(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: Lemna gibba.

4. **CITATION:** Hughes, J.S. 1987. The Toxicity of AC 243,997 (Lot No. AC 4866-62) to Lemna gibba. 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:** 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 plant test. With a 14-day EC50 value of 0.024 mg/L and NOEC value of 0.01 mg/L nominal concentration, AC 243,997 is considered highly toxic and is expected to exert a detrimental effect on Lemna gibba, when applied at maximum application rates up to 1.25 lbs a.i./acre.

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



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9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Species:** Lemna gibba used in this test came from laboratory stock cultures. The original culture was obtained from Dr. C.F. Cleland, Smithsonian Institute Radiation Biology Laboratory, Rockville, MD. Stock cultures were maintained in a synthetic twenty-strength algal assay procedure nutrient medium (20X-AAP) in Erlenmeyer flasks under constant illumination of approximately 390-540 foot-candles (4198-5813 lumens/$m^2$) and temperature of 25 $\pm$ 2°C. Transfers were made regularly into fresh medium, using aseptic technique.

B. **Dosage:** Fourteen-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. Twenty-fold strength synthetic algal assay procedure nutrient medium (20X-AAP) was prepared with deionized water and the pH was adjusted to 7.5 $\pm$ 0.1.

Based on a range-finding test, five nominal concentrations of AC 243,997 (10, 18, 32, 56, and 100 µg/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 20X-AAP medium in 1000-ml volumetric flasks. After thoroughly mixing, 200 ml of each concentration were added to each of three replicate test vessels. The control contained only 200 ml medium in each of three replicate flasks. Approximately 250 ml of each test concentration and the control were retained for analysis of initial test concentrations.

The test was initiated when three four-frond colonies and one three-frond colony (total of 15 fronds) of 7-day-old stock cultures were aseptically added to each test vessel. Flasks were kept in a Sherer Model RI-32LLTP Incubator at a temperature of 25 $\pm$ 2°C. Temperature was recorded daily. Continuous illumination of 4196-5810 lumens/$m^2$ was provided by overhead warm-white fluorescent lights. Flasks were randomly repositioned each working day to minimize spatial differences in the incubator.

Frond counts were made using a lighted magnifying lens, on test days 2, 4, 7, 9, 11, and 14. In order to eliminate subjective decisions on frond maturity, every frond visibly projecting beyond the edge of the parent frond was counted. Fronds were not removed from the test vessels for counting. 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 14).

E. Statistics: Nominal test concentrations were used as the basis for the data analysis. Mean frond count values at test termination on day 14 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:

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

where: C = mean growth in the control,
O = original inoculum level,
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 author reported that "due to the very low test concentrations and interferences from the sample matrix, analysis of the day-0 and day-14 test concentrations was largely unsuccessful." All results were, therefore, based on the nominal concentrations.

Table 2 (attached) presents mean frond counts during the assay. Mean frond 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 degree of inhibition of population growth increased with increasing test concentration.

Effects of the test material on mean standing crop on day 14 relative to the control ranged from 15.1% to 95.0% inhibition (Table 3, attached). As determined by inverse

estimation least squares linear regression, the 14-day EC25 and 14-day EC50 values were 0.013 (95% C.L. = 0.009-0.019) and 0.024 (95% C.L. = 0.016-0.033) 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 pesticide contains 4 lbs of acid/gallon and the application rate is 2.5 pint/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 20X-AAP medium rather than M-Hoagland's medium was used in the test. Furthermore, 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 for Lemna gibba.

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. In addition, pH of the nutrient used was $7.5 \pm 0.1$, instead of $5.0 \pm 0.1$ as recommended by the Subdivision J guidelines.

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

B. Statistical Analysis: The reviewer recalculated EC50 and EC25 values using a regression analysis (attached) and obtained the same results as those calculated by the author. Analysis of variance was performed to



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compare cell counts at each treatment level to those of the controls (attached). The results showed that all test concentrations of AC 2433,997, except the lowest concentration (0.01 mg/L), significantly reduced the number of frond counts in Lemna gibba at test termination (day 14).

C. **Discussion/Results:** The 14-day EC25 and EC50 values of AC 243,997 for Lemna gibba were 0.013 and 0.024 mg/L nominal concentration, respectively. Based on the reduction of frond counts at 0.018 mg/L, the no-observed-effect concentration (NOEC) was determined to be 0.01 mg/L nominal concentration. Therefore, AC 243,997 is considered highly toxic and is expected to exert a detrimental effect on Lemna gibba following application 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.

PC 128821 40811802

LEONA GIBBA

Page _____ is not included in this copy.

Pages 6 through 9 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

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_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

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_____ The product confidential statement of formula.

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_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Analysis of Variance File: lemna 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 | Nominal Conc. (mg/L) | N | Mean | S.D. |
|---|---|---|---|---|
| * | | 18 | 278.2778 | 199.2636 |
| 1 | 0 | 3 | 539.0000 | 19.5192 |
| 2 | 0.010 | 3 | 459.6667 | 21.9393 |
| 3 | 0.018 | 3 | 364.0000 | 123.8870 |
| 4 | 0.032 | 3 | 192.0000 | 10.5830 |
| 5 | 0.056 | 3 | 74.0000 | 24.6374 |
| 6 | 0.100 | 3 | 41.0000 | 4.5826 |

Fmax for testing homogeneity of between subjects variances: 730.86
Number of variances= 6 df per variance= 2.

Analysis of Variance Dependent variable: COUNTS

| Source | df | SS (H) | MSS | F | P |
|---|---|---|---|---|---|
| Between Subjects | 17 | 675001.6200 | | | |
| C (CONC) | 5 | 641100.9400 | 128220.1880 | 45.387 | 0.0000 |
| Subj w Groups | 12 | 33900.6880 | 2825.0574 | | |

Post-hoc tests for factor C (CONC)

| Level | Mean | Level | Mean |
|---|---|---|---|
| 1 | 539.000 | 6 | 41.000 |
| 2 | 459.667 | | |
| 3 | 364.000 | | |
| 4 | 192.000 | | |
| 5 | 74.000 | | |

| Comparison | Scheffe' | Tukey-A* | Tukey-B* | Newman -Keuls* | Bon-ferroni | T-test | Dunnett |
|---|---|---|---|---|---|---|---|
| 1 > 2 | | | | | | | |
| 1 > 3 | 0.0434 | 0.0500 | 0.0100 | 0.0100 | 0.0255 | 0.0017 | 0.0100 |
| 1 > 4 | 0.0002 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | 0.0100 |
| 1 > 5 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | 0.0100 |
| 1 > 6 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | 0.0100 |
| 2 > 3 | | | | 0.0500 | | 0.0478 | N.A. |
| 2 > 4 | 0.0020 | 0.0100 | 0.0100 | 0.0100 | 0.0009 | 0.0001 | N.A. |
| 2 > 5 | 0.0001 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 2 > 6 | 0.0000 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 3 > 4 | 0.0480 | 0.0500 | 0.0100 | 0.0100 | 0.0288 | 0.0019 | N.A. |
| 3 > 5 | 0.0010 | 0.0100 | 0.0100 | 0.0100 | 0.0004 | 0.0000 | N.A. |
| 3 > 6 | 0.0004 | 0.0100 | 0.0100 | 0.0100 | 0.0000 | 0.0000 | N.A. |
| 4 > 5 | | | | 0.0500 | | 0.0187 | N.A. |
| 4 > 6 | | 0.0500 | 0.0500 | 0.0500 | | 0.0046 | 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).

Chemical Name Arsenal (AC 243,997)

Accession No. 408118-02

Validation Status: Core
PK/11-29-88

Species: Navicula pelliculosa

7-Day EC50 = > 41 ppm* (95% C.L. = — ppm)

Test Concentrations (ppm*)/Percent Inhibition:
8.2 (-17.0), 10.5 (-1.6), 23.5 (-11.1),
55.8 (-13.5), 41.0 (-11.1)

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Comments * = mean measured concentration
Negative % inhibition indicates stimulation.

Accession No. 408118-02

Validation Status: Core
PK/11-29-88

Species: Selenastrum capricornutum

7-Day EC50 = 71 ppm* (95% C.L. = — ppm)

Test Concentrations (ppm*)/Percent Inhibition:
9.4 (-0.6), 16.8 (-4.8), 30.8 (-0.9), 50.9 (-5.8),
101.2 (99.9)

Performing Laboratory Malcolm Pirnie

Comments * = mean measured concentration
NOEC = 50.9 ppm
Negative inhibition indicates stimulation

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Chemical Name Arsenal (AC 243,997)

Accession No. 408118-02 Validation Status: Core
PK/11-29-88.

Species: Skeletonema costatum

7-Day EC50 = 92 ppm* (95% C.L. = — ppm)

Test Concentrations/Percent Inhibition:
8.9 (4.0), 15.9 (0.7), 28.7 (27.9), 47.4 (24.1),
90.5 (54.0)

Performing Laboratory

Comments * = mean measured concentration
NOEC = 15.9 ppm.

Accession No.

Species:

-Day EC50 = ppm (95% C.L. = ppm)

Test Concentrations/Percent Inhibition:

Performing Laboratory

Comments

Chemical Name Arsenal (AC 243,997)

Accession No. 408118-02

Validation Status: Core
PK/11/29/88

Species: Anabaena flos-aquae

7-Day EC50 = 12.2 ppm* (95% C.L. = — pp__)

Test Concentrations (ppm*)/Percent Inhibition:
5.26 (-4.8), 9.6 (19.6), 17.6 (71.5),
30.20 (99.2), 55.1 (99.4), 103.0 (99.3)

Performing Laboratory Malcolm Pirnie

Comments * = mean measured concentration
NOEC = 9.6 ppm mean measured concentration.
Negative % inhibition indicates stimulation

Accession No. 408118-02

Validation Status: Core
PK/11-29-88

Species: Lemna gibba

14-Day EC50 = 0.024 ppm* (95% C.L. = — pp__)

Test Concentrations (ppm*)/Percent Inhibition:
0.010 (15.1), 0.018 (33.4), 0.032 (66.2),
0.056 (88.7), 0.100 (95.0)

Performing Laboratory Malcolm Pirnie

Comments * = nominal concentration
NOEC = 0.01 ppm