DATA EVALUATION RECORD

1. **CHEMICAL:** Trisulfuron. Shaughnessey No. 128969-3.

2. **TEST MATERIAL:** CGA-131036 (FL-861740); Sulfonyl Urea herbicide; N-(6-methoxy-4-methyl-1,3,5-triazin-2-yl-amino-carbonyl)-2-(2- chloroethoxy)-benzenesulfonamide. 93.9% active ingredient (a.i.), a colorless crystalline solid. CAS Registry No. 83097-50-5.

3. **STUDY TYPE:** Non-target plants: Seed Germination/Seedling Emergence Tier 2. Species Tested: Soybean, Lettuce, Carrot, Tomato, Cucumber, Cabbage, Oat, Perennial Ryegrass, Corn, and Onion.

4. **CITATION:** Canez, V. M. 1988. Nontarget Phytotoxicity Test Seed Germinaton/Seedling Emergence Tier 2. EPA Guidelines No. 123-1. Conducted by Pan Agricultural Labs., Inc. Madera, CA. Submitted by Ciba-Geigy Corporation, Greensboro, NC. MRID No. 407283-21.

5. **REVIEWED BY:**

Robin Hart
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Robin Hart
Date: August 30, 1989
Chenda Lenn 9/12/89

6. **APPROVED BY:**

Michael Whitten, M.S.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: Michael L. Whitten
Date: 8-31-89

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

Signature: Henry T. Craven
Date: 10/4/89

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements for a Tier 2 seedling germination/seedling emergence study using non-target plants. Seed germination was not affected for any crop by any treatment level tested. Seedling emergence was not affected with the exception of ryegrass and onion which had no-effect concentrations of 5.0 and 40.0 g tech/ha, respectively. Lettuce was the most

sensitive species with no-effect concentrations of 5.0, 0.15, 0.30, and 0.075 g tech/ha for radicle length, height, phytotoxicity rating, and shoot dry weight, respectively. Oat was the least sensitive species with no-effect concentrations of 160, 40.0, 10.0, and 80.0 g tech/ha for radicle length, height, phytotoxicity rating, and shoot dry weight, respectively. All crops showed a detrimental effect $\geq$ 25% for at least one parameter at the maximum application rate.

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

9. **BACKGROUND:** N/A.

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

11. **MATERIALS AND METHODS:**

A. **Test Plants:** Dicotyledon plants were represented by soybean, lettuce, carrot, tomato, cucumber, and cabbage. Monocotyledon plants were represented by oat, ryegrass, corn, and onion. The seed source, cultivars, germination ratings, and lot numbers are provided in the report.

B. **Test System:** Seed germination. Seven milliliters of test solution was added to plastic petri plates (100 x 15 mm) that had two pieces of Whatman #3 filter paper in the top portion. After the test solution was added, ten seeds of each crop were added to each plate. Petri plates were placed in plastic boxes in which the lid was sealed with parafilm to prevent moisture loss. The petri plates were incubated in the dark at 25 $\pm$ 1° C for five to seven days.

Seedling emergence. Ten seeds of each crop were sown in plastic pots of sterilized loamy sand soil and placed in a greenhouse where environmental conditions were monitored. Photoperiod, tempeature, and relative humidity during the experiment are provided in the report. The report also contains an analysis of the soil. The pots were watered daily as needed using an irrigation system to provide the same daily amount for all pots. Test duration was 21 or 28 days depending upon the rate of seedling emergence.

C. **Dosage:** The test compound was prepared by dissolving 0.342 g technical grade CGA-131036 (FL-861740) in 10 ml acetone and diluting with distilled water to a final volume of 190 ml. The concentration of active

ingredient (a.i.) was 171.0 mg/l, equivalent to a 100% application rate of 80 g a.i./ha. The technical material was assumed to be 100% a.i. for the calculated concentrations, but subsequently was found to be 93.9% a.i. Therefore, the actual test concentrations were 6.1% lower than the calculated concentrations.

CGA-131036 was applied to the petri plates for the seed germination test at the calculated rates of 0, 2.5, 5.0, 10.0, 40.0, 80.0 and 160.0 g technical substance/ha (tech/ha). In order to determine the no-effect levels for crops with the exception of soybean and corn, application rates for a subsequent test were 0, 0.188, 0.375, 0.75, 1.5, and 3.0 g tech/ha. In addition cucumber and cabbage were treated with 0.625 g tech/ha.

Application rates to the soil for the seedling emergence test were 0, 1.25, 2.5, 5.0, 10.0, 20.0, 40.0, and 80.0 g/technical substance/ha. Rates of application in a second experiment designed to detect a "no-effect level" and to allow for probit analysis were 0, 0.188, 0.375, 0.75, 1.5, and 3.0 g technical substance/ha. Lettuce and cabbage were also treated with 0.019, 0.038, 0.075, 0.150, and 0.30 g tech/ha. Application was performed with a belt sprayer at a nozzle height of 12 inches and a nozzle pressure of 42 psi.

D. <u>Design:</u> Seedling germination. Each crop/treatment combination was replicated three times (10 seeds/pot, 3 pots/treatment). After six days of incubation, the seeds were removed from the petri plates. The percent germination was calculated and the radicle lengths of germinated seeds were measured to the nearest millimeter. Seeds were considered germinated if the radicle was 5 mm in length or greater.

Seedling emergence. Each crop/treatment combination was replicated three times. After treatment the pots were randomized within crops and among treatments and placed in the greenhouse. Percent seedling emergence, seedling height and phytotoxicity ratings were recorded at 7, 14, and 21 days after treatment. For crops in which the control pots exhibited less than 50% seedling emergence, the study was continued until 28 days after treatment. Twenty-one or twenty-eight days after treatment, the plants within treatment replicates were cut at the soil level and dried in a pre-weighed paper

bag at 70°C for 48 hours. After drying, the dry weight of the plant material from each pot was recorded.

Phytoxicity ratings measured five observable toxic effects: 0 = no injury or effect; 1 = slight plant effect or restriction to one leaf; 2 = moderate effect engrossing the entire plant; 3 = severe effect; and 4 = total effect or plant death.

E. **Statistics:** Treatment means were used to calculate the percent deterimental effect resulting from the treatment as follows:

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

A one-way analysis of variance model for data with equal subsamples was used to analyze radicle length, percent seed germination, and percent seedling emergence. A one-way analysis of variance model for data with unequal subsamples was used to analyze the seedling height data. The percent detrimental effect values were input into a MSTAT probit analysis program. Duncan's New Multiple Range Test was used to determine statistical no-effect levels. Statistical significance was $p \leq 0.05$.

12. **REPORTED RESULTS:** Treatment with CGA-13106 did not have a statistically significant effect on percent seed germination regardless of crop or treatment concentration used within the range from 2.5 to 160 g tech/ha (Table 10, attached). Treatment with CGA-13106 did not have a statistically significant effect on radicle lengths of soybean, carrot, oat, and corn radicle lengths within the treatment range of 2.5 to 160 g tech/ha. No effect concentrations on other crops tested are shown in Table 9 (attached) and range from <0.18 for onion to 10 g tech/ha for cabbage. EC_{25} and EC_{50} values ranged from 0.05 and 10.1, respectively for onion to 238 and 2635 g tech/ha, respectively, for corn.

Treatment with CGA-13106 generally did not affect percent seedlings emerged (Table 56, attached). Only ryegrass and onion had no-effect concentrations less than 80 g tech/ha. Ryegrass had a no-effect concentration of 5.0 and onion had a no-effect concentration of 40 for percent seedling emergence. No-effect concentrations on emerged seedling plant height ranged from 0.15 g tech/ha for lettuce to 40.0 g tech/ha for oat (Table 55, attached). All species showed >25% detrimental effect at the maximum application rate of 80 g tech/ha. EC_{25} and EC_{50} values ranged from 0.095 and

0.722, respectively, for lettuce to 26.5 and 73.9 g tech/ha, respectively, for oat.

Statistical no-effect concentrations for phytotoxicity ratings ranged from 0.3 for lettuce to 10.0 g tech/ha for oat and corn (Table 57, attached). Treatment with CGA-13106 had a >25% detrimental effect on shoot dry weight for all crops except oat (Table 58, attached). No-effect concentrations ranged from 0.075 for lettuce to 80.0 for oat. The attached table from the report, numbered Table 1 by the reviewer, shows the lowest observed no-effect concentrations, EC_{25} and EC_{50} values, along with the parameter in which these concentrations were observed.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Treatment with CGA-13106 at the maximum application rate did not have a significant effect on seed germination and affected percent seedling emergence for ryegrass and onion, only. Oat was generally the plant species least sensitive to CGA-131036 based on the parameters measured. All species showed EC_{25} values less than the maximum application rate of 80 g tech/ha for at least one parameter measured.

A good laboratory practice certification and a quality assurance statement were included in the report.

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

A. **Test Procedure:** The test procedure was in accordance with EPA Subdivision J and M guidelines for a Tier 2 seed germination/seedling emergence test on non-target plants. However, in the dry weight study, the total weight of plants and the tare was less than the weight of the tare for several crops tested at the 0 to 80 g tech/ha rates, indicating that there was a problem in procedure. However, these tests were repeated successfully at lower treatment ranges and there are several parameters in addition to weight to evaluate treatment effects. Therefore, this problem does not affect the overall results and conclusions of the study.

B. **Statistical Analysis:** Analysis of variance of radicle length, plant height, and germination was done by the reviewer to confirm the results of the study (attached). Our analysis of radicle length differed from the author in that we treated radicle lengths of seeds that did not germinate as missing values, not as length = 0. This is a very important difference where seed germination of the control is somewhat low, e.g.

carrot and ryegrass. A much lower variance resulted and the effect of treatment on carrot was significant and indicated a no-effect concentration of <10 g tech/ha. Ryegrass showed a lower no-effect concentration than reported by the author for the same reason.

The analysis of emerged seedling plant height generally agreed with author's analysis. However, there were two tests of corn. The first test had a treatment range of 0 to 80 and a second test had a range of 0 to 3.0 g tech/ha. They showed no-effect levels of 10 and 0.75, respectively. The author showed 10 as the no-effect level on the summary Table 55 whereas we preferred to be conservative and show the lower no-effect level obtained in the second test.

C. **Discussion/Results:** This study is scientifically sound and fulfills the guideline requirements for a Tier 2 seed germination/seedling emergence test on non-target plants. All crops tested showed a greater than 25% detrimental effect at the application rate of 80 g tech/ha for CGA-13106. Most crops showed dose-response relationships with treatment. Lettuce was the most sensitive crop tested with no-effect concentrations of 5.0, 0.15, 0.30, and .075 g tech/ha for radicle length, height, phytotoxicity rating, and shoot dry weight, respectively. Oat was the least affected crop of the species that were tested with no-effect concentrations of 160.0, 40.0, 10,0, and 80.0 g tech/ha for radicle length, height, phytotoxicity rating, and shoot dry weight, respectively.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: This study follows the approved protocol for a Tier 2 test of toxicity on seed germination and seedling emergence of non-target plants.

(3) Repairability: N/A

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

Trisulfuron RIN: 6870-93

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