3-21-91 2 File Copy : 121011

MRID No. 416851-02

DATA EVALUATION RECORD

1. **CHEMICAL:** Clethodim.
Shaughnessey No. 059639.

2. **TEST MATERIAL:** Select (Clethodim); CAS No. 99129-21-2; 82.4% purity; a brown-amber liquid.

122-1a)

3. **STUDY TYPE:** Non-Target Plants: Seed Germination/Seedling Emergence Phytotoxicity Test - Tier 1. Species Tested: Soybean, Lettuce, Carrot, Tomato, Cucumber, Cabbage.

4. **CITATION:** Maggio, R.M. 1990. Tier 1 Seed Germination/ Seedling Emergence Nontarget Phytotoxicity Study Using SELECT (Clethodim). Laboratory Project No. LR 89-54B. Conducted by Pan-Agricultural Laboratories, Inc., Fresno, CA. Submitted by Valent, USA, Corporation, Walnut Creek, CA. EPA MRID No. 416851-02.

5. **REVIEWED By:**

Richard C. Petrie
Agronomist
EEB/EFED

Signature: Richard C. Petrie
Date: 3/21/91

6. **APPROVED BY:**

Charles Lewis, Acting Head,
Section 3,
EEB/EFED

Signature: Charles Lewis
Date: 3/22/91

7. **CONCLUSIONS:**

Seed Germination: This study meets the requirements for a Tier 1 seed germination test using non-target plants. Treatment of the seeds with clethodim up to the maximum

Soybean $EC_{25} > 0.25$ lb/A
Lettuce " "
Carrot " " NOEL = 0.25 lb/A
Tomato " "
Cucumber " "

Cabbage $EC_{25} > 0.25$ lb/A
NOEL < 0.25 lb/A

application rate (0.25 lb ai/A) did not have any significant effect on any plant species tested. A significant increase was observed in tomato and lettuce radicle length.

Seedling Emergence: Cabbage was the most sensitive species to clethodim for seedling emergence. For all other parameters, all species tested demonstrated no significant effects due to clethodim application.

8. **RECOMMENDATIONS:** No further Tier I non-target phytotoxicity studies for seed germination/seedling emergence are required.

9. **BACKGROUND:** Because clethodim is active in grasses only, the registrant was given the opportunity to demonstrate this by performing Tier I tests on dicot species only; as long as Tier II tests were conducted on all required monocot species.

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). Cultivars, lot number, and germination ratings were provided in the report.

B. **Test System:**
Seed Germination: Two circles of blue blotter were placed in the bottom of a glass petri plate (100 x 15 mm). The test solutions were prepared with deionized water and acetone. Fifteen milliliters of the test solution were added to each plate of soybean and cucumber. Ten milliliters were added to plates of lettuce, carrot, tomato, and cabbage.

Ten seeds of each crop were added to each petri plate after the test solution was absorbed into the paper. The plates were then randomly placed in plastic boxes (12.25 x 9.0 x 4.1 inches) with tightly fitting lids to prevent moisture loss. The petri plates were incubated in the dark at 25 ±1°C for 7 days, except lettuce, which was incubated at 18 ±1°C.

Seedling Emergence: 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. An analysis 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 ft^2).

Soybean and cucumber were planted at a depth of 2.5 cm, while the remaining four 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 clethodim technical in deionized water and acetone. 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.

C. **Dosage:** Clethodim was applied at a rate of 0.25 lb ai/A to all plant species. Treatment application rates were adjusted for the percent purity of the test material (82.4%).

D. **Design:**

Seed Germination: Each treatment/crop combination was replicated five times (i.e., 10 seeds/plate, 5 plates/treatment). After 7 days of incubation, the seeds were removed from the petri plates and the radicle lengths were measured to the nearest millimeter. Percent seed germination and mean radicle length were calculated for all germinated seeds. Seeds were considered germinated if the radicle was at least 5 mm long.

Seedling Emergence: Each crop/treatment combination was replicated five times (i.e., 10 seeds/pot, 5 pots/treatment level). The percentage of the ten seeds planted in each pot which emerged was calculated for each treatment. After treatment, the 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. Seedling height and phytotoxicity ratings were recorded

at 7, 14, and 21 days after treatment for all species except carrot, which was evaluated after 28 days. Twenty-one days after treatment (28 for carrot), 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.

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 table was constructed using the Lotus 1-2-3 raw data spreadsheet. A one-way analysis of variance (ANOVA) model for data with equal subsamples was used to analyze data from the seed germination (radicle length and percent germination) and seedling emergence (percent emergence, phytotoxicity, plant height and plant dry weight) tests. The F-value from the analysis of variance table and a F table were used to determine if the treatment means were significantly different ($p < 0.05$).

12. **REPORTED RESULTS:**

Seed Germination: Treatment of seeds with clethodim at the rate of 0.25 lb ai/A did not result in a significant decrease in percent germination ($p < 0.05$) between the control and any of the six treated plant species. The percent effect on seed germination ranged between 0% on lettuce, tomato, and cucumber to -6% on soybean and cabbage.

Treatment of seeds with clethodim resulted in a significant increase ($p< 0.05$) in radicle lengths of lettuce and tomato. The percent effect on radicle length ranged between -10% on cucumber to 20% on tomato.

Seedling Emergence:
Percent germination: Statistical analysis of the 21 day (28 day for carrot) percent emergence data shows a significant difference ($p< 0.05$) between the control and cabbage seedlings emerging from the soil surface treated with clethodim at the maximum rate of 0.25 lb ai/A. The percent effect on percent emergence ranged from -13% on cabbage to 0% on cucumber.

Phytotoxicity rating: Statistical analysis of the 21 day (28 day for carrot) phytotoxicity ratings show no significant difference ($p< 0.05$) on mean phytotoxicity ratings between the control plants and treatments with clethodim at the rate of 0.25 lb ai/A for all crops listed.

Plant height: Treatment of the soil surface with clethodim did not result in a significant effect on plant height between the control and treatment with clethodim at the 21 day (28 day for carrot) observation period for all crops tested. Percent effect on plant height ranged from -5% on soybean to 9% on cabbage.

Plant dry weight: Statistical analysis of the plant dry weight data show no significant diffference ($p< 0.05$) between the control plants and treatment with clethodim. Dry weights ranged from 0% difference for soybeans to a 30% increase for carrot. After treatment with clethodim, carrot produced some vigorous plants.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
Clethodim applied at the maximum label rate of 0.25 lb ai/A did not result in a greater than 25% detrimental effect on any of the parameters measured in all systems.

The Quality Assurance Unit of Pan-Agricultural Labs, Inc., was responsible for the assurance of compliance with Good Laboratory Practice (GLP) Standards.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures followed the SEP and Subdivision J guidelines.

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.

B. Statistical Analysis: Statistical analyses were conducted on cabbage (the most sensitive species) data for percent germination and seedling emergence (attached). The results are in agreement with the author's.

C. Discussion/Results:

Seed Germination: This study is scientifically sound and meets the requirements for Tier 1 testing. Treatment of the seeds with clethodim at the maximum application rate of 0.25 lb ai/A did not have any significant effects on any plant species tested. Radicle length was also not negatively impacted by clethodim application.

Seedling Emergence:
Phytotoxicity rating: Based on phytotoxicity ratings, all crops demonstrated no effect from clethodim application.
Percentage of emerged seedlings: Only cabbage was significantly ($p < 0.05$) affected by clethodim application after 21 days. The remainder of the species were not affected by the herbicide.

Plant height: Plant height was not significantly affected in all species.

Plant dry weight: Plant dry weight was not significantly affected in all species.

These studies are scientifically sound.

D. **Adequacy of the Study:**

(1) **Classification:** CORE

(2) **Rationale:**

(3) **Repairability:**

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

MRID No. 416851-02

KBN Duplicate

DATA EVALUATION RECORD

1. **CHEMICAL:** Clethodim.
Shaughnessey No. 059639.

2. **TEST MATERIAL:** Select (Clethodim); CAS No. 99129-21-2; 82.4% purity; a brown-amber liquid.

3. **STUDY TYPE:** Non-Target Plants: Seed Germination/Seedling Emergence Phytotoxicity Test - Tier 1. Species Tested: Soybean, Lettuce, Carrot, Tomato, Cucumber, Cabbage.

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5. **REVIEWED By:**

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

Signature: Mark A. Mossler
Date: 2/26/91

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 2/26/91

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

Signature:
Date:

20 hrs

8

7. **CONCLUSIONS:**

Seed Germination: This study does not meet the requirements for a Tier 1 seed germination test using non-target plants due to the absence of the four monocot species requried. Treatment of the seeds with clethodim up to the maximum application rate (0.25 lb ai/A) did not have any significant effect on any plant species tested. A significant increase was observed in tomato and lettuce radicle length.

Seedling Emergence: This study does not meet the requirements for a Tier 1 seedling emergence test using non-target plants due to the absence of the four monocot species required. Cabbage was the most sensitive species to clethodim for seedling emergence. For all other parameters, all species tested demonstrated no significant effects due to clethodim application.

8. **RECOMMENDATIONS:** Tests need to be performed that include the monocot species outlined in the SEP for Tier 1 testing.

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). Cultivars, lot number, and germination ratings were provided in the report.

B. **Test System:**
Seed Germination: Two circles of blue blotter were placed in the bottom of a glass petri plate (100 x 15 mm). The test solutions were prepared with deionized water and acetone. Fifteen milliliters of the test solution were added to each plate of soybean and cucumber. Ten milliliters were added to plates of lettuce, carrot, tomato, and cabbage.

Ten seeds of each crop were added to each petri plate after the test solution was absorbed into the paper. The plates were then randomly placed in plastic boxes (12.25 x 9.0 x 4.1 inches) with tightly fitting lids to prevent moisture loss. The petri plates were incubated

in the dark at 25 ±1°C for 7 days, except lettuce, which was incubated at 18 ±1°C.

Seedling Emergence: 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. An analysis 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 ft^2).

Soybean and cucumber were planted at a depth of 2.5 cm, while the remaining four 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 clethodim technical in deionized water and acetone. 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.

C. **Dosage:** Clethodim was applied at a rate of 0.25 lb ai/A to all plant species. Treatment application rates were adjusted for the percent purity of the test material (82.4%).

D. **Design:**

Seed Germination: Each treatment/crop combination was replicated five times (i.e., 10 seeds/plate, 5 plates/treatment). After 7 days of incubation, the seeds were removed from the petri plates and the radicle lengths were measured to the nearest millimeter. Percent seed germination and mean radicle length were calculated for all germinated seeds. Seeds were considered germinated if the radicle was at least 5 mm long.

Seedling Emergence: Each crop/treatment combination was replicated five times (i.e., 10 seeds/pot, 5 pots/treatment level). The percentage of the ten seeds planted in each pot which emerged was calculated for each treatment. After treatment, the 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. Seedling height and phytotoxicity ratings were recorded at 7, 14, and 21 days after treatment for all species except carrot, which was evaluated after 28 days. Twenty-one days after treatment (28 for carrot), 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.

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 table was constructed using the Lotus 1-2-3 raw data spreadsheet. A one-way analysis of variance (ANOVA) model for data with equal subsamples was used to analyze data from the seed germination (radicle length and percent germination) and seedling emergence (percent emergence, phytotoxicity, plant height and plant dry weight) tests. The F-value from the analysis of variance table and a F table were used to determine if the treatment means were significantly different ($p < 0.05$).

12. **REPORTED RESULTS:**
Seed Germination: Treatment of seeds with clethodim at the

11

rate of 0.25 lb ai/A did not result in a significant decrease in percent germination ($p < 0.05$) between the control and any of the six treated plant species. The percent effect on seed germination ranged between 0% on lettuce, tomato, and cucumber to -6% on soybean and cabbage.

Treatment of seeds with clethodim resulted in a significant increase ($p < 0.05$) in radicle lengths of lettuce and tomato. The percent effect on radicle length ranged between -10% on cucumber to 20% on tomato.

Seedling Emergence:

Percent germination: Statistical analysis of the 21 day (28 day for carrot) percent emergence data shows a significant difference ($p < 0.05$) between the control and cabbage seedlings emerging from the soil surface treated with clethodim at the maximum rate of 0.25 lb ai/A. The percent effect on percent emergence ranged from -13% on cabbage to 0% on cucumber.

Phytotoxicity rating: Statistical analysis of the 21 day (28 day for carrot) phytotoxicity ratings show no significant difference ($p < 0.05$) on mean phytotoxicity ratings between the control plants and treatments with clethodim at the rate of 0.25 lb ai/A for all crops listed.

Plant height: Treatment of the soil surface with clethodim did not result in a significant effect on plant height between the control and treatment with clethodim at the 21 day (28 day for carrot) observation period for all crops tested. Percent effect on plant height ranged from -5% on soybean to 9% on cabbage.

Plant dry weight: Statistical analysis of the plant dry weight data show no significant diffference ($p < 0.05$) between the control plants and treatment with clethodim. Dry weights ranged from 0% difference for soybeans to a 30% increase for carrot. After treatment with clethodim, carrot produced some vigorous plants.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Clethodim applied at the maximum label rate of 0.25 lb ai/A did not result in a greater than 25% detrimental effect on any of the parameters measured in all systems. Therefore, a Tier 2 study is not required.

The Quality Assurance Unit of Pan-Agricultural Labs, Inc., was responsible for the assurance of compliance with Good Laboratory Practice (GLP) Standards.

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:

no monocots were used in this experiment. According to the SEP, a Tier 1 germination and seedling emergence test must contain ten species: six dicots and four monocots.

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.

The Herbicide Handbook (WSSA '89) lists the maximum label rate of clethodim as 0.6 lb ai/A. If this is the case, these tests need to be conducted again at this maximum rate.

B. **Statistical Analysis:** Statistical analyses were conducted on cabbage (the most sensitive species) data for percent germination and seedling emergence (attached). The results are in agreement with the author's.

C. **Discussion/Results:**

Seed Germination: This study is scientifically sound but does not meet the requirements for Tier 1 testing. The studies conducted must include four species of monocots. Treatment of the seeds with clethodim at the maximum application rate of 0.25 lb ai/A did not have any significant effects on any plant species tested. Radicle length was also not negatively impacted by clethodim application.

Seedling Emergence:
Phytotoxicity rating: Based on phytotoxicity ratings,

application.

Percentage of emerged seedlings: Only cabbage was significantly ($p < 0.05$) affected by clethodim application after 21 days. The remainder of the species were not affected by the herbicide.

Plant height: Plant height was not significantly affected in all species.

Plant dry weight: Plant dry weight was not significantly affected in all species.

These studies are scientifically sound, however, monocot species were not included in testing as outlined by the SEPs for a Tier 1 non-target seedling emergence test.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** The study did not follow the approved protocol for Tier 1 toxicity tests on seed germination/seedling emergence of non-target plants as listed in the SEP.

(3) **Repairability:** Additional tests should be conducted to include the four monocot species needed to comply with the SEP for Tier 1 testing.

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

OBS	TRT	REP	GERMI
1	1	1	100
2	1	2	100
3	1	3	100
4	1	4	80
5	1	5	100
6	2	1	80
7	2	2	100
8	2	3	90
9	2	4	80
10	2	5	100

Analysis of Variance Procedure

Dependent Variable: GERMI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	90.00000000	90.00000000	1.00	0.3466
Error	8	720.00000000	90.00000000		
Corrected Total	9	810.00000000			

R-Square	C.V.	Root MSE	GERMI Mean
0.111111	10.20090	9.4868330	93.00000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	1	90.00000000	90.00000000	1.00	0.3466

OBS	TRT	REP	GERMI
1	1	1	90
2	1	2	80
3	1	3	90
4	1	4	100
5	1	5	90
6	2	1	70
7	2	2	70
8	2	3	80
9	2	4	80
10	2	5	90

Analysis of Variance Procedure

Dependent Variable: GERMI

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	360.00000000	360.00000000	6.00	0.0400
Error	8	480.00000000	60.00000000		
Corrected Total	9	840.00000000			

R-Square	C.V.	Root MSE	GERMI Mean
0.428571	9.221389	7.745967	84.00000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	1	360.00000000	360.00000000	6.00	0.0400