US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD

3/11/1994

1. **CHEMICAL:** Metolachlor.
Shaughnessey No. 108801.

2. **TEST MATERIAL:** CGA 24705 technical; Batch No. P. 605025; 96.4% active ingredient; a brown liquid.

3. **STUDY TYPE:** 72-4. Life-Cycle (21-day Renewal) Chronic Toxicity Test. Species Tested: *Daphnia magna*.

4. **CITATION:** Rufli, H. 1989. CGA 24705 Technical - Daphnia Reproduction Test. Study No. 89 11 03. Conducted by Ciba-Geigy Ltd., Basle, Switzerland. Submitted by Ciba-Geigy Corporation, Greensboro, NC. EPA MRID No. 430446-03.

5. **REVIEWED BY:**

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Date: 3/11/94

6. **APPROVED BY:**

Harry Craven
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Signature: H. T. Craven
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7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for a chronic, static-renewal toxicity test using the freshwater invertebrate, *Daphnia magna*. The number of young Daphnids produced was very low, this is sign of stress. The concentrations of the fresh solutions were not measured. See Section 14 A for the other deviations.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

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

11. MATERIALS AND METHODS:

A. **Test Animals:** *Daphnia magna* (<24 hours old) were obtained from in-house cultures. Adult daphnids were housed in glass vessels containing 2.5 l of dilution water (20 ±1°C).

The adult daphnids were fed a combination of green algae (*Scenedesmus subspicatus*) and flake fish food suspension three times weekly.

B. **Test System:** The test vessels were 100-ml beakers containing 50 ml of test solution. The beakers were covered with watch glasses and maintained at 20 ±1°C. The photoperiod was 16-hour light/8-hour dark with a light intensity of 1.5 klux.

The dilution water was reconstituted water which was characterized as having a pH of 7.9 ±0.1, and a hardness of 240 mg/l as $CaCO_3$. The water was aerated with filtered air for at least 24 hours before use.

A stock solution of between 14.5 and 16 mg/l was prepared in water on each renewal day. The stock was used to prepare the treatment solutions. The test solutions were not aerated.

C. **Dosage:** Twenty-one-day, static-renewal, life-cycle toxicity test. Based on a range finding test, five nominal concentrations (0.024, 0.12, 0.60, 3.0, and 15 mg/l) and a dilution water control were selected for the test.

D. **Design:** Each test concentration and control consisted of ten replicate beakers containing one daphnid each. Five extra replicates per test level were maintained from days 0-13 to account for the occurrence of males. The solutions were renewed every Monday, Wednesday, and Friday. Six days a week, the daphnids were fed the same combination of food as in culturing.

The number of immobilized adult daphnids was determined on days 1, 2, 4, 7, 14, and 21. The number of young (both dead and alive) were determined on days 7, 9, 12, 14, 16, 19, and 21.

The dissolved oxygen concentration (DO) was measured three times a week immediately before renewal. The pH in the control and highest-concentration solutions was

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5. **REVIEWED BY:**

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7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for a chronic, static-renewal toxicity test using the freshwater invertebrate, *Daphnia magna*. The test design was not scientifically sound, particularly with respect to the elimination of five daphnids from each test group on day 13 of the test. The author did not state how the selection for elimination was made. The dilution factor used in this test was very low (0.2), which may have led to an underestimation of the chronic effects of the test material. Additionally, growth was not measured. Based on mean measured concentrations and the most sensitive parameters (survival and average number of young produced per adult reproductive day), the MATC was >0.5 mg/l and <2.8 mg/l (geometric mean MATC = 1.2 mg/l).

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measured at test initiation, in the new and old solutions at each renewal, and at termination. Temperature was measured continuously.

Samples were taken from newly prepared treatment solutions on day 0 and from old solutions (after 48 hours) on days 7, 14, and 21. The concentration of test material was determined using high pressure liquid chromatography.

E. **Statistics:** The EC_{50} values for adult immobilization were determined using probit analysis. Immobilization and length of time to appearance of the first brood were analyzed using the Cochran-Armitage trend test or a generalization of this test. Reproduction and fraction of dead young were analyzed using Jonckheere's test.

12. **REPORTED RESULTS:** Measured concentrations of metolachlor in the treatment solutions were presented in Table 6 (attached). The overall mean measured concentrations ranged from 90 to 98% of nominal and were 0.023, 0.12, 0.54, 2.78, and 13.9 mg/l. Results are based on nominal concentrations.

The 21-day EC_{50} for adult survival was reported to be 6.8 mg/l with a 95% confidence interval of 3.4-15 mg/l. The 21-day NOEC and LOEC were 3.0 and 15 mg/l, respectively.

The 21-day NOEC, LOEC, and EC_{50} for reproduction in terms of total number of young produced were 3.0, 15, and >3.0 mg/l, respectively. In terms of fraction of total young dead, the NOEC and LOEC were 0.6 and 3.0 mg/l, respectively.

The NOEC and LOEC for length of time for appearance of first brood were 3.0 and 15 mg/l, respectively.

The pH of the test solutions ranged from 7.6 to 8.6 and the DO ranged between 93 and 128% of saturation.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No other information other than that provided in the results section was provided in the author's conclusions.

Quality Assurance documentation was provided in the report. The Good Laboratory Practice (GLP) statement included in the report indicated that the study was conducted in compliance with Swiss GLPs, which are in essence consistent with USEPA GLPs (40 CFR Part 160).

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

A. Test Procedure: The test procedures deviated substantially from the SEP and ASTM (1988) guidelines. The deviations are as follows:

The history of the brood stock (time in culture, number of previous broods) was not reported.

Test vessels (100-ml) were smaller than recommended (250-ml).

The hardness (240 mg/l as $CaCO_3$) was greater than recommended (160-180 mg/l as $CaCO_3$).

The results of the temperature measurements were not reported.

The light intensity (1.5 klux) was greater than recommended (0.4-0.8 klux).

Weekly measurements of alkalinity, hardness, and conductance were not made.

Three replicate containers containing 5 daphnids each to assess survival were not included in the test.

It was not stated whether the daphnids were randomly assigned or if the five daphnids excluded on day 13 were excluded by random draw.

No length or weight measurements were made.

Control solutions were not analyzed for the test material.

Chemical analysis was performed on treatment solutions only on day 0. The subsequent measurements (on days 7, 14, and 21) were performed only on old treatment solutions. The ASTM guidelines require a weekly measurement of both fresh and old solutions.

ASTM guidelines require that control Daphnids that lived for 21 days produce , on the average, at least 60 young. The average for the control was 17.6.

B. Statistical Analysis: N/A

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C. **Discussion/Results:** This study is not scientifically sound and does not meet the guideline requirements for a Life Cycle Aquatic Invertebrate. The study shows many deficiencies that cannot be repaired.

The number of young produced by the control was very low. On the average 17.6 young were produced at the end of the study. This show that the organisms were stressed.

Of much concern to the reviewer is the elimination of the extra five replicates of daphnids for each control and treatment group. The author indicated that this was done in case of the occurrence of males. Daphnids under proper culture conditions should not develop into males, as this is a sign of stress. The author did not reported the observations made to those extra five replicates.

The concentrations on the fresh solutions were not measured. This is a major deviation since we do not know the concentration at the time of the renewal.

The results of this study cannot be used because it is uncertain if the Daphnids were under stress conditions and because the concentration at the time of renewal are not known.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** See Sections 14 A and C.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 2-22-94.

REFERENCES: ASTM. 1991. Standard Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*. E 1193-91.

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