

DATA EVALUATION RECORD

1. **CHEMICAL:** Profenofos
Shaughnessey No. 111401
2. **TEST MATERIAL:** Profenofos Technical; Batch Code P707240; ID # FL-881610 ARS-9312; 90.4% active ingredient; an amber-colored oily liquid.
3. **STUDY TYPE:** Freshwater Invertebrate Static Acute Toxicity Test. Species, Tested: Daphnia magna
4. **CITATION:** Bellantoni, D.C. 1990. Profenofos Technical: A 48-Hour Static Acute Toxicity Test with the Cladoceran (Daphnia magna). Laboratory Study No. 108A-106. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Agricultural Division, Ciba-Geigy Corporation, Greensboro, NC. EPA MRID No. 416273-04.

5. **REVIEWED BY:**

Louis M. Rifici, M.S.
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Signature: *Louis M Rifici*
Date: *4/8/91*

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
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Signature: *P. Kosalwat*
Date: *4/8/91*

Henry T. Craven, M.S.
Supervisor, EEB/HED
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Signature: *Henry T. Craven*
Date: *6/11/91*

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute static toxicity test for freshwater invertebrates. Based on nominal concentrations, the 48-hour EC₅₀ of Profenofos for Daphnia magna was 0.93 µg a.i./L. Therefore, Profenofos is classified as very highly toxic to Daphnia magna. The NOEC, based on the lack of sublethal effects, was 0.72 µg a.i./L.

8. **RECOMMENDATIONS:** N/A

6/11/91

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

- A. Test Animals: The daphnids (*Daphnia magna*) used in the test were obtained from in-house cultures. The adult daphnids were fed a mixture of yeast, Cerophyll®, and trout chow. Daphnids were cultured under the test conditions listed in Section 11.B. The daphnid cultures were judged as in good health and showed no signs of disease or stress. Neonates were obtained for testing by transferring individual adult daphnids to dilution water 24 hours prior to test initiation. Less than 24-hour old, first instar larvae from at least three different adults were chosen for the test.
- B. Test System: The test chambers were 250-mL glass beakers containing 150 mL of test solution. The test solution depth was approximately 5 cm. The test chambers were randomly positioned in a temperature-controlled water bath designed to maintain $20 \pm 1^\circ\text{C}$. The laboratory environment was maintained on a 16-hour daylight photoperiod with 30-minute dawn and dusk simulations. Medium-hard well water with the characteristics listed in Table 1 (attached) was aerated and filtered ($0.2 \mu\text{m}$) before use as dilution water.
- C. Dosage: Forty-eight-hour static test. Based on a preliminary test, five nominal concentrations (0.26, 0.43, 0.72, 1.2 and 2.0 $\mu\text{g/L}$), a dilution water control and a solvent control (0.0004 mL acetone/L) were used. The concentrations made were based on the percent active ingredient in the test material.
- D. Design: A stock solution was prepared by diluting 0.6 g of Profenofos to 100 mL with acetone. Two serial dilutions were made in reverse-osmosis water. Aliquots of the resultant stock were pipetted directly into appropriate amounts of dilution water. The test solutions were stirred and distributed to the test vessels.

Daphnids were impartially removed from holding tanks in groups of two and distributed to each test beaker for a total of 10 individuals per concentration. Observations of mortality and immobility were made

every 24 hours. The daphnids were not fed during the test.

The dissolved oxygen (D.O.), temperature, and pH were measured in all concentrations and the controls at the beginning and end of the test. The temperature was also monitored continuously in one control replicate.

E. **Statistics:** The median effective concentration (EC_{50}) and associated 95% confidence interval (C.I.) for each 24-hour interval were calculated using a computer program developed by Stephan et al. (1978).

12. **REPORTED RESULTS:** The responses of *Daphnia magna* are given in Table 3 (attached). The 48-hour EC_{50} based on nominal concentrations was 1.4 $\mu\text{g a.i./L}$ (95% C.I. = 0.72-2.0 $\mu\text{g a.i./L}$). The no-observed-effect concentration (NOEC) was given as 0.72 $\mu\text{g a.i./L}$.

Dissolved oxygen ranged from 7.8 to 8.4 mg/L. The pH values were 8.3 at the beginning of the test and 8.6 at the end of the test. The temperature was 20.0°-20.5°C throughout the test.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
The author concluded that the 48-hour EC_{50} of Profenofos for daphnids was 1.4 $\mu\text{g a.i./L}$ (95% C.I. = 0.72-2.0 $\mu\text{g a.i./L}$) based on nominal concentrations and the NOEC was 0.72 $\mu\text{g a.i./L}$.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

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

A. **Test Procedure:** The test procedures were generally in accordance with protocols recommended by the guidelines, but deviated as follows:

Ten daphnids were tested per concentration. At least 20 daphnids per concentration should be tested to help ensure reliable results.

The results of preliminary studies were not given in the report.

The period between test solution preparation and the initiation of the test was not stated in the report. Tests should be initiated within 30 minutes of solution preparation.

- B. Statistical Analysis:** The reviewer used EPA's Toxanal Program to calculate the EC₅₀ value (see attached printout). Based on the total number of daphnids affected, the 48-hour EC₅₀ was 0.93 µg a.i./L by the binomial method. The 95% C.I. was 0.72-1.2 µg a.i./L. The EC₅₀ value is different from the author's value (1.4 µg a.i./L). Based on visual inspection of the data, it is believed that the author's value is in error. The author probably did not include the eight individuals exhibiting hyperexcitability at the 1.2 µg a.i./L level in the analysis.
- C. Discussion/Results:** An adequate number of test organisms per test level should be used to help ensure reliable results. This study used ten organism per level. The SEP recommends 20 Daphnia magna per test level but ASTM (1980) states that as few as 10 organisms per level can be used. It does not appear that the number of organisms used per level in the study affected the results.

This study is scientifically sound and meets the guideline requirements for a static acute freshwater invertebrate toxicity study. The 48-hour EC₅₀ of 0.93 µg a.i./L (based on nominal concentrations) classifies Profenofos as very highly toxic to Daphnia magna. The NOEC, based on the lack of sublethal effects, was 0.72 µg a.i./L.

- D. Adequacy of the Study:**
- (1) **Classification:** Core
 - (2) **Rationale:** N/A
 - (3) **Repairability:** N/A

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 03-21-91.

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Table 1. Dilution Water Chemistry Parameters for the 4-Week Period Immediately Preceding the Study		
Client: CIBA-GEIGY Corporation Test Substance: Profenofos Test Organism: Daphnid, <u>Daphnia magna</u> Dilution Water: Well Water		
	Mean	Range
Conductivity (μ mhos/cm)	336	330 - 350
Hardness (mg/L as CaCO ₃)	151	132 - 159
Alkalinity (mg/L as CaCO ₃)	191	160 - 200
pH	8.1	8.0 - 8.4

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Table 3. Cumulative Percent Mortality and Observed Effects.				
Client: CIBA-GEIGY Corporation				
Test Substance: Profenofos				
Test Organism: Daphnid, <u>Daphnia magna</u>				
Dilution Water: Well Water				
Nominal Concentration (mg active ingredient/L)	24 Hours		48 Hours	
	Mortality	Effects	Mortality	Effects
Negative Control	0% (0/10)	NUO	0% (0/10)	NUO
Solvent Control	0% (0/10)	NUO	0% (0/10)	NUO
0.00026 mg/L	0% (0/10)	NUO	0% (0/10)	NUO
0.00043 mg/L	0% (0/10)	NUO	0% (0/10)	NUO
0.00072 mg/L	0% (0/10)	NUO	0% (0/10)	NUO
0.00120 mg/L	0% (0/10)	10B	20% (2/10)	8B
0.002 mg/L	50% (5/10)	5E	100% (10/10)	N/A

Observed Effects:

A = Surfacing	J = Mucus Shedding
B = Hyperexcitability	K = Curved Spine
C = Lethargy	L = Ulcer
D = Discoloration	M = Moribund
E = Erratic Swimming	N = Loss of Equilibrium
F = Hyperventilation	O = Opaque Eyes
G = Gulping Air	P = Molting
H = Hemorrhaging	NUO = Nothing Unusual Observed
I = Excessive Mucus	N/A = Not Applicable

LOUIS M. RIFICI PROFENOFOS DAPHNIA MAGNA 3-21-91

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
2	10	10	100	9.765625E-02
1.2	10	10	100	9.765625E-02
.72	10	0	0	9.765625E-02
.43	10	0	0	9.765625E-02
.26	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT .72 AND 1.2 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 .9295159

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.

Haughnessy No. 110 11401

Chemical Name Peledenobis Chemical Class _____ Page 1 of 1

Study/Species/Lab/ Accession _____ Chemical g.a.i. Reviewer/Date _____ Validation Status _____

4-Day Single Dose Oral LD₅₀ Results

LD₅₀ = mg/kg (95% C.L.) Contr. Mort.(X) = _____

Species _____ Slope = # Animals/Level = _____ Age(Days) = _____ Sex = _____

Lab _____ 14-Day Dose Level mg/kg/(X Mortality) _____

Acc. _____ Comments: _____

14-Day Single Dose Oral LD₅₀ Results

LD₅₀ = mg/kg. (95% C.L.) Contr. Mort.(X) = _____

Species _____ Slope = # Animals/Level = _____ Age(Days) = _____ Sex = _____

Lab _____ 14-Day Dose Level mg/kg/(X Mortality) _____

Acc. _____ Comments: _____

8-Day Dietary LC₅₀ Results

LC₅₀ = ppm (95% C.L.) Contr. Mort.(X) = _____

Species _____ Slope = # Animals/Level = _____ Age(Days) = _____ Sex = _____

Lab _____ 8-Day Dose Level ppm/(X Mortality) _____

Acc. _____ Comments: _____

8-Day Dietary LC₅₀ Results

LC₅₀ = ppm (95% C.L.) Contr. Mort.(X) = _____

Species _____ Slope = # Animals/Level = _____ Age(Days) = _____ Sex = _____

Lab _____ 8-Day Dose Level ppm/(X Mortality) _____

Acc. _____ Comments: _____

48-Hour LC₅₀ Results

Species Daphnia magna LC₅₀ = 0.93 ^{*}ppm (95% C.L. binomial) Contr. Mort.(X) = 0

Lab Wildlife International Ltd. Slope = N/A # Animals/Level = 10 Sol. Contr. Mort.(X) = 0

Acc. MRID 411,272-04 90.4% Temperature = 20 ± 1°C LR Che

3/26/91

0.26 (0) 0.43 (0) 0.72 (0) 1.12 (no) 2.0 (100)

Comments: * nominal concentrations

96-Hour LC₅₀ Results

LC₅₀ = pp (95% C.L.) Con. Mort.(X) = _____

Species _____ Sol. Con. Mort.(X) = _____

Lab _____ Temp. = _____

Acc. _____ 96-Hour Dose Level pp/(X Mortality) _____

Comments: _____

96-Hour LC₅₀ Results

LC₅₀ = pp (95% C.L.) Con. Mort.(X) = _____

Species _____ Sol. Con. Mort.(X) = _____

Lab _____ Temp. = _____

Acc. _____ 96-Hour Dose Level pp/(X Mortality) _____

Comments: _____

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