8-8-93

MRID No. 427702-29

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:** 71-2. Avian Dietary LC_{50} Test. Species Tested: Mallard duck (*Anas platyrhynchos*).

4. **CITATION:** Gagne, J.A. and J.P. Sullivan. 1993. 8-Day Acute Dietary LC_{50} Test with AC 303,630 in the Mallard Duck (*Anas platyrhynchos*). Laboratory Project ID No. 105-010-02. Performed by Bio-Life Associates, Ltd., Neillsville, WI. Submitted by American Cyanamid Company, Princeton, NJ. EPA MRID No. 427702-29.

5. **REVIEWED BY:**

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

Signature:

Date: 7/1/93

6. **APPROVED BY:**

Michael Whitten, M.S.
Wildlife Toxicologist
KBN Engineering and
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Signature:

Date: 7/1/93

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

Signature:

Date: 8/18/93

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an avian dietary LC_{50} toxicity test. The LC_{50} of the test material was 8.6 ppm, which classifies AC 303,630 technical as very highly toxic to the mallard duck. The NOEC could not be determined.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

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

5.5

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Mallard ducklings (*Anas platyrhynchos*) were obtained at one day of age from a commercial supplier in Hanover, IL. All birds were phenotypically indistinguishable from wild birds and were acclimated to the testing conditions for 4 days. No deaths occurred during the acclimation period. The birds were 5 days of age at test initiation.

B. **Test System:** The birds were housed in brooder pens in a thermostatically controlled room. The pens measured 91 x 36 x 28 cm. During the test, the average minimum and maximum temperatures were 26 and 38°C, respectively, and the relative humidity ranged between 54 and 73%. A 16-hour fluorescent lighting photoperiod was used throughout the study.

For each treatment diet, test material was dissolved in acetone (covered and stirred on a hot plate) and added to Purina® Game Bird Startena. The diets were allowed to blend for 15 minutes and the acetone was allowed to evaporate from all diets before administration to the birds. Water and food were supplied *ad libitum*. Prior to and following the 5-day exposure period, all birds were placed on regular feed.

C. **Dosage:** Eight-day dietary LC_{50} test. Dosage levels selected for the study were 4, 6, 9, 13.5, and 20.3 ppm. The amount of test material added to the diets was not adjusted for percent purity. A vehicle control diet was also prepared.

D. **Design:** Ten ducklings per treatment level and control were randomly assigned to pens. Observations were made twice daily for clinical signs indicative of test material effect. Inspections were made twice daily for mortalities and abundance of food and water.

The 26 birds that died during the test and the ten birds sacrificed from each of the control, 4, and 6 ppm treatment groups at the termination of the project were subjected to gross pathological examinations. The surviving four birds at the 9 ppm level were also inspected.

Individual bodyweights were measured at 0-hour on day 1 and on test days 5 and 8. Average feed consumption was determined daily by group for days 1-5 (the exposure period) and days 6-8 (the observation period).

Stability and homogeneity samples were collected from a trial diet. Stability samples were allowed to remain at room temperature for 5 and 10 days. Immediately after diet preparation for the definitive test, concentration verification samples were collected from the control and each treatment diet. The samples were frozen and sent to the sponsor for analysis using high performance liquid chromatography.

E. **Statistics:** The acute median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) were calculated using the simplified method of Litchfield and Wilcoxon. The no-observed-effect concentration (NOEC) was based on Dunnett's test ($p \leq 0.05$) and clinical observations.

12. **REPORTED RESULTS:** No mortality was observed in the control or two lowest treatment groups during the study. Sixty, 100, and 100% mortality was observed in the 9, 13.5, and 20.3 ppm groups, respectively (Table 1, attached). The 8-day dietary LC_{50} of AC 303,630 technical for mallard ducklings was determined to be 8.6 ppm (95% C.I.= 7.4-10.1 ppm). Based on this value, AC 303,630 technical would be classified as very highly toxic to the mallard duck.

No clinical signs of toxicity were noted for the control, 4, and 6 ppm treatment birds. Various effects including lethargy, circling backwards, lack of coordination, lying on the side with legs kicking in the air, rapid breathing, placing head on side of body, lack of peeping, loss of righting reflex, labored breathing, and pale green and chalky white excreta were observed for the three highest treatment levels (Table 3, attached). Complete remission of these signs was achieved by the beginning of test day 5.

Mean percent change in bodyweight of birds at the three lowest treatment levels was significantly reduced in comparison to the vehicle control birds for the exposure and entire test periods (Table 5, attached). Statistical analysis of the bodyweights of the two highest concentration birds was not undertaken due to complete mortality by the afternoon of day 5.

Feed consumption appeared to be reduced at the two highest treatment levels during the first two days of the test (20.3 ppm birds) and days 2 through 4 of the test (13.5 ppm birds). All other values were equal or greater to the vehicle control values (Table 6, attached).

Gross changes were noted in all birds that died and two birds which were sacrificed. The majority of these changes were noted in birds that died during the night, and therefore, the changes were believed to be due to postmortem autolysis (Table 7, attached). However, green gizzard contents and enlarged gallbladders may have been treatment-related changes.

The NOEC was determined to be <4 ppm, based on the reduction in bodyweight gain at the lowest treatment level.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions other than those stated above were presented by the authors.

Quality Assurance and Good Laboratory Practice (GLP) compliance statements were included in the report, indicating that the study was conducted in accordance with GLPs as set forth in 40 CFR Part 160. A Quality Assurance statement was also included in the analytical appendix.

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

A. **Test Procedure:** The test procedures were in accordance with Subdivision E, ASTM, and SEP guidelines with the following exception:

The pen dimensions (91 x 36 cm = 3,276 cm^2) were smaller than recommended (70 x 100 cm = 7,000 cm^2).

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to determine the LC_{50} value and 95% confidence interval (see attached printout). The value was similar to that of the authors.

C. **Discussion/Results:** Results of chemical analyses indicated that the material was homogeneously mixed, stable, and present at the desired levels. The results are summarized in Appendix II, Tables I, IIB, IIIA, and IIIB (attached). Although the stability and homogeneity data were derived from trial diets, the reviewer believes that the definitive diets would have demonstrated these same qualities.

After review of the mortality, weight, and feed consumption data, the reviewer noted that there were food consumption reductions at the 9 ppm level throughout the study, in addition to the reductions noted by the authors at the 13.5 and 20.3 ppm levels.

This study is scientifically sound and meets the guideline requirements for an avian dietary LC_{50} toxicity test. The LC_{50} of the test material for mallard ducklings was 8.6 ppm, which classifies AC 303,630 technical as very highly toxic to the mallard duck. The NOEC could not be determined, due to a significant reduction in bodyweight gain at all treatment levels.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

15. **COMPLETION OF ONE-LINER:** Yes, 6-29-93.

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MOSSLER AC 303630 ANAS PLATYRHYNCHOS 6-29-93

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
20.3	10	10	100	9.765625E-02
13.5	10	10	100	9.765625E-02
9	10	6	60.00001	37.69531
6	10	0	0	9.765625E-02
4	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 6 AND 13.5 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 8.548964

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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