

11.12.92

MRID No. 152739

DATA EVALUATION RECORD

1. **CHEMICAL:** Cypermethrin
Shaughnessey No. 109702
2. **TEST MATERIAL:** 3-Phenoxybenzoic Acid (3-PBA); Lot No. 36601; 99% active ingredient.
3. **STUDY TYPE:** Freshwater Invertebrate Static Acute Toxicity Test. Species Tested: Daphnia magna
4. **CITATION:** Everett, C.J., M.J. Hamer, and I.R. Hill. 1983. 3-Phenoxybenzoic acid: Toxicity to first instar Daphnia magna (II). Report Series RJ 0318B. Prepared and submitted by ICI Plant Protection Division, Bracknell, Berkshire, England. EPA MRID No. 152739.

5. **REVIEWED BY:**

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Signature:

Date:

Louis M. Rifici

4/11/91

*Gene Lambert
12/18/92*

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
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7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute static toxicity test for freshwater invertebrates. Based on measured concentrations, the average 48-hour EC_{50} from two tests of 3-Phenoxybenzoic Acid for Daphnia magna was 89.0 mg/L. Therefore, 3-Phenoxybenzoic Acid is classified as slightly toxic to Daphnia magna. The NOEC was estimated as 52.2 mg/L.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

- A. Test Animals: The Daphnia magna used were less than 24 hours old and were cultured by the methods outlined in Doma (1979) except that the organisms were permanently kept in a dense algal suspension.
- B. Test System: Vessels used in the test were 250-mL glass beakers containing 200 mL of dilution water (control) or test solution. A 16-hour light photoperiod was provided by two warm-white fluorescent tubes with a light intensity of 700 lux at the solution surface. The vessels were held in a temperature controlled water bath ($20^{\circ}\pm 1^{\circ}\text{C}$). Reconstituted water was used as dilution water. The quantities of salts used were 192 mg NaHCO_3/L , 120 mg $\text{CaSO}_4 \times 2\text{H}_2\text{O}/\text{L}$, 245 mg $\text{MgSO}_4 \times 7\text{H}_2\text{O}/\text{L}$, and 8 mg KCl/L .

The daphnids were not fed during the test.

- C. Dosage: Forty-eight-hour static test. Based on an earlier test, five nominal concentrations (25, 50, 100, 200, and 400 mg/L) and a dilution water control were used. Two separate tests were performed within 6 days of each other.
- D. Design: Two 3-PBA solutions, 400 and 200 mg/L, were prepared in reconstituted water. The solutions were shaken overnight followed by sonication for 10 minutes before use. The 200 mg/L solution was serially diluted in dilution water to give the three remaining test concentrations. Three beakers were used for each concentration and ten daphnids were used per beaker. All concentrations were observed once at 3, 9, 24, and 48 hours for mortality and immobilization. The dissolved oxygen (D.O.) and pH were measured in all concentrations at the beginning and end of the test. The temperature of the water bath was recorded at each observation time.
- 3-PBA concentrations were measured by HPLC analysis from samples taken at test initiation and termination.
- E. Statistics: The 48-hour median effective concentration (EC_{50}) and associated 95% confidence interval (C.I.)

were calculated using weighted linear regression or simple logarithmic interpolation.

12. **REPORTED RESULTS:** The mean measured concentrations in test I were 24.0, 52.2, 104, 220, and 425 mg/L and in test II were 25.2, 50.7, 104, 216, and 404 mg/L. The mean measured concentrations from the two tests ranged from 96 to 110% of nominal test concentrations (Table 1, attached).

The responses of Daphnia magna are given in the Appendix (attached). The 48-hour LC_{50} value based on mean measured concentrations was 85 mg/L (95% C.I. = 52-104 mg/L for test I and 51-104 mg/L for test II) for both tests. A no-observed-effect concentration (NOEC) was not given by the authors.

Dissolved oxygen levels were greater than 9.0 mg/L throughout both tests. The pH values ranged from 7.8 to 8.2. The temperature was within the $20 \pm 1^\circ C$ range at each observation time.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The authors suggested that the breakdown of Cypermethrin in the environment to 3-PBA and dihalovinyl cyclopropane acid greatly reduces the toxicity.

A Quality Assurance Unit statement was included in the report.

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:

The hardness of the dilution water was not given in the report. The hardness is estimated by the reviewer to be approximately 168 mg/L as $CaCO_3$.

The 200 and 400 mg/L test solutions were made the night before the test. Test solutions should be prepared within 30 minutes of test initiation.

The length of time between solution preparation and test initiation was not given and the method used to transfer daphnids to test solutions was not included in the report.

The test temperature was not monitored every six hours as recommended. No raw temperature data were provided.

No acclimation period to the test water was reported.

The report did not state whether the recommended 15-30 minute transition period between light and dark was used.

Each nominal concentration was approximately 50% of the next highest concentration. The guidelines recommend that each concentration be at least 60% of the next highest concentration.

Observations of the daphnid cultures such as adult mortality, stress, and the presence of ephippia were not given in the report.

First instar Daphnia magna used in tests should be from the fourth or later broods of a given parent. The authors did not indicate which brood was the source of the test animals.

- B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the EC₅₀ values and obtained similar results (see attached printout).
- C. **Discussion/Results:** These two tests were performed to determine the toxicity of 3-PBA, a breakdown product of the pesticide Cypermethrin. The tests were performed with the pure chemical, not the mixture of breakdown products which would probably result from Cypermethrin application.

The hardness of the reconstituted water was not given in the report. The authors describe the reconstituted water as "hard", but the hardness had to be estimated from the quantity of calcium and magnesium salts added. When preparing reconstituted water, basic water chemistry determinations, such as hardness and alkalinity, should be performed to ensure the quality of the water. In addition, guidelines recommend limits for hardness which should be addressed prior to test initiation.

The EC₅₀ values from the two tests were in agreement with each other. The 48-hour EC₅₀ of 89.0 mg/L (based on measured concentrations) classifies 3-PBA as

slightly toxic to Daphnia magna. The NOEC was 52.2 mg/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, 03-24-91.

AUTHOR'S REFERENCES: Doma, S. 1979. Ephippia of Daphnia magna Straus - a Technique for Their Mass Production and Quick Revival. Hydrobiologia 67(2):183-188.

Cypermethrin Review

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Test 1

LOUIS M. RIFICI CYPERMETHRIN DAPHNIA MAGNA 3-22-91

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
425	30	30	100	9.313227E-08
220	30	30	100	9.313227E-08
104	30	21	70	2.138698
52.2	30	0	0	9.313227E-08
24	30	0	0	9.313227E-08

THE BINOMIAL TEST SHOWS THAT 52.2 AND 104 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 89.21508

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.

Test 2

LOUIS M. RIFICI CYPERMETHRIN DAPHNIA MAGNA 3-22-91

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
404	30	30	100	9.313227E-08
216	30	30	100	9.313227E-08
104	30	21	70	2.138698
50.7	30	0	0	9.313227E-08
25.2	30	0	0	9.313227E-08

THE BINOMIAL TEST SHOWS THAT 50.7 AND 104 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 88.63832

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.
