DATA EVALUATION RECORD

1. **CHEMICAL:** XRD-498.
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:** Growth and Reproduction of Aquatic Plants -- Tier 2. Species Tested: *Selenastrum capricornutum*.

4. **CITATION:** Hughes, J.S. 1991. The Toxicity of DE-498 Herbicide to *Selenastrum capricornutum*. Laboratory Project No. B460-11-1. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 419317-43.

5. **REVIEWED BY:**

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Date: 11/6/91

6. **APPROVED BY:**

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7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. The 5-day EC_{25} and EC_{50} values for *S. capricornutum* are 1.29 and 3.21 µg ai/l based on mean measured concentrations. The NOEC was determined to be 0.36 µg ai/l.

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

9. **BACKGROUND:**

6 hrs

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The alga used in the test, *Selenastrum capricornutum*, came from laboratory stock cultures originally obtained from the University of Texas Culture Collection, Austin, TX. Stock cultures were maintained in synthetic algal assay procedure nutrient medium (AAP) under continuous cool-white illumination (4306 ±646 lux) and a temperature of 24 ±2°C. The culture flasks were shaken continuously at 100 oscillations per minute. Transfers were made to maintain logarithmic growth. The culture used as inoculum had been transferred to fresh medium seven days before test initiation.

B. **Test System:** All glassware was cleaned according to EPA methods 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 with the pH adjusted to 7.5 ±0.1 and filtered (0.22 µm).

The test vessels were kept in an incubator with environmental conditions like those employed in culturing.

C. **Dosage:** Five-day growth and reproduction test. Based on preliminary tests, eight nominal concentrations (0.1, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, and 32.0 µg ai/l), a control and a solvent control [N,N-dimethylformamide (DMF)] were used.

A primary stock solution of 8.0 mg ai/ml was prepared by adding 0.0803 g of DE-498 to 10 ml of solvent. The material went into solution after inverting the flask several times. Secondary stocks were prepared from the primary stock. The test solutions were prepared by diluting appropriate volumes of the secondary stock solutions in 500 ml of sterile medium. A medium and solvent (0.4 ml DMF/l) control were also prepared.

D. **Test Design:** Fifty milliliters of the appropriate test solution were placed into each flask (3 replicate flasks per treatment and the controls).

An inoculum of *Selenastrum capricornutum* cells calculated to provide 3,000 cells/ml was aseptically introduced into each flask. The inoculum volume was

0.214 ml per flask. The flasks were 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, using sample volumes of 0.1 to 2.0 ml, were made on each counting day.

Test temperature was recorded daily. The pH was measured at test initiation (initial test solution) and termination (replicates combined). Samples obtained at these same time periods were analyzed for the test material by high performance liquid chromatography (HPLC).

E. **Statistics:** Percent inhibition was computed by comparing the treatment cell densities with those of the solvent control using the following formula:

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

where C = mean growth in the solvent control and
T = mean growth in treated culture.

The EC values and 95% confidence intervals (C.I.) were determined using the weighted least squares nonlinear regression of the log of test concentration against cell counts. The NOEC was determined using analysis of variance (ANOVA) and Dunnett's test (p= 0.05).

12. **REPORTED RESULTS:** The results of the analysis for DE-498 are given in Table 3 (attached). The measured concentrations yielded from 113 to 144% of nominal on day 0 and from non-detectable to 122% on day 5. The lowest test concentration (0.1 µg/l) was at the limit of detection. The mean measured concentrations were 0.06, 0.36, 0.92, 2.12, 4.54, 9.70, 19.30, and 37.09 µg ai/l.

Cell counts and percent inhibition for the control, solvent control, and exposure concentrations are given in Tables 4 and 5 (attached).

Effects on growth of *Selenastrum capricornutum* ranged from 0.6% stimulation to 99.3% inhibition when exposed to the test concentrations. The cell growth in the medium control and the two lowest test concentrations was not significantly different from growth in the solvent control.

The pH at initiation and termination ranged from 7.31 to 7.40 and from 7.60 to 8.43, respectively, in the test solutions and the controls.

The 5-day EC_{25} was determined to be 2.86 µg ai/l (95% C.I.= 2.25-3.62 µg ai/l). The 5-day EC_{50} was determined to be 4.93 µg ai/l (95% C.I.= 4.17-5.82 µg ai/l). The two lowest rates of DE-498 were not significantly different from the solvent control. Therefore, the NOEC was determined to be 0.36 µg ai/l.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusion other than those stated were made by the author.

Quality Assurance and Good Laboratory Practice 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:

~~The dissolved oxygen and conductivity of the test solution were not measured.~~ mrp

Light intensity during the test was 4.306 klux. The recommended light intensity is 4 klux.

The temperature in the incubator was given as 24 ±2°C The author did not report the results of the daily temperature measurements.

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

The lowest test concentration was 5 times less than the second lowest. All dilution progressions should be two-fold.

B. **Statistical Analysis:** The 5-day cell density data were analyzed using ANOVA with Dunnett's test to determine the NOEC value. The results obtained by the reviewer are in agreement with the author. The EC_{25} and EC_{50} were determined by probit analysis to be 1.29 and 3.21 µg ai/a, respectively. These values are slightly lower than those calculated by the author.

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. The 5-day EC_{25} and EC_{50} values for *S. capricornutum* are 1.29 and 3.21 µg ai/l based on mean measured concentrations, respectively. The NOEC was determined to be 0.36 µg ai/l.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

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

15. **COMPLETION OF ONE-LINER:** Yes, 10/8/91.

RIN 7767-93

REVIEWS FOR BROADSTRIKE (FLUMETSULAM 129016)

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Selenastrum cell density

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.1393	0.0248	0.3435
EC 5.00	0.3492	0.0957	0.7040
EC10.00	0.5700	0.1950	1.0422
EC15.00	0.7935	0.3132	1.3671
EC50.00	3.2107	1.9892	5.0182
EC85.00	12.9917	7.8490	29.6494
EC90.00	18.0838	10.3599	47.3192
EC95.00	29.5183	15.4233	95.8549
EC99.00	73.9979	31.7849	368.7487

$Y = 4.14 + 1.71(x)$

Y = probit % inhibition

x = log(rate)

Raw data from Table 5 (attached)

$EC_{25} = 1.29$ mg ai/l

Selenastrum cell density
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	51069741999872.000	6383717749984.000	80.018
Within (Error)	18	1436016000000.000	79778666666.625	
Total	26	52505757999872.000		

Critical F value = 2.51 (0.05,8,18)
Since F > Critical F REJECT Ho:All groups equal

Selenastrum cell density
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DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	3620000.000	3620000.000		
2	0.06 ug ai/l	3640000.000	3640000.000	-0.087	
3	0.36	3166666.667	3166666.667	1.966	
4	0.92	2886666.667	2886666.667	3.180	*
5	2.12	2553333.333	2553333.333	4.625	*
6	4.54	2063333.333	2063333.333	6.750	*
7	9.70	613333.333	613333.333	13.037	*
8	19.30	133000.000	133000.000	15.120	*
9	37.09	25666.667	25666.667	15.585	*

Dunnett table value = 2.58 (1 Tailed Value, P=0.05, df=18,8)

NOEC = 0.36 ug ai/l

Raw data from Table 4 (attached)

nastrum cell density
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DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	3			
2	0.06 ug ai/l	3	595000.682	16.4	-20000.000
3	0.36	3	595000.682	16.4	453333.333
4	0.92	3	595000.682	16.4	733333.333
5	2.12	3	595000.682	16.4	1066666.667
6	4.54	3	595000.682	16.4	1556666.667
7	9.70	3	595000.682	16.4	3006666.667
8	19.30	3	595000.682	16.4	3487000.000
9	37.09	3	595000.682	16.4	3594333.333