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: *Lemna gibba*.

4. **CITATION:** Hughes, J.S. and M.M. Alexander. 1992. The Toxicity of DE-498 Herbicide to *Lemna gibba* G3. Laboratory Project ID No. B460-13-4. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 424731-04.

5. **REVIEWED BY:**

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

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
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KBN Engineering and
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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. Based on mean measured concentrations, the 14-day NOEC, LOEC, and EC_{50} for *L. gibba* exposed to DE-498 were 1.4, 3.9, and 3.1 µg ai/l, respectively.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** *Lemna gibba* G3 used in the test came from laboratory stock cultures originally obtained from the Smithsonian Institution Radiation Biology Laboratory, Rockville, MD. Stock cultures were maintained in synthetic twenty-strength algal assay procedure nutrient medium (20X-AAP) under 4198-5813 lux illumination, and a temperature of 25 ±2°C. Transfers were made regularly to provide 6 to 11 day old cultures. The culture used as inoculum in this test had been transferred to fresh medium nine 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 under environmental conditions like those employed in culturing with continuous warm-white fluorescent illumination.

A 0.1 mg active ingredient (ai)/ml stock solution was prepared by dissolving 20.1 mg of the test material in nutrient medium to a volume of 200 ml. Secondary stocks were prepared by serial dilution of the primary stock with nutrient medium. The test solutions were created by addition of appropriate volumes of the stocks to 1 l of nutrient medium.

C. **Dosage:** Fourteen-day growth and reproduction test. Based on a range-finding test, six nominal concentrations of 1.00, 3.12, 6.25, 12.5, 25, and 50 µg ai/l, and a medium control were selected for the definitive test.

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

The plants were aseptically added to nutrient medium within 24 hours of solution preparation. An inoculum

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of *Lemna gibba* consisted of three plants per flask, each with four fronds. The flasks were randomly repositioned each working day to minimize spatial differences in the incubator. Frond counts were performed on test days 3, 5, 7, 10, 12, and 14. Every frond that visibly projected beyond the edge of the parent frond was counted and counting was done at approximately the same time each counting day.

Temperature in the incubator was measured continuously using an automated system and also measured manually each day. The pH was measured at test initiation and termination. Samples were taken at test initiation and at termination for analysis of the test material by high pressure liquid chromatography.

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 frond counts (expressed as inhibition compared to the control) at each concentration against the log of the test concentrations. Since inhibition was based on the amount of fronds added to each flask initially, percent inhibition greater than 100% was possible due to death (and subsequent decay) of original fronds. 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 89 and 102% of nominal on day 0 and between not-detectable and 50% on day 14. The mean measured concentrations (non-detected samples were considered as 0) were 0.5, 1.4, 3.9, 8.7, 17.7, and 36 µg/l (Table 3, attached). Results of a stability study indicated that approximately 50% of the test material was lost over the 14-day study period.

Frond counts and percent inhibition for each concentration after fourteen days are given in Tables 4, 5, and 6 (attached). Increasing concentrations of DE-498 above 3.9 µg/l resulted in increasingly inhibitory effects on frond production.

Since algal contamination was noted in two of the control replicates, data were analyzed by using all three replicate values of the control or just the value from the one uncontaminated flask. Based on all three replicates, 14-day responses ranged from 26.7% stimulation to 102.2% inhibition. Based on the one uncontaminated control

replicate, 14-day responses ranged from 19.8 to 101.5% inhibition.

Based on all three control replicates, the 14-day EC_{25} was 3.8 µg/l (95% C.I. = 2.7-5.3 µg/l) and the 14-day EC_{50} was 5.6 µg/l (95% C.I. = 4.7-7.1 µg/l). The NOEC was determined to be 3.9 µg/l.

Based on the one uncontaminated control replicate, the 14-day EC_{25} was 3.3 µg/l (95% C.I. = 2.6-4.2 µg/l) and the 14-day EC_{50} was 5.1 µg/l (95% C.I. = 4.3-6.0 µg/l). The NOEC was determined to be 1.4 µg/l.

The pH ranged from 7.64 to 7.73 in all test solutions and the control at test initiation. The pH values on day 14 ranged from 8.62 to 9.59.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors made no conclusions.

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:

The results of the temperature measurements were not reported.

The light intensity (4.2-5.8 klux) was occasionally lower or higher than recommended (5 klux).

The medium (20X-AAP) used in the study had a pH of 7.5 ±0.1. The guidelines state that the pH of the medium should be 5.0 ±0.1.

Three plants with four fronds each were used as the inoculum rather than the recommended five plants with 3 fronds each.

B. **Statistical Analysis:** Since two control flasks were the only vessels that were contaminated with algae, the reviewer believes that using the one uncontaminated control replicate value represents a valid and conservative approach to determining the EC and NOEC

values. Additionally, results from the chemical analyses of the test solutions (Table 3) and the quality control samples (Table C-3, attached) indicated that approximately 50% of the compound degraded over the 14-day study period. The values derived by the authors for the two lowest concentrations (0.5 and 1.4 µg/l) are believed to be representative of actual concentrations.

Using the one control value and mean measured concentrations, the reviewer determined the EC_{50} using EPA's Toxanal program. 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 for the NOEC (see attached printouts). A slightly more conservative value was determined using the moving average method and this will be the value reported for the EC_{50}. The 14-day EC_{50} and associated 95% confidence interval were determined to be 3.1 and 2.6-3.5 µg 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 14-day NOEC, LOEC, and EC_{50} for *L. gibba* exposed to DE-498 were 1.4, 3.9, and 3.1 µg 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, 10-27-92.

RIN 7767-93

REVIEWS FOR BROADSTRIKE
(FLUMETSULAM 129016)

Page_____ is not included in this copy.

Pages 6e through 11 are not included.

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.

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____ Sales or other commercial/financial information.

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____ The product confidential statement of formula.

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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.

Lemna frond number

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	1	778.0000	.0000	.0
2 0.5	3	626.6667	73.2826	11.7
3 1.4	3	650.3333	45.9819	7.1
4* 3.9	3	418.6667	49.2375	11.8
5* 8.7	3	146.3333	62.3244	42.6
6* 17.1	3	12.0000	8.8882	74.1
7* 36.6	3	.6667	1.1547	173.2

Group concentration (mg/l *)

NOEC = 1.4 mg/l *

LOEC = 3.9 mg/l *

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by a t - test with Bonferroni adjustment of alpha level

* mean measured conc.

Minumum detectable difference for
t-tests with Bonferroni adjustment = -189.057431
This difference corresponds to -24.30 percent of control

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*                                                *
* Note - the above value for the minimum         *
* detectable difference is approximate as        *
* the sample sizes are not the same for all of   *
* the groups.                                    *
*                                                *
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Between groups sum of squares = 1525843.824561 with 6 degrees of freedom.

Error mean square = 2312.277778 with 12 degrees of freedom.

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*                                                *
* Warning - the test for equality of variances   *
* could not be computed as 1 or more of the      *
* variances is zero.                             *
*                                                *
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MOSSLER DE 498 LEMNA GIBBA 10-27-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
36	100	100	100	0
17.7	100	100	100	0
8.7	100	82	82	0
3.9	100	47	47	0
1.4	100	17	17	0
.5	100	20	20	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 4.157559

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	1.458794E-02	3.073959	2.650715	3.534949

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.3755616	10.79255	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 = 1.884656
95 PERCENT CONFIDENCE LIMITS = .7296808 AND 3.039631

LC50 = 2.78799
95 PERCENT CONFIDENCE LIMITS = .9147101 AND 6.221601

LC10 = .5907833
95 PERCENT CONFIDENCE LIMITS = 2.682278E-02 AND 1.473605

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