(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: Navicula pelliculosa.

4. **CITATION:** Hughes, J.S. 1987. The Toxicity of AC 243,997 (Lot No. AC 4866-62) to Navicula pelliculosa. 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
Chuta Sun
12/5/91

6. **APPROVED BY:**

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

Signature: Isabel C. Johnson
Date: November 30, 1988

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 freshwater diatom test. With a 7-day EC50 value of greater than 41 mg/L and NOEC value of 41 mg/L mean measured concentration, AC 243,997 is not expected to exert a detrimental effect on the freshwater diatom (Navicula pelliculosa) 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:** Navicula pelliculosa 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 nutrient medium with silicon in Erlenmeyer flasks under constant illumination of approximately 400 foot-candles (4304 lumens/m^2) and a temperature regime of 20 ± 2°C. Flasks were continuously shaken at 100 oscillations/min. 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 250-ml sterile Erlenmeyer flasks fitted with foam stoppers to permit gas exchange. Synthetic algal assay procedure nutrient medium with silicon (AAP/Si) 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 (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/Si 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 0.176 ml of 7-day-old stock culture (containing 852,000 cells/ml) was aseptically added to 50 ml of medium in each flask, yielding a nominal initial concentration of 3000 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 continuously shaken at 100 oscillations/minute and a continuous illumination of 4304 ± 650 lumens/m^2 was provided by overhead cool-white fluorescent lights.

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.

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 typically plotted against percent inhibition expressed as probit (y-axis). However, in this toxicity test, insufficient inhibition was obtained to permit the determination of EC values by inverse estimation least squares linear regression.

12. REPORTED RESULTS: The test concentrations of AC 243,997 measured on day 0 ranged from 44.5 to 105.5% of the nominal concentrations, and on day 7 from 38.2 to 93.6% 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 shapes of the growth curves, the author determined that exposure to the test material had no appreciable effect upon the population growth of Navicula pelliculosa.

Effects of the test material on mean standing crop on day 7 relative to the control ranged from 1.6% stimulation to 17.0% stimulation (Table 3, attached). Because all test concentrations were stimulatory, EC values could not be determined.

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 temperature during the test was controlled at 20 $\pm$ 2^{o}C. Subdivision J recommends 24 $\pm$ 2^{o}C as the test temperature for a freshwater diatom.

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 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:** No statistical analysis was performed to estimate EC25 and EC50 because all test concentrations had no negative effects on the diatom tested. Analysis of variance was used to compare cell counts at each treatment level to those of the controls (attached). The results indicate that cell counts in all treated solutions, except the lowest concentration (8.2 mg/L), were comparable to those in the control solution. Dunnett's test showed a significantly higher cell counts at 8.2 mg/L ($p = 0.05$), when compared to the controls.

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C. **Discussion/Results:** EC values could not be determined since the range of concentrations tested did not include any inhibitory effect levels. Therefore, the 7-day EC25 and EC50 values of AC 243,997 for Navicula pelliculosa were determined to be greater than 41.0 mg/L mean measured concentration. A no-observed-effect concentration (NOEC) was 41.0 mg/L, the highest concentration tested. Therefore, AC 243,997 is not expected to exert a detrimental effect on the freshwater diatom (Navicula pelliiculosa) 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 toxicity results.

(3) **Repairability:** N/A.

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

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Analysis of Variance File: NAVICULA 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	4194444.5000	316808.0300
1	0	3	3853333.2000	70237.6880
2	8.2	3	4506666.5000	140475.3910
3	10.5	3	3913333.2000	390042.7500
4	23.5	3	4240000.0000	283548.9400
5	55.8	3	4373333.5000	302875.0900
6	41.0	3	4280000.0000	52915.0270

A total of 1 observations had missing data on a dependent variable or covariate or inappropriate factor level codes.

Fmax for testing homogeneity of between subjects variances: 54.33
Number of variances= 6 df per variance= 2.

Analysis of Variance Dependent variable: COUNTS

Source	df	SS (H)	MSS	F	P
Between Subjects	17	%1706244440000.0000			
C (CONC)	5	%1002777740000.0000	%200555544000.0000	3.421	0.0373
Subj w Groups	12	%703466700000.0000	%58622226000.0000		

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

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	3853333.200	6	4280000.000
2	4506666.500		
3	3913333.200		
4	4240000.000		
5	4373333.500		

Comparison	Scheffe'	Tukey-A*	Tukey-B*	Newman -Keuls*	Bon-ferroni	T-test	Dunnett
1 < 2						0.0063	0.0500
1 < 3							
1 < 4							
1 < 5						0.0220	
1 < 6							
2 > 3						0.0111	N.A.
2 > 4							N.A.
2 > 5							N.A.
2 > 6							N.A.
3 < 4							N.A.
3 < 5							N.A.
3 < 6						0.0383	N.A.
4 < 5							N.A.
4 < 6							N.A.
5 > 6							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).