12-16-92

MRID No. 424731-02

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: *Navicula pelliculosa*.

4. **CITATION:** Hughes, J.S. and M.M. Alexander. 1992. The Toxicity of DE-498 Herbicide to *Navicula pelliculosa*. Laboratory Project ID No. B460-13-2. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 424731-02.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Agronomist
KBN Engineering and
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Signature: [signature]
Date: 11/18/92

6. **APPROVED BY:**

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

Henry T. Craven, M.S.
Supervisor, EEB/EFED
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Date: 12-4-92

Henry T Craven 12/14/92

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 *N. pelliculosa* exposed to DE-498 were 21.8, 44.2, and 41.6 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, *Navicula pelliculosa*, 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 with added silicon (AAP/Si) under 4306 lux illumination, and a temperature of 24 ±2°C. The cultures were continuously shaken at 100 oscillations per minute. 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 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 (4306 ±646 lux).

A 0.09 mg active ingredient (ai)/ml primary stock was prepared by dissolving 45.2 mg of the test material in nutrient medium to the volume of 500 ml. A second stock solution (0.045 mg ai/ml) was prepared with the same amount of test material in 1 l of nutrient medium. 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, six nominal concentrations of 2.81, 5.62, 11.25, 22.5, 45, and 90 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 four replicate flasks (4 per treatment level and the control) within 24 hours of solution preparation.

A 3-ml aliquot of an *N. pelliculosa* culture was diluted with 7 ml of nutrient medium. This was conducted twice and the densities of the two solutions were determined. An inoculum of cells calculated to provide 3,000 cells/ml was aseptically introduced into each flask. The inoculum volume was either 0.303 or 0.431 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 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 100% of nominal on day 0 and between 95 and 97% on day 5. The mean measured concentrations were 2.72, 5.44, 10.9, 21.8, 44.2, and 87.6 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 highest concentration. This effect at the highest concentration may have been due to a lowered pH, which occurred due to test material addition at this concentration. Five-day responses ranged from 15.8% stimulation to 99.7% inhibition.

The five-day EC_{25} was 44.7 mg/l (95% C.I. = 40.1-49.8 mg/l) and the five-day EC_{50} was 51.1 mg/l (95% C.I. = 46.3-56.3 mg/l). The NOEC was determined to be 44.2 mg/l.

The pH ranged from 5.25 to 7.64 in all test solutions and the control at test initiation. The pH values on day 5 ranged from 5.63 to 7.32.

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 41.6 mg ai/l (95% C.I.= 37.8-46.1 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 there was 26% inhibition at the second highest test concentration (44.2 mg ai/l), the NOEC and LOEC will be reported as 21.8 and 44.2 mg ai/l, respectively.

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 *N. pelliculosa* exposed to DE-498 were 21.8, 44.2, and 41.6 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.

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RIN 1948-94

FLUMETSULAM REVIEWS (129016)

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Navicula cell density

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
concentration (mg/l*)				
1 = control	4	1716325.0000	108073.4125	6.3
2 2.72	4	1987650.0000	351307.8849	17.7
3 5.44	4	1577200.0000	338957.7260	21.5
4 10.9	4	1523075.0000	392738.8307	25.8
5 21.8	4	1473850.0000	243499.7673	16.5
6 44.2	4	1268750.0000	101285.2572	8.0
7* 87.6	4	5375.0000	1007.7864	18.7

NOEC = 44.2, however 26% inhibition at this level:

NOEC = 21.8 mg/l *

LOEC = 44.2 mg/l *

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

* real mean done,

Minumum detectable difference for Dunnett's test = -450705.393680
This difference corresponds to -26.26 percent of control

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

Error mean square = ********** with 21 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 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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