12-18-92

MRID No. 424651-02

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** DE-498; N-(2,6-difluorophenyl)-5-methyl-1,2,4-triazolo [1,5a] pyrimidine-2-sulfonamide; CAS No. 98967-40-9; ID No. AGR 240043; 99.6% purity; a light tan powder.

3. **STUDY TYPE:** 72-4. *Daphnia magna* Life-Cycle (21-Day Renewal) Chronic Toxicity Test. Species Tested: *Daphnia magna*.

4. **CITATION:** Milazzo, D.P., M.F. Servinski, D.L. Rick, M.D. Martin, and D.C. Stahl. 1992. DE-498 Herbicide: *Daphnia magna* Straus Life-Cycle (21-Day Renewal) Toxicity Test. Laboratory Study No. DECO-ES-DR-0238-5651-24. Conducted by The Dow Chemical Company, Midland, MI. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 424651-02.

5. **REVIEWED BY:**

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

Signature: [signature]
Date: 11/18/92

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 11/18/92

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

Signature: [signature] 12/4/92
Date: [signature] 12-10-92
Henry T. Craven 12/8/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a daphnid life-cycle test. The MATC of DE-498 herbicide for *Daphnia magna* was between 111 and 200 mg/l, mean measured concentrations.

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

9. **BACKGROUND:**

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

33
1

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Instar *Daphnia magna* (<24 hours old) were obtained from in-house cultures. The adults (all of which had produced at least 4 broods) were isolated the day before the test to ensure that all neonates collected for testing were <24 hours old. The brood stock were held at 20 ±1°C under a 16-hour light photoperiod of 2.5 klux illumination. The adults were fed *Selenastrum capricornutum* and *Nitzschia frustulum* three times weekly.

B. **Test System:** The test chambers were covered 250-ml borosilicate jars containing 200 ml of test solution. The test was conducted in a growth chamber maintained on a 16-hour light/8-hour dark photoperiod with cool-white fluorescent tubes (400-800 lux). The test temperature was maintained at 20 ±2°C.

The dilution water was Lake Huron water obtained from the City of Midland Water Treatment Plant prior to treatment. The hardness of the water was adjusted to 170 mg/l as $CaCO_3$ before autoclaving. The water was monitored for selected potential contaminants annually.

The test solutions were prepared prior to test initiation and on renewal days (every other day of the test). A stock solution was prepared by dissolving the test material in water with a pH of 8.0-8.5 (adjusted with 1M Na_2CO_3).

C. **Dosage:** Twenty-one-day, static-renewal, life-cycle toxicity test. Five nominal concentrations (12.5, 25, 50, 100, and 200 mg/l) and a dilution water control were tested.

D. **Design:** Each treatment was replicated ten times. Seven replicates with one daphnid in each replicate chamber were used for survival, reproduction, and growth. The remaining three replicates contained 5 daphnids/chamber and were used for survival observations only. The daphnids were impartially distributed to the test chambers. The test solutions were renewed every other day and daphnids were fed a mixture of *S. capricornutum* and *N. frustulum* at this same time.

Survival and reproduction (number of neonates produced, alive or dead) were determined at each renewal and at

test termination. Individual daphnid length was measured at the end of the test. Average weight of the daphnids was determined for each treatment (group weight divided by number of surviving daphnids in that group).

The dissolved oxygen concentration (DO), pH, and temperature were measured in each test vessel at test initiation and at each renewal before addition of new solution. The temperature of the air in the incubator and in one representative vessel was monitored continuously. The hardness, alkalinity, pH, and conductivity of the dilution water and highest test concentration solution were determined at initiation, and on test days 7, 14, and 21.

The concentration of DE-498 in the freshly prepared solutions was determined at test initiation and on renewal days using high performance liquid chromatography.

E. **Statistics:** The responses in the treatment concentrations were compared to those of the control daphnids. The percent survival, reproduction (mean total young/adult) and adult length and dry weight data were analyzed using Dunnett's test ($p \leq 0.05$). Survival data were arcsine square root transformed.

12. **REPORTED RESULTS:** Preliminary work with DE-498 indicated that the compound would be stable under the conditions used in the test. Measured concentrations in the test solutions are presented in Table 6 (attached). The reported mean measured concentrations were 13.2, 27.3, 56.1, 111, and 200 mg/l. These values represented 107.6 $\pm$4.8% of nominal concentrations.

Biological observations are presented in Tables 7 and 8 (attached). There was no mortality in the control group. The first brood in the controls was observed on day 7. The average cumulative number of young per female in the control group was 101 by day 21 and no ephippia were produced by any of the organisms.

No toxic response was achieved through the highest exposure concentration of 200 mg/l. The MATC was not determined. The NOEC for survival, reproduction (mean total young/female), and growth (weight and length) was 200 mg/l.

In the freshly prepared solutions, pH ranged from 7.3 to 8.1, temperature ranged from 19.0 to 21.8°C, and DO was

greater than 89% of saturation during the study. In solutions with daphnids before renewal, pH ranged from 7.0 to 7.5, temperature ranged from 19.0 to 22.0°C, and DO was greater than 66% of saturation. Light intensity ranged from 538 to 753 lux. The hardness, alkalinity, and conductivity of the control and highest concentration test solutions were 152-172 mg/l as $CaCO_3$, 47-56 mg/l as $CaCO_3$, and 350-400 µmhos/cm, respectively.

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

Quality Assurance and Good Laboratory Practice (GLP) Statements were included in the report indicating adherence to EPA GLP Regulations (40 CFR Part 160).

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were generally in accordance with the SEP and ASTM guidelines, except for the following:

Fifteen to 30 minute transition periods between light and dark are recommended by ASTM. Transition periods did not appear to be used in this study.

The highest concentration solution (200 mg/l) was prepared at greater than 4 hours before testing.

Daphnid length was measured to the nearest 0.1 mm; 0.01 mm is recommended.

B. **Statistical Analysis:** Length of the adult daphnids was the only parameter in which greater than 5% effect was observed (10-12% reduction in comparison to the control at the two highest concentrations). Length data were analyzed using one-way analysis of variance (ANOVA) and Dunnett's test (see attached printout). Length was not significantly different from the control at any test concentration. In most cases, treatment means were greater than control means (Tables 7 and 8).

C. **Discussion/Results:** Concentrations of DE-498 herbicide tested did not adversely affect survival, reproduction, or length of the daphnids. Although weight measurement was not required, the authors determined the average daphnid weight for each treatment. The average daphnid weight at the highest test level is slightly less than that of the control (17% reduction). To be

conservative, the highest test concentration in this test (200 mg/l) is considered to be the lowest-observed-effect concentration (LOEC). The MATC was therefore between 111 and 200 mg/l, mean measured concentrations. This study is scientifically sound and fulfills the guideline requirements for a daphnid life-cycle test.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 11-4-92.

~~27~~ 5

RIN 1948-94

FLUMETSULAM REVIEWS (129016)

Page_____ is not included in this copy.

Pages 6 through 8 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

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.

Daphnia length

Summary Statistics and ANOVA

Transformation = None

Group (concentration (mg/l *))	n	Mean	s.d.	cv%
1 = control	7	5.1714	.7017	13.6
2 13.2	7	5.1143	.7105	13.9
3 27.3	7	5.3857	.6543	12.1
4 56.1	7	4.7714	.3352	7.0
5 111	7	4.6143	.2673	5.8
6 200	7	4.7286	.2289	4.8

NOEC = 200 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

Minumum detectable difference for Dunnett's test = -.652558
This difference corresponds to -12.62 percent of control

Between groups sum of squares = 3.207857 with 5 degrees of freedom.

Error mean square = .276905 with 36 degrees of freedom.

Bartlett's test p-value for equality of variances = .022