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

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Trifluralin Data Submission, ID# 36101.

FROM: Douglas Urban, Acting Branch Chief
Ecological Effects Branch
Environmental Fate and Effects Division

Douglas Urban 11/6/91

TO: Walter Waldrop
PM 71, H7508W
Special Review and Reregistration Division

EEB has completed a review of the vegetative and aquatic plant studies submitted by Dow-Elanco for reregistration requirements. The following is a summary of those studies:

1. Waldrep, T. 1990. "Influence of Trifluralin on the Germination of Seeds of Ten Plant Species". DowElanco Laboratory, Global Herbicide Discovery Research, P.O. Box 708, Greenfield, IN 46140-0708. Laboratory No. 61990002. Submitted by DowElanco, 9002 Purdue Road, Indianapolis, IN 46268-1189. EPA MRID No. 419345-01.

This study is scientifically sound and fulfills the requirements of Guideline 122-1 for Seedling Germination. Data from this study show that Tier 2 Germination testing is required for cabbage and onions (an adverse effect of greater than 25 percent resulted).

2. Adams, E. and P. Cocke. 1990. "Toxicity of Trifluralin to a Freshwater Green Alga (Selenastrum capricornutum) in a Static Test System". Study performed by Lilly Research Laboratories, Eli Lilly and Company, Greenfield, Indiana. Laboratory Project ID. J00989. submitted by DowElanco, 9002 Purdue Road, Indianapolis, IN 46268-1189. EPA MRID No. 419345-02.

This study does not fulfill the guideline requirements for a Tier 2 Aquatic Plant Growth and Reproduction Study. The measured concentrations at test termination had decreased to <5% of initial level; DowElanco states that the decrease was due to volatilization and photolysis. Samples were collected for trifluralin analysis at test initiation and at test termination, not during the interim. Therefore, the actual concentrations that the algae was subjected

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remain uncertain. This study has been classified as invalid. It is EEB's recommendation that the test be repeated utilizing a renewal test system.

3. Waldrep, T.W. 1990. "Influence of Trifluralin Post Emergence Spray on the Vegetative Vigor of Ten Plant Species". DowElanco Laboratory, Global Herbicide Discovery Research, P.O. Box 708, Greenfield, IN 46140-0708. Laboratory No. 61990003. Submitted by DowElanco, 9002 Purdue Road, Indianapolis, IN 46268-1189. EPA MRID No. 419345-03.

This study is scientifically sound and fulfills the requirements of Guideline 123-1 for Vegetative Vigor. Greater than 25% reduction in weight occurred in corn, cucumber, and radish crops. Greater than 25% reduction in height occurred in corn, cucumber, onion and radish crops. The EC25 determined using the data of the most sensitive species is .796 lb a.i./A, the NOEL is .125 lb a.i./A.

4. Waldrep, T. 1990. "Influence of Trifluralin Preemergence Spray on Seedling Emergence and Vegetative Vigor of Ten Plant Species". DowElanco Laboratory, Global Herbicide Discovery Research, P.O. Box 708, Greenfield, IN 46140-0708. Laboratory No. 61990004. Submitted by DowElanco, 9002 Purdue Road, Indianapolis, IN 46268-1189. EPA MRID No. 419345-04.

This study does not meet guideline requirements for a Seedling Emergence and Vegetative Vigor Study. The emergence testing had no effects to any crops at any of the test concentrations; therefore an EC25 could not be determined. The effects data for the vegetative vigor portion of the test is inconclusive and lacks a clear dose response relationship. The Seedling Emergence portion of the study must be resubmitted using higher concentrations of trifluralin to ensure an effect level. The Vegetative Vigor portion has been fulfilled by another submittal (MRID No. 419345-03).

As per your request, the following is a list of unfulfilled requirements for the reregistration of Trifluralin as of 10/21/91:

72-3 Acute Toxicity to Estuarine and Marine Organisms;
- a) Fish
- b) Mollusc

123-1 Tier 2 Seed Germination (cabbage and onion only)
Seedling Emergence

123-2 Tier 2 Aquatic Plant Growth and Reproduction
The following species are required:
- Selenastrum capricornutum
- Anabaena flos-aquae
- Skeletonema costatum
- Lemna gibba
- Freshwater Diatom

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72-7 Aquatic Field Testing
Laboratory data has not been submitted.
EEB has determined that Field Testing is still required.

Use pattern with aerial application requires the following studies be submitted to the Agency:

201-1 Droplet Size Spectrum

201-2 Drift Field Evaluation

Questions concerning these reviews or list of unfulfilled reregistration requirements, contact Dana Lateulere at 557-4365, 7347.

DATA EVALUATION RECORD

1. **CHEMICAL:** Trifluralin
Shaughnessey No. 36101.

2. **TEST MATERIAL:** Trifluralin (TREFLAN), technical material - 95.7%. Lot No. 531AP6.

3. **STUDY TYPE:** Non-Target Plants: Seed Germination - Tier 1; Guideline No. 122-1. Species tested: corn, soybean, sunflower, cotton, cucumber, cabbage, radish, onion, wheat and sorghum.

4. **CITATION:** Waldrep, T. 1990. "Influence of Trifluralin on the Germination of Seeds of Ten Plant Species". DowElanco Laboratory, Global Herbicide Discovery Research, P.O. Box 708, Greenfield, IN 46140-0708. Laboratory No. 61990002. Submitted by DowElanco, 9002 Purdue Road, Indianapolis, IN 46268-1189. EPA MRID No. 419345-01.

5. **REVIEWED BY:**

Dana Lateulere, Biologist
Ecological Effects Branch
Environmental Fate and
Effects Division

Signature: Dana Lateulere
Date: 10/21/91

6. **APPROVED BY:**

Ann Stavola, Section Head, 5
Ecological Effects Branch
Environmental Fate and
Effects Division

Signature: Ann Stavola
Date: 11/6/91

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements of Guideline 122-1 for Seedling Germination. Data from this study shows that Tier 2 germination testing is required for cabbage and onions (an adverse effect of greater than 25 percent resulted).

8. **RECOMMENDATIONS:**

9. **BACKGROUND:** This study was submitted as part of reregestration requirements.

10. **DISCUSSION OF INDIVIDUAL TESTS:**

11. **MATERIALS AND METHODS:**

A. **Test Plants:**

Four monocots:

Corn (Zea mays, L.)
Onion (Allium cepa, L.)
Wheat (Triticum aesitivum, L.)
Sorghum (Sorghum bicolor, L.

Six dicots (including one required root crop):

Soybean (Glycine max, L.)
Sunflower (Helianthus annus, L.)
Cotton (Gossypium hirsutum, L).
Cucumber (Cucumis sativus, L.)
Cabbage (Brassica oleracea, L.)
Radish (Raphanus sativus, L.) - root crop

B. **Test System:** For each of the crops ten replicates with ten seeds each were utilized. A control with ten replicates having ten seeds each was also set up for each crop.

James River blue blotter paper was utilized as the germination paper. Each sheet was soaked in a tray which contained the 24.0 ppm solution of trifluralin. After soaking, the blotter paper was held over the tray so any excess solution could run off before the seeds were added. All seeds were counted and placed on one-half of the blotter paper. The seeds were distributed as evenly as possible over one-half of the blotter paper to give each seed the maximum amount of area to germinate and grow. The blotter paper was then folded over into halves so the seeds were contained between two halves of paper. The edges of the paper were folded so the seeds could not fall out when being transferred to the germinators. Untreated controls were soaked in solution containing the appropriate amount of solvent and then seeded as described above. After being seeded, the blotter paper and crop seeds were transferred to the seed germinators where they remained until evaluated.

The seed germinators were maintained at a constant temperature of 27°C and in complete darkness 24 hours each day. Due to some drying out of the blotter paper it was necessary to add more solution to the treated and untreated paper and seeds on day four after treatment. The solution was added to the blotter paper by spraying with a hand-held atomizer. The paper was sprayed until it appeared to be completely moist again. The spraying was accomplished without unfolding the blotter paper.

C. **Dosage:** The dose level of trifluralin was 8.0 lb a.i./A, the maximum labeled rate recommended for use. For the purpose of calculation, the guidelines state that 3.0 ppm of compound in solution should be considered equal to 1.0 lb/A of active ingredient. Since 8.0 lb/A is the maximum recommended rate, this would be equal to 24.0 ppm.

D. **Design:** All blotter papers and seedlings were removed from the germinators after 5 days and the number of seeds that germinated for each crop was counted and recorded. Number of seeds that did not germinate were also recorded. Further, seeds that germinated were further divided into normal seedlings and abnormal seedlings. All these counts are recorded in Tables 1 and 2.

E. **Statistics:** The germination data were calculated as the proportion of seed germinated. For the analysis these data were than transformed using an arc sine transformation. An ANOVA test was performed to determine whether the treated seed differed in germination rate from the control.

12. **REPORTED RESULTS:** Germination of corn, cotton, cucumber, radish, soybean, wheat, and sunflower seeds was not inhibited when treated with a 24.0 ppm solution of trifluralin. Germination of sorghum seeds was reduced slightly, while onion and cabbage seeds were severely inhibited with the trifluralin treatment. The number of seeds that germinated, and did not germinate, in the 24.0 ppm trifluralin treatment were recorded after 5 days of treatment and are shown in Table 1; control germination is shown in Table. 2.

The estimated percent decreases with 95% confidence intervals are shown:

a. cabbage 59.7 (44.3, 74.1)
b. onion 83.1 (63.0, 96.3)
c. sorghum 5.2 (.2, 16.7)

Ten seedlings of each species were randomly selected and the shoots measured from the mesocotyl of the monocots and from the cotyledon of the dicots to the tip of leafy growth. For the roots, the length of the primary root was measured in both the monocots and dicots. These shoot and root measurements are shown in Table 3. Shoots and roots of cotton and sunflower seedlings were slightly stunted, but looked normal in other respects. The roots of cucumber and radish seedlings were slightly stunted also, but the shoots of these seedlings were severely stunted. The shoots and roots of all other seedlings were severely stunted and swollen in size when compared to untreated controls.

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13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Trifluralin at 24.0 ppm did not interfere with the germination of corn, cotton, cucumber, radish, soybean, sunflower or wheat seeds. Mean percent germination for cabbage, onion, and sorghum was reduced at the 5% level of probability.

A Good Laboratory Practice Statement was included with the study. However, the statement notes that during the time of the study it was not subject to the Good Laboratory Practices standards and was, therefore, not monitored by the Quality Assurance Unit.

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

A. **Test Procedure:** The test procedures were generally in accordance with Subdivision J Guidelines and recommended protocols for a Tier 1 Seed Germination Study.

B. **Statistical Analysis:** The germination data was analyzed by ANOVA and Dunnett's test. Percent difference comparisons were made to determine if >25% germination inhibition occurred in any of the ten species. (25% is the determination value for Tier 2 testing).

C. **Discussion/Results:** The reviewer's results corresponded with the study authors in that cabbage, onion and sorghum showed significant inhibition; however, the reviwer also found significant inhibition in wheat and soybean (See attached). The percent difference in germination for those crops found to be significantly different from the control are as follows (with control germination in parentheses):

onion

germinated	8	(90)
not germinated	92	(10)

percent difference = 82%

cabbage

germinated	35	(98)
not germinated	65	(2)

percent difference = 63%

sorghum

germinated	71	(85)
not germinated	29	(15)

percent difference = 14%

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wheat

germinated	90	(97)
not germinated	10	(3)

percent difference = 7%

soybean

germinated	93	(98)
not germinated	7	(2)

percent difference = 5%

Because >25% inhibition resulted in the cabbage and onion tests, Tier 2 studies are required.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale:

(3) Repairability:

Trifluralin Science Reviews

Page _____ is not included in this copy.

Pages __6__ through _10__ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

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__X__ FIFRA registration data.

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_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

TRIFLURALIN SUNFLOWER GERMINATION TIER 1
File: TRIFSUN 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	0	9.800	9.800		
2	24	9.400	9.400	1.644	
3	98	0.000	0.000	40.278	*

Dunnett table value = 2.01 (1 Tailed Value, P=0.05, df=24,2)

TRIFLURALIN SUNFLOWER GERMINATION TIER 1
File: TRIFSUN 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	0	10			
2	24	10	0.489	5.0	0.400
3	98	10	0.489	5.0	9.800

TRIFLURALIN GERMINATION FOR WHEAT
File: WHEATTRIF 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	0	9.700	9.700		
2	24	9.000	9.000	2.339	*
3	99	0.000	0.000	32.405	*

Dunnett table value = 2.01 (1 Tailed Value, P=0.05, df=24,2)

TRIFLURALIN GERMINATION FOR WHEAT
File: WHEATTRIF 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	0	10			
2	24	10	0.602	6.2	0.700
3	99	10	0.602	6.2	9.700

trifluralin tier 1 cabbage
File: trifcab 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	0	9.800	9.800		
2	24.0	3.500	3.500	13.807	*
3	98	0.000	0.000	21.478	*

Dunnett table value = 2.01 (1 Tailed Value, P=0.05, df=24,2)

trifluralin tier 1 cabbage
File: trifcab 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	0	10			
2	24.0	10	0.917	9.4	6.300
3	98	10	0.917	9.4	9.800

RIFLURALIN CORN TIER 2 GERMINATION
ile: TRIFCORN Transform: NO TRANSFORM

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

ROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	9.400	9.400		
2	24	9.600	9.600	-0.891	
3	98	0.000	0.000	41.871	*

unnett table value = 2.01 (1 Tailed Value, P=0.05, df=24,2)

RIFLURALIN CORN TIER 2 GERMINATION
ile: TRIFCORN Transform: NO TRANSFORM

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

ROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	0	10			
2	24	10	0.451	4.8	-0.200
3	98	10	0.451	4.8	9.400

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TRIFLURALIN TIER 1 GERMINATION OF COTTON
File: TRIFCOTTON 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	0	9.000	9.000		
2	24	9.300	9.300	-1.097	
3	98	0.000	0.000	32.907	*

Dunnett table value = 2.01 (1 Tailed Value, P=0.05, df=24,2)

TRIFLURALIN TIER 1 GERMINATION OF COTTON
File: TRIFCOTTON 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	0	10			
2	24	10	0.550	6.1	-0.300
3	98	10	0.550	6.1	9.000

TRIFLURALIN CUCUMBER GERMINATION TIER 1
File: TRIFCUKE 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	0	9.700	9.700		
2	24	9.700	9.700	0.000	
3	98	0.000	0.000	45.226	*

Dunnett table value = 2.01 (1 Tailed Value, P=0.05, df=24,2)

TRIFLURALIN CUCUMBER GERMINATION TIER 1
File: TRIFCUKE 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	0	10			
2	24	10	0.431	4.4	0.000
3	98	10	0.431	4.4	9.700

TRIFLURALIN ONION GERMINATION TIER 1
File: TRIFONION 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	0	9.000	9.000		
2	24	0.800	0.800	18.137	*
3	98	0.000	0.000	19.907	*

Dunnett table value = 2.01 (1 Tailed Value, P=0.05, df=24,2)

TRIFLURALIN ONION GERMINATION TIER 1
File: TRIFONION 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	0	10			
2	24	10	0.909	10.1	8.200
3	98	10	0.909	10.1	9.000

TRIFLURALIN RADISH GERMINATION TIER 1
File: TRIFRAD 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	0	9.600	9.600		
2	24	9.100	9.100	1.727	
3	98	0.000	0.000	33.163	*

Dunnett table value = 2.01 (1 Tailed Value, P=0.05, df=24,2)

TRIFLURALIN RADISH GERMINATION TIER 1
File: TRIFRAD 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	0	10			
2	24	10	0.582	6.1	0.500
3	98	10	0.582	6.1	9.600

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	8.500	8.500		
2	24	7.100	7.100	3.000	*
3	98	0.000	0.000	18.213	*

Dunnett table value = 2.01 (1 Tailed Value, P=0.05, df=24,2)

TRIFLURALIN SORGHUM GERMINATION TIER 1
File: TRIFSORG 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	0	10			
2	24	10	0.938	11.0	1.400
3	98	10	0.938	11.0	8.500

TRIFLURALIN SOYBEAN GERMINATION TIER 1
File: TRIFSOY 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	0	9.800	9.800		
2	24	9.300	9.300	2.434	*
3	98	0.000	0.000	47.706	*

Dunnett table value = 2.01 (1 Tailed Value, P=0.05, df=24,2)

TRIFLURALIN SOYBEAN GERMINATION TIER 1
File: TRIFSOY 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	0	10			
2	24	10	0.413	4.2	0.500
3	98	10	0.413	4.2	9.800

DATA EVALUATION RECORD

1. **CHEMICAL**: Trifluralin
   Shaughnessy No. 36101

2. **TEST MATERIAL**: Trifluralin, (TREFLAN), 99.86%

3. **STUDY TYPE**: Growth and Reproduction of Aquatic Plants, Tier 2. Species Tested: Selenastrum capricornutum.

4. **CITATION**: Adams, E. and P. Cocke. 1990. "Toxicity of Trifluralin to a Freshwater Green Alga (Selenastrum capricornutum) in a Static Test System". Study performed by Lilly Research Laboratories, Eli Lilly and Company, Greenfield, Indiana. Laboratory Project I.D.#. J00989. Submitted by DowElanco, 9002 Purdue Road, Indianapolis, IN 46268-1189. MRID No. 419345-02.

5. **REVIEWED BY**:

   Dana S. Lateulere, Biologist
   Ecological Effects Branch
   Environmental Fate and
   Effects Division

   Signature: Dana Lateulere
   Date: 10/21/91

6. **APPROVED BY**:

   Ann Stavola, Section Head, 5
   Ecological Effects Branch
   Environmental Fate and
   Effects Division

   Signature: Ann Stavola
   Date: 11/6/91

7. **CONCLUSIONS**: This study does not fulfill the guideline requirements for a Tier 2 Aquatic Plant Growth and Reproduction study. The measured concentrations at test termination had decreased to <5% of initial level; DowElanco assumes the decrease was due to volatilization and photolysis. Samples were collected for trifluralin analysis at test initiation and at test termination, not the interim. Therefore, the actual concentrations that the algae was subjected remain uncertain. This study has been classified invalid. It is EEB's recommendation that the test be repeated utilizing a renewal test system.

8. **RECOMMENDATIONS:** Repeat the test with a renewal system so the concentrations may be maintained.

9. **BACKGROUND:** This study is submitted as part of reregestration requirements for trifluralin.

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Selenastrum capricornutum used in this test came from laboratory stock cultures. The original culture was obtained from the University of Texas Culture Collection (UTEX #1648), Austin, TX. Stock Cultures were maintained in algal nutrient medium in an environmental growth chamber.Cultures were held at about 25°C and continuously illuminated at 4 klux.

B. **Test System:** The test vessels were 500 ml Erlenmeyer flasks containing 100 ml of the appropriate test solution. An environmental chamber was used to maintain the desired test temperature and lighting conditions throughout the test period. Flasks were shaken by hand at least once a day to minimize the clumping of cells. Aluminum foil was used as a flask closure to prevent outside contamination while still allowing free gas exchange.

The medium was prepared by adding each of six macronutrient stock solutions and 8 micronutrient stock solutions to 500 ml of sterile water; mixing after each addition. The volume was brought to 10 L.

Treatment concentrations of trifluralin were based on trials from a 7-day pilot study that indicated that the no-observed-effect-concentration (NOEC) was less than or equal to a nominal trifluralin level of 100 ug/L and the median effective concentration was between 100 and 500 ug.L.

C. **Dosage:** The nominal exposure concentrations of trifluralin selected for this study were 0.0 (water and acetone controls), 10, 80, 160, 320, and 640 ug/L. Three replicates wee used at each treatment level. At test initiation, analyzed trifluralin concentrations were 87% to 102% of nominal levels. Due to the susceptibility of trifluralin to volatilization and photolytic decomposition, analyzed concentration had

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decreased to 0.46% to 4.5% of nominal levels by day 7. The geometric means of analyzed trifluralin concentrations were 2.12, 5.37, 11.7, 21.9, and 62.1 ug/L at nominal levels of 10, 80, 160, 320, and 640 ug/L, respectively.

D. **Design:** Reproduction in the algal cultures was determined by quantifying cell populations on Days 1, 2, 3, 4, 5, and 7. A compound microscope and hemocytometer were used to enumerate the algal cells. Cell counts were expressed as number of algal cells per milliliter of solution (cells/ml). To obtain a direct measure of biomass, dry weight of the algal cells in each flask was determined on day 7. A measured volume of solution from each flask was passed through a preweighed glass-fiber filter. Each filter was dried at approximately 103°C for 2 hours and reweighed. Dry weight of the algal cells was determined by calculation and expressed as milligrams of dry weight per milliliter of test solution (mg/ml). The temperature and pH were measured in each replicate at test initiation and at test termination. Total alkalinity, total hardness, and conductivity of the aqueous nutrient medium were determined on Day 0.

Samples were analyzed for the actual concentration of Trifluralin present in the test solution on Day 0 and Day 7. Samples for Day 0 analyses were portions of the test treatments used to begin the test. At test termination, samples were collected by filtering each test solution through a .7 um glass-fiber membrane filter to remove algal cells. Filtrates from treatment replicates were pooled and submitted for analysis of trifluralin.

E. **Statistics:** A one-tailed Dunnett's t-test was used to detect treatment responses that were significantly different from those of the acetone control. To define the NOEC, individual Dunnett's t-tests were performed on specific growth rates, on algal cell count data from Day 7, and on the algal biomass data obtained from dry weight measurements on Day 7. The median effective concentration was defined as the concentration of trifluralin that caused 50% inhibition of the specific growth rate of treated algal populations relative to acetone control populations.

12. **REPORTED RESULTS:** Results from this study indicated that the NOEC of trifluralin for the green alga, S. capricornutum, was 5.37 ug/L. Terminal cell count and algal

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biomass were significantly reduced relative to acetone control cultures at mean analyzed trifluralin concentrations >11.7 ug/L. Using the specific growth rate during the logarithmic phase of reproduction as an indicator of algal growth, the median effective concentration was 12.2 ug/L.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Quality Assurance Inspection was conducted for compliance verification by the Quality Assurance Unit. It was also stated that this study was conducted in compliance with the FIFRA Good Laboratory Practice Standards, 40 CFR Part 160.

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

A. **Test Procedure:** The test procedures were in accordance with Subdivision J, and SEP guidelines except for the following deviations:

-the concentrations decreased over the duration of the test to less than 5% of the nominal.

B. **Statistical Analysis:** Statistical analysis was not performed because the final test concentrations were unacceptable.

C. **Discussion/Results:**

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** Test concentrations decreased to <5% of the original concentrations.

(3) **Repairability:** This test cannot be repaired; a renewal test system is recommended when this test is repeated.

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TABLE 1. ANALYZED CONCENTRATIONS OF TRIFLURALIN IN THE TEST SOLUTIONS DURING A 7-DAY EXPOSURE OF Selenastrum capricornutum. STUDY J00989.

Nominal Trifluralin Concentration (µg/L)	Analyzed Trifluralin Concentration (µg/L)		
	Day 0	Day 7	Geometric Mean
0.0 (Water Control)	ND[a]	--[b]	--
0.0 (Acetone Control)	ND	--[b]	--
10	10.0	0.45	2.12
80	78.0	0.37	5.37
160	164	0.83	11.7
320	295	1.62	21.9
640	554	6.97	62.1

[a] ND = None Detected. The analytical limit of quantitation was 0.3 µg/L for Day-0 samples and 0.08 µg/L for Day-7 samples.

[b] Low levels of trifluralin were detected in control solutions at test termination due to contamination on glassware used at the end of the study for biomass determinations.

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DATA EVALUATION RECORD

1. **CHEMICAL:** Trifluralin
Shaughnessey No. 36101

2. **TEST MATERIAL:** Trifluralin (TREFLAN), lot number 531AP6, 95.7% active ingredient.

3. **STUDY TYPE:** Non-Target Plants: Vegetative Vigor Phytotoxicity Test - Tier 2. Species Tested: corn, soybean, sunflower, cotton, cucumber, cabbage, radish, onion, wheat, and sorghum.

4. **CITATION:** Waldrep, T. W. 1990. "Influence of Trifluralin Post Emergence Spray on the Vegetative Vigor of Ten Plant Species". DowElanco Laboratory, Global Herbicide Discovery Research, P.O. Box 708, Greenfield, IN 46140-0708. Laboratory No. 61990003. Submitted by DowElanco, 9002 Purdue Road, Indianapolis, IN 46268-1189. EPA MRID No. 419345-03.

5. **REVIEWED BY:**

Dana Lateulere, Biologist
Ecological Effects Branch
Environmental Fate
and Effects Division

Signature: Dana Lateulere

Date: 10/21/91

6. **APPROVED BY:**

Ann Stavola, Section Head, 5
Ecological Effects Branch
Environmental Fate
and Effects Division

Signature: Ann Stavola

Date: 11/6/91

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements of Guideline 123-1 for Vegetative Vigor. Greater than 25% reduction in weight occurred in corn, cucumber, and radish crops. Greater than 25% reduction in height occurred in corn, cucumber, onion and radish crops. The EC25 determined using the data of the most sensitive species is .796 lb a.i./A, the NOEL is .125 lb a.i./A.

8. **RECOMMENDATIONS:**

9. **BACKGROUND:** This study was submitted as part of reregestration requirements.

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

11. **MATERIALS AND METHODS:**

A. **Test Plants:**
Four monocots:
Corn (Zea mays,L.)
Onion (Allium cepa,L.)
Wheat (Triticum aesitivum,L.)
Sorghum (Sorghum bicolor,L.)
Six dicots (including one required root crop):
Soybean (Glycine max,L.)
Sunflower (Helianthus annuus,L)
Cotton (Gossypium hirsutum,L.)
Cucumber (Cucumis sativus,L.)
Cabbage (Brassica oleracea,L.)
Radish (Raphanus sativus,L.) - root crop.

B. **Test System:** Plastic containers were used for seeding; five crops per container in rows 8.7" long. Cabbage, sorghum, cucumber, onion, and radish in one container, and cotton, wheat, sunflower, corn and soybean in a second container. For each of the crops four replicates were utilized, the number per replicate varied with crop; all plants were thinned before spraying. Six plants per replicate were sprayed for corn, cotton, cucumber, soybean, and sunflower; 25 per replicate for radish and sorghum; 30 per replicate for cabbage and onion; and 50 per replicate for wheat. Cabbage plants were 5-6 cm tall and cotton plants were 8-9 cm tall and both species had the cotyledons plus two true leaves exposed at time of spraying. Cucumber plants were 8-9 cm tall an radish plants were 7-8 cm tall an both of these species had the cotyledons plus two true leaves exposed. Soybean plants were 11-12 cm tall with the cotyledons plus two leaves and one set of Trifoliate leaves exposed, while sunflower plants were 14-15 cm tall and had the cotyledons plus four leaves exposed at time of spraying. Onion plants were 7-8 cm tall with two leaves, wheat plants 21-22 cm tall with three leaves, sorghum plants 15-16 cm tall with four leaves, and corn plants 29-30 cm tall with four leaves at time of spraying.

The containers were filled 2.5" deep with Terra-Lite metro mix growing medium (Canadian sphagnum peat moss, vermiculite, processed bark ash, and washed granite sand-all essentially sterile ingredients) manufactured by W.R. Grace and Company, Cambridge, MA 02140. The containers had holes in the bottom to facilitate bottom

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watering and drainage.

C. **Dosage:** The application rates were 0.0, .125, .25, .50, 1.0, and 2.0 lb a.i./A. The solvent used was Toximul R and S surfactants and an acetone:ethanol (1:1) mixture. An 80.0 ml solution was prepared of the solvents (equal to the highest concentration used in the treatments); 10 ml of this solution was sprayed on each container of control plants.

D. **Design:** The trifluralin solution was sprayed overtop of the young and succulent plants in each container with a hand-held compressed air sprayer operated at low pressure (5 psi). Immediately after spraying, the plants were placed in a greenhouse (80-85°F day temperature and 70-75°F night temperature with 12 hours light and 12 hours darkness) where they were bottom watered and fertilized as needed until the conclusion of the test.
Visual injury ratings were made on days 7 and 14. Height and fresh weight measurements were taken on day 14.

E. **Statistics:** An ANOVA test was performed, along with Dunnett's test for comparing treatment means to a control, to determine whether any of the treatments differed from the control and, if they did, whether the difference between any one treatment and the control could be equal to a 25% decrease from the control. If there were any differences, regression lines of a plateau-linear type (Anderson and Nelson, 1975) were fit using either the treatment levels or a log transformation of the treatment levels. The fitted lines were used to predict the EC25 and its 95% confidence interval.

12. **REPORTED RESULTS:** Based on visual observations, trifluralin at .5 lb a.i./A caused slight injury to all the plants tested except wheat, which was not injured. At 1.0 lb a.i./A trifluralin caused slight injury to all plants except cucumber, which was injured moderately. At 2.0 lb ai. /A trifluralin caused moderated injury to all the plants tested. Corn and cucumber were the most sensitive plants tested and cucumber was injured slightly with trifluralin at 0.25 lb a.i./A. Injury symptoms observed were stunting of plants, crinkled leaves, and some slightly twisted plants. Postemergence sprays of trifluralin at 0.125 lb a.i./A did not cause visible injury to any of the plants tested and the .125 lb a.i./A rate only injured cucumber.

Based on height measurements, trifluralin at 0.5 lb a.i./A caused slight stunting of all plants except corn and wheat, neither of which was stunted. At 1.0 lb a.i./A trifluralin caused slight stunting to all plants tested except radish, which was moderately stunted. At 2.0 lb a.i./A trifluralin caused slight to moderate stunting to all plants tested. Corn, radish, and cucumber were the most sensitive and cucumber was slightly stunted at .25 lb a.i./A. Based on statistical analysis, the average height of plants 14 days after treatment with trifluralin was significantly reduced in all plants tested; however the decrease in soybean and wheat was significantly less than 25%. For the rest of the plants, the estimated EC25's and their respective confidence intervals are as follows:

Plant	EC25 (lb a.i.)	95% C.I.
cucumber	0.668	(0.440-1.028)
radish	0.733	(0.480-1.139)
onion	1.429	(.8850-2.450)
corn	1.585	(1.355-1.829)
cotton	2.267	(1.158-4.205)
sunflower	2.476	(1.943-3.162)
cabbage	2.644	(1.103-9.00)
sorghum	2.648	(2.005-3.527)

Fresh weight of the above ground shoots was recorded 14 days after treatment and these data are presented in Table 3. The fresh wight of the crop plants varied much more than their heights. In cotton and onion none of the treatments could be distinguished as different from the control; however, all of these treatments showed the possibility of having decreased in weight be 25% or more. Cabbage, sorghum, soybean, sunflower, and wheat did not show any decrease in fresh weight due to trifluralin. Corn, cucumber, and radish all showed significant decreases in weight. Their estimated EC25's are listed below accompanied by the 95% confidence intervals:

Plant	EC25 (lb a.i.)	95% C.I.
cucumber	0.721	(0.195-5.815)
corn	1.027	(0.655-1.699)
radish	1.140	(0.793-1.468)

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** It is concluded that trifluralin postemergence spray at 2.0 lb a.i./A caused slight to moderate stunting of plants but did not significantly reduce fresh weight of cabbage, cotton, onion, sorghum, soybean, sunflower, or wheat plants. Corn, cucumber, and radish were the most sensitive plants tested

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and these plants were injured with trifluralin sprays at 0.25 to 0.50 lb a.i./A. Injury symptoms observed were stunting, crinkled leaves, and some slightly twisted plants. A no effect level (NOEL) was reached at 0.125 lb a.i./A as trifluralin did not significantly injure any of the plants tested at this concentration.

The study author included a Good Laboratory Practices Statement in this report.

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

A. **Test Procedure:** The vegetative vigor study followed SEP and Subdivision J Guidelines.

B. **Statistical Analysis:** The reviewer determined from the reported data those crops which showed a 25% or greater reduction in height or weight at the completion of the test. The data for those crops was used in 'Toxanol' to determine the LC50; ANOVA and Dunnett's test were used to determine the NOEL. The LC50 and slope derived from Toxanol was used in a statistical program, "EC25", to determine the EC25. (See attached).

C. **Discussion/Results:** Utilizing the weight data, corn, cucumber, and radish crops had a greater than 25% reduction over the 14 day test period. (See Table 2). The following EC25's and NOEL's were determined:

Plant	EC25	NOEL
corn	1.09	.50 lb a.i.
cucumber	.796	.25 lb a.i.
radish	1.23	.50 lb a.i.

Utilizing the height data, corn, cucumber, onion and radish had a greater than 25% reduction over the 14 day test period. (See Table 3). The following EC25's were determined:

Plant	EC25	NOEL
corn	1.47	.125 lb a.i.
cucumber	.800	.50 lb a.i.
onion	1.45	.25 lb a.i.
radish	.936	.25 lb a.i.

Utilizing the most sensitive species data, a NOEL of .125 lb a.i. and the EC25 is .796 lb a.i. has been determined for trifluralin.

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D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:**

(3) **Repairability:**

Trifluralin Science Reviews

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LATEULERE TRIFLURALIN CORN WEIGHT

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
2	100	45	45	0
1	100	23	23	0
.5	100	0	0	0
.25	100	7	7	0
.125	100	8	8	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 0

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	1.906255	9.108928	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 = 1.301037
95 PERCENT CONFIDENCE LIMITS =-.4952686 AND 3.097343

LC50 = 3.586932
95 PERCENT CONFIDENCE LIMITS = 1.001717 AND +INFINITY

LC10 = .3789473
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LATEULERE TRIFLURALIN CUCUMBER WT

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
2	100	37	37	0
1	100	26	26	0
.5	100	21	21	0
.25	100	15	15	0
.125	100	8	8	0

THE BINOMIAL TEST SHOWS THAT 2 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 0

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
2	.137536	1	.9266558

SLOPE = .8303333

95 PERCENT CONFIDENCE LIMITS = .5223972 AND 1.138269

LC50 = 5.167282

95 PERCENT CONFIDENCE LIMITS = 2.697651 AND 19.48574

LC10 = .1526708

95 PERCENT CONFIDENCE LIMITS = 6.003198E-02 AND .2485939

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LATEULERE TRIFLURALIN RADISH WEIGHT

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
2	100	37	37	0
1	100	29	29	0
.5	100	3	3	0
.25	100	0	0	0
.125	100	0	0	0

THE BINOMIAL TEST SHOWS THAT 2 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 0

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.5766547	3.646937	1.205027E-02

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED

USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 2.436649
95 PERCENT CONFIDENCE LIMITS = .5863116 AND 4.286987

LC50 = 2.322446
95 PERCENT CONFIDENCE LIMITS = 1.393256 AND 28.16725

LC10 = .69942
95 PERCENT CONFIDENCE LIMITS = .1193079 AND 1.13443
****

Height

LATEULERE TRIFLURALIN VEGVIG.CORN.HT

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
2	100	43	43	0
1	100	11	11	0
.5	100	0	0	0
.25	100	0	0	0
.125	100	0	0	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 0

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	7.771852E-02	1	.7865218

SLOPE = 3.924073

95 PERCENT CONFIDENCE LIMITS = 2.830118 AND 5.018027

LC50 = 2.181052

95 PERCENT CONFIDENCE LIMITS = 1.908012 AND 2.658677

LC10 = 1.035196

95 PERCENT CONFIDENCE LIMITS = .853583 AND 1.18078

****

LATEULERE TRIFLURALIN CUCUMBER

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
2	100	39	39	0
1	100	31	31	0
.5	100	21	21	0
.25	100	12	12	0
.125	100	1	1	0

THE BINOMIAL TEST SHOWS THAT 2 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 0

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET
BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT
BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND
100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	7.087344E-02	1	.1470714

SLOPE = 1.267349
95 PERCENT CONFIDENCE LIMITS = .9299545 AND 1.604744

LC50 = 2.725402
95 PERCENT CONFIDENCE LIMITS = 1.905351 AND 4.793722

LC10 = .2712307
95 PERCENT CONFIDENCE LIMITS = .1743187 AND .3649573

****

LATEULERE TRIFLURALIN ONION

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
2	100	27	27	0
1	100	24	24	0
.5	100	10	10	0
.25	100	1	1	0
.125	100	1	1	0

THE BINOMIAL TEST SHOWS THAT 2 AND +INFINITY CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 0

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET
BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT
BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND
100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	8.294326E-02	1	.1219903

SLOPE = 1.516213
95 PERCENT CONFIDENCE LIMITS = 1.079546 AND 1.95288

LC50 = 4.047149
95 PERCENT CONFIDENCE LIMITS = 2.713008 AND 7.955142

LC10 = .5882148
95 PERCENT CONFIDENCE LIMITS = .4304527 AND .7471905
****

LATEULERE TRIFLURALIN RADISH
****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
2	100	41	41	0
1	100	35	35	0
.5	100	16	16	0
.25	100	0	0	0
.125	100	0	0	0

THE BINOMIAL TEST SHOWS THAT 2 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 0

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.5514668	4.290795	4.92096E-03

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.989804
95 PERCENT CONFIDENCE LIMITS = .5121596 AND 3.467449

LC50 = 2.04872
95 PERCENT CONFIDENCE LIMITS = 1.130682 AND 27.65138

LC10 = .4712256
95 PERCENT CONFIDENCE LIMITS = 5.328084E-02 AND .8366164
****

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

LATEULERE TRIFLURALIN CORNAVEREV
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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
2	100	57	57	0
1	100	58	58	0

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	43.375	43.375		
2	.125	39.935	39.935	1.018	
3	.25	40.393	40.393	0.883	
4	.5	43.640	43.640	-0.078	
5	1.0	33.393	33.393	2.955	*
6	2.0	24.027	24.027	5.728	*

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

WEIGHT OF CORN FOR TRIFLURALIN VIGOR SYUDY
File: CORNWET 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	0	4			
2	.125	4	8.141	18.8	3.440
3	.25	4	8.141	18.8	2.982
4	.5	4	8.141	18.8	-0.265
5	1.0	4	8.141	18.8	9.982
6	2.0	4	8.141	18.8	19.348

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	14.500	14.500		
2	.125	14.250	14.250	0.469	
3	.25	12.750	12.750	3.281	*
4	.50	11.500	11.500	5.624	*
5	1.0	10.000	10.000	8.437	*
6	2.0	8.750	8.750	10.780	*

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

CUCUMBER VIGOR HEIGHT TRIFLURALIN
File: CUKEVIG 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	0	4			
2	.125	4	1.285	8.9	0.250
3	.25	4	1.285	8.9	1.750
4	.50	4	1.285	8.9	3.000
5	1.0	4	1.285	8.9	4.500
6	2.0	4	1.285	8.9	5.750

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	35.615	35.615		
2	.125	36.335	36.335	-0.264	
3	.25	35.785	35.785	-0.062	
4	.5	34.665	34.665	0.348	
5	1.0	25.345	25.345	3.763	*
6	2.0	22.320	22.320	4.871	*

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

RADISH VIGOR-WEIGHT STUDY FOR TRIFLURALIN
File: RADWET 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	0	4			
2	.125	4	6.578	18.5	-0.720
3	.25	4	6.578	18.5	-0.170
4	.5	4	6.578	18.5	0.950
5	1.0	4	6.578	18.5	10.270
6	2.0	4	6.578	18.5	13.295

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	35.615	35.615		
2	.125	36.335	36.335	-0.264	
3	.25	35.785	35.785	-0.062	
4	.5	34.665	34.665	0.348	
5	1.0	25.345	25.345	3.763	*
6	2.0	22.320	22.320	4.871	*

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

RADISH VIGOR-WEIGHT STUDY FOR TRIFLURALIN
File: RADWET 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	0	4			
2	.125	4	6.578	18.5	-0.720
3	.25	4	6.578	18.5	-0.170
4	.5	4	6.578	18.5	0.950
5	1.0	4	6.578	18.5	10.270
6	2.0	4	6.578	18.5	13.295

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	40.500	40.500		
2	.125	43.250	43.250	-1.535	
3	.25	42.250	42.250	-0.977	
4	.5	41.500	41.500	-0.558	
5	1.0	36.000	36.000	2.512	*
6	2.0	23.000	23.000	9.770	*

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

TRIFLURALIN VEGETATIVE VIGOR CORN, HEIGHT
File: VIGCORN 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	0	4			
2	.125	4	4.317	10.7	-2.750
3	.25	4	4.317	10.7	-1.750
4	.5	4	4.317	10.7	-1.000
5	1.0	4	4.317	10.7	4.500
6	2.0	4	4.317	10.7	17.500

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	15.750	15.750		
2	.125	16.000	16.000	-0.344	
3	.25	16.000	16.000	-0.344	
4	.5	13.250	13.250	3.441	*
5	1.0	10.250	10.250	7.569	*
6	2.0	9.250	9.250	8.945	*

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

TRIFLURALIN RADISH HEIGHT FOR VIGOR
File: RADVIG 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	0	4			
2	.125	4	1.751	11.1	-0.250
3	.25	4	1.751	11.1	-0.250
4	.5	4	1.751	11.1	2.500
5	1.0	4	1.751	11.1	5.500
6	2.0	4	1.751	11.1	6.500

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	0	15.500	15.500		
2	.125	15.250	15.250	0.433	
3	.25	15.250	15.250	0.433	
4	.50	14.000	14.000	2.597	*
5	1.0	11.750	11.750	6.494	*
6	2.0	11.250	11.250	7.359	*

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

TRIFLURALIN VIGOR, ONION HEIGHT
File: ONIONVIG 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	0	4			
2	.125	4	1.392	9.0	0.250
3	.25	4	1.392	9.0	0.250
4	.50	4	1.392	9.0	1.500
5	1.0	4	1.392	9.0	3.750
6	2.0	4	1.392	9.0	4.250

SLOPE = 1.086903	1.301037 LC50 =	3.586932 LC25 =	Corn (Weight)
SLOPE = .7958098	.8303333 LC50 =	5.167282 LC25 =	Cucumber (Weight)
SLOPE = 1.227623	2.436649 LC50 =	2.322446 LC25 =	Radish (Weight)
SLOPE = 1.46797	3.924073 LC50 =	2.181052 LC25 =	Corn (Height)
SLOPE = .80009	1.267349 LC50 =	2.725402 LC25 =	Cucumber (Height)
SLOPE = 1.452719	1.516213 LC50 =	4.047149 LC25 =	Onion (Height)
SLOPE = .9385304	1.989804 LC50 =	2.04872 LC25 =	Radish (Height)

DATA EVALUATION RECORD

1. **CHEMICAL:** Trifluralin
Shaughnessey No. 36101.

2. **TEST MATERIAL:** Trifluralin (TREFLAN), technical material 95.7 %, lot number 531AP6.

3. **STUDY TYPE:** Non-Target Plants: Seedling Emergence & Vegetative Vigor Phytotoxicity Test - Tier 2. Species Tested: corn, onion, sorghum, wheat, cabbage, cotton, cucumber, radish, soybean. and sunflower.

4. **CITATION:** Waldrep, T. 1990. "Influence of Trifluralin Preemergence Spray on Seedling Emergence and Vegetative Vigor of Ten Plant Species". DowElanco Laboratory, Global Herbicide Discovery Research, P.O. Box 708, Greenfield, IN 46140-0708. Laboratory No. 61990004. Submitted by DowElanco, 9002 Purdue Road, Indianapolis, IN 46268-1189. EPA MRID No. 419345-04.

5. **REVIEWED BY:**

Dana Lateulere, Biologist
Ecological Effects Branch
Environmental Fate and
Effects Division

Signature: Dana Lateulere

Date: 10/21/91

6. **APPROVED BY:**

Ann Stavola, Section Head, 5
Ecological Effects Branch
Environmental Fate and
Effects Division

Signature: Ann Stavola

Date: 11/6/91

7. **CONCLUSIONS:** This study does not meet guideline requirements for a Seedling Emergence and Vegetative Vigor Study. The emergence testing had no effects to any crops at any of the test concentrations; therefore an EC25 could not be determined. The effects data for the vegetative vigor portion of the test is inconclusive and lacks a clear dose response relationship. The Seedling Emergence portion of the study must be resubmitted using higher concentrations of trifluralin to ensure an effect level. The vegetative vigor portion has been fulfilled by another submittal (MRID No. 419345-03).

8. **RECOMMENDATIONS:** Retest with higher concentrations of trifluralin to ensure an EC25 for seedling emergence.

9. **BACKGROUND:** This study was submitted as part of reregestration requirements.

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

11. **MATERIALS AND METHODS:**

A. **Test Plants:**
Four monocots:
Corn (Zea mays,L.
Onion (Allium cepa,L.)
Wheat (Triticum aesitivum,L.)
Sorghum (Sorghum bicolor,L.)

Six dicots (including one required root crop):
Soybean (Glycine max,L.)
Sunflower (Helianthus annus,L.)
Cotton (Gossypium hirsutum,L.)
Cucumber (Cucumis sativus,L.)
Cabbage (Brassica oleracea,L.)
Radish (Raphanus sativus,L.) - root crop

B. **Test System:** Each crop had four replications with the number of plants per replicate depending on the crop. Corn, cotton, cucumber and soybean had 10 plants per replicate. Onion, sorghum, wheat, cabbage and radish had 25 plants per replicate. The growth medium was a 1:1 mixture of top soil and sand. All plantings were made in plastic containers approximately 31.5 cm long, 21.5 cm wide, and 8.0 cm deep. The plastic containers had holes in the bottom to facilitate bottom watering and drainage.
The label recommends that trifluralin be incorporated into the soil for maximum herbicidal efficacy. Therefore, each rate of trifluralin in each container was incorporated in the soil before seeding. Approximately 5500 grams of soil were measured and placed in metal containers held in a modified cement mixer that rotates the containers and thoroughly mixes the trifluralin into the soil. Each rate of trifluralin was sprayed on to the tumbling soil with a hand-held compressed air sprayer operated at low pressure (5 psi). Each batch of soil was sprayed with 10.0 ml of solution diluted accordingly. After spraying, the soil was allowed to tumble and mix for five minutes before being prepared for seeding.

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After the trifluralin was sprayed on the soil and mixed thoroughly, approximately 5000 grams of treated soil was added to a plastic container. Each container was seeded with 1) corn, onion, sorghum, and wheat, or 2) cabbage, cotton, cucumber, radish, soybean, and sunflower. After seeding, the seeds were covered with the remaining 500 grams of treated soil. The soil in each container was top watered immediately after seeding and the containers were placed in a greenhouse where they remained during the course of the study.

After seeding, all containers were moved into a greenhouse that was equipped with supplemental artificial lighting which was set to give a 12 hour day and 12 hour night. The approximate day temperature was 75-80°F and the approximate night temperature was 70-75°F. After the initial top watering, all containers were bottom watered as needed thereafter for good plant growth.

C. **Dosage:** .0156, .0312, .0625, .125 and .25 lb a.i./A for the monocot plants (corn, onion, sorghum, and wheat) and .0312, .0625, .125 .25 and .50 lb a.i./A for the dicot plants (cabbage, cotton, cucumber, radish, soybean and sunflower). The reason for this difference is that monocot plants are more susceptible to trifluralin than dicot plants.

D. **Design:** The number of seedlings that emerged from each crop were counted weekly during the 21 day test. In addition to seedling count, a vegetative vigor rating was made visually for each crop to determine if the seedlings were injured in any way. Further, if any seedling injury occurred, the type of injury symptoms observed were recorded. Average plant height and above ground fresh weight of crop plants were recorded at the end of three weeks when the test was terminated.

E. **Statistics:** The emergence data was calculated as number of plants emerged per number of seeds planted; this number was then transformed using arcsine transformation. An ANOVA test was performed for the emergence, height, an fresh weight data, along with Dunnett's test for comparing treatment means to a control, to determine whether any of the treatments differed from the control and, if they did, whether the difference between any one treatment and the control could be equal to or greater than 25%. There were some cases, especially in the weight data, where the variability in the data within the treatments was so large that for certain treatments the tests indicated

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that the difference from the control could be both 0% or 25% decrease. In these cases the highest treatment level which definitely did not show a 25% decrease from the control was noted.

12. **REPORTED RESULTS:** The number of plant seedlings that emerged was recorded 1, 2 and 3 weeks after treatment and these numbers are presented in Table 1. Twenty-one days after treatment with trifluralin, seedling emergence was not significantly reduced from the untreated control for any of the ten test plants. Due to the wide range in emergence rates a 25% reduction was still possible at rates of .0312 lb a.i./A or greater for corn, soybean, and sunflower.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Seedling emergence was not significantly reduced from the untreated control for any of the ten test plants. The average height of the plants 3 weeks after treatment with trifluralin was not reduced from the control in cabbage and sunflower. The other eight plants all showed at least a 25% decrease in height due to one or more treatment levels of trifluralin. The fresh weights of the ten plants did not differ significantly from the control for cabbage, cotton, onion, radish, and sunflower; however, due to a large variation in the fresh weight a 25% reduction was still possible at levels greater than 0.0625 lb a.i./A in cabbage and cotton, 0.0312 lb a.i./A in onion and .5 lb a.i./A in sunflower. The fresh weight of the other plants was significantly reduced when compared to the untreated control.

    The study author included a Good Laboratory Practices Statement with the report.

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

    A. **Test Procedure:** The test procedures were in accordance with Subdivision J Guidelines and recommended protocols for a Tier 2 Seedling Emergence and Vegetative Vigor Study; however, the data reported cannot be used for statistical analysis because of poor dose response relationships.

    B. **Statistical Analysis:** Statistical analysis could not be performed due to inconclusive data.

C. **Discussion/Results:** Seedling emergence testing showed no effect at all concentrations. See Table 1. In order to determine an EC25 there has to be at least 25% reduction in emergence compared to the controls. Vegetative vigor testing showed no clear dose response. See Tables 4 and 5.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** An EC25 for emergence could not be determined; poor dose response relationship for vegetative vigor data.

(3) **Repairability:** This study cannot be repaired; new testing needs to be done for seedling emergence to fulfill requirement. Vegetative vigor requirement has been fulfilled with another submission (MRID 419345-03).

15. **COMPLETION OF ONE-LINER:** N/A.

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