RECORD NUMBER: 211201
SCHAUGHNESSEY NO: 006418-8

EEB REVIEW

DATE IN: 1-11-88          OUT: 2-18-88

FILE OR REG. NO: 55638-L
PETITION OR EXP. NO:
DATE OF SUBMISSION:
DATE RECEIVED BY HED: 1-11-88
RD REQUESTED COMPLETION DATE: 2-8-88
EEB ESTIMATED COMPLETION DATE: 2-8-88
RD ACTION CODE/TyPE OF REVIEW: 117
TYPE PRODUCT(S): Fungicide
DATA ACCESSION NO(S): 403848-14 to 403848-23
PRODUCT MANAGER AND NO: 21
PRODUCT NAME(S) Dagger
COMPANY NAME: Ecogen Incorporated
SUBMISSION PURPOSE: Registration of this biofungicide as an in-furrow treatment for damping-off in cotton

SCHAUGHNESSEY NUMBER CHEMICAL AND FORMULATION % A.I.
----------------------------------------
006418-8 Pseudomonas fluorescens 20 *

* Contains at least 1 billion colony-forming units per 40 lb bag
ECOLOGICAL EFFECTS BRANCH REVIEW

100 SUBMISSION PURPOSE AND LABEL INFORMATION

100.1 SUBMISSION PURPOSE AND PESTICIDE USE

Ecogen Inc. has submitted an application for full registration of its biofungicide Dagger™, containing Pseudomonas fluorescens for use on cotton to control "damping-off, seed rot and seedling rot. This microorganism is a soil isolate and has not been altered or improved.

100.2 FORMULATION INFORMATION (excerpt from label)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percent (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active:</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>20*</td>
</tr>
<tr>
<td>Inert</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

* Contains at least 1 billion colony forming units (cfu) per bag.

100.3 APPLICATION METHODS, DIRECTIONS, RATES

There were no directions for use or recommended application rates on the label.

100.4 TARGET ORGANISM

Pythium spp.
Rhizoctonia solani

100.5. PRECAUTIONARY LABELING

"Keep out of lakes, ponds or streams. Do not contaminate water by cleaning of equipment or disposal of wastes."

101. HAZARD ASSESSMENT

101.1 DISCUSSION

Dagger™, which is composed of a naturally occurring, nonmanipulated microorganism, is a candidate for registration as a biofungicide for use on cotton as an in-furrow treatment at planting time. There were no application rates specified on the label, thus, it is not possible to determine the per-acre concentration of this microbe.

The species known as Pseudomonas fluorescens comprise a rather diverse complement of microorganisms that occur in both
soil and aquatic microhabitats. They can be isolated from these sources using enrichment with appropriate media. The optimum growth temperature is reported to be 25–30 °C. The saprophytic species are classified on their biochemical characteristics and G + C ratios into five biovars, A, B, C, F and G. The varieties comprising these biovars are not known to be plant pathogens. P. fluorescens can be readily differentiated from the fluorescent, phytopathogenic species P. syringae, P. virisflava and P. cichorii.

P. fluorescens has been associated with disease in fish. In most cases identification was confidently established at the genus level but identification to the species level was accomplished in only about half of the cases. Whether the isolated strains were the primary infecting species or secondary invaders is moot.

Bacteria have been shown to be transported, both from soil surfaces through runoff and through the subsoil by hydraulic pressure, to aquatic systems. The details, such as speed, death rate and regrowth have not been clearly elucidated. Therefore, it is not possible to predict whether or not P. fluorescens will move from the application site, at what speed or what will happen to its numbers if it reaches an aquatic ecosystem.

101.2 LIKELIHOOD OF ADVERSE EFFECTS TO NONTARGET ORGANISMS

There was no application rate or application method for Dagger™ on the label that was submitted with the application for registration. However, the plant studies performed in support of this registration were said to be done at levels equivalent to the application rate in support of this registration, the application rate, at least 15 lb/A. Using the potency stated on the label (1 x 10⁹ colony forming units/40 lb), one can calculate the approximate number of cfu per acre. This value is 3.8 x 10⁸ cfu/A.

Terrestrial wildlife.

Avian testing on the active ingredient, P. fluorescens, by both the oral and injection route showed no adverse effects at levels of 6.9 x 10⁴ and 4.6 x 10⁴ colony forming units per bird. At these levels, especially when administered via a parenteral route, coupled with the fact that EEB knows of no avian pathogens in the genus Pseudomonas lead EEB to believe with very high confidence that there will not be any adverse effects to avian species through the use of Dagger™ on cotton. Likewise, injection studies using mice show no pathogenic or toxic signs attributable to P. fluorescens. Therefore, EEB believes that use of this product will not cause adverse effects to feral mammals.

Aquatic organisms.

2
Because of the inability to predict with some measure of confidence the number of bacteria reaching aquatic ecosystems from terrestrial applications and what, in terms of death or regrowth, will occur once they reach the ecosystem, the effect on aquatic organisms that may be affected by *P. fluorescens* must be rigorously tested.

The major concern in aquatic systems is fish. *P. fluorescens* has been associated with disease in fish. EEB is not aware of disease occurrences with *Pseudomonas* in aquatic invertebrates. Because no acceptable fish studies have been submitted, EEB cannot complete an aquatic risk assessment at this time.

**Nontarget insects.**

Since this is an in-furrow treatment to be applied at planting time, no insects are expected to be exposed to the active ingredient. Therefore, EEB does not expect any adverse effects on beneficial insects.

**Nontarget plants.**

Testing on nontarget plants showed that, in most cases, the active ingredient in *Dagger™* caused an increase in growth after germination. The exceptions were cabbage, which showed a significant decrease in seedling height, and lettuce, which showed a significant decrease in fresh weight at the end of four weeks. No phytotoxic effects were seen in any of the species tested.

The data taken as a whole indicate that, in general, *Dagger™* should not pose a threat to most plants. There is the possibility that some crop species may be affected by the application of *Dagger™*. Tier II studies may confirm and quantify this negative effect.

**101.3 ENDANGERED SPECIES CONSIDERATION**

EEB does not feel that there will be a "may effect" situation for endangered terrestrial wildlife, insects or plants through the use of *Dagger™* on cotton. No determination of "may affect" can be made for endangered fish until EEB receives a pathogenicity study for fish.

**101.4 ADEQUACY OF THE TOXICITY DATA**

**Avian Studies**

Both the avian oral and injection tests are considered to be "core" and are usable for hazard assessment. The results of these tests show that *P. fluorescens* is not pathogenic or toxic to avian species.
insufficient test duration which, EEB feels did not allow time for a pathogenic process, if one existed, to become evident. Thus, no assessment of hazard to aquatic organisms can be made at this time.

Nontarget insect testing

None of the tests submitted under this category are suitable for hazard assessment.

Nontarget plant testing

The studies submitted are not classified as "core" because the identity and concentration of the "biocontrol agent" used was not unequivocally reported. These tests do show, however, that, as a whole, Dagger™ is not phytotoxic or phytopathogenic to plants. In fact, the peat substrate may provide nutrients that will assist many species of plants.

101.5 ADEQUACY OF LABELING

In order to be consistent with current EEB Precautionary Labeling, the precautionary statement on the draft label should be reworded as follows:

"Keep out of lakes, ponds or streams. Do not contaminate water when disposing of equipment washwaters."

102 CLASSIFICATION NA

103 CONCLUSIONS

Ecological Effects Branch has reviewed the application by Ecogen Inc to register Dagger™ biofungicide, containing Pseudomonas fluorescens, for use on cotton as an in-furrow treatment. EEB cannot complete its risk assessment at this time due to a lack of toxicity/pathogenicity data. The most critical of these data are the toxicity/pathogenicity study on freshwater fish (Guideline Ref. No: 154-19). EEB recommends against any form of registration for this product until the results of this test are submitted.

After submission and review of the above-mentioned test, EEB is inclined to either recommend against registration, if the data show pathogenicity, or a conditional registration. The condition being that all flawed (invalid) Tier I testing be repeated or repaired so that EEB can have a complete toxicity/pathogenicity profile of this microorganism. These tests and repairability are:

1. 154-20 Toxicity/Pathogenicity to Freshwater Aquatic Invertebrates

This test is not repairable and must be repeated.
1. 154-20 Toxicity/Pathogenicity to Freshwater Aquatic Invertebrates

This test is not repairable and must be repeated.

2. 121-1 Target Area Phytotoxicity Tests (2)

These tests may be upgraded to core if the percent active ingredient is reported and the test microorganism is confirmed as the same one being proposed for registration.

3. 122-1 Seedling Germination/Vegetative Vigor (Tier I)

This test may be upgraded to core if the microorganism under test is confirmed as the same as the one being registered.

4. 154-23 Nontarget insect testing

These studies, although invalid, will not have to be resubmitted. The in-furrow use pattern will not result, in EEB's opinion in exposure to nontarget insects. However, if the product will be registered for an above-ground use, full nontarget insect testing will be required. The Agency has interim protocols available, upon request, which may be used to develop appropriate testing protocols for nontarget insect testing.

Lastly, EEB recommends that any registration for this product be conditional on performance of Tier II Seedling Germination/Vegetative Vigor Tests in order to quantify the effects seen in Tier I testing.

Robert W. Pilucki, Microbiologist
Ecological Effects Branch
Hazard Evaluation Division (TS-769C)

Raymond W. Matheny, Head, Section 1
Ecological Effects Branch
Hazard Evaluation Division (TS-769C)

Harry T. Craven, Acting Chief
Ecological Effects Branch
Hazard Evaluation Division (TS-769C)
1. **CHEMICAL**: *Pseudomonas fluorescens*  
   **SN**: 006418-8

2. **TEST MATERIAL**:  
   The test material used in this study was a washed suspension of *Pseudomonas fluorescens* strain 1053. The concentration of the cell suspension was $4.6 \times 10^8$ CFU/mL.

3. **STUDY/ACTION TYPE**: Nontarget insect test

4. **STUDY IDENTIFICATION**:  
   **Study sponsor**: Ecogen Inc. **Study location**: Easton, MD. EPA Acc. No: 403848-18.

5. **REVIEWED BY**:  
   Robert W. Pilsucki, Microbiologist  
   Ecological Effects Branch  
   Hazard Evaluation Division  
   **Signature**:  
   **Date**: 2/18/88

6. **APPROVED BY**:  
   Raymond W. Matheny, Head, Section 1  
   Ecological Effects Branch  
   Hazard Evaluation Division  
   **Signature**:  
   **Date**: 2/18/88

7. **CONCLUSIONS**:  
   This study is considered to be invalid because the study duration was only 2 days instead of the recommended 30 days. This study does not fulfill the guideline requirement for a honey bee test.

8. **RECOMMENDATION**:  
   This test should be repeated.
9. **BACKGROUND:** NA

10. **DISCUSSION OF INDIVIDUAL STUDIES OR TESTS:** NA

11. **METHODS AND MATERIALS:**

   **Species.** Honey bee (*Apis mellifera*)

   **Source and handling.**

   Wildlife International's Bee Colony
   Easton, MD

   Honey bee pupae were placed in an incubator seven days prior to test initiation. At the beginning of the study, all bees were immobilized with nitrogen and 25 bees were placed in each test chamber.

   **Test chambers.**

   The test chambers were paper containers measuring 87 mm in diameter x 85 mm in height. Each container was covered by a petri dish. Each test chamber contained a 20 mL vial containing a 50% sucrose/water solution. This served as a food source for the bees throughout the study.

   **Treatment levels and group size.**

   There were 5 treatment levels of 0.1, 0.2, 0.42, 0.88 and 2 mg of test material per bee. There were two replicates of 25 bees for each treatment group.

   **Dosing.**

   The test material was weighed out and a solution containing 1 mg/μL. Treatment chambers were selected by random draw. Bees were immobilized using nitrogen. A 2 μL dose was delivered to the thorax/abdominal area using a digital micropipet.

   **Controls.**

   There were two control groups, negative and solvent, containing two replicates of 25 beetles were used. The controls were manipulated identically to the treatment group except that they were not dosed.

   **Number of insects/concentration.** 50
Environmental conditions.

Temperature: 22 - 26 °C

Humidity: 78%

Observations.

Bees were observed daily for mortality and signs of toxicity.

Statistical analysis.

An estimated LD50 was made by visual inspection of the data.

12. REPORTED RESULTS:

The authors reported no control mortality. Mortalities in the treatment groups did not exceed 2%. The estimated LD50 was reported as greater than 2 mg/bee. The no-observed effect level was reported as 2 mg/bee.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The authors concluded that the mortalities in the treatment group were not treatment-related. They also concluded that the test material was relatively nontoxic to the ladybird beetle, according to the toxicity categories of Atkins (1).

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

A. Test Procedure. The procedure used in this study deviated significantly from that recommended in EPA's Pesticide Assessment Guidelines: Subdivision M. Specifically, the test was only carried out for 2 days rather than the recommended 30 days.

B. Statistical Analysis. These data are not amenable to statistical treatment.

C. Results/Discussion. The main problem with this study is that the observation period was not long enough to provide a high level of confidence that P. fluorescens is not pathogenic to the honey bee. P. fluorescens is not known to be a frank pathogen of

insects. Thus, when testing such a microorganism, every effort should be made to allow pathogenesis to occur. One of the ways is to observe the insects for an extended period of time after dosing. While 2 days may be long enough for chemical toxicity to occur, it is not enough time for a weakly pathogenic process to manifest itself.

Closely connected with pathogenesis is the route of exposure. It is unlikely that contact dosing would show a positive response except for microorganisms, such as fungi, that elaborate extracellular chitinases to allow penetration of the exoskeleton. A more appropriate route of dosing, in this reviewer's opinion, would be some method of oral/feeding exposure.

D. Adequacy of the Study,

1. Category: Invalid

2. Rationale: The study was not carried out for a duration sufficiently long to rule out \textit{P. fluorescens} as a pathogen of the honey bees.

3. Repairability: None

15. \textbf{COMPLETION OF ONE-LINER}

One-liner not done
DATA EVALUATION RECORD

1. CHEMICAL: Pseudomonas fluorescens       SN: 006418-8

2. TEST MATERIAL:

   The test material used in this study was a washed suspension of Pseudomonas fluorescens strain 1053. The concentration of the cell suspension was 4.6 x 10^8 CFU/mL.

3. STUDY/ACTION TYPE: Nontarget insect test

4. STUDY IDENTIFICATION:


5. REVIEWED BY:

   Robert W. Pilsucki, Microbiologist
   Ecological Effects Branch
   Hazard Evaluation Division
   Signature: __________________________  Date: 2/18/88

6. APPROVED BY:

   Raymond W. Matheny, Head, Section 1
   Ecological Effects Branch
   Hazard Evaluation Division
   Signature: __________________________  Date: 2/18/88

7. CONCLUSIONS:

   This study is considered to be invalid because the study duration was only 2 days instead of the recommended 30 days. This study does not fulfill the guideline requirement for a nontarget insect test.

8. RECOMMENDATION:

   This test should be repeated.
9. BACKGROUND: NA

10. DISCUSSION OF INDIVIDUAL STUDIES OR TESTS: NA

11. METHODS AND MATERIALS:

Species. Brachymeria ovata

Source and handling.

Colorado Department of Agriculture
Palisade, CO

Adult wasps were placed in individual 87 mm x 85 mm high paper containers upon receipt. Each container was covered by a petri dish. A 20 mL vial of honey was placed in the containers and served as a food source for the wasps.

Test chambers.

The test chambers were the same type of paper container as described above.

Treatment levels and group size.

There were five treatment levels of 0.1, 0.2, 0.42, 0.88 and 2 mg of test material per wasp. There were two replicates of 25 wasps for the treatment group.

Dosing.

The test material was weighed out and a solution containing 1 mg/µL. Treatment chambers were selected by random draw. The appropriate volume for a particular dose was delivered to the thorax/abdominal area using a digital micropipet.

Controls.

A control group containing three replicates of 10 wasps was used. The controls were manipulated identically to the treatment group except that they received a volume of acetone.

Environmental conditions.

Temperature: 24 °C

Humidity: 78%

Wasps were misted daily to increase the humidity in the test chambers.
Number of insects/concentration. 50

Observations.

Wasps were observed daily for mortality and signs of toxicity.

Statistical analysis.

An estimated LD50 was made by visual inspection of the data.

12. REPORTED RESULTS:

The authors reported a negative and solvent control mortalities of 10\% (5/50) and 6\% (3/50) respectively. Mortalities in the treatment group were:

<table>
<thead>
<tr>
<th>Concentration (mg/bee)</th>
<th>Number Exposed</th>
<th>Number Dead</th>
<th>Percent Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>50</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>0.88</td>
<td>50</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0.42</td>
<td>50</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0.2</td>
<td>50</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>0.1</td>
<td>50</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

The estimated LD50 was reported as greater than 2 mg/wasp.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The authors concluded that the mortalities in the treatment group were not treatment-related. They also concluded that the test material was relatively nontoxic to the parasitic wasp, Brachymera ovata according to the toxicity categories of Atkins (1).

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

A. Test Procedure. The procedure used in this study deviated significantly from that recommended in EPA's Pesticide Assessment Guidelines: Subdivision M. Specifically, the test was only carried out for 2 days rather than the recommended 30 days.

B. **Statistical Analysis.** These data are not amenable to statistical treatment.

C. **Results/Discussion.** The main problem with this study is that the observation period was not long enough to provide a high level of confidence that *P. fluorescens* is not pathogenic to the parasitic hymenoptera, *Brachymeria ovata*. *P. fluorescens* is not known to be a frank pathogen of insects. Thus, when testing such a microorganism, every effort should be made to allow pathogenesis to occur. One of the ways is to observe the insects for an extended period of time after dosing. While 2 days may be long enough for chemical toxicity to occur, it is not enough time for a weakly pathogenic process to manifest itself.

Closely connected with pathogenesis is the route of exposure. It is unlikely that contact dosing would show a positive response except for microorganisms, such as fungi, that elaborate extracellular chitinases to allow penetration of the exoskeleton. A more appropriate route of dosing, in this reviewer’s opinion, would be some method of oral/feeding exposure.

D. **Adequacy of the Study.**

1. Category: Invalid

2. Rationale: The study was not carried out for a duration sufficiently long to rule out *P. fluorescens* as a pathogen of the parasitic hymenoptera, *Brachymeria ovata*.

3. Repairability: None

**15. COMPLETION OF ONE-LINER**

One liner not done
DATA EVALUATION RECORD

1. **CHEMICAL:** *Pseudomonas fluorescens*  
   **SN:** 006418-8

2. **TEST MATERIAL:**
   The test material used in this study was, ECONEG Pf G, a granular formulation of *Pseudomonas fluorescens*. The percent active ingredient was not reported.

3. **STUDY/ACTION TYPE:** Target area phytotoxicity study

4. **STUDY IDENTIFICATION:**

5. **REVIEWED BY:**
   Robert W. Pilsucki, Microbiologist  
   Ecological Effects Branch  
   Hazard Evaluation Division

   **Signature:**  
   **Date:** 2/15/85

6. **APPROVED BY:**
   Raymond W. Matheny, Head, Section 1  
   Ecological Effects Branch  
   Hazard Evaluation Division

   **Signature:**  
   **Date:** 2/15/88

7. **CONCLUSIONS:**
   This test is classified as supplemental because the percent active ingredient in the test material was not reported. This test does not fulfill the guideline requirement for nontarget plant testing.

8. **RECOMMENDATION:**
   This test may be upgraded to core status if the percent active ingredient in the test material is submitted.
9. **BACKGROUND:** NA

10. **DISCUSSION OF INDIVIDUAL STUDIES OR TESTS:** NA

11. **METHODS AND MATERIALS:**

    **Test plant species.**


    **Test area description.**

    See attachment.

    **Controls.**

    There was a control (check) row of untreated plants within each 3-row replicate.

    **Application methods.**

    The test material was applied, by hand, as a furrow application at planting time. Rates of application were 15 lb/A and 30 lb/A.

    **Test duration.** 46 days

    **Environmental conditions.**

    Temperature (air/soil): 85/78 °F

    Humidity: 60%, soil was moist.

    First rain: 8/3/87  Amount: 0.40 inch

    There was no supplemental irrigation.

    **Observations.**

    Plants were observed at 7, 14, 28 and 46 days after planting for phytotoxicity, including injury or stress symptoms, and for effects on growth, development and morphology. Effects were scored on a scale of 0 to 10 (0 = no effect; 10 = maximum effect). In addition, the number of plants emerging in each plot was determined.

    **Statistical analysis.**

    The data were analyzed using Analysis of Variance.
12. REPORTED RESULTS:

The authors reported that there were no phytotoxic symptoms or effects on growth, development or morphology exhibited by the treated plants during the test. All plants appeared to be healthy. In addition, the stand was not significantly affected by the treatments. They pointed out, however, that there appeared to be a reduction in stand for variety Acala SJ-2 but the high variability resulted in no significant differences when analyzed statistically.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

There were no formal conclusions. There was a quality assurance statement attached to the study.

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

A. Test Procedure. The procedure used in this study generally follows that outlined in the Standard Evaluation Procedure for Nontarget Plants: Target Area Testing except the percent active ingredient in the formulation was not reported.

B. Statistical Analysis.

Results of EEB's validation of the statistical analysis using an ANOVA coupled with the Duncan's Multiple Range Test is in close agreement with that of the authors.

C. Results/Discussion.

These results indicate that the test material is neither phytotoxic or phytopathogenic to the varieties of cotton tested. The effect on Acala SJ-2 may or may not be biologically relevant; the variability is too large to make a determination.

D. Adequacy of the Study.

1. Category: Supplemental

2. Rationale: The percent active ingredient in the test material was not reported.

3. Repairability: If information on the percent active ingredient in the test material is reported, this study may be upgraded to core status.

15. COMPLETION OF ONE-LINER

No one-liner done.
DATA EVALUATION RECORD

1. CHEMICAL: Pseudomonas sp.  
   SN: 006418-8

2. TEST MATERIAL:  
The test material used in this study was Pseudomonas sp that was formu- 
This is a formulated product. The concentration of the Pseudomonas was not 
 stated.

3. STUDY/ACTION TYPE: Seedling emergence/Vegetative vigor test

4. STUDY IDENTIFICATION:  
Canez, V.M. and P.A. Jones. Nontarget Phytotoxicity Study Seed 
Germination/Seedling Emergence Stewart Agricultural Research 

5. REVIEWED BY:  
Robert W. Pilucki, Microbiologist  
Ecological Effects Branch  
Hazard Evaluation Division  
Signature:  
Date: 2/17/88

6. APPROVED BY:  
Raymond W. Matheny, Head, Section 1  
Ecological Effects Branch  
Hazard Evaluation Division  
Signature:  
Date: 2/18/88

7. CONCLUSIONS:  
This study is considered to be supplemental until proper 
identification of the species of Pseudomonas (with regard to the 
material that is to be registered) is supplied. This study does 
not fulfill the guideline requirement for a nontarget plant 
study. In addition, Tier II dose response testing for seed 
germination and seedling emergence is recommended.

8. RECOMMENDATION:  
A) Submit proper microbial identification of the test 
material  
B) Perform Tier II Seed germination and Seedling Emergence 
Testing

*NOT INGREDIENT INFORMATION IS NOT INCLUDED*
9. **BACKGROUND:** NA

10. **DISCUSSION OF INDIVIDUAL STUDIES OR TESTS:** NA

11. **METHODS AND MATERIALS:**

   **Test plant species.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean (<em>Glycine max</em>)</td>
<td>Bradley Seed Service</td>
</tr>
<tr>
<td>Lettuce (<em>Lactuca sativa</em>)</td>
<td>Asgrow Seed Co.</td>
</tr>
<tr>
<td>Carrot (<em>Daucus carota</em>)</td>
<td>Germain's</td>
</tr>
<tr>
<td>Tomato (<em>Lycopersicon esculentum</em>)</td>
<td>Petoseed</td>
</tr>
<tr>
<td>Cucumber (<em>Cucumis sativus</em>)</td>
<td>Asgrow Seed Co.</td>
</tr>
<tr>
<td>Cabbage (<em>Brassica oleracea</em>)</td>
<td>Asgrow Seed Co.</td>
</tr>
<tr>
<td>Oat (<em>Avena sativa</em>)</td>
<td>Germain's</td>
</tr>
<tr>
<td>Perennial Ryegrass (<em>Lolium perenne</em>)</td>
<td>Valley Seed Co.</td>
</tr>
<tr>
<td>Corn (<em>Zea mays</em>)</td>
<td>Asgrow Seed Co.</td>
</tr>
<tr>
<td>Onion (<em>Allium cepa</em>)</td>
<td>Germain's</td>
</tr>
</tbody>
</table>

   **Test container.**
   Size/Volume: 7.5 x 7.5 x 6.0 cm

   **Number of seeds/container:** 10

   **Controls.**
   There was a negative control (n=10) performed concurrently with each species of plant.

   **Exposure method for seed germination test.**
   A concentration of test material equivalent to the application rate (15 lb/A) was added to filter paper in a plastic petri dish. Ten seeds, replicated 3 times, were placed on the filter paper. Each group was incubated at 25 °C in the dark for five days.

   **Potting material used in the seedling emergence test.**
   The material used was sterilized loamy sand soil that had been previously screened. Moisture determinations were made prior to the study.

   **Formulation and incorporation of test material into the potting material for the seedling emergence test.**
   All treatment groups in the seedling emergence test had the test material (*Pseudomonas sp*) incorporated into the soil at a rate equivalent to the application rate, 15 lb/A,
the application rate. After the additions, the soil was mixed in a cement mixer for 5 minutes.

**Test duration.**

Seed germination: 5 days  
Seedling emergence: 4 weeks

**Environmental conditions for the seedling emergence test.**

All of the study was carried out in a greenhouse.

Misting: 3 times/day

Temperature: 19 - 38 °C

Humidity: 40 - 100%  
Lighting: The greenhouse was covered with a 54% shade cloth to simulate average sunshine. Light measurements at canopy level were 1424, 1985 and 2038 at 9:00 AM, 12:00 PM and 3:00 PM respectively. Sunrise ranged from 5:53 to 6:12 AM and sunset ranged from 7:44 to 8:05 PM.

**Observations.**

Seed Germination: At the end of 5 days, the length of the radical was measured. Germination was scored as positive if the radicle was at least 5 mm long.

Seedling emergence: Treatment groups were observed weekly for the number of seedlings emerging and phytotoxic signs. At the end of the period, shoot length and fresh weight was determined for each plant.

**Statistical analysis.**

Using mean values for each treatment group, the percent effect for seed germination, number of seedlings emerged, final plant height and fresh weight were determined. Results were then analyzed using Duncan's multiple range test.

12. **REPORTED RESULTS:**

**Seed germination**

See appendix for tabular material. The authors reported that the test material had no significant detrimental effect on seed germination or radicle of any species of plant. The percent detrimental effect for seed germination ranged from 15% in ryegrass to 8% in cabbage. A significant increase in radicle length was observed in lettuce and cabbage. Greater than 25%
increases in radicle length were observed in ryegrass, carrot, lettuce, and soybean.

Seedling emergence

See appendix for tabular material. There were no phytotoxic effects exhibited during the test. Treatment did not result in a significant difference in emerged seeds except in cabbage where an increase of 26% was noted. A significant decrease in plant height was noted in cabbage and a significant increase was noted in ryegrass. Oat, cucumber and tomato showed greater than 25% increases in plant height, although these increases were not statistically significant.

Statistically significant changes in fresh weight occurred on cucumber, oat and ryegrass. Species showing 25% or greater decreases in fresh weight were carrot and lettuce. Species showing 25% or greater increases in fresh weight were oat and ryegrass. The authors reported that addition of the test material did not produce any visible phytotoxic effect during the growing period.

3. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The authors concluded that treatment with the test material did not affect the percentage of germinated and emerged seeds although significant changes in radicle length, plant height and fresh weight were noted. Lettuce was the only species to show a greater than 25% decrease in plant height and fresh weight.

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

A. Test Procedure. The procedure used in this study generally follows that outlined in the Standard Evaluation Procedure for Nontarget Plants; Seed germination and Seedling Emergence except that the identity to species level of the Pseudomonas use was not reported.

B. Statistical Analysis.

Results of EEB's validation of the statistical analysis using an ANOVA coupled with the Duncan's Multiple Range Test is in close agreement with that of the authors.

C. Results/Discussion.

It appears that treatment of the plant species under study caused either detrimental or overstimulation effects, depending on species. According the EPA's Standard Evaluation Procedure for Nontarget Plants: Seed Germination and Seedling Emergence, if

INERT INGREDIENT INFORMATION IS NOT INCLUDED
either of those situations occur, then progression to Tier II testing (dose-response testing) is required. Therefore, Tier II testing for Seed Germination and Seedling Emergence is recommended.

The major deviation found in this study, namely the failure to identify, to the species level, is necessary to allow application of these results to the microorganism presently under review for registration.

D. Adequacy of the Study.

1. Category: Supplemental

2. Rationale: The microorganism in the test material was not identified to the species level.

3. Repairability: This study may be upgraded to core status if the full identity of the microorganism in the test material is supplied.

15. COMPLETION OF ONE-LINER

   No one-liner done.
DATA EVALUATION RECORD

1. CHEMICAL: Pseudomonas sp. SN: 006418-8

2. TEST MATERIAL:
   The test material used in this study was Pseudomonas sp that This is a formulated product. The concentration of the Pseudomonas was not stated.

3. STUDY/ACTION TYPE: Target area phytotoxicity study

4. STUDY IDENTIFICATION:

5. REVIEWED BY:
   Robert W. Pilsucki, Microbiologist
   Ecological Effects Branch
   Hazard Evaluation Division
   Signature: [Signature]
   Date: 2/18/88

6. APPROVED BY:
   Raymond W. Matheny, Head, Section 1
   Ecological Effects Branch
   Hazard Evaluation Division
   Signature: [Signature]
   Date: 2/18/88

7. CONCLUSIONS:
   This study is classified as supplemental because the microorganism in the test material was not adequately identified. This study does not fulfill the guideline requirement for nontarget plant testing.

8. RECOMMENDATION:
   The full identity of the microorganism in the test material should be reported.

SECRET INGREDIENT INFORMATION IS NOT INCLUDED
9. BACKGROUND: NA

10. DISCUSSION OF INDIVIDUAL STUDIES OR TESTS: NA

11. METHODS AND MATERIALS:

Test plant species. Cotton, var Acala

Test container.
Size/Volume: 7.5 x 7.5 x 6.0 cm
Number of seeds/container: 10

Potting material.

The material used was sterilized loamy sand soil that had been previously screened. Moisture determinations were made prior to the study.

Controls.

There was a negative control performed concurrently with the treatment groups. The control group contained 40 plants.

Formulation and incorporation of test material into the potting material.

All treatment groups had the test material (Pseudomonas sp) incorporated into the soil at a rate equivalent to the application rate, 15 lb/A, as well as 2X and 4X the application rate. One treatment group had the test material only and the other was co-inoculated with Rhizoctonia solani, the target pest. After additions, the soil was mixed in a cement mixer for 5 minutes.

Preparation and inoculation of the soil with the target pest.

Sterile medium (cornmeal, vermiculite, water; 1:1:2) was inoculated with a plug from a one-week-old R. solani culture and was incubated for 5 weeks. Eight and one-half grams of inoculant was then added to each 1000 grams (dry weight) of soil.

Test duration. 4 weeks

Environmental conditions.

All of the study was carried out in a greenhouse.

Misting: 3 times/day
Temperature: 19 - 38 °C

Humidity: 40 - 100%
Lighting: The greenhouse was covered with a 54% shade cloth to simulate average sunshine. Light measurements at canopy level were 1424, 1985 and 2038 at 9:00 AM, 12:00 PM and 3:00 PM respectively. Sunrise ranged from 5:53 to 6:12 AM and sunset ranged from 7:44 to 8:05 PM.

Toxic signs.
Phytotoxic signs were recorded weekly. At the end of the study, all plants were measured for fresh weight.

Statistical analysis.
Mean values for final plant height and fresh weight were analyzed using Duncan's Multiple Range Test.

12. REPORTED RESULTS:

The authors reported that all seeds in soils without R. solani germinated within one week and appeared normal throughout the study. Seeds planted in infested soil germinated more slowly and showed signs of damping off one to two weeks after planting. By two weeks after planting, plants in the infested soil ceased to grow and fungal mycelia were visible.

In noninfested soils (no R. solani), there were no significant differences (p<.05) in plant height or fresh weight between any groups containing Pseudomonas and control plants.

<table>
<thead>
<tr>
<th>Conc. of Pseudomonas (ppm)</th>
<th>Infested/Noninfested (N/I)</th>
<th>Mean 1 Height (cm)</th>
<th>Mean Fresh Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>N</td>
<td>20.2</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>0.0</td>
<td>NR</td>
</tr>
<tr>
<td>112.5</td>
<td>N</td>
<td>19.6</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>0.0</td>
<td>NR</td>
</tr>
<tr>
<td>225.0</td>
<td>N</td>
<td>18.8</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>0.0</td>
<td>NR</td>
</tr>
<tr>
<td>450.0</td>
<td>N</td>
<td>19.6</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>0.0</td>
<td>NR</td>
</tr>
</tbody>
</table>

1 = All seedlings destroyed by R. solani
NR = Not Reported

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:
The authors concluded that treatment of sandy loam soil with *Pseudomonas* sp for the control of *R. solani* damping-off fungus did not affect the seedling emergence and growth of Acala cotton seeds. They further concluded that the lack of control by *Pseudomonas* was due to an overabundance of *R. solani* in the infested soil and possible specificity of the biocontrol agent.

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

A. Test Procedure. The procedure used in this study generally follows that outlined in the Standard Evaluation Procedure for Nontarget Plants; Target Area Testing except that the identity to species level of the *Pseudomonas* use was not reported.

B. Statistical Analysis.

Results of EEB's validation of the statistical analysis using an ANOVA coupled with the Duncan's Multiple Range Test is in close agreement with that of the authors.

C. Results/Discussion.

It appears that treatment of cotton seeds with the biofungicide, *Pseudomonas* sp, had no phytopathogenic or phytotoxic effect on the emergence and growth of Acala cotton seedlings, even at rates four times the effective application rate.

The major deviation found in this study, namely the failure to identify, to the species level, is necessary to allow application of these results to the microorganism presently under review for registration.

D. Adequacy of the Study.

1. Category: Supplemental

2. Rationale: The microorganism in the test material was not identified to the species level.

3. Repairability: This study may be upgraded to core status if the full identity of the microorganism in the test material is supplied.

15. COMPLETION OF ONE-LINER No one-liner done
DATA EVALUATION RECORD

1. CHEMICAL: Pseudomonas strain 1053

2. TEST MATERIAL:
   The test material used in this study was identified as TGAI-PSEUDOMONAS STRAIN 1053 (WASHED(218,266),(983,286). The concentration of the test material was 4.6 x 10^8 cells per mL.


4. STUDY IDENTIFICATION:

5. REVIEWED BY:
   Robert W. Pilsucki, Microbiologist
   Ecological Effects Branch
   Hazard Evaluation Division

6. APPROVED BY:
   Raymond W. Matheny, Head, Section 1
   Ecological Effects Branch
   Hazard Evaluation Division

7. CONCLUSIONS:
   This study is classified as core and, as such, fulfills the guideline requirement for an avian injection pathogenicity test.

8. RECOMMENDATION:
   NA
9. BACKGROUND: NA

10. DISCUSSION OF INDIVIDUAL STUDIES OR TESTS: NA

11. METHODS AND MATERIALS:

  Species. Mallard duck (Anas platyrhynchos)

Age. 16 days

Source and rearing history.

   Whistling Wings
   Hanover, IL

   The birds were obtained from Whistling Wings at one day
   of age. They were examined for physical injury and
   acclimated to laboratory conditions until test initiation.
   Each bird was identified with a leg band or web tag.

Selection of test birds.

   Sixty birds were assigned to ten groups without regard
   to sex. It was not reported whether or not the assignment
   was random.

Housing conditions.

   Temperature: 27.2 °C

   Humidity: 71%

   Lighting: 16 hours light/8 hours darkness

   Pen size: 72 x 90 x 24 - first 14 days
             72 x 90 x 33 - balance of study

Food consumption and weight gain. See attached tables.

Diluent.

   There was no diluent used.

Controls.

   Two control groups were run concurrently with the
   treatment groups. Both control groups received sterile
   saline. In addition, there was one contact control bird in
   each of the treatment groups. The contact controls received
   no injections.
Number of birds/concentration. 30

**Dosing method.**

The birds were individually weighed and dosed with enough test material, attenuated strain or saline to achieve a dose of 10,000 mg/Kg or 1% of body weight. The dosed was administered to the peritoneum or air sac using a 3 mL syringe fitted with a 20 ga. needle.

**Observation period.**

The birds were observed twice daily for mortalities, toxic signs and abnormal behavior for a period of 30 days.

**Necropsies.**

All birds were subjected to gross necropsy at the end of the study.

**Statistical analysis.**

No statistical analysis of the data was performed.

12. **REPORTED RESULTS:**

No treatment-related mortalities occurred in any of the groups. Two birds in the treated group were found dead at day 7 with crushed craniums. It was theorized that their heads were caught between the refuse pan and back of the cage during cleaning operations. One bird in the treatment group was observed to have a broken carpal joint and another, wing droop. Neither of these observations were attributed to the treatment. All other birds appeared normal throughout the study.

Gross necropsy revealed no treatment-related findings.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The author concluded that administration of the test material at a dose of 10,000 mg/Kg by intraperitoneal injection showed no apparent pathogenicity or effect on the survival of young mallards.

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

A. **Test Procedure.** The procedure used in this study generally followed that outlined in EPA's Pesticide Assessment Guidelines: Subdivision M. There were no major deviations that would have altered the outcome of the study.
B. Statistical Analysis.

The data are not amenable to statistical analysis.

C. Results/Discussion.

It appears, from the data, that *Pseudomonas fluorescens*, when injected by the intraperitoneal route, had no adverse effects on mallard ducklings. The dose, 10000 mg/Kg, expressed as cfu/mL, is approximately $4.6 \times 10^6$ per bird. While this is probably not the maximum hazard dose that could have been given, EEB feels that, when given by the injection route, this dose is sufficient to assess hazard. The only other comment on this test is that the number of viable cells in the dose should have been checked by microbiological plating. This is not required by present guidelines but will be in the revised guidelines.

D. Adequacy of the Study.

1. Category: Core

2. Rationale: This study is in follows the procedures outlined in EPA's *Pesticide Assessment Guidelines: Subdivision M*.

3. Repairability: NA

15. COMPLETION OF ONE-LINER

One-liner completed 1-28-88.
Page____ is not included in this copy.
Pages __31__ through __32__ are not included.

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___ Identity of product impurities.
___ Description of the product manufacturing process.
___ Description of quality control procedures.
___ Identity of the source of product ingredients.
___ Sales or other commercial/financial information.
___ A draft product label.
___ The product confidential statement of formula.
___ Information about a pending registration action.
___ FIFRA registration data.
___ The document is a duplicate of page(s) ______.
___ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.
DATA EVALUATION RECORD

1. CHEMICAL: *Pseudomonas* strain 1053  
   SN: 006418-8

2. TEST MATERIAL:

   The test material used in this study was identified as TGAI-
   *Pseudomonas* strain 1053 (washed). The strain was not identified at
   the species level. The concentration was 4.6 x 10^8 cells per mL.

3. STUDY/ACTION TYPE: Avian Acute Oral Toxicity/Pathogenicity Test

4. STUDY IDENTIFICATION:

   Beavers, J.B. TGAI-PSEUDOMONAS STRAIN 1053 (WASHED): An Avian
   Acute Oral LD₅₀ Pathogenicity Study in the Mallard. 1987. Project
   No: 235-105. Wildlife International Ltd. Study sponsor: Ecogen

5. REVIEWED BY:

   Robert W. Pilsucki, Microbiologist  
   Ecological Effects Branch  
   Hazard Evaluation Division
   Signature:  
   Date: 3/9/88

6. APPROVED BY:

   Raymond W. Matheny, Head, Section 1  
   Ecological Effects Branch  
   Hazard Evaluation Division
   Signature:  
   Date: 2/18/88

7. CONCLUSIONS:

   This study is classified as core. As such, it fulfills the
   guideline requirement for an Avian Oral Toxicity/Pathogenicity Test.

8. RECOMMENDATION:

   None
9. **BACKGROUND:** NA

10. **DISCUSSION OF INDIVIDUAL STUDIES OR TESTS:** NA

11. **METHODS AND MATERIALS:**

   **Species.** Mallard duck (*Anas platyrhynchos*)

   **Age.** 16 days

   **Source and rearing history.**
   
   Whistling Wings
   Hanover, IL

   The ducklings were purchased from Whistling Wings at one day of age. The ducklings were examined for physical injuries, identified with a leg band or web clip and then acclimated until study initiation.

   **Selection of test birds.**

   Sixty birds were assigned to ten groups without regard to sex. Whether or not this assignment was random was not reported.

   **Housing conditions.**

   Temperature: 27.2° C

   Humidity: 71%

   Lighting: 16 hours light/8 hours darkness

   Pen size: 72 x 90 x 24 cm - first 14 days
   72 x 90 x 33 cm - balance of study

   **Food consumption and weight gain.** See attached tables.

   **Fasting.**

   A fasting period was not reported.

   **Diluent.**

   There was no diluent used.
Controls.

Two control groups were used. One received saline only and the other received attenuated TGAI - Pseudomonas strain 1053 (washed). In addition, a single bird, serving as a contact control, was placed in with each test group.

Number of birds/concentration. 30

Dosing method.

An amount of test material from the stock, supplied by Ecogen, equal to 1.5% body weight was given by gavage. The material was placed in the crop or proventriculus via a stainless steel cannula. After dosing, birds were given food and water ad libitum.

Observation period.

The birds were observed for a period of thirty days after dosing, twice daily. They were observed for mortalities, toxic signs and abnormal behavior.

Necropsy

All birds were subjected to necropsy at the termination of the study.

Statistical analysis.

No statistical analysis was performed.

12. REPORTED RESULTS:

The author reported that there were no mortalities in any of the groups (treated, saline control and attenuated control) and none of the contact controls died. One bird in the control group, one bird in the attenuated group and two birds in the treatment group were found to have a broken or dislocated carpal joint. One bird in the treatment group had wing droop. Regurgitation was noted in one bird in the attenuated group and two birds in the treatment group. None of these observations was determined to be treatment-related.

Upon necropsy, two treated birds and two contact controls were observed to have lung lesions ranging from a single abscess to multiple abscesses in both lungs. These abscesses were not considered to be treatment-related.
13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The author concluded that administration of TGAI-PSEUDOMONAS STRAIN 1053 (WASHED) at a dose of 15,000 mg/Kg showed no pathogenicity or effect on survival of young mallards.

There was a quality assurance statement attached to the study.

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

A. Test Procedure. The procedure used in this study generally follows EPA's Pesticide Assessment Guidelines: Subdivision M. There were no major deviations that would affect the outcome of the test.

B. Statistical Analysis.

The data are not amenable to statistical analysis.

C. Results/Discussion.

It appears, from the data, that *Pseudomonas fluorescens*, when given by gavage, had no adverse effects on mallard ducklings. The dose, 15,000 mg/Kg, expressed as cfu/mL, is approximately $6.9 \times 10^8$ per bird. While this is probably not the maximum hazard dose that could have been given, EEB feels that this dose is sufficient to assess hazard. The only other comment on this test is that the number of viable cells in the dose should have been checked by microbiological plating. This is not required by present guidelines but will be in the revised guidelines.

D. Adequacy of the Study.

1. Category: Core

2. Rationale: This study generally follows the procedures outlined in EPA's Pesticide Assessment Guidelines: Subdivision M.

3. Repairability: NA

15. COMPLETION OF ONE-LINER

One-liner completed 1-28-88.
Pseudomonas

Page____ is not included in this copy.
Pages 37 through 38 are not included.

The material not included contains the following type of information:

___ Identity of product inert ingredients.
___ Identity of product impurities.
___ Description of the product manufacturing process.
___ Description of quality control procedures.
___ Identity of the source of product ingredients.
___ Sales or other commercial/financial information.
___ A draft product label.
___ The product confidential statement of formula.
___ Information about a pending registration action.
___ FIFRA registration data.

The document is a duplicate of page(s) ________.
___ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.
DATA EVALUATION RECORD

1. **CHEMICAL**: *Pseudomonas fluorescens* strain 1053  SN:006418-8

2. **TEST MATERIAL**:
   
The test material used in this study was a washed cell suspension of *P. fluorescens*. The cell density was $4.6 \times 10^6$ cells per mL.

3. **STUDY/ACTION TYPE**: Freshwater fish toxicity/pathogenicity test

4. **STUDY IDENTIFICATION**:
   

5. **REVIEWED BY**:
   
Robert W. Pilsucki, Microbiologist
Ecological Effects Branch
Hazard Evaluation Division

Signature: [Signature]
Date: 2/18/88

6. **APPROVED BY**:

Raymond W. Matheny, Head, Section 1
Ecological Effects Branch
Hazard Evaluation Division

Signature: [Signature]
Date: 2/18/88

7. **CONCLUSIONS**:

This study is considered invalid because the test duration was only 96 hours instead of thirty days as recommended by Guidelines. This study does not fulfill the guidelines requirement for a Freshwater Fish Toxicity/Pathogenicity Test.

8. **RECOMMENDATION**:

This test must be repeated.
9. **BACKGROUND:** NA

10. **DISCUSSION OF INDIVIDUAL STUDIES OR TESTS:** NA

11. **METHODS AND MATERIALS:**

    **Species.** Rainbow trout (*Salmo gairdneri*)

    **Size.**
    - Mean length: 32 mm
    - Mean weight: 0.28 g.

    **Fish source.**
    - Fish were obtained from "a commercial supplier in California".

    **Fish holding period.**
    - Fish were held in a 300 L. fiberglass tank for a minimum of 14 days. The water temperature was maintained at 13 - 14 °C during holding and the fish were fed a commercial dry fish food. There were no mortalities during the 48 hour immediately preceding test initiation.

    **Food withholding.** 96 hours

    **Test vessel.**
    - Size/Volume: The test vessel size was 18.9 L., containing 15 L. of test solution.
    - Construction: Glass
    - Loading: 0.18 g.

    **Test water.**
    - Temperature: 12 1 °C
    - Water source and chemistry: The test water was reconstituted from deionized water and had the following characteristics: hardness (as CaCO₃), 48 mg/L; alkalinity (as CaCO₃), 32 mg/L; pH, 7.0; specific conductivity, 180 umhos/cm.
    - Aeration: None

    **Solvent.** None

    **Controls.**
There was a negative, untreated control group of 30 fish in three replicates run concurrently with the treatment group.

**Number of fish/concentration:** 30

**Dosing method.**

The test substance was added directly to the water to achieve a nominal concentration of 1000 mg/L. At \(4.6 \times 10^8\) cells per mL, and assuming an approximate density of the test material solution of 1 g/mL, the final nominal concentration should have been approximately \(4.6 \times 10^5\) cells per mL.

**Test duration:** 96 hours

**Observations.**

Fish were observed every 24 hour throughout the test duration. Observations on both the fish and physical characteristics of the test vessels were recorded at each observation.

**Statistical analysis.**

No statistics were performed. Estimates of the LC50 and no-effect level were made each at each observation.

12. **REPORTED RESULTS:**

The author reported that there were no mortalities in either the control or treatment group. There was no visible sign of undissolved material in the treatment vessels. The 96-hour LC50 was estimated to be greater than 1000 mg/L. The no-effect level was reported as 1000 mg/L. Additionally, it was reported that water quality parameters were not affected by the test material.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The author concluded that the test material was practically nontoxic to rainbow trout.

There was a quality assurance statement attached to the study.

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

A. Test Procedure. This study generally followed the procedures outlined in EPA's *Pesticide Assessment Guidelines: Subdivision M* except that the test duration was far shorter than that
recommended in the guidelines. This is a major deviation and invalidates the test.

B. **Statistical Analysis.** These data are not amenable to statistical analysis.

C. **Results/Discussion.** *Pseudomonas fluorescens* have been isolated from diseased fish (1). Whether these isolates were primary pathogens or secondary invaders is not clear. Thus, it is extremely important that *Pseudomonas* species be carefully tested with respect to fish pathogenicity. It is the opinion of Ecological Effects Branch that 96 hours is insufficient time to allow for expression of a pathogenic process at temperatures normally maintained in test vessels during aquatic testing.

D. **Adequacy of the Study.**

1. **Category: Invalid**

2. **Rationale:** The test duration was too short to assess the pathogenic potential of the test material.

3. **Repairability:** None

15. **COMPLETION OF ONE-LINER**

One-liner not done
DATA EVALUATION RECORD

1. CHEMICAL: Pseudomonas fluorescens  
   SN: 006418-8

2. TEST MATERIAL:
   The test material used in this study was a washed cell suspension of Pseudomonas fluorescens. The concentration of cells in the material was $4.6 \times 10^8$ cells per mL.

3. STUDY/ACTION TYPE: Freshwater aquatic invertebrate LC50

4. STUDY IDENTIFICATION:

5. REVIEWED BY:
   Robert W. Piluckski, Microbiologist  
   Ecological Effects Branch  
   Hazard Evaluation Division
   Signature:
   Date: 2/18/88

6. APPROVED BY:
   Raymond W. Matheny, Head, Section 1  
   Ecological Effects Branch  
   Hazard Evaluation Division
   Signature:
   Date: 2/18/88

7. CONCLUSIONS:
   This study is considered to be invalid because the test duration was insufficient. This study does not fulfill the guideline requirement for a Freshwater Aquatic Invertebrate Toxicity/Pathogenicity study.

8. RECOMMENDATION:
   This test should be repeated.
9. BACKGROUND: NA

10. DISCUSSION OF INDIVIDUAL STUDIES OR TESTS: NA

11. METHODS AND MATERIALS:

**Species.** Daphnia magna

**Age.** First instar

**Source.** Springborn cultures

**Test vessel.**

- **Size/Volume:** The test was carried out in 1000 mL beakers containing 1000 mL of water.
- **Construction:** Glass
- **Aeration:** None

**Test water.**

- **Temperature:** 20 °C
- **Water source and chemistry:** The water was obtained from Springborn's well and was fortified to meet ASTM's formula for hard water. Chemical and physical characteristics were: hardness (as CaCO₃), 170 mg/L; alkalinity (as CaCO₃), 130 mg/L; pH, 8.0; specific conductivity, 500 umhos/cm.
- **Aeration:** None

**Solvent.** None

**Controls.**

Three replicates of ten daphnids each were not treated and served as the negative control.

**Number of invertebrates/concentration.** 30

**Observations.**

Immobilized daphnids were noted and recorded at 24 and 48 hours. Physical characteristics of the test system were noted and recorded at 0, 24 and 48 hours.
Statistical analysis.

A statistical analysis was not performed. An EC50 and no-effect level was estimated by inspection of the data.

12. REPORTED RESULTS:

The author reported that there were no immobilized daphnids during the 48 hours of the test. There was no sign of undissolved material in the treatment vessels. The estimated EC50 and no-effect level were both reported as greater than 1000 mg/L. Additionally, the author noted that water quality parameters were not affected by the test material.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The author concluded that the test material was practically nontoxic to *Daphnia magna*.

There was a quality assurance statement attached to the study.

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

A. Test Procedure. The procedure used in this study generally follows that outlined in EPA's *Pesticide Assessment Guidelines: Subdivision M* except that the test duration was two days instead of the recommended thirty days. This is a major deviation and, as a result, the test must be invalidated.

B. Statistical Analysis.

These data are not amenable to statistical analysis.

C. Results/Discussion. Although *Pseudomonas fluorescens* is not known to be a frank pathogen of invertebrates, other, more subtle effects such as lowered fecundity cannot be assessed in a short-term (2-day) test. Therefore, there is little useful information that can be gained from this study.

D. Adequacy of the Study.

1. Category: Invalid

2. Rationale: The test duration was too short for the test to be of use in risk analysis.

3. Repairability: None

15. COMPLETION OF ONE-LINER

One-liner not completed
DATA EVALUATION RECORD

1. CHEMICAL: *Pseudomonas fluorescens*  
   SN: 006418-8

2. TEST MATERIAL:

   The test material used in this study was a washed suspension of *Pseudomonas fluorescens* strain 1053. The concentration of the cell suspension was $4.6 \times 10^8$ CFU/mL.

3. STUDY/ACTION TYPE: Nontarget insect test

4. STUDY IDENTIFICATION:


5. REVIEWED BY:

   Robert W. Pilsocki, Microbiologist  
   Ecological Effects Branch  
   Hazard Evaluation Division

   Signature:  
   Date: 2/18/88

6. APPROVED BY:

   Raymond W. Matheny, Head, Section 1  
   Ecological Effects Branch  
   Hazard Evaluation Division

   Signature:  
   Date: 2/18/88

7. CONCLUSIONS:

   This study is considered to be invalid because the study duration was only 2 days instead of the recommended 30 days. This study does not fulfill the guideline requirement for a nontarget insect test.

8. RECOMMENDATION:

   This test should be repeated.
9. **BACKGROUND:** NA

10. **DISCUSSION OF INDIVIDUAL STUDIES OR TESTS:** NA

11. **METHODS AND MATERIALS:**

   **Species.** Green Lacewing larvae (*Chrysopa carnea*)

   **Source and handling.**

   Rincon-Vitova Insectaries
   Oakview, CA

   Larvae were placed in individual containers upon receipt to prevent cannibalism. They were fed Angoumois moth eggs. All larvae chosen for the study were at least 6 mm long.

   **Test chambers.**

   The test chambers were one and one-quarter plastic cups with semitransparent lids.

   **Treatment levels and group size.**

   There was a single treatment level of 2 mg of test material per larva. There were four replicates of 25 larvae for the treatment group.

   **Dosing.**

   The test material was weighed out and a solution containing 1 mg/uL. Treatment chambers were selected by random draw. A 2 uL dose was delivered to the thorax/abdominal area using a digital micropipet.

   **Controls.**

   A control group containing three replicates of 10 larvae was used. The controls were manipulated identically to the treatment group except that they were not dosed.

   **Environmental conditions.**

   Temperature: 21 - 25 °C
   Humidity: 73%

   **Number of insects/concentration.** 100
Observations.

Larvae were observed daily for mortality and signs of toxicity. In addition, ten larvae from each group were drawn at random and their length from the anterior point of the mandibles to the posterior end of the abdomen was measured.

Statistical analysis.

An estimated LD50 was made by visual inspection of the data.

12. REPORTED RESULTS:

The authors reported a control mortality of 2% (2/50) and a 12% (12/100) mortality in the treatment group. The estimated LD50 was reported as greater than 2 mg/larva.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The authors concluded that the mortalities in the treatment group were not treatment-related. They also concluded that the test material was relatively nontoxic to the green lacewing larva, according to the toxicity categories of Atkins (1).

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

A. Test Procedure. The procedure used in this study deviated significantly from that recommended in EPA's Pesticide Assessment Guidelines: Subdivision M. Specifically, the test was only carried out for 2 days rather than the recommended 30 days.

B. Statistical Analysis. These data are not amenable to statistical treatment.

C. Results/Discussion. The main problem with this study is that the observation period was not long enough to provide a high level of confidence that P. fluorescens is not pathogenic to the green lacewing larva. P. fluorescens is not known to be a frank pathogen of insects. Thus, when testing such a microorganism, every effort should be made to allow pathogenesis to occur. One of the ways is to observe the insects for an extended period after dosing. While 2 days may be long enough for chemical toxicity to occur, it is not enough time for a weakly pathogenic process to manifest itself.

Closely connected with pathogenesis is the route of exposure. It is unlikely that contact dosing would show a positive response except for microorganisms, such as fungi, that elaborate extracellular chitinases to allow penetration of the exoskeleton. A more appropriate route of dosing, in this reviewer's opinion, would be some method of oral/feeding exposure.

D. Adequacy of the Study.

1. Category: Invalid

2. Rationale: The study was not carried out for a duration sufficiently long to rule out \textit{P. fluorescens} as a pathogen of the green lacewing larva.

3. Repairability: None

15. \textbf{COMPLETION OF ONE-LINER}

One-liner not done
DATA EVALUATION RECORD

1. CHEMICAL: Pseudomonas fluorescens  SN: 006418-8

2. TEST MATERIAL:
   The test material used in this study was a washed suspension of Pseudomonas fluorescens strain 1053. The concentration of the cell suspension was 4.6 x 10^6 CFU/mL.

3. STUDY/ACTION TYPE: Nontarget insect test

4. STUDY IDENTIFICATION:

5. REVIEWED BY:
   Robert W. Pilsucki, Microbiologist
   Ecological Effects Branch
   Hazard Evaluation Division
   Signature: [Signature]
   Date: 2/18/88

6. APPROVED BY:
   Raymond W. Matheny, Head, Section 1
   Ecological Effects Branch
   Hazard Evaluation Division
   Signature: [Signature]
   Date: 2/18/88

7. CONCLUSIONS:
   This study is considered to be invalid because the study duration was only 2 days instead of the recommended 30 days. This study does not fulfill the guideline requirement for a nontarget insect test.

8. RECOMMENDATION:
   This test should be repeated.
9. **BACKGROUND:** NA

10. **DISCUSSION OF INDIVIDUAL STUDIES OR TESTS:** NA

11. **METHODS AND MATERIALS:**

   **Species.** Ladybird beetle

   **Source and handling.**
   
   Vitova Insectaries
   Oakview, CA
   
   Beetles were kept under refrigeration until test initiation whereupon they were transferred to the test chambers. There was no mention of a pretest acclimation period.

   **Test chambers.**
   
   The test chambers were paper containers measuring 87 mm in diameter x 85 mm in height. Each container was covered by a petri dish.

   **Treatment levels and group size.**
   
   There was a single treatment level of 2 mg of test material per beetle. There were five replicates of ten beetles for the treatment group.

   **Dosing.**
   
   The test material was weighed out and a solution containing 1 mg/μL. Treatment chambers were selected by random draw. Beetles were immobilized using nitrogen. A 2 μL dose was delivered to the thorax/abdominal area using a digital micropipet.

   **Controls.**
   
   A control group containing three replicates of 10 beetles was used. The controls were manipulated identically to the treatment group except that they were not dosed.

   **Environmental conditions.**
   
   Temperature: 24 - 26 °C
   
   Humidity: 78%

   **Number of insects/concentration.** 50
Observations.

Beetles were observed daily for mortality and signs of toxicity.

Statistical analysis.

An estimated LD50 was made by visual inspection of the data.

12. REPORTED RESULTS:

The authors reported a control mortality of 3% (1/30) and an 8% mortality in the treatment group. The estimated LD50 was reported as greater than 2 mg/beetle.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The authors concluded that the mortalities in the treatment group were not treatment-related. They also concluded that the test material was relatively nontoxic to the ladybird beetle, according to the toxicity categories of Atkins (1).

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

A. Test Procedure. The procedure used in this study deviated significantly from that recommended in EPA's Pesticide Assessment Guidelines: Subdivision M. Specifically, the test was only carried out for 2 days rather than the recommended 30 days.

B. Statistical Analysis. These data are not amenable to statistical treatment.

C. Results/Discussion. The main problem with this study is that the observation period was not long enough to provide a high level of confidence that P. fluorescens is not pathogenic to the ladybird beetle. P. fluorescens is not known to be a frank pathogen of insects. Thus, when testing such a microorganism, every effort should be made to allow pathogenesis to occur. One of the ways is to observe the insects for an extended period of time after dosing. While 2 days may be long enough for chemical toxicity to occur, it is not enough time for a weakly pathogenic process to manifest itself.

Closely connected with pathogenesis is the route of exposure. It is unlikely that contact dosing would show a positive response except for microorganisms, such as fungi, that

elaborate extracellular chitinases to allow penetration of the exoskeleton. A more appropriate route of dosing, in this reviewer’s opinion, would be some method of oral/feeding exposure.

D. Adequacy of the Study.

1. Category: Invalid

2. Rationale: The study was not carried out for a duration sufficiently long to rule out *P. fluorescens* as a pathogen of the ladybird beetle.

3. Repairability: None

15. COMPLETION OF ONE-LINER

One-liner not done