12-20-93

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Trifluralin Aquatic Plant Studies, DP# D194090.

To: Walter Waldrop, PM 71
Special Review and Reregistration Division, 7505C

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division, 7507C 12/20/93

EEB has reviewed the aquatic plant studies submitted by DowElanco for Trifluralin. The studies were submitted under FIFRA 6(a)(2) because previous aquatic phytotoxicity had not been determined for these species. The data shows phytotoxicity to be similar to that of another species previously submitted; therefore, this data would not likely change conclusions of a trifluralin risk assessment.

The following is a summary of the review:

1) 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.

This study is scientifically sound and meets 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.

2) 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.

This study is scientifically sound but does not meet the guideline requirements for a Tier 2 non-target aquatic plant study because a 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.

3) 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.

This study is scientifically sound and meets 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 *A. flos-aquae* exposed to trifluralin were 89, 162, and >339 ug/l, respectively.

4) Milazzo, D.P., M.F. Servinski, R.P. Brown, J.M. Hugo, and M.D. Martin. 1993. Trifluralin Technical Grade 95%: Toxicity to the Aquatic Plant, Duckweed, *Lemna gibba* L. G-3. Laboratory Study No. DECO-ES-2653. Conducted by The Dow Chemical Company, Midland, MI. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 428341-04.

This study is scientifically sound but does not meet the guideline requirements for a Tier 2 aquatic plant growth and reproduction study because a NOEC could not be determined. Based on initial measured concentrations of Trifluralin, the 14-day EC_{50} for *L. gibba* was 43.5 μg/l. The NOEC could not be determined due to growth inhibition at all test levels. The LOEC was 2.53 μg/l.

Questions regarding this review, please contact Dana Lateulere of my staff at 308-2856.

DATA EVALUATION RECORD

1. **CHEMICAL:** Trifluralin.
Shaughnessey No. 036101.

2. **TEST MATERIAL:** Trifluralin Technical 95%; CAS No. 1582-09-8; Identification No. AGR291669; 97.9% purity; an orange crystal.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: Duckweed (*Lemna gibba*).

4. **CITATION:** Milazzo, D.P., M.F. Servinski, R.P. Brown, J.M. Hugo, and M.D. Martin. 1993. Trifluralin Technical Grade 95%: Toxicity to the Aquatic Plant, Duckweed, *Lemna gibba* L. G-3. Laboratory Study No. DECO-ES-2653. Conducted by The Dow Chemical Company, Midland, MI. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 428341-04.

5. **REVIEWED BY:**

Joe Aufmuth, B.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Joe Aufmuth
Date: 10/14/93

Dana Spatz EFED 11/18/93

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 10/14/93

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

Signature: Woodyard Henry Craven 12/15/93
Date: 12/17/93

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a Tier 2 aquatic plant growth and reproduction study. Due to instability of trifluralin in the test medium, all test concentrations were below the limit of quantitation after day 3. The test should have been conducted using static-renewal procedures. Based on initial measured concentrations of Trifluralin, the 14-day EC_{50} for *L. gibba* was 43.5 µg/l. The NOEC could not

be determined due to growth inhibition at all test levels. The LOEC was 2.53 µg/l. (Study is supplemental solely due to lack of NOEC). DL 11/18/93

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** *Lemna gibba* L. G-3 used in the test came from axenic laboratory stock cultures originally obtained from the Smithsonian Institution Radiation Biology Laboratory, Rockville, MD. Stock cultures were maintained axenically by weekly transfer into fresh sterile twenty-strength algal assay medium (AAM). The cultures were maintained under a continuous illumination (5382 ±1076 lux), at a temperature of 25 ±2°C, and a pH of 7.5 ±0.2.

B. **Test System:** Sterile, 250-ml borosilicate Erlenmeyer flasks with Shimadzu closures were conditioned by rinsing with the appropriate test solution. One-hundred ml of the appropriate test solution were placed into each flask.

The flasks were placed in a growth chamber maintained under the same conditions as those used in culturing.

C. **Dosage:** Fourteen-day growth and reproduction test. Based on the results of a range-finding test, six nominal exposure concentrations of 101, 50.4, 25.2, 12.6, 6.26, and 3.13 µg/L were selected for the definitive test. Two control groups, algal assay medium (AAM) with acetone and AAM without acetone were used.

D. **Test Design:** The test consisted of 3 replicate flasks per treatment level and control. *Lemna gibba* (5 plants; three fronds each) was aseptically introduced into each flask. On test days 3, 6, 9, 12, and 14, fronds and plants were counted and observations were made.

The pH in each concentration and control were measured every three days. The temperature of a representative test solution was continuously monitored during the test. Light intensity was recorded daily.

Samples on days 0, 3, 7 and 14 were removed from each replicate flask for analysis by gas chromatography.

E. **Statistics:** Growth in the control and solvent control was not significantly different; therefore, the data were combined.

Regression equations were determined based on the mean values of the number of plants and fronds and were calculated for plant growth and inhibition of growth (Table 7, attached). EC_{25} and EC_{50} with 95% confidence intervals (C.I.) were estimated from the equations.

The no-observed-effect concentration (NOEC) was determined using analysis of variance (ANOVA) and Dunnett's test.

12. **REPORTED RESULTS:** The mean measured exposure concentrations of trifluralin ranged from 80.8% to 102% of nominal with an overall average for all dose levels of 93% on day 0 (Table 5, attached). Measured concentrations decreased to 2% of nominal values on day 3 and below the limit of quantitation by day 7 due to photodegradation. All test results were calculated based on the initial mean measured concentrations.

Mean number of plants and fronds at test termination is presented in Table 6 (attached). The 14-day EC_{50} values and their 95% confidence intervals for plants and fronds were 55.9 and 53.9 µg/l, respectively, for plant growth, and 47.8 and 43.5 µg/l, respectively, for growth inhibition (Table 8, attached). The statistically derived 14-day NOECs for plants and fronds were 12.9 µg/L and <2.53 µg/L, respectively, for growth.

During the test, the temperature ranged from 25.2 to 26.0°C, light intensity from 4300 to 5500 lux, and average pH from 8.3 to 8.4 without growth and from 8.8 to 9.1 with growth.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The biological interpretation of comparing individual mean values for fronds for each dose group and controls indicated no biological significance between controls and exposure concentrations through 12.9 µg/L. Due to test material instability, dose-response of duckweed to trifluralin was assessed using only day 0 analyzed dose levels.

The study director confirmed that this study was conducted in compliance with Good Laboratory Practice (GLP) Standards following U.S. EPA regulations (40 CFR Part 160). A Quality Assurance statement was included in the report.

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 with the exception that the test medium had a higher pH (7.5 ±0.2) than recommended (5.0).

B. Statistical Analysis: Based on initial measured concentrations, the 14-day EC_{50} and 95% C.I. were calculated using EPA's Toxanal computer program. The results are similar to the authors'.

The reviewer used Williams' test to determine the lowest-observed-effect concentration (LOEC) and NOEC based on frond number. The statistical analyses showed significant difference between control and all treatment levels; therefore, NOEC could not be determined. The LOEC was 2.53 µg/l, the lowest test level.

C. Discussion/Results: Due to instability of trifluralin in the test medium, the concentrations in test solutions decreased to 2% of nominal values by day 3 and below the limit of quantitation by day 7. This test should have been conducted using static-renewal procedures, with test solutions renewed daily.

This study is scientifically sound but does not meet the guideline requirements for a Tier 2 aquatic plant growth and reproduction study. Based on initial measured concentrations, the 14-day EC_{50} (95% C.I.) of trifluralin for *Lemna gibba* was 43.5 (4.16-454.7) µg/l. The NOEC could not be determined due to growth inhibition at all test levels. The LOEC was 2.53 µg/l.

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: The test material was not stable in the test medium and all concentrations were below the limit of quantitation after day 3. A NOEC was not determined; depletion of test concentrations is acceptable for trifluralin based on prior correspondence, DP# D178396.

DC 1.4

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, 9-27-93.

JOE AUFMUTH TRIFLURALIN LEMNA GIBBA 09-24-93

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
91.3	100	80	80	0
45.5	100	46	46	0
25.3	100	24	24	0
12.9	100	4	4	0
5.91	100	4	4	0
2.53	100	7	7	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 49.15114

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	2.406858E-02	46.91666	41.718	53.60335

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.780981	13.60412	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 = 1.816703

95 PERCENT CONFIDENCE LIMITS = .2112256 AND 3.422181

LC50 = 48.7989

95 PERCENT CONFIDENCE LIMITS = 20.22207 AND 4612.69

LC10 = 9.757564

95 PERCENT CONFIDENCE LIMITS = 1.692679E-03 AND 22.78094

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TRIFLURALIN DOW CHEMICAL
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	CONTROL	3	531.000	531.000	531.000
2	1	3	492.333	492.333	498.333
3	2	3	494.333	494.333	498.333
4	3	3	508.333	508.333	498.333
5	4	3	402.000	402.000	402.000
6	5	3	286.333	286.333	286.333
7	6	3	103.667	103.667	103.667

TRIFLURALIN DOW CHEMICAL
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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
CONTROL	531.000				
1	498.333	2.004	*	1.76	k= 1, v=14
2	498.333	2.004	*	1.85	k= 2, v=14
3	498.333	2.004	*	1.88	k= 3, v=14
4	402.000	7.913	*	1.89	k= 4, v=14
5	286.333	15.008	*	1.90	k= 5, v=14
6	103.667	26.212	*	1.91	k= 6, v=14

s = 19.967
Note: df used for table values are approximate when v > 20.