Supplem. but upgradable

1-13-94
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MRID No. 416138-08

DATA EVALUATION RECORD

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

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

3. **STUDY TYPE:** 122-1(a) Non-Target Plants: Seed Germination Phytotoxicity Test - Tier 1. Species Tested: Soybean, Sunflower, Cotton, Cabbage, Cucumber, Radish, Sorghum, Wheat, Corn, and Onion.

4. **CITATION:** Waldrep, T.W. 1989. Influence of Benefin on the Germination of Seeds of Ten Crop Plants. Laboratory Report ID. No. 61989010. Conducted and submitted by DowElanco, Greenfield, IN. EPA MRID No. 416138-08.

5. **REVIEWED BY:**

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Date: 6/22/92

6. **APPROVED BY:**

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Signature: Michael Dow 1-13/94
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7. **CONCLUSIONS:** This study is scientifically sound, but does not meet the guideline requirements for a Tier 1 seed germination non-target plant phytotoxicity test. The criteria used to judge seed germination was not given and division of seeds into abnormal and normal groups was not presented.

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:** Sheets of blue blotter paper were soaked in the test solution or solvent control solution and the excess was allowed to drain by gravity. Ten seeds of each crop were placed on one-half of the blotter paper. The paper was folded over the seeds and the edges of the paper were folded again. The blotter paper was transferred to germinators that kept the seeds at 27°C in total darkness. Blotter paper was re-moistened with the appropriate solution using a hand-atomizer when it became dry.

C. **Dosage:** A stock solution was prepared by adding 47.1 mg of the test material to 10 ml of 1:1 acetone:ethanol. Two milliliters of the stock solution were brought to a volume of 1.0 l in deionized water. The resulting concentration was 9.0 ppm benefin with a 1:1 acetone:ethanol concentration of 2 ml/l. This concentration corresponds to a treatment rate of 3.0 lb active ingredient/A. The control solution contained 2 ml of 1:1 acetone:water/l.

D. **Design:** Each treatment/crop combination was replicated ten times (10 seeds/blotter, 10 blotters/treatment or control). After 5 days of incubation, the seeds were removed from the blotter paper and the number of germinated seeds was determined. Seeds that germinated were divided into normal and abnormal seedlings.

E. **Statistics:** The control and treatment germination were compared to each other using a one-way analysis of variance on double arcsine transformed data.

12. **REPORTED RESULTS:** Tables 1 & 2 (attached) summarize results for the germination test. No significant difference in percent germination existed for any of the test species except cabbage and wheat. Roots of cabbage, cotton, and soybean were stunted slightly. Roots and shoots of corn, sorghum, and wheat were swollen in size and severely

stunted, reaching about one-third the length of the control seedlings.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Benefin at 9.0 ppm did not interfere with the germination of corn, cotton, cucumber, onion, radish, sorghum, soybean, or sunflower seeds. Seedlings of cotton, cabbage, cucumber, onion, radish, soybean, and sunflower were stunted very slightly, but appeared normal in every other respect, whereas roots and shoots of corn, wheat, and sorghum were severly stunted and swollen in size. Mean percent germination for cabbage and wheat was reduced at the 5% level of probability.

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 therefore not monitored by the Quality Assurance Unit. However, 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 with the following exceptions:

A dilution water control was not included in the test.

The criteria (i.e., radicle length) used to judge whether a seed was germinated were not included in the report.

B. **Statistical Analysis:** A t-test was conducted on cabbage percent germination (the species with the largest difference between treatment and control). The reviewer obtained the same results as the author (attached).

C. **Discussion/Results:** The SEP and Subdivision J guidelines state that a seedling must have a radicle length of $\geq$5 mm to be considered germinated. The author did not include any information as to what criteria were used to judge seed germination. The author did include visual observations that indicated that the test material damaged roots and shoots of corn, sorghum, and wheat. If radicle length was investigated, data would probably indicate significant effects on these species as well as cabbage and wheat.

The author stated in the methodology section that the seeds were divided between normal and abnormal seedlings. In the results section, the author stated that the seedlings of corn, wheat, and sorghum were severely stunted and swollen. Results summarized in Table 1 did not exhibit division of the abnormal and normal seedlings and the reviewer questions why this information was not submitted.

No significant decreases in germination were observed between the control seeds and seeds treated with 3.0 lb ai/A benefin except for cabbage and wheat.

This study is scientifically sound, but does not meet the guideline requirements for a Tier 1 seed germination non-target plant phytotoxicity test.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** This study could be upgraded to "core" upon submission of information concerning the radicle lengths of the treated seed. Data concerning the division of the abnormal seeds and subsequent analyses would also need to be submitted.

(3) **Repairability:** Yes, submit the aforementioned data.

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