DATA EVALUATION RECORD

1. **CHEMICAL:** Profenofos.
Shaughnessey Number: 111401.

2. **TEST MATERIAL:** O-(4-bromo-2-chlorophenyl)-O-ethyl-s-propyl phosphorothioate; 89.4% purity; Lot No. FL 851177; CAS # 41198-08-7; an amber-colored, oily liquid with a sulfur-like odor.

3. **STUDY TYPE:** Avian Dietary LC_{50} Test.
Species Tested: Bobwhite quail (Colinus virginianus).

4. **CITATION:** Pedersen, C.A. 1990. Profenofos Technical: 8-Day Acute Dietary LC_{50} Study in Bobwhite Quail. Study performed by Bio-Life Associates, Ltd., Neillsville, Wisconsin. Laboratory study #89 QC 146. Submitted by Ciba-Geigy Corporation, Greensboro, NC. EPA MRID No 416273-03.

5. **REVIEWED BY:**

Richard W. Felthousen
EFED/EEB

Signature: R. W. Felthousen
Date: 6/14/91

6. **APPROVED BY:**

Norm Cook, Head Section 2
EFED/EEB

Signature: for Norm Cook
Date: 6/18/91

Harry Craven
Supervisor, EEB/HED
USEPA

Signature: Harry T. Craven
Date: 6/18/91

7. **CONCLUSIONS:**

As a result of a telephone conversation with an analytical chemist from the USDA/APHIS at Denver, Colorado, the EEB has learned that the dietary test rations prepared by Bio-Life have so much variability in measured concentrations that it is impossible to determine actual treatment levles (See attached telephone conversation sheet and EEB files for review conducted by R. Felthuosen on 7/20/90). In so much

as the design deficiency was not corrected for this study, the EEB must conclude that the results reported for this study may not be representative of the dietary toxicity of Profenofos to the bobwhite quail. As such, the study must be considered as inadequate to support the data requirement.

The study may be upgraded to Core status provided the test concentrations were actually measured. This information must be submitted to the EEB along with a reanalysis of the results. **(Note: The KBN review also mentioned that a chemical analysis of the test diets was not performed.)**

8. **RECOMMENDATIONS:** (See Section 7.)

9. **BACKGROUND:**

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) hatched at Bio-Life Associates, Ltd. from eggs obtained from Oak Ridge Game Farm, Gravette, Arkansas. All test birds were phenotypically indistinguishable from wild birds. The birds were acclimated to laboratory conditions and observed daily for a 3-day quarantine period. Well water was supplied ad libitum during quarantine. Prior to initiation of the project, all birds were examined and their suitability for testing (based on general physical condition) was determined. The healthiest birds were selected for the study.

B. **Test System:** All birds were housed indoors in wire pens maintained over concrete. Each pen measured approximately 45.7 cm X 61.0 cm X 45.7 cm. Lighting was provided by fluorescent lights left on 24 hours per day. Maximum and minimum temperatures, as well as the relative humidity of the animal room were recorded daily. The room temperatures during the test period ranged from 37°C (reported as 98°F) to 43°C (reported as 110°F). The relative humidity during the test period ranged between 25% and 54%.

C. **Dosage:** 8-day dietary LC_{50} test. All dosages and the LC_{50} value are reported as parts per million (ppm) active ingredient (a.i.). Nominal dosages were 9.8 (T-

I), 19.5 (T-II), 39 (T-III), 78 (T-IV) and 156 (T-V) ppm a.i.

D. **Design:** Groups of chicks were not formally randomized, but rather arbitrary selections were made from the entire population of male and female birds. Ten birds per group were then assigned to each of the five treatment groups (T-1 through T-V) and the five vehicle control groups.

The test material was dispersed in acetone. The five vehicle control diets were prepared by mixing an amount of acetone equivalent to that in the test solution (98.255 grams) into 10 kg basal diet.

Test diets were fed to the chicks for five consecutive days. After this five-day test period, treated diets were removed and birds were offered untreated feed for a three-day recovery period. All birds were fed Purina® Game Bird Starter as the basal diet.

Birds were weighed by groups (Table 4, attached) at 0 hour on test day 1 and on test day 8. Food consumption (Table 4, attached) was recorded for each group for the quarantine period, the five-day test period, and the three-day recovery period.

All birds were observed daily to ascertain the presence (or absence) of clinical signs indicative of test material effect. Inspections were made daily for mortalities, abundance of food and water, and food spillage. All birds which died during the study were subjected to gross pathological examinations. Additionally, four arbitrarily selected birds from the vehicle control groups and from each of the 9.8, 19.5, and 39 ppm a.i. test groups, as well as the three surviving 78 ppm a.i. test birds were subjected to gross pathological examinations at the end of the test.

E. **Statistics:** At the end of the 8-day study period, the LC_{50} was calculated by employing the simplified method of Litchfield and Wilcoxon (Table 2, attached).

12. **REPORTED RESULTS:**

The LC_{50} of the test material in this study was 70 ppm a.i. with 95% confidence limits of 58 to 85 ppm a.i. (Table 2, attached). Seven deaths were recorded in the 78 ppm a.i.

test group and ten deaths were recorded in the 156 test group (Table 3, attached). No mortalities occurred in the three lowest concentrations or in any of the five vehicle control groups.

No abnormal behavioral reactions or systemic signs of toxicity were noted in the five vehicle control groups or the two lowest test concentrations throughout the investigation. The first deaths occurred within approximately 46 1/3 hours post-administration. Signs of toxicity noted in the three highest test groups included lethargy and anorexia. Total remission of all clinical signs was achieved by the end of the test period.

Gross pathological examinations of the seventeen birds that died and nineteen of the surviving birds revealed no abnormal pathological findings.

The vehicle control groups' average body weights on test day 8 ranged from 42 to 49 g. Test groups T-I and T-II demonstrated weights similar to that of the controls. Groups T-III and T-IV values (34 g and 37 g, respectively) were depressed in comparison to control values.

Food consumption values during the quarantine period ranged from 3 to 4 grams/bird/day. The vehicle control groups' food consumption values during the test period ranged from 5 to 6 grams/bird/day while T-III and T-IV test groups' values illustrated a dose-related reduction. The vehicle control groups' food consumption values during the recovery period ranged from 6 to 7 grams/bird/day; the test groups demonstrated similar consumption rates.

The no-observed-effect level was 19.5 ppm a.i.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The no-observed-effect level was 19.5 ppm a.i. The 8-day acute dietary LC_{50} was 70 ppm a.i. with 95% confidence limits of 58 to 85 ppm a.i.

The report stated that the study was conducted in conformance with Good Laboratory Practice regulations. Quality assurance audits were conducted and the final report was signed by the Quality Assurance Officer and Study director of Bio-Life Associates, Ltd.

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

4

A. **Test Procedure:** The test procedures were in accordance with Subdivision E - Hazard Evaluation: Wildlife and Aquatic Organisms, ASTM and SEP guidelines except for the following deviations:

The room temperatures during the test period ranged from 37°C (reported as 98°F) to 43°C (reported as 110°F) and are considerably higher than temperatures recommended in the guidelines. Recommended temperatures are 35°C (brooder) and 22-27°C (ambient).

The pen dimensions (45.7 cm x 61.0 cm = 2788 cm^2) were smaller than the recommended dimensions (35 cm x 100 cm = 3500 cm^2) for bobwhite quail chicks.

The birds were assigned to pens by "arbitrary selections." Guidelines state that birds must be randomly assigned to pens.

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

The test concentrations resulted in only one "partial kill", i.e., only one concentration where the mortality was between 0 and 100%. In order to provide statistically reliable results, the study should attempt to produce three "partial kills" surrounding the estimated LC_{50}.

Although lethargy and anorexia were reported in the T-II, T-III, and T-IV Test groups, the report did not indicate when the signs of toxicity were first noted or how long the signs were present.

The amounts of food and water available to the birds during the test was not stated. These should be available ad libitum.

The concentration of the test substance in the diet was not confirmed by chemical analysis.

B. **Statistical Analysis:** The reviewer calculated the LC_{50} using EPA's Toxanal computer program (attached). Due to only one partial kill in the test, neither the moving average nor the probit method can give statistically sound results for the LC_{50} test. The approximate LC_{50} calculated using the Binomial Test is 66.6 ppm a.i. with 95% confidence limits of 39 to 156. The LC_{50} value presented by the author is similar, and

the confidence interval is narrower, therefore the author's values are accepted.

C. **Discussion/Results:** This study has several deviations from the recommended guidelines. The most serious deviation is the lack of data regarding the time period in which symptoms of toxicity were present. The report states that "total remission of all clinical signs was achieved by study termination." Since the report did not indicate when the signs first appeared or precisely when they ended it is assumed that the signs were present throughout the test period. The registrant should provide a more detailed description of behavioral symptoms in future studies.

Chemical analysis of the test diets was not performed. The concentration , homogeneity, and stability of the chemical in the diets should have been verified to determine actual treatment concentrations.

Test environmental conditions (i.e., pen size, temperature) did not conform to the SEP, ASTM, and Subdivision E guidelines. Although it can not be determined that these deviations have adversely affected the results of this study, the registrant should initiate procedures to more closely follow recommended guidelines in order to avoid potential confounding effects. It appears that the study is scientifically sound and meets the intent of the guidelines.

The NOEC was 19.5 ppm a.i., based on lethargy, anorexia, and reduced body weight gains at all higher concentrations. The study appears to be scientifically sound and meets the requirements for an avian dietary LC_{50} study.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid

(2) **Rationale:** See Conclusion Section

(3) **Repairability:** Yes

15. **COMPLETION OF ONE-LINER:** Yes; April 10, 1991.

TELEPHONE RECORD: June 26, 1990

TELG. NO: (303) 236-7872

Beth Michalanie Analytical Chemist

Research Chemist -

Preliminary batches of feed from Bio-Life showed tremendous variation in residues in feed.

—— 10 – 40% Coefficient of variation

Feed - 6000 feed assayed:

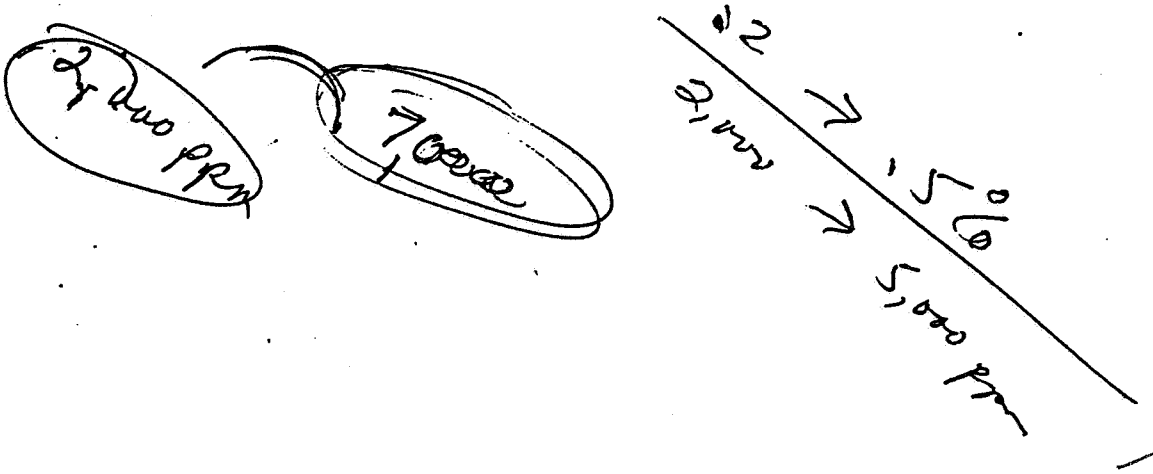
	Actual Values	Strychnine
Coarse grain	3,500 ppm	"
Medium grain	4,000 ppm	"
Fine	11,000 ppm	
	10 – 12 ppm	

~~3~~ preliminary batches.

Precision -

Biological variability too large

* Don't know how to analyze the material



1

TABLE 2

LC_{50} CALCULATIONS

PROFENOFOS TECHNICAL

Group	Nominal Concentration (ppm a.i.)	Observed Response	Expected Response	Residual	Contribution to Chi Square
T-I	9.8	0.0	0.0	0.0	0.0000
T-II	19.5	0.0	0.0	0.0	0.0000
T-III	39	0.0	0.3	0.3	0.0032
T-IV	78	70.0	70.0	0.0	0.0000
T-V	156	100.0	100.0	0.0	0.0000

Total 0.0032
x10

0.032

Total Number of Animals = 50
Number of Groups = 5

Total contributions to Chi square = 0.032

Chi square (P=0.05) for 3 degrees of freedom is 7.82

The data are not significantly heterogeneous.

LC_{16} = 57 ppm a.i. Slope Function = 1.24

LC_{50} = 70 ppm a.i. N' = 10

LC_{84} = 87 ppm a.i. $F(LC_{50})$ = 1.21

95% confidence limits of LC_{50}

LC_{50} = 70 ppm a.i. (58 to 85)

8

TABLE 3

CUMULATIVE MORTALITIES

PROFENOFOS TECHNICAL

Group	Nominal Concentration (ppm a.i.)	Number Dead/Number Exposed Day of Death 1	2	3	4	5	6	7	8
V. Control-I	0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
V. Control-II	0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
V. Control-III	0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
V. Control-IV	0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
V. Control-V	0	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-I	9.8	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-II	19.5	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-III	39	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-IV	78	0/10	0/10	0/10	4/10	6/10	7/10	7/10	7/10
T-V	156	0/10	1/10	1/10	8/10	10/10	10/10	10/10	10/10

The LC_{50} value was determined to be 70 ppm a.i. with 95% confidence limits of 58 to 85 ppm a.i.

TABLE 4

AVERAGE BODY WEIGHT AND ESTIMATED FOOD CONSUMPTION

PROFENOFOS TECHNICAL

Group	Nominal Concentration (ppm a.i.)	Avg. Body Weight (g) Test 0 Hour	Test Day 8	Day 8 - 0 hr	Total Estimated Food Consumption (g) 0 Hour-Test Day 5	Test Days 6-8	Estimated Food Consumption Per Bird Per Day (g) 0 Hour-Test Day 5	Test Days 6-8
V. Control - I	0	23	45	22	304	195	6	7
V. Control - II	0	24	48	24	306	210	6	7
V. Control - III	0	23	44	21	295	198	6	7
V. Control - IV	0	23	42	19	238	187	5	6
V. Control - V	0	24	49	25	294	210	6	7
T-I	9.8	22	43	21	263	213	5	7
T-II	19.5	24	41	17	260	201	5	7
T-III	39	24	37	13	179	190	4	6
T-IV	78	23	34	11	90	61	2	7
T-V	156	23	-		-	-	-	-

- indicates that there were no survivors in this group at the end of this interval.

MARISE ROBBINS PROFENOFOS BOBWHITE QUAIL

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
156	10	10	100	9.765625E-02
78	10	7	70	17.1875
39	10	0	0	9.765625E-02
19.5	10	0	0	9.765625E-02
9.8	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 39 AND 156 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 66.58505

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.

****

Shaughnessey # 111401 Chemical Name Profenofos Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
14-Day Single Oral LD_{50}		LD_{50} = mg/kg (95% C.L.) Control Mortality (%) =		
Species		Slope = # Animals/Level = Age (Days) =		
Lab		Sex =		
MRID #		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
		Comments:		

8-Day Dietary LC_{50}	89.4%	LC_{50} = 70 ppm (95% C.L. 58, 85) Control Mortality (%) = 0%		
Species	Colinus virginianus	Slope = 1.2 # Animals/Level = 10 Age (Days) = 10	RGM 4/10/91	Core
Lab	Biolife Associates, Ltd.	Sex = ND		
MRID #	416273-03	8-Day Dose Level ppm ai/(% Mortality) 9.8 (0%), 19.5 (0%), 39 (0%), 78 (70%), 156 (100%)		
		Comments: Based Concentrations + LC_{50} based on nominal concentrations.		