

US EPA ARCHIVE DOCUMENT

Seed emergence

DATA EVALUATION RECORD

1. **CHEMICAL:** AC 243,997 (ARSENAL)  
Shaughnessey No. 128821
2. **TEST MATERIAL:** Technical grade AC 243,997 dissolved in 1:1 (v/v) solution of acetone and water. Maximum label rate is 1,680 g/ha.
3. **STUDY TYPE:** Non-target plants: Seedling Emergence Tier I and II. Species Tested: Cucumber, Pea, Soybean, Sunflower, Sugarbeet, Tomato, Oat, Onion, Corn, and Wheat.
4. **CITATION:** American Cyanamid Company. 1988. The Effect of ARSENAL on Seedling Emergence and Vegetative Vigor of Non-target Terrestrial Plants. Tier II Guideline No. 123-1. Conducted and submitted by American Cyanamid Company, Agricultural Research Division, P.O. Box 400, Princeton, N.J. 08540. Accession No. 408118-01.
5. **REVIEWED BY:**  

Robin Hart, Ph.D. Senior Scientist KBN Engineering and Applied Sciences, Inc.	Signature: <i>Robin Hart</i> Date: <i>December 16, 1988</i> <i>Charles Liu</i> <i>12/5/91</i>
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6. **APPROVED BY:**  

James R. Newman, Ph.D. Project Manager/ Principal Scientist KBN Engineering and Applied Sciences, Inc.	Signature: <i>James R Newman</i> Date: <i>12/7/88</i>
Henry T. Craven, M.S. Supervisor, EEB/HED USEPA	Signature: Date:
7. **CONCLUSIONS:** The study was conducted in a scientifically sound manner in accordance with EPA guidelines for Tier II



non-target terrestrial plants and fulfills the requirements for a seed emergence toxicity study using non-target plants. AC 243,997 had a detrimental effect on all species tested. Seedling emergence was significantly affected in tomato, onion, and pea with EC50's of 35, 1120, and 280 g/ha, respectively. EC25 for emergence of onion and pea were 280 and 70 g/ha and tomato was not dose-responsive. EC25's for height of corn, wheat, sunflower, oat, onion, soybean, and pea were 7.4, 7.6, 11.1, 25.9, 7.8, 14.3, and 47.6 g/ha respectively. EC50 for crop injury of sugarbeet was 560 g/ha; EC25 and EC50 for crop injury of cucumber were 4.4 and 70 g/ha, respectively. Most crops showed detrimental effects for several parameters (See Table 1 of this review). Since the maximum recommended rate of AC 243,997 is 1680 g/ha, and this is greater than the EC25 for all the crops tested, a Tier 3 test is required.

8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:** N/A.
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.
11. **MATERIALS AND METHODS:**
  - A. **Test Plants:** Six dicotyledon plants represented by pea, sugarbeet, sunflower, soybean, tomato, and cucumber. Four monocotyledon plants represented by onion, corn, wheat, and oat. Cultivars and lot numbers are provided in the report.
  - B. **Test System:** Crop species were planted in 4 inch dixie cups filled with washed quartz sand. Ten seeds of each species were planted in each cup. Cups were placed in a growth chamber at 29° C. Quantity and quality of light, watering schedule, and relative humidity in the growth chamber were not described.
  - C. **Dosage:** Technical grade AC 243,997 dissolved in 1:1 (v/v) solution of acetone and water was applied preemergence at rates of 1120, 560, 280, 140, 70, 35, 17.5, 8.75, 4.38, 2.19, and 0 g/ha using a laboratory belt sprayer.
  - D. **Design:** The number of emerged seedlings was recorded at 14 days after treatment. Injury ratings, height and fresh weight of seedlings were recorded at 14 and at 28 days after treatment. Crop injury ratings take into

account visual condition such as plant vigor, color, turgidity and height and ranged from 0 (no injury) to 100 (death). Injury ratings were divided into 10 categories, but there was no explanation of the criteria for each category. There were three replicates of each dose, including a control, for each crop.

E. **Statistics:** Means for each type of data were calculated and analyzed using the LSD test to calculate differences at the 95% confidence level. Heights at 14 days after treatment were analyzed by linear regression to predict the levels that result in 25% (EC25) and 50% (EC50) inhibition of growth in comparison to the control.

12. **REPORTED RESULTS:** The author reported the doses that resulted in significant differences (95% confidence level)  $I_{25}$  (25% inhibition) and  $I_{50}$  (50% inhibition for seedling emergence, injury, height, and fresh weight in comparison to the control for the crops tested. All doses are in g/ha. The author's results are summarized in Table 1.

Corn. **Emergence.** At 14 DAT (days after treatment) only 1120 and 2.19 resulted in significant difference and >25% reduction in emergence. No treatment caused a 50% reduction. **Injury.** At 14 DAT plants showed slight foliar anthocyanin at 2.19-17.5; some chlorosis at 8.75 or higher; and stunting and necrosis at 35. At 28 DAT plant growth was completely inhibited at >140; stunted and some mortality at 35; normal at 4.38-17.5.

**Height.** At 14 DAT all doses resulted in heights significantly lower than the control; the predicted  $I_{25}$  dose was 2.34 and  $I_{50}$  was 49.87. At 28 DAT heights were significantly different at >17.5;  $I_{50}$  >17.5. **Weight.** At 28 DAT weights were significantly different at >17.5.

Wheat. **Emergence.** At 14 DAT only 2.19, 35, and 140 were significantly different. All doses caused 25% reduction except 70, 280, and 560. Only 2.19 caused 50% reduction. Seedling emergence did not appear dosage related. **Injury.** At 14 DAT severe stunting and interveinal chlorosis or cessation of growth occurred at doses >8.75; stunting only occurred at 8.75 or lower. At 28 DAT 4.38 showed no significant difference, but 2.19 was more injurious than the control. Greater than 93% injury occurred at  $\geq 70$  and slight stunting at 4.38. Plants had fewer and shorter leaves and significant stunting at 2.19-17.5 and stopped growing at

35. Height. At 14 DAT height reduced at  $> 8.75$ ;  $I_{25}$  was predicted at 2.41 and  $I_{50}$  at 5.83. At 28 DAT there was NSD at  $\geq 8.75$  and greater than 50% reduction at  $>8.75$ . Weight. At 28 DAT 4.38 showed NSD. Fresh weights were negatively correlated with herbicide dose.

Sugarbeet. Emergence. At 14 DAT emergence was NSD at all doses except 35. Doses of 2.19-560 showed a non-significant 25% inhibition. Injury. At 14 DAT  $> 40\%$  injury resulted from doses of 35 or higher. Doses lower than 35 caused less than 25% injury. At 28 DAT all doses of  $\geq 35$  caused significant injury. Rates higher than 35 caused  $>60\%$  crop injury. Height. At 14 DAT heights were NSD at  $<35$ . At 28 DAT treatments of 70, 140, 560, and 1120 were significantly different. Height was not correlated with dose, so no linear regression could be calculated to predict percent inhibitions. Weight. Doses  $< 17.5$  resulted in NSD in fresh weight.

Sunflower. Emergence. At 14 DAT all doses showed NSD except for 560 which resulted in a 25% inhibition. Injury. At 14 DAT there was NSD at  $\geq 17.5$ . Injury ratings were  $\geq 50\%$  at doses  $>17.5$ . At 28 DAT there was NSD  $\geq 35$ . Injury ratings were 30% or greater at  $>70$ . Height. At 14 DAT only 2.15 showed NSD compared to the control. Regression analysis predicted  $I_{25}$  and  $I_{50}$  at 2.73 and 7.46, respectively. At 28 DAT height shows NSD at 17.5. Weight. There is NSD in fresh weight at  $<280$ .

Tomato. Emergence. At 14 DAT 2.19, 8.75, 140, 560 and 1120 showed NSD from control seedling emergence. A 50% reduction resulted from 4.38, 17.5, 35, and 70 and a 25% reduction resulted from 2.19, 140, and 280. Injury. At 14 DAT only 1120, 560, and 140 caused significant injury. Injured seedlings were stunted and yellow. At 28 DAT only 2.19 and 8.75 were NSD. Injury levels approached 30% at  $>17.5$ , except for 140 and 560 which showed less than 30% injury. Height. At 14 DAT and at 28 DAT there was NSD at any dose. Weight. At 28 DAT there was NSD at 140, 17.50, 8.75, 4.38, and 2.19 from the control. Fresh weight was less at 35, 70, 280, 560, and 1120.

Cucumber. Emergence. At 14 DAT there was NSD at any dose except 560. Injury. At 14 DAT there was NSD in injury at 2.15, 8.75, and 280 compared to the control. All other doses caused significant stunting and yellowing. At 28 DAT all doses showed significant injury. Less than 30% inhibition was shown by 2.15, 4.38, 8.75, 17.5, and 280.

Height. At 14 DAT NSD resulted from doses of 2.19, 4.38, 8.75, 35, 140, and 280. At 28 DAT only 2.19 and 8.75 showed NSD. Dosage response was not sufficiently correlated to calculate a linear regression at either 14 or 28 DAT.  
Weight. At 28 DAT treatment resulted in significant reduction in plant fresh weight with only a few exceptions.

Oat. Emergence. There was NSD in seedling emergence at DAT. Injury. At 14 DAT visual observations of stunting, interveinal chlorosis, and inhibition of the growing point were pronounced at  $\geq 35$ . At 28 DAT doses  $\geq 17.5$  showed NSD. Doses  $> 280$  caused almost total growth inhibition and 90% crop injury. Height. At 14 DAT there was NSD control at  $< 35$ . Regression analysis indicated  $I_{25}$  at 4.11 and  $I_{50}$  at 16.87. At 28 DAT only 4.38 and 2.19 showed NSD from control. Other treatments showed a negative correlation between dose and height. Weight. Doses of 17.5 and 2.19 showed NSD in fresh weight from the control. All other doses showed significant inhibition.

Onion. Emergence. At 14 DAT there was NSD in seedling emergence among the doses. Differences  $\geq 25\%$  were observed at doses exceeding 280. Injury. At 14 DAT there was NSD at  $\leq 140$ . Stunting and plant chlorosis were observed at higher doses. At 28 DAT doses of 2.19 and 35 were significantly different. Treatments  $\geq 70$  caused  $> 50\%$  injury. Height. At 14 DAT doses  $< 140$  were NSD from the control. Height was significantly reduced at higher doses. At 28 DAT only 2.19 and doses  $\geq 70$  caused significant differences. Doses exceeding 70 caused  $> 57\%$  height reduction.  $I_{25}$  was predicted to be 7.80 and  $I_{50}$  was predicted to be 81.01. Weight. Doses  $< 140$  resulted in NSD in fresh weight.

Soybean. Emergence. There was NSD at any dose. Injury. At 14 DAT  $I_{25}$  occurred at doses  $> 17.5$  and  $I_{50}$  occurred at doses  $> 35$ . Plants were very stunted, chlorotic with necrotic vascular tissue at  $\geq 280$ . At 28 DAT  $I_{25}$  occurred at 17.5 and  $I_{50}$  at 35. Height. At 14 DAT  $I_{25}$  was observed when doses exceeded 35 and  $I_{50}$  at approximately 280. Regression analysis predicted  $I_{25}$  at 14.33 and  $I_{50}$  at 205.42. At 28 DAT  $I_{25}$  was observed at 17.5 and  $I_{50}$  at approximately 70. Weight.  $I_{25}$  occurred at  $> 35$  and  $I_{50}$  at approximately 70.

Pea. Emergence.  $I_{25}$  and  $I_{50}$  occurred at  $> 70$  and  $> 280$ , respectively. Injury. At 14 DAT  $I_{25}$  and  $I_{50}$  occurred at  $> 70$ . All doses injured plants. At 21 DAT  $I_{25}$  occurred at approximately 17.5 and  $I_{50}$  at 70. Height. At 14 DAT  $I_{25}$

and  $I_{50}$  occurred at doses  $>280$ . Regression analysis predicted  $I_{25}$  and  $I_{50}$  at 213.75 and 430.5, respectively. At 28 DAT  $I_{25}$  occurred at  $>140$  and  $I_{50}$  at  $>280$ . Weight.  $I_{25}$  and  $I_{50}$  occurred at  $>70$ . However, 17.5 showed a 30% reduction.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author concluded that AC 243,997 was detrimental to all species tested, but was more injurious to oats and wheat than any of the other crops tested. Of all parameters seedling emergence was least affected. Growth inhibition usually increased with increasing dose. Quality assurance measures were not described.
14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**
  - A. **Test Procedure:** The test procedure was generally in accordance with EPA Subdivision J guidelines. Growth chamber conditions were not described, except for temperature. The plants were grown in quartz sand with no added nutrients. Poor growth of sugarbeet, tomato, and cucumber across all treatments, including the control, may have been due to lack of nutrients. Fresh weight was recorded rather than dry weight; since fresh weight is a function of plant moisture status as well as biomass, the use of fresh weight to measure effects is not as reliable as dry weight.
  - B: **Statistical Analysis:** LSD tests were used to compare means for all parameters even when analysis of variance was not significant. EC25 and EC50 values were estimated by inspection of response to treatment levels for all parameters except seedling height. EC25 and EC50 for seedling height was predicted by linear regression of height vs. log dose. Values of 0 for height and weight were included in the LSD tests and in the linear regressions. Apparently these represented seedlings that had died. Seedling mortality in response to dose level was measured by percent seedling emergence. It is not appropriate to include dead seedlings in height and weight regressions by assigning them values of 0. Percent injury scores were not transformed to arcsine square roots to normalize their distribution; however, percent injury was so high in comparison to the control that the analysis of untransformed values yielded similar results (See

attached analyses of untransformed vs. transformed data for sugarbeet injury).

Corrections of statistical analyses are shown in Table 1. I ran statistical tests for seedling emergence and linear regressions for seedling height. Seedling injury results were analyzed if height and emergence showed no EC25. No tests were done of fresh weight because of the inherent unreliability of those data and because effects were already indicated on other parameters. Computer printouts of the tests are attached.

C. Discussion/Results: Although environmental growth conditions and criteria used to scale crop injury were not completely described, the results can be extrapolated to the natural environment. The report did not include a Tier 3 decision. However, since all species tested showed a greater than EC<sub>25</sub> effect when ARSENAL was applied at less than the maximum label rate (1,680 g/ha), Tier 3 tests are called for.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: This study follows the approved protocol for a test of toxicity of a pesticide on seedling emergence.

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER: N/A



**Table 1.** EC25 and EC50 Doses of Arsenal for Seedling Emergence Test as determined by author of report. Reviewer's corrections are footnoted. NSD = no significant difference. No growth means that all plants, including control were less than 3-4 cm and weighed less than 1 g.

	EMERGENCE PERCENT		INJURY		HEIGHT		FRESH WEIGHT	
	<u>EC25</u>	<u>EC50</u>	<u>EC25</u>	<u>EC50</u>	<u>EC25</u>	<u>EC50</u>	<u>EC25</u>	<u>EC50</u>
Corn	<sup>1</sup> 1120	none	none	35	<sup>2</sup> 2.3	49.9	none	35
Wheat	<sup>3</sup> none	2.2	2.2	8.8	<sup>4</sup> 2.4	5.8	none	8.8
Sugar-beet	<sup>5</sup> 2.2	none	8.8	<sup>6</sup> 35	no growth			
Sun-flower	<sup>7</sup> 560	none	none	140	<sup>8</sup> 2.7	7.5	140	560
Tomato	none	35	<sup>9</sup> 140	1120	no growth			
Cucumber	none	none	4.4	70	no growth			
Oats	<sup>10</sup> 4.4	none	none	52	<sup>11</sup> 4.1	16.9	4.4	70
Onion	280	1120	35	70	7.8	81	0	280
Soybean	NSD		17.5	35	14.3	205	35	70
Pea	<sup>12</sup> 70	280	17.5	70	<sup>13</sup> 213.8	430.5	none	140

1. NSD
2. 7.4, 55
3. NSD
4. 7.6, 58
5. NSD
6. EC50 = 560
7. NSD
8. EC25 = 11.1  
EC50 = 124
9. NSD
10. NSD
11. EC25 = 25.9  
EC50 = 669

12. EC25 = 140  
EC50 = 560
13. EC25 = 47.6  
EC50 = 2265