5-17-89

Accession No. 407009-10

DATA EVALUATION RECORD

1. CHEMICAL: Ethyltrianol

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

3. STUDY TYPE: Avian Reproduction on the Bobwhite Quail
Species Tested: Colinus virginianus

4. CITATION: Toll, P. A. 1988. Effects of HWG 1608 (FOLICUR^{TM}) on Bobwhite Quail Reproduction. Study No. 87-675-02. 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. 407009-10.

5. REVIEWED BY:

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

Signature:
Date: 11/17/88

6. APPROVED BY:

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

Signature: James R Newman
Date: 11/23/88

Henry T. Craven
Chief EEB/HED
USEPA

Signature:
Date: 5/17/89 Henry T. Craven 5/17/89

7. CONCLUSIONS The submitted study is scientifically sound and concluded that feeding Ethyltianol (97.4% a.i.) up to 73.5 ppm did not produce any treatment-related effects in bobwhite quail (Colinus virginianus). 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 bobwhite quail (Colinus virginianus) that were apparently healthy and phenotypically indistinguishable from wild birds were obtained from Sand Prairie Quail Farm, Maquoketa, Iowa. All birds were from the same hatch and were approximately 17 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 20 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 wing 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 - six weeks.
3. Pre-egg laying (with photostimulation) - seven 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. One hundred and forty-four (72 cocks and 72 hens) were randomly distributed into four treatment groups as shown below.

Nominal Dose Concentration	Number of Pens	Birds per Pen Drakes	Hens
Control	18	1	1
5 ppm	18	1	1
20 ppm	18	1	1
80 ppm	18	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 18 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, 20 and 25 were determined... The mean measured concentrations (4.96, 18.4 and 73.5 ppm) were 99, 92 and 92 percent of nominal respectively for the 5, 20 and 80 ppm nominal dose groups.

"Mortality

"Two female quail were found dead in each of the control and 18.4 ppm dietary groups, and one female was found dead in each of the 4.96 and 73.5 ppm dietary groups during the course of the study. These mortalities were due to cage mate aggression and not compound-related.... Two female quail from the 18.4 ppm group and one male from the 4.96 ppm treatment level were sacrificed **in extremis** due to fractured legs. The mates from all these birds which were either found dead or were sacrificed due to injuries were also sacrificed.

"Clinical Observations

"No overt signs of toxicity were noted during the study. Occasional occurrences of feather loss, lacerations, et., all associated with normal laboratory pen housing were observed. There was one female in each of the 4.96 and 18.4 ppm groups and one male in the 73.5 ppm group that had a nervous disorder affecting the carriage of the head and at times affected the birds general coordination. This effect was not considered compound-related since this same activity has been observed in birds which have not been exposed to pesticides. These birds appeared to have normal reproduction tendencies and there were no grossly observable causes for these actions when these birds were examined.

"Gross Necropsy

"...There were no grossly observable compound-related or dose-related lesions in any of the birds found dead or sacrificed when examined at time of necropsy.

"Adult Body Weight and Feed Composition

"...There was a slight reduction in body weight in both males and females of the 73.5 ppm dietary group...on week 24, the termination of the adult phase of the study. This difference; however, was not statistically significant. There was not statistical difference between groups in terms of feed consumption...

"Reproductive Results

"...The data from cages where the adults were either found dead or sacrificed, prior to or during egg laying, were not used for statistical purposes. There were no statistically significant differences between the control birds and the treated groups in any of the reproductive parameters examined. There was a slightly lower percentage of 21-day embryo survivors and slightly lower hatch percentage at the 75.8 ppm dietary level...These differences, however, were **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 significant treatment-related differences shown by any of the treatment levels in either shell strength or shell thickness."

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

"Homogeneity studies conducted with HWG-1608 showed the material to be homogeneous and stable when mixed with the avian ration. The measured dietary concentrations closely approximated nominal concentrations with a range of 92 to 99 percent nominal.

"Incidental mortality occurred on every level including control due to cage mate aggression. 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. There was one female in each of the 4.96 and 18.4 ppm groups and one male in the 73.5 ppm group that had a nervous disorder affecting the carriage of the head and at times

affected the birds [sic] general coordination. This effect was not considered compound-related since this same observation has been noted in previously untreated birds. These birds appeared to have normal reproduction tendencies and when the postmortem examination was conducted there were no observable compound-related or treatment-related lesions in these or any of the other birds examined.

"The 73.5 ppm dietary level male and female birds showed a slight weight reduction at the time of the adult sacrifice. This was not; however, a statistically significant difference. There was no differences [sic] between control and treatment groups in terms of 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 slightly lower percentage of 21-day embryo survivors and slightly lower hatch percentage 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 treatment-related differences shown by any of the treatment levels in either shell strength or shell thickness.

"Based on the results of this study, the no-effect concentration (NOEC) for technical HWG-1608 on bobwhite quail reproduction is 73.5 ppm, the highest level test."

"Seven audits were conducted prior to the experimental termination of this study. In addition, a draft of the final report was audited. 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 Practices regulations."

"In compliance with the Good Laboratory Practice regulations, this final report for study number 87-675-02 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 homogeneity and stability, feed consumption, weight gain and reproductive parameters supported text.

(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.

(c) SEP (4) requires that birds be maintained for first eight weeks under a regime of seven hours of light per day. Study kept birds under 7 hours light/day for the first six weeks and then increased it one hour per week through week 10, at which time it was increased to 17 hours light/day.

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 bobwhite quail, which received technical HWG-1608, at nominal concentrations of 0, 5, 20 and 80 ppm for 24 weeks, showed no statistically significant differences between the control group and treatment groups in any of the parameters; however the 73.5 mean dietary male and female quail showed a slight reduction in body weight compared to controls at adult termination.

Effects on reproduction and first generation offspring were monitored by egg production, viability (fertility), 21-day embryo survival, hatchability and hatchling body weight, 14-day survival and survivor body weight and eggshell strength/thickness

measurements. There were no significant differences in any of the reproductive parameters. The 73.5 ppm dietary group had a slight reduction in percent 21-day embryos and percent hatched; however, these differences were not statistically significant.

Based on the results of this study, the no-effect concentration (NOEC) for technical HWG-1608 on bobwhite quail reproductive is 73.5 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 11/17/88

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
		Group	Dose (ppm)	Effected/Parameters	Mort.(%)	% CHe Inh.		
Avian Reproduction Species: Bobwhite		Control	0	0/All	5.5	N/A		
	97.4	Treatment I	5	0/All	2.8	N/A	Lincer/ 11/17/88	Core
Lab: Mobay Project #: 87-675-02 AC #: 407009-10		Treatment II	20	0/All	5.5	N/A		
		Treatment III	80	0/All	2.8	N/A		
		Study Duration:	31 weeks					

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