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: Vegetative Vigor Nontarget Phytotoxicity Study - Tier 2. Species Tested: Ryegrass, Corn, Oat, Onion, Soybean, Lettuce, Carrot, Tomato, Cucumber, Cabbage.

4. **CITATION:** Chetram, R.S. 1990. Tier 2 Vegetative Vigor Nontarget Phytotoxicity Study Using Prometon Technical. Laboratory Project No. LR90-03. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by Ciba-Giegy Corporation, Greensboro, NC. EPA MRID No. 417253-04.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Agronomist
KBN Engineering and
Applied Sciences, Inc.

Signature: Mark A. Mossler
Date: 5/29/91

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 5/29/91

Cynthia A. Monlux 12.3.91

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

Signature:
Date: Henry T. Craven 12/4/91

7. **CONCLUSIONS:**

This study is scientifically sound and meets the requirements for a Tier 2 vegetative vigor test using non-target plants. With respect to phytotoxicity, cucumber was the most sensitive species tested. The NOEC for cucumber is 0.012 lb ai/A.

10.1.5

Lettuce and cabbage were equally the most sensitive to prometon with respect to plant height. The resulting NOEC value for these two species is 0.012 lb ai/A. Cabbage has the lowest EC_{25} and EC_{50}, reported as 0.019 and 0.030 lb ai/A, respectively.

Lettuce, cabbage, and cucumber were equally the most sensitive species with respect to plant dry weight. The NOEC value for these three species is 0.0047 lb ai/A. Dry weight was the most sensitive parameter tested in this study. Cabbage has the lowest EC_{25} and EC_{50}, reported as 0.003 and 0.013 lb ai/A, respectively.

The EC values for each section of the vegetative vigor test are listed in Table 16 (attached).

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 six families (i.e., soybean, lettuce, carrot, 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 soil mix. A plexiglass template was used to create planting holes in the soil, thus 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. An analysis of the soil was provided in the report. After emergence, each pot was thinned to five plants/pot. The plant species were allowed to grow for 6-18 days before treatment to allow each species to attain the 1-3 true leaf stage. 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).

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. Approximately 2 drops (50 ppm) of Triton X-100 was added to each spray solution as a surfactant. 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 24-31 ml of water was used to irrigate each pot per day for the initial and first continuation study. Approximately 27-42 ml of water was used to irrigate the plants for the second continuation study.

C. **Dosage:** Prometon was applied at rates of 30, 15, 7.5, 3.75, 1.88, 0.938, 0.469, and 0.0 lb ai/A to all plant species for the initial study. For the first continuation study, prometon was applied at rates of 0.75, 0.375, 0.188, 0.094, 0.047, 0.023, 0.012, and 0.0 lb ai/A. A second continuation study was required in which rates of 0.0188, 0.0094, 0.0047, 0.0023, 0.0012, 0.0006, 0.0003, and 0.0 lb ai/A were applied to soybean, lettuce, cucumber, and cabbage. 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., 5 plants/pot, 4 pots/treatment level). After treatment, the pots were randomized within crops and among treatments and placed in an on-site greenhouse.

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 calculated at 0 and 14 (21 for the study continuations) days after application.

Plant phytotoxicity was monitored at 7 and 14 (and 21 for the continuation studies) 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; and 4-indicates a total effect or plant death.

Fourteen (21 for the continuation studies) days after treatment, the plants within 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. After drying, the dry weight of the plant material was recorded.

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 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 as a baseline to calculate the percent effect on growth at the 14 or 21 day observation period. The percent increase in height from the 0 day reading was calculated using the following equation:

$$\% \text{ increase} = \frac{(\text{21 (or 14) day mean} - \text{0 day mean})}{\text{0 day mean}} \times 100$$

The percent effect on growth was calculated for each treatment using the following equation:

$$\% \text{ effect} = \frac{(\text{treat. \% increase} - \text{cont. \% increase})}{\text{control \% increase}} \times 100$$

An analysis of variance table was constructed using the Lotus 1-2-3 raw data spreadsheet. A one-way analysis of variance (ANOVA) model for data with equal sub-samples was used to analyze the data. The F-value from the analysis of variance table and an F table were used to determine if the treatment means were significantly different ($p < 0.05$). Treatment mean separation was achieved using Lotus 1-2-3 spreadsheet. Means were separated by using Duncan's New Multiple Range Test.

The percent detrimental effect values were input into a SAS probit analysis program. The program ignores positive values and transforms the dose by natural

logarithms. For plant height and dry weight, the probit is calculated using all data points; for all other parameters, the probit is calculated using replicate means.

12. **REPORTED RESULTS:**

Phytotoxicity rating: All ten crops were sensitive to prometon at all rates in the initial study. A continuation study was required to determine NOEC values for phytotoxicity. By the end of 21 days, all ten plant species demonstrated evidence of phytotoxicity at some rate of prometon. The NOEC value (in lb ai/A) for the test species, in increasing sensitivity, are:

corn (0.375) < ryegrass (0.094) < carrot = tomato = onion (0.047) < soybean = lettuce = cabbage = oat (0.023) < cucumber (0.012).

No EC values were determined from the phytotoxicity data.

Plant height: All ten crops were sensitive to prometon at all rates in the initial study. A continuation study was required to determine NOEC values for plant height. By the end of 21 days, all ten plant species demonstrated reduced height when compared to untreated controls at some rate of prometon tested. The NOEC values (in lb ai/A) for the test species, in increasing sensitivity, are:

corn (0.375) < ryegrass (0.094) < tomato (0.047) < soybean = lettuce = carrot = oat = onion (0.023) < cucumber = cabbage (0.012).

Except for corn, probit analysis was conducted on all species to determine EC values. The EC values are listed in Table 16 (attached).

Plant dry weight: All ten crops were sensitive to prometon at all rates in the initial study. Two continuation studies were conducted to determine NOEC values for dry weight. Although corn dry weight was not statistical different from the control at the 0.375 lb ai/A rate, the weight was inhibited by 26%, therefore, the NOEC for corn was taken to be 0.188 lb ai/A. The NOEC values (in lb ai/A) for the ten test species, in increasing sensitivity, are:

corn (0.188) < carrot (0.023) < tomato = oat = ryegrass = onion (0.012) < soybean (0.0094) < lettuce = cucumber = cabbage (0.0047).

lettuce dry weight

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0019	0.0011	0.0029
EC 5.00	0.0033	0.0021	0.0046
EC10.00	0.0045	0.0030	0.0059
EC15.00	0.0055	0.0039	0.0069
EC50.00	0.0126	0.0106	0.0145
EC85.00	0.0290	0.0250	0.0351
EC90.00	0.0353	0.0298	0.0444
EC95.00	0.0473	0.0385	0.0634
EC99.00	0.0819	0.0615	0.1252

$Y = 10.43 + 2.86(x)$

Y = % probit inhibition

X = log (rate)

$EC_{25} = 0.00716$ ai/A.

lettuce dry weight

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
rate (lb ai/A)				
1 = control	4	163.2500	5.5603	3.4
2 - .0003	4	151.5000	8.3865	5.5
3 - .0006	4	197.0000	33.0555	16.8
4 - .0012	4	177.7500	12.5266	7.0
5 - .0024	4	184.7500	9.3229	5.0
6 - .0047	4	134.2500	12.3119	9.2
7 - .0094	4	129.7500	21.8842	16.9
8* - .0188	4	108.7500	32.6943	30.1

NOEC = 0.0094 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 = -34.726458
This difference corresponds to -21.27 percent of control

Between groups sum of squares = 25792.000000 with 7 degrees of freedom.

Error mean square = 392.145833 with 24 degrees of freedom.

Bartlett's test p-value for equality of variances = .041

Table 16 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 Prometon Technical at the 21 day observation period.

Crop	Phytotoxicity[x]		Plant Height (mm)			Dry Weight (mg)		
	NOEC[y]	Mean Rating	NOEC	EC_{25}	EC_{50}	NOEC	EC_{25}	EC_{50}
Soybean	0.023	0.0	0.023	0.051	0.156	0.0094	0.015	0.037
Lettuce	0.023	0.5	0.023	0.022	0.030	0.0047	0.008	0.014
Carrot	0.047	0.8	0.023	0.040	0.094	0.023	0.034	0.069
Tomato	0.047	0.0	0.047	0.050	0.114	0.012	0.018	0.047
Cucumber	0.012	0.2	0.012	0.023	0.036	0.0047	0.008	0.016
Cabbage	0.023	0.0	0.012	0.019	0.030	0.0047	0.003	0.013
Oat	0.023	0.4	0.023	0.027	0.078	0.012	0.016	0.031
Ryegrass	0.094	0.0	0.094	0.096	0.380	0.012	0.032	0.099
Corn	0.375	0.0	0.375	ND[z]	ND	0.188	ND	ND
Onion	0.047	0.3	0.023	0.043	0.091	0.012	0.021	0.046

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

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

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

* EC_{25} and EC_{50} values for plant height and dry weight were determined by using a treatment range of 0.012 - 0.75 lb ai/A (first study continuation).

Corn was the only species that did not demonstrate a dose response, therefore, EC values could not be determined for this species. The EC values for the remaining nine species are listed in Table 16.

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

The Quality Assurance Unit of Pan-Agricultural Laboratories, Inc., was responsible for the assurance of compliance with Good Laboratory Practice (GLP) Standards. Statements of compliance to GLP and QA were enclosed in the report.

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 to the study report), the report did not restate if the control plants were treated with the appropriate amount of acetone and water.

B. **Statistical Analysis:** Probit and mean comparison (Dunnett's) analyses were conducted on lettuce (one of the most sensitive species) data for plant dry weight (attached). The reviewer obtained a slightly less conservative estimate than the author for the NOEC value. Therefore, the author's value of 0.0047 lb ai/A will be taken to be the NOEC for lettuce dry weight. The reviewer's EC values are in general agreement with the author's.

C. **Discussion/Results:**
This study is scientifically sound and meets the requirements for Tier 2 vegetative vigor test using non-target plants.

Phytotoxicity: All plant species were significantly affected by all rates of prometon in the initial study. A continuation study was undertaken to determine the NOEC values. Cucumber was the most sensitive species with an NOEC value of 0.012 lb ai/A.

Plant Height: All plant species demonstrated significant reductions in height at all rates of prometon in the initial study. A continuation study was undertaken to determine the NOEC values for the ten test species. Cucumber and cabbage were equally the most sensitive species with respect to height with an NOEC value of 0.012 lb ai/A. With respect to EC values, cabbage was the most sensitive species, with EC_{25} and EC_{50} values of 0.019 and 0.030 lb ai/A, respectively.

Plant Dry Weight: Two study continuations were required to determine NOEC values for all ten test species. Lettuce, cabbage, and cucumber were equally the most sensitive species with respect to weight. The NOEC value for these three species was 0.0047 lb ai/A. With respect to EC values, cabbage was the most sensitive species, with EC_{25} and EC_{50} values of 0.003 and 0.013 lb ai/A, respectively.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

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