

DATA EVALUATION RECORD

- 1. **CHEMICAL:** Acetochlor.
Shaughnessey No. 121601.
- 2. **TEST MATERIAL:** Acetochlor technical; Batch No. QUE-9001-1482-T; 92.07% purity; a dark brown liquid.
- 3. **STUDY TYPE:** 123-1. Non-Target Plants: Vegetative Vigor Nontarget Phytotoxicity Study - Tier 2. Species Tested: Ryegrass, Corn, Oat, Onion, Soybean, Lettuce, Radish, Tomato, Cucumber, Cabbage.
- 4. **CITATION:** White, T.L. 1992. Tier 2 Vegetative Vigor Nontarget Phytotoxicity Study Using Acetochlor. Laboratory Study No. BL91-465. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by The Agricultural Group of Monsanto Company, St. Louis, MO. EPA MRID No. 427131-19.

5. **REVIEWED BY:**

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

Signature: *[Handwritten Signature]*
Date: 6/10/93

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 6/10/93

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

Signature: *William S. Robert*
Date: *H. T. Craven* 10/19/93
12/2/93 *[Handwritten Initials]*

- 7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements for a Tier 2 vegetative vigor test using non-target plants.

Phytotoxicity: The most sensitive monocot species was ryegrass, with an NOEL and LOEL of 0.005 and 0.009 lb ai/A, respectively. The most sensitive dicot species were equally lettuce and cucumber, with an NOEL and LOEL of 0.33 and 1.0

lb ai/A, respectively. No EC values were determined from the phytotoxicity ratings.

Percent survival: The most sensitive species were equally all ten test crops, with a 21-day NOEL, LOEL, EC₂₅, and EC₅₀ of 3.0, >3.0, >3.0, and >3.0 lb ai/A, respectively.

Plant height: Ryegrass was the most sensitive monocot species, with a 21-day NOEL, LOEL, EC₂₅, and EC₅₀ of 0.005, 0.009, 0.007, and 0.031 lb ai/A, respectively. Lettuce was the most sensitive dicot species, with a 21-day NOEL, LOEL, EC₂₅, and EC₅₀ of 0.110, 0.330, 0.510, and 1.700 lb ai/A, respectively.

Plant dry weight: Ryegrass was again the most sensitive monocot species, with a 21-day NOEL, LOEL, EC₂₅, and EC₅₀ of 0.002, 0.005, 0.005, and 0.013 lb ai/A, respectively. Lettuce was again the most sensitive dicot species, with a 21-day NOEL, LOEL, EC₂₅, and EC₅₀ of 0.110, 0.330, 0.320, and 1.400 lb ai/A, respectively.

8. RECOMMENDATIONS: N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Plants: Monocotyledon plants were represented by four species from two families (i.e., ryegrass, oat, corn, and onion). Dicotyledon plants were represented by six species from five families (i.e., soybean, lettuce, radish, cabbage, tomato, and cucumber). Cultivars, seed sources, lot numbers, and germination ratings were provided in the report.

B. Test System: Seeds of each crop were planted in plastic pots (7.5 x 7.5 x 6.0 cm) filled with a sterilized sandy loam soil (pH 7.9, 0.3% organic matter) and perlite. A plexiglass template was used to create planting holes in the soil, allowing for uniform planting depth and seed distribution. Oat, soybean, cucumber, and corn were planted at a depth of 2.5 cm, while the remaining six species were planted at a depth of 1.3 cm. After emergence, each pot was thinned to five plants of uniform height per pot. The plant species were allowed to grow for 10-30 days before treatment to allow each species to attain the 1-3 true

leaf stage. Each treatment replicate was placed on an aluminum tray which was placed in the spray plot. The spray plot was 45.5 in. x 15.5 in. (i.e., 4.9 ft²).

All applications were performed in a spray booth equipped with a single nozzle. A nozzle height of 10.5 inches and a nozzle pressure of 35 psi were used. The test spray solutions were prepared by dissolving acetochlor in a 75% acetone/25% deionized water solution. The plants were sprayed at the equivalent of 468 l/ha (50 GPA) of diluent.

Pots were watered by hand (avoiding foliage) for the first 48 hours of the study and thereafter were watered four times a day. A total of 17 and 37 ml of water was used to irrigate each pot per day for the initial and continuation studies, respectively.

- C. **Dosage:** Acetochlor was applied at nominal rates of 0.037, 0.11, 0.33, 1.0, and 3.0 lb active ingredient (ai)/acre (A) to all plant species for the initial study. For the continuation study, acetochlor was applied to selected species at rates ranging from 0.0023 to 0.037 lb ai/A. Treatment application rates were adjusted for the percent purity of the test material (92.07%). Treatments were applied within two hours of solution preparation.
- D. **Design:** Each crop/treatment combination was replicated four times (i.e., 5 plants/pot, 4 pots/treatment level). After treatment, the pots were randomized in an on-site greenhouse and rotated 180° twice weekly to reduce phototropism.

Plant height was measured by extending the seedling to its maximum height and recording the height to the nearest millimeter. The mean plant height was measured at 0 and 21 days after application.

Plant phytotoxicity was monitored at 7, 14, and 21 days after treatment. The phytotoxicity ratings evaluated five observable toxic effects: 0-indicates no effect; 1-indicates slight plant effect; 2-indicates a moderate effect (e.g., mild stunting or chlorosis); 3-indicates a severe effect with recovery possible; 4-indicates a total effect (very poor vigor); and 5-moribund or plant death.

Twenty-one days after treatment, the plants within treatment replicates (pots) were cut at the soil level (except radish, which was cut beneath the cotyledons) and dried in pre-weighed aluminum sheets at 100°C for a minimum of 48 hours.

Temperature, relative humidity, photoperiod, and illuminance during the period of growth were provided in the report.

Samples of the spray solutions were analyzed for acetochlor content using gas chromatography.

E. Statistics: All calculations are based on nominal rates. All data were entered into a Lotus 1-2-3 spreadsheet. The spreadsheet calculated replicate means, treatment means, standard deviations, percent effect, and analysis of variance tables. Treatment means were used to calculate the percent effect resulting from the treatment. The percent effect was calculated using the following equation:

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

Plant heights taken prior to treatment were used to uniformly distribute pots to treatment groups. The pretreatment percent difference was determined using the following equation:

$$\% \text{ difference} = \frac{(\text{pretreat. height} - \text{control height})}{\text{control plant height}} \times 100$$

A randomized complete block analysis of variance (ANOVA) was performed on treatment level x replicate means. Prior to analysis, phytotoxicity data were converted to the proportion of the maximum rating. When the ANOVA indicated a significant difference from the control, treatment means were subjected to a one-tailed comparison test (Dunnett's) to determine which treatments were significantly different (p < 0.05) from the control. The no-effect-level (NOEL) was determined as the highest treatment rate not statistically different from the control or the rate below which 25% inhibition was witnessed.

The percent detrimental effect values were input into a computer program which fit the data to various mathematical equations. The least squares error of fit and F-value were used as criteria to judge which

equation provided the best representation of the response. The selected equation was used to determine the EC₂₅ and EC₅₀ values.

12. **REPORTED RESULTS:** The author based all reported results on nominal concentrations. The percent recoveries of acetochlor in the base and continuation studies ranged between 82 and 121% (Tables I-III, attached).

Phytotoxicity: By the end of 21 days, nine of the ten test species demonstrated evidence of phytotoxicity at some rate of acetochlor. The NOELs (in lb ai/A) for the test species, in increasing sensitivity, are:

radish (3.0) < soybean = cabbage = onion (1.0) < lettuce = cucumber = corn (0.33) < tomato (0.11) < oat (0.037) < ryegrass (0.0046).

No EC values were determined from the phytotoxicity data.

Percent survival: By the end of 21 days, none of the test species demonstrated evidence of reductions in plant survival. The NOEL for all test species was 3.0 lb ai/A.

Regression analysis was not conducted for any species due to the lack of dose response relationships.

Plant height: Six of the ten crops were sensitive to acetochlor at some tested rate. The NOELs (in lb ai/A) for the test species, in increasing sensitivity, are:

soybean = radish = cucumber = onion (3.0) < cabbage (1.0) < tomato (0.33) < lettuce = corn (0.11) < oat (0.019) < ryegrass (0.0046).

Regression analysis was conducted for five species. The EC values are listed in Table X.

Plant dry weight: Seven crops were sensitive to acetochlor at some tested rate. The NOELs (in lb ai/A) for the test species, in increasing sensitivity, are:

radish = cabbage = onion (3.0) < corn (1.0) < soybean = tomato = cucumber (0.33) < lettuce (0.11) < oat (0.037) < ryegrass (0.0046).

Regression analysis was conducted for six species. The EC values are listed in Table X.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A no-effect level was reached for each parameter measured for all crops tested. The lowest NOEL for each parameter were as follows: phytotoxicity - ryegrass (0.0046 lb ai/A); percent survival - all crops (3.0 lb ai/A); plant height - ryegrass (0.0046 lb ai/A); plant dry weight - ryegrass (0.0046 lb ai/A).

The Quality Assurance Unit of Pan-Agricultural Laboratories, Inc. was responsible for the assurance of compliance with Good Laboratory Practice (GLP) Standards as outlined in 40 CFR Part 160. GLP and QA statements were enclosed in the report and the analytical appendix.

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

A. Test Procedure: The test procedures followed the SEP and Subdivision J guidelines, except for the following:

It was not stated if the control plants were sprayed with a 75% acetone/25% deionized water solution. The protocol submitted with the study indicated that control plants would be sprayed with deionized water and an appropriate solvent if necessary. Consequently, a negative control was not included in the test design.

The rate dilution progression for all studies other than the continuation study was three-fold, rather than the recommended two-fold.

B. Statistical Analysis: Probit and mean comparison (Dunnett's) analyses were conducted on ryegrass (the most sensitive monocot species) and lettuce (the most sensitive dicot species) data for plant dry weight. The reviewer obtained similar values as the author (see attached printouts). However, since the NOEL was the same as the EC₂₅, the NOEL and LOEL for ryegrass dry weight were determined to be 0.002 and 0.005 lb ai/A, respectively.

C. Discussion/Results: Results of the chemical analyses indicated that the percentage recoveries in the spray solutions were near those of the spiked samples. The reviewer therefore believes that the nominal concentrations are representative of actual rates applied and accepts the results in terms of nominal concentrations.

Phytotoxicity: The most sensitive monocot species was ryegrass, with an NOEL and LOEL of 0.005 and 0.009 lb ai/A, respectively. The most sensitive dicot species were equally lettuce and cucumber, with an NOEL and LOEL of 0.33 and 1.0 lb ai/A, respectively. No EC values were determined from the phytotoxicity ratings.

Percent survival: The most sensitive species were equally all ten test crops, with a 21-day NOEL, LOEL, EC₂₅, and EC₅₀ of 3.0, >3.0, >3.0, and >3.0 lb ai/A, respectively.

Plant height: Ryegrass was the most sensitive monocot species, with a 21-day NOEL, LOEL, EC₂₅, and EC₅₀ of 0.005, 0.009, 0.007, and 0.031 lb ai/A, respectively. Lettuce was the most sensitive dicot species, with a 21-day NOEL, LOEL, EC₂₅, and EC₅₀ of 0.110, 0.330, 0.510, and 1.700 lb ai/A, respectively.

Plant dry weight: Ryegrass was again the most sensitive monocot species, with a 21-day NOEL, LOEL, EC₂₅, and EC₅₀ of 0.002, 0.005, 0.005, and 0.013 lb ai/A, respectively. Lettuce was again the most sensitive dicot species, with a 21-day NOEL, LOEL, EC₂₅, and EC₅₀ of 0.110, 0.330, 0.320, and 1.400 lb ai/A, respectively.

This study is scientifically sound and meets the requirements for a Tier 2 vegetative vigor test using non-target plants.

D. Adequacy of the Study:

- (1) **Classification:** Core.
- (2) **Rationale:** N/A.
- (3) **Repairability:** N/A.

15. **COMPLETION OF ONE LINER:** Yes, 5-13-93.

ACETOCHLOR

Page _____ is not included in this copy.

Pages 8 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.
 - Identity of the source of product ingredients.
 - Sales or other commercial/financial information.
 - A draft product label.
 - The product confidential statement of formula.
 - Information about a pending registration action.
 - FIFRA registration data.
 - The document is a duplicate of page(s) _____.
 - 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.

lettuce dry weight

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	4	.5120	.0371	7.2
2 0.037	4	.4373	.0421	9.6
3 0.11	4	.4560	.0590	12.9
4* 0.33	4	.3665	.0672	18.3
5* 1.0	4	.2733	.0322	11.8
6* 3.0	4	.2103	.0427	20.3

NOEL = 0.11 16 μ A
LOEL = 0.33 16 μ A

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minimum detectable difference for Dunnett's test = -.082369
This difference corresponds to -16.09 percent of control

Between groups sum of squares = .267073 with 5 degrees of freedom.

Error mean square = .002336 with 18 degrees of freedom.

Bartlett's test p-value for equality of variances = .824

lettuce dry weight

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence	Upper Limits
EC 1.00	0.0066	0.0012	0.0180
EC 5.00	0.0323	0.0103	0.0646
EC10.00	0.0754	0.0318	0.1290
EC15.00	0.1336	0.0675	0.2077
EC50.00	1.5001	1.0783	2.3381
EC85.00	16.8435	8.2203	55.1625
EC90.00	29.8486	13.0421	118.7611
EC95.00	69.6785	25.7540	371.2086
EC99.00	341.6986	91.7199	3166.2295

$$y = 4.83 + 0.99(x)$$

$y = \text{probit } \% \text{ inhibition}$

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

$$EC_{25} = 0.31216 \text{ ai/A}$$

ryegrass dry weight

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	4	.1185	.0158	13.3
2 0.0023	4	.1025	.0236	23.1
3 0.0046	4	.1033	.0077	7.4
4*0.0093	4	.0603	.0151	25.0
5*0.019	4	.0430	.0042	9.9
6*0.037	4	.0298	.0090	30.1

NOEL = 0.0046 $\mu\text{g ai/A}$
LOEL = 0.0093 $\mu\text{g ai/A}$

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minimum detectable difference for Dunnett's test = -.024020
This difference corresponds to -20.27 percent of control

Between groups sum of squares = .026908 with 5 degrees of freedom.

Error mean square = .000199 with 18 degrees of freedom.

Bartlett's test p-value for equality of variances = .132

ryegrass dry weight

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence	Upper Limits
EC 1.00	0.0007		
EC 5.00	0.0017		
EC10.00	0.0026		
EC15.00	0.0036		
EC50.00	0.0131		
EC85.00	0.0478		
EC90.00	0.0649		
EC95.00	0.1022		
EC99.00	0.2398		

$$y = 8.47 + 1.84(x)$$

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

$y =$ predict Σ inhibition

$$\bar{EC}_{25} = 0.00616 \text{ ai/A}$$