalent to that in the treated diets (%). Each of the four groups of adult birds was fed the appropriate diet from test initiation until terminal sacrifice.

Fresh batches of diet were prepared weekly and stored in the freezer until used. After one week all uneaten diet was destroyed by incineration and fresh feed was offered to the birds.

Samples of the control and each of the test diets were taken weekly immediately after mixing and frozen. Samples taken on weeks 1, 5, 10, 15 and 20 were analyzed from HWG-1608 diet concentration. Concentrations were determined using high pressure liquid and gas chromatographic analysis (1).

HWG-1608 homogeneity in the diet was determined at 5 and 80 ppm by analyzing three samples of ration taken from three layers - top, middle and bottom (total of nine samples) of the mixing bowl. The concentrations from each layer were compared, using Duncan's Multiple Range test for homogeneous distribution of test article throughout the mixing bowl.

c. Statistics: The following end-points were subjected to statistical analysis.

Adult Body Weight	Survivor Body Weight
Adult Feed Consumption	Eggshell Strength
Eggs Laid	Eggshell Thickness
Eggs Cracked	Normal Hatchlings
Eggs Set	Hatchling Body Weight
Viable Embryos	Survivors
Viable Three Week Embryos	

Prior to analysis, all ratio data (i.e., percentage data) were transformed using a square root arcsin transformation (2). This was done to stabilize the variance of values and to more closely approximate a normal distribution. Bartlett's test of equal variance (2) was performed on the data for each end-point to determined if the dose groups have equal variances. If the variances were equal, subsequent analysis was conducted using parametric techniques; otherwise, nonparametric techniques were used.

For the parametric procedures, a standard one-way analysis of variance (ANOVA) using the F distribution to assess significance was used (2). If significant differences among the means were indicated, William's test was used to determined which treatment groups differed significantly from controls (3, 4).

For nonparametric procedures, the test of equality of means was performed using Kruskal-Wallis test (5). If significant differences among the means were indicated, Dunn's Summed Rank test was used to determine which treatment groups differed significantly from control (5).

The test for equal variance (Bartlett's test) was conducted at the 1% level of significance. All other tests were conducted at the 5% level of significance. All statistical analyses were conducted using software supplied by SAS Institute Inc., Cary, North Carolina.

12. REPORTED RESULTS:

"Diet Analysis

"Homogeneity studies conducted with HWG-1608 showed the material to be homogeneous in the gamebird ration at nominal concentrations of 5 and 80 ppm. The coefficient of variation for the 5 and 80 ppm nominal dietary groups was 6%

and 7% respectively. Duncan's Multiple Range test indicated no significant difference in concentration throughout the mixing bowl at either dietary level...

"HWG-1608 was stable in the avian ration with no real declines in the concentration for the 5 and 80 ppm nominal test levels. Recoveries ranged from 92 to 116% of nominal...

"The dietary concentrations for weeks 0, 5, 10, 15 and 20 were determined... The mean measured concentrations (4.6, 18.8 and 75.8 ppm) were 93, 94 and 95 percent of nominal respectively for the 5, 20 and 80 ppm nominal dose groups...

"Mortality

"There was no compound related or dose related mortality over the course of the study. One pair ... on the 18.8 ppm dietary level and two pair... from the 75.8 ppm group were sacrificed because the females began producing eggs prior to being on treated diets for the required 10 weeks.

"Clinical Observations

"No overt signs of toxicity were noted during the study. Occasional occurrences of feather loss, lacerations, etc., all associated with normal laboratory pen housing were observed.

"Gross Necropsy

"...There were no grossly observable compound-related or dose-related lesions seen in any of the birds.

"Adult Body Weight and Feed Consumption

"...There were no statistically significant differences between the control group and the treated levels in terms of body weight or feed consumption.

"Reproductive Results

"...There were no statistically significant differences between the control birds and the treated groups in any of the reproductive parameters examined. There was a slight dose-related trend to a lower hatch percentage... This difference; however, was not statistically different from controls.

"Offspring Body Weights and Survival

"...There were no statistically significant differences between the control group and treated groups in mean hatch weights, 14-day survivor weights or percent survival.

"Eggshell Strength and Thickness

"...There were no statistically differences shown by any of the treatment levels."

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

"Homogeneity studies conducted with HWG-1608 showed the material to be homogeneous and stable in the avian ration. The mean measured concentrations (4.6, 18.8 and 75.8 ppm) were 93, 94 and 95 percent of nominal respectively for the 5, 20 and 80 ppm nominal dose groups.

"There was no compound related or dose related mortality over the course of the study. One pair ... on the 18.8 ppm dietary level and two pair ... from the 75.8 ppm group were sacrificed because the females began producing eggs prior to being on treated diets for the required 10 weeks.

"No overt signs of toxicity were noted during the study. Occasional occurrences of feather loss, lacerations, etc., all associated with normal laboratory pen housing were observed. When postmortem examinations were performed there were no grossly observable compound-related or dose-related lesions seen in any of the birds.

"There were no significant statistical differences between the control group and the treated levels in terms of body weight or feed consumption.

"There were no statistically significant differences between the control birds and the treated groups in any of the reproductive parameters examined. There was a slight dose-related trend to a lower hatch percentage with the greatest difference from controls at the 75.8 ppm dietary level. This difference; however, was not statistically difference from controls. There were no statistically significant differences between the control group and treated groups in mean hatch weights, 14 day survivor weights or percent survival. There were no significant differences shown by any of the treatment levels in mean eggshell strength or thickness.

"Based on the results of this study, the no-effect concentration (NOEC) for technical HWG-1608 on mallard duck reproductive is 75.8 ppm, the highest level test."

"All phases of the study conducted for this study type have been inspected once every three months by the Quality Assurance Unit. Audit reports have been submitted to laboratory management and the study director, documenting the status of compliance with departmental standard procedures, the study protocol and Good Laboratory Practice regulations." A total of sixteen (16) audits were performed during this study.

"In compliance with the Good Laboratory Practice regulations, this final report for study number 87-675-01 has been reviewed by the Quality Assurance Unit. The results presented in this report accurately describe the methods and standard procedures and reflect the raw data collected during the conduct of the study."

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure(s):

(1) Raw data for feed consumption, weight gain and reproductive parameters supported text.

Of note, however, was a dose-related increase in "eggs cracked of eggs laid", going from 10% for the control to 24% for the 75.8 ppm group, which was not discussed by author. EEB/HED has indicated a 10% figure for percentage of cracked eggs in control groups is unusual. Applicant should be requested to respond to this point.

(2) Study followed guidelines, with the following exceptions:

(a) SEP (pg. 8) requires inspection of several specific organs. The author did not indicate adequate methodology details of gross necropsy in order to determine if these organs were systematically examined.

(b) SEP (pg. 8) requires that the day of death/effects must be reported. It was not.

B. Statistical Analysis: The reviewer reanalyzed the data using an ANOVA and Duncan's multiple range test (i.e., EPA's Bigbird computer program) and obtained the same conclusion. The printouts are attached.

C. Discussion/Results: Adult mallard ducks, which received technical HWG-1608 at nominal concentrations of 5, 20 and 80 ppm for 20 weeks, showed no compound related or dose related mortality over the course of the study. No overt signs of toxicity were noted during the study. Occasional occurrences of feather loss, lacerations, etc., all associated with normal laboratory pen housing were observed. When postmortem examinations were performed there were no grossly observable compound-related or dose-related lesions seen in any of the birds.

There were no statistically significant differences between control group and the treated levels in terms of body weight, feed consumption, or in any of the reproductive parameters examined. There was a slight dose-related trend in hatch percentage and "eggs cracked of eggs laid" with the greatest difference from controls at the 75.8 ppm dietary level. This difference; however, was not statistically different from controls. There were no statistically significant differences between the control group and treated groups in mean hatch weights, 14-day survivor weights or percent survival. There were no significant differences shown by any of the treatment levels in mean eggshell strength or thickness.

Based on the results of this study, the no-effect concentration (NOEC) for technical HWG-1608 on mallard duck reproduction is 75.8 ppm, the highest level test.

E. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: The study followed the SEP guidelines.

(3) Reparability: N/A.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, on 11/13/88

References

(1) Moore, K. D. 1988. The Determination of HWG-1608 (FOLICUR) in Avian Ration. Mobay Agricultural Chemicals Division Report No. 95693.

(2) Snedecor and Cochran, Statistical Methods, 6th Edition, The Iowa State Press, Ames, Iowa, 1971.

(3) Williams, D. A. "A Test for Differences Between Treatment Means When Several Dose Levels are Compared with a Zero Control", Biometrics, (27), pg. 103, 1971.

(4) Williams, D. A. "The Comparison of Several Dose Levels with a Zero Control", (28), pg. 519, 1972.

(5) Hollander, M. and Wolfe, D. A., Nonparametric Statistical Methods, John Wiley and Sons, New York, 1973.

ONE LINER SHEET

Shaughnessey No. _____ Chemical Name Ethyltrianol Chemical Class _____ Page _____ of _____

Study/Species/Lab Accession #	Chemical % a.i.	Results				Reviewer/ Date	Validation Status
Avian Reproduction Species: Mallard		Group	Dose (ppm)	Effected/Parameters	Mort.(%)	% CHe Inh.		
		Control	0	0/All	0	N/A		
	97.4	Treatment I	5	0/All	0	N/A	Lincer/ 11/13/88	Core
Lab: Mobay Project #: 87-675-01 MRID #: 407009-09		Treatment II	20	0/All	0	N/A		
		Treatment III	80	0/All	0	N/A		
		Study Duration:	28					

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