MRID # 408837-40 2-6-90

Accession No. 408837-40

**DATA EVALUATION RECORD**

1. **CHEMICAL:** HLO 516-88. Shaughnessey Number: 129006.

2. **TEST MATERIAL:** H # 16,429; Phosphorothioic acid, O,O-diethyl O-(1,2,2,2-tetrachloroethyl) ester; 86% active ingredient; a pale yellow liquid.

3. **STUDY TYPE:** Avian Reproduction Study.
Species Tested: Mallard duck (Anas platyrhynchos).

4. **CITATION:** Beavers, J.B., K. Hoxter, and M. Jaber. 1988. H # 16,429: A One-Generation Reproduction Study with the Mallard (Anas platyrhynchos). Laboratory Project No. 112-191. Prepared by Wildlife International Ltd., Easton, Maryland. Submitted by E. I. duPont de Nemours and Company, Inc., Newark, Delaware. EPA Accession Number: 408837-40.

5. **REVIEWED BY:**

Jeffrey Bigler
Fishery Biologist
Ecological Effects Branch

**Signature:** JBig
**Date:** 2-6-90

6. **APPROVED BY:**

Ann Stavola
Acting Section Head
Ecological Effects Branch

**Signature:** Ann Stavola
**Date:** 2/6/90

7. **CONCLUSIONS:** Nominal dietary concentrations of H # 16,429 at 1 and 5 ppm did not result in treatment-related mortality, effects upon reproductive performance, overt signs of toxicity, or effects upon body weight among adult mallards during the 19 week exposure period. In the 25 ppm group, there were no treatment related mortalities, or effects upon reproductive performance. However, overt signs of toxicity were observed in two birds in the 25 ppm group, as well as differences ($P<0.05$) in female body weight when compared to the control group. The effects of H # 16,429 on food consumption were unclear, but there appears to have been a reduction in food consumption at 1, 5 and 25 ppm. The no-observed-effect concentration for H # 16,429 in this study was 5 ppm.

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5. **REVIEWED BY:**

Michael L. Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
Applied Sciences, Inc.

**Signature:** Michael L. Whitten
**Date:** 6-20-89

6. **APPROVED BY:**

James R. Newman, Ph.D.
Project Manager/
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

**Signature:** James R Newman
**Date:** 6/24/89

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

**Signature:**
**Date:**

7. **CONCLUSIONS:** Nominal dietary concentrations of H # 16,429 at 1 and 5 ppm did not result in treatment-related mortality, overt signs of toxicity or effects upon body weight or reproductive performance in adult mallards during the 19 week exposure period. At 25 ppm, there were no treatment related mortalities, and no effects upon body weight or reproductive performance. However, overt signs of toxicity were observed in two birds in the 25 ppm group. The effects of H # 16,429 on food consumption were unclear, but there appears to have been a reduction in food



2

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:** Submitted in support of an experimental use permit for Fortress 5G on corn.

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds employed in this study were unmated 22 week-old mallards received from Whistling Wings, Hanover, Illinois. All birds were acclimated to the facilities for 6 weeks prior to initiation of the test. Birds that did not appear healthy at test initiation were discarded.

B. **Dose/Diet Preparation/Food Consumption:** Test diets were prepared by mixing H # 16,429 into a pre-mix which was used for weekly preparation of the final diet. Control diet and three test concentrations (1, 5, and 25 ppm) were prepared weekly and presented to the birds on Wednesday of each week. The control diet contained an amount of the carrier (corn oil) and solvent (acetone) equal to that in the treated diets. Adults were fed a game bird ration formulated for breeding birds. All offspring received a game bird ration formulated for young growing birds. The test substance was not mixed into the diet of the offspring. Water and feed were supplied ad libitum during acclimation and during the testing in all pens except one. "In an attempt to discourage egg laying prior to photostimulation by one pen, water was withheld from that pen for periods of approximately 24 hours."

Samples of the control diet and each of the test diets were taken after mixing and during weeks 1 and 19. The samples were frozen immediately after collection and later analyzed for stability, homogeneity, and concentration of test substance. Food consumption in each pen was determined weekly throughout the study.

C. **Design:** The birds were randomly distributed into four groups as follows:

| Nominal Concentration | Number Of Pens | Birds Per Pen Males | Females |
|---|---|---|---|
| Control (0 ppm) | 16 | 1 | 1 |
| 1 ppm | 16 | 1 | 1 |
| 5 ppm | 16 | 1 | 1 |
| 25 ppm | 16 | 1 | 1 |

"Treatment levels were based upon a pilot reproduction study (WIL Project No. 112-185), expected field residue exposure levels, and consultation with the client." Adult birds were identified by individual leg bands. The primary phases of the study and their approximate durations were as follows:

1. Acclimation - 6 weeks.
2. Pre-photostimulation - 8 weeks.
3. Egg laying - 11 weeks.
4. Post-adult sacrifice (final incubation, hatching, 14-day offspring rearing period) - 6 weeks.

D. **Pen Facilities**: Adult birds were housed indoors in 75 cm x 90 cm x 45 cm high galvanized wire pens. The average temperature in the adult study room was 18.7°C ± 1.9°C (SD) with an average relative humidity of 44%.

The photoperiod during the first 8 weeks of the study was eight hours of light per day. The photoperiod was then increased to 17 hours of light per day, and was maintained at that length until terminal sacrifice. Birds received approximately 130 lux of illumination throughout the study.

E. **Adult Observations/Gross Pathology**: All adult birds were observed at least once daily throughout the study for signs of toxicity or abnormal behavior. All birds that died during the study were necropsied. At study termination, all surviving birds were sacrificed and necropsied. Adult birds were weighed at test initiation, at the end of weeks 2, 4, 6, 8, and at study termination.

F. **Eggs/Eggshell Thickness**: Eggs were collected daily, marked according to pen of origin, and washed to prevent pathogen contamination. The eggs were then stored at 10.1°C ± 1.7°C (SD) and 80% relative humidity until incubated. Eggs were removed from the storage room weekly and candled. Cracked or abnormal eggs were discarded. All eggs that were not cracked, abnormal or used for egg shell thickness measurements were placed in



4

an incubator at 37.4°C and 56% relative humidity. Eggs were candled again on day 14 of incubation to determine embryo viability and on day 21 to determine embryo survival. All eggs were turned automatically while in the incubator and placed in hatching trays on incubation day 24. Temperature in the hatcher was 37.2°C with a relative humidity of 73%.

Weekly throughout the egg laying period, one egg was collected, when available, from each of the odd numbered pens during the odd numbered weeks, and from each of the even numbered pens during the even numbered weeks. These eggs were used for egg shell thickness measurements. The average thickness of the dried shell plus membrane was determined by measuring (to the nearest 0.005 mm) five points around the waist of the egg using a micrometer.

G. **Hatchlings**: All hatchlings and unhatched eggs were removed from the hatcher on day 26 or 27 of incubation. The average body weight of the hatchlings by pen was then determined. Hatchlings were toe and web clipped for identification by pen of origin and then placed in galvanized wire mesh brooding pens until 14 days of age. Each brooding pen measured 72 cm x 90 cm x 24 cm high. Brooder temperatures were maintained at 38°C until the birds were 5-7 days of age, and 26°C thereafter. Ambient room temperature was 23°C ± 2.1°C (SD). The photoperiod was maintained at 17 hours of light per day. Hatchlings were fed untreated diet. At 14 days of age the average body weight by parental pen of all survivors was determined.

H. **Statistics**: Upon completion of the study, Dunnett's method was used to determine statistically significant differences between the control group and each of the treatment groups. Sample units were the individual pens within each experimental group. Percentage data were examined using Dunnett's method following arcsine transformation. The pens in which mortality occurred were not used in statistical comparisons of the data.

Each of the following parameters was analyzed statistically:

Adult Body Weight

Offspring's Body Weight

Adult Feed Consumption
Eggs Laid of Maximum Laid
Eggs Cracked of Eggs Laid
Viable Embryos of Eggs Set
Live 3-Week Embryos of Viable Embryos
Hatchlings of 3-Week Embryos
Hatchlings of Eggs Set
Hatchlings of Maximum Set
14-Day Old Survivors of Maximum Set
14-Day Old Survivors of Eggs Set
14-Day Old Survivors of of Hatchlings
Egg Shell Thickness

12. **REPORTED RESULTS**

A. **Diet Analysis**: The mean measured concentration of H #16,429 in the diets was 90%, 90%, and 89% of nominal concentration for the 1, 5, and 25 ppm groups, respectively.

B. **Mortality and Behavioral Reactions**: There was one mortality during the study: a female in the 25 ppm group was found dead during week 13. The bird displayed no abnormal behavior prior to death. Necropsy revealed extensive egg yolk peritonitis and air saculitis with a regressing reproductive tract. The mortality was considered unrelated to treatment.

No overt signs of toxicity were observed in the 1 ppm group. One female in the 5 ppm group was observed walking stiff-legged from week 6 until week 9. The bird eventually displayed lesions of bumblefoot, which may have contributed to the abnormal gait.

Two birds in the 25 ppm group displayed overt signs of toxicity. A female exhibited stiff-legged ataxia from the second day of the study until study termination. A male was noted with leg tremors from week 3 until the bird was sacrificed during week 13. No foot lesions or leg abnormalities were noted in either bird upon necropsy. An additional female in the 25 ppm group displayed a stiff-legged gait during week 14. This bird suffered from bumblefoot which may have contributed to the abnormal gait.

All other birds at all concentrations appeared normal throughout the study.

Necropsy of all surviving adults was conducted at study termination. All lesions observed were considered to be incidental and not related to treatment.

C. **Adult Body Weight and Food Consumption**: No significant



6

differences in body weights between the control and any treatment group were noted throughout the investigation.

"Due to excessive wastage by some birds, feed consumption was variable between pens." When compared to the control group, at 1 ppm there were significant decreases in food consumption during weeks 13, 17 ($p<0.05$) and 18 ($p<0.01$). At 5 ppm there was a significant decrease ($p<0.05$) during week 18. "These differences were considered to be incidental to treatment."

"At 25 ppm, when compared to the control group, there may have been a slight treatment related reduction in feed consumption that was statistically significant at $p<0.05$ during weeks 2, 3, 13 and 17, and at $p<0.01$ during weeks 1, 6 and 18" (Table 2 and Figure 3, attached).

D. **Reproduction**: When compared to the control group, there were no significant differences in reproductive parameters at any concentration tested (Tables 3 & 3A, attached).

E. **Egg Shell Thickness**: When compared to the control group, there were no significant differences in egg shell thickness at any concentration.

F. **Offspring Body Weight**: There were no significant differences between groups in body weights of offspring at hatching or at 14 days of age.

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

"Dietary concentrations of H # 16,429 at 1 and 5 ppm did not result in treatment-related mortality, overt signs of toxicity or effects upon body weight or feed consumption among adult mallards during the 19 week exposure period. In the 25 ppm treatment group, there were no treatment related mortalities or effects upon body weight. However, overt signs of toxicity were observed in two birds and there may have been a slight reduction in feed consumption. No treatment related effects upon reproductive performance were noted at any concentration. The no-observed-effect concentration for H # 16,429 in this study was 5 ppm." The study was conducted in conformance with Good Laboratory Practice regulations. The data were inspected and the final report signed by the Quality Assurance Officer of Wildlife International, Ltd.

14. **Reviewer's Discussion and Interpretation of the Study:**

A. **Test Procedures:** The test procedures were in accordance with the SEP and Subdivision E guidelines except for the following deviations:

Eggs were stored at a temperature of approximately 10°C and a relative humidity of approximately 81%; 16°C and 65% are recommended.

Observations on food palatability were not reported.

Behavioral observations of offspring were not reported.

B. **Statistical Analysis:** Statistical procedures differed from recommended methods. Specifically, there is no basis for transforming the number of eggs laid and the number of hatchlings to percentile values of the maximum number of eggs laid or set in any test group.

Analyses of reproductive parameters were verified (attached) and found to match those reported by the authors.

C. **Discussion/Results:** The causes of differences in food consumption between groups are difficult to ascertain. Table 2 and Figure 3 (attached) show that food consumption in the control group was higher than in any treatment group. This suggests that the birds were repelled by the test material. The authors state that food consumption varied between pens due to excessive wastage by some birds. This explains between pen variation but not between group variation. If these differences were due simply to wastage, then treatment means both above and below control means would be expected.

The authors did not report observations on reduced food palatability nor provisions for minimizing food spillage. Both items should have been discussed in this situation. Though not significant at $P<0.05$, there are indications of food repellancy at all three test levels when compared to the controls and should be considered treatment related.

Gross pathological observations conducted at the termination of the study indicate an abnormally high (50%) occurance of "Bumblefoot" amoung the females in all treatment levels, including the control group. Though this problem is evidently not treatment related,

questions could arise concerning the overall health of the females used in the study.

Nominal dietary concentrations of H # 16,429 at 1 and 5 ppm did not result in treatment-related mortality, effects upon reproductive performance, overt signs of toxicity, or effects upon body weight among adult mallards during the 19 week exposure period. In the 25 ppm group, there were no treatment related mortalities, or effects upon reproductive performance. However, overt signs of toxicity were observed in two birds in the 25 ppm group, as well as differences ($P<0.05$) in female body weight when compared to the control group. The effects of H # 16,429 on food consumption were unclear, but there appears to have been a reduction in food consumption at 1, 5 and 25 ppm. The no-observed-effect concentration for H # 16,429 in this study was 5 ppm.

D. **Adequacy of the Study:**

**(1) Classification:** Core

**(2) Rationale:** N/A

**(3) Repairability:** N/A

15. **COMPLETION OF ONE-LINER:** N/A.

Fortress

Page_____ is not included in this copy.

Pages 10 through 12 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Shaughnessey No. 129006 Chemical Name HLO-516-88 Chemical Class _____ Page 1 of 1

| Study/Species/Lab/ Succession | Chemical % Active | Results | | | | | Reviewer/ Date | Valid Stat |
|---|---|---|---|---|---|---|---|---|
| | | Group | Dose(ppm) | Effected/Parameters | Mort.(%) | ChE Inh. | | |
| Avian Reproduction, | | Control | 0 | NONE | 0 | NONE | | |
| Species: Mallard (Anas platyrhynchos) | 86% | Treatment I | 1 | NONE | 0 | | ML. WHITTEN CORE 6-15-89 | |
| Lab: Wildlife International Ltd. | | Treatment II | 5 | NONE | 0 | | | |
| Acc 408837-40 | | Treatment III | 25 | FOOD CONSUMPTION, BEHAVIOR | 3% | | | |
| | | Study Duration: 19 WEEKS | | | | | | |
| | | Comments: | | | | | | |

| Field Study(Simulated/Actual) Species: | Group | Rate(ai/a) | Treatment Interval | Total # Treatments | Mor.(%) | | |
|---|---|---|---|---|---|---|---|
| | Control | | | | | | |
| | Treatment I | | | | | | |
| Lab: | Treatment II | | | | | | |
| Acc. | Treatment III | | | | | | |
| | Crop/Site: | | Study Duration: | | | | |
| | Comments: | | | | | | |

| Chronic fish, Species | Concentrations Tested (pp__)= | | | |
|---|---|---|---|---|
| | MATC = >____ <____ pp__. | Effected Parameter = | | |
| Lab: | Contr. Mort.(%)= | Sol. Contr. Mort.(%)= | | |
| Acc. | Comments: | | | |

| Chronic invertebrate Species | Concentrations Tested (pp__)= | | | |
|---|---|---|---|---|
| | MATC =>____< ____pp__. | Effected Parameter(s) | | |
| Lab | Contr. Mort.(%)= | Sol. Contr. Mort.(%)= | | |
| Acc. | Comments: | | | |