(12-5-91)

Accession No. 408118-02

DATA EVALUATION RECORD

1. **CHEMICAL:** Arsenal.
Shaughnessey No. 128821.

2. **TEST MATERIAL:** AC 243,997; Lot No. 4866-62; 99.5% active ingredient; a white powder.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants.
Species Tested: Anabaena flos-aquae.

4. **CITATION:** Hughes, J.S. 1987. The Toxicity of AC 243,997 (Lot No. AC 4866-62) to Anabaena flos-aquae. Prepared by Malcolm Pirnie, Inc., White Plains, NY. Submitted by American Cyanamid Company, Princeton, NJ. EPA Accession No. 408118-02.

5. **REVIEWED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
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Signature: P. Kosalwat

Date: 11/29/88

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature:

Date: 12/5/91

6. **APPROVED BY:**

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Isabel C. Johnson

Date: 11/30/88

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier 2 growth and reproduction of a non-target bluegreen alga test. AC 243,997 with a 7-day EC50 value of 12.2 mg/L and NOEC value of 9.6 mg/L mean measured concentration, is not expected to exert a detrimental effect on the bluegreen alga (Anabaena flos-aquae) when applied at maximum application rates up to 1.25 pounds active ingredient per acre.

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

9. BACKGROUND:

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, Austin, TX. Stock cultures were maintained in a synthetic algal assay procedure (AAP) nutrient medium in Erlenmeyer flasks under constant illumination of approximately 200 foot-candles and temperature of 24 ± 2°C. Flasks were manually shaken 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 500-ml sterile Erlenmeyer flasks fitted with foam stoppers to permit gas exchange. The AAP nutrient medium was prepared with deionized water and the pH was adjusted to 7.5 ± 0.1.

Based on a range-finding test, five nominal concentrations of AC 243,997 (5.6, 10, 18, 32, 56, and 100 mg/L) were selected for the definitive test. Test concentrations were prepared by adding the appropriate volumes of the stock solution (5000 mg a.i/L) to AAP medium in 500-ml volumetric flasks. After thoroughly mixing, 100 ml of each concentration were added to each of three replicate test vessels. The control contained only 100-ml medium in each of three replicate flasks. Approximately 200 ml of each test concentration and the control were retained for analysis of initial test concentrations.

The test was initiated when 0.136 ml of a sonicated, 7-day-old stock culture (containing 2,210,000 cells/ml) was aseptically added to 100 ml of medium in each flask, yielding a nominal initial concentration of 3000 cells/ml. Flasks were kept in a Sherer Model RI-32-LLTP Incubator, at a temperature of 24 ± 2°C. Temperature was recorded daily. Flasks were manually shaken each working day and a continuous illumination of 2153 ± 323

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lumens/m^2 was provided by overhead cool-white fluorescent lights. Flasks were randomly distributed each working day to minimize spatial differences in the incubator.

Cell counts were made using a Coulter Counter (Model ZBI) on test days 2, 3, 4, and 7. Three counts per replicate were made. Samples were analyzed for the actual concentrations of AC 243,997 in the test solutions on day 0 and at the end of the assay.

E. Statistics: Mean cell count values at test termination on day 7 for each mean measured 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.

Note: A negative percent inhibition indicated stimulation.

To determine the EC25 and EC50 values, the log of test concentration (x-axis) was plotted against percent inhibition expressed as probit (y-axis). Inverse estimation least squares linear regression was used to determine the line of best fit, the concentrations corresponding to 25 and 50 percent inhibition and the associated 95% confidence limits. Parameters of the regression line were determined using the SAS statistical package. The value for the lowest test concentration, which was stimulatory, was omitted from the regression analysis.

12. REPORTED RESULTS: The test concentrations of AC 243,997 measured on day 0 ranged from 102 to 108% of the nominal concentrations, and on day 7 from 84 to 98% of the nominal concentrations.

Table 2 (attached) presents mean cell counts during the assay. Mean cell counts were plotted against time for each test concentration in Figure 1 (attached). From the growth curves in Figure 1, the author determined that all test concentrations except the lowest had inhibitory effects upon the population growth of A. flos-aquae, with very little growth in the three highest test concentrations.

Effects of the test material on mean standing crop on day 7 relative to the control ranged from 4.8% stimulation to 99.4% inhibition (Table 3, attached). The 7-day EC25 and 7-day EC50 values were 7.3 mg/L (95% C.L. = <0.0001 - 51.4 mg/L) and 11.7 mg/L (95% C.L. = <0.0001 - 105.5 mg/L), respectively.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: No conclusion was made by the author. Inspections had been conducted during the course of study by the Quality Assurance Unit of Malcolm Pirnie, Inc., for compliance with EPA Good Laboratory Practice Standards under the Federal Insecticide, Fungicide, and Rodenticide Act and the Toxic Substances Control Act (Fed. Reg. Vol. 48, No. 230, 11/29/83).

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, except for the following deviations:

o The maximum label rate was not provided in the report. However, according to the EEB, the test substance contains 4 lbs of acid/gallon and the application rate is 2.5 pints/acre or 1.25 lbs active ingredient/acre. Therefore, if the test substance were directly applied to the surface of a 15-cm or 6-inch water column, the resulting concentration in the water would be approximately 0.92 mg/L.

o The micronutrient stock solution used to prepare the AAP nutrient medium contained 300 mg/L of $Na_2EDTA.2H_2O$. According to Subdivision J guidelines, EDTA should not be used in the experimental medium.

o The pH measurement was made in only freshly prepared medium (without test chemical). The pH should have been measured in all test solutions at test initiation and termination.

o Cell counts at each treatment level were not statistically compared to the control values.

o Observations were made only on days 2, 3, 4, and 7. Therefore, it could not be determined whether the data provided for day 7 were the maximum standing crop of the controls. Daily observations should have been taken during the test period.

B. Statistical Analysis: The reviewer recalculated EC50 and EC25 values using a regression analysis (attached) and obtained slightly different results from those calculated by the author. The differences were due to the transformation of percent inhibition into probit before performing regression analysis by the author, while arcsine square-root transformation of percent inhibition was used by the reviewer. Analysis of variance was performed to compare cell counts at each treatment level to those of the controls (attached). The results showed that test concentrations of 17.6 mg/L and higher significantly reduced the cell counts of Anabaena flos-aquae at test termination (day 7).

C. Discussion/Results: The recalculated, 7-day EC25 and EC50 values of AC 243,997 for Anabaena flos-aquae were 6.62 and 12.2 mg/L mean measured concentration, respectively. Based on the reduction of cell counts at concentrations $\geq$ 17.6 mg/L, the no-observed-effect concentration (NOEC) was determined to be 9.6 mg/L. Therefore, AC 243,997 is not expected to exert a detrimental effect on the bluegreen alga (Anabaena flos-aquae) following normal application methods at rates up to 1.25 lbs active ingredient/acre.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: Although the test procedures deviated from the guidelines, the reviewer does not believe they significantly affected the validity of the toxicity results.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, November 29, 1988.

Anabaena flos-aquae

DATA POINT	X	Y
1	.9823	.4586
2	1.2455	1.0076
3	1.48	1.4812
4	1.7412	1.4933
5	2.0128	1.487

REGRESSION EQUATION:
Y=-.285134 + .9854688 X

COEFFICIENT OF CORRELATION= .8731549

ACTUAL VERSUS ESTIMATED VALUES
X=LOG CONCENTRATION Y=INHIBITION (Arcsin SQRT transformation)

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	.9823	.4586	.6828919	-.2242919
2	1.2455	1.0076	.9422672	6.533271E-02
3	1.48	1.4812	1.17336	.3078404
4	1.7412	1.4933	1.430764	6.253576E-02
5	2.0128	1.487	1.698417	-.2114174

Arcsine SQRT transformation of 50% and 25%
= 0.7854 and 0.5236, respectively.

If $Y = 0.7854$, $X = 1.0863$

$\therefore$ $EC_{50} = 12.20$ mg/L

If $Y = 0.5236$, $X = 0.8207$

$\therefore$ $EC_{25} = 6.62$ mg/L

Analysis of Variance File: anabaena Date: 01-12-1988

FILTER: None

N's, means and standard deviations based on dependent variable: COUNTS

* Indicates statistics are collapsed over this factor

Factors: C	Mean measured Conc. (mg/L)	N	Mean	S.D.
*		21	690142.8800	727658.6900
1	0	3	1530000.0000	141067.3590
2	5.26	3	1603333.3800	574485.2500
3	9.6	3	1230000.0000	134536.2500
4	17.6	3	436000.0000	74726.1640
5	30.2	3	12000.0000	2000.0000
6	55.1	3	9666.6670	577.3503
7	103.0	3	10000.0000	1732.0508

Fmax for testing homogeneity of between subjects variances:%990099.94
Number of variances= 7 df per variance= 2.

Analysis of Variance Dependent variable: COUNTS

Source	df	SS (H)	MSS	F	P
Between Subjects	20%	10589743.1000E+06			
C (CONC)	6%	9842493200000.0000%	1640415.4900E+06	30.734	0.0000
Subj w Groups	14%	747249860000.0000%	53374988000.0000		

Analysis of Variance File: anabaena Date: 01-12-1988

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1530000.000	6	9666.667
2	1603333.380	7	10000.000
3	1230000.000		
4	436000.000		
5	12000.000		

Comparison	Scheffe'	Tukey-A*	Tukey-B*	Newman -Keuls*	Bon-ferroni	T-test	Dunnett
1 < 2							
1 > 3							
1 > 4	0.0037	0.0100	0.0100	0.0100	0.0011	0.0001	0.0100
1 > 5	0.0001	0.0100	0.0100	0.0100	0.0000	0.0000	0.0100
1 > 6	0.0001	0.0100	0.0100	0.0100	0.0000	0.0000	0.0100
1 > 7	0.0001	0.0100	0.0100	0.0100	0.0000	0.0000	0.0100
2 > 3						0.0678	N.A.
2 > 4	0.0021	0.0100	0.0100	0.0100	0.0006	0.0000	N.A.
2 > 5	0.0001	0.0100	0.0100	0.0100	0.0000	0.0000	N.A.
2 > 6	0.0001	0.0100	0.0100	0.0100	0.0000	0.0000	N.A.
2 > 7	0.0001	0.0100	0.0100	0.0100	0.0000	0.0000	N.A.
3 > 4	0.0442	0.0500	0.0100	0.0100	0.0189	0.0009	N.A.
3 > 5	0.0014	0.0100	0.0100	0.0100	0.0004	0.0000	N.A.
3 > 6	0.0014	0.0100	0.0100	0.0100	0.0004	0.0000	N.A.
3 > 7	0.0014	0.0100	0.0100	0.0100	0.0004	0.0000	N.A.
4 > 5						0.0413	N.A.
4 > 6						0.0403	N.A.
4 > 7						0.0404	N.A.
5 > 6							N.A.
5 > 7							N.A.
6 < 7							N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.1000).
A blank means the P-value is greater than 0.1000.

For Dunnett's test only the P-values .05 and .01 are possible
and only for comparisons with the control mean (level 1).

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