

DATA EVALUATION RECORD

1. **CHEMICAL:** Quinclorac.
Shaughnessey Number: not available.
2. **TEST MATERIAL:** BAS 514 H Technical (Quinclorac); Lot Number 150732N32; 3,7-dichloro-8-quinolinecarboxylic acid; CAS Number 84087-01-4; 96.5% active ingredient (a.i.), a white powder.
3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants, Tier 2. Species Tested: Anabaena flos-aquae.
4. **CITATION:** Hughes, J.S. 1989. The Toxicity of BAS 514H Lot No. 150732N32 to Anabaena flos-aquae. Laboratory Project ID. 0445-04-1100-2. Prepared by Malcolm Pirnie, Inc., White Plains, NY. Submitted by BASF Corporation, Research Triangle Park, NC. MRID No. 410635-74d.
5. **REVIEWED BY:**

Robin Hart, Ph.D.
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Signature: Robin Hart
Date: September 1, 1989
Charles Lee 7/13/90
6. **APPROVED BY:**

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Signature: Michael L. Whitten
Date: 9-5-89

Henry T. Craven, M.S.
Supervisor, EEB/HED
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Date:
7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements for a Tier 2 growth and reproduction of a non-target green alga test. The 7-day EC25 and EC50 values are greater than the highest test concentration, 500 ug/L. The highest test concentration in which growth is not significantly different from that in the control, the no observed effect concentration, is 500 ug/L.

8. RECOMMENDATIONS: N/A.
9. BACKGROUND: N/A.
10. DISCUSSION OF INDIVIDUAL TESTS: N/A.
11. MATERIALS AND METHODS:

A. Test Species: *Anabaena flos-aquae* used in this test came from laboratory stock cultures. The original culture was obtained from the University of Texas Culture Collection (UTEX 1444), Austin, TX. Stock cultures were maintained in synthetic algal assay procedure nutrient medium in Erlenmeyer flasks under illumination of approximately 2153 lumens/m² and temperature of 24 ± 2° C. Flasks were manually shaken once on each working day. Transfers were made regularly into fresh medium to provide 6 to 8-day old cultures for assay inoculations.

B. Dosage: Seven-day growth and reproduction test.

C. Test System and Design: Test vessels used were sterile chemically clean 250-ml Erlenmeyer flasks fitted with foam stoppers to permit gas exchange. Synthetic algal assay procedure medium was prepared with Type I water and the pH was adjusted to 7.5 ± 0.1 with 0.1N sodium hydroxide or hydrochloric acid. Medium was stored in the dark at 4° C and brought to room temperature prior to use.

Based on a range-finding test, nominal test concentrations of 3.125 to 100 ug/L were selected for definitive testing. However, insufficient inhibition was obtained during this test; therefore, a supplemental test was conducted using test concentrations up to 500 ug/L. Test concentrations were prepared by preparing a stock solution of 0.01 mg a.i./mL using 2.6 mg BAS 514H Technical, to the nearest 0.1 mg, and diluting to 250 ml in a volumetric flask with Type I water. After sonication for 15 to 45 minutes to put the test material into solution, a 1 ug/mL stock solution was prepared by appropriate dilution. Test concentrations were prepared by adding required volumes of stock solution to algal assay nutrient medium in volumetric flasks. The control contained medium only. Nominal test concentrations of

0, 3.125, 6.25, 12.5, 25, 50, and 100 ug/L were prepared. For the supplemental test, nominal concentrations of 0, 125, 250, and 500 ug/L were prepared. The pH of each treatment was recorded. Three replicates of each test concentration were used.

Algal inoculum was aseptically added to 100 mL of medium in each flask for the first test and the supplemental test to yield nominal initial concentrations of 3,000 cells/mL. Flasks were kept in a Psycrotherm Controlled Environment Incubator Shaker, Model G-27, at a temperature of $24 \pm 2^\circ \text{C}$. Temperature was recorded daily. Flasks were manually shaken once each working day. Continuous illumination of $2153 \pm 323 \text{ lumens/m}^2$ was provided by overhead cool-white fluorescent lights. Flasks were randomly repositioned each working day to minimize effects of spatial differences in the incubator.

Cell counts were made using a Coulter Counter on test days 2, 3, 4, and 7 of each test. The filaments were sonicated before counting to reduce the length of the filaments to a size that was consistent between samples without rupturing the cells. Three counts per replicate were made. For each test, a sample of the stock solution used to begin the test, the highest test concentration on Day 0 and Day 7 and the control on Day 7 were provided to the sponsor for the determination of the actual concentration of test material.

- D. **Statistics:** Mean cell count values at test termination for each test concentration were expressed as a percent relative to that in the control. Percent inhibition I was calculated according to the following formula:

$$\%I = \frac{C - T}{C} \times 100$$

where C = mean growth in the control

T = mean growth in treated culture.

A negative percent inhibition indicates stimulation. The no observed effect concentration was determined from an analysis of variance and Dunnett's test. Statistical analyses were performed using an SAS software package. The level of significance was at 0.05.

12. **REPORTED RESULTS:** Plots of mean cell counts vs. time show that exposure to BAS 514H had little effect upon the population growth of Anabaena flos-aquae (Figure 1 and Figure 2, attached). Effects of the test material on mean standing crop on day 7, relative to the control, ranged from 10.0% stimulation to 34.7% inhibition in the first test (Table 5, attached). Effects ranged from 11.7% to 15.9% inhibition in the supplemental test (Table 6, attached). Insufficient inhibition was attained to allow calculation of EC25 and EC50 values. The 7-day EC25 and the 7-day EC50 values are greater than the highest test concentration, 500 ug/L. The results of the ANOVA and Dunnett's test, conducted separately for each test, indicate that in both instances, the mean standing crop values on day 7 in all the test concentrations were not significantly different from that in the control. Therefore, the highest test concentration in which growth is not significantly different from that in the control, the NOEC, is 500 ug/L.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The results indicate that Tier 3 aquatic field testing is not necessary. The maximum label application rate of BAS 514H is 0.5 lbs a.i./acre. Direct application of 0.5 lbs a.i./acre to a 1 acre, 6 inch deep pond would result, under "worst-case" conditions, in an estimated environmental concentration of 367 ug/L, which is less than the estimated EC50 of greater than 500 ug/L.

A GLP Quality Assurance Compliance Statement was included in the report indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards (40 CFR Part 792 and 40 CFR Part 160).

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

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines.

B. **Statistical Analysis:** Although reference was made to an analysis of variance and Dunnett's test indicating that there was no significant difference between treated plants and the controls, the analysis was not presented with the report. However, an analysis performed by the

reviewer (attached) indicated that the mean counts were not significantly different between treatments.

C. Discussion/Results: The treated algae showed no difference in cell count from that of the control algae and, therefore, BAS 514H had no effect on this species at the rates that were applied.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: The study is scientifically sound and has been conducted in accordance with Subdivision J guidelines of a Tier 2 growth and reproduction of a non-target green alga test.

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER: N/A

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