Accession No. 404766-02C

**DATA EVALUATION RECORD**

1. **CHEMICAL:** Pyridate Technical
Shaughnessey Number: 128834

2. **TEST MATERIAL:** Pyridate Technical, 93.3% active ingredient.

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

4. **CITATION:** Beavers, J.B., K.A. Hoxter, and M.J. Jaber. 1987. Pyridate Technical: A One-Generation Reproduction Study with the Mallard (Anas platyrhynchos). Laboratory Project No. 217-103. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Gilmore, Inc., Memphis, TN. EPA Accession No. 404766-02C.

5. **REVIEWED BY:**

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

**Signature:** Michael L. Whitten
**Date:** 2-28-89

6. **APPROVED BY:**

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

**Signature:** James R Newman
**Date:** 3/1/89

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

**Signature:** Daniel Rieder 4-25-89
**Date:** Henry T. Craven 3/25/89

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for an avian reproductive test. There were no treatment related effects upon adult mallards exposed to nominal dietary concentrations of 256, 640, or 1600 ppm pyridate technical. The number of 14-day survivors was significantly lower ($p < 0.05$) in the 1600 ppm group compared to the control group. The NOEL was 640 ppm.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds employed in this study were unmated 23-week old mallards received from Whistling Wings, Hanover, Illinois. All birds had been under observation for a 7-week pre-test period for laboratory acclimation. Birds that did not appear healthy at test initiation were discarded.

B. **Dose/Diet Preparation/Food Consumption:** Test diets were prepared by mixing pyridate technical into a pre-mix which was used for preparation of the final diet. Control diet and three test concentrations (256, 640, and 1600 ppm) were prepared weekly and presented to birds on Monday of each week. The control diet contained an amount of the carrier (corn oil) and solvent (acetone) equal to that in the treated diets. Dietary concentrations were not adjusted for purity of the test material. Adults were fed a game bird ration formulated for breeding birds. All offspring received a game bird ration formulated for young growing birds. Water and feed were supplied ad libitum during acclimation and during the test. Exceptions occurred in six pens where it was necessary to to temporarily remove water to discourage pre-photostimulation egg production (see Section 12D).

Samples of the control and test diets were taken for analysis weekly after mixing and frozen immediately after collection.

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 |
| 256 ppm | 16 | 1 | 1 |
| 640 ppm | 16 | 1 | 1 |
| 1600 ppm | 16 | 1 | 1 |

Treatment levels "were based on known toxicity 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 - 7 weeks.
2. Pre-photostimulation - 9 weeks.
3. Egg laying (post-photostimulation) - 9 weeks.
4. Post-adult sacrifice (final incubation, hatching, 14-day offspring rearing period) - 5 weeks.

D. **Pen Facilities**: Adult birds were housed indoors in 75 cm x 90 cm x 45 cm high wire pens. The average temperature in the adult study room was 19.0°C ± 1.7°C (SD) with an average relative humidity of 40%.

During acclimation and in the first five weeks of the study, the birds were maintained under a photoperiod of eight hours of light per day. The photoperiod was decreased to seven hours of light per day at the beginning of week 6. During week 10, the photoperiod was increased to 17 hours of light per day and was maintained at that lenght until terminal sacrifice. Birds received approximately 12 footcandles 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. A record was maintained of all mortalities and observations. At study termination, all 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 11°C and 75% 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 an incubator at 37.5°C and 54% relative humidity. Eggs were candled again on day 14 of incubation to determine embryo viability and on day 21 to determine embryo surrvival. All eggs were turned automatically while in the incubator and placed in hatching trays on incubation day 24. Temperature in the hatcher was 37°C with a relative humidity of 70%.

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 27 or 28 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 to 7 days of age and 26°C thereafter. Ambient room temperature was maintained at 23.8°C ± 2.5°C. The photoperiod was maintained at 16 hours of light per day. Hatchlings were fed untreated diet. At 14 days of age the average body weight by parental pen of all surviving chicks 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 Feed Consumption
Adult Body Weight
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
Offspring's Body Weight
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
Eggshell Thickness

12. **REPORTED RESULTS**

A. **Diet Analysis**: One shipment of diet samples, containing diets collected from weeks 1-3, were misplaced by the carrier. These samples were shipped on January 6, 1987, but were not received by the analytical laboratory until June 1987. Analysis of diets (excluding those delayed in shipment) yielded values that ranged from 79% to 109% of nominal with an average of 94% (Table 6, attached). Nominal and mean measured concentrations were as follows:

| Nominal Concentration (ppm) | Mean Measured Concentration (ppm) |
|---|---|
| 256 | 245 |
| 640 | 595 |
| 1600 | 1479 |

B. **Mortality and Behavioral Reactions**: There were no adult mortalities during the course of the study in either the control, 256, 640 or 1600 ppm treatment groups.

No overt signs of toxicity were observed in any group. "Aside from lesions or observations normally associated with pen wear and/or interaction among pen mates and other incidental clinical signs which were considered to be unrelated to treatment, all birds at all concentrations appeared normal throughout the study."

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

C. **Adult Body Weight and Food Consumption**: No significant differences in body weights between the control and treatment groups were noted throughout the investigation.

Food consumption varied between pens due to excessive feed wastage by some birds. However, there were no significant differences in food consumption between the control and any treatment group during the study.

D. **Reproduction**: During week 5, one hen from the 640 ppm group was noted to be in egg production. The photoperiod was then lowered from eight to seven hours of light per day to discourage further egg production. Eggs were subsequently laid, prior to photostimulation, by two hens in the control group, one additional hen in

the 640 ppm group, and two hens in the 1600 ppm group. To augment the reduction in photoperiod, water was periodically withdrawn for no more than 24 hours at a time from pens with hens in production. Eggs collected prior to photostimulation were considered to be aberrant and were not used in egg production data for that hen.

When compared to controls, there was a significant decrease in the ratio of cracked eggs to eggs laid at 640 ppm ($p < 0.05$) and 1600 ppm ($p < 0.01$). These differences were not attributed to treatment and appeared to be a result of a high percent of cracked eggs in the control group.

Reproduction in the 1600 ppm group tended to be lower than the control and other treatment groups in three parameters: hatchlings/live 3 week embryos, hatchlings/ eggs set, and 14-day survivors/eggs set (Table 3A, attached). However, these differences were not statistically significant. Other reproductive parameters showed no apparent or significant differences between the control and treatment groups.

E. **Egg Shell Thickness:** No significant differences in egg shell thickness were noted between treatment groups and the control group.

F. **Offspring Body Weights:** There were no significant differences in body weights of hatchlings or 14-day old survivors at any concentration tested.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
Dietary concentrattions of pyridate technical at 256, 640, and 1600 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 18 week exposure period. While not statistically significant, at 1600 ppm there appeared to be a slight reduction in hatchlings of 3-week embryos, hatchlings of eggs set, and 14-day survivors of eggs set. The no-observed-effect concentration for pyridate technical in this study was greater than 640 ppm and less than 1600 ppm.

A quality assurance audit was performed by the Quality Assurance Manager. The final report was determined to be an accurate reflection of the obtained results.

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

A. **Test Procedures:**

The percentage of cracked eggs in the control group (9%, Appendix VII) is noted. Values ranged from 0 to 29%. According to the Technical Support Document of Subdivision E - Hazard Evaluation: Wildlife and Aquatic Organisms, the percentage of cracked eggs for caged mallards is normally 0.6% to 6.0%. Eight cages in the control group exceeded 6.0%. No explanation was provided by the study author for these unusually large values. Since the cracked egg percentages in the two highest treatment groups (640 ppm and 1600 ppm) fell within the normal range, it appears that differences among groups were not treatment related.

Other deviations from required or recommended procedures are as follows:

The average relative humidity in adult pens was 40%. The SEP recommends 55%.

Adult birds were exposed to 12 foot-candles of illumination; 6 foot-candles is recommended.

Eggs were stored at a temperature of 11°C and a relative humidity of approximately 75%; 16°C and 65% are recommended.

B. **Statistical Analysis**: Statistical procedures for analyses of eggs laid, hatchlings per hen and 14-day survivors differed from recommended methods. Specifically, there is no basis for transforming these values to percentiles of the maximum number of eggs laid or set in any test group, which were then used in statistical procedures. The SEP is clear concerning how these parameters should be analyzed. The reviewer's analysis (attached) of eggs laid and number hatched, however, showed no significant differences between control and treatment groups. The analysis of 14-day survivors showed a significant ($p < 0.05$) decrease in the 1600 ppm group when compared to the control group.

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

C. **Discussion/Results**: There were no treatment related effects upon adult mallards exposed to dietary concentrations of 256, 640, or 1600 ppm pyridate technical. The number of 14-day survivors was

significantly lower ($p < 0.05$) in the 1600 ppm group compared to the control group. The NOEL was 640 ppm.

D. **Adequacy of the Study:**

**(1) Classification:** Core

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

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

15. **COMPLETION OF ONE-LINER:** Yes, February 28, 1989.

TABLE 6

DIET ANALYSES SUMMARY OF FEED SAMPLES FOR PYRIDATE TECHNICAL

PYRIDATE TECHNICAL - PROJECT NO. 217-103

Diet Analyses - Day 0

| Week | Nominal Concentration (ppm) | | | |
|---|---|---|---|---|
| | 0 | 256 | 640 | 1600 |
| 1 | 0 | 43.9 | 143.8 | 693.9 |
| 2 | 0 | 64.3 | 156.6 | 562.5 |
| 3 | 0 | 50.4 | 179.5 | 608.0 |
| 4 | 0 | 245.3 | 590.4 | 1463.3 |
| 5 | 0 | 225.2 | 557.6 | 1416.3 |
| 6 | 0 | 203.4 | 539.2 | 1379.4 |
| 7 | 0 | 264.4 | 590.3 | 1493.7 |
| 8 | 0 | 271.8 | 627.1 | 1555.7 |
| 9 | 0 | 252.7 | 700.5 | 1635.9 |
| 10 | 0 | 242.4 | 603.5 | 1581.5 |
| 11 | 0 | 244.7 | 595.2 | 1412.5 |
| 12 | 0 | 260.5 | 588.4 | 1493.4 |
| 13 | 0 | 252.3 | 629.2 | 1498.3 |
| 14 | 0 | 256.7 | 571.1 | 1426.2 |
| 15 | 0 | 233.8 | 604.5 | 1500.8 |
| 16 | 0 | 247.1 | 577.0 | 1524.4 |
| Extra | 0 | 244.9 | 622.6 | 1500.6 |
| 17 | 0 | 242.0 | 564.8 | 1533.9 |
| 18 | 0 | 231.4 | 581.7 | 1555.6 |
| Extra | 0 | 244.9 | 569.6 | 1402.7 |

TABLE 3

REPRODUCTIVE DATA - MALLARD

PYRIDATE TECHNICAL - PROJECT NUMBER 217-103

| | | PYRIDATE TECHNICAL | | |
|---|---|---|---|---|
| | 0 PPM | 256 PPM | 640 PPM | 1600 PPM |
| Eggs Laid | 629 | 740 | 678 | 667 |
| Eggs Cracked | 50 | 43 | 24 | 19 |
| Eggs Set | 614 | 629 | 591 | 587 |
| Viable Embryos | 484 | 594 | 555 | 536 |
| Live 3-Week Embryos | 483 | 592 | 554 | 535 |
| Hatchlings | 395 | 500 | 447 | 360 |
| 14-Day Old Survivors | 390 | 497 | 441 | 356 |
| Eggs Laid/Hen | 39 | 46 | 42 | 42 |
| Eggs Laid/Hen/Day @ | 0.70 | 0.83 | 0.76 | 0.74 |
| 14-Day Old Survivors/Hen | 24 | 31 | 28 | 22 |

@ - Based on 56 days.

TABLE 3A

REPRODUCTIVE DATA - (%) - MALLARD

PYRIDATE TECHNICAL - PROJECT NUMBER 217-103

| | | PYRIDATE TECHNICAL | | |
|---|---|---|---|---|
| | 0 PPM | 256 PPM | 640 PPM | 1600 PPM |
| Eggs Laid | 629 | 740 | 678 | 667 |
| Eggs Laid/Max. Laid (%) | 67 | 78 | 72 | 71 |
| Eggs Cracked/Eggs Laid (%) | 9 | 6 | 4* | 3** |
| Viable Embryos/Set (%) | 94 | 94 | 94 | 90 |
| Live 3-Week Embryos/Viable (%) | 100 | 100 | 100 | 100 |
| Hatchlings/3-Week (%) | 80 | 84 | 81 | 67 |
| 14-Day Old Survivors/Hatch (%) | 99 | 99 | 99 | 99 |
| Hatchlings/Set (%) | 76 | 79 | 76 | 61 |
| 14-Day Old Survivors/Set (%) | 75 | 78 | 75 | 60 |
| Hatchlings/Max. Set (%) | 45 | 57 | 51 | 41 |
| 14-Day Old Survivors/Max. Set (%) | 44 | 56 | 50 | 40 |

* Difference from the control statistically significant at $p < .05$.
** Difference from the control statistically significant at $p < .01$.

APPENDIX VII
PAGE 3
REPRODUCTIVE DATA - MALLARD
PYRIDATE TECHNICAL - PROJECT NUMBER 217-103
EGGS CRACKED/EGGS LAID (%)

| | 0 PPM | | | | 256 PPM | | | | 640 PPM | | | | 1600 PPM | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pen | No. Crack | No Laid | % | Arcsin Trans. | No. Crack | No. Laid | % | Arcsin Trans. | No. Crack | No. Laid | % | Arcsin Trans. | No. Crack | No. Laid | % | Arcsin Trans. |
| 1 | 3 | 56 | 5 | 13.38 | 2 | 36 | 6 | 13.63 | 2 | 49 | 4 | 11.66 | 0 | 38 | 0 | 0.00 |
| 2 | 0 | 41 | 0 | 0.00 | 1 | 46 | 2 | 8.48 | 0 | 59 | 0 | 0.00 | 3 | 42 | 7 | 15.50 |
| 3 | 1 | 43 | 2 | 8.77 | 3 | 46 | 7 | 14.80 | 0 | 39 | 0 | 0.00 | 4 | 43 | 9 | 17.76 |
| 4 | 1 | 42 | 2 | 8.88 | 3 | 45 | 7 | 14.96 | 1 | 39 | 3 | 9.21 | 0 | 44 | 0 | 0.00 |
| 5 | 8 | 43 | 19 | 25.55 | 0 | 57 | 0 | 0.00 | 2 | 44 | 5 | 12.31 | 1 | 50 | 2 | 8.13 |
| 6 | 5 | 54 | 9 | 17.72 | 4 | 46 | 9 | 17.15 | 2 | 42 | 5 | 12.60 | 1 | 49 | 2 | 8.21 |
| 7 | 4 | 34 | 12 | 20.06 | 1 | 40 | 3 | 9.10 | 1 | 32 | 3 | 10.18 | 0 | 38 | 0 | 0.00 |
| 8 | 4 | 52 | 8 | 16.10 | 2 | 51 | 4 | 11.42 | 1 | 32 | 3 | 10.18 | 0 | 40 | 0 | 0.00 |
| 9 | 4 | 14 | 29 | 32.31 | 7 | 39 | 18 | 25.07 | 0 | 48 | 0 | 0.00 | 2 | 46 | 4 | 12.04 |
| 10 | 2 | 41 | 5 | 12.76 | 1 | 40 | 3 | 9.10 | 0 | 44 | 0 | 0.00 | 2 | 44 | 5 | 12.31 |
| 11 | 1 | 16 | 6 | 14.48 | 5 | 57 | 9 | 17.23 | 5 | 53 | 9 | 17.89 | 1 | 31 | 3 | 10.35 |
| 12 | 1 | 32 | 3 | 10.18 | 2 | 44 | 5 | 12.31 | 4 | 42 | 10 | 17.98 | 2 | 39 | 5 | 13.09 |
| 13 | 2 | 41 | 5 | 12.76 | 6 | 46 | 13 | 21.17 | 1 | 40 | 3 | 9.10 | 1 | 45 | 2 | 8.57 |
| 14 | 4 | 28 | 14 | 22.21 | 3 | 48 | 6 | 14.48 | 2 | 44 | 5 | 12.31 | 0 | 51 | 0 | 0.00 |
| 15 | 7 | 46 | 15 | 22.96 | 2 | 46 | 4 | 12.04 | 1 | 35 | 3 | 9.73 | 0 | 24 | 0 | 0.00 |
| 16 | 3 | 46 | 7 | 14.80 | 1 | 53 | 2 | 7.90 | 2 | 36 | 6 | 13.63 | 2 | 43 | 5 | 12.45 |
| Total | 50 | 629 | | | 43 | 740 | | | 24 | 678 | | * | 19 | 667 | | ** |
| Mean | 3 | 39 | 9 | 15.81 | 3 | 46 | 6 | 13.05 | 2 | 42 | 4 | 9.17 | 1 | 42 | 3 | 7.40 |
| sd | 2 | 12 | 7 | 7.69 | 2 | 6 | 5 | 5.81 | 1 | 7 | 3 | 6.05 | 1 | 7 | 3 | 6.41 |

* Difference from the control statistically significant at $p < .05$.
** Difference from the control statistically significant at $p < .01$.

(Continued)

SH. No. 128834 Chemical Name Pyridate Chemical Class _____ Page 1 of 1

Study/Species/Lab/ Succession _____ Chemical % Active

Avian Reproduction,

Species:
Anas platyrhynchos 93.3

Lab:
Wildlife International Ltd

Acc
404766-62C

Results

| Group | Dose(ppm) | Effected/Parameters | Mort.(%) | %Chg Inh. | Reviewer/ Date | Validation Status |
|---|---|---|---|---|---|---|
| Control | 0 | NONE | 0 | N/A | | |
| Treatment I | 256 | NONE | 0 | / | MLW 2-28-89 | CORE |
| Treatment II | 640 | NONE | 0 | / | | |
| Treatment III | 1600 | #14 DAY-OLD SURVIVORS | 0 | N | | |

Study Duration: 18 WEEKS

Comments: NOEL: 646 ppm

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Field Study(Simulated/Actual)
Species:

_____

Lab:

Acc.

| Group | Rate(ai/a) | Treatment Interval | Total # Treatments | Mor.(%) |
|---|---|---|---|---|
| Control | | | | |
| Treatment I | | | | |
| Treatment II | | | | |
| Treatment III | | | | |

Crop/Site: Study Duration:

Comments:

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Chronic fish,
Species

_____

Lab:

Acc.

Concentrations Tested (pp__)= _____

MATC = >____ <____ pp__. Effected Parameter = _____

Contr. Mort.(%)= _____ Sol. Contr. Mort.(%)= _____

Comments:

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Chronic invertebrate
Species

_____

Lab

Acc.

Concentrations Tested (pp__)= _____

MATC =>____< ____pp__. Effected Parameter(s) _____

Contr. Mort.(%)= _____ Sol. Contr. Mort.(%)= _____

Comments:

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