

DATA EVALUATION RECORD

1. **CHEMICAL:** Profenofos.
Shaughnessey No. 111401.

2. **TEST MATERIAL:** Profenofos technical (Curacron); 0-(4-bromo-2-chlorophenyl)-0-ethyl-s-propyl phosphorothioate; CAS No. 108-90-7; 90.4% purity; an amber oily liquid.

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 Profenofos Technical (Curacron). Laboratory Report No. LR90-410. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by Ciba-Giegy Corporation, Greensboro, NC. EPA MRID No. 416273-07.

5. **REVIEWED BY:**

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10 hrs

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, all ten tested species were equally tolerant of profenofos after 14 days when the maximum rate of 1.0 lb ai/A was applied to the soil. After 21 days, (28 days for lettuce) only cucumber was affected by the maximum rate of profenofos. The subsequent NOEC, EC₂₅, and EC₅₀ values for cucumber are 0.333, 0.272, and 0.867 lb ai/A, respectively. The NOEC value for the other nine species is 1.0 lb ai/A.

Only cucumber was affected by the maximum rate of profenofos with respect to phytotoxicity. The resulting NOEC value is 0.333 lb ai/A. The other nine species were equally tolerant to the maximum rate of 1.0 lb ai/A.

Cucumber and cabbage were equally sensitive to the maximum rate of profenofos with respect to plant height. The resulting NOEC value for these two species is 0.333 lb ai/A. Due to a lack of rate response, EC values could not be computed for cabbage. The EC₂₅ and EC₅₀ values for cucumber are 0.242 and 0.766 lb ai/A, respectively. The remaining eight species were equally tolerant to profenofos with an NOEC value of 1.0 lb ai/A.

Lettuce, cabbage, and cucumber dry weight were all affected by some rate of profenofos below the maximum application rate. Cucumber was the most sensitive species with an NOEC value of 0.111 lb ai/A. Lettuce and cabbage were equally the next most sensitive with an NOEC value of 0.333 lb ai/A. The remaining seven species have an NOEC value of 1.0 lb ai/A. Cucumber was the only species that demonstrated a dose response and the subsequent EC₂₅ and EC₅₀ values are 0.13 and 0.521 lb ai/A, respectively.

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 3.21 x 1.67 ft (i.e., 5.36 ft²).

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 40-45 psi were used. The test spray solutions were prepared by dissolving profenofos technical in deionized water and acetone, then diluting with water from a well located at the testing facility. 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 53 ml of water was used to irrigate each pot per day.

C. **Dosage:** Profenofos was applied at the rates of 0, 0.0123, 0.037, 0.111, 0.333, and 1.0 lb ai/A to the soil in which all species were planted. Treatment application rates were adjusted for the percent purity of the test material (90.4%).

D. **Design:** Each crop/treatment combination was replicated three times (i.e., 10 seeds/pot, 3 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 lettuce) after treatment for all species. Twenty-one days (28 days for lettuce) 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:

Percent emergence and survival: Results are summarized in Table 9 (attached). Through day 14, percent emergence for all species did not differ significantly ($p < 0.05$) from the control. The 1.0 lb ai/A rate is the NOEC value for all species. Due to a lack of dose related responses, no EC values could be estimated.

By the end of the testing period (21 days) only cucumber demonstrated any significant differences in percent emergence. The NOEC values for cucumber and the remaining nine species are 0.333 and 1.0 lb ai/A, respectively. Due to the lack of a rate response by any species except cucumber, EC values could not be determined for nine of the tested species. The EC_{25} and EC_{50} values for cucumber were 0.272 and 0.867 lb ai/A, respectively.

Phytotoxicity rating: Based on 21-day observations, only cucumber demonstrated a significant difference between the control and the highest rate of profenofos tested. The subsequent NOEC values for cucumber and the remaining nine species are 0.333 and 1.0 lb ai/A, respectively.

Plant height: All species tested, except cucumber, demonstrated no significant decreases in plant height when compared to the controls. Cabbage height was reduced by 27% when compared to the control but was not significantly different when compared to the controls due to variability in replications. Therefore, the NOEC for cabbage and cucumber is 0.333 lb ai/A. The NOEC value for the remaining eight species is 1.0 lb ai/A. Only cucumber demonstrated a dose related response curve. The EC_{25} and EC_{50} values for cucumber are 0.242 and 0.766 lb ai/A, respectively.

Plant dry weight: All species tested except cabbage and cucumber demonstrated no significant reductions in weight when compared to the controls. Oat demonstrated a 28% reduction at the 0.0123 lb ai/A rate, however, this was not significant and the next four higher rates did not affect oat to the same magnitude. At the 0.037 lb ai/A rate, lettuce demonstrated a 41% reduction in dry weight but this too was not significant due to the variability in replicates and delayed emergence. This same trend for lettuce occurred at the 1.0 lb ai/A rate of profenofos. Since the amount of dry weight reduction was over 25% (i.e., 33%), the NOEC for lettuce is 0.333 lb ai/A. Cabbage was significantly affected with respect to dry weight at the 1.0 lb ai/A rate while cucumber was affected at the 0.333 lb ai/A rate. The most sensitive species was therefore cucumber with an NOEC

value of 0.111 lb ai/A. Cabbage has an NOEC value of 0.333 lb ai/A. The remaining seven species have an NOEC value of 1.0 lb ai/A.

Cucumber was the only crop that demonstrated a dose related response, therefore, EC values could be computed. The resulting EC₂₅ and EC₅₀ values for cucumber are 0.13 and 0.521 lb ai/A, respectively.

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. EC₂₅ and EC₅₀ values were determined for percent survival, plant height, and dry weight of cucumber."

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 VIII 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 cucumber (most sensitive species) data for dry weight. The reviewer's results for the cucumber data are in near agreement with the author's.

- C. **Discussion/Results:**
Percent emergence and survival: At the 14 day

observation period, all ten species demonstrated no significant differences in emergence in comparison to the controls. The NOEC value is therefore 1.0 lb ai/A. By 21 days after application, only cucumber demonstrated significant differences in percent emergence at the highest rate of profenofos. The NOEC value is therefore 0.333 lb ai/A. The NOEC value for percent survival for the remaining nine species is 1.0 lb ai/A. A lack of dose related response precluded EC values from being determined for any species tested, except cucumber. The EC₂₅ and EC₅₀ values for cucumber are 0.272 and 0.867 lb ai./A, respectively.

Phytotoxicity rating: By the end of the study, the only species that was affected by profenofos was cucumber. The NOEC value was determined to be 0.333 lb ai/A. For the remaining nine species, the NOEC value is 1.0 lb ai/A.

Plant height: Plant height of all species except cucumber and cabbage was not affected by profenofos application. Cabbage and cucumber were equally sensitive with an NOEC value of 0.333 lb ai/A. The remaining species all have an NOEC value of 1.0 lb ai/A. Cucumber was the only species that exhibited a dose response and the subsequent EC₂₅ and EC₅₀ values are 0.242 and 0.766 lb ai/A, respectively.

Plant dry weight: The only species that demonstrated significant dry weight reductions were cucumber and cabbage. Although not significant, lettuce demonstrated greater than 25% reduction in dry weight when compared to controls. Cucumber was the most sensitive species with an NOEC value of 0.111 lb ai/A. The NOEC value for cabbage and lettuce is 0.333 lb ai/A. The other seven species have an NOEC value of 1.0 lb ai/A. Cucumber exhibited a dose response, and the subsequent EC₂₅ and EC₅₀ values are 0.13 and 0.521 lb ai/A, respectively.

The seedling emergence 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 9

Statistical no-effect concentration (NOEC) (lb ai/A), EC₂₅ and EC₅₀ values (lb ai/A) for parameters measured during a nontarget plant study with Profenofos Technical (Curacron) at 21 days after treatment.

Plant Species	Seedling Emergence			Seedling Survival		
	NOEC ^x	EC ₂₅	EC ₅₀	NOEC	EC ₂₅	EC ₅₀
Soybean	1.0	ND ^y	ND	1.0	ND	ND
Lettuce	1.0	ND	ND	1.0	ND	ND
Carrot	1.0	ND	ND	1.0	ND	ND
Tomato	1.0	ND	ND	1.0	ND	ND
Cucumber	1.0	ND	ND	0.333	0.272	0.867
Cabbage	1.0	ND	ND	1.0	ND	ND
Oat	1.0	ND	ND	1.0	ND	ND
Ryegrass	1.0	ND	ND	1.0	ND	ND
Corn	1.0	ND	ND	1.0	ND	ND
Onion	1.0	ND	ND	1.0	ND	ND

Plant Species	Phytotoxicity ^z		Plant Height			Dry Weight		
	NOEC	Mean Rating	NOEC	EC ₂₅	EC ₅₀	NOEC	EC ₂₅	EC ₅₀
Soybean	1.0	0.0	1.0	ND	ND	1.0	ND	ND
Lettuce	1.0	0.1	1.0	ND	ND	0.333	ND	ND
Carrot	1.0	0.0	1.0	ND	ND	1.0	ND	ND
Tomato	1.0	0.0	1.0	ND	ND	1.0	ND	ND
Cucumber	0.333	0.3	0.333	0.242	0.766	0.111	0.13	0.521
Cabbage	1.0	0.7	0.333	ND	ND	0.333	ND	ND
Oat	1.0	0.0	1.0	ND	ND	1.0	ND	ND
Ryegrass	1.0	0.0	1.0	ND	ND	1.0	ND	ND
Corn	1.0	0.0	1.0	ND	ND	1.0	ND	ND
Onion	1.0	0.2	1.0	ND	ND	1.0	ND	ND

^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, EC₂₅ and EC₅₀ values could not be determined.

^z EC₂₅ and EC₅₀ values are not normally determined for mean phytotoxicity rating.

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cucumber dry weight

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0064	0.0001	0.0239
EC 5.00	0.0231	0.0018	0.0596
EC10.00	0.0459	0.0069	0.1006
EC15.00	0.0729	0.0165	0.1483
EC50.00	<u>0.5163</u>	0.2578	1.9896
EC85.00	3.6570	1.1852	90.7107
EC90.00	5.8114	1.6412	232.0135
EC95.00	11.5431	2.6372	940.3757
EC99.00	41.8150	6.3254	13171.8857

$$y = 5.35 + 1.22x$$

$y =$ probit % inhibition

$x =$ log (rate)

$$EC_{25} = 0.15 \text{ lb ai/A.}$$

cucumber dry weight

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	3	2.3927	.0807	3.4
2 0.0123	3	2.1753	.2194	10.1
3 0.037	3	2.0260	.1867	9.2
4 0.111	3	2.1117	.2926	13.9
5* 0.353	3	1.2193	.9355	76.7
6* 1.0	3	.3427	.4741	138.4

NOEC = 0.111 16 ai/A.

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minimum detectable difference for Dunnett's test = -.941014
This difference corresponds to -39.33 percent of control

Between groups sum of squares = 9.162316 with 5 degrees of freedom.

Error mean square = .212522 with 12 degrees of freedom.

Bartlett's test p-value for equality of variances = .058

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