

US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD
SEEDLING EMERGENCE EC₂₅ TEST
VEGETATIVE VIGOR EC₂₅ TEST
§122-1(a & b) (TIER I)

DRAFT COPY

1. **CHEMICAL:** Hallcomid M-8-10 PC Code No.: 800435

2. **TEST MATERIAL:** Hallcomid M-8-10 Purity: Not reported

3. **CITATION:**

Author: Feutz, E.

Title: Evaluating the Effects of Hallcomid M-8-10 on the Germination, Emergence, and Vegetative Vigor of Non-Target Terrestrial Plants

Study Completion Date: December 13, 1991

Laboratory: ABC Laboratories, Inc.
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Laboratory Report ID: 38712R

MRID No.: 45369707

DP Barcode: D284964

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature:

Date: 6/9/03

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Date: 6/9/03

5. **APPROVED BY:** Jim Goodyear, OPP/EFED/ERB III

Signature:

Date:

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6. STUDY PARAMETERS:

Scientific Name of Test Organism: Dicots: *Lactuca sativa*, *Raphanus sativus*

Definitive Study Duration: Germination: 7 days
Seedling emergence: Approximately 14 days
Vegetative vigor: Approximately 30 days

Type of Concentrations: Nominal

7. CONCLUSIONS:

Germination, percent emergence, shoot and root length, and shoot weight were studied for the test species, lettuce and radish. The test substance, Hallcomid M-8-10, was applied at the nominal test concentration of 100 ppm. The actual application rate for the seedling emergence and vegetative vigor tests was 113.9 ppm, based on soil percent moisture.

Germination was significantly reduced for both radish and lettuce when exposed to treatment with Hallcomid M-8-10. Percent germination in the 100 ppm treatment group was 0% for lettuce, the more sensitive species. Percent germination was 26.4% for radish in the 100 ppm treatment group.

Percent emergence was significantly reduced for lettuce when exposed to treatment with Hallcomid M-8-10 (19% from the solvent control). Radish seedling emergence was not significantly affected by treatment with Hallcomid M-8-10.

During the **vegetative vigor** portion of the test, no species or endpoint was significantly reduced by treatment with Hallcomid M-8-10. For lettuce, shoot and root length were stimulated in the 100 ppm treatment group and shoot weight was reduced 19% from the solvent control group. For radish, shoot and root length were reduced 7% and 8%, and shoot weight was reduced 11% when compared to the solvent control group.

This study is classified as INVALID. It is scientifically sound but it does not fulfill the US EPA guideline requirements for seedling emergence and vegetative vigor studies (Subdivision J, §122-1, TIER I). There were significant deviations from the US EPA guideline 122-1, including testing with only two dicot plant species and the purity of the test chemical was not provided.

Results Synopsis—INVALID (results not reported)

Germination

Most sensitive dicot/monocot:

Most sensitive parameter:

EC₀₅: 95% C.I.:
EC₂₅: 95% C.I.:
NOEC:

Seedling Emergence.

Most sensitive dicot/monocot:

Most sensitive parameter:

EC₀₅: 95% C.I.:
EC₂₅: 95% C.I.:
NOEC:

Vegetative Vigor

Most sensitive dicot/monocot:

Most sensitive parameter:

EC₀₅: 95% C.I.:
EC₂₅: 95% C.I.:
NOEC:

8. ADEQUACY OF THE STUDY:

A. Classification: INVALID

B. Rationale: There were significant deviations from the US EPA guideline 122-1, including the testing of only two (dicot) plant species and the purity of the test chemical was not provided.

C. Repairability: The purity of the test material is mandatory for a risk assessment. This information should be reported for the potential upgrade of this study's classification to Supplemental.

9. GUIDELINE DEVIATIONS:

1. Only two dicot test species were used in the study. No monocot species were tested.
2. The purity of the test chemical was not provided.

10. SUBMISSION PURPOSE: This study was submitted to provide data on the effects of

Hallcomid M-8-10 to the seedling emergence and vegetative vigor of non-target terrestrial plants for the purpose of chemical registration.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
<p>Species: 6 dicots in 4 families, including soybean and a rootcrop; 4 monocots in 2 families, including corn.</p>	<p><u>Dicots:</u> lettuce and radish</p>
<p>Number of plants per repetition:</p>	<p><u>Germination:</u> 25 seeds per replicate, five replicates per control and treatment group <u>Seedling emergence:</u> 16 seeds per replicate, 4 replicates per control and treatment group <u>Vegetative vigor:</u> 5 plants per replicate, 4 replicates per control and treatment group</p>
<p>Source of seed and historical % germination of seed:</p>	<p>See pp. 25-26 for seed source information; historical % germination was 85-90%.</p>

B. Test System

Guideline Criteria	Reported Information
<p>Solvent:</p>	<p>Acetone, volatilized with nitrogen gas.</p>
<p>Site of test:</p>	<p><u>Germination:</u> Incubator <u>Seedling emergence:</u> Environmental chamber <u>Vegetative vigor:</u> Greenhouse #1 Tests were performed at the Analytical Bio-Chemistry (ABC) Labs, Columbia, Missouri</p>
<p>Planting method/type of pot:</p>	<p><u>Germination:</u> 9 cm diameter petri dishes with filter paper <u>Seedling emergence:</u> Nupots (4.5 x 4.5 x 5 inches deep). Planting depths were 3/8 inches using a planting template.</p>

Guideline Criteria	Reported Information
	<u>Vegetative vigor</u> : Same planted seeds as used in the seedling emergence test.
Method of application:	<u>Germination</u> : The test solutions (5 mL) were applied to the petri dish with a pipette. <u>Seedling emergence</u> : Test solutions were mixed into sand carrier (pp. 22-23), then mixed into the sandy loam test soil with pH 7.6 and 0.7% organic matter (pp. 20-21). <u>Vegetative vigor</u> : The same treated plants from the seedling emergence test were used.
Method of watering:	Misters and drip emitters were used to irrigate the plants (pp. 121-123). A 20-20-20 fertilizer was added twice during irrigation.
Growth stage at application:	<u>Germination</u> : seed <u>Seedling emergence</u> : seed <u>Vegetative vigor</u> : seedling (number of leaves not reported)

C. Test Design

Guideline Criteria	Reported Information
Dose range: 2x or 3x	Only one dose tested (Tier I)
Doses: At least 5	<u>Germination</u> : 100 ppm <u>Seedling emergence</u> : 100 ppm <u>Vegetative vigor</u> : 100 ppm
Controls: Negative and solvent	Negative control (deionized water) and Solvent (acetone)
Replicates per dose: At least 3	<u>Germination</u> : 5 replicates <u>Seedling emergence</u> : 4 replicates <u>Vegetative vigor</u> : 4 replicates
Test duration: 14 days	<u>Germination</u> : July 10-16, 1990 <u>Seedling emergence</u> : October 26, 1990 to November 9, 1990

Guideline Criteria	Reported Information
	<u>Vegetative vigor</u> : November 9, 1990 to December 5, 1990
Were observations made at least weekly?	<u>Germination</u> : Test initiation, day 5, and test termination <u>Seedling emergence</u> : 2 to 3 times per week <u>Vegetative vigor</u> : 2 to 3 times per week
Maximum dosage rate:	Not reported

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Was a NOEC observed for each species?	No, a NOEC could not be determined for lettuce and radish germination and lettuce % emergence.
Phytotoxic observations:	See pp. 91-96 for descriptions of vegetative vigor phytotoxicity.
Were initial chemical concentrations measured? (Optional)	No
Were adequate raw data included?	Replicate data were provided.

Results for the most sensitive parameter of each speciesMorphological ObservationsGermination

Lettuce: Negative control percent germination ranged from 84 to 100% and the solvent control percent germination ranged from 88 to 100%. The percent germination for the 100 ppm treatment group was 0% in all replicates.

Radish: Negative control percent germination ranged from 96 to 100% and the solvent control percent germination ranged from 96 to 100%. The percent germination for the 100 ppm treatment group ranged from 20 to 36%.

Percent emergence

Lettuce: Negative control percent emergence ranged from 81 to 100% and the solvent control percent emergence ranged from 88 to 100%. The percent emergence for the 100 ppm treatment group ranged from 63 to 88%.

Radish: Negative control percent emergence was 100% for all replicates and the solvent control percent emergence ranged from 94 to 100%. The percent emergence for the 100 ppm treatment group ranged from 81 to 100%.

Vegetative vigor

Lettuce: The negative control, solvent control, and 100 ppm average shoot lengths were 180, 179, and 181 mm, respectively. The negative control, solvent control, and 100 ppm average root lengths were 166, 161, and 173 mm, respectively. The negative control, solvent control, and 100 ppm average shoot weights were 0.419, 0.545, and 0.442 g, respectively.

There were no toxicological effects observed in the controls or treatment groups

Radish: The negative control, solvent control, and 100 ppm average shoot lengths were 122, 121, and 113 mm, respectively. The 100 ppm treatment group had an 8% reduction in shoot length. The negative control, solvent control, and 100 ppm average root lengths were 212, 223, and 206 mm, respectively. The 100 ppm treatment group had a 3% reduction in root length. The negative control, solvent control, and 100 ppm average shoot weights were 0.351, 0.377, and 0.337 g, respectively. The 100 ppm treatment group had a 4% reduction in shoot weight.

During the toxicological effect observations, the negative control had constricted plants and wilted leaves. One constricted plant was observed in the 100 ppm treatment group.

Statistical Results

Statistical Method: Analysis of variance, Bartlett' test for homogeneity, and Duncan multiple range test were performed on emergence data (pp. 71-73). The vegetative vigor study data (shoot length, root length, and shoot weight) were analyzed using analysis of variance (pp. 111-114). Because the EC₂₅ value was not reported, sensitivity (below) was determined based on NOEC values.

Germination

More sensitive dicot: Lettuce and radish

EC_{05 & 25}: Not reported

NOEC: <100 ppm

Percent Emergence

More sensitive dicot: Lettuce

EC_{05 & 25}: Not reported

NOEC: <100 ppm (lettuce); 100 ppm (radish)

Shoot Length

More sensitive dicot: None

EC_{05 & 25}: Not reported

NOEC: 100 ppm (lettuce and radish)

Root Length

More sensitive dicot: None

EC_{05 & 25}: Not reported

NOEC: 100 ppm (lettuce and radish)

Shoot Weight

More sensitive dicot: None

EC_{05 & 25}: Not reported

NOEC: 100 ppm (lettuce and radish)

13. REVIEWER'S VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Percent germination, percent emergence, shoot and root length, and shoot weight data were statistically analyzed when there

was a reduction in the group treated with Hallcomid M-8-10 (when compared to the solvent control group). Comparisons between the solvent control group and the Hallcomid M-8-10-treated group were made using a Student's t-test. The most sensitive parameter was based on the EC₂₅ value. The reviewer's results are not reported in the Conclusions section because this study is classified as INVALID.

Results synopsis

Germination and Seedling Emergence

Crop	% germination			% emergence			Most sensitive parameter
	NOEC*	EC ₀₅ *	EC ₂₅ *	NOEC*	EC ₀₅ *	EC ₂₅ *	
Lettuce	<100	<100	<100	<100	<100	>100	Germination
Radish	<100	<100	<100	≥100	100	>100	Germination

*Units = ppm.

Vegetative Vigor

Crop	Shoot length			Root length			Shoot weight			Most sensitive parameter
	NOEC*	EC ₀₅ *	EC ₂₅ *	NOEC*	EC ₀₅ *	EC ₂₅ *	NOEC*	EC ₀₅ *	EC ₂₅ *	
Lettuce	100	>100	>100	100	>100	>100	100	<100	>100	None
Radish	100	<100	>100	100	<100	>100	100	<100	>100	None

*Units = ppm.

Germination

More sensitive dicot: Lettuce and Radish

EC₀₅: <100 ppm (lettuce and radish)

EC₂₅: <100 ppm (lettuce and radish)

NOEC: <100 ppm (lettuce and radish)

Percent Emergence

More sensitive dicot: None

EC₀₅: <100 ppm (lettuce); ≥100 ppm (radish)

EC₂₅: >100 ppm (lettuce and radish)
NOEC: <100 ppm (lettuce); 100 ppm (radish)

Shoot Length

More sensitive dicot: None
EC₀₅: >100 ppm (lettuce); <100 ppm (radish)
EC₂₅: >100 ppm (lettuce and radish)
NOEC: 100 ppm (lettuce and radish)

Root Length

More sensitive dicot: None
EC₀₅: >100 ppm (lettuce); <100 ppm (radish)
EC₂₅: >100 ppm (lettuce and radish)
NOEC: 100 ppm (lettuce and radish)

Shoot Weight

More sensitive dicot: None
EC₀₅: <100 ppm (lettuce and radish)
EC₂₅: >100 ppm (lettuce and radish)
NOEC: 100 ppm (lettuce and radish)

14. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's; however, the reviewer also estimated EC₀₅ and EC₂₅ values. Because this is a Tier I study, a range could only be estimated for these values. A NOEC and EC₀₅ value could not be determined for lettuce and radish germination and lettuce percent emergence because significant reductions (greater than 5%) occurred in the treated group. For percent emergence, shoot and root length, and shoot weight, a 25% reduction was not exhibited. As a result, a Tier II test is not required for these species. There were several deviations from US EPA guidelines (Subdivision J, §122-1), including the testing of only two (dicot) species and the failure to report the purity of the test material. The purity of the test material is mandatory for a risk assessment (US EPA Pesticide Reregistration Rejection Rate Analysis: Ecological Effects, p. 64), so it should be provided by the registrant for the potential upgrade of this study to Supplemental.

The environmental chamber (October 26, 1990 to November 5, 1990) had an average temperature range of 21.4-25.7° and mean relative humidity range of 34.1-50.5%. The climatological data for the greenhouse dates November 14, 1990 to November 27, 1990 were lost. Light intensity data presented on pages 126-132. The temperature chart for

plant drying incubator presented on page 134.

The test soil had a moisture content of 15.8-16.4%. Bases on the percent moisture the actual application rate was calculated as 113.9 ppm.

This study was conducted in accordance with USEPA Good Laboratory Practice Standards and includes a Quality Assurance statement.

APPENDIX I. OUTPUT FROM REVIEWER'S STATISTICAL VERIFICATION:**SEEDLING EMERGENCE****Radish**

t-Test: Two-Sample Assuming Equal Variances

	<i>Control</i>	<i>100 ppm</i>
Mean	95.5	90.75
Variance	9	66.25
Observations	4	4
Pooled Variance	37.625	
Hypothesized Mean Difference	0	
df	6	
t Stat	1.095142	
P(T<=t) one-tail	0.157728	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.315456	
t Critical two-tail	2.446914	

Lettuce

t-Test: Two-Sample Assuming Equal Variances

	<i>Control</i>	<i>100 ppm</i>
Mean	95.5	77
Variance	33	167.3333
Observations	4	4
Pooled Variance	100.1667	
Hypothesized Mean Difference	0	
df	6	
t Stat	2.614118	
P(T<=t) one-tail	0.01995	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.0399	
t Critical two-tail	2.446914	

SHOOT LENGTH**Radish**

t-Test: Two-Sample Assuming Equal Variances

	<i>Control</i>	<i>100 ppm</i>
Mean	120.75	112.5
Variance	128.25	53.66667
Observations	4	4
Pooled Variance	90.95833	
Hypothesized Mean Difference	0	
Difference		

df	6
t Stat	1.223341
P(T<=t) one-tail	0.133531
t Critical one-tail	1.943181
P(T<=t) two-tail	0.267062
t Critical two-tail	2.446914

ROOT LENGTH

Radish

t-Test: Two-Sample Assuming Equal Variances

	<i>Control</i>	<i>100 ppm</i>
Mean	223	206
Variance	34.66667	264.6667
Observations	4	4
Pooled Variance	149.6667	
Hypothesized Mean Difference	0	
df	6	
t Stat	1.965176	
P(T<=t) one-tail	0.048498	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.096997	
t Critical two-tail	2.446914	

SHOOT WEIGHT

Radish

t-Test: Two-Sample Assuming Equal Variances

	<i>Control</i>	<i>100 ppm</i>
Mean	0.3775	0.3365
Variance	0.001226	0.000827
Observations	4	4
Pooled Variance	0.001026	
Hypothesized Mean Difference	0	
df	6	
t Stat	1.8099	
P(T<=t) one-tail	0.060146	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.120292	
t Critical two-tail	2.446914	

SHOOT WEIGHT

Lettuce

t-Test: Two-Sample Assuming Equal Variances

	<i>Control</i>	<i>100 ppm</i>
Mean	0.54525	0.44225
Variance	0.004873	0.003279
Observations	4	4
Pooled Variance	0.004076	
Hypothesized Mean Difference	0	
df	6	
t Stat	2.2816	
P(T<=t) one-tail	0.031331	
t Critical one-tail	1.943181	
P(T<=t) two-tail	0.062662	
t Critical two-tail	2.446914	