393105
RECORD NO.

SHAUGHNESSEY NO. REVIEW NO.

EEB REVIEW

DATE: IN 4-1-91 OUT SEP 6 1991

FILE OR REG. NO 063950-R

PETITION OR EXP. NO.

DATE OF SUBMISSION 1-31-91

DATE RECEIVED BY EFED 4-1-91

RD REQUESTED COMPLETION DATE 6-30-91

EEB ESTIMATED COMPLETION DATE

RD ACTION CODE/TYPE OF REVIEW 130

TYPE PRODUCT(S) : I, D, H, F, N, R, S Biological Fungicide

DATA ACCESSION NO(S). 415466-05,-06,-07,-08,-09,416248-03

PRODUCT MANAGER NO. C. Grable/S. Lewis (PM-21)

PRODUCT NAME(S) Blue Circle™

Blue Circle™, Inoculant SMP-1

COMPANY NAME Stine Microbial Products

SUBMISSION PURPOSE Section 3 Registration

SHAUGHNESSEY NO.	CHEMICAL, & FORMULATION	
006419	Pseudomonas cepacia type Wisconsin	3.8 %

EEB REVIEW

Pesticide Name: Blue Circle™ Pseudomonas cepacia SMP-1

100.0.0 Submission Purpose and Label Information

100.1.0 Submission Purpose and Pesticide Use

Stine Microbial Products has requested a Section 3 Registration for Pseudomonas cepacia as the active ingredient for control of a variety of fungi and nematodes on several grain and vegetable crops.

100.2.0 Formulation Information

INERT INGREDIENT INFORMATION IS NOT INCLUDED

A. Blue Circle™

ACTIVE INGREDIENT:
Pseudomonas cepacia type Wisconsin.....3.8% (by wt.)
INERT INGREDIENTS:.....96.2% (by wt.)
TOTAL.....100.0% (by wt.)

B. Blue Circle™ Inoculant SMP-1

ACTIVE INGREDIENT:
Pseudomonas cepacia type Wisconsin.....3.8% (by wt.)
(contains at least 50 million viable cells/lb)
(10^5 cells/gram)

[redacted]

100.3.0 Application Methods, Directions, Rates

Application Site: seed coating at planting

Target Pests: plant pathogenic fungi and nematodes

Dosage Rate:

A. Blue Circle™

field corn, sweet corn, popcorn.....2.8oz./50lbs seed
melons, tomatoes, lettuce, squash,
cole crops, sunflower, sugar beets,
carrots, canola.....0.5oz./50lbs seed

alfalfa, clovers, grain sorghum,
cotton.....4.3oz./50lbs seed
soybeans, snap beans, dry beans.....2.6oz./50lbs seed
potatoes.....2.2oz./50lbs seed

B. Blue Circle™ Inoculant SMP-1

field corn, sweet corn, popcorn,
cotton, squash, melons, tomatoes,
lettuce, cole crops, sunflower,
grain sorghum, sugar beets, carrots,
canola, alfalfa, clovers.....1-2oz./acre
soybeans, snap beans, dry beans.....2-4oz./acre
potatoes.....2-4lb/acre

100.4.0 Target Organisms

fungi that cause damping-off disease (Rhizoctonia, Pythium, and Fusarium); lesion (Helicotylenchus spp.), spiral (Pratylenchus), lance (Hopolamus), cyst (Heterodera glycines), and root knot (Meloidogyne incognita) nematodes

100.5.0 Precautionary Labeling

The label contains the following precautions:

CAUTION: KEEP OUT OF REACH OF CHILDREN

HAZARDS TO HUMANS (AND DOMESTIC ANIMALS): (adequate)

ENVIRONMENTAL HAZARD STATEMENT: Keep out of lakes, ponds or streams. Do not contaminate water when disposing of equipment washwater.

(Pesticide and container disposal directions are adequate)

101.0.0 Hazard Assessment

101.1.0 Discussion

The studies supplied with this submission indicate that Blue Circle™ is practically nontoxic to bobwhite quail, honey bee, and the following beneficial insect species: parasitic hymenoptera, green lacewing, and ladybird beetles. The non-target aquatic organism testing and the acute avian study using the mallard duck were waived for this product because the method of application was in-furrow, Pseudomonas cepacia has never been reported as a pathogen of fish, and this fungus ia a common colonizer

of natural water bodies.

101.2.0 Likelihood at Adverse Effects to Nontarget Organisms

Avian Studies

A submitted study entitled "An Avian Oral Pathogenicity and Toxicity Study in the Bobwhite" (MRID #415466-05) shows that the acute oral LD_{50} value for northern bobwhite exposed to P. cepacia as a single encapsulated oral dose was greater than 5,000 mg/kg ($1x10^{10}$ cfu/kg) the highest dose tested. The LD_{50} was greater than the maximum hazard dosage level. These results indicate that this product is practically nontoxic to birds and should not cause any adverse effects to avian species.

Fish Studies

No studies were submitted because the registrant was granted waivers for all aquatic testing (see discussion).

Mammalian Wildlife

The data submitted to the toxicology branch indicate that there is no significant toxicity to rodents from acute oral testing at the maximum hazard dose. In light of the above results risk to mammalian wildlife is expected to be minimal to nonexistent.

Aquatic Invertebrate Studies

No studies were submitted because the registrant was granted waivers for all aquatic testing (see discussion).

Nontarget Plant Studies

A submitted study entitled "Blue Circle Inoculant: Tier I Non-Target Plant Studies" (MRID #416284-03) tested the effect of P. cepacia type Wisconsin on 12 plant species. The results indicated that this organism is not pathogenic to any of the plant species tested. Therefore, this product should not cause any adverse effects to nontarget plant species.

Nontarget Insect Studies

Three submitted studies entitled "A Dietary and Toxicity Study with the Parasitic Hymenoptera (Uga menoni); A Dietary and Toxicity Study with Green Lacewing Larvae; and A Dietary and Toxicity Study with Ladybird Beetles" (MIRD #415466-06,-07,-08) demonstrated that P. cepacia type Wisconsin was practically nontoxic to parasitic

wasps, green lacewings and ladybird beetles. Therefore, this product should not cause any adverse effects to nontarget insect species.

Honey Bee Studies

A submitted study entitled "A Dietary Pathogenicity and Toxicity Study with the Honey Bee" (MRID #415466-09) demonstrated that P. cepacia type Wisconsin was practically nontoxic to honey bee. Therefore, this product should not cause adverse effects to honey bee.

101.3.0 Endangered Species Considerations

EEB feels that there will not be a "may effect" situation for endangered mammals, birds, plants and aquatic species from the proposed uses of this product.

101.4.0 Adequacy of Toxicity Data

(See the Generic Data Table)

The registrant has addressed the data requirements outlined in the Pesticide Assessment Guidelines, Subdivision M. The wavier requests and studies submitted by the registrant are adequate to address the data requirements for the registration of a microbial pesticide and can be used to make a risk assessment.

Generic Data Requirements For Blue Circle™

Data Requirement	Test[1] Substance	Use[2] Patterns	Does EPA Have Data?	Bibliographic Citation	Must Additional Data Be Submitted?
§158.740 Microbial Pesticide Nontarget Organism - Tier I					
Avian Testing					
154-16 Avian Acute Oral					
- upland gamebird	TGAI	A	Yes	415466-05	No
- waterfowl	TGAI	A	No	--	No
Aquatic Organism Testing					
154-19 Freshwater Fish LC_{50}					
- rainbow trout	TGAI	A	No	--	No
154-20 Freshwater Invertebrate Testing					
- Daphnia magna	TGAI	A	No	--	No
154-22 Nontarget plant studies					
- terrestrial	TGAI	A	Yes	416284-03	No
- aquatic	TGAI	A	No	--	No

154-23	Nontarget insect testing					
	- parasitic wasps	TGAI	A	Yes	415466-06	No
	- green lacewing	TGAI	A	Yes	415466-07	No
	-ladybird beetle	TGAI	A	Yes	415466-08	No
154-24	Honey bee testing					
	- Dietary	TGAI	A	Yes	415466-09	No

1/ TGAI = Technical Grade of the Active Ingredient; TEP = Typical End-Use Product.

2/ The use patterns are coded as follows: A = Terrestrial, Food Crop; B = Terrestrial, Nonfood; C = Aquatic, Food Crop; D = Aquatic, Nonfood; E = Greenhouse, Food Crop; F = Greenhouse, Nonfood; G = Forestry; H = Domestic, Outdoor; I = Indoor.

101.5.0 Adequacy of Labeling

The precautionary labeling (see sec. 100.5.0) needs to have the following additions/modifications:

For MANUFACTURING-USE PRODUCTS:

"Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or public water unless this product is specifically identified and addressed in an NPDES permit. Do not discharge effluent containing this product to sewer systems without previously notifying the sewage treatment plant authority. For guidance, contact your State Water Board or Regional Office of the EPA."

For END-USE PRODUCTS:

" Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high-water mark. Do not contaminate water when disposing of equipment washwater or rinsate."

Endangered Species Labeling:

Endangered species labeling is deferred until the Technical Bulletin information is made available by OPP.

102.0.0 Conclusions

EEB has reviewed the proposed Section 3 Registration of Blue Circle™ by Stine Microbial Products for the control of a variety of fungi and nematodes on field crops and vegetables. The registrant has adequately addressed all of the ecological effects testing requirements.

All of the aquatic testing requirements, including the acute avian test using the mallard duck, have been waived for this product due to a lack of exposure to aquatic organisms. The results of the acute avian study using quail indicate that this product is practically nontoxic to birds. The nontarget plant studies demonstrate that P. cepacia is not pathogenic to a number of commercially important crop plants and selected plants that had been reported to be susceptible to this organism in the open literature. The nontarget insect testing demonstrated that this product did not have an adverse effect on 3 beneficial insect species (parasitic wasps, green lacewings, and ladybird beetles). No adverse effects to honey bee were observed with this product. Due to a lack of toxicity in mouse studies submitted to the Health Effects division, the product should not pose a risk to wild mammal species.

Therefore, EEB concludes that the proposed uses of Blue Circle™ will lead to a minimal risk to nontarget organisms or endangered species.

David Bays, Microbiologist 9/6/91
Ecological Effects Branch
Ecological Fate and Effects Division (H7507C)

Leslie W. Touart, Head Section 1 9/13/91
Ecological Effects Branch
Ecological Fate and Effects Division (H7507C)

Douglas J. Urban, Acting Chief 9.17.91
Ecological Effects Branch
Ecological Fate and Effects Division (H7507C)

DATA EVALUATION REPORT

1. Chemical: Pseudomonas cepacia - SMP-1

2. Test Material: Technical

3. Study/Action Type: Nontarget Honey Bee (Apis mellifera) Testing (154A-24)

4. Study Identification: SMP-1: A Dietary Pathogenicity and Toxicity Study with the Honey Bee. By M. M. Thompson, K. A. Hoxter and M. Jaber. Prepared By Wildlife International LTD, June 1990. Project No. 275-102C. Submitted By Stine Microbial Products. Madison, WI. EPA Acc. No. 415466-09.

5. Reviewed By: David C. Bays
Microbiologist
EFED/EEB

Signature: David C. Bays
Date: 9/6/91

Les W. Touart
Head, Section 1
EFED/EEB

Signature: L. Touart
Date: 9/13/91

6. Conclusions: The study is scientifically sound and demonstrated an $LC_{50} > 10^9$ cfu/ml. This indicates that SMP-1 is practically nontoxic to Honey Bee. The study fulfills EPA Guideline requirements for a nontarget insect pathogenicity/toxicity test.

7. Recommendations: N/A

8. Background: This study was submitted to support the request for the registration of Pseudomonas cepacia - SMP-1.

10. Materials and Methods:

A. Test Organisms: The test bees were obtained from the Wildlife International Ltd. hives located in Easton, Maryland. One frame of pupae was taken from the hives (7 days before test initiation) and placed in a Marsh Roll-X automatic incubator for 7 days to allow the adult bees to emerge. The bees used in the test were 1 to 7 days old and were healthy in appearance.

B. Dosage Form: The test diets were prepared by the registrant and received as a cloudy liquid in the following different formulations: SMP-1 Attenuated Control in 12.5% sucrose (equal to highest concentration used); 12.5% sucrose control; SMP-1 10^5 cfu/ml; SMP-1 10^7 cfu/ml; and SMP-1 10^9 cfu/ml. The solutions were shaken for approximately 2 minutes before use.

C. Referenced Protocol: The test insects were placed in disposable one pint rolled paper containers (87 mm in diameter/85 mm high) that were covered with a disposable plastic petri dish (90 mm in diameter). The test diet (available ad libitum) was placed in a 20 ml glass vial which was covered with cheese cloth, and then inserted into the container's cover. A moist sponge, which was misted daily, was placed on the top of each container to increase humidity within the test chamber.

Three replicates, containing 10 insects each, were randomly assigned to each of 3 treatment levels (10^5, 10^7, 10^9 cfu/ml of feed) along with the attenuated (equal to highest test concentration used) and negative (untreated sugar water) controls. The bees were immobilized with nitrogen at the start of the study and the test diets were placed atop the test chambers. The test insects were observed for mortality and signs of toxicity twice on the day the experiment started (first observation immediately following the introduction of the test diets) and once a day thereafter until the end of the study. The environmental conditions were as follows: 8 hours of light/day, a temperature of 22-24C, and an average relative humidity of 53%.

D. Statistical Analysis: After study completion, an estimation of the LC^{50} value was made by visual inspection of the mortality data. A calculation of the LC^{50} value was not necessary because of the lack of mortalities found in this study.

12. Reported Results:

Dosage	cfu/g	Replicate	Number Dead/Number Exposed (At 5 Days After Dosing)
Negative control	0	A	2/10
		B	4/10
		C	2/10
Attenuated control	0	A	0/10
		B	5/10
		C	2/10

Treatment			
	10^5	A	3/10
		B	3/10
		C	3/10
	10^7	A	5/10
		B	4/10
		C	5/10
	10^9	A	2/10
		B	4/10
		C	3/10

$LC_{50} > 10^9$ cfu/ml

Mortalities occurred in both of the control groups (negative and attenuated) and in all 3 of the treatment groups. The study was terminated after the negative control exceeded 20% mortality (after 5 days). The mortalities in the negative and attenuated control groups were 27% and 23%, respectively, while those in the 10^5, 10^7, and 10^9 cfu/g feed concentrations averaged 30%, 47% and 30%, respectively. The pattern of mortality was found not to be dose responsive and did not appear to be treatment related. The LC_{50} was determined to be greater than 10^9 cfu/ml feed and the no effects concentration was 10^9 cfu/ml feed which was the highest concentration tested.

13. Study Author's Conclusions/Quality Assurance Measures:

$LC_{50} > 10^9$ cfu/ml feed

"This study was conducted so as to conform with Good Laboratory Practices as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160." Signed by study director, Kimberly A. Hoxter.

14. Reviewer's Discussion and Interpretation of the Study:

A. Test Procedures: The procedures used follow those recommended by EPA in the 1989 Pesticide Testing Guidelines for Microbial and Biochemical Pest Control Agents, Subdivision M.

B. Statistical Analysis: None was needed since the pattern of mortality did not facilitate the calculation of an LC_{50} value.

C. Discussion/Results: An $LC_{50} > 10^9$ indicates that Pseudomonas cepacia SMP-1 is practically non-toxic to

Honey Bee.

D. Adequacy of the Study:

1. Validation Category: Core

2. Rationale: Meets EPA Guideline requirements

15. Completion of the One-liner:

DATA EVALUATION REPORT

1. Chemical: Pseudomonas cepacia - SMP-1

2. Test Material: Technical

3. Study/Action Type: Nontarget Insect-Green Lacewing Larvae (Chrysopa carnea) (154A-23)

4. Study Identification: SMP-1: A Dietary Pathogenicity and Toxicity Study with Green Lacewing Larvae. By M. M. Thompson, K. A. Hoxter and M. Jaber. Prepared By Wildlife International LTD, June 1990. Project No. 275-103B. Submitted By Stine Microbial Products. Madison, WI. EPA Acc. No. 415466-07.

5. Reviewed By: David C. Bays
Microbiologist
EFED/EEB
Signature: David C. Bays
Date: 9/6/91

Les W. Touart
Head, Section 1
EFED/EEB
Signature: L. Touart
Date: 9/13/91

6. Conclusions: The study is scientifically sound and demonstrated an $LC_{50} > 2x10^8$ cfu/g feed. This indicates that SMP-1 is practically nontoxic to Green Lacewing Larvae. The study fulfills EPA Guideline requirements for a nontarget insect pathogenicity/toxicity test.

7. Recommendations: N/A

8. Background: This study was submitted to support the request for the registration of Pseudomonas cepacia - SMP-1.

10. Materials and Methods:

A. Test Organisms: Apparently healthy, Green Lacewing Larvae (Chrysopa carnea) were used in the study and were obtained from the Rincon-Vitova Insectaries, Inc. located in Oakview, California.

B. Dosage Form: The test diets were prepared by the registrant and received as a cloudy liquid in the following different concentrations: 10^9 cfu/ml feed and the attenuated control (equal to the highest concentration administered to the

larvae).

C. Referenced Protocol: The test insects were placed in one ounce semi-transparent plastic cups with semi-transparent lids (1 replicate-individual/cup). The test diet was prepared by mixing together weighed amounts of test substance, pollen substitute and distilled water, if necessary.

Thirty larvae were randomly assigned to each of 3 treatment levels ($2x10^6$, $2x10^7$, $2x10^8$ cfu/g of feed) along with the attenuated (equal to highest test concentration used) and negative (did not receive viable or attenuated test substance) controls. Each test group of 30 larvae was divided into 3 subgroups of 10 individuals to facilitate record keeping.

For the first 5 days of the study, the larvae were fed the test diets. From Day 5 until the end of the study, the test diets were replaced with untreated eggs of the Angoumois grain moth (Sitotroga cerealella as a food source. The test larvae were observed for mortality and signs of toxicity immediately following introduction of the test diets and continued twice a day until the end of the study. The environmental conditions were as follows: 8 hours of light/day, a temperature of 20-28C and an average relative humidity of 54%.

D. Statistical Analysis: After study completion, an estimation of the LC^{50} value was made by visual inspection of the mortality data. A calculation of the LC^{50} value was not necessary because of the lack of mortalities found in this study.

12. Reported Results:

Dosage	cfu/g	Replicate	Number Dead/Number Exposed (At 14 Days After Dosing)
Negative control	0	A	3/10
		B	3/10
		C	4/10
Attenuated control	0	A	6/10
		B	10/10
		C	8/10
Treatment	$2x10^6$	A	4/10
		B	3/10
		C	1/10

$2x10^7$	A	2/10
	B	5/10
	C	3/10
$2x10^8$	A	3/10
	B	7/10
	C	3/10

$LC_{50} > 2x10^8$ cfu/g feed

Mortalities occurred in both of the control groups (negative and attenuated) and in all 3 of the treatment groups. The study was terminated after the negative control exceeded 20% mortality (after 14 days). The mortalities in the negative and attenuated control groups were 33% and 80%, respectively, while those in the $2x10^6$, $2x10^7$, and $2x10^8$ cfu/g feed concentrations averaged 27%, 33% and 45%, respectively. The pattern of mortality was found to be dose responsive, appeared to be treatment related, and was highest in the high treatment group and attenuated control. A disproportionate number of larvae used in the attenuated control were observed to be of small size and less hardy. This was thought to have contributed to the high mortality observed with the attenuated control group. Since mortality was observed with the attenuated control, it was suggested that the mortality was due to toxicity and not pathogenicity.

13. Study Author's Conclusions/Quality Assurance Measures:

$LC_{50} > 2x10^8$ cfu/g feed

"This study was conducted so as to conform with Good Laboratory Practices as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160." Signed by study director, Kimberly A. Hoxter.

14. Reviewer's Discussion and Interpretation of the Study:

A. Test Procedures: The procedures used follow those recommended by EPA in the 1989 Pesticide Testing Guidelines for Microbial and Biochemical Pest Control Agents, Subdivision M.

B. Statistical Analysis: None was needed since the pattern of mortality did not facilitate the calculation of an LC_{50} value.

C. Discussion/Results: An $LC_{50} > 2x10^8$ indicates that Pseudomonas cepacia SMP-1 is practically non-toxic to Green Lacewing Larvae.

D. Adequacy of the Study:

1. Validation Category: Core

2. Rationale: Meets EPA Guideline requirements

15. Completion of the One-liner:

DATA EVALUATION REPORT

1. Chemical: Pseudomonas cepacia - SMP-1

2. Test Material: Technical

3. Study/Action Type: Nontarget Insect-Ladybird Beetles (Hippodamia convergens) (154A-23)

4. Study Identification: SMP-1: A Dietary Pathogenicity and Toxicity Study with Ladybird Beetles. By M. M. Thompson, K. A. Hoxter and M. Jaber. Prepared By Wildlife International LTD, March 1990. Project No. 275-104A. Submitted By Stine Microbial Products. Madison, WI. EPA Acc. No. 415466-08.

5. Reviewed By: David C. Bays
Microbiologist
EFED/EEB
Signature: David C. Bays
Date: 9/6/91

Les W. Touart
Head, Section 1
EFED/EEB
Signature: L W T
Date: 9/13/91

6. Conclusions: The study is scientifically sound and demonstrated an $LC_{50} > 10^9$ cfu/ml. This indicates that SMP-1 is practically nontoxic to Ladybird Beetles. The study fulfills EPA Guideline requirements for a nontarget insect pathogenicity/toxicity test.

7. Recommendations: N/A

8. Background: This study was submitted to support the request for the registration of Pseudomonas cepacia - SMP-1.

10. Materials and Methods:

A. Test Organisms: Apparently healthy, Ladybird Beetles (Hippodamia convergens) were used in the study and were obtained from the Rincon-Vitova Insectaries, Inc. located in Oakview, California.

B. Dosage Form: The test diets were prepared by the registrant and received as a cloudy liquid in the following different concentrations: 10^5, 10^7, and 10^9 cfu/ml feed, and the attenuated control (equal to the highest concentration

administered to beetles).

C. Referenced Protocol: The test insects were placed in disposable one pint rolled paper containers (87 mm in diameter/85 mm high) that were covered with a disposable plastic petri dish (90 mm in diameter). The test diet (available ad libitum) was placed in a 20 ml glass vial which was covered with cheese cloth, and then inserted into the container's cover. A moist sponge, which was misted daily, was placed on the top of each container to increase humidity within the test chamber.

Two replicates, containing 25 insects each, were randomly assigned to each of 3 treatment levels (10^5, 10^7, 10^9 cfu/ml of feed) along with the attenuated (equal to highest test concentration used) and negative (12.5% sucrose mixture) controls. Fresh diet was given to the wasps and the average feed consumption for each test concentration and control group was determined on a weekly basis. The wasps were immobilized with nitrogen at the start of the study and when the test diet was introduced. The test insects were observed for mortality and signs of toxicity twice on the day the experiment started (first observation immediately following the introduction of the test diets) and once a day thereafter until the end of the study. The environmental conditions were as follows: the test beetles were maintained in the dark except when dosing and making observations, a temperature of 19-28C and an average relative humidity of 70%.

D. Statistical Analysis: After study completion, an estimation of the LC^{50} value was made by visual inspection of the mortality data. A calculation of the LC^{50} value was not necessary because of the lack of mortalities found in this study.

12. Reported Results:

Dosage	cfu/ml	Replicate	Number Dead/Number Exposed (At 11 Days After Dosing)
Negative control	0	A	6/25
		B	6/25
Attenuated control	10^9	A	9/25
		B	6/25
Treatment	10^5	A	4/25
		B	3/25

10^7	A	4/25
	B	3/25
10^9	A	3/25
	B	7/25

LC_{50} > 10^9 cfu/ml feed

Mortalities occurred in both of the control groups (negative and attenuated) and in all 3 of the treatment groups. The mortalities in the negative and attenuated control groups were 24% and 30%, respectively, while those in the 10^5, 10^7, and 10^9 cfu/ml feed concentrations averaged 14%, 14% and 20%, respectively. The mortality in the treatment groups was found to be less than, or equal to the control mortality and did not appear to be treatment related. No additional signs of toxicity were observed during the test.

13. Study Author's Conclusions/Quality Assurance Measures:

LC_{50} > 10^9 cfu/ml feed

"This study was conducted so as to conform with Good Laboratory Practices as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160." Signed by study director, Kimberly A. Hoxter.

14. Reviewer's Discussion and Interpretation of the Study:

A. Test Procedures: The procedures used follow those recommended by EPA in the 1989 Pesticide Testing Guidelines for Microbial and Biochemical Pest Control Agents, Subdivision M.

B. Statistical Analysis: None was needed since the pattern of mortality did not facilitate the calculation of an LC_{50} value.

C. Discussion/Results: An LC_{50} > 10^9 indicates that Pseudomonas cepacia SMP-1 is practically non-toxic to Ladybird Beetles.

D. Adequacy of the Study:

1. Validation Category: Core

2. Rationale: Meets EPA Guideline requirements

15. Completion of the One-liner:

DATA EVALUATION REPORT

1. Chemical: Pseudomonas cepacia - SMP-1

2. Test Material: A suspension of Pseudomonas cepacia cells in distilled water

3. Study/Action Type: Nontarget plant testing (154A-22)

4. Study Identification: Blue Circle Inoculant: TIER I Non-Target Plant Studies. By Janice A. Kimpel, Consultant to Stine Microbial Products. June, 1990. Project ID. XI. Submitted By Stine Microbial Products, Madison, WI. EPA Acc. No. 416284-03.

5. Reviewed By: David C. Bays
Microbiologist
EFED/EEB
Signature: David C. Bays
Date: 9-C-91

Les W. Touart
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EFED/EEB
Signature: L. Touart
Date: 9-13-91

6. Conclusions:

The studies are scientifically sound and demonstrated that Pseudomonas cepacia is not pathogenic to a number of terrestrial crop plants. However, the study will be considered supplemental because guidelines for Good Laboratory Practices (GLP) were not followed completely. Even though the studies did not fully conform to GLP the information is sufficient to fulfill EPA Guideline requirements for nontarget plant testing.

7. Recommendations: N/A

8. Background:

This study was submitted to meet the requirements for nontarget plant testing which is required for the registration of this microbial pesticide.

10. Materials and Methods:

A. Test Organisms: The test plants used in this study were chosen because of their economic importance (EPA Subdivision M, Table 3, pg 146) and in selected cases because of previous reports in the literature of susceptibility to this organism. The plants screened in the studies included yellow onion (Allium cepa cv. Utah Yellow Sweet Spanish), garlic (Allium sativa cv. Elephant), orchid (Cymbidium sp.), maize (Zea mays), sunflower (Helianthus annuus), green bean (Phaseolus vulgaris), soybean (Glycine max), African violets (Saintpulia ionantha), alfalfa (Medicago sativa), easter

lilies (Lilium longiflorum), sugar beet (Beta vulgaris), and cotton (Gossypium hirsutum).

B. Dosage Form: The test material was a bacterial cell suspension in distilled water or phosphate buffered saline, depending on the test. The bacterium was grown up on a standard nutrient broth (NBY medium) and the suspension was prepared by centrifuging the bacterial cells out of the NBY medium, washing them twice, and resuspending the pellet in distilled water or buffer. The bacterial concentration was determined using optical density (A_{425}) and calibration curves, and plating on NBY agar.

C. Referenced Protocol: The onion maceration assay was initiated by cutting slices from the onion and placing them in sterile Petri dishes. The onions were inoculated by spreading 0.1 ml of a 10^3, 10^5 or 10^8 cfu/slice bacterial suspension transversely across each slice. The Petri plates were sealed, incubated at 30C for 72 hours, and observed after 24, 48 and 72 hours. The onion leaf assay was initiated by wound-inoculating 3-week old onion plants (cv. Stuttgarter) by pricking the leaves with a hypodermic syringe near the tip, middle and base of the leaf blade (3 leaves/plant/treatment). A bacterial suspension at a concentration of 10^5 or 10^8 cfu was applied to each wound site. The plants were grown for 2 days on a misted greenhouse bench at 24C (day)/21C (night) and then transferred to a non-misted greenhouse bench for 4 days.

The garlic assay was very similar to the onion maceration test described above. Five transverse sections/clove, which had been surface sterilized with 70% isopropanol, were cut and placed in Petri plates. The slices were inoculated by spreading 0.1 ml of a 10^3, 10^5 or 10^8 cfu/slice bacterial suspension over the surface of each slice. The Petri plates were incubated at 25-30C for 72 hours and observed after 24, 48 and 72 hours.

The orchid study included both an intact plant and excised leaf assay. For the intact plant assay, leaves were wound-inoculated by pricking the leaves several times with a hypodermic syringe and then applying a bacterial suspension (10^5 or 10^8 cfu). The plants were grown for 2 days on a misted greenhouse bench at 24C (day)/21C (night) and then transferred to an unmisted greenhouse bench for 12 days. The leaf assay involved placing surface sterilized leaf sections (2 inches long) in Petri dishes (3/treatment) and wound-inoculating them by pricking the leaf sections several times with a hypodermic syringe, then applying a bacterial suspension (10^5 or 10^8) to the wound sites. The Petri plates were sealed in plastic and incubated at 24C for 7 days.

The final studies involved evaluating the pathogenicity of SMP-1 on a selected group of plant species that encompasses the major agronomic species. In the seedling assay, inoculated (1.0 ml of a bacterial suspension) or coated [redacted] containing 10^7 cells of P. cepacia) seeds sown in pots containing a soil/sand mixture and germinated in growth chambers. Observations were taken for 2-3 weeks and roots were harvested to determine the amount of colonization. In addition, small scale field studies were performed using a number of the crop species to assess the efficacy of SMP-1 in controlling natural or introduced infections and the potential for pathogenicity. The plots were in a complete randomized block design (several replications/treatment) and were observed weekly throughout the growing season to measure % germination, standability counts, plant weights, and colonization.

D. Statistical Analysis: The results were calculated as counts, percentages, and averages with standard deviations.

12. Reported Results:

	Inoculum Dose Applied (cfu/slice)									
	Onion Slice Maceration			Onion Leaf		Garlic Pathogenicity			Cymbidium Path.	
Strain	10^3	10^5	10^8	10^5	10^8	10^3	10^5	10^8	10^5	10^8
Onion Pathogenic:										
72-20	0,1,1[a]	3,3,3	5,5,5	nd	nd	nd	nd	nd	nd	nd
74-34	nd[e]	5,5,5	5,5,5	nd	nd	nd	nd	nd	nd	nd
64-22	3,3,3	2,5,5	3,3,3	1,1,1[b]	2,2,2	2,2,2[c]	2,2,2	0,0,0	1,1,0[d]	2,2,3
72-60	nd	nd	nd	1,1,2	2,2,2	2,2,2	0,0,0	0,0,0	1,1,2	2,2,3
Type Wisconsin:										
J82	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	2,2,2
J51	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	1,1,1	0,0,0	0,0,0	2,1,2
SG17	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	1,0,0	0,0,0	1,1,1
M36	0,0,0	0,0,0	0,1,2	0,0,0	0,0,0	1,1,1	1,0,0	1,2,1	1,1,0	1,2,2
Other Strains:										
P. fluorescens 608	0,0,0	0,0,0	0,0,0	0,0,0	1,2,1	0,0,0	0,0,0	0,0,0	0,1,0	2,1,1
P. multivorans (ATCC 17759)	nd	0,1,0	0,1,1	1,1,1	1,2,1	nd	nd	nd	nd	nd
P. syringae 301	nd	nd	nd	1,1,1	1,2,1	nd	nd	nd	1,2,2	2,2,1
Buffer Control	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	1,0,0	0,0,0	0,0,0	0,0,0	0,0,0

[a]Results were scored on a scale of 0-5 after 72 hours: 0=no maceration; 1=slight maceration; 2=maceration with brown-yellow discoloration; 5=severe yellowing or browning and complete maceration.
[b]Results were scored on a scale of 0-2: 0=necrosis confined to wound site (i.e. no expanding lesion); 1=chlorosis and necrosis expanding beyond the wound site; 2=completely infected and collapsed leaf.
[c]Results were scored on a scale of 0-5: 0=no discoloration; 1=slight browning; 2=brown-yellow discoloration; 5=severe yellowing or browning with complete maceration other the tissue.
[d]Results were scored on a scale of 0-3: 0=no discoloration and no halo formation around the wound site; 1=necrosis confined to the wound site; 3=discoloration and an expanded lesion or halo beyond the wound site.
[e]nd=not determined

The results of the studies presented in the above table demonstrated that the Wisconsin type isolates of P. cepacia were not pathogenic to onion, garlic, and Cymbidium. The results of the pathogenicity testing on selected plant species demonstrated that P. cepacia isolates were not pathogenic to the plants tested. No signs of rot or disease were found in maize (Zea mays) root samples. No incidences of treatment related disease were found in sunflower (Helianthus annuus), green bean (Phaseolus vulgaris), soybean (Glycine max), African violets (Saintpaulia ionantha), alfalfa (Medicago sativa), Easter lilies (Lilium longiflorum), sugar beet (Beta vulgaris) and cotton (Gossypium hirsutum).

13. Study Author's Conclusions/Quality Assurance Measures:

"This study was done in compliance with EPA Good Laboratory Practices as published in 40 CFR 160, with the following exceptions. This study did not comply with 160.35 (a), (b), (c) or 160.120 because these experiments were undertaken initially as basic exploratory studies to determine the overall feasibility of this product." Signed by study director, Thomas McLoughlin, Ph.D.

14. Reviewer's Discussion and Interpretation of the Study:

A. Test Procedures: The procedures used followed those recommended by EPA in Section 158.170 of the EPA Registration Guidelines (Pesticide Testing Guidelines, Subdivision M, Microbial and Biochemical Control Agents).

B. Statistical Analysis: Results were calculated as counts, percentages and averages with standard deviations.

C. Discussion/Results: The results of these studies indicate that the P. cepacia isolates to be used by the registrant to formulate this product is not pathogenic to the plant species tested.

D. Adequacy of the Study:

1. Validation Category: Supplemental

2. Rationale: The studies were scientifically valid and are sufficient to demonstrate that these isolates were not pathogenic to the plant species known to be susceptible to other strains of P. cepacia and to selected major crop species. However, GLP guidelines were not followed, so even though the data can be used to make a risk assessment, the studies themselves will be considered supplemental.

15. Completion of the One-Liner:

DATA EVALUATION REPORT

1. Chemical: Pseudomonas cepacia - SMP-1

2. Test Material: Technical

3. Study/Action Type: An Avian Oral Pathogenicity and Toxicity Study, Species: Bobwhite Quail (Colinus virginianus)

4. Study Identification: An Avian Oral Pathogenicity and Toxicity Study, By J. Grimes, K. A. Hoxter and M. Jaber. Prepared By Wildlife International LTD, March 1990. Project No. 275-101. Submitted By Stine Microbial Products. Madison, WI. EPA Acc. No. 415466-05.

5. Reviewed By: David C. Bays
Microbiologist
EFED/EEB
Signature: David C. Bays
Date: 9/6/91

Les Touart
Supervisory Biologist
EFED/EEB
Signature: L. Touart
Date: 9/13/91

6. Conclusions:

The study is scientifically sound and demonstrated an LD_{50} > 5,000 mg/kg. This indicates that SMP-1 is practically nontoxic to birds. The study fulfills EPA Guideline requirements for an avian oral pathogenicity/toxicity test.

7. Recommendations: N/A

8. Background: This study was submitted to support the request for the registration of Pseudomonas cepacia - SMP-1.

10. Materials and Methods:

A. Test Organisms: Healthy day 2 old bobwhite, phenotypically indistinguishable from wild birds, were obtained from Fritts Quail Farm in Phillipsburg, NJ and acclimated until they were 21 days old. The bobwhite were distributed into 14 test groups of 5 birds each, without regard for the sex of the bird. The average body weights of the test birds at the beginning of the study ranged from 29±2 to 40±5 grams depending on test group. Water and feed, a game bird ration

formulated by Wildlife International Ltd, were provided ad libitum during the acclimation and testing periods.

B. Dosage Form: The test ($2x10^9$ cfu/ml), attenuated test, sterile filtrate test, and nutrient broth test substances, all yellow broths, were administered neat as received. The suspension was given to the birds at a dose of 0.5% of body weight (5,000 mg/kg of body weight or $1x10^{10}$ cfu/kg) each day for 5 days. The test substance was administered directly into the crop or proventriculus using a stainless steel cannula.

C. Referenced Protocol: The total concentration of the test substance given to each bird was 25,000 mg/kg or approximately $5x10^{10}$ cfu/kg of body weight. Two groups of birds were orally gavaged with each of the control suspensions (attenuated, nutrient broth and sterile filtrate). The negative control consisted of distilled water administered to 2 groups of birds (5,000 mg/kg of body weight). The remaining 6 test groups were dosed with SMP-1.

During acclimation and testing, all birds were assigned to breeding pens (23x72x90 cm) by random draw and housed indoors. Average ambient room temperature for the study was 26±2C with an average relative humidity of 51±14%. The photoperiod (monitored by a time clock) was 16 hours of light per day during acclimation and throughout the study. The light was provided by Chroma 50 fluorescent lights (5000 Kelvin) which closely approximated noon-day sunlight (4870 Kelvin). The birds received approximately 12 footcandles of illumination. Housing and husbandry practices were based upon the "Guide for the Care and Use of Laboratory Animals", NIH Publication No. 85-23, 1985.

All birds were observed daily during acclimation and any exhibiting abnormal behavior or physical injury were not used. After test initiation and continuing until termination, all birds were observed at least twice daily with all mortality, signs of toxicity or abnormal behavior being recorded. Body weights of the test birds were recorded individually prior to dosing and on days 0, 1, 2, 3, 4, 11, 18, 25 and 30. Average estimated feed consumption was measured for days 0-4, 4-11, 11-18, 18-25 and 25-30.

D. Statistical Analysis: None was needed due to the lack of mortalities observed in the study.

12. Reported Results:

Dosage	Replicate	Number Dead/Number Exposed (At 30 Days After Dosing)
Negative control	NC1	0/5
	NC2	0/5
Attenuated control	AC1	0/5
	AC2	0/5
Sterile filtrate	SFC1	0/5
	SFC2	0/5
Nutrient broth control	NBC1	0/5
	NBC2	0/5
5,000 mg/kg	T1	0/5
	T2	0/5
	T3	0/5
	T4	0/5
	T5	0/5
	T6	0/5

LD_{50} > 5,000 mg/kg

No mortalities occurred in any of the control groups (negative, attenuated, sterile filtrate or nutrient broth) or among the treated birds (5,000 mg/kg or approximately $9x10^8$ cfu/kg per day for five days). All birds were normal in appearance and behavior throughout the test period, except for 4 birds in the control groups and 3 birds in the treatment groups which had toe-picking, and one treated bird which had numersous symptoms. These conditions were not thought to be treatment related. There were no apparent effects on body weight or feed consumption between the control and the treated groups. All birds were euthanized using carbon dioxide at the termination of the study and then subjected to gross necroscopy. The results were not found to be remarkable except for evidence of toe-picking which was not considered treatment related.

13. Study Author's Conclusions/Quality Assurance Measures:

LD_{50} > 5,000 mg/kg

"This study was conducted so as to conform with Good Laboratory Practices as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160. Signed by study director, Kimberly A. Hoxter.

14. Reviewer's Discussion and Interpretation of the Study:

A. Test Procedures: The procedures used follow those recommended by EPA in the 1989 Pesticide Testing Guidelines for Microbial and Biochemical Pest Control Agents, Subdivision M.

B. Statistical Analysis: None was needed since there were no mortalities.

C. Discussion/Results: An LD_{50} > 5,000 mg/kg indicates Pseudomanas cepacia SMP-1 is practically non-toxic, on an acute basis, to birds.

D. Adequacy of the Study:

1. Validation Category: Core

2. Rationale: Meets EPA Guideline requirements

DATA EVALUATION REPORT

1. Chemical: Pseudomonas cepacia - SMP-1

2. Test Material: Technical

3. Study/Action Type: Nontarget Insect-Parasitic Hymenopteran (Uga menoni) (154A-23)

4. Study Identification: SMP-1: A Dietary Pathogenicity and Toxicity Study with the Parasitic Hymenopteran Uga menoni. By M. M. Thompson, K. A. Hoxter and M. Jaber. Prepared By Wildlife International LTD, March 1990. Project No. 275-105. Submitted By Stine Microbial Products. Madison, WI. EPA Acc. No. 415466-06.

5. Reviewed By: David C. Bays
Microbiologist
EFED/EEB

Signature: D. C. Bays
Date: 9/6/91

Les W. Touart
Head, Section 1
EFED/EEB

Signature:
Date: 9/13/91

6. Conclusions:

The study is scientifically sound and demonstrated an $LC_{50} > 10^7$ cfu/ml. This indicates that SMP-1 is practically nontoxic to parasitic wasps. The study fulfills EPA Guideline requirements for a nontarget insect pathogenicity/toxicity test.

7. Recommendations: N/A

8. Background:

This study was submitted to support the request for the registration of Pseudomonas cepacia - SMP-1.

10. Materials and Methods:

A. Test Organisms: Apparently healthy, parasitic wasps (Uga menoni) were used in the study and were obtained from the New Jersey department of Agriculture located in Trenton, New Jersey.

B. Dosage Form: The test substances were received as a cloudy liquid in the following different concentrations and formulations:

1. 100 ml 2/9/90 Attenuated SMP-1
2. 10^5 SMP-1 in 12.5% sucrose 2/12/90
3. 10^7 SMP-1 in 12.5% sucrose 2/12/90
4. 10^9 SMP-1 in 12.5% sucrose 2/12/90
5. 100 ml 2/9/90 12.5% (w/v) sucrose
6. 12.5% sucrose (w/v) 2/19/90
7. SMP-1 Attenuated Control (in 12.5% sucrose) 2-19-90
8. SMP-1 10^5 cfu/ml 2-19-90
9. SMP-1 10^7 cfu/ml 2-19-90
10. SMP-1 10^9 cfu/ml 2-19-90
11. SMP-1 Attenuated in 12.5 % sucrose 2/23 Parasitic Wasp
12. 2-26-90 12.5% Sucrose Negative Control
13. SMP-1 10^5 in 12.5% sucrose 2-26-90
14. SMP-1 10^7 in 12.5% sucrose 2-26-90
15. SMP-1 10^9 in 12.5% sucrose 2-26-90
16. 12.5% sucrose 3-2-90
17. Attenuated Control in 12.5% sucrose
18. SMP-1 in 12.5% sucrose 10^5
19. SMP-1 in 12.5% sucrose 10^7
20. SMP-1 in 12.5% sucrose 10^9
21. 12.5% sucrose
22. SMP-1 Attenuated Control in 12.5% sucrose.
23. SMP-1 10^5
24. SMP-1 10^7
25. SMP-1 10^9

C. Referenced Protocol: The test insects were placed in disposable one pint rolled paper containers (87 mm in diameter/85 mm high) that were covered with a disposable plastic petri dish (90 mm in diameter). The test diet (available ad libitum) was placed in a 20 ml glass vial which was covered with cheese cloth, and then inserted into the container's cover. A moist sponge, which was misted daily, was placed on the top of each container to increase humidity within the test chamber.

Two replicates, containing 25 insects each, were randomly assigned to each of 3 treatment levels (10^5, 10^7, 10^9 cfu/ml of feed) along with the attenuated (equal to highest test concentration used) and negative (12.5% sucrose mixture) controls. Fresh diet was given to the wasps and the average feed consumption for each test concentration and control group was determined on a weekly basis. The wasps were immobilized with nitrogen at the start of the study and when the test diet was introduced. The test insects were observed for mortality and signs of toxicity twice on the day the experiment started (first observation immediately following the introduction of the test diets) and once a day

thereafter until the end of the study. The environmental conditions were as follows: 8 hours of light/day, a temperature of 21-28C, and an average relative humidity of 51%.

D. Statistical Analysis: After study completion, an estimation of the LC^{50} value was made by visual inspection of the mortality data. A calculation of the LC^{50} value was not necessary because of the lack of mortalities found in this study.

12. Reported Results:

Dosage	cfu/ml	Replicate	Number Dead/Number Exposed (At 30 Days After Dosing)
Negative control	0	A	6/25
		B	1/25
Attenuated control	10^9	A	4/25
		B	3/25
Treatment	10^5	A	2/25
		B	3/25
	10^7	A	3/25
		B	3/25
	10^9	A	5/25
		B	2/25

$LC_{50} > 10^9$ cfu/ml feed

Mortalities occurred in both of the control groups (negative and attenuated) and in all 3 of the treatment groups. The mortalities in the control groups averaged 14%, while those in the 10^5, 10^7, and 10^9 cfu/ml feed concentrations averaged 10%, 12% and 14%, respectively. The mortality in the treatment groups was found to be less than, or equal to the control mortality and did not appear to be treatment related. No additional signs of toxicity were observed during the test.

13. Study Author's Conclusions/Quality Assurance Measures:

$LC_{50} > 10^9$ cfu/ml feed

"This study was conducted so as to conform with Good Laboratory Practices as published by the U.S. Environmental Protection Agency, Office of Pesticide Programs in 40 CFR Part 160." Signed by study director, Kimberly A. Hoxter.

14. Reviewer's Discussion and Interpretation of the Study:

A. Test Procedures: The procedures used follow those recommended by EPA in the 1989 Pesticide Testing Guidelines for Microbial and Biochemical Pest Control Agents, Subdivision M.

B. Statistical Analysis: None was needed since the pattern of mortality in this study did were not facilitate the calculation of an LC_{50} value.

C. Discussion/Results: An $LC_{50} > 10^9$ indicates that Pseudomonas cepacia is practically non-toxic to parasitic wasps.

D. Adequacy of the Study:

1. Validation Category: Core

2. Rationale: Meets EPA Guideline requirements

15. Completion of the One-liner:

Pseudomonas cepacia

Tox Chem. No. 714BC File last updated _____ Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	Tox Catagory	CORE Grade/ Doc. No
1/ Acute Oral Toxicity Microbiological Assoc G-7247.222 21 February 1990	TGAI	415466-01	Not pathogenic or infective at an oral dose of 2×10^8 CFU	—	Acceptable
2/ Acute Dermal Toxicity Microbiological Associates G-7254.232 21 February 1990	TGAI in [redacted]	415466-02	not toxic for rats when 2g material was dermally administered	—	Acceptable
3/ Acute Pulmonary Toxicity (152A-12) Microbiological Associates G-7247.225 21 February 1990	TGAI	415466-03	not infective or pathogenic for rats when dosed with 1.9×10^8 CFU	—	Acceptable
4/ Acute Intravenous Toxicity (152A-13) Microbiological Associates G-7247.224 21 February, 1990	TGAI	415466-04	not infective, pathogenic nor toxic for rats when dosed with 1.2×10^7 CFU	—	Acceptable

INERT INGREDIENT INFORMATION IS NOT INCLUDED

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