12-15-93

MRID No. 428341-02

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: *Navicula pelliculosa*.

4. **CITATION:** Hughes, J.S. and T.L. Williams. 1993. The Toxicity of Trifluralin to *Navicula pelliculosa*. Laboratory Study ID No. B460-153-2. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 428341-02.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
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KBN Engineering and
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Signature: [signature]
Date: 10/18/93 [signature] 11/18/93 EFED/EEB

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 not meet 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 and the NOEC was not determined. Based on initial measured concentrations, the 5-day LOEC and EC_{50} for *N. pelliculosa* exposed to trifluralin were 7.7 and 15.3 µg ai/l, respectively. (Test is classified as supplemental solely for lack of a NOEC) D.R.

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 Carolina Biological, Inc. Stock cultures were maintained in synthetic algal assay procedure nutrient medium with silicon (AAP/Si) under 4306 lux illumination, at a temperature of 24 ±2°C. The cultures were continuously shaken at 100 rpm. 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 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 fluorescent illumination (4306 ±646 lux).

A 0.439 mg active ingredient (ai)/ml stock solution was prepared by dissolving 11.2 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 6.35, 12.7, 25.3, 50.6, 101, and 202 µg ai/l were selected for the definitive test. A medium and solvent control were also prepared. The DMF concentration in the solvent control (0.46 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 four replicate flasks (4 per treatment level and the controls).

The cellular density of a 4-day old *N. pelliculosa* culture was determined. An inoculum of cells

calculated to provide 3,000 cells/ml was aseptically introduced into each flask. The inoculum volume was 0.227 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 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 107 and 121% of nominal (Table 3, attached). The samples for the nominal 25.3 µg ai/l solution were lost. The initial measured concentrations were 7.7, 15.4, 25.3 (nominal), 54.3, 118, and 238 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 48.4 to 96.5% inhibition.

The 5-day EC_{25} was determined to be 4.6 µg ai/l (95% C.I.= 1.3-15.9 µg ai/l). The 5-day EC_{50} was determined to be 15.3 µg ai/l (95% C.I.= 6.7-34.7 µg ai/l). Cell density at all treatment levels was determined to be significantly reduced. Therefore, the NOEC was less than 7.7 µg ai/l.

The pH ranged from 7.38 to 7.55 in all treatment solutions and the controls at test initiation. The pH values on day 5 ranged from 7.46 to 7.53. Temperature ranged from 22.7 to 24.7°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 NOEC was not determined.

B. **Statistical Analysis:** T-test indicated no significant difference between the control and solvent control cell density; therefore, the controls were pooled. Initial measured concentrations and the inhibition values derived from pooled 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 results for the NOEC and less conservative results than the authors for the EC_{50} (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 21% 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 LOEC and EC_{50} for *N. pelliculosa* exposed to trifluralin were 7.7 and 15.3 µg ai/l, respectively. The NOEC could not be determined.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** Concentrations of the test material decreased to non-detectable levels between day 1 and test termination and the NOEC was not determined. Depletion of test concentrations is acceptable for trifluralin based on chemical properties - see DP# D178396, 9/22/92. DL, 11/10/93

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, 10-1-93.

Page_____ is not included in this copy.

Pages 10 through 11 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.

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Navicula pelliculosa cell density
File: nav Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	pooled control	8	806590.000	888.874	888.874
2	7.7	4	340597.500	531.913	588.717
3	15.4	4	382647.500	595.367	588.717
4	25.3	4	416195.000	638.872	588.717
5	54.3	4	261437.500	501.330	501.330
6	118	4	61357.500	241.924	241.924
7	238	4	28002.500	156.942	156.942

Navicula pelliculosa cell density
File: nav 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
pooled control	888.874				
7.7	588.717	3.238	*	1.71	k= 1, v=25
15.4	588.717	3.238	*	1.79	k= 2, v=25
25.3	588.717	3.238	*	1.82	k= 3, v=25
54.3	501.330	4.181	*	1.83	k= 4, v=25
118	241.924	6.980	*	1.84	k= 5, v=25
238	156.942	7.897	*	1.84	k= 6, v=25

s = 151.359
Note: df used for table values are approximate when v > 20.

NOEC = could not be determined
LOEC = 7.7 mg ai/l

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¤ STUDENT'S T-TEST (two-tailed) ¤
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      Enter the name of the DATAFILE you wish to analyze:  nav
   (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 =       666680                        946500
Variances =      4.317648E+10                  3.860553E+10

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

        T =  1.95695                          df =  6
                          p =  9.810919E-02
     The MEANS of these 2 samples are NOT significantly different.

The confidence limits on the DIFFERENCE between the means of these samples
can be calculated as:
                        279820 +/- T(6) *  142987.8


              Do you want another T-TEST using this datafile?
```

MOSSLER TRIFLURALIN NAVICULA PELLICULOSA 10-1-93

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
202	100	97	97	0
101	100	92	92	0
50.6	100	68	68	0
25.3	100	48	48	0
12.7	100	53	53	0
6.35	100	58	58	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 24.16603

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.4681935	27.07902	13.05888	34.37977

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.9489524	9.410936	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 = 1.015703

95 PERCENT CONFIDENCE LIMITS = 2.626413E-02 AND 2.005142

LC50 = 9.569127

95 PERCENT CONFIDENCE LIMITS = 1.601199E-17 AND 28.49903

LC10 = .537659

95 PERCENT CONFIDENCE LIMITS = 5.877472E-39 AND 4.408113

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