

US EPA ARCHIVE DOCUMENT

12-15-93
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MRID No. 428341-03

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Trifluralin.
Shaughnessey No. 036101.
- 2. **TEST MATERIAL:** Trifluralin; α, α, α -trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine; CAS No. 001582-09-8; AGR 291669; 97.92% active ingredient; a bright orange crystalline solid.
- 3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Anabaena flos-aquae*.
- 4. **CITATION:** Hughes, J.S. and T.L. Williams. 1993. The Toxicity of Trifluralin to *Anabaena flos-aquae*. Laboratory Study ID No. B460-153-1. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 428341-03.

5. **REVIEWED BY:**

Nancy M. Gourlie, M.S.
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Signature: *Nancy G*
Date: *9/27/93*
Deane J. ... 11/18/93

6. **APPROVED BY:**

Mark A. Mossler, M.S.
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Signature: *M.A. Mossler*
Date: *9/21/93*
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Henry T. Craven, M.S.
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Signature: *Henry T. Craven*
Date: *12/17/93*
12/15/93

7. **CONCLUSIONS:** This study is scientifically sound ~~but does not~~ *and* ~~meets~~ the guideline requirements for a Tier 2 non-target aquatic plant study. Concentrations of trifluralin at all test levels decreased to non-detectable levels by test termination on day 5. Based on initial measured concentrations, the 5-day NOEC, LOEC, and EC₅₀ for *A. flos-aquae* exposed to trifluralin were 89, 162, and >339 ug/l, respectively. *Depletion of test concentrations is acceptable*

8. **RECOMMENDATIONS:** N/A. *for trifluralin based on*

9. **BACKGROUND:** *its chemical properties - see prior correspondence in DP# D178396, 9/22/92.*

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10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Species: The alga used in the test, *Anabaena flos-aquae*, came from laboratory stock cultures originally obtained from the American Type Culture Collection, Rockville, MD. Stock cultures were maintained in synthetic algal assay procedure nutrient medium (AAP) under 2153 lux illumination, and a temperature of $24 \pm 2^\circ\text{C}$. The cultures were manually shaken each working day. Transfers were made regularly to provide logarithmically-growing cultures.

B. Test System: All glassware was 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 ($0.22 \mu\text{m}$) prior to inoculation.

The test vessels were kept in an incubator with environmental conditions like those employed in culturing with continuous cool-white fluorescent illumination (2153 ± 323 lux).

A 642 ug/ml stock solution was prepared by dissolving 16.4 mg of the test material in N,N-dimethylformamide (DMF) to a final volume of 25 ml. Secondary stocks were prepared by serial dilution of the primary stock with DMF. The test solutions were created by addition of an appropriate volume of the stocks to the final volume of 500 ml in nutrient medium.

C. Dosage: Five-day growth and reproduction test. Six nominal concentrations of 9.70, 19.3, 38.6, 77.3, 154, and 308 ug/l were selected for the definitive test. The concentrations were corrected for the percent purity of the test material. A medium and solvent control were also prepared. The DMF concentration in the solvent control (0.48 ml/l) was the same as that in all treatment solutions.

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

A sample of a 7-day old *A. flos-aquae* culture was sonicated for five minutes and diluted with AAP medium, and the cellular density was determined. An inoculum of cells calculated to provide 3,000 cells/ml was aseptically introduced into each flask. The inoculum volume was 0.457 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. The samples were sonicated for approximately 5 minutes to break the algal filaments into consistent lengths. Three counts were made per replicate.

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 solutions after centrifuging for four minutes at 3,700 rpm.

E. Statistics: All calculations were based on initial 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 pooled 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 at $\alpha = 0.05$.

12. REPORTED RESULTS: Initial measured concentrations ranged between 105 and 129% of nominal (Table 3, attached). The initial measured concentrations were 12.5, 21.6, 45.7, 89.3, 162, and 339 ug/l. No test material was detected in any of the test solutions on day 5. Additional tests conducted with the study material indicated that it was unstable under the conditions of the test, with no detectable amounts of trifluralin found at any treatment level at the end of day 5 (Appendix C, Table C-5, attached).

Cell counts and mean percent inhibition for each concentration after five days are given in Tables 4 and 5 (attached). Five-day responses ranged from 2.94 to 20.0% inhibition.

Based on these results, the EC25 and EC50 were determined to be greater than the highest test concentration of 339 ug/l. As determined by weighted least squares nonlinear regression, 5-day EC25 and EC50 values were extrapolated to be 688 ug/l (95% confidence limits 63.7 - 7,426 ug/l) and 16,623 ug/l (95% confidence limits 54.6 - 5.06 X 10⁶). Statistical analyses indicated that standing crop values in all test concentrations were not significantly different from control values. Thus, the NOEC was determined to be 339 ug/l, the highest concentration tested.

The pH ranged from 7.39 to 7.63 in all treatment solutions and the controls at test initiation. The pH values on day 5 ranged from 7.72 to 8.44. Temperature ranged from 23.3 to 23.8°C.

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 deviation:

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

B. **Statistical Analysis:** Due to the lack of an adequate dose-response, regression analysis could not be conducted. The lowest-observed-effect concentration (LOEC) and NOEC were determined using Williams' test. The reviewer obtained more conservative results than the authors. The 5-day NOEC and LOEC were 89 and 162 µg/l, respectively (see attached printouts).

C. **Discussion/Results:** The authors indicated that the test material was unstable. However, they also indicated that the material had a propensity for adhering to the glassware. This was evident in an average 20% loss of material in solution at time 0. Therefore, silanized glassware should be used with the inclusion of a silanized control.

Although the algae were sonicated for five minutes prior to counting, this procedure did not damage the

plants, as evidenced by the growth after inoculation of sonicated algae. Therefore, this study is scientifically sound but does not meet the guideline requirements for a Tier 2 non-target aquatic plant study. Based on initial measured concentrations, the 5-day NOEC, LOEC, and EC₅₀ for *A. flos-aquae* exposed to trifluralin were 89, 162, and >339 ug/l, respectively.

D. Adequacy of the Study:

- (1) **Classification:** ~~Supplemental.~~ *Core.*
- (2) **Rationale:** Concentrations of the test material decreased to non-detectable levels by test termination; *this is acceptable for trifluralin based on chemical properties; see DPH D178396, 9/22/92.*
- (3) **Repairability:** ~~NO.~~

15. **COMPLETION OF ONE-LINER:** Yes, 9-14-93.

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Page _____ is not included in this copy.

Pages 1 through 10 are not included.

The material not included contains the following type of information:

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 - Description of the product manufacturing process.
 - Description of quality control procedures.
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Anabaena cell density

File: ana Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	pooled cont	6	351000.000	351000.000	351000.000
2	12.5	3	340666.667	340666.667	340666.667
3	21.6	3	326000.000	326000.000	326000.000
4	45.7	3	292000.000	292000.000	303333.333
5	89.3	3	314666.667	314666.667	303333.333
6	162	3	294000.000	294000.000	294000.000
7	339	3	280666.667	280666.667	280666.667

Anabaena cell density

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
pooled cont	351000.000				
12.5	340666.667	0.367		1.74	k= 1, v=17
21.6	326000.000	0.889		1.82	k= 2, v=17
45.7	303333.333	1.695		1.85	k= 3, v=17
89.3	303333.333	1.695		1.87	k= 4, v=17
162	294000.000	2.027	*	1.87	k= 5, v=17
339	280666.667	2.501	*	1.88	k= 6, v=17

s = 39774.364

Note: df used for table values are approximate when v > 20.

NOEC = 89.3 µg/l

LOEC = 162 µg/l