DATA EVALUATION RECORD

1. **CHEMICAL:** Diphacinone.
Shaughnessey No. 067701.

2. **TEST MATERIAL:** Diphacinone Technical; Batch No. T-988; Sample No. 5766; 96.9% active ingredient; a yellow powder.

3. **STUDY TYPE:** 71-2. Avian dietary LC_{50} test. Species Tested: Bobwhite quail (*Colinus virginianus*).

4. **CITATION:** Long, R.D., J. Foster, K.A. Hoxter, G.J. Smith, and S.M. Campbell. 1992. Diphacinone Technical: A Dietary LC_{50} Study with the Northern Bobwhite. Project No. 284-101A. Conducted by Wildlife International, Ltd., Easton, MD. Submitted by Bell Laboratories, Inc., Madison, WI. EPA MRID No. 424088-01.

5. **REVIEWED BY:**

Charles G. Nace Jr., M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten For C.G. Nace
Date: 2/7/94

Goodyear 9-15-94

6. **APPROVED BY:**

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

Signature: Michael L. Whitten
Date: 2/7/94

James J. Goodyear, Ph.D.
Project Officer, EEB/EFED
USEPA

Signature: James Goodyear
Date: 9-15-94

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements for a dietary LC_{50} study using bobwhite quail (*Colinus virginianus*). The LC_{50} was greater than 5000 ppm (nominal concentration), which classifies Diphacinone technical as practically non-toxic to the bobwhite quail. The no-observed-effect concentration (NOEC) was 62 ppm, based upon mortality and behavioral signs of toxicity at 185 ppm.

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

9. **BACKGROUND:**

5.5 hrs

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds used in the study were 10-day old bobwhite quail (*Colinus virginianus*) obtained from in-house flocks. All of the birds were from the same hatch, pen-reared, and phenotypically indistinguishable from wild birds. The birds could not be differentiated by sex. The birds were acclimated to the caging and test facilities from the day of hatch and appeared to be in good health at test initiation.

B. **Test System:** The birds were housed indoors in pens constructed of galvanized steel wire and sheeting (72 x 90 x 23 cm). A photoperiod of 16 hours of daylight and 8 hours of darkness was maintained with fluorescent lights at an intensity of 247 lux. The average brooder temperature was maintained at 38 ±1°C, average ambient temperature was 25 ±1°C, and relative humidity averaged 31 ±9%.

C. **Dosage:** Twenty-two day dietary LC_{50} test. Based on known toxicity data, six nominal concentrations of 21, 62, 185, 556, 1667, 5000 parts per million (ppm) were selected for the test. Test concentrations were not adjusted for purity of the test material.

D. **Design:** Groups of ten birds were assigned by indiscriminate draw, without regard to sex, to each of six treatment groups and four control groups. All birds were fed a game bird ration formulated to in-house standards. Food and water were supplied *ad libitum* throughout the test.

The test diets were prepared by dissolving the test material in acetone and mixing the solution into the diet with corn oil. The concentration of corn oil in the treated and control diets was 2%. The diets were prepared on the day of test initiation. The birds were fed the appropriate diet for 5 days (exposure period) and untreated food for 17 days (post-exposure period).

Samples of the test diets were taken to verify the test concentrations administered. The samples were sent to Bell Laboratories, Madison, WI for analysis using high performance liquid chromatography (HPLC).

Mortality and symptoms of toxicity were recorded at least twice daily throughout the study. Birds were

weighed by group at initiation, on days 5, 8, 15, and at study termination (day 22). Food consumption was recorded by group for days 0-5, 6-8, 9-15, and 16-22.

E. **Statistics:** The level of mortality was insufficient to determine an LC_{50} using statistical analysis. An estimation of the was made by visual inspection of the mortality data.

12. **REPORTED RESULTS:** Measured test concentrations at 185, 556, 1667, and 5000 ppm were 109.2, 84.9, 109.2, and 102.2% of nominal concentrations, respectively. Test concentrations below 185 ppm (62 and 21 ppm) were unable to be analyzed due to inseparable interference on the HPLC (Appendix III, attached).

There were no mortalities in the control groups. All birds were normal in appearance and behavior throughout the study, except for one bird with sub-orbital eye swelling on day 19.

There were no mortalities at 21, 62, or 556 ppm and all birds at these levels appeared normal in appearance and behavior throughout the test period. There was one mortality at 185 ppm (day 15), three at 1667 ppm (days 12, 13, and 18), and one at 5000 ppm (day 4) (Table 1, attached). Symptoms of toxicity were noted in the three groups that experienced mortality. These symptoms included ruffled appearance, lethargy, depression, reduced reaction to external stimuli, wing droop, loss of coordination, and lower limb weakness. There were also lesions in these groups that were associated with hock picking and toe picking, cannibalistic forms of aggression. The bird at 5000 ppm that was found dead on day 4 exhibited subcutaneous hemorrhage over the ear and submandibular region. Additionally, bile staining of the ventriculus and evidence of dehydration in the lower intestinal tract were noted. All other mortalities exhibited lesions normally associated with cannibalism, including abrasions and hemorrhage on the hocks and/or legs. Due to the timing of the mortalities and the nature of the lesions and clinical signs observed, the most likely cause of the mortalities seen at 185 and 1667 ppm was related to cannibalism. However, since Diphacinone is an anticoagulant, the test substance may have been a contributing factor in the mortalities.

When compared to the control group, there appeared to be a treatment-related reduction in body weight gain at 5000 ppm during the exposure period. Reductions in body weight gain and feed consumption observed at 1667 from day 8 until study termination appeared to be related to cannibalism. There

were no other apparent effects on body weight or feed consumption at any of the concentrations tested (Tables 3 and 4, attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "The dietary LC_{50} value for northern bobwhite exposed to Diphacinone Technical was determined to be greater than 5000 ppm, the highest concentration tested. The no-mortality level was 62 ppm. The no-observed-effect level was 62 ppm."

The report stated that the study was conducted in conformance with Good Laboratory Practice (GLP) regulations (40 CFR Part 160). Quality assurance audits were conducted during the study and the final report was signed by a Quality Assurance Officer for Wildlife International, Ltd. An additional statement of conformance with GLP (40 CFR part 160) guidelines was included in the analytical report.

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

A. **Test Procedure:** This study followed procedures outlined in the SEP, ASTM, and Subdivision E Guidelines, except for the following deviations:

Body weights were measured by group. Individual body weights should have been measured.

The birds were not randomly assigned to pens. Instead, they were assigned by indiscriminate draw.

Diet samples were not analyzed to determine the homogeneity of the test substance in the diet.

B. **Statistical Analysis:** The reviewer's LC_{50} value was the same as the authors' (>5000 ppm), based on the mortality data.

C. **Discussion/Results:** The study is scientifically sound and fulfills the requirements for a dietary LC_{50} study using bobwhite quail. The LC_{50} was greater than 5000 ppm (nominal concentration), which classifies Diphacinone technical as practically non-toxic to the bobwhite quail. The NOEC was 62 ppm, based upon mortality and behavioral signs of toxicity at 185 ppm.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes; 01/18/94.

TABLE 1

CUMULATIVE MORTALITIES OF NORTHERN BOBWHITE
EXPOSED TO DIPHACINONE TECHNICAL FOR FIVE DAYS
Number Dead/Number Exposed

Experimental Group	Day of Study											
Control (ppm)	0	1	2	3	4	5	6	7	8	9	10	11
0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Treatment (ppm)												
21	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
62	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
185	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
556	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
1667	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
5000	0/10	0/10	0/10	0/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10

The LC50 value was determined to be greater than 5000 ppm, the highest concentration tested.

TABLE 1 (continued)

CUMULATIVE MORTALITIES OF NORTHERN BOBWHITE
EXPOSED TO DIPHACINONE TECHNICAL FOR FIVE DAYS
Number Dead/Number Exposed

Experimental Group	Day of Study										
Control (ppm)	12	13	14	15	16	17	18	19	20	21	22
0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Treatment (ppm)											
21	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
62	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
185	0/10	0/10	0/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10
556	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
1667	1/10	2/10	2/10	2/10	2/10	2/10	3/10	3/10	3/10	3/10	3/10
5000	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10	1/10

The LC50 value was determined to be greater than 5000 ppm, the highest concentration tested.

TABLE 3

AVERAGE BODY WEIGHTS OF NORTHERN BOBWHITE
EXPOSED TO DIPHACINONE TECHNICAL FOR FIVE DAYS

Experimental Group	Average Body Weight (Grams)									
Control (ppm)	Day 0	Change	Day 5	Change	Day 8	Change	Day 15	Change	Day 22	Total Change
0	20	11	31	8	39	23	62	24	86	66
0	18	11	29	7	36	21	57	22	79	61
0	20	9	29	7	36	20	56	23	79	59
0	19	10	29	8	37	22	59	24	83	64
Treatment (ppm)										
21	19	10	29	9	38	23	61	24	85	66
62	19	10	29	8	37	22	59	24	83	64
185	19	9	28	9	37	17	54	19	73	54
556	19	10	29	7	36	21	57	23	80	61
1667	19	8	27	7	34	9	43	8	51	32
5000	19	4	23	6	29	18	47	25	72	53

TABLE 4

ESTIMATED FEED CONSUMPTION OF NORTHERN BOBWHITE EXPOSED TO DIPHACINONE TECHNICAL FOR FIVE DAYS

Experimental Group	Feed Consumption (Grams Per Bird Per Day)			
Control (ppm)	Days 0 - 5	Days 6 - 8	Days 9 - 15	Day 16 - 22
0	11	16	12	16
0	9	12	14	14
0	8	11	12	15
0	8	14	10	17
Treatment (ppm)				
21	9	14	11	15
62	11	16	13	15
185	9	15	10	10
556	10	12	13	13
1667	9	12	7	9
5000	9	10	12	15

APPENDIX III

DIET ANALYSIS

ANALYTICAL REPORT

Following are the results of the quality control analyses on the Mallard diet samples. Samples with a concentration of 40ppm and lower could not be analyzed due to inseparable interference on the HPLC.

Sample Number	Nominal Concentration	Date of Analysis	Analyzed Concentration
S-284-101A-50	5000ppm	11/28/90	5116ppm
S-284-101A-49	1667ppm	12/06/90	1820ppm
S-284-101A-48	556ppm	12/07/90	472 ppm
S-284-101A-47	185ppm	12/07/90	202ppm

The following samples could not be separated on our HPLC:

S-284-101A-46	62ppm
S-284-101A-45	21ppm
S-284-101A-44	0ppm

Principal Investigator: [signature]

Date: 7 Jan 91

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