MRID No. 424731-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Flumetsulam.
Shaughnessey No. 129016.

2. **TEST MATERIAL:** DE-498; N-(2,6-difluorophenyl)-5-methyl (1,2,4) triazolo (1,5-a) pyrimidine-2-sulfonamide; CAS No. 0098967-40-9; AGR 240043; 99.6% active ingredient; a tan powder.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Anabaena flos-aquae*.

4. **CITATION:** Hughes, J.S. and M.M. Alexander. 1992. The Toxicity of DE-498 Herbicide to *Anabaena flos-aquae*. Laboratory Project ID No. B460-13-1. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 424731-01.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Agronomist
KBN Engineering and
Applied Sciences, Inc.

Signature:
Date: 11/16/92

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
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Signature: P. Kosalwat
Date: 11/18/92

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

Signature: Michael Davy
Date: 12-4-92 12/10/92

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a Tier 2 non-target aquatic plant growth and reproduction study. A precise NOEC was not determined. Based on mean measured concentrations, the 5-day LOEC and EC_{50} for *A. flos-aquae* exposed to DE-498 were 0.12 and 0.16 mg ai/l, respectively.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The alga used in the test, *Anabaena flos-aquae*, came from laboratory stock cultures originally obtained from the American Type Culture Collection, Rockville, MD. Stock cultures were maintained in synthetic algal assay procedure nutrient medium (AAP) under 2153 lux illumination, and a temperature of 24 ±2°C. The cultures were manually shaken each working day. Transfers were made regularly to provide logarithmically-growing cultures. The culture used as inoculum in this test had been transferred to fresh medium seven days before test initiation.

B. **Test System:** All glassware were cleaned and autoclaved before use. Test vessels used were 500-ml Erlenmeyer flasks fitted with foam stoppers which permitted gas exchange. The test medium was the same as that used for culturing with the pH adjusted to 7.5 ±0.1. The medium was filter sterilized (0.22 µm) prior to inoculation.

The test vessels were kept in an incubator with environmental conditions like those employed in culturing with continuous cool-white illumination (2153 ±323 lux).

A 0.04 mg active ingredient (ai)/ml primary stock was prepared by dissolving 10 mg of the test material in nutrient medium to a volume of 250 ml. A secondary stock solution (0.004 mg ai/ml) was prepared from the primary stock. Test solutions were created by addition of appropriate volumes of the stocks to nutrient medium.

C. **Dosage:** Five-day growth and reproduction test. Based on the results of preliminary tests, six nominal concentrations of 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0 mg ai/l, and a medium control were selected for the definitive test.

D. **Test Design:** One-hundred ml of the appropriate test or control solution were placed into each of three replicate flasks (3 per treatment level and the control) within 24 hours of solution preparation.

A 5-ml aliquot of an *A. flos-aquae* culture was sonicated for 5 minutes and diluted with 10 ml of nutrient medium and the density was determined. An inoculum of cells calculated to provide 3,000 cells/ml was aseptically introduced into each flask. The inoculum volume was 0.266 ml per flask. The flasks were shaken and randomly repositioned each working day to minimize spatial differences in the incubator. Cell counts were performed using an electronic particle counter on test days 3, 4, and 5. The samples were sonicated for five minutes prior to counting and three counts per replicate were made on each counting day.

The pH was measured at test initiation and termination. Temperature was monitored manually daily and continuously with a recording device.

Samples were collected at test initiation and termination for analysis of the test material by high pressure liquid chromatography. The terminal samples were taken from the supernatant of the test solutions after 4 minutes of centrifugation.

E. **Statistics:** All calculations were based on mean measured concentrations. The EC values and associated 95% confidence intervals (C.I.) were computed using weighted least squares non-linear regression of the cell counts (expressed as inhibition compared to the control) at each concentration against the log of the test concentrations. The no-observed-effect concentration (NOEC) was estimated using analysis of variance (ANOVA) and Dunnett's test. The level of significance was $p \leq 0.05$.

12. **REPORTED RESULTS:** Measured concentrations ranged between 101 and 109% of nominal on day 0 and between 94 and 115% on day 5. The mean measured concentrations were 0.122, 0.258, 0.536, 1.12, 2.08, and 4.07 mg/l (Table 3, attached).

Cell counts and percent inhibition for each concentration after five days are given in Tables 4 and 5 (attached). Increasing concentrations of DE-498 resulted in increased cellular growth inhibition. Five-day responses ranged from 30.7 to 99.1% inhibition.

The five-day EC_{25} was 0.117 mg/l (95% C.I. = 0.099-0.139 mg/l) and the five-day EC_{50} was 0.167 mg/l (95% C.I. = 0.148-0.189 mg/l). The NOEC was determined to be <0.122 mg/l.

The pH ranged from 7.42 to 7.43 in all test solutions and the control at test initiation. The pH values on day 5 ranged from 7.41 to 7.65.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the study authors.

Good Laboratory Practice and Quality Assurance statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 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, except for the following deviations:

Cell growth measurements were not taken daily. Measurements were made on days 3, 4, and 5 only.

The results of the daily or continuous temperature measurements were not reported.

B. **Statistical Analysis:** The reviewer determined the EC_{50} using EPA's Toxanal program as 0.16 mg ai/l (95% C.I.= 0.14-0.18 mg ai/l). The lowest-observed-effect concentration (LOEC) and NOEC were determined using EPA's Dunnett's test program. The reviewer obtained similar results as the authors (see attached printouts). Since the reviewer's model includes a slope for the probit curve (4.2), it will be used to determine the reported EC value.

C. **Discussion/Results:** This study is scientifically sound but does not meet the guideline requirements for a Tier 2 non-target aquatic plant study. Based on mean measured concentrations, the 5-day LOEC and EC_{50} for *A. flos-aquae* exposed to DE-498 were 0.12 and 0.16 mg ai/l, respectively. A precise NOEC could not be determined.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** The NOEC was not determined.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, 10-22-92.

RIN 7767-93

REVIEWS FOR BROADSTRIKE (FLUMETSULAM 129016)

Page____ is not included in this copy.

Pages 6 through 9 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

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Anabaena cell density

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	3	474000.0000	21633.3077	4.6
2*	3	328666.6667	38591.8817	11.7
3*	3	103000.0000	25119.7134	24.4
4*	3	4333.3333	577.3503	13.3
5*	3	5333.3333	1527.5252	28.6
6*	3	5000.0000	1000.0000	20.0
7*	3	4666.6667	577.3503	12.4

*) the mean for this group is significantly less than
the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minumum detectable difference for Dunnett's test = -39753.126781
This difference corresponds to -8.39 percent of control

Between groups sum of squares =********** with 6 degrees of freedom.

Error mean square = 370333333.333322 with 14 degrees of freedom.

Bartlett's test p-value for equality of variances = .001

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*                                              *
* Warning - the test for equality of variances *
  is significant (p less than 0.01).  The      *
  results of this analysis should be inter-    *
* preted with caution.                         *
*                                              *
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MOSSLER DE 498 ANABAENA FLOS AQUAE 10-22-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.536	100	99	99	0
.258	100	78	78	0
.122	100	31	31	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .1641514

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	8.066679E-02	.1641514	.1455441	.1826302

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.0440802	1	.5132908

SLOPE = 4.153304

95 PERCENT CONFIDENCE LIMITS = 3.281306 AND 5.025302

LC50 = .1628346

95 PERCENT CONFIDENCE LIMITS = .1451023 AND .1801488

LC10 = 8.053158E-02

95 PERCENT CONFIDENCE LIMITS = 6.244749E-02 AND 9.594638E-02

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