

US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Chlorpyrifos (Dursban ME 20).
Shaughnessey No. 041701.
- 2. **TEST MATERIAL:** Dursban® 20 MEC (XRM-5160); Identification:
AGR 286398; Dow Registry No. DR-0320-1647; 25.6% active
ingredient chlorpyrifos; a milk-colored liquid.
- 3. **STUDY TYPE:** Freshwater Invertebrate Static Acute Toxicity
Test. Species Tested: Daphnia magna.
- 4. **CITATION:** Mayes, M.A., M.F. Servinski, S.J. Gorzinski, and
R.B. Potter. 1991. XRM 5160 (Microencapsulated
Insecticide): The Response of Daphnia magna in a 48 Hour
Static Acute Test. Laboratory Project Study ID. ES-DR-0320-
1647-4. Prepared by Environmental Toxicology & Chemistry
Research Laboratory, The Dow Chemical Company, Midland, MI.
Submitted by DowElanco, Indianapolis, IN. EPA MRID No.
418852-02.

5. **REVIEWED BY:**

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Date: 7/19/91

6. **APPROVED BY:**

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Signature: *Henry T Craven*
Date: 10/2/91

- 7. **CONCLUSIONS:** This study is not scientifically sound. The
analysis for chlorpyrifos was highly variable and suggests
that the actual concentrations of chlorpyrifos the daphnids
were exposed to are unknown. Under the conditions of the
test, the 48-hour LC₅₀ of 0.45 mg/L (based on nominal
concentrations of formulation) classifies XRM-5160 as highly
toxic to Daphnia magna. Observations of sublethal effects,
if any, were not given in the report, therefore, the NOEC
could not be determined.

8. RECOMMENDATIONS: N/A.
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 cultures were fed Selenastrum capricornutum three times weekly. On the day before the test, adults were isolated from the mixed (adults and neonates) cultures by filtering on nylon mesh screens and placed in new rearing tanks. On the day of the test, the adults were screened out of the 24-hour-old cultures leaving less than 24-hour-old daphnids for use in testing.

B. Test System: The test vessels were 250-mL glass beakers containing 200 mL of test solution and were covered with watch glasses. The vessels were positioned in an incubator designed to maintain $20 \pm 1^\circ\text{C}$. A 16-hour light photoperiod was maintained using cool-white fluorescent tubes.

The dilution water was obtained from the City of Midland Water Treatment Plant before treatment. The hardness was adjusted to approximately 170 mg/L as CaCO_3 . The water was autoclaved and aerated before use and had a pH of 7.8, an alkalinity of 53 mg/L as CaCO_3 , and a conductivity of 340 $\mu\text{mhos/cm}$.

C. Dosage: Forty-eight-hour static test. Seventeen nominal concentrations (0.008, 0.015, 0.03, 0.06, 0.12, 0.24, 0.49, 0.98, 2.0, 3.9, 7.8, 15.6, 31.3, 62.5, 125, 252, and 502 mg/L) and a dilution water control were used. The concentrations made were based on the total product (tested as a formulation).

D. Design: Within 30 minutes on test solution addition, daphnids were impartially added to the test vessels for a total of 10 individuals per vessel, three vessels per concentration. Observations of mortality and sublethal effects (immobility or floating) were made daily. Dead individuals were not removed from the test vessels. 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 test

initiation, 24, and 48 hours. The temperature was also monitored continuously in a representative test vessel.

The concentrations of chlorpyrifos in six test solutions (0.015, 0.06, 0.49, 3.91, 62.5, and 250 mg/L nominal concentration) after 0 and 48 hours were measured using gas chromatography .

E. **Statistics:** The 96-hour median lethal concentration (LC_{50}) and associated 95% confidence interval (C.I.) was calculated using a computer program developed by Stephan et al.

12. **REPORTED RESULTS:** "Results of the analysis of XRM-5160 can be found in Table 5 (attached). The erratic analyses and high variability among replicates at hour 0 and the reduced analyzed concentrations of chlorpyrifos observed at 48 hours, especially at the low concentrations, indicate that some of the XRM-5160 microcapsules and/or chlorpyrifos which may have leached from the capsules sorbed to test vessels reducing the availability of chlorpyrifos for sampling. In addition, the distribution of capsules within the solution appeared to be unevenly dispersed."

The responses of Daphnia magna are given in Table 7 (attached). The 48-hour LC_{50} value, based on nominal concentrations, was 0.45 mg/L (95% C.I. = 0.11-1.7 mg/L). "The very flat response curve suggests that consistent low levels of chlorpyrifos were being leached into the water over a wide range of XRM-5160 concentrations."

Dissolved oxygen ranged from 8.6 to 9.4 mg/L. The pH was 7.1-7.9 and the temperature was 18.4°-20.6°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
The authors stated that XRM-5160 was highly toxic to Daphnia magna.

Quality Assurance and Good Laboratory Practice 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:

The test was conducted with a formulated product. The technical grade is required for testing.

An inert or carrier ingredient control was not included in the test design.

The test concentrations were approximately 50% of the next highest concentration. The SEP recommends that the concentrations be approximately 60% of the next highest.

The test temperature was as low as 18.4°C. The recommended test temperature for Daphnia magna is 20°C.

The procedures used to prepare the toxicant stock solutions were not reported.

No observations on pretest mortality and the health of the source culture(s) were given in the report.

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

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

The method used to transfer daphnids to test solutions was not described in the report.

- B. **Statistical Analysis:** The reviewer used EPA's Toxanal Program to calculate the LC₅₀ value and obtained the same results (see attached printout). Since only 6 out of 17 test concentrations were measured during the test, nominal concentrations were used to calculate the LC₅₀.
- C. **Discussion/Results:** As the authors indicated, the analysis of chlorpyrifos in solution gave erratic and highly variable results. In the words of the authors, "chlorpyrifos which may have leached from the capsules sorbed to test vessels reducing the availability of chlorpyrifos for sampling." In the reviewer's opinion, the availability of the toxic component, chlorpyrifos, to the daphnids must have also been reduced, therefore making uncertain the actual concentrations the daphnids were exposed to.

It is doubtful the these test results are reproducible. XRM-5160 is obviously a difficult material to work with and a static system is probably not adequate for testing this formulation.

This study is not scientifically sound. The physical properties of the formulation (microencapsulation) caused the analysis for chlorpyrifos to be highly variable and suggests that the actual concentrations of chlorpyrifos the daphnids were exposed to are unknown. Under the conditions of the test, the 48-hour LC₅₀ of 0.45 mg/L (based on nominal concentrations) classifies XRM-5160 as highly toxic to Daphnia magna. Observations of sublethal effects, if any, were not given in the report, therefore, the no-observed-effect-concentration (NOEC) could not be determined.

D. Adequacy of the Study:

- (1) **Classification:** Invalid.
- (2) **Rationale:** The actual concentrations of chlorpyrifos the daphnids were exposed to are unknown.
- (3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 07-11-91.

MSD # 418852-02

Page is not included in this copy.

Pages 6 through 7 are not included.

The material not included contains the following type of information:

- Identity of product inert ingredients.
- Identity of product impurities.
- Description of the product manufacturing process.
- Description of quality control procedures.
- Identity of the source of product ingredients.
- Sales or other commercial/financial information.
- A draft product label.
- The product confidential statement of formula.
- Information about a pending registration action.
- FIFRA registration data.
- The document is a duplicate of page(s) .
- The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

LOU RIFICI CHLORPYRIFOS DAPHNIA MAGNA 7-11-91

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
502	30	20	66.66667	4.936858
252	30	24	80	7.154533E-02
125	30	24	80	7.154533E-02
62.5	30	26	86.66666	2.973807E-03
31.3	30	21	70	2.138698
15.6	30	18	60.00001	18.07973
7.8	30	20	66.66667	4.936858
3.9	30	17	56.66667	29.23324
2	30	13	43.33333	29.23324
.98	30	19	63.33333	10.02442
.49	30	22	73.33334	.8062402
.24	30	18	60.00001	18.07973
.12	30	16	53.33334	42.77678
.06	30	7	23.33334	.261144
.03	30	10	33.33334	4.936858
.015	30	7	23.33334	.261144
8.000001E-03	30	9	30	2.138698

2.138698

LK 7/11/91

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 1.745484

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
12	.3980959	.4468572	.1097556	1.62428

Study/Species/Lab/ MRID # _____ Chemical % a.i. _____ Results _____ Reviewer/ Date _____ Validation Status _____

48-Hour EC₅₀ LC₅₀ * 95% C.L. MOVING AVERAGE
LC₅₀ 25.6 ppm 0.11 - 1.62 Control Mortality (%) - 0
 Slope - N/A # Animals/Level - 30 Solvent Control Mortality (%) - N/A
 Species: Daphnia magna Temperature - 20°C
 Lab: Environ. tox. + Chem. Dew Chemical Co.
 MRID # 418852-02 * based on nominal concentrations of the formulation
48-Hour Dose Level ppm / (% Effect)
0.008 (30), 0.015 (23), 0.03 (33), 0.06 (23), 0.12 (53), 0.27 (60), 0.54 (60), 1.08 (60)
2.0 (43), 3.9 (57), 7.8 (67), 15.6 (60), 31.3 (70), 62.5 (80), 125 (100)
 Comments: * based on nominal concentrations of the formulation

96-Hour LC₅₀ _____ 95% C.L. _____ Control Mortality (%) - _____
 Slope - _____ # Animals/Level - _____ Solvent Control Mortality (%) - _____
 Species: _____ Temperature - _____
 Lab: _____
 MRID # _____ 96-Hour Dose Level ppm / (% Mortality)
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Comments: