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MRID No. 427702-28

DATA EVALUATION RECORD

1. **CHEMICAL:** Pirate® (AC 303,630).
Shaughnessey No. 129093.
2. **TEST MATERIAL:** AC 303,630 technical; CAS No. 122453-73-0;
Batch No. AC-7504-59A; 94.5% purity; a tan powder.
3. **STUDY TYPE:** 71-1A. Avian Acute Oral LD₅₀ Test. Species
Tested: Bobwhite quail (*Colinus virginianus*).
4. **CITATION:** Helsten, B.R. and J.P. Sullivan. 1993. 21-Day
Acute Toxicity Test with AC 303,630 Technical in the
Northern Bobwhite (*Colinus virginianus*). Laboratory Project
No. 105-013-03. Performed by Bio-Life Associates, Ltd.,
Neillsville, WI. Submitted by American Cyanamid Company,
Princeton, NJ. EPA MRID No. 427702-28.
5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Mark A. Mossler*
Date: 7/23/93

Andre Spindel
8/4/93
6. **APPROVED BY:**

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

Signature: *Michael L. Whitten*
Date: 7/23/93

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

Signature: *Henry T. Craven*
Date: 8/6/93

Angela Savola
8/6/93
7. **CONCLUSIONS:** This study is scientifically sound and meets
the guideline requirements for an avian acute oral LD₅₀
toxicity test. The LD₅₀ value of the test material for
bobwhite quail was 34 mg/kg of body weight. Therefore, AC
303,630 technical is classified as highly toxic to the
bobwhite quail. The NOEL was 2 mg/kg.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. MATERIALS AND METHODS:

- A. **Test Animals:** Bobwhite quail (*Colinus virginianus*) were obtained from a commercial supplier in Maquoketa, IA. All birds were from the same hatch and were phenotypically indistinguishable from wild birds. The birds were acclimated to test conditions for 19 days and six deaths were noted during this time period. The birds were 27 weeks of age at test initiation and were normal and active.
- B. **Test System:** The birds were housed in wire pens in a thermostatically controlled room. The pens measured 53 x 61 x 38 cm. During the test, the temperature ranged from 50 to 84°F and the relative humidity ranged from 66 to 90%. A 10-hour light photoperiod was used throughout the study.
- A test solution (40 mg/ml) was prepared by suspending the test material in acetone. The dose for each bird was volumetrically measured into two gelatin capsules based on the bodyweight of the bird. The capsules were allowed to remain uncapped for three hours to allow the acetone to evaporate. All capsules were then capped prior to dosing. The birds were fasted 21-22 hours before dosing (with water allowed). After dosing, birds were fed a commercial bird diet and water *ad libitum*.
- C. **Dosage:** Twenty-one day acute oral LD₅₀ test. Based on results of a preliminary study, dosage levels selected for the study were 1, 2, 4, 8, 16, 32, 64, and 128 mg/kg of body weight. The vehicle control birds received two gelatin capsules (containing acetone which was evaporated). The dosages were not corrected for purity of the test material.
- D. **Design:** Five female and five male birds were randomly assigned to each treatment or control group. Observations were made daily to monitor clinical signs indicative of test material effect. Inspections were made daily for mortalities, abundance of food and water, and food spillage.

The 24 birds that died during the study were subjected to gross necropsy as soon as they were found dead. Of the surviving birds, four arbitrarily selected birds (two males and two females) from each of the vehicle control, 1, 2, 4, 8, and 16 mg/kg groups, and all the surviving birds from the 32 and 64 mg/kg groups were subjected to gross pathological examinations.

Individual body weights were measured at 9 hours prior to dosing on test day 1 and on test days 3, 7, 14, and 21. Average feed consumption was determined by group for days 1-3, 4-7, 8-14, and 15-21.

E. Statistics: Analysis of variance was used to analyze the body weight data. The LD₅₀ was determined using the simplified version of Litchfield and Wilcoxon.

12. REPORTED RESULTS: No mortality occurred in the control or four lowest dosage groups. Mortality at the 16, 32, 64, and 128 mg/kg treatment levels was 10, 40, 90, and 100%, respectively. All deaths were recorded within the first seven days of the test. The LD₅₀ was determined to be 34 mg/kg (95% confidence interval of 24-49 mg/kg), which classifies AC 303,630 technical as highly toxic to the bobwhite quail.

No signs of toxicity were apparent in the vehicle control or the 1, 2, 4, and 8 mg/kg groups. Clinical signs of toxicity at higher treatment levels consisted of loose green and chalky excreta, convulsions, wing beat convulsions, and lethargy. Complete remission of clinical signs of toxicity was achieved in survivors of all treatment groups by test day 9. A toe injury for a 2 mg/kg dosage bird and a foot injury for a vehicle control bird were noted but were not believed to be treatment-related.

Gross changes were noted in 18 of the 24 birds found dead during the test. The majority of the gross changes seen in birds found dead were in birds that had died during the night and were found the next morning. All of these changes, except for firm pectoral muscles and green-colored gizzard contents, were probably due to postmortem autolysis and were therefore not believed to be treatment-related. Gross necropsy of the 29 surviving birds revealed no abnormal findings.

There was a significant reduction in body weight for the three highest dosage level birds for the first three days of the study. No other significant reductions were observed for any of the other weighing periods (Table 7, attached). Statistical analysis was not conducted for groups containing two or less survivors. Food consumption was reduced in the 16, 32, 64, and 128 mg/kg groups for days 1 through 3. Food consumption was also reduced in the 64 mg/kg group for days 4 through 7. With these exceptions, all other control and treatment values were similar (Table 7).

The no-observed-effect level (NOEL) was determined to be 8 mg/kg, based on the lack of mortality or signs of toxicity at this level.

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

No conclusions other than those stated previously were made by the study authors.

A Quality Assurance statement indicating that the study was conducted in accordance with Good Laboratory Practices (GLP) was included in the report. A separate GLP compliance statement was also included in the report. The compliance statement indicated that the feed component analysis and water contaminant analysis were not performed under GLPs.

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

A. **Test Procedure:** The test procedures were in accordance with Subdivision E and SEP guidelines.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to determine the LD₅₀ and obtained the same value as the authors. Based on nominal dosage levels, the LD₅₀ and 95% confidence interval were 34 mg/kg and 25-47 mg/kg, respectively. The slope of the probit curve was 4.5 (see attached printout).

C. **Discussion/Results:** Upon review of the body weight and feed consumption data, the reviewer believes that a treatment-related reduction in body weight and feed consumption occurred during days 1-3 for the 4 and 8 mg/kg groups (Table 7). Therefore, the NOEL was 2 mg/kg.

This study is scientifically sound and meets the guideline requirements for an avian acute oral LD₅₀ toxicity test. The LD₅₀ value of the test material for bobwhite quail was 34 mg/kg of body weight. Therefore, AC 303,630 technical is classified as highly toxic to the bobwhite quail. The NOEL was 2 mg/kg.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

15. **COMPLETION OF ONE-LINER:** Yes, 7-18-93.

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MOSSLER AC 303630 COLINUS VIRGINIANUS 7-16-93

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
128	10	10	100	9.765625E-02
64	10	9	90	1.074219
32	10	4	40	37.69531
16	10	1	10	1.074219
8	10	0	0	9.765625E-02
4	10	0	0	9.765625E-02
2	10	0	0	9.765625E-02
1	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 16 AND 64 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 36.30858

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	.1144044	33.65171	24.34765 47.11994

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	.2368037	1	.9993729

SLOPE = 4.490919
95 PERCENT CONFIDENCE LIMITS = 2.305527 AND 6.676312

LC50 = 34.01446
95 PERCENT CONFIDENCE LIMITS = 24.83725 AND 46.6134

LC10 = 17.73661
95 PERCENT CONFIDENCE LIMITS = 8.614484 AND 24.39551

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