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: *Skeletonema costatum.*

4. **CITATION:** Hughes, J.S. and M.M. Alexander. 1992. The Toxicity of DE-498 Herbicide to *Skeletonema costatum.* Laboratory Project ID No. B460-13-3. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 424731-03.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
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Date: 11/16/92

6. **APPROVED BY:**

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Date: 12/4/92 12-10-92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. Based on mean measured concentrations, the 5-day NOEC, LOEC, and EC_{50} for *S. costatum* exposed to DE-498 were 29.5, 59.4, and 54.7 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 diatom used in the test, *Skeletonema costatum*, came from laboratory stock cultures originally obtained from the EPA Environmental Research Laboratory in Gulf Breeze, FL. Stock cultures were maintained in synthetic marine algal assay nutrient medium under 4306 lux illumination, and a temperature of 20 ±2°C. The cultures were manually shaken each day and 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 250-ml Erlenmeyer flasks fitted with foam stoppers which permitted gas exchange. The test medium was the same as that used for culturing (excluding EDTA). The pH was adjusted to 8.1 ±0.1 and the medium was filter-sterilized (0.22 µm) prior to inoculation. The salinity of the medium was 30 parts per thousand.

The test vessels were kept in an incubator with environmental conditions like those employed in culturing with 14 hours of cool-white illumination (4306 ±646 lux) per day.

A 0.12 mg active ingredient (ai)/ml primary stock was prepared by dissolving 30.1 mg of the test material in nutrient medium to a volume of 250 ml. A second stock solution (0.06 mg ai/ml) was prepared by dissolving 60.2 mg of the test material in nutrient medium to a volume of 1 l. These two stocks were used as the two highest concentration solutions. Lower concentration test solutions were created by addition of appropriate volumes of the second stock to nutrient medium.

C. **Dosage:** Five-day growth and reproduction test. Based on the results of preliminary tests, five nominal concentrations of 7.5, 15, 30, 60, and 120 mg ai/l, and a medium control were selected for the definitive test.

D. **Test Design:** Fifty 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.

An inoculum of *S. costatum* cells calculated to provide 10,000 cells/ml was aseptically introduced into each flask. The inoculum volume was 0.397 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. 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 97 and 101% of nominal on day 0 and between 80 and 99% on day 5. The mean measured concentrations were 7.37, 14.6, 29.5, 59.4, and 107 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 little effect on cellular growth inhibition except at the two highest concentrations. The effects at these levels may have been due to a lowered pH (5.41-6.47), which occurred due to test material addition at these concentrations. Five-day responses ranged from 5.9% stimulation to 96.8% inhibition.

The five-day EC_{25} was 50.0 mg/l (95% C.I. = 46.7-53.5 mg/l) and the five-day EC_{50} was 61.3 mg/l (95% C.I. = 58.4-64.3 mg/l). The NOEC was determined to be 29.5 mg/l.

The pH ranged from 5.41 to 8.01 in all test solutions and the control at test initiation. The pH values on day 5 ranged from 5.43 to 7.71.

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.

A photoperiod of 16 hours is recommended rather than 14 hours of light per day.

B. **Statistical Analysis:** The reviewer determined the EC_{50} using EPA's Toxanal program as 54.7 mg ai/l (95% C.I.= 50.5-59.6 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).

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. Based on mean measured concentrations, the 5-day NOEC, LOEC, and EC_{50} for *S. costatum* exposed to DE-498 were 29.5, 59.4, and 54.7 mg ai/l, respectively.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 10-26-92.

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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Skeletonema cell density

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	3	275000.0000	11532.5626	4.2
2 7.4	3	285333.3333	18929.6945	6.6
3 14.6	3	291333.3333	12503.3329	4.3
4 29.5	3	248333.3333	15275.2523	6.2
5* 59.4	3	151000.0000	12000.0000	7.9
6* 107	3	8666.6667	1154.7005	13.3

Concentrations (mg/l*)

NOEC = 29.5 mg/l*

LOEC = 59.4 mg/l*

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

* mean measured concentrations

Minumum detectable difference for Dunnett's test = -26697.031323
This difference corresponds to -9.71 percent of control

Between groups sum of squares =********** with 5 degrees of freedom.

Error mean square = 171055555.555552 with 12 degrees of freedom.

Bartlett's test p-value for equality of variances = .190

MOSSLER DE 498 NAVICULA PELLICULOSA 10-26-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
87.6	100	100	100	0
44.2	100	26	26	0
21.8	100	14	14	0
10.9	100	11	11	0
5.44	100	8	8	0
2.72	100	0	0	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 52.51078

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	1.379993E-02	41.56981	37.83288	46.05268

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	1.050428	21.75449	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.380081

95 PERCENT CONFIDENCE LIMITS =-5.927301E-02 AND 4.819434

LC50 = 42.17185

95 PERCENT CONFIDENCE LIMITS = 9.412501 AND +INFINITY

LC10 = 12.34319

95 PERCENT CONFIDENCE LIMITS = 0 AND 29.23136

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