DATA EVALUATION RECORD

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

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

3. **STUDY TYPE:** 71-2. Avian dietary LC_{50} test. Species Tested: Mallard duck (*Anas platyrhynchos*).

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 Mallard. Project No. 284-102B. Conducted by Wildlife International, Ltd., Easton, MD. Submitted by Bell Laboratories, Inc., Madison, WI. EPA MRID No. 424088-02.

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 J. Goodyear
Date: 9 15 94

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements for a dietary LC_{50} study using mallard ducks (*Anas platyrhynchos*). Based on nominal concentrations, the LC_{50} was 906 ppm with 95% confidence limits of 187 and 35107 ppm, which classifies Diphacinone technical as moderately toxic to the mallard duck. The no-observed-effect concentration (NOEC) was not established due to overt signs of toxicity at 1.6 ppm, the lowest concentration tested.

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

9. **BACKGROUND:**

6.0 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 mallard ducklings (*Anas platyrhynchos*) obtained from a commercial supplier in Hanover, IL. 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 receipt and appeared to be in good health at test initiation.

B. **Test System:** The birds were housed indoors in pens constructed of vinyl coated wire mesh (62 x 92 x 25.5 cm). A photoperiod of 16 hours of daylight and 8 hours of darkness was maintained with fluorescent lights at an intensity of 130 lux. The average brooder temperature was maintained at 33 ±2°C, average ambient temperature was 24 ±1°C, and relative humidity averaged 65 ±13%.

C. **Dosage:** Twenty-five day dietary LC_{50} test. Based upon known toxicity data, six nominal concentrations of 1.6, 8, 40, 200, 1000, and 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 three 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 resulting 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 20 days (post-exposure period).

Samples of the 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, day 5, 8, 15, 22 and at study termination (day 25). Food consumption was recorded by group for days 0-5, 6-8, 9-15, 16-22, and 23-25.

E. **Statistics:** Mortality data were analyzed by probit analysis using the computer program of C.E. Stephan.

12. **REPORTED RESULTS:** Measured test concentrations at 200, 1000, and 5000 ppm were 103, 115.2, and 99.8% of nominal concentrations, respectively. Test concentrations below 200 ppm (40, 8, and 1.6 ppm) were unable to be analyzed due to inseparable interference on the HPLC (Appendix III, attached).

There were no mortalities in the control groups (Table 1, attached). All birds were normal in appearance and behavior throughout the test period.

There were no mortalities at the 1.6 ppm test concentration. There was 20% mortality at 8 ppm, 30% at 40 ppm, 40% mortality at 200 ppm, 50% mortality at 1000 ppm, and 60% mortality at 5000 ppm. All mortalities were considered to be treatment-related and the observed mortality pattern was dose responsive.

There were overt signs of toxicity at all levels tested. These signs included lower limb weakness, depression, reduced reaction to external stimuli, loss of coordination, prostrate posture, convulsions, ruffled appearance, swollen legs, lethargy, wing droop, and the loss of righting reflex.

Sufficient birds to characterize the nature of the lesions associated with the mortalities were necropsied. Findings were consistent with anticoagulant ingestion, and included subcutaneous and/or internal hemorrhages.

When compared with the control groups, during the exposure period there was a reduction in body weight gain at concentrations of 40, 200, and 1000 ppm and a marked reduction in body weight gain at 5000 ppm (Table 3, attached). A reduction in body weight gain continued to be observed at 200 and 1000 ppm through day 8, and at 5000 ppm through day 15. A loss of body weight was noted at 40 and 5000 ppm from day 22 to 25 while a slight reduction in body weight gain was noted at 1.6 ppm during the same time period. Feed consumption appeared to be reduced at 5000 ppm through day 15 (Table 4, attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "The dietary LC50 value for mallards exposed to Diphacinone Technical was determined to be 906 ppm, with a 95% confidence interval of 187 to 35107 ppm. The slope of the dose response curve was 0.5. The no-mortality level was 1.6 ppm. The no-observed-effect level was less than 1.6 ppm, the lowest concentration tested, based upon overt signs of toxicity."

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 used the probit method in EPA's Toxanal program to verify the authors' LC_{50}. The reviewer agrees with authors' LC_{50} of 906 ppm (see attached sheet).

C. **Discussion/Results:** The study is scientifically sound and fulfills the requirements for a dietary LC_{50} study using mallard ducks. With an LC_{50} of 906 ppm (nominal concentration), the test material is classified as moderately toxic to mallard ducks. The NOEC was not established due to overt signs of toxicity at 1.6 ppm, the lowest concentration tested.

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 MALLARDS
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	12	13	14
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	0/10	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	0/10	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	0/10	0/10	0/10
Treatment (ppm)															
1.6	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
8	0/10	0/10	0/10	1/10	1/10	2/10	2/10	2/10	2/10	2/10	2/10	2/10	2/10	2/10	2/10
40	0/10	0/10	0/10	1/10	1/10	1/10	3/10	3/10	3/10	3/10	3/10	3/10	3/10	3/10	3/10
200	0/10	0/10	0/10	0/10	0/10	1/10	2/10	4/10	4/10	4/10	4/10	4/10	4/10	4/10	4/10
1000	0/10	0/10	0/10	0/10	2/10	2/10	4/10	4/10	5/10	5/10	5/10	5/10	5/10	5/10	5/10
5000	0/10	0/10	0/10	2/10	2/10	2/10	3/10	3/10	4/10	4/10	4/10	4/10	4/10	4/10	4/10

The LC50 value was determined to be 906 ppm, with a 95% confidence interval of 187 ppm to 35107 ppm.

TABLE 1 (continued)

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

Experimental Group	Day of Study											
Control (ppm)	15	16	17	18	19	20	21	22	23	24	25	Total
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)												
1.6	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
8	2/10	2/10	2/10	2/10	2/10	2/10	2/10	2/10	2/10	2/10	2/10	2/10
40	3/10	3/10	3/10	3/10	3/10	3/10	3/10	3/10	3/10	3/10	3/10	3/10
200	4/10	4/10	4/10	4/10	4/10	4/10	4/10	4/10	4/10	4/10	4/10	4/10
1000	5/10	5/10	5/10	5/10	5/10	5/10	5/10	5/10	5/10	5/10	5/10	5/10
5000	4/10	6/10	6/10	6/10	6/10	6/10	6/10	6/10	6/10	6/10	6/10	6/10

The LC50 value was determined to be 906 ppm, with a 95% confidence interval of 187 ppm to 35107 ppm.

TABLE 3

AVERAGE BODY WEIGHTS OF MALLARDS
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	Change	Day 25	Total Change
0	197	162	359	127	486	142	628	159	787	44	831	634
0	187	171	358	108	466	128	594	169	763	28	791	604
0	185	171	356	114	470	152	622	93	715	43	758	573
Treatment (ppm)												
1.6	191	182	373	119	492	133	625	161	786	15	801	610
8	182	155	337	122	459	186	645	167	812	42	854	672
40	184	127	311	107	418	186	604	104	708	-3	705	521
200	175	133	308	66	374	213	587	183	770	72	842	667
1000	179	113	292	49	341	206	547	193	740	64	804	625
5000	189	9	198	62	260	92	352	236	588	-53	535	346

TABLE 4

ESTIMATED FEED CONSUMPTION OF MALLARDS
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	Days 16 - 22	Days 23 - 25
0	98	163	140	143	118
0	96	122	120	134	125
0	86	143	128	117	126
Treatment (ppm)					
1.6	94	144	130	141	124
8	75	123	139	152	151
40	67	92	116	117	121
200	63	63	117	136	142
1000	64	58	114	118	128
5000	38	45	68	106	148

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-284-102B-50	5000ppm	12/06/90	4990ppm
S-284-102B-49	1000ppm	12/06/90	1152ppm
S-284-102B-48	200ppm	12/07/90	206ppm

The following samples could not be separated on our HPLC:

S-284-102B-47	40ppm
S-284-102B-46	8ppm
S-284-102B-45	1.6ppm
S-284-102B-44	0ppm

Principal Investigator: _____

Date: 3 Jan 92

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CHUCK NACE DIPHACINONE MALLARD DUCK 01/19/94

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
5000	10	6	60.00001	37.69531
1000	10	5	50	62.30469
200	10	4	40	37.69531
40	10	3	30	17.1875
8	10	2	20	5.46875
1.6	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY 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 999.9999

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	2.352835	999.9999	0	+INFINITY

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.3763084	1	.8399181

SLOPE = .5154888
95 PERCENT CONFIDENCE LIMITS = .1992675 AND .8317101

LC50 = 905.7718
95 PERCENT CONFIDENCE LIMITS = 187.3474 AND 35109.62

LC10 = 3.114135
95 PERCENT CONFIDENCE LIMITS = 3.660907E-03 AND 22.60299