12-1593

MRID No. 428341-01

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: *Skeletonema costatum*.

4. **CITATION:** Hughes, J.S. and T.L. Williams. 1993. The Toxicity of Trifluralin to *Skeletonema costatum*. Laboratory Study ID No. B460-153-3. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 428341-01.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
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Signature: [signature]

Date: 10/18/93

[signature] EEB/OPP 11/18/93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
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Signature: P. Kosalwat

Date: 10/18/93

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

Signature: [signature] 12/15/93

Date: 12/12/93

7. **CONCLUSIONS:** This study is scientifically sound ~~but does~~ and ~~not~~ 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 between day 1 and test termination on day 5. Based on initial measured concentrations, the 5-day NOEC, LOEC, and EC_{50} for *S. costatum* exposed to trifluralin were 4.6, 18.3, and 28 µg ai/l, respectively. Was previously determined due to trifluralins chemical properties - that this is acceptable - See DP# D178396, 9-22-92 for verification. DL 11/18/93

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, *Skeletonema costatum*, came from laboratory stock cultures originally obtained from the EPA Environmental Research Laboratory, Gulf Breeze, FL. Stock cultures were maintained in synthetic marine algal assay nutrient medium (MAA) under 14 hours of 4306-lux illumination per day, at a temperature of 20 ±2°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 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 8.1 ±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 14 hours of cool-white fluorescent illumination (4306 ±646 lux) per day.

A 2.16 mg active ingredient (ai)/ml stock solution was prepared by dissolving 22.1 mg of the test material in N,N-dimethylformamide (DMF) to a final volume of 10 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 250 ml in nutrient medium.

C. Dosage: Five-day growth and reproduction test. Six nominal concentrations of 3.13, 6.25, 12.5, 24.9, 49.8, and 99.5 µg ai/l were selected for the definitive test. A medium and solvent control were also prepared. The DMF concentration in the solvent control (0.5 ml/l) was the same as that in all treatment solutions.

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

The cellular density of a 6-day old *S. costatum* culture was determined. An inoculum of cells calculated to provide 10,000 cells/ml was aseptically introduced into each flask. The inoculum volume was 0.862 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 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 solvent 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 74 and 146% of nominal (Table 3, attached). The initial measured concentrations were 2.5, 4.6, 18.3, 24.6, 44.0, and 94.2 µg ai/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 1.7% stimulation to 96.5% inhibition.

The 5-day EC_{25} was determined to be 19.6 µg ai/l (95% C.I.= 15.9-24.2 µg ai/l). The 5-day EC_{50} was determined to be 28.0 µg ai/l (95% C.I.= 24.2-32.5 µg ai/l). Cell density at the four highest concentration levels was significantly

reduced when compared to the solvent control density. Therefore, the NOEC was determined to be 4.6 µg ai/l.

The pH ranged from 8.02 to 8.05 in all treatment solutions and the controls at test initiation. The pH values on day 5 ranged from 8.09 to 8.59. Temperature ranged from 18.2 to 20.4°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 procedures 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 photoperiod (14 hours of light per day) was less than recommended (16 hours of light per day).

B. **Statistical Analysis:** Initial measured concentrations and the inhibition values derived from solvent control comparison were used to determine the EC_{50}. The lowest-observed-effect concentration (LOEC) and NOEC were determined using Williams' test. The reviewer obtained the same or similar results as the authors' (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 23% loss of material in solution at time 0. Therefore, silanized glassware should be used with the inclusion of a silanized control.

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 EC_{50} for *S. costatum* exposed to trifluralin were 4.6, 18.3, and 28 µg ai/l, respectively.

D. **Adequacy of the Study:**

(1) **Classification:** ~~Supplemental.~~ Core.

(2) **Rationale:** Concentrations of the test material decreased to non-detectable levels between day 1 and test termination. Based on trifluralins chemical properties it has been determined that this is acceptable; see DP# D178396, 9/22/92.

(3) **Repairability:** ~~No.~~

15. **COMPLETION OF ONE-LINER:** Yes, 9-30-93.

DL 11/8/93

Page_____ is not included in this copy.

Pages 6 through 10 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

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_____ Description of quality control procedures.

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skeletonema costatum cell density
File: skl Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	sol. control	3	427333.333	653.677	656.366
2	2.5	3	434666.667	659.055	656.366
3	4.6	3	400000.000	632.300	632.300
4	18.3	3	374666.667	611.374	611.374
5	24.6	3	284666.667	533.317	533.317
6	44	3	43333.333	206.921	206.921
7	94.2	3	15000.000	122.429	122.429

skeletonema costatum cell density
File: skl Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
sol. control	656.366				
2.5	656.366	0.151		1.76	k= 1, v=14
4.6	632.300	1.204		1.85	k= 2, v=14
18.3	611.374	2.382	*	1.88	k= 3, v=14
24.6	533.317	6.778	*	1.89	k= 4, v=14
44	206.921	25.159	*	1.90	k= 5, v=14
94.2	122.429	29.917	*	1.91	k= 6, v=14

s = 21.749
Note: df used for table values are approximate when v > 20.

NOEL= 4.6 µg ai/l
LOEL = 18.3 µg ai/l

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º STUDENT'S T-TEST (two-tailed) º
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Enter the name of the DATAFILE you wish to analyze: skl
(Press RETURN if you wish to skip directly to T evaluation)

What are the SAMPLE NUMBERS of the 2 variables you want to compare?

	1 `control'	2 `sol cont'
Means =	388666.7	427333.4
Variances =	2.533334E+07	1.013334E+08

Are these INDEPENDENT or PAIRED samples? (I or P) i

T = 5.950724 df = 4

p = 4.001618E-03

The MEANS of these 2 samples are significantly different.

The confidence limits on the DIFFERENCE between the means of these samples can be calculated as:

38666.69 +/- T(4) * 6497.813

Do you want another T-TEST using this datafile?

MOSSLER TRIFLURALIN SKELETONEMA COSTATUM 9-30-93

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
94.2	100	97	97	0
44	100	90	90	0
24.6	100	33	33	0
18.3	100	12	12	0
4.6	100	6	6	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 28.8318

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	1.820604E-02	29.16065	26.61946	31.71047

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	2.138259	27.83009	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 = 3.264937
95 PERCENT CONFIDENCE LIMITS =-1.509311 AND 8.039185

LC50 = 27.53953
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 11.2454
95 PERCENT CONFIDENCE LIMITS = 0 AND 26.43882

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