5/24/91

MRID No. 408450-01

DATA EVALUATION RECORD D159571 122-2 Section 5

1. CHEMICAL: Mancozeb.
Shaughnessey Number: 14504.

2. TEST MATERIAL: Dithane Ultra; 80% active ingredient as Mancozeb.

3. STUDY TYPE: Growth and Reproduction of Aquatic Plants - Tier II. Species tested: Scenedesmus subspicatus.

4. CITATION: Ellgenhausen, H. 1982. Determination of the Toxicity of Dithane Ultra to the Green Algae Scenedesmus subspicatus Chodat: Evaluation of EC10 and EC50. Project ID Number 002125. Prepared by RCC Research & Consulting, Itingen, Switzerland. Submitted by Rohm and Haas France S.A., Basel, Switzerland. EPA MRID Number 408450-01.

5. REVIEWED BY:

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6. APPROVED BY:

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7. CONCLUSIONS: This study does not fulfill the guideline requirements for a Tier 2 growth and reproduction of a non-target green alga test because the exposure was not at least 5 days and chemistry was not performed to verify concentrations. The 96-hour EC_{25} and EC_{50} values based upon nominal concentrations of Mancozeb to Scenedesmus subspicatus were 1.38 and 2.76 mg/L, respectively.
This study is classified as invalid and is not repairable. DK.

8. RECOMMENDATIONS: N/A.

6 hrs

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9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: The test organism was the unicellular freshwater green alga, Scenedesmus subspicatus, cultured according to the "Pruefrichtlinie des Umweltbundesamtes" (see reference 1).

B. Test System: The test was conducted under static conditions in cotton-plugged 50-mL Erlenmeyer flasks containing 20 mL of algal suspension. The flasks were incubated in a shaking waterbath (200 strokes/min.) at 22 ± 2°C under continuous illumination with an intensity of 8200 Lux. The illumination source was 20 cm above the flasks.

C. Dosage: Ninety-six-hour static test. Six nominal concentrations of the test substance were tested: 1, 2, 4, 8, 12, and 16 mg/L. A culture medium control was maintained currently. It was unclear from the report whether test concentrations reported were based upon whole material or active ingredient. It was assumed that the concentrations reported were as whole material. The test concentrations were prepared directly in the nutrient solutions. No solvents were used.

D. Design: Six test substance concentrations and a culture medium control were tested. Treatments were duplicated. The test was initiated with 13,000 cells/mL. Algal density determinations were conducted at 72 and 96 hours of exposure by filtration through a 0.45-µm filter, resuspending in 0.5 mL of water, and then determining cell density by means of a Neubauer counting cell.

The report does not mention if any water quality parameters or test material concentrations were measured.

E. Statistics: The mean cell densities were measured at each time period and were plotted as growth curves. The area under the growth curve (days 3 to 4) were

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calculated from each replicate culture according to the following formula:

$$\text{Area} = \frac{(N1-N0)}{2} \times T1 + \frac{(N1+N2) - 2N0}{2} \times (T2-T1)$$

Where:

N0 = Number of cells/mL at test initiation,
N1 = Number of cells/mL after 72 hours, and
N2 = Number of cells/mL after 96 hours.

Inhibition was then determined as follows:

$$I = \frac{\text{Area (Control)} - \text{Area (Treatment)}}{\text{Area (Control)}} \times 100\ \%$$

Percentage inhibition was plotted against test substance concentrations as logarithms and the EC_{10} and EC_{50} values determined from the curve.

Logit analysis was also used to determine an EC_{50} estimate with 95% confidence limits.

12. REPORTED RESULTS: Results of the test are presented in Table 1 (attached).

The reported 96-hour EC_{10} and EC_{50} values derived from the graphical method were 0.82 and 2.8 mg/L, respectively. Logit analysis produced an EC_{50} of 2.77 mg/L with 95% confidence limits of 2.63 and 2.92 mg/L. No NOEC was reported. All values were calculated using nominal concentrations.

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

The author states that this compound is "by a factor of 2.5 less toxic than potassium dichromate." Also, it is stated that due to its low solubility in water and its affinity for particulates, that it would be doubtful that this compound would be present in the aquatic environment for a long period of time.

No GLP compliance statement was included in the report.

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

A. Test Procedure: Test procedures deviated from the SEP and Subdivision J guidelines as follows:

- Neither the maximum label rate nor the maximum environmental quantity for this compound was reported.
- No description of pesticide application was given.
- The application rate of the product was not provided.
- Measurements of cell density were made only on days 3 and 4 of exposure. The guidelines specify that the length of the exposure should be at least 5 days and that cell density measurements should be made daily.
- No water quality parameters were reported.
- The EC_{25} was not calculated and reported as required by the SEP.
- Measurements of compound concentrations were not conducted.
- Initial inoculum was 13,000 cells/mL and not 3,000 cells/mL.
- No indication was provided if the study was conducted under GLP.
- Test temperature range for a test of a green alga species should be 24 ± 2°C; 22 ± 2°C was used in this study.
- The light intensity used was twice that recommended in the SEP.

B. Statistical Analysis: The reviewer used the EPA's probit analysis computer program to calculate the 96-hour EC_{25} and EC_{50} values (attached). Analysis of variance was not performed due to the lack of replicate growth values, therefore, a 96-hour NOEC could not be determined.

C. Discussion/Results: The study results as presented do not meet the requirements for a Tier 2 test due to the lack of replication and information describing the test conditions. Due to the facts that: 1) the test material had extremely low aqueous solubility (almost unsoluble), 2) the solvent was not used in this test, and 3) test concentrations were not measured, the actual concentrations the algae were exposed to are unknown.

The reviewer used a probit computer program to calculate the 96-hour EC_{25} and EC_{50} using values given for the area under the curves (attached). The 96-hour EC_{50} based upon nominal concentrations of Mancozeb was 2.76 mg/L with 95% confidence limits of 2.39 to 3.14 mg/L. The 96-hour EC_{25} calculated based upon nominal concentrations was 1.38 mg/L.

D. Adequacy of the Study:

(1) Classification: Invalid.

(2) Rationale: The test procedures significantly deviated from the guidelines, as presented in Section 14 A.

(3) Repairability: None.

15. COMPLETION OF ONE-LINER: Yes, April 12, 1991.

16. AUTHOR'S REFERENCES:

The following was referenced as containing the methods used by this facility for culturing and testing Scenedesmus subspicatus:

Pruefrichtlinie: "Hemmung der Zellvermehrung bei der Gruenalge Scenedesmus subspicatus Chodat", Berlin, 6.05.1982.

PROBIT ANALYSIS
USED FOR CALCULATING EC VALUES
IN ALGAL TESTS

Results calculated using area under curve.

CHI SQUARED NOT SIGNIFICANT. EXPECTED NUMBERS NOT CHECKED
Chi Square = 2.386414

P R O B I T T A B L E

CONCENTRATION	MEAN CELL COUNT	PERCENT INHIBITION	PREDICTED PROPORTION RESPONDING
1.00	5.9E+07	16.90	0.1639
2.00	4.2E+07	40.85	0.3784
4.00	2.9E+07	59.15	0.6402
8.00	1.2E+07	83.10	0.8480
12.00	5000000	92.96	0.9220
16.00	2000000	97.18	0.9551

THE CONSTANTS USED IN THIS PROBLEM WERE ;
HETEROGENEITY FACTOR = .5966034
NUMBER OF POINTS = 6
DEGREES OF FREEDOM = 4
DEVIATE = 1.96
G = 1.956264E-02
NUMBER OF CYCLES = 3
THE STATISTICS ARE;
AVG Y = 5.309927
AVG X = .5799683
AVG T = 1.395953
SLOPE = 2.221561 WITH A STANDARD ERROR = .1585316
INTERCEPT = 4.021493

EC POINT	CONCENTRATION	95% CONFIDENCE LIMITS LOWER	UPPER
EC 1	0.25	0.16	0.35
EC10	0.73	0.54	0.92
EC25	1.38	1.11	1.64
EC50	2.76	2.39	3.14
EC75	5.52	4.85	6.39
EC90	10.41	8.76	12.87
EC95	15.09	12.27	19.60