DATA EVALUATION RECORD

1. **CHEMICAL:** Prometon.
Shaughnessey No. 080804.

2. **TEST MATERIAL:** Prometon technical; 2,4-bis(isopropylamino)-6-methoxy-s-triazine; CAS No. 1610-18-0; ID No. F1-872050; 98.5% purity; a white powder.

3. **STUDY TYPE:** Non-Target Plants: Seedling Emergence Phytotoxicity Test - Tier 2. Species Tested: Soybean, Lettuce, Carrot, Tomato, Cucumber, Cabbage, Corn, Oat, Ryegrass, Onion.

4. **CITATION:** Chetram, R.S. 1990. Tier 2 Seedling Emergence Nontarget Phytotoxicity Study Using Prometon Technical. Laboratory Report No. LR90-05. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by Ciba-Giegy Corporation, Greensboro, NC. EPA MRID No. 417253-03.

5. **REVIEWED BY:**

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7. **CONCLUSIONS:**

This study is scientifically sound and meets the requirements for a Tier 2 seedling emergence test using non-target plants. With respect to percent emergence, lettuce and carrot were equally the most sensitive to prometon after

10 DS.

14 days, with a subsequent NOEC value of 7.5 lb ai/A. After 28 days, lettuce, cucumber, and cabbage were equally the most sensitive species affected by the prometon application. The subsequent NOEC value for these three species is 0.0235 lb ai/A.

Cabbage was affected the most by prometon with respect to plant phytotoxicity after 21 days. The resulting NOEC value is 0.0188 lb ai/A.

Lettuce and cabbage were equally the most sensitive to prometon with respect to plant height. The resulting NOEC value for these two species is 0.0188 lb ai/A.

Oat was the most sensitive species with respect to plant dry weight. The NOEC value for oat is 0.0047 lb ai/A.

The EC values for each section of the seedling emergence test are listed in Table 17 (attached). In some cases, the EC_{50} value cannot be determined because the percent detrimental effect is not greater than 50% at the highest rate of prometon tested.

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 six species from six families (i.e., soybean, lettuce, carrot, tomato, cucumber, and cabbage). Monocotyledon plants were represented by four species from two families (i.e., corn, oat, ryegrass, and onion). Cultivars, seed sources, lot number, and germination ratings were provided in the report.

B. **Test System:**
Ten seeds of each crop were planted in plastic pots (7.5 x 7.5 x 6.0 cm), filled with sterilized soil obtained from the laboratory facility. A plexiglass template was used to create planting holes in the soil, thus allowing for uniform planting depth and seed distribution. A description of the soil was provided in the report. Each treatment replicate was placed on an aluminum tray (6.125 x 31.125 cm). The spray plot was 38.5 in x 20 in (i.e., 5.35 ft^2).

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. All applications were performed with a belt sprayer equipped with a single nozzle. A nozzle height of 12 inches and a nozzle pressure of 40-50 psi were used. The test spray solutions were prepared by dissolving prometon technical in deionized water and acetone, then diluting with water. The plants were sprayed at the equivalent of 468 l/ha (50 gpa) of water.

The pots were watered three times a day and a total of 18 ml of water was used to irrigate each pot per day for the initial study. The first and second study continuations provided the plants with 23 ml and 27-42 ml of water, respectively.

C. **Dosage:** Prometon was applied at the rates of 0, 0.469, 0.938, 1.88, 3.75, 7.5, 15.0, and 30.0 lb ai/A to the soil in which all species were planted. A study continuation was conducted for all ten species with rates of 0, 0.0118, 0.0235, 0.0469, 0.0938, 0.188, 0.375, and 0.75 lb ai/A. A second study continuation was required in which rates of 0, 0.0003, 0.0006, 0.0012, 0.0023, 0.0047, 0.0094, and 0.0188 lb ai/A were applied to lettuce, cabbage, and oat. Treatment application rates were adjusted for the percent purity of the test material (98.5%).

D. **Design:**
Each crop/treatment combination was replicated four times (i.e., 10 seeds/pot, 4 pots/treatment level). After treatment, all pots were randomized within crops and among treatments and placed in an on-site greenhouse. The percentage of the ten seeds planted in each pot which emerged was calculated for each treatment. Emerged seedlings and phytotoxicity ratings were recorded at 10, 14, and 21 days (28 days for the study continuations) after treatment for all species. Twenty-one days (28 days for the study continuations) after treatment, plant height was recorded and treatment replicates (pots) were cut at the soil level and dried in a pre-weighed paper bag at 70°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; and 4-indicates a total effect or plant death.

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

E. **Statistics:** All data were entered into a Lotus 1-2-3 spreadsheet. The spreadsheet calculated replicate means, treatment means, standard deviations, and analysis of variance tables. Treatment means were used to calculate the percent detrimental effect resulting from the treatment. The percent detrimental effect was calculated using the following equation:

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

An analysis of variance was performed using the raw data spreadsheet. A one way analysis of variance model for data with equal subsamples was used to analyze the percentage seedling emergence data. A one-way analysis of variance model for data with unequal subsamples was used to analyze the seedling height data. Treatment separation was achieved by using Duncan's New Multiple Range Test.

The percent effect values were input into a probit analysis program. The program ignored positive values and transformed the dose by natural logarithms. For seedling emergence, the probit was calculated using all data points; for all other parameters, the probit was calculated using replicate means.

12. **REPORTED RESULTS:** Results are summarized in Table 17 (attached).
Percent emergence and survival: Through day 14, percent emergence for soybean, cabbage, oat, ryegrass, corn and onion did not differ significantly ($p < 0.05$) from the control. However, onion demonstrated a -37% effect. Therefore, the NOEC value for onion was taken to be 15 lb ai/A. The remainder of the tested species demonstrated a significant reduction in emergence at some lower rate of prometon. Species listed in order of increasing sensitivity to prometon based on emergence NOEC (in lb ai/A) are:

soybean = cabbage = oat = ryegrass = corn (30.0) < tomato = cucumber = onion (15.0) < lettuce = carrot (7.5).

Lettuce, tomato, cucumber, and onion demonstrated dose related response curves, and subsequently EC values could be determined. The EC values are listed in Table 17. Sometimes EC_{50} values were not determined because the percent response did not reach beyond the 50% inhibition point.

By the end of the testing period (21 days), only soybean, oat, and corn demonstrated no significant reductions in percent emergence, resulting in an NOEC value of 30.0 lb ai/A. By the end of 28 days (the first study continuation), the remaining seven species were affected by some rate less than 0.75 lb ai/A of prometon. The NOEC values (in lb ai/A) for the ten tested species, in increasing sensitivity, are:

soybean = oat = corn (30.0) < ryegrass = onion (0.375) < carrot (0.188) < tomato (0.0938) < lettuce = cucumber = cabbage (0.0235).

Due to the lack of a rate response, EC values could not be determined for four of the tested species. The EC_{25} and EC_{50} values for the remaining six species are listed in Table 17.

Phytotoxicity rating: Based on observations throughout 28 days, only corn survived through the base study period and was unaffected by any rate of prometon in the first continuation study. The NOEC for corn was therefore 0.75 lb ai/A. The remaining nine species were affected at some rate of prometon between 0.75-0.0118 lb ai/A. The NOEC values (in lb ai/A) for the ten species, in increasing sensitivity, are:

ryegrass = corn (0.75) < carrot (0.375) < soybean = tomato = oat = onion (0.0938) < cabbage (0.0469) < lettuce (0.0235) < cucumber (0.0118).

No EC values were determined from the phytotoxicity data.

Plant height: The height of all species tested was affected by prometon at the lowest rate in the initial study. In the first study continuation (prometon rates of 0.75-0.0118 lb ai/A), all species reached a concentration in which no effect was evident on plant height. The NOEC values (in lb ai/A) for the ten test species, in increasing sensitivity, are:

corn (0.75) < soybean = carrot = oat = ryegrass (0.0938) < tomato = onion (0.0469) < cucumber (0.0235 < lettuce = cabbage (0.0118).

Due to a lack of dose responses, EC values were not determined for lettuce, tomato, ryegrass, and corn. Soybean, carrot, cucumber, cabbage, oat, and onion demonstrated dose related response curves and EC values were determined for these six species. However, the EC_{50} for onion was not reported because the percent detrimental effect was less than 50% at the highest rate of prometon (0.75 lb ai/A). The EC values are listed in Table 17.

Plant dry weight: The weight of all species tested was affected by prometon at the lowest rate in the initial study. An NOEC value was determined for corn, carrot, soybean, tomato, ryegrass, cucumber, and onion in the first continuation study (prometon rates of 0.75-0.0118). A second study continuation was needed to determine NOEC values for lettuce, cabbage, and oat. The prometon rates in this study were between 0.0188 and 0.0003 lb ai/A. The NOEC values (in lb ai/A) for the ten species, in increasing sensitivity, are:

corn (0.375) < carrot (0.0938) < soybean = tomato = ryegrass (0.0235) < cucumber = onion (0.0118) < lettuce = cabbage (0.0094) < oat (0.0047).

All species except corn demonstrated dose related responses. Both EC_{25} and EC_{50} values were determined for carrot, onion, soybean, tomato, and cucumber. Only EC_{25} values were determined for ryegrass, oat, cabbage, and lettuce because the percent detrimental effect was less than 50% at the highest rate of prometon tested. The EC values are listed in Table 17.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
"A no-effect concentration was achieved for soybean, lettuce, carrot, tomato, cabbage, cucumber, oat, ryegrass, corn, and onion in every parameter measured. EC_{25} and EC_{50} values were determined for lettuce (seedling emergence), carrot (seedling survival, plant height, dry weight), tomato (seedling survival, dry weight), cabbage (plant height), oat (plant height), ryegrass (seedling survival), and onion (seedling survival, plant height, dry weight). EC_{25} value was determined for lettuce, cabbage, ryegrass (dry weight), tomato, and onion (seedling emergence)."

The Quality Assurance Unit of Pan-Agricultural Laboratories, Inc., stated that Good Laboratory Practice (GLP) Standards were employed. Statements of Compliance with GLPs and Quality Assurance were provided.

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:

All plants in each replicate were weighed together, then the total weight was divided by the total number of plants to obtain each replicate mean value. The plants should have been individually weighed so the variation among plants within each replicate could be accounted for in the statistical analysis of the data.

Although stated in the protocol (attached as Appendix B in the study report), it was not restated in the report as to whether the control plants were treated with control water that contained the appropriate amount of acetone. This factor should have been mentioned in the report.

B. Statistical Analysis: Probit and Dunnett's analysis were conducted on oat (the most sensitive species) data for dry weight. The reviewer's results for the oat data are in near agreement with the author's.

C. Discussion/Results:

Percent emergence: At the 14 day observation period, soybean, cabbage, oat, ryegrass, and corn demonstrated no significant differences in emergence at any test rate in comparison to the controls. The NOEC value for these species is therefore 30.0 lb ai/A. The NOEC values for the other five species were less, with lettuce and carrot being equally the most sensitive. By 21 days after application, soybean, oat, and corn were still unaffected by any rate of prometon. Subsequently, the NOEC value for these three species is 30.0 lb ai/A. The other seven species were affected by some rate of prometon with lettuce, cucumber, and cabbage being equally sensitive (NOEC = 0.0235 lb ai/A).

Phytotoxicity rating: By the end of the initial study, all species except corn were affected by all rates of prometon. A continuation study was undertaken to determine NOEC values for the other nine species. Because NOEC values should be determined from 21 day data, the following values (in lb ai/A) reflect, in increasing sensitivity to prometon, the NOECs for the ten species at this time:

corn (0.75) < carrot = ryegrass (0.188) < tomato (0.0938) < soybean = oat = onion (0.0469) < lettuce = cucumber (0.0235) < cabbage (0.0188).

Plant height: Plant height of all species was affected by all rates of prometon in the initial study. A continuation study was undertaken to determine the NOEC value for the ten test species. Lettuce and cabbage were equally the most sensitive species with respect to plant height with an NOEC value of 0.0188 lb ai/A, rather than 0.0118 lb ai/A because the former value is higher than the latter and this better reflects the true NOEC.

Plant dry weight: Two study continuations were required to determine NOEC values for all ten species. Oat was the most sensitive species with respect to plant dry weight. The NOEC value for oat was 0.0047 lb ai/A.

This study is scientifically sound and fulfills the guideline requirements for the Tier 2 seedling emergence test using non-target plants.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

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

Table 17 Statistical no-effect concentration (NOEC) (lb ai/A), EC_{25} and EC_{50} values (lb ai/A) for parameters measured during a nontarget plant study with Prometom Technical.

Crop	Seedling Emergence[w] NOEC[x]	EC_{25}	EC_{50}	Seedling Survival[w] NOEC	EC_{25}	EC_{50}
Soybean	30.0	ND	ND	30.0	ND	ND
Lettuce	7.5	10.243	95.283	0.0235	ND	ND
Carrot	7.5	ND	ND	0.188	0.179	0.766
Tomato	15.0	39.176	ND*	0.0938	0.082	0.153
Cucumber	15.0	19.518	55.851	0.0235	0.037	0.046
Cabbage	30.0	ND	ND	0.0235	0.041	0.131
Oat	30.0	ND	ND	30.0	ND	ND
Ryegrass	30.0	ND	ND	0.375	0.263	0.916
Corn	30.0	ND	ND	30.0	ND	ND
Onion	15.0	22.254	ND*	0.375	0.222	1.664

Crop	Phytotoxicity[wz] NOEC	Mean Rating	Plant Height[w] NOEC	EC_{25}	EC_{50}	Dry Weight[w] NOEC	EC_{25}	EC_{50}
Soybean	0.0938	0.0	0.0938	0.133	0.256	0.0235	0.057	0.111
Lettuce	0.0235	0.1	0.0118	ND	ND	0.0094	0.010	ND*
Carrot	0.375	0.3	0.0938	0.157	0.452	0.0938	0.051	0.164
Tomato	0.0938	0.8	0.0469	ND	ND	0.0235	0.031	0.077
Cucumber	0.0118	0.0	0.0235	0.033	0.047	0.0118	0.017	0.028
Cabbage	0.0469	2.0	0.0118	0.016	0.066	0.0094	0.014	ND*
Oat	0.0938	0.5	0.0938	0.172	0.515	0.0047	0.027	ND*
Ryegrass	0.75	0.3	0.0938	ND	ND	0.0235	0.053	ND*
Corn	0.75	0.0	0.75	ND	ND	0.375	ND	ND
Onion	0.0938	0.4	0.0469	0.217	ND*	0.0118	0.040	0.126

[w] Seedling emergence based on 14-day readings; seedling survival 21-day readings (base study), 28 day (first and second study continuation) phytotoxicity, plant height, and dry weight based on 28-day readings.

[x] Highest treatment concentration which was not statistically different from the control ($p < 0.05$).

[y] ND = Not determined. If a dose response was not evident or the highest treatment concentration tested did not result in a significant effect, EC_{25} or EC_{50} values could not be determined.

[z] EC_{25} and EC_{50} values are not normally determined for mean phytotoxicity rating.

* EC_{50} value calculated, but not reported because detrimental effect at maximum concentration was less than 50%.

oat dry weight

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
rate (lb ai/A)				
1 = control	4	192.0000	25.2587	13.2
2. 0003	4	172.5000	13.3292	7.7
3. 0006	4	190.0000	24.0970	12.7
4. 0012	4	163.7500	22.2467	13.6
5. 0024	4	170.7500	13.0990	7.7
6. 0047	4	182.5000	19.4850	10.7
7*. 0094	4	149.7500	15.4569	10.3
8*. 0188	4	138.7500	10.4363	7.5

NOEC = 0.0047 lb ai/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 = -32.750636
This difference corresponds to -17.06 percent of control

Between groups sum of squares = 9891.000000 with 7 degrees of freedom.

Error mean square = 348.791667 with 24 degrees of freedom.

Bartlett's test p-value for equality of variances = .811

oat dry weight

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0000		
EC 5.00	0.0003		
EC10.00	0.0014		
EC15.00	0.0044		
EC50.00	0.5652		
EC85.00	72.7356		
EC90.00	229.5262		
EC95.00	1259.7929		
EC99.00	30706.7344		

$y = 5.12 + 0.49(x)$

y = % probit inhibition

x = log (rate)

$EC_{25} = 0.024$ lb ai/A.