Text Searchable Document

DATA EVALUATION RECORD
SEEDLING EMERGENCE TEST
§ 122-1 (TIER I)

1. **CHEMICAL:** Thiophanate-methyl PC Code No.: 102001

2. **TEST MATERIAL:** Thiophanate-methyl technical - 96.2% purity
^{14}C-Topsin - radiochemical purity of 89.4%

3. **CITATION:**

Author: D. Teixeira
Title: Thiophanate-methyl - Determination of Effects on Seed Germination and Seedling Emergence of Two Plant Species
Study Completion Date: September 29, 1997
Laboratory: Springborn Laboratories, Inc., Wareham, MA
Sponsor: ELF Atochem North America Agrichemicals, Group, Philadelphia, PA
Laboratory Report ID: 97-7-7033
MRID No.: 444041-01
DP Barcode: D240729

4. **REVIEWED BY:** Mark Mossler, M.S., Toxicologist, Golder Associates Inc.

Signature: Mark L Mossler **Date:** 1/30/98

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist Golder Associates Inc.

Signature: P. Kosalwat **Date:** 1/30/98

5. **APPROVED BY:** Richard C Petersen

Signature: **Date:** 2/23/00

6. **STUDY PARAMETERS:**

Definitive Study Duration: 14 days

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier I seedling emergence study with terrestrial plants. Application at the maximum rate of 1.4 lb ai/A did not advervsely effect the emergence of soybean or onion plants.

8. **ADEQUACY OF THE STUDY:**

A. Classification: Core.

2004936

B. **Rationale:** The results presented for onion and soybean, once added to the results from the original study, complete the seedling emergence testing requirement.

C. **Repairability:** N/A.

9. **GUIDELINE DEVIATIONS:** No guideline deviations of consequence were noted.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. **Test Organisms**

Guideline Criteria	Reported Information
Species 6 dicots in 4 families, including soybean and a rootcrop; 4 monocots in 2 families, including corn.	Dicot: soybean Monocot: onion
Number of seeds per rep 10	10
Source of Seed	Untreated seed obtained from commercial suppliers
Historical % Germination of Seed	≥94%

B. **Test System**

Guideline Criteria	Reported Information
Solvent	Acetone
Site of test	Growth chamber
Planting method / type of pot	Planted at 1.0-cm depth/ polypropylene pots (13-cm top diameter)
Method of application	200 mL of solution applied to the soil surface of each pot
Method of watering	Subirrigation

Guideline Criteria	Reported Information
Growth stage at application Seed or plant.	Seed

C. **Test Design**

Guideline Criteria	Reported Information
Dose range 2x or 3x	Soybean and onion tested at Tier I level
Doses At least 5	1.4 lb ai/A
Controls Negative and solvent	Negative (deionized water) and solvent (10% acetone) controls
Replicates per dose At least 3	3
Duration of test 14 days	14 days
Were observations made at least weekly?	Observations made on days 10 and 14
Maximum labeled rate	1.4 lb ai/A

12. <u>REPORTED RESULTS</u>:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Was an NOEL observed for each species?	Yes
Phytotoxic observations	Yes
Were initial chemical concentrations measured? (Optional)	The measured concentration of the application solution averaged 95% of nominal.
Were adequate raw data included?	Yes

Results for the most sensitive parameter* of each species

Species	Parameter	Percentage Inhibition
Soybean	shoot length	7
Onion	none	treatment values greater than control values

*The most sensitive parameter is based on the amount of inhibition in comparison to the solvent control.

Observations: No treatment-related symptoms of test material toxicity were noted.

Statistical Method: No statistical methods were employed.

13. **VERIFICATION OF STATISTICAL RESULTS:** Results for soybean and onion indicated that these plants were not injured by 25% or more for any parameter evaluated.

14. **REVIEWER'S COMMENTS:** It was indicated that this study was a supplement to a previously submitted study (MRID No. 421235-04). Within the previous report, onion and soybean data were judged to be invalid. With the inclusion of the results presented herein, the entire emergence study can be classified as "core." Consequently, this study is scientifically sound and fulfills the guideline requirements. The study is classified as **Core**.

2-EEB-17

Reviewer: Richard Petrie 3/26/92

MRID No. 421235-04

DATA EVALUATION RECORD

1. **CHEMICAL:** Thiophanate.
Shaughnessey No. 102001.

2. **TEST MATERIAL:** 1) Thiophanate-methyl (Topsin-M); Lot No. TIF-01016; 97.57% active ingredient; an off-white powder. 2) ^{14}C-Thiophanate-methyl; Lot No. Amersham, CFQ.4519GTW/C2/52; radiopurity of 95.3% and chemical purity of 90.1%.

3. **STUDY TYPE:** Non-Target Plants: Seed Germination, Seedling Emergence & Vegetative Vigor Phytotoxicity Test - Tiers 1 & 2. Species Tested: Soybean, Lettuce, Radish, Tomato, Cucumber, Cabbage, Oat, Ryegrass, Corn, and Onion.

4. **CITATION:** Hoberg, J.R. 1991. (Thiophanate-methyl) - Determination of Effects on Seed Germination, Seedling Emergence and Vegetative Vigor of Ten Plant Species. Laboratory Report No. 91-7-3854. Conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by ATOCHEM North America, Philadelphia, PA. EPA MRID No. 421235-04.

5. **REVIEWED BY:**

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

Signature: Mark A. Mossler
Date: 3/26/92

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 3/26/92

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

Signature: Richard C. Petrie
Date: 2/23/00

7. **CONCLUSIONS:**

Seed Germination:

Percent Germination and Radicle Length: Treatment of the seeds with thiophanate-methyl up to the maximum application rate (1.4 lb ai/A, nominal concentration) did not have any significant effects on percent germination or radicle length. The subsequent NOEC for the test species was the

33

L D

maximum rate tested (1.2-1.3 lb ai/A, measured concentration).

Seedling Emergence:
Percentage of Emerged Seedlings: The most sensitive species was cucumber. The NOEC, LOEC, and EC_{25} for cucumber emergence were 0.58, 1.2, and 0.8 lb ai/A, respectively.

Shoot Length: Shoot length for the ten species did not differ from the controls at the highest rate tested. The NOEC for all species was 1.2 lb ai/A.

Vegetative Vigor:
Shoot Length: The most sensitive species with respect to shoot length was soybean. The NOEC, LOEC, and EC_{25} for soybean shoot length were 1.6, 3.2, and 3.2 lb ai/A, respectively.

Shoot Weight: The most sensitive species with respect to shoot weight was again soybean. The NOEC, LOEC, and EC_{25} for soybean shoot weight were 1.6, 3.2, and 2.3 lb ai/A, respectively.

Root Weight: The most sensitive species with respect to root weight was soybean. The NOEC, LOEC, and EC_{25} for soybean root weight were 1.6, 3.2, and 3.2 lb ai/A, respectively.

The seed germination study is scientifically sound (for all species except onion and soybean) but does not fulfill the guideline requirements for a Tier 1 or 2 seed germination test. The seeds were not tested to the maximum labeled rate (1.4 lb ai/A).

The seedling emergence study is scientifically sound (for all species except onion and soybean) but does not fulfill the guideline requirements for a Tier 1 or 2 seedling emergence test. The test material was not applied to the maximum labeled rate (1.4 lb ai/A).

The vegetative vigor study is scientifically sound but does not meet the guideline requirements for a Tier 1 or 2 vegetative vigor test. The NOEC (and possibly EC values as well) needs to be determined for tomato shoot weight and information as to the stage of development of the test plants needs to be submitted.

8. **RECOMMENDATIONS:** Submit data concerning the vegetative vigor test.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

Test Plants: Dicotyledon plants were represented by six species from five families (i.e., soybean, lettuce, radish, tomato, cucumber, and cabbage). Monocotyledon plants were represented by four species from two families (i.e., corn, oat, ryegrass, and onion). Cultivars, lot number, and germination ratings (except for oat) were provided in the report.

B. Test System:

Seed Germination: Two circles of filter paper were placed in the bottom of a glass petri plate (150 mm in diameter x 20 mm in height). To initiate each germination test, the paper was saturated with the appropriate test solutions (5 ml). Plates were placed under a fume hood to allow the solvent to evaporate. Solvent control plates received the same amount of solvent as the treatment plates (5 ml). All plates then received 15 ml of deionized water. Twenty seeds of each crop were impartially added to each petri plate after the water was absorbed into the paper. The plates were randomly placed in an incubator set at 30 ±1°C for corn and cucumber and 22 ±1°C for the remaining species. Each plate was wrapped with moistened paper towel. Plates were randomly placed in an incubator and the seeds were allowed to germinate for five to six days in the dark.

Seedling Emergence: Ten seeds of each crop were planted in plastic pots (13 cm tall, 13 cm top-9 cm bottom diameter) filled with sand (1.5 kg). The sand had a pH of 6.2 and contained 0.11% organic matter. Each pot was moistened with 200 ml of nutrient solution (control), 200 ml of 10% acetone nutrient solution (solvent control) or treatment nutrient solution. All seeds were planted at a depth of 1 cm. Nutrient solution at one-quarter-strength was applied during the course of the study by sub-irrigation to all species. The plants were allowed to emerge in a growth chamber supplied with incandescent and fluorescent lights (16 hours of light/day, 1,100 footcandles). For each species, pots from each treatment were randomly placed within a block. The temperature was maintained between 21 and 29°C and the mean relative humidity was 74%.

Vegetative Vigor: Approximately 200 seeds of each species were germinated in moist towels that were placed in a sealed wooden box and kept in a growth chamber. Corn and soybean

seeds were allowed to germinate for three days. Radish seed was allowed to germinate for four days. Cabbage, lettuce, oat, and ryegrass seeds were allowed to germinate for five days, tomato seed for six days, and onion seed for seven days. After germination but prior to planting, all plants were exposed to 170-450 footcandles of artificial light for one photoperiod (16-hour light/8-hour dark) to allow for proper orientation of root and shoot portions of the seedlings and to initiate photosynthesis. Five seedlings were transplanted per pot (13 cm tall, 13 cm top-9 cm bottom diameter). The sand (1.5 kg) had a pH of 6.2 and an organic matter of 0.11%.

Appropriate thiophanate-methyl solutions (30 ml) were applied to established plants 7-8 days after transplantation. Applications were made from lowest to highest concentration with a hand spraying apparatus. Treatments included both a control and a solvent control (10% acetone). The test material was allowed to dry and the plants were randomized within a growth chamber. The chamber was illuminated by fluorescent and incandescent lighting (16 hours of light/day, 1,600 footcandles) and set to maintain a temperature of 21-30°C. The mean relative humidity was 81%. Nutrient solution at one-quarter-strength was applied during the course of the study by sub-irrigation to all species.

C. Dosage: Based on preliminary tests, thiophanate-methyl was applied at the nominal rate of 1.4 lb active ingredient (ai)/A to all plant species for Tier 1 tests. Where Tier 2 testing was indicated, the nominal rates were 0.088, 0.18, 0.35, 0.7, and 1.4 lb ai/A. Stock, nutrient, and spray solutions were measured for thiophanate-methyl by liquid scintillation counting for the germination, emergence and vigor tests, respectively. The measured test concentrations were recorded (Tables 2, 24, and 46, attached). Treatment application rates were adjusted for the percent purity of the test material.

D. Design:

Seed Germination: Each treatment/crop combination was replicated three times (i.e., 20 seeds/plate, 3 plates/ treatment level). After 5 or 6 days of incubation, the seeds were removed from the petri plates and the radicle lengths were measured. Percent seed germination and mean radicle length were calculated for all germinated seeds. Seeds were considered germinated if the radicle was $\geq$5 mm long. Seeds were observed for morphological abnormalities such as discoloration, swelling or lesions.

Temperature during the test period in the growth chamber was provided in the report.

Seedling Emergence: Each treatment/crop combination was replicated three times (i.e., 10 seeds/pot, 3 pots/treatment level). At 10 and 14 days, each replicate pot was observed for abnormalities such as chlorosis, leaf blotch, foliar lesions and necrosis. The percentage of the ten seeds planted in each pot which emerged was calculated for each treatment. The shoot length was also recorded at 14 days (test termination).

Temperature, relative humidity, light intensity, and carbon dioxide ranges measured in the growth chamber during the period of growth were provided in the report.

Vegetative Vigor: Each treatment/crop combination was replicated three times (i.e., 5 plants/pot, 3 pots/treatment level). Individual shoot lengths were measured and recorded on the day of but prior to the application of thiophanate-methyl and weekly thereafter. Observations of morphological abnormalities were also made on the same days. Fourteen days after treatment, the plants within treatment replicates (pots) were cut at the soil level and dried at 70°C for several days. After drying, the dry weights of the individual shoots were recorded. Roots were removed from sand, rinsed in deionized water, oven dried and weighed in the same manner as the shoots. Roots were combined as a group dry weight per replicate pot.

Temperature, relative humidity, light intensity, and carbon dioxide ranges measured in the growth chamber during the period of growth were provided in the report.

E. **Statistics:** For Tier 1 tests, the solvent control data were first compared to the control data using a t-test ($p < 0.05$). If control and solvent data were significantly different, then treatment data were compared to the solvent control data. If no significant difference was found, control and solvent control data were pooled before further analysis with treatment data. If $\geq$25% detrimental response at the treatment level was observed, a t-test was used to compare the percent germination, radicle length, percent seedling emergence, emergent shoot length, seedling shoot length, seedling root weight and seedling shoot weight of the exposed plants with the data obtained from either the solvent control or pooled control plants. Percentage data were transformed using the arcsine-square-root transformation before the t-test was performed.

For Tier 2 tests, the solvent control and control were treated as mentioned above. Data were then tested for normality by a Chi-square test and for homogeneity of variance using Bartlett's test. If the assumptions of normality and homogeneity of variance were not met, the data were transformed and analyzed further if those assumptions could then be satisfied. Data not meeting the assumption of homogeneity of variance or normality were statistically analyzed for significant effects using the non-parametric Kruskal-Wallis test. If data met the assumption of homogeneity of variance and normality using the pooled control data, then Bonferroni's t-test was performed in order to determine statistically significant reduction effects. If solvent control data were used, then Dunnett's test was performed. These statistical procedures were conducted using data on a per replicate basis from each measured parameter to determine the no-observed-effect concentration (NOEC) for each parameter. All statistical conclusions were made at the 95% level of certainty.

For all Tier 2 tests, the EC_{25} and EC_{50} values were calculated by linear regression of response versus exposure concentration. Four linear regression curves were computed based on (a) untransformed data, (b) untransformed response vs. logarithm transformed concentration, (c) probit transformed response vs. untransformed concentration, and (d) probit transformed response vs. logarithm transformed concentration. Based on the highest coefficient of determination, the regression line that provided the best fit of the untransformed or transformed data was selected. This regression equation was then applied to calculate the EC_{25} and EC_{50} values and their 95% confidence limits, using the method of inverse prediction.

The calculated NOEC values determined for some species were greater than the corresponding EC_{25} values, due to the variability within the data such that the mean measured values of some treatment parameters were not significantly different from the control data. Though a parameter may have been reduced by >25% during a test as compared to the solvent control or pooled control, the reduction may not have been statistically significant.

12. **REPORTED RESULTS:**

Seed Germination: All rates reported are adjusted to percentage recovery of stock (test) solutions (Table 2). No morphological abnormalities were observed among the germinating seeds. No significant ($p < 0.05$) difference in percent germination or radicle length existed for any of the

test species. The NOEC for percent germination and radicle length for corn and cucumber was 1.2 lb ai/A. The NOEC for percent germination and radicle length for the remaining eight species was 1.3 lb ai/A. The EC_{25} and EC_{50} values for both percent germination and radicle length were determined to be greater than either 1.2 or 1.3 lb ai/A for each group, respectively.

Seedling Emergence: All rates reported are adjusted to percentage recovery of nutrient solution concentrations (Table 24). By day 14 (test termination), percent emergence did not differ significantly from the controls at the highest rate tested (1.5 lb ai/A, measured concentration) for all test species. The NOEC was determined to be 1.5 lb ai/A. A rate response was observed for cucumber emergence and the EC_{25} and EC_{50} were determined to be 1.2 and 2.1 lb ai/A, respectively. The EC_{25} and EC_{50} values for the remaining species were determined to be greater than 1.5 lb ai/A.

Similar to percent emergence, none of the test species demonstrated significant reductions in shoot length at the maximum application rate of thiophanate-methyl. The NOEC for all test species for shoot length was 1.5 lb ai/A. The EC_{25} and EC_{50} values were determined to be greater than 1.5 lb ai/A.

Vegetative Vigor: All rates reported are adjusted to percentage recovery of thiophanate-methyl in the spray solutions (Table 46). At test termination (14 days), shoot length was only significantly affected in soybean at the maximum application rate. The NOEC for soybean shoot length was 0.69 lb ai/A. The NOEC for the remaining test species (except tomato) was 1.4 lb ai/A. The author reported that the NOEC for tomato shoot length was <1.4 lb ai/A. The EC_{25} and EC_{50} for soybean shoot length were 1.4 and 3.2 lb ai/A, respectively. The EC values for the remaining species were determined to be greater than 1.4 lb ai/A (Table 77, attached).

Results from shoot weight reflect similar trends. By test termination, shoot weight was only significantly affected in soybean at the maximum application rate. The NOEC for soybean shoot weight was 0.69 lb ai/A. The NOEC for the remaining test species (except tomato) was 1.4 lb ai/A. The author reported that the NOEC for tomato shoot weight was <1.4 lb ai/A. The EC_{25} and EC_{50} for soybean shoot weight were 1.0 and 2.4 lb ai/A, respectively. The EC values for

the remaining species were determined to be greater than 1.4 lb ai/A.

Again, results from the root weight data were similar to trends seen in the shoot length and weight data. However, none of the ten test species were significantly affected by the maximum application rate of thiophanate-methyl. The NOEC for all species (except tomato) was determined to be 1.4 lb ai/A. The author reported that the NOEC for tomato root weight was <1.4 lb ai/A. The EC_{25} and EC_{50} for soybean root weight were 0.69 and 2.77 lb ai/A, respectively. The EC values for the remaining species were determined to be greater than 1.4 lb ai/A, except tomato, for which the author stated that the EC_{25} was approximately 1.4 lb ai/A.

Occasional signs of toxicity noted in the emergence and vegetative vigor tests were decreased size, brown leaf tips, necrosis, and chlorosis.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author concluded that the test material demonstrated either no toxicity or an insignificant impact on the test species at or below the recommended application rate.

The Quality Assurance Unit of Springborn Laboratories, Inc., was responsible for the assurance of compliance with Good Laboratory Practice (GLP) Regulations. Both statements of compliance with GLPs and QA were enclosed. The GLP statement indicated that maintenance of records pertaining to the stability, characterization, and verification of the test substance are the responsibility of the sponsor. Additionally, routine water analyses were conducted at a laboratory that did not operate under GLPs.

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

A. **Test Procedure:** In the germination studies, the measured concentrations only extended to 1.2 or 1.3 lb ai/A, rather than to the maximum application rate of 1.4 lb ai/A. Additionally, no raw data were submitted for the percent germination portion of the test.

In the seedling emergence test, the amount of test material applied to each pot in 200 ml of nutrient solution was only 78% of what should have been theoretically applied. Based on the application rate of 1.4 lb ai/A, and determining the area of each pot in relation to an acre, it was determined that 256 ml of the test nutrient solutions should have been applied to each pot rather than 200 ml of the concentrations

listed herein. Because of this, the maximum application rate was 1.2 lb ai/A, rather than 1.5 lb ai/A. Therefore, the concentrations only extended to 1.2 lb ai/A, rather than 1.4 lb ai/A. Additionally, no raw data were submitted for the shoot length portion of the test.

In the vegetative vigor study, no mention was made of the number of days that cucumber plants were allowed to germinate. Also, the number of true leaves on the "established" test plants was not reported.

Although not stated by the author, the reviewer assumes that the vegetative vigor test plants were sprayed with 30 ml of each solution as were the control plants (sprayed with 30 ml of deionized water). If this is the case, the plants in the vegetative vigor test were exposed to rates 2.3 times greater than those stated by the author.

B. **Statistical Analysis:** Statistical analyses were conducted on the most sensitive species and parameter for all three tests (see attached printouts). Means were separated (using analysis of variance coupled with Dunnett's test) by comparison to the solvent control for the germination and emergence tests. Comparison was made to the negative control for the vegetative vigor test due to an outlying value in replicate three of the solvent control. These analyses determined both the NOEC and lowest-observed-effect concentration (LOEC). The results are generally in agreement with the author's except that the NOEC for cucumber emergence was determined to be 0.58 lb ai/A, rather than 1.5 lb ai/A. Due to a lack of dose response, EC values could not be determined using probit analysis or linear regression. The EC_{25} values for lettuce radicle length, cucumber emergence, and soybean root weight were >1.3, 0.8, and 3.2 lb ai/A, respectively, using visual interpretation.

C. **Discussion/Results:**

Seed Germination:

Percent Germination and Radicle Length: Treatment of the seeds with thiophanate-methyl up to the maximum application rate (1.4 lb ai/A, nominal concentration) did not have any significant effects on percent germination or radicle length. The subsequent NOEC for the test species was the maximum rate tested (1.2-1.3 lb ai/A, measured concentration). However, the results for onion and soybean are invalid due to the poor germination (<70%) of the test seeds. Because there were no dose responses, the LOEC, EC_{25}, and EC_{50} values could not be predicted and were

determined to be greater than the maximum rate tested (either 1.2 or 1.3 lb ai/A).

Seedling Emergence:
Percentage of Emerged Seedlings: By test termination, percent emergence for the ten species did not differ from the controls at the highest rate tested except for cucumber. The NOEC for this species was 0.58 lb ai/A (based on the reviewer's statistical analysis and measured concentration adjusted for lack of 100% addition, see section 14 A.). The NOEC for the remaining species was 1.2 lb ai/A based on this same adjustment. However, the results for onion and soybean are again invalid due to the poor emergence (<70%) of the test seeds. The LOEC and EC_{25} for cucumber emergence were 1.2 and 0.8 lb ai/A, respectively. The EC_{50} determined for cucumber emergence by the author is invalid due to a detrimental response that only extends to 40%. Because there were no dose responses for the remaining species, the LOEC, EC_{25}, and EC_{50} values could not be predicted and were determined to be greater than the maximum rate tested (1.2 lb ai/A).

Shoot Length: By test termination, shoot length for the ten species did not differ from the controls at the highest rate tested. The NOEC for all species was 1.2 lb ai/A (measured concentration adjusted for lack of 100% addition). Again, the results for onion and soybean are invalid due to the poor emergence (<70%) of the test seeds. Because there were no dose responses, the LOEC, EC_{25}, and EC_{50} values could not be predicted and were determined to be greater than the maximum rate tested (1.2 lb ai/A).

Vegetative Vigor:
Shoot Length: The most sensitive species with respect to shoot length was soybean. The NOEC, LOEC, and EC_{25} for soybean shoot length were 1.6, 3.2, and 3.2 lb ai/A, respectively, based on measured concentrations corrected for overapplication (see section 14 A.). The EC_{50} determined for soybean shoot length by the author is invalid due to a detrimental response that only extends to 25%. The NOEC for the remaining species was 3.2 lb ai/A, based on these same calculations. Because there were no dose responses for the other nine species, the LOEC, EC_{25}, and EC_{50} values could not be predicted and were determined to be greater than the maximum rate tested (3.2 lb ai/A).

Shoot Weight: The most sensitive species with respect to shoot weight was again soybean. The NOEC, LOEC, and EC_{25} for soybean shoot weight were 1.6, 3.2, and 2.3 lb ai/A,

respectively, based on measured concentrations corrected for overapplication. The EC_{50} determined for soybean shoot weight by the author is invalid due to a detrimental response that only extends to 33%. The NOEC for the remaining species was 3.2 lb ai/A, with the exception of tomato, in which case the NOEC was <3.2 lb ai/A. Because there were no dose responses for the other nine species, the LOEC, EC_{25}, and EC_{50} values could not be predicted and were determined to be greater than the maximum rate tested (3.2 lb ai/A).

Root Weight: The most sensitive species with respect to root weight was soybean. The NOEC, LOEC, and EC_{25} for soybean root weight were 1.6, 3.2, and 3.2 lb ai/A, respectively, based on measured concentrations corrected for overapplication. The EC_{50} determined for soybean root weight by the author is invalid due to a detrimental response that only extends to 25%. The NOEC for the remaining species was 3.2 lb ai/A (except tomato, see following discussion).

The author stated that the NOEC for tomato shoot length and root weight were less than 1.4 lb ai/A (3.2 lb ai/A, corrected), however, the reviewer feels that these are typographical errors and that the NOEC for both of these parameters is 3.2 lb ai/A. The NOEC for tomato shoot weight is <3.2 lb ai/A due to a significant reduction in comparison to the control. The author also stated that the EC_{25} for tomato root weight was approximately 1.4 lb ai/A (3.2 lb ai/A, corrected). However, the response at this rate is only 6%, and the reviewer questions this statement. In the reviewer's opinion, there were no dose responses for the other nine species and the LOEC, EC_{25}, and EC_{50} values could not be predicted. Therefore, these values were determined to be greater than the maximum rate tested (3.2 lb ai/A) for root weight.

The seed germination study is scientifically sound (for all species except onion and soybean) but does not fulfill the guideline requirements for a Tier 1 or 2 seed germination test. The seeds were not tested to the maximum labeled rate (1.4 lb ai/A).

The seedling emergence study is scientifically sound (for all species except onion and soybean) but does not fulfill the guideline requirements for a Tier 1 or 2 seedling emergence test. The test material was not applied to the maximum labeled rate (1.4 lb ai/A).

The vegetative vigor study is scientifically sound but does not meet the guideline requirements for a Tier 1 or 2 vegetative vigor test. The NOEC (and possibly EC values as well) needs to be determined for tomato shoot weight and information as to the stage of development of the test plants needs to be submitted.

D. Adequacy of the Study:

(1) **Classification:** Seed Germination - Invalid for onion and soybean, supplemental for the remaining species.

Seedling Emergence - Invalid for onion and soybean, supplemental for the remaining species.

Vegetative Vigor - Supplemental.

(2) **Rationale:** Germination and emergence of onion and soybean controls were below (<70%) the percentage considered for the term "good viability." For the germination and emergence tests, the test material was not applied to the maximum rate. For the vegetative vigor test, no mention as to how many days cucumber was allowed to germinate or the stage of development of the test plants was reported.

(3) **Repairability:** No, for the germination and emergence tests. Submit stage of development for all plants and cucumber germination data for the vegetative vigor test.

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

Table 2a. Concentrations of Thiophanate-methyl measured in the stock solution at test initiation of the seed germination tests with corn and cucumber.

Nominal Concentration (lbs A.I./A)	Nominal Stock Concentration (mg A.I./L)		Measured Stock Concentration (mg A.I./L)	Mean Stock[a] Recovery (%)	Measured Concentration[b] (lbs A.I./A)
1.4	13	A	11	87	1.2
		B	11		
		C	11		
Solvent Control	Solvent Control	A	< 0.076		
		B	< 0.076		
		C	< 0.076		
Control	Control	A	< 0.0070		
		B	< 0.0070		
		C	< 0.0070		
QC #1[c]			6.84 (6.37)[d]	107	
QC #2			7.70 (7.28)	106	
QC #3			8.51 (8.19)	104	

[a] Mean values were calculated from original raw data and not the rounded values presented in this table.
[b] The nominal concentration in lb A.I./A was multiplied by the percent recovery (as a decimal) of the stock solution analysis to yield the measured concentration in lb A.I./A.
[c] QC = Quality Control sample
[d] Value in parentheses is the nominal fortified concentration for the corresponding QC sample.

Table 2b. Concentrations of Thiophanate-methyl measured in the stock solutions at test initiation of the seed germination tests with cabbage, lettuce, oat, onion, radish, ryegrass, soybean and tomato.

Nominal Concentration (lbs A.I./A)	Nominal Stock Concentration (mg A.I./L)		Measured Stock Concentration (mg A.I./L)	Mean Stock[a] Recovery (%)	Measured Concentration[b] (lbs A.I./A)
1.4[cd]	13	A	12	91	1.3
		B	11		
		C	12		
0.70[d]	6.3	A	6.4	99	0.69
		B	6.1		
		C	6.2		
0.35[d]	3.3	A	2.8	88	0.31
		B	2.9		
		C	3.1		
0.18[d]	1.6	A	1.5	89	0.16
		B	1.4		
		C	1.4		
0.088[d]	0.78	A	0.69	89	0.078
		B	0.69		
		C	0.71		
Solvent Control	Solvent Control	A	< 0.079		
		B	< 0.079		
		C	< 0.079		
Control	Control	A	< 0.0072		
		B	< 0.0071		
		C	< 0.0071		
QC # 1[e]			4.71 (4.55)[f]	103	
QC # 2			48.1 (45.5)	106	
QC # 3			86.4 (8.19)	106	

a Mean values were calculated from original raw data and not the rounded values presented in this table.
b The nominal concentration in lb A.I./A was multiplied by the percent recovery (as a decimal) of the stock solution analysis to yield the measured concentration in lb A.I./A.
c Cabbage, oat, onion, radish, ryegrass and tomato exposed to 1.4 lb A.I./A.
d Lettuce and soybean exposed to 1.4 - 0.088 lb A.I./A.
e QC = Quality Control sample
f Value in parentheses is the nominal fortified concentration for the corresponding QC sample.

Table 24. Concentrations of Thiophanate-methyl measured in the test solutions prior to test initiation of the seedling emergence tests.

Nominal Concentration (lbs A.I./A)	Nominal Concentration (mg A.I./L)		Measured Concentration (mg A.I./L)	Mean Percent[a] Recovery (%)	Measured Concentration[b] (lbs A.I./A)
1.4[cd]	8.2	A	9.0	105	1.5
		B	8.7		
		C	8.3		
0.70[d]	4.1	A	4.0	105	0.74
		B	4.6		
		C	4.3		
0.35[d]	2.0	A	2.2	109	0.38
		B	2.1		
		C	2.2		
0.18[d]	1.1	A	1.1	104	0.19
		B	1.2		
		C	1.1		
0.088[d]	0.51	A	0.53	107	0.094
		B	0.56		
		C	0.55		
Solvent Control	Solvent Control	A	< 0.047		
		B	< 0.047		
		C	< 0.047		
Control	Control	A	< 0.042		
		B	< 0.042		
		C	< 0.042		
QC # 1[e]			0.434 (0.455)[f]	95.4	
QC # 2			4.68 (4.55)	103	
QC # 3			8.39 (8.19)	102	

NA = Not applicable.

a Mean values were calculated from original raw data and not the rounded values presented in this table.
b The nominal concentration in lb A.I./A was multiplied by the percent recovery (as a decimal) of the stock solution analysis to yield the measured concentration in lb A.I./A.
c Cabbage, corn, lettuce, oat, onion, radish, ryegrass, soybean and tomato were exposed to 1.4 lb A.I./A.
d Cucumber was exposed to 1.4 - 0.088 lb A.I./A.
e QC = Quality Control sample
f Value in parentheses is the nominal fortified concentration for the corresponding QC sample.

Table 46. Concentrations of Thiophanate-methyl measured in the test solutions prior to test initiation of the vegetative vigor tests.

Nominal Concentration (lbs A.I./A)	Nominal Concentration (mg A.I./L)		Measured Concentration (mg A.I./L)	Mean Percent[a] Recovery (%)	Measured Concentration[b] (lbs A.I./A)
1.4[cd]	163	A	166	100	1.4
		B	160		
		C	163		
0.70[d]	82	A	81.4	99	0.69
		B	79.0		
		C	83.7		
0.35[d]	41	A	39.4	99	0.35
		B	41.2		
		C	41.2		
0.18[d]	20	A	22.2	110	0.20
		B	21.9		
		C	22.0		
0.088[d]	10	A	9.97	104	0.092
		B	10.6		
		C	10.5		
Solvent Control	Solvent Control	A	< 1.01		
		B	< 1.01		
		C	< 0.982		
Control	Control	A	< 0.917		
		B	< 0.917		
		C	< 0.917		
QC #1[e]			22.1 (20.0)[f]	111	
QC #2			40.5 (40.0)	101	
QC #3			51.5 (50.0)	103	

NA = Not applicable.

a Mean values were calculated from original raw data and not the rounded values presented in this table.
b The nominal concentration in lb A.I./A was multiplied by the percent recovery (as a decimal) of the stock solution analysis to yield the measured concentration in lb A.I./A.
c Corn, cucumber, oat, onion, radish and tomato were exposed to a nominal concentration range of 1.4 lb A.I./A.
d Cabbage, lettuce, ryegrass and soybean were exposed to a nominal concentration range of 1.4 - 0.088 lb A.I./A.
e QC = Quality Control sample
f Value in parentheses is the nominal fortified concentration for the corresponding QC sample.

Table 77. Summary of EC25, EC50 and NOEC values determined during a vegetative vigor test exposing ten plant species to Thiophanate-methyl.

Species	Parameter	EC25[a] (lb A.I./A)	EC50[a] (lb A.I./A)	r^2	NOEC[ab]
Cabbage	Shoot length	> 1.4	> 1.4	NA	1.4
	Shoot weight	> 1.4	> 1.4	NA	1.4
	Root weight	> 1.4	> 1.4	NA	1.4
Corn	Shoot length	> 1.4	> 1.4	NA	1.4
	Shoot weight	> 1.4	> 1.4	NA	1.4
	Root weight	> 1.4	> 1.4	NA	1.4
Cucumber	Shoot length	> 1.4	> 1.4	NA	1.4
	Shoot weight	> 1.4	> 1.4	NA	1.4
	Root weight	> 1.4	> 1.4	NA	1.4
Lettuce	Shoot length	> 1.4	> 1.4	NA	1.4
	Shoot weight	> 1.4	> 1.4	NA	1.4
	Root weight	> 1.4	> 1.4	NA	1.4
Oat	Shoot length	> 1.4	> 1.4	NA	1.4
	Shoot weight	> 1.4	> 1.4	NA	1.4
	Root weight	> 1.4	> 1.4	NA	1.4

NOTE: 1.4 lb A.I./A = Maximum application rate
NA = Not applicable
[a] Based on measured concentration (see Table 46)
[b] NOEC = No Observed Effect Concentration

Table 77. Continued.

Species	Parameter	EC25[a] (lb A.I./A)	EC50[a] (lb A.I./A)	r^2	NOEC[ab]
Onion	Shoot length	> 1.4	> 1.4	NA	1.4
	Shoot weight	> 1.4	> 1.4	NA	1,4
	Root weight	> 1.4	> 1.4	NA	1.4
Radish	Shoot length	> 1.4	> 1.4	NA	1.4
	Shoot weight	> 1.4	> 1.4	NA	1.4
	Root weight	> 1.4	> 1.4	NA	1.4
Ryegrass	Shoot length	> 1.4	> 1.4	NA	1.4
	Shoot weight	> 1.4	> 1.4	NA	1.4
	Root weight	> 1.4	> 1.4	NA	1.4
Soybean	Shoot length[c]	1.4	3.2	0.90	0.69
	Lower 95% C.I.[d]	0.89	1.9		
	Upper 95% C.I.	2.6	11		
	Shoot weight[e]	1.0	2.4	0.58	0.69
	Lower 95% C.I.	0.027	1.4		
	Upper 95% C.I.	2.4	4.8		
	Root weight[e]	0.69	2.77	0.24	1.4
	Lower 95% C.I.	---[f]	---		
	Upper 95% C.I.	---	---		
Tomato	Shoot length	> 1.4	> 1.4	NA	< 1.4
	Shoot weight	> 1.4	> 1.4	NA	< 1.4
	Root weight	~ 1.4	> 1.4	NA	< 1.4

NOTE: 1.4 lb A.I./A = Maximum application rate
NA = Not applicable
[a] Based on measured concentration (see Table 46)
[b] NOEC = No Observed Effect Concentration
[c] Test concentrations used in EC25 and EC50 calculations are 1.4 and 0.69 lb A.I./A.
[d] C.I. = Confidence interval
[e] Test concentrations used in EC25 and EC50 calculations are 1.4, 0.69, 0.35, 0.20 and 0.092 lb A.I./A.
[f] Confidence intervals could not be calculated for this data set.

lettuce radicle length

Summary Statistics and ANOVA

Transformation = None

Group rate (lb ai/A)*	n	Mean	s.d.	cv%
1 = control	3	19.2000	.8000	4.2
2 0.078	3	19.6333	1.0066	5.1
3 0.16	3	20.2333	1.1015	5.4
4 0.31	3	21.0667	.8963	4.3
5 0.69	3	19.1667	2.6502	13.8
6 1.3	3	18.6667	2.2030	11.8

1 = solvent control

NOEC = 1.3 lb ai/A *

LOEC = >1.3 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

* based on measured concentrations.

Minumum detectable difference for Dunnett's test = -3.285772
This difference corresponds to -17.11 percent of control

Between groups sum of squares = 11.249444 with 5 degrees of freedom.

Error mean square = 2.591111 with 12 degrees of freedom.

Bartlett's test p-value for equality of variances = .490

cucumber emergence

Summary Statistics and ANOVA

Transformation = None

Group rate (lb ai/A)*	n	Mean	s.d.	cv%
1 = control	3	90.0000	10.0000	11.1
2 0.07	3	83.3333	11.5470	13.9
3 0.15	3	100.0000	.0000	.0
4 0.30	3	93.3333	5.7735	6.2
5 0.58	3	93.3333	5.7735	6.2
6* 1.2	3	56.6667	5.7735	10.2

1 = solvent control

NOEC = 0.58 lb ai/A*

LOEC = 1.2 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

* - based on measured concentrations transformed by 0.78 (percent of theoretically applied).

Minumum detectable difference for Dunnett's test = -15.214515
This difference corresponds to -16.91 percent of control

Between groups sum of squares = 3561.111111 with 5 degrees of freedom.

Error mean square = 55.555556 with 12 degrees of freedom.

```
**********************************************
*                                            *
* Warning - the test for equality of variances *
* could not be computed as 1 or more of the  *
* variances is zero.                         *
*                                            *
**********************************************
```

Soybean root weight

Summary Statistics and ANOVA

Transformation = None

Group rate (lb ai/A)*	n	Mean	s.d.	cv%
1 = control	3	.1029	.0213	20.7
2 0.2	3	.0967	.0139	14.4
3 0.5	3	.0955	.0161	16.9
4 0.8	3	.0930	.0218	23.5
5 1.6	3	.0944	.0087	9.2
6 3.2	3	.0771	.0058	7.5

1 = negative control
25% inhibition at 3.2 lb ai/A
∴ NOEC = 1.6 lb ai/A*
LOEC = 3.2 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

* based on measured concentrations transformed by 2.3 (amount over applied to each pot).

Minumum detectable difference for Dunnett's test = -.032220
This difference corresponds to -31.31 percent of control

Between groups sum of squares = .001121 with 5 degrees of freedom.

Error mean square = .000249 with 12 degrees of freedom.

Bartlett's test p-value for equality of variances = .590