7-20-94

431955-02

MRID No. 426614-25

UPGRADE TO DATA EVALUATION RECORD
(MRID 426614-25 upgraded by MRID 431955-02 of 3/14/93)

1. CHEMICAL: MON 12000.
Shaughnessey No. 128721.

2. TEST MATERIAL: MON 12000; Batch No. SIB-9104-3106-T; 99.3% purity; a white powder.

3. STUDY TYPE: 123-1(6) Non-Target Plants: Vegetative Vigor Nontarget Phytotoxicity Study - Tier 2. Species Tested: Ryegrass, Corn, Oat, Onion, Soybean, Lettuce, Radish, Tomato, Cucumber, Cabbage.

4. CITATION: Chetram, R.S. 1992. Tier 2 Vegetative Vigor Nontarget Phytotoxicity Study Using MON 12000. Laboratory Study No. BL91-463. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by The Agricultural Group of Monsanto Company, St. Louis, MO. EPA MRID No. 426614-25.

5. REVIEWED BY:

Alvaro A. Yamhure, Aquatic Biologist (7507-C), Signature:
Section 2, Ecological Effects Branch,
Environmental Fate and Effects Division Date: 6/25/94

6. APPROVED BY:

Norm Cook, Head Section 2, (7507-C) Signature:
Ecological Effects Branch,
Environmental Fate and Effects Division Date: 07.20.94

7. CONCLUSIONS: This study is scientifically sound and meets the requirements for a Tier 2 vegetative vigor test using non-target plants. Addendum MRID 431955-02 submitted by the registrant gives data on test plants that were sprayed with a 10% THF -- 90% acetonitrile mixture. **This new data is found acceptable and this study is upgraded to core.** The addendum shows that only lettuce (at 3.3% acetonitrile and 0.37% THF for a 12% detrimental effect) and cucumber (at 90% acetonitrile -- 10% THF mixture for 11% detrimental effect) plants showed low adverse effects on plant height. The NOELs for tomato and oat dry weight were below the lowest treatment level and were not determined.

Phytotoxicity: The most sensitive plant species was radish, with an NOEL and LOEL of 0.00007 and 0.00014 lb ai/A, respectively. No EC values were determined from the phytotoxicity ratings.

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Percent survival: All species except oat were affected by some tested rate of MON 12000. The most sensitive plant species was radish, with a 21-day NOEL, LOEL, EC_{25}, and EC_{50} of 0.00085, 0.0026, 0.0011, and 0.0026 lb ai/A, respectively.

Plant height: Radish was the most sensitive species, with a 21-day NOEL, LOEL, EC_{25}, and EC_{50} of 0.00007, 0.00014, 0.00018, and 0.0012 lb ai/A, respectively.

Plant dry weight: Radish was again the most sensitive species, with a 21-day NOEL, LOEL, EC_{25}, and EC_{50} of 0.000018, 0.000035, **0.000057**, and 0.00019 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 7.5, 0.7% 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 10-24 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 ft^2).

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 MON 12000 in a 10% tetrahydrofuran (THF)/90% acetonitrile 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 and a total of between 18 and 52 ml of water was used to irrigate each pot per day for the initial and continuation studies.

C. Dosage: MON 12000 was applied at nominal rates of 0.023, 0.070, 0.21, 0.63, and 1.9 lb active ingredient (ai)/acre (A) to all plant species for the initial study. For the continuation studies, MON 12000 was applied to selected species at rates of ranging from 0.000018 to 0.069 lb ai/A. Treatment application rates were adjusted for the percent purity of the test material (99.3%). Treatments were applied within 40-120 minutes 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 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 MON 12000 content using liquid 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, percent effect, standard deviations, 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 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 EC_{25} and EC_{50} values.

12. REPORTED RESULTS: The author based all reported results on nominal concentrations. The percent recoveries of MON 12000 in the base and continuation studies ranged between 82 and 128% (Tables I-IV, attached).

Phytotoxicity: By the end of 21 days, all ten test species demonstrated evidence of phytotoxicity at some rate of MON 12000. The NOELs (in lb ai/A) for the test species, in increasing sensitivity, are:

tomato = oat (0.070) < cucumber = corn = ryegrass (0.0077) < soybean = lettuce (0.00085) < cabbage = onion (0.00014) < radish (0.00007).

No EC values were determined from the phytotoxicity data.

Percent survival: By the end of 21 days, all species except oat demonstrated evidence of reductions in plant survival. The NOELs (in lb ai/A) for the test species, in increasing sensitivity, are:

oat (1.9) < cucumber = ryegrass (0.21) < corn = tomato (0.070) < soybean (0.023) < lettuce = cabbage (0.0077) < radish = onion (0.00085).

Regression analysis was conducted for all species except cabbage and oat. The EC values are listed in Table XVII (attached). The EC_{25} for lettuce was not presented due to the lack of a definite dose response relationship.

Plant height: All of the ten crops were sensitive to MON 12000 at some tested rate. The NOELs (in lb ai/A) for the test species, in increasing sensitivity, are:

oat (0.21) < tomato = cucumber (0.070) < corn (0.023) < ryegrass (0.0077) < soybean = lettuce = onion (0.00085) < cabbage (0.00028) < radish (0.00007).

Regression analysis was conducted for all species. The EC values are listed in Table XVII. The EC_{25} for ryegrass was not presented due to the lack of a definite dose response relationship.

Plant dry weight: All ten crops were sensitive to MON 12000 at some tested rate. The NOELs (in lb ai/A) for the test species, in increasing sensitivity, are:

tomato = oat (0.023) < cucumber (0.0077) < ryegrass = corn (0.0026) < soybean (0.00014) < cabbage (0.00007) < lettuce = radish = onion (0.000035).

Regression analysis was conducted for all species. The EC values are listed in Table XVII.

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 - radish (0.00007 lb ai/A), percent survival - radish and onion (0.00085 lb ai/A), plant height - radish (0.00007 lb ai/A), plant dry weight - lettuce, radish, and onion (0.000035 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:

Originally it was not stated if the control plants were sprayed with a 10% THF/90% acetonitrile solution. Addendum MRID No. 431955-02 has satisfied this requirement. The protocol submitted with the study indicated that control plants would be sprayed with deionized water and an appropriate solvent if necessary.

The rate dilution progression for all studies other than the second continuation study was 3x, rather than the recommended 2x.

The NOELs for tomato and oat dry weight were not determined [significant effects at the lowest tested rate (pp. 53-54, attached)].

B. Statistical Analysis: Probit and mean comparison (Dunnett's) analyses were conducted on radish data for plant dry weight (see attached). The reviewer obtained a more conservative estimate than the author for the NOEL. The EC values were in general agreement. Therefore, the NOEL and lowest-observed-effect level (LOEL) for radish dry weight are 1.8 $x10^{-5}$ and 3.5 $x10^{-5}$ lb ai/A, respectively.

C. Discussion/Results: Results of the chemical analyses indicated that the actual concentrations were near nominal concentrations. The reviewer therefore believes that the nominal concentrations are

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representative of actual rates applied and accepts the results in terms of nominal concentrations.

The phytotoxicity ratings indicated that control ryegrass, cucumber, and radish plants were injured by the diluent (solvents) used to apply the test material in the initial study only. The NOEL and EC values for cucumber and ryegrass survival were determined from the initial study. However, the reviewer determined that the other monitored parameters (height and weight) were more sensitive measures of the test material's effect than percent survival. Therefore, the reviewer did not invalidate the results from these two species.

Phytotoxicity: The most sensitive plant species was radish, with an NOEL and LOEL of 0.00007 and 0.00014 lb ai/A, respectively. No EC values were determined from the phytotoxicity ratings.

Percent survival: The EC_{25} value for lettuce should have been presented. The reviewer performed linear interpolation and calculated an EC_{25} value of 0.013 lb ai/A for lettuce. All species except oat were affected by some tested rate of MON 12000. The most sensitive plant species was radish, with a 21-day NOEL, LOEL, EC_{25}, and EC_{50} of 0.00085, 0.0026, 0.0011, and 0.0026 lb ai/A, respectively.

Plant height: The reviewer determined that the NOEL for cucumber height should be 0.023 lb ai/A, rather than 0.07 lb ai/A. The EC_{25} value for ryegrass should have been presented. The reviewer performed linear interpolation and calculated an EC_{25} value of 0.017 lb ai/A for ryegrass. Radish was the most sensitive species, with a 21-day NOEL, LOEL, EC_{25}, and EC_{50} of 0.00007, 0.00014, 0.00018, and 0.0012 lb ai/A, respectively.

Plant dry weight: Radish was again the most sensitive species, with a 21-day NOEL, LOEL, EC_{25}, and EC_{50} of 0.000018, 0.000035, 0.000057, and 0.00019 lb ai/A, respectively.

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

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D. Adequacy of the Study:

(1) **Classification:** Core.

(2) **Rationale:** The control plants were spayed with a 10% THF/90% acetonitrile mixture in the new study (Addendum MRID 431955-02). The author has fulfilled EEB's request and this study is therefore upgraded to core. The NOELs for tomato and oat dry weight remain undetermined but the other other suffice to show the toxicological characteristics of MON 12000.

(3) **Repairability:** This study has been upgraded to "core" for all species except tomato and oat since the has submitted the additonal data requested by EEB.

15. COMPLETION OF ONE LINER: Yes, 4-7-93. Upgraded 6/25/94