7-20-94

431955-01

MRID No. 426614-24

UPGRADE TO DATA EVALUATION RECORD
(MRID 426614-24 and Addendum MRID 431955-01 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. Non-Target Plants: Seed Germination/ Seedling Emergence Phytotoxicity Test - Tier 2. Species Tested: Soybean, Lettuce, Radish, Tomato, Cucumber, Cabbage, Oat, Ryegrass, Corn, and Onion.

4. CITATION: Chetram, R.S. 1992. Tier 2 Seed Germination/ Seedling Emergence Nontarget Phytotoxicity Study Using MON 12000. Laboratory Study No. BL91-464.. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by The Agricultural Group of Monsanto Company, St. Louis, MO. EPA MRID No. 426614-24.

5. REVIEWED BY:

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

6. APPROVED BY:

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

7. CONCLUSIONS: These studies are scientifically sound and meet the requirements for Tier-2 seed germination and seedling emergence tests using non-target plants and is upgraded to core . For the emergence study, the solvent system negatively impacted only the growth of the control cabbage plants by 7%.

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The most sensitive species for seedling emergence was lettuce with a dry weight EC_{25} of $2X^{10-5}$ lb ai/A. Because the registrant clarified the most important data gaps with Addendum MRID 431955-01 about the effects of the solvent mixture for the germination study and the composition of said mixture, these sudies are upgraded to core.

Seed Germination: The most sensitive species was cabbage. The 6-day NOEL, LOEL, EC_{25}, and EC_{50} for cabbage germination were 0.015, 0.030, 0.011, and 0.040 lb ai/A, respectively.

Seedling Emergence:
Seedling Emergence and Survival: Ryegrass appeared to be the most sensitive valid species with respect to seedling emergence. The NOEL, LOEL, EC_{25}, and EC_{50} were 0.07, 0.21, 0.20, and >1.90 lb ai/A, respectively.

By 21 days after treatment, radish appeared to be the most sensitive species with respect to survival (based on overall values). The NOEL, LOEL, EC_{25}, and EC_{50} were 0.023, 0.070, 0.090, and 1.50 lb ai/A, respectively.

Plant Phytotoxicity: The most sensitive species with regard to plant phytotoxicity was lettuce. The NOEL and LOEL for this species were 0.000094 and 0.00028 lb ai/A, respectively.

Plant Height: The most sensitive species was lettuce, with NOEL, LOEL, EC_{25}, and EC_{50} values of 0.000012, 0.000024, 0.000099, and 0.00024 lb ai/A, respectively.

Plant Dry Weight: Lettuce was again the most sensitive species with respect to dry weight. The NOEL, LOEL, EC_{25}, and EC_{50} were 0.000012, 0.000024, 0.00002, and 0.0001 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, lettuce, radish, tomato, cucumber, and cabbage). Monocotyledon plants were represented by four species from two families (i.e., corn, oat, ryegrass, and onion). Cultivars, lot numbers, seed sources, and germination ratings were provided in the report.

B. Test System:
Seed Germination: Two circles of blue blotter were placed in the bottom of a glass petri plate (100 x 15 mm). The highest concentration test solution for the

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base study was prepared in a 1% acetonitrile/deionized water solution and then diluted serially with this same solvent mixture to achieve the lower concentration solutions. The highest concentration test solution for the continuation study was prepared in a 0.25% acetonitrile/deionized water solution and subsequently serially diluted. Twelve milliliters of the test solution were added to each plate of soybean, cucumber, oat, and corn. Ten milliliters were added to plates of lettuce, radish, tomato, cabbage, ryegrass, and onion.

Ten seeds of each crop were added to each petri plate within 169 minutes of test solution preparation. The plates containing crops with the same concentration were then impartially placed in plastic boxes (31 x 23 x 10 cm) with raised mesh bottoms and tight-fitting lids to prevent moisture loss. Water was added to the bottom of each box to increase humidity. The petri plates were incubated in the dark at 24-27°C, except lettuce, which was incubated at 18-20°C. All crops were incubated for six days.

Seedling Emergence: Ten seeds of each crop were planted in plastic pots (7.5 x 7.5 x 6.0 cm), filled with sterilized sandy loam soil (pH 7.5, 0.7% organic matter) and perlite obtained from the laboratory facility. A plexiglass template was used to create planting holes in the soil, allowing for uniform planting depth and seed distribution. Soybean, cucumber, oat, 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. Each treatment replicate was placed on an aluminum tray which was placed in the spray plot. The spray plot was 45.5 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 the material in a 10% tetrahydrofuran/90% acetonitrile solution. The plants were sprayed at the equivalent of 468 l/ha (50 gpa) within 31 to 60 minutes after solution preparation.

The pots were hand watered (10-14 ml/pot) during the first 48 hours to facilitate movement of the test material to the seed zone. Lettuce plants were kept in an area maintained at 19-22°C. After 48 hours, the

pots were watered four times a day and a total of 11-22 ml of water was used to irrigate each pot per day.

C. Dosage: In both seed germination and seedling emergence tests, Mon 12000 was applied at the 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 germination test, one continuation study was performed. Two continuation studies were conducted for the emergence test. Rates ranging from 0.0000059 to 0.030 lb ai/A were applied to selected test species. The test solutions were corrected for the percent purity of the test material (99.3%).

D. Design:

Seed Germination: Each treatment/crop combination was replicated four times (i.e., 10 seeds/plate, 4 plates/ treatment). After 6 days of incubation, the percentage of germinated seeds was determined by counting the number of seeds which had radicle lengths of 5 mm or greater.

Seedling Emergence: Each crop/treatment combination was replicated four times (i.e., 10 seeds/pot, 4 pots/ treatment level). After treatment, the pots were randomized in a greenhouse. Trays were rotated 180° twice weekly to reduce phototropism.

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

The percentage of the ten seeds planted in each pot which emerged was calculated for each treatment at 10 and 14 days after treatment. Seedling height and survival were measured 21 days after treatment and phytotoxicity ratings were recorded 10, 14, and 21 days after treatment for all species. Twenty-one days after treatment, the plants within treatment replicates (pots) were cut at the soil level and dried in pre-weighed foil sheets at 100°C for a minimum of 48 hours.

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.

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:

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

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 ($p < 0.05$) 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 81 and 136% (Tables I-V, attached).

Seed Germination: No significant difference in percent germination existed between controls and each treatment level for soybean, cucumber, oat, ryegrass, corn, and onion. The NOELs for radish and tomato, lettuce, and cabbage were 0.21, 0.023, and 0.015 lb ai/A, respectively. The NOEL for the remaining six species was 1.9 lb ai/A. No EC_{25} or EC_{50} values were computed due to the lack of definite dose-response relationships except for lettuce and cabbage. The EC_{50} values for these two species are 0.11 and 0.030 lb ai/A, respectively.

Seedling Emergence:
Percent Emergence and Survival: By the end of 14 days, three of the species tested demonstrated significant reductions in emergence. The NOELs for percent emergence (in lb ai/A) for the test species, in increasing sensitivity, are:

soybean = lettuce = radish = tomato = cucumber = cabbage = oat (1.9) < corn (0.63) < ryegrass (0.070) < onion (0.0077).

Due to lack of definite dose-response relationships, regression analysis was only conducted for ryegrass, corn, and onion. The EC values for these species are presented in Table XX (attached).

By the end of 21 days, corn demonstrated increased emergence, and seedling survival was not significantly reduced at any of the treatment rates, as was the case with the seven species that were unaffected at 14 days after application (except for radish and lettuce). Four species demonstrated significant reductions in survival at some rate of MON 12000. The NOELs for percent survival (in lb ai/A) for the test species, in increasing sensitivity, are:

soybean = tomato = cucumber = cabbage = oat = corn (1.9) < ryegrass (0.63) < lettuce (0.21) < radish (0.023) < onion (0.0077).

Due to lack of significant rate effects, regression analysis was only conducted for lettuce and radish. The EC values for these species are presented in Table XX.

Plant Phytotoxicity: *By the end of the 21 day test period, every species demonstrated significant signs of phytotoxicity at some rate of MON 12000 tested.* The NOELs for phytotoxicity (in lb ai/A) for the test species, in increasing sensitivity, are:

oat (0.023) < tomato = cucumber (0.0077) < soybean = ryegrass = corn (0.0026) < radish = cabbage = onion (0.00028) < lettuce (0.000094).

No EC values were computed from the phytotoxicity data.

Plant Height: By the end of 21 days, all of the test species demonstrated significant reductions in height at some rate of MON 12000. The NOELs for plant height (in lb ai/A) for the test species, in increasing sensitivity, are:

oat (0.0077) < soybean = tomato = cucumber = corn (0.0026) < radish = ryegrass (0.00085) < cabbage = onion (0.00028) < lettuce (0.000012).

Regression analysis was conducted for all test species. The EC values are presented in Table XX.

Plant Dry Weight: By the end of 21 days, all of the test species demonstrated significant reductions in weight at some rate of Mon 12000. The **NOELs** for plant weight (in lb ai/A) for the test species, in increasing sensitivity, are:

soybean = cucumber = oat = corn (0.0026) < radish = cabbage = ryegrass (0.00028) < onion (0.000094) < tomato (0.000047) < lettuce (0.000012).

Regression analysis was conducted for all test species. The EC values are presented in Table XX.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: A no-effect concentration was reached for each parameter measured for all crops tested. The lowest NOEL for each parameter were as follows: percent germination - cabbage (0.015 lb ai/A), percent emergence - onion (0.0077), phytotoxicity - lettuce (0.000094 lb ai/A), percent survival - onion (0.0077 lb ai/A), plant height - lettuce (0.000012 lb ai/A), plant dry weight - lettuce (0.000012 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 generally adhered to the SEP and Subdivision J guidelines, except for the following:

Only one parameter (germination) was measured or recorded for the germination study.

The protocol submitted with the study indicated that control plants would be sprayed with deionized water, with an appropriate solvent if necessary. Consequently, a negative control was not included in the test design.

The rate dilution progression for the base study for both the emergence and germination studies and the first emergence continuation study was 3x, rather than the recommended 2x.

B. Statistical Analysis: Probit and mean comparison (Dunnett's test) analyses were conducted on cabbage germination and lettuce dry weight data for the germination and emergence studies, respectively (see attached printouts). The reviewer obtained the same or similar results as the author for the NOEL and EC values.

C. Discussion/Results: Results of the chemical analyses indicated that the actual concentrations were near nominal concentrations (attached). The reviewer therefore believes that the nominal concentrations are representative of actual rates applied and accepts the results in terms of nominal concentrations.

Seed Germination: The most sensitive species was cabbage. The 6-day NOEL, lowest-observed-effect level (LOEL), EC_{25}, and EC_{50} for cabbage germination were 0.015, 0.030, 0.011, and 0.040 lb ai/A, respectively. A rate response was observed for lettuce, and the EC_{25} should have been determined for this species.

Seedling Emergence: The solvent system used in this study detrimentally affected the development of onion plants (>25% phytotoxicity in the control). The results for this species are therefore invalid.

Seedling Emergence and Survival: Ryegrass appeared to be the most sensitive valid species with respect to seedling emergence. The NOEL, LOEL, EC_{25}, and EC_{50} were 0.07, 0.21, 0.20, and >1.90 lb ai/A, respectively.

By 21 days after treatment, radish appeared to be the most sensitive species with respect to survival (based on overall values). The NOEL, LOEL, EC_{25}, and EC_{50} were 0.023, 0.070, 0.090, and 1.50 lb ai/A, respectively.

Plant Phytotoxicity: The most sensitive species with regard to plant phytotoxicity was lettuce. The NOEL and LOEL for this species were 0.000094 and 0.00028 lb ai/A, respectively.

Plant Height: The most sensitive species was lettuce, with NOEL, LOEL, EC_{25}, and EC_{50} values of 0.000012,

0.000024, 0.000099, and 0.00024 lb ai/A, respectively. Although not computed by the author, reasonable estimates of the EC_{50} for tomato and oat are 0.023 and 1.9 lb ai/A, respectively.

Plant Dry Weight: Lettuce was again the most sensitive species with respect to dry weight. The NOEL, LOEL, EC_{25}, and EC_{50} were 0.000012, 0.000024, 0.00002, and 0.0001 lb ai/A, respectively. A rate response was observed for tomato and cabbage, and the EC_{25} should have been determined for these species (the reviewer had to).

These studies are scientifically sound and meet the requirements for Tier-2 seed germination and seedling emergence tests using non-target plants. The results for onion from the emergence test are not scientifically sound and do not meet the requirements.

D. Adequacy of the Study:

(1) **Classification:** Core. According to the upgrade for this study, MRID431955-01, non of the tested plants showed adverse effects to the solvent treatment in the emergence study. Only cabbage showed a dry weight of 7%.

(2) **Repairability:** Study has been repaired and upgraded to core. The author confirmed that the solvent controls were treated with the appropriate mixtures and the appropriate EC values were determined with only dry weight for cabbage seedling emergence showing a detrimental effect (7%) at 21 day when treated with 90% acetonitrile and 10% THF so the results for all species except onion for the emergence test are upgraded to the "core" category.

15. COMPLETION OF ONE-LINER: Yes, 3-26-93. Updated 6/25/94