1-13-94 VV

MRID No. 416138-11

DATA EVALUATION RECORD

1. **CHEMICAL:** Benefin.
Shaughnessey No. 084301.

2. **TEST MATERIAL:** Benefin (Balan®); N-butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-p-toluidine; Lot No. 317EF2; 95.6% active ingredient.

3. **STUDY TYPE:** 123-1(b) Non-Target Plants: Vegetative Vigor Phytotoxicity Test - Tier 2. Species Tested: Soybean, Sunflower, Cotton, Cabbage, Cucumber, Radish, Sorghum, Wheat, Corn, and Onion.

4. **CITATION:** Waldrep, T.W. 1989. Influence of Benefin Postemergence Spray on the Vegetative Vigor of Ten Crop Plants. Laboratory Report ID. No. 61989011. Conducted and submitted by DowElanco, Greenfield, IN. EPA MRID No. 416138-11.

5. **REVIEWED BY:**

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Signature: Mark Mossler
Date: 6/22/92

6. **APPROVED BY:**

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Signature: Michael Barry
Date: Goodyear 1-13-94
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7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for a Tier 2 vegetative vigor non-target plant phytotoxicity test. Multiple species were planted per tray, leading to competition between species. Cucumber was the most sensitive species with respect to plant height and weight based on the author's EC_{25} values. Based on visual effects, corn was the most sensitive species with respect to phytotoxicity. The NOEC, LOEC, EC_{25}, and EC_{50} for cucumber height (the most sensitive species parameter) were 0.50, 1.0, 0.75, and 3.24 lb ai/A, respectively.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Plants:** Dicotyledon plants were represented by six species from five families (i.e., soybean, sunflower, cotton, cabbage, cucumber, and radish). Monocotyledon plants were represented by four species from two families (i.e., corn, sorghum, wheat, and onion). Cultivars, lot number, and seed source were provided in the report.

B. **Test System:** Plastic trays (30.5 x 22 cm) were filled with 6.4 cm of a commercial growing medium. One tray was seeded with cabbage, sorghum, cucumber, onion, and radish. A second tray was planted with cotton, wheat, sunflower, corn, and soybean. The seeds were covered with 1.3 cm of soil medium and allowed to emerge in a greenhouse where the pots were watered by subirrigation. After emergence, trays were thinned to a constant number of plants (5 plants for corn, cotton, cucumber, soybean, and sunflower; 25 for radish, cabbage, and sorghum; and 35 for onion and wheat).

Plants were allowed to grow for 12 days after planting. All plants had between 1 and 3 true leaves upon application, except cotton, which only had cotyledonary leaves. The plants were sprayed with a hand-held compressed air sprayer at 5 psi.

After application, the trays were placed in a greenhouse with a 14-hour photoperiod and a temperature of 21-29°C. Irrigation was accomplished by bottom-watering and plants were fertilized as needed.

C. **Dosage:** The highest test solution was prepared by diluting 502 mg of the test material to 16 ml of 1:1 acetone:ethanol. To this, 16 ml of Toximul R and S (a mixture of surfactants and emulsifiers) was added as well as 128 ml deionized water for a total of 160 ml of solution. Ten ml of this solution was sprayed per tray for the highest test concentration (equivalent to 160 gallons/A). The remaining 80 ml of this solution were used to create the lower spray concentrations by serial dilution. The nominal rates were 0.25, 0.5, 1.0, 2.0,

and 4.0 lb ai/A. Control plants were sprayed with a 10% acetone:ethanol and 10% Toximul R and S solution.

D. **Design:** Each treatment/crop combination was replicated four times (i.e., 5 to 35 plants/tray, 4 trays/ treatment or control). The plants were rated for damage (using a 0-100 scale) 7 and 14 days after application (DAA). At 14 DAA, plant shoot height was measured and fresh weight per pot assessed.

E. **Statistics:** Analysis of variance (ANOVA) and Dunnett's test were used to determine significant treatment differences. If differences were observed, regression analysis was used to predict EC_{25} values and 95% confidence intervals.

12. **REPORTED RESULTS:** Corn, soybean, and cucumber appeared to be the most sensitive plants and were injured moderately to severely at 2.0-4.0 lb ai/A. Moderate injury was observed at the 2.0-4.0 lb ai/A rate in cabbage, cotton, wheat, and sunflower. Onion, radish, and sorghum were slightly injured at the 2.0-4.0 lb ai/A rate. Injury symptoms observed were stunting of plants, crinkled leaves, and some curled and twisted plants. These symptoms were not observed at rates below 2.0 lb ai/A.

Benefin significantly reduced the height of all test species at some tested rate except for onion and sorghum. The EC_{25} values, in order of increasing sensitivity, for the ten test species (in lb ai/A), are:

radish (9.4) < soybean (5.1) < sunflower (4.6) < cotton (3.6) < cabbage (3.5) < corn (2.5) < cucumber (0.7).

Fresh weight data was variable, resulting in results that indicated that the difference from the control could be both 0 and 25%. In these cases the highest treatment level which definitely did not show a 25% decrease from the control was noted. It was impossible to detect any differences from the controls in the treated plants of wheat, cabbage, cotton, onion, radish, sorghum, and soybean. However, there were treatments in which a 25% reduction was also possible. A significant reduction did occur for corn, cucumber, and sunflower. The EC_{25} values, in order of increasing sensitivity, for the test species (in lb ai/A), are:

corn (3.7) < sunflower (2.8) < cucumber (1.1).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** It was concluded that benefin postemergence spray at 4.0 lb ai/A did not interfere with either height or weight of onion, radish, and sorghum plants. Cucumber was the most sensitive species tested and this plant was injured with 0.5 lb ai/A or higher rates of benefin. A no-effect level was reached at 0.25 and 0.5 lb ai/A, because no plants were injured at these rates except cucumber.

A statement was included in the report indicating that the study was not subject to Good Laboratory Practices at the time of performance and was not monitored by the Quality Assurance Unit. However, it was stated that the study was conducted within the principles of good laboratory practices.

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:

The NOEC and EC_{50} values were not reported if determined.

A negative control was not included in the test to determine the effect of solvents and surfactants on the test plants.

Cotton plants had only cotyledons rather than 1-3 true leaves.

B. **Statistical Analysis:** Dunnett's test and probit analysis were conducted on cucumber height data (the most sensitive species and parameter). The reviewer obtained a slightly less conservative estimate of the EC_{25} than the author. However, since an EC_{50} was capable of being determined, the reviewer's values will be presented (see attached printouts). The NOEC, lowest-observed-effect concentration (LOEC), EC_{25}, and EC_{50} for cucumber height were 0.50, 1.0, 0.75, and 3.2 lb ai/A, respectively.

C. **Discussion/Results:** Five species were planted per tray. This situation not only leads to crop competition but probably interferes with spray deposition on the foliage of the plants. Each species should be planted in separate pots with adequate dimensions to prevent competition.

It was not stated by the author whether the solution used to make serial dilutions contained 10% acetone:ethanol and 10% Toximul R and S. All spray solutions should have contained these amounts of solvents and surfactants.

Corn was the most sensitive species with respect to plant damage. However, NOEC values were not determined by the author.

Cucumber had the lowest EC_{25} with respect to plant height (i.e., 0.657 lb ai/A). However, corn demonstrated the greatest percent inhibition (52%) at the highest rate of benefin. Both corn and cucumber demonstrated a greater than 50% inhibition and an EC_{50} could have been determined. The highest percent inhibition for sunflower, radish, and soybean was less than 25%; therefore, the EC_{25} values for these species are invalid.

Cucumber also had the lowest EC_{25} for plant weight (1.057 lb ai/A). The highest percent inhibition for corn was less than 25%; therefore, the EC_{25} value for this species is invalid.

This study is not scientifically sound and does not meet the guideline requirements for a Tier 2 vegetative vigor non-target plant phytotoxicity test.

D. **Adequacy of the Study:**

(1) Classification: Invalid.

(2) Rationale: Improper design (i.e., multiple plants per tray) precluded valid results from being drawn from the data due to competition.

(3) Repairability: No.

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

cucumber height

Summary Statistics and ANOVA

Transformation = None

Group (rate (lb a.i/A))	n	Mean	s.d.	cv%
1 = control	4	19.5000	3.1091	15.9
2 0.25	4	17.7500	4.7871	27.0
3 0.50	4	15.0000	4.0825	27.2
4*1.0	4	13.7500	2.8723	20.9
5*2.0	4	10.7500	2.2174	20.6
6*4.0	4	9.7500	1.7078	17.5

NOEC = 0.50 lb a.i/A

LOEC = 1.0 lb a.i/A

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

Minumum detectable difference for Dunnett's test = -5.623333
This difference corresponds to -28.84 percent of control

Between groups sum of squares = 291.833333 with 5 degrees of freedom.

Error mean square = 10.888889 with 18 degrees of freedom.

Bartlett's test p-value for equality of variances = .604

cucumber height

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper Confidence Limits
EC 1.00	0.0195	0.0039	0.0490
EC 5.00	0.0872	0.0307	0.1607
EC10.00	0.1938	0.0909	0.3059
EC15.00	0.3321	0.1872	0.4772
EC50.00	3.2365	2.3417	5.2912
EC85.00	31.5437	14.8998	115.3539
EC90.00	54.0579	22.8330	241.7968
EC95.00	120.0837	42.8871	725.4175
EC99.00	536.5494	139.3734	5716.2915

$y = 4.46 + 1.05(x)$

y = probit % inhibition

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

$EC_{25} = 0.7516$ ai/A