(12-5-91)

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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: Skeletonema costatum.

4. **CITATION:** Hughes, J.S. 1987. The Toxicity of AC 243,997 (Lot No. AC 4866-62) to Skeletonema costatum. 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
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 11/29/88 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

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

Signature:
Date:

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier 2 growth and reproduction of a non-target marine diatom test. With a 7-day EC50 value of 92 mg/L and NOEC value of 15.9 mg/L mean measured concentration, AC 243,997 is not expected to exert a detrimental effect on the marine diatom (Skeletonema costatum) when applied at maximum application rates up to 1.25 lbs a.i./acre.

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

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Species: Skeletonema costatum used in this test came from laboratory stock cultures. The original culture was obtained from the Culture Collection of Marine Phytoplankton at the Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME. Stock cultures were maintained in a synthetic marine algal assay nutrient medium in Erlenmeyer flasks under illumination of approximately 400 foot-candles (4306 lumens/m^2) and temperature of 20 $\pm$ 2°C. Flasks were manually shaken each working day. The photoperiod was 14-hour light: 10-hour dark. Transfers were made regularly into fresh medium to provide 9- to 12-day old cultures for assay inoculations.

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

C. Test System and Design: Test vessels used were 250-ml sterile Erlenmeyer flasks fitted with foam stoppers to permit gas exchange. Filtered synthetic seawater with a salinity of approximately 30 parts per thousand was used to prepare nutrient medium for the test. Stock culture medium contained EDTA whereas that used for toxicity tests did not.

Based on a range-finding test, five nominal concentrations of AC 243,997 (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 nutrient medium in 250 or 500-ml volumetric flasks. After thoroughly mixing, 50 ml of each concentration were added to each of three replicate test vessels. The control contained only 50 ml medium in each of three replicate flasks. Approximately 100 ml of each test concentration and the control were retained for analysis of initial test concentrations.

The test was initiated when 0.635 ml of a 14-day-old stock culture (containing 788,000 cells/ml) was aseptically added to 50 ml of medium in each flask, yielding a nominal initial concentration of 10,000 cells/ml. Flasks were kept in a Psycrotherm Controlled Environment Incubator Shaker, Model G-27, at a

temperature of 20 ± 2°C. Temperature was recorded daily. Flasks were manually shaken each working day. Illumination of 4306 ± 650 lumens/m^2 was provided by overhead cool-white fluorescent lights, with a photoperiod of 14-hour light: 10-hour dark. Flasks were randomly repositioned 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 (day 7).

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.

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 calculated using the SAS statistical package.

12. **REPORTED RESULTS**: The test concentrations of AC 243,997 measured on day 0 ranged from 89 to 105% of the nominal concentrations, and on day 7 from 74 to 90% 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 shape of the growth curves, the author determined that the two lowest test concentrations had no appreciable effect upon the population growth of *S. costatum*, while inhibition was observed in cultures exposed to the three highest test concentrations.

Effects of the test material on mean standing crop on day 7 relative to the control ranged from 0.7% to 54.0% inhibition (Table 3, attached). As determined by inverse estimation least squares linear regression, the 7-day EC25 was 42.2 mg/L and the 7-day EC50 was 85.5 mg/L. The 95% confidence limits for these EC values could not be determined from the data since an error condition arised in the calculations as a result of an attempt to take the square root of a negative number.

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 pH of the medium was not reported. Subdivision J recommends a pH of 8.0. Also, the pH should have been measured in all test solutions at test initiation and termination.

o The photoperiod employed during the test was 14-hour light: 10-hour dark. A 16/8-hour day/night photoperiod should have been used.

o The average light intensity used during the test was approximately 4.3 Klux, which is slightly higher than the intensity of 4.0 Klux recommended by the guidelines.

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

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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 as those calculated by the author. The differences were probably due to the transformation of percent inhibition to probits by the author before performing the regression analysis, while the reviewer used arcsine square-root transformation in place of probits. Analysis of variance was performed to compare cell counts at each treatment level to those of the controls (attached). The results showed that AC 243,997 at concentrations 28.7 mg/L and higher significantly reduced the number of cell counts of S. costatum at test termination (day 7).

C. **Discussion/Results**: The recalculated, 7-day EC50 and EC25 values of AC 243,997 for S. costatum were 92 and 38 mg/L mean measured concentration, respectively. Based on the reduction of cell counts at 28.7 mg/L, the no-observed-effect concentration (NOEC) was determined to be 15.9 mg/L mean measured concentration. Therefore, AC 243,997 is not expected to exert a detrimental effect on the marine diatom (S. costatum) following normal application methods at rates up to 1.25 lbs a.i./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.

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Skeletonema costatum

DATA POINT	X	Y
1	.9494	.2014
2	1.2014	.0838
3	1.4579	.5565
4	1.6758	.5131
5	1.9566	.8254

REGRESSION EQUATION:
Y=-.5463586 + .678349 X

COEFFICIENT OF CORRELATION= .9013888

PRESS ENTER TO CONTINUE.?

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

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	.9494	.2014	9.766591E-02	
.1037341				
2	1.2014	.0838	.2686099	-.1848099
3	1.4579	.5565	.4426064	.1138936
4	1.6758	.5131	.5904186	-7.731855E-02
5	1.9566	.8254	.7808989	4.450107E-02

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

If $Y = 0.7854$, $X = 1.9632$.

$\therefore$ EC50 = 91.88 mg/L

If $Y = 0.5236$, $X = 1.5770$.

$\therefore$ EC25 = 37.78 mg/L

Skeletonema costatum

DATA POINT	X	Y
1	.9494	4
2	1.2014	.7
3	1.4579	27.9
4	1.6758	24.1
5	1.9566	54

REGRESSION EQUATION:
Y=-50.35355 + 50.057 X

COEFFICIENT OF CORRELATION= .9187204

ACTUAL VERSUS ESTIMATED VALUES
X=LOG CONCENTRATION Y=% INHIBITION

DATA POINT	X	Y	ESTIMATED Y	ERROR
1	.9494	4	-2.829433	6.829433
2	1.2014	.7	9.784931	-9.084931
3	1.4579	27.9	22.62455	5.27545
4	1.6758	24.1	33.53197	-9.431967
5	1.9566	54	47.58798	6.412026

If $Y = 50\%$, $X = 2.0048$

$\therefore$ $EC50 = 101.11$ mg/L mean measured conc.

If $Y = 25\%$, $X = 1.5054$

$\therefore$ $EC_{25} = 32.02$ mg/L mean measured conc.

Analysis of Variance File: SKELETON Date: 01-19-1988

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.
*		18	162555.5620	41179.9800
1	0	3	199333.3280	8326.6641
2	8.9	3	191333.3280	11015.1416
3	15.9	3	198000.0000	15620.4990
4	28.7	3	143666.6720	18009.2578
5	47.4	3	151333.3280	10785.7930
6	90.5	3	91666.6640	11015.1416

Fmax for testing homogeneity of between subjects variances: 4.68
Number of variances= 6 df per variance= 2.

Analysis of Variance Dependent variable: COUNTS

Source	df	SS (H)	MSS	F	P
Between Subjects	17	28828445000.0000			
C (CONC)	5	26835112000.0000	5367022600.0000	32.310	0.0000
Subj w Groups	12	1993332730.0000	166111056.0000		

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	199333.328	6	91666.664
2	191333.328		
3	198000.000		
4	143666.672		
5	151333.328		

Comparison	Scheffe'	Tukey-A*	Tukey-B*	Newman -Keuls*	Bon-ferroni	T-test	Dunnett
1 > 2							
1 > 3							
1 > 4	0.0068	0.0100	0.0100	0.0100	0.0031	0.0002	0.0100
1 > 5	0.0199	0.0100	0.0100	0.0100	0.0102	0.0007	0.0100
1 > 6	0.0000	0.0100	0.0100	0.0100	0.0000	0.0000	0.0100
2 < 3							N.A.
2 > 4	0.0208	0.0100	0.0100	0.0100	0.0108	0.0007	N.A.
2 > 5		0.0500	0.0100	0.0100	0.0384	0.0026	N.A.
2 > 6	0.0000	0.0100	0.0100	0.0100	0.0000	0.0000	N.A.
3 > 4	0.0082	0.0100	0.0100	0.0100	0.0038	0.0003	N.A.
3 > 5	0.0240	0.0100	0.0100	0.0100	0.0127	0.0008	N.A.
3 > 6	0.0000	0.0100	0.0100	0.0100	0.0000	0.0000	N.A.
4 < 5							N.A.
4 > 6	0.0113	0.0100	0.0100	0.0100	0.0055	0.0004	N.A.
5 > 6	0.0039	0.0100	0.0100	0.0100	0.0018	0.0001	N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.0500).
A blank means the P-value is greater than 0.0500.

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