Accession No. 404766-03C

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: Bobwhite quail
(Colinus virginianus)

4. **CITATION:** Beavers, J.B., K.A. Hoxter, and M.J. Jaber. 1987. Pyridate Technical: A One-Generation Reproduction Study with the Bobwhite (Colinus virginianus). Laboratory Project No. 217-102. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Gilmore, Inc., Memphis, TN. EPA Accession No. 404766-03C.

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:

7. **CONCLUSIONS:** Nominal dietary concentrations of pyridate technical at 256, 640, or 1600 ppm did not result in effects on bobwhite quail reproductive parameters. The study is scientifically sound and meets the requirements for an avian reproductive test. The NOEL was 1600 ppm, the highest concentration tested.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

35

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds employed in this study were unmated 38-week old Bobwhite quail received from Fritts Quail Farm, Phillipsburg, New Jersey. All birds had been under observation for a 13-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 as well as a water soluble vitamin mix in their water from day of hatch until 14 days of age. Water and feed were supplied ad libitum during acclimation and during the test.

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

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 - 13 weeks.
2. Pre-photostimulation - 7 weeks.
3. Pre-egg laying (with photostimulation) - 3 weeks.
4. Egg laying - 9 weeks.
5. Post-adult sacrifice (final incubation, hatching, 14-day offspring rearing period) - 5 weeks.

D. **Pen Facilities**: Adult birds were housed indoors in 30 cm x 51 cm galvanized wire pens. The pens had sloping floors resulting in a ceiling height of 21 to 26 cm. The average temperature in the adult study room was 20.0°C ± 2.1°C (SD) with an average relative humidity of 39%.

During acclimation and in the first eight weeks of the study, the birds were maintained under a photoperiod of eight hours of light per day. From week 8 until terminal sacrifice, the photoperiod was 17 hours of light per day. 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. All birds that died during the study were necropsied. At study termination, all remaining 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 stored at 11°C and 81% relative humidity until incubated. Eggs were removed from the storage room weekly and candled. Cracked eggs were discarded. All eggs that were not cracked or used for egg shell thickness measurements were then fumigated to prevent pathogen contamination. After fumigation, the eggs were placed in an incubator at 37.5°C and 54% relative humidity. Eggs were candled again on day 11 or 12 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 21. Temperature in the hatcher was 37°C with a relative humidity of 54%.

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.

G. **Hatchlings:** All hatchlings and unhatched eggs were removed from the hatcher on day 25 or 26 of incubation. The average body weight of the hatchlings by pen was then determined. Hatchlings were leg banded 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 23 cm high and was maintained at 38°C. 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	243
640	592
1600	1484

B. **Mortality and Behavioral Reactions**: Two adult mortalities occurred during the study; both appeared to be incidental to treatment. One male from the 256 ppm group was discovered with a broken neck following study initiation. No other lesions were observed. A female from the 640 ppm group was found dead at the end of week 4. Necropsy revealed bloodied carpal joints and a broken neck. No other lesions were observed. Pen mates of both mortalities were sacrificed and necropsied, with unremarkable results.

No overt signs of toxicity were observed in any group. One female in the control group exhibited a ruffled appearance and slight wing droop. One female in the 640 ppm group exhibited lower limb weakness associated with lameness. Neither bird's symptoms were attributed to treatment.

Necropsy of all surviving adults was conducted at study termination (Appendix IV, attached). 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 or food consumption between treatment groups and the control group were noted throughout the investigation.

D. **Reproduction**: A significant ($p < 0.05$) decrease in the number of viable embryos, hatchlings, and 14-day old survivors as percentages of the number of eggs set was noted in the 256 ppm group when compared to the control group (Table 3A, attached). "However, the reductions were primarily the result of one pen from which no viable embrys resulted. The reductions were not considered to be treatment related." Other reproductive parameters showed no 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 upon hatching at any concentration tested. There was a significant ($p < 0.05$) decrease in body weight of 14-day old survivors at the 1600 ppm concentration when compared to controls (Appendix X, attached). However, the difference was not considered to be treatment related.

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 bobwhite during the 20 week exposure period. There were no treatment related effects upon reproductive parameters at the 256, 640, or 1600 ppm concentrations. The no-observed effect concentration for pyridate technical in this study was 1600 ppm, the highest concentration tested.

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:** Deviations from required or recommended procedures are as follows:

Neither palatability nor feed spillage was reported.

The average relative humidity in adult pens was 39%. 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 81%; 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 percentile values 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 these parameters, however, showed no significant differences between control and treatment groups.

Other analyses of reproductive parameters were verified (attached) and found to match those reported by the author except in the following areas:

No significant difference was found between groups in the ratio of viable embryos to eggs set, contrary to the difference reported by the study author.

The ANOVA program recommended by EPA showed significant differences between groups in the number hatched/eggs set ($p=0.0113$) and 14 day survivors/eggs set ($p=0.0073$) but subsequent analyses using Duncan's multiple range test showed no significant differences between pairs.

C. **Discussion/Results**: There were no treatment related effects upon bobwhite quail exposed to nominal dietary concentrations of 256, 640, or 1600 ppm pyridate technical. No treatment related effects upon reproductive performance were noted. The NOEL was 1600 ppm, the highest concentration tested.

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. 217-102

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
19	0	215.6	572.2	1520.5
20	0	239.9	562.0	1549.4

APPENDIX IV

GROSS PATHOLOGICAL OBSERVATIONS

PYRIDATE TECHNICAL - PROJECT NUMBER 217-102

BIRDS SACRIFICED AT TERMINATION OF THE STUDY

	MALES				FEMALES			
	PPM				PPM			
	CONTROL	256	640	1600	CONTROL	256	640	1600
Head Lesion(s)	0/16	0/15	0/15	0/16	0/16	0/15	2/15	0/16
Foot Lesion(s)	3/16	1/15	3/15	0/16	0/16	2/15	4/15	2/16
Feather Loss	0/16	0/15	0/15	0/16	1/16	0/15	1/15	0/16
Slight Egg Yolk Peritonitis	0/16	0/15	0/15	0/16	1/16	0/15	0/15	0/16
Regressed Ovary	0/16	0/15	0/15	0/16	0/16	1/15	1/15	0/16
Regressing Testes	0/16	0/15	1/15	1/16	0/16	0/15	0/15	0/16
Pale Yellow Liver	1/16	0/15	0/15	0/16	0/16	0/15	0/15	0/16
Ear Infection	0/16	0/15	0/15	0/16	0/16	0/15	1/15	0/16
Old Fracture - Left Metatarsus	0/16	0/15	0/15	0/16	0/16	0/15	1/15	0/16
Not Remarkable	12/16	14/15	11/15	15/16	14/16	12/15	8/15	14/16

APPENDIX X
PAGE 1
MEAN 14-DAY SURVIVORS BODY WEIGHT DATA PER PEN BY WEEK LOT - (g)
BOBWHITE
PYRIDATE TECHNICAL - PROJECT NUMBER 217-102
0 PPM

	WEEK												
Pen	A (n)	B (n)	C (n)	D (n)	E (n)	F (n)	G (n)	H (n)	I (n)	MEAN	sd	TOTAL 14-DAY
601	(0)	35 (4)	31 (3)	34 (6)	30 (3)	30 (5)	33 (6)	31 (4)	34 (6)	32	2	37
602	(0)	25 (1)	29 (3)	30 (5)	24 (4)	27 (3)	27 (6)	26 (6)	30 (6)	27	2	34
603	(0)	32 (3)	29 (1)	34 (4)	29 (3)	(0)	(0)	(0)	(0)	31	2	11
604	(0)	(0)	27 (3)	28 (1)	26 (3)	29 (1)	31 (4)	27 (1)	31 (5)	28	2	18
605	(0)	29 (3)	30 (4)	35 (4)	33 (2)	29 (3)	30 (5)	30 (4)	35 (4)	31	3	29
606	34 (1)	32 (3)	30 (7)	30 (4)	31 (4)	29 (3)	31 (6)	30 (6)	33 (6)	31	2	40
607	(0)	34 (2)	29 (4)	34 (4)	30 (3)	31 (5)	33 (6)	29 (4)	32 (4)	31	2	32
608	(0)	(0)	34 (3)	35 (3)	37 (3)	36 (4)	35 (8)	35 (5)	34 (5)	35	1	31
609	(0)	(0)	28 (3)	33 (4)	31 (5)	31 (5)	32 (5)	32 (6)	31 (4)	31	2	32
610	(0)	(0)	(0)	(0)	(0)	(0)	35 (4)	31 (2)	35 (7)	34	2	13
611	30 (1)	29 (1)	32 (4)	26 (5)	25 (3)	32 (3)	29 (6)	27 (7)	30 (4)	29	3	34
612	(0)	(0)	29 (5)	33 (4)	28 (5)	31 (4)	30 (6)	28 (1)	29 (6)	30	2	31
613	(0)	30 (2)	28 (3)	30 (5)	26 (2)	26 (4)	30 (5)	27 (6)	26 (5)	28	2	32
614	(0)	35 (1)	30 (3)	31 (2)	27 (4)	30 (2)	31 (6)	30 (4)	36 (5)	31	3	27
615	24 (1)	26 (4)	28 (2)	23 (4)	26 (3)	25 (6)	26 (4)	26 (6)	26 (7)	25	1	37
616	21 (1)	26 (2)	25 (4)	26 (3)	22 (4)	20 (2)	23 (4)	26 (2)	29 (3)	24	3	25
										30	3	463

Overall mean and sd are based on pen mean body weight by parental pen and not on pen means.
sd = Standard deviation of mean body weight by parental pen by week.

(Continued)

APPENDIX X
PAGE 4
MEAN 14-DAY SURVIVORS BODY WEIGHT DATA PER PEN BY WEEK LOT - (g)
BOBWHITE
PYRIDATE TECHNICAL - PROJECT NUMBER 217-102
1630 PPM

	WEEK												
Pen	A (n)	B (n)	C (n)	D (n)	E (n)	F. (n)	G (n)	H (n)	I (n)	MEAN	sd	TOTAL 14-DAY
649	(0)	(0)	28 (1)	28 (4)	24 (1)	27 (5)	29 (4)	30 (4)	29 (3)	28	2	22
650	(0)	30 (2)	23 (4)	26 (3)	25 (4)	27 (1)	29 (4)	26 (4)	27 (5)	27	2	27
651	(0)	(0)	(0)	(0)	(0)	(0)	(0)	31 (1)	(0)	31		1
652	28 (1)	33 (1)	28 (4)	33 (3)	25 (4)	27 (4)	27 (4)	31 (3)	29 (6)	29	3	30
653	(0)	(0)	(0)	(0)	(0)	23 (2)	27 (2)	24 (3)	26 (1)	25	2	8
654	(0)	(0)	29 (4)	31 (2)	27 (3)	27 (1)	28 (6)	25 (4)	30 (5)	28	2	25
655	(0)	(0)	(0)	32 (4)	23 (1)	24 (3)	33 (1)	29 (4)	(0)	28	4	13
656	(0)	32 (2)	28 (2)	27 (2)	28 (6)	24 (3)	30 (7)	23 (3)	28 (3)	27	3	28
657	(0)	27 (2)	25 (3)	33 (4)	22 (4)	24 (4)	29 (4)	25 (3)	28 (5)	26	3	29
658	(0)	(0)	(0)	(0)	(0)	27 (3)	29 (6)	27 (5)	27 (5)	27	1	19
659	27 (1)	32 (5)	27 (4)	34 (5)	23 (4)	27 (5)	30 (4)	27 (7)	30 (5)	29	3	40
660	(0)	(0)	(0)	(0)	(0)	(0)	(0)	21 (3)	27 (2)	24	4	5
661	(0)	22 (1)	(0)	31 (5)	21 (2)	23 (3)	26 (3)	24 (4)	31 (5)	25	4	23
662	(0)	(0)	(0)	(0)	(0)	(0)	25 (2)	(0)	32 (1)	29	5	3
663	24 (1)	28 (3)	26 (4)	29 (5)	26 (4)	29 (5)	28 (5)	27 (5)	29 (4)	27	2	36
664	32 (2)	23 (1)	25 (4)	29 (4)	25 (4)	21 (3)	28 (6)	27 (6)	31 (6)	27	4	36
										27 *	3	345

* Difference from the control statistically significant at p < .05.
Overall mean and sd are based on pen mean body weight by parental pen and not on pen means.
sd = Standard deviation of mean body weight by parental pen by week.

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

Study/Species/Lab/ Succession	Chemical % Active	Group	Dose(ppm)	Effected/Parameters	Mort.(%)	%Cke Inh.	Reviewer/ Date	Validation Status
Avian Reproduction,		Control	0	NONE	0	N.A.		
Species: Colinus virginianus	93.3%	Treatment I	256	NONE	3%		MLW 2-28-89	CORE
Lab: Wildlife International, Ltd		Treatment II	640	NONE	3%			
Acc. 404766-03C		Treatment III	1600	NONE	0			

Study Duration: 20 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

Lab:

Acc.

Concentrations Tested (pp__)= _____

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

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

Comments:

Chronic invertebrate

Species

Lab

Acc.

Concentrations Tested (pp__)= _____

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

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

Comments:

TABLE 3

REPRODUCTIVE DATA - BOBWHITE

PYRIDATE TECHNICAL - PROJECT NUMBER 217-102

	PYRIDATE TECHNICAL			
	0 PPM	256 PPM	640 PPM	1600 PPM
Eggs Laid	594	586	404	495
Eggs Cracked	8	10	7	7
Eggs Set	517	509	348	426
Viable Embryos	496	448	337	394
Live 3-Week Embryos	492	448	335	393
Hatchlings	475	426	325	361
14-Day Old Survivors	463	407	316	345
Eggs Laid/Hen	37	39	27	31
Eggs Laid/Hen/Day @	0.59	0.62	0.43	0.49
14-Day Old Survivors/Hen	29	27	21	22

@ - Based on 63 days.

TABLE 3A

REPRODUCTIVE DATA - (%) - BOBWHITE

PYRIDATE TECHNICAL - PROJECT NUMBER 217-102

	PYRIDATE TECHNICAL			
	0 PPM	256 PPM	640 PPM	1600 PPM
Eggs Laid	594	586	404	495
Eggs Laid/Max. Laid (%)	63	66	46	52
Eggs Cracked/Eggs Laid (%)	1	2	1	2
Viable Embryos/Set (%)	96	81 *	96	89
Live 3-Week Embryos/Viable (%)	99	100	100	99
Hatchlings/3-Week (%)	97	96	97	90
14-Day Old Survivors/Hatch (%)	98	96	96	95
Hatchlings/Set (%)	92	78 *	92	79
14-Day Old Survivors/Set (%)	90	75 *	88	76
Hatchlings/Max. Set (%)	55	53	40	42
14-Day Old Survivors/Max. Set (%)	54	50	39	40

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