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

OCT 4 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Trifluralin Tier II Seed Germination Testing, DP #D189443.

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

From: Anthony Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division, H7507C

EEB has completed a review of the Tier II Seed Germination Testing for Trifluralin; the following is a summary of that review:

Schwab, D. 1993. Evaluating the Effects of Trifluralin on the Germination of Non-Target Terrestrial Plants. Laboratory Project ID No. 40619. Conducted by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 426956-01.

This study is scientifically sound and fulfills the guideline requirements for a Tier 2 germination study; Tier I testing had previously determined that only cabbage and onion needed to be tested at the Tier 2 level. Onion radicle length was the most sensitive parameter with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.13, 0.25, 0.33, and 4.3 lb ai/A, respectively. The EC_{25} and EC_{50} for cabbage germination and the EC_{50} for onion germination should be considered as >4.0 lb ai/A Table 4 (attached). The NOEC and reasonable EC_{25} estimate for onion germination are 2 and 4 lb ai/A, respectively.

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

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** Trifluralin technical; Lot No. 428APO; 95% active ingredient; an orange solid.

3. **STUDY TYPE:** 123-1. Non-Target Plants: Seed Germination Phytotoxicity Test - Tier 2. Species Tested: Cabbage and Onion.

4. **CITATION:** Schwab, D. 1993. Evaluating the Effects of Trifluralin on the Germination of Non-Target Terrestrial Plants. Laboratory Project ID No. 40619. Conducted by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 426956-01.

5. **REVIEWED BY:**

Dana Lateulere, Biologist
EFED/EEB
[Edited KBN submittal:
Mark Mossler, 4/26/93.]

Signature: Dana Lateulere
Date: 9/30/93

6. **APPROVED BY:**

Ann Stavola, Section Head
EFED/EEB

Signature: Ann Stavola
Date: 9/30/93

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier 2 germination study; Tier I testing had previously determined that only cabbage and onion needed to be tested at the Tier 2 level. Onion radicle length was the most sensitive parameter with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.13, 0.25, 0.33, and 4.3 lb ai/A, respectively. The EC_{25} and EC_{50} for cabbage germination and the EC_{50} for onion germination should be considered as >4.0 lb ai/A Table 4 (attached). The NOEC and reasonable EC_{25} estimate for onion germination are 2 and 4 lb ai/A, respectively.

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

9. **BACKGROUND:**

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

DATA EVALUATION RECORD

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

2. **TEST MATERIAL:** Trifluralin technical; Lot No. 428APO; 95% active ingredient; an orange solid.

3. **STUDY TYPE:** 123-1. Non-Target Plants: Seed Germination Phytotoxicity Test - Tier 2. Species Tested: Cabbage and Onion.

4. **CITATION:** Schwab, D. 1993. Evaluating the Effects of Trifluralin on the Germination of Non-Target Terrestrial Plants. Laboratory Project ID No. 40619. Conducted by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. Submitted by DowElanco, Indianapolis, IN. EPA MRID No. 426956-01.

5. **REVIEWED BY:**

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

Signature: Mark A. Mossler
Date: 4/26/93

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 4/26/93

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

Signature: Henry T. Craven
Date: 10/5/93

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements. The required number of test species (i.e., 10 species) was not treated with the test material. Onion radicle length was the most sensitive parameter with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.13, 0.25, 0.33, and 4.3 lb ai/A, respectively.

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

9. **BACKGROUND:**

11. **MATERIALS AND METHODS:**

A. **Test Plants:** Dicotyledon plants were represented by one species from one family (i.e., cabbage) and monocotyledon plants were represented by one species from one family (i.e., onion). Cultivars, seed sources, lot numbers, and germination ratings were provided in the report.

B. **Test System:** Two circles of filter paper were placed in the bottom of a 9-cm disposable petri plate. Seven milliliters of the test solution were added to each plate. Ten seeds of each crop were added to each petri plate after the test solution was absorbed into the paper. The plates were wrapped with moistened filter paper and randomly placed in an environmental chamber set to maintain the temperature at 25 ±2°C. The relative humidity in the chamber was greater than 90%. Seeds were incubated for 5 days.

C. **Dosage:** The test material was applied at the rates of 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0 lb active ingredient (ai)/acre (A). Onion was also treated at the rate of 0.0313 and 0.0625 lb ai/A.

The test solutions were prepared in a 2.8% acetone/ deionized water mixture. The maximum application rate of trifluralin was reported to be 8 lb ai/A.

D. **Design:** Each crop/treatment combination was replicated four times (i.e., 10 seeds/plate, 4 plates/treatment level). After incubation, germinated seeds were removed from the petri plates and radicle length determined. A seed with a radicle length of 5 mm was considered germinated. Observations of seedling health were also made.

E. **Statistics:** All data were entered into a computer spreadsheet. The spreadsheet calculated replicate means, treatment means, and standard deviations. Treatment means were used to calculate the percent effect resulting from the treatment. The percent effect was calculated using the following equation:

$$\% \text{ effect} = \frac{(\text{control mean} - \text{treatment mean})}{\text{control mean}} \times 100$$

Percent germination and root length data were analyzed using analysis of variance (ANOVA). Treatment means were compared to control means by Dunnett's comparison

test (95% confidence level). The percentage data were arcsine transformed before the analysis.

If a significant difference was detected by Dunnett's test, and a dose related response of 50% or more was observed, then probit analysis or non-linear regression was used to determined the EC values.

12. **REPORTED RESULTS:** Root lesions and necrosis were observed sporadically on cabbage exposed to trifluralin, but these observations were not considered to be treatment-related. For onion, swelling of the roots and shoots were observed in plants in the three highest concentration solutions. Fungus was observed in all treatments, but was not considered to be treatment-related.

Percent Germination: The no-observed-effect level (NOEL) for cabbage and onion were determined to be 6 and >12 μg ai/ml (2 and >4 lb ai/A), respectively. The EC values could not be calculated due the lack of a dose response for both species.

Radicle Length: The NOEC, EC_{25}, and EC_{50} for radicle length of cabbage were 3.0, 4.8, and 13 μg ai/ml (1, 1.6, and 4.3 lb ai/A), respectively.

The NOEC, EC_{25}, and EC_{50} for radicle length of onion were 0.38, 1.0, and 13 μg ai/ml (0.13, 0.33, and 4.3 lb ai/A), respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions other than those previously stated were made by the author.

The Quality Assurance Unit of ABC Laboratories, Inc., indicated that the data collection and report were accurate reflections of the study as it was conducted. A statement of Compliance with Good Laboratory Practice Standards was provided.

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

A. **Test Procedure:** The test procedures followed the SEP and Subdivision J guidelines.

Although a negative control was not included in the study design, the percentage germination (88-93%) indicated that the solvent system had little effect on control seed germination.

B. **Statistical Analysis:** Probit analysis was conducted on onion radicle length (the most sensitive species) data to determine the EC values. The ANOVA (coupled with Dunnett's test) was used to verify the NOEL and lowest-observed-effect level (LOEL). Since the author's model predicted a more conservative EC_{25}, the results from this model will be used rather than the reviewer's. Results from ANOVA were in agreement with the author's (see attached printouts).

C. **Discussion/Results:** The EC_{25} and EC_{50} for cabbage germination and the EC_{50} for onion germination should be considered as >4.0 lb ai/A Table 4 (attached). The NOEC and reasonable EC_{25} estimate for onion germination are 2 and 4 lb ai/A, respectively. Onion radicle length was the most sensitive parameter with NOEC, LOEC, EC_{25}, and EC_{50} values of 0.13, 0.25, 0.33, and 4.3 lb ai/A, respectively.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** n/a.

(3) **Repairability:** n/a.

TABLE 4 EC_{25}, and EC_{50} and NOEC Values in μg a.i./mL Obtained from the Germination Test with Trifluralin

Species	EC_{25} (μg a.i./mL)	EC_{50} (μg a.i./mL)	NOEC (μg a.i./mL)
Cabbage Germination	>12 ND	>12 ND	12 ND
Onion Germination	~12 ND	>12 ND	6.0
Cabbage Root Length	4.8	13	3.0
Onion Root Length	1.0	13	0.38

ND = Not Determined

onion radicle length
File: oni Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	8	341.222	42.653	9.597
Within (Error)	27	120.000	4.444	
Total	35	461.222		

Critical F value = 2.31 (0.05,8,27)
Since F > Critical F REJECT Ho:All groups equal

onion radicle length
File: oni Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	17.000	17.000		
2	0.031	15.750	15.750	0.839	
3	0.063	17.000	17.000	0.000	
4	0.125	15.000	15.000	1.342	
5	0.25	11.250	11.250	3.857	*
6	0.5	11.250	11.250	3.857	*
7	1	14.500	14.500	1.677	
8	2	9.000	9.000	5.367	*
9	4	8.750	8.750	5.534	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=24,8)

NOEL = 0.125 lb ai/A
LOEL = 0.25 lb ai/A

onion radicle length
File: oni Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	0.031	4	3.772	22.2	1.250
3	0.063	4	3.772	22.2	0.000
4	0.125	4	3.772	22.2	2.000
5	0.25	4	3.772	22.2	5.750
6	0.5	4	3.772	22.2	5.750
7	1	4	3.772	22.2	2.500
8	2	4	3.772	22.2	8.000
9	4	4	3.772	22.2	8.250

onion radicle length

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0041	0.0006	0.0128
EC 5.00	0.0304	0.0089	0.0648
EC10.00	0.0886	0.0364	0.1563
EC15.00	0.1824	0.0927	0.2880
EC50.00	3.8610	2.4576	7.5184
EC85.00	81.7305	30.4040	420.6632
EC90.00	168.2777	54.3730	1105.2138
EC95.00	490.6064	128.2860	4637.2642
EC99.00	3650.3201	638.4584	68653.8910

$y = 4.54 + 0.78(x)$

y = probit % inhibition

$x = \log(\text{rate})$

$EC_{25} = 0.5416$ ai/A