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: Mallard duck (Anas platyrhynchos).

4. **CITATION:** Pedersen, C.A. 1990. Profenofos Technical: 8-Day Acute Dietary LC_{50} Study in Mallard Ducklings. Study performed by Bio-Life Associates, Ltd., Neillsville, Wisconsin. Laboratory study #89 DC 142. Submitted by Ciba-Geigy Corporation, Greensboro, NC. EPA MRID No 416273-02.

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: E.C. Fite for Norm Cook
Date: 6/18/91

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

Signature: Henry 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 Associates, Inc, (BLAL) have so much variability in measured concentrations that is is impossible to determine actual treatment levels (See attached telephone conversation sheet and EEB files for review conducted by R.Felthousen on 7/20/90). In so much as the design deficiency was not

corrected for in this study the EEB must conclude that the results reported for this study may not be representative of the dietary toxicity of Profenfos to the mallard duck. 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:** N/A

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** The birds used in the study were 8-day-old mallard ducklings (Anas platyrhynchos) obtained from Whistling Wings, Hanover, Illinois. All test birds were phenotypically indistinguishable from wild birds. The birds were acclimated to laboratory conditions and observed daily for a 6-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 22°C (reported as 71°F) to 26°C (reported as 79°F). The relative humidity during the test period ranged between 46% and 83%.

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 78 (T-I), 156 (T-II), 312 (T-III), 625 (T-IV) and 1250 (T-V) ppm a.i.

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D. **Design:** Groups of mallards 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 (193 grams) into 13 kg basal diet.

Test diets were fed to the ducklings 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 last four days of 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 dying during the study were subjected to gross pathological examinations. Additionally, four arbitrarily selected birds from the vehicle control groups and from the 78 ppm a.i. group, as well as the four surviving 156 ppm a.i. test birds underwent 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 150 ppm a.i. with 95% confidence limits of 128 to 176 ppm a.i. (Table 2, attached). Six deaths were recorded in the 156 ppm a.i. test group and ten deaths were recorded in each of the 312, 625 and 1250 test groups (Table 3, attached). No mortalities occurred in the lowest concentration test group (78 ppm a.i.) 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 throughout the investigation. The first deaths occurred within approximately 19 1/4 hours post-administration. Signs of toxicity noted in the test groups included lethargy, anorexia, reduced water consumption, the appearance of weakness, and smallness in size. Total remission of all clinical signs was achieved by the end of test day 6.

Gross pathological examinations of the thirty-six birds that died during the investigation revealed abnormal pathological findings in two birds. The tops of both liver lobes in one 156 ppm a.i. test bird were pale. A pale, light tan-colored, left liver lobe was noted in one 625 ppm a.i. test bird. Gross pathological examinations of twelve selected survivors at termination revealed no abnormal pathological findings.

The vehicle control groups' average body weights on test day 8 ranged from 106 to 178 grams while the 78 and 156 ppm a.i. test groups' values were 126 and 165 grams, respectively.

Food consumption values during the quarantine period ranged from 14 to 20 grams/bird/day. The vehicle control groups' food consumption values during the test period ranged from 22 to 35 grams/bird/day while the 78, 156, and 312 ppm a.i. test groups' values ranged from 10 to 24 grams/bird/day. Anorexia was noted in the 312 ppm a.i. test group during the test period. The vehicle control groups' food consumption values during the recovery period ranged from 14 to 38 grams/bird/day while the 78 and 156 ppm a.i. test groups' values were 35 and 45 grams/bird/day, respectively.

A no-observed-effect level was not achieved in this study.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
An NOEC was not achieved in this study. The 8-day acute dietary LC_{50} was 150 ppm a.i. with 95% confidence limits of 128 to 176 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:**

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 brooder temperature was not reported.

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}.

The pen dimensions (45.7 cm x 61.0 cm = 2788 cm^2) were smaller than the recommended dimensions (70 cm x 100 cm = 7000 cm^2) for mallard ducks. Overcrowding as a result of small pens should be avoided.

The birds were assigned by 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 report did not indicate when signs of toxicity were first noted.

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

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

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 143 ppm a.i. with 95% confidence limits of 78 to 312. Since the author's value is similar to, and has a narrower confidence interval than the reviewer's LC_{50}, the reviewer accepts the author's value (150 ppm a.i.) as the estimated value to be used in risk assessment.

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C. **Discussion/Results:** This study has several procedural deviations. The most serious deviation being that the report did not indicate the time period in which signs of toxicity occurred. The report does state that signs of toxicity were gone by the end of day 6. The report does not indicate when the symptoms initially appeared. However, since "the first deaths occurred within approximately 19 1/4 hours post administration," it is safe to assume that behavioral signs of toxicity were present in the initial hours of the test. Therefore, for purposes of risk assessment, it should assumed that behavioral signs of toxicity were present from day 1 through day 6, The registrant should ensure that in future reports, a more detailed description is provided regarding the time period in which signs of toxicity were present.

The pen dimensions were much smaller than those recommended. Overcrowding as a result of small pens could potentially result in abnormal behavior. Although it can not be determined that overcrowding 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.

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

The study is scientifically sound and meets the requirements for an avian dietary LC_{50} study. The LC_{50} (150 ppm a.i.) indicates that the test substance is highly toxic to mallard ducklings. The NOEC could not be determined due to behavioral signs of toxicity at all treatment concentrations.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid

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

(3) **Repairability:** Yes

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

TELEPHONE RECORD: June 26, 1990

TELE. 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	

~~35~~ preliminary batches.

Precision -

Biological variability too large

* Don't know how to analyze the material

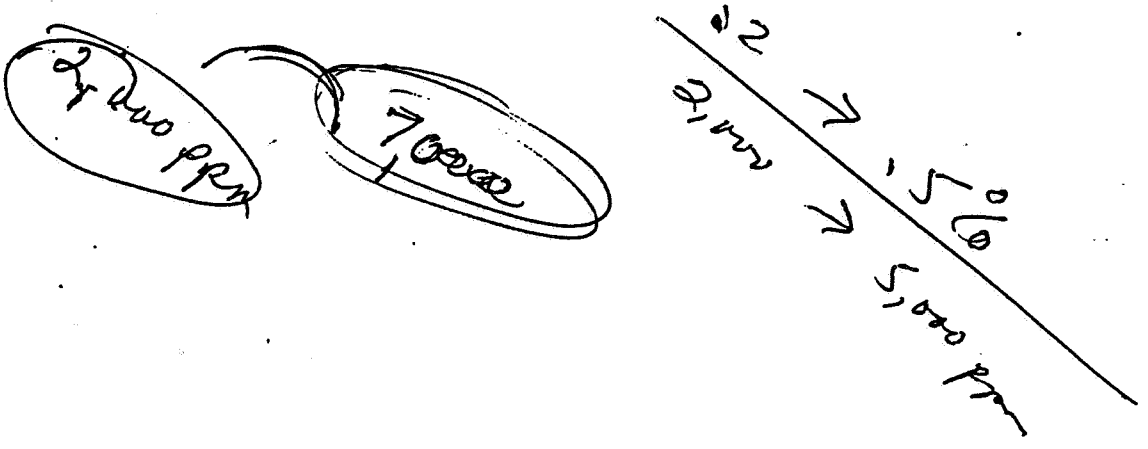

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TABLE 2

LC_{50} CALCULATIONS

PROFENOFOS TECHNICAL

Group	Nominal Concentration (ppm a.i.)	Observed Response	Expected Response	Residual	Contribution to Chi Square
T-I	78	0.0	0.0	0.0	0.0000
T-II	156	60.0	60.0	0.0	0.0000
T-III	312	100.0	100.0	0.0	0.0000
T-IV	625	100.0	100.0	0.0	0.0000
T-V	1,250	100.0	100.0	0.0	0.0000

Total 0.0000
x10
0.0000

Total Number of Animals = 50
Number of Groups = 5

Total contributions to Chi square = 0.000

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

The data are not significantly heterogeneous.

LC_{16} = 125 ppm a.i.

LC_{50} = [illegible] ppm a.i.

LC_{84} = [illegible] ppm a.i.

Slope Function = 1.20

N' = 10

$F(LC_{50})$ = 1.17

95% confidence limits of LC_{50}

LC_{50} = 150 ppm a.i. (128 to 176)

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	78	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
T-II	156	0/10	0/10	2/10	5/10	6/10	6/10	6/10	6/10
T-III	312	0/10	0/10	2/10	7/10	10/10	10/10	10/10	10/10
T-IV	625	0/10	2/10	5/10	10/10	10/10	10/10	10/10	10/10
T-V	1,250	0/10	4/10	8/10	10/10	10/10	10/10	10/10	10/10

The LC_{50} value was determined to be 150 ppm a.i. with 95% confidence limits of 128 to 176 ppm a.i.

TABLE 4

AVERAGE BODY WEIGHT AND ESTIMATED FOOD CONSUMPTION

PROFENOFOS TECHNICAL

Group	Nominal Concentration (ppm a.i.)	Avg. Body Weight (g) 0 Hour	Test Day 8	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	92	106 +14 15%	1089	422	22	14
V. Control - II	0	98	178 +80 82%	1731	1128	35	38
V. Control - III	0	91	129 +38 42%	1213	503	24	17
V. Control - IV	0	97	109 +12 12%	1176	654	24	22
V. Control - V	0	81	115 +34 42%	1211	673	24	22
T-I	78	90	126 +36 40%	1195	1038	24	35
T-II	156	87	165 +78 89%	791	535	20	45
T-III	312	91	-	338	-	10	-
T-IV	625	88	-	-	-	-	-
T-V	1,250	93	-	-	-	-	-

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

MARISE ROBBINS PROFENOFOS MALLARD DUCK

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
1250	10	10	100	9.765625E-02
625	10	10	100	9.765625E-02
312	10	10	100	9.765625E-02
156	10	6	60.00001	37.69531
78	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 78 AND 312 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 142.8738

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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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} = 150 ppm a.i. (95% C.L. 128, 176) Control Mortality (%) = 0%		
Species	Anas platyrhynchos	Slope = 12 # Animals/Level = 10 Age (Days) = 8	Rem 4/10/91	Core
Lab	BioLife Associates, Ltd	Sex = ND		
MRID #	41627-02	8-Day Dose Level ppm a.i./(% Mortality) 78 (0%), 156 (60%), 312 (100%), 625 (100%), 1250 (100%)		
		Comments: ~~Data reqm~~ LC_{50} + all concentrations based on nominal values.		