Pseudomonas aureofaciens Strain Tx-1 (006473) Technical Document

Issued: 04/00

I. Overview

A. Microbial Pesticide Overview

The following active ingredient is covered by this registration decision:

- **Common name:** *Pseudomonas aureofaciens*
- **Isolate name:** *Pseudomonas aureofaciens* strain Tx-1
- **OPP Chemical code:** 006473
- **Trade and other names:** Spot-LessT Biofungicide
- **Registrant:** Eco Soils Systems, Inc., San Diego, Ca

B. Use Profile

The following information details the proposed use sites and application methods for *Ps. aureofaciens* Tx-1, as applied through the BioJect® System.

- **Type of pesticide:** biological fungicide
- **Terrestrial crops:** turf
- **Target pests:** *Sclerotinia homeocarpa* (dollar spot), *Collectotrichum graminicola* (anthracnose), *Pythium aphanadermatum* (pythium), *Michrodochium nivale* (pink snow mold).
- **Formulation type:** fermenter product into irrigation lines, initially at 1 x 108- 1 x 109 cfu/mL, diluted > 1000 fold at application site.
- **Method of application:**
- **Equipment:** BioJect® Fermentation and Injection System
- **Method:** spray through sprinkler system after fermentation (approx. 12h) and dilution into water line on-site (golf course)
- **Timing:** at night after golfers have vacated the course; can be applied on a nightly basis after each fermentation cycle.
- **Use practice limitations:** label limits use to turf only and application using BioJect® System only

C. Estimated use of Pesticide

There are approximately 10,000 golf course in the United States currently and Eco Soils estimates that 20% of these are potential customers of the BioJect System. The potential customers are located primarily in the eastern, midwestern and southern states. On a typical course approximately 50 acres will be treated with the BioJect System, hence, a maximum of 100,000 acres could be anticipated as being treated. However the registration is currently limited to 50,000 acres until the outstanding non-target organism data are satisfied.

D. Data Requirements

Eco Soil Systems will submit the following data within the specified time-frames:

The following data must be submitted within the specified time-frames.

Eco Soil Systems Data Requirements

Study and Guideline	Test Substance	Due Date
Avian Oral Toxicity/Pathogenicity Bobwhite Quail Harmonized Test Guideline 885.4050 (PDF) (6 pp, 15 K)	Undiluted BioJect® Fermented Pseudomonas aureofaciens strain Tx-1 Fermentation Broth and Solids	February 9, 2000
Avian Oral Toxicity/ Pathogenicity Mallard Duck Harmonized Test Guideline 885.4050 (PDF) (6 pp, 15 K)	Undiluted BioJect® Fermented *Pseudomonas aureofaciens* strain Tx-1 Fermentation Broth and Solids	February 9, 2000
Freshwater Fish Toxicity/Pathogenicity Rainbow Trout Harmonized Test Guideline 885.4200 (PDF) (6 pp, 15 K)	Undiluted BioJect® Fermented Pseudomonas aureofaciens strain Tx-1 Fermentation Broth and Solids	February 9, 2000
Freshwater Fish Toxicity/ Pathogenicity Bluegill Sunfish Harmonized Test Guideline 885.4200 (PDF) (6 pp, 15 K)	Undiluted BioJect® Fermented Pseudomonas aureofaciens strain Tx-1 Fermentation Broth and Solids	February 9, 2000
Freshwater Aquatic Invertebrate Toxicity/ Pathogenicity/ *Daphnia magna*/	Undiluted BioJect® Fermented	February 9, 2000

Harmonized Test Guideline 885.4240 (PDF) (7 pp, 16 K)	Pseudomonas aureofaciens strain Tx-1 Fermentation Broth and Solids	
Non-Target Insect Toxicity/ Pathogenicity/ Green Lacewing Harmonized Test Guideline 885.4340 (PDF) (6 pp, 16 K)	Undiluted BioJect® Fermented Pseudomonas aureofaciens strain Tx-1 Fermentation Broth and Solids	February 9, 2000
Non-Target Insect Toxicity/ Pathogenicity/ Parasitic Wasp Harmonized Test Guideline 885.4340 (PDF) (6 pp, 16 K)	Undiluted BioJect® Fermented Pseudomonas aureofaciens strain Tx-1 Fermentation Broth and Solids	February 9, 2000
Non-Target Insect Toxicity/ Pathogenicity/ Ladybird Beetle Harmonized Test Guideline 885.4340 (PDF) (6 pp, 16 K)	Undiluted BioJect® Fermented Pseudomonas aureofaciens strain Tx-1 Fermentation Broth and Solids	February 9, 2000
Honey Bee Toxicity/pathogenicity Harmonized Test Guideline 885.4380 (4 pp, 10 K)	Undiluted BioJect® Fermented Pseudomonas aureofaciens strain Tx-1 Fermentation Broth and Solids	February 9, 2000
Quantitative analysis of 5 batches for phenazine-1-carboxylic acid and other Pseudomonas antibiotics along with a complete literature search and copies of relevant publications regarding antibiotic(including 1-phenazine-carboxylic acid) production in Pseudomonas.	Undiluted BioJect® Fermented Pseudomonas aureofaciens strain Tx-1 Fermentation Broth and Solids	February 9, 2000

E. Regulatory History

Eco Soil Systems applied to register Pseudomonas aureofaciens Strain Tx-1 on June 23, 1997 for use on turf. Their application was received on July 15, 1997 and EPA published a notice of receipt in the Federal Register on December 9, 1997. No comments were received as of February 8, 1999.

F. Food Clearances / Tolerances

None.

II.

III. Science Assessment

A. Product Characterization Assessment

All product characterization data requirements are satisfied for Pseudomonas aureofaciens strain Tx-1.

Ps. aureofaciens is a naturally occurring bacterium that is ubiquitous in soil and plant rhizospheres. Strains of this species have been used for biological control of several fungal plant diseases attacking the root zone of crop species. A phenazine antibiotic is produced by *Ps. aureofaciens* and is known to have antifungal properties that are useful in limiting the spread of fungal phytopathogens. This organism will be used in an on site fermentation and irrigation application system to target *Sclerotinia homeocarpa*, a significant fungal pathogen of bentgrass and ryegrass as used in golf course situations.

The physical state of this product will be the fermented culture of Ps. aureofaciens Tx-1 following 12 to 14 h of culture (approx. 108 cfu/mL final concentration; spent culture medium; pH 7.5) in the BioJect Fermentation and Injection System diluted with municipal (potable) water by injection into a sprinkler irrigation system. On some solid media (e.g., brain-heart infusion (BHIA), trypticase soy (TSA)) the bacterium produces circular orange colonies that are smooth, with an entire margin, convex, and translucent in opacity. On nutrient agar the colonies are cream colored. A green fluorescent pigment is produced upon culture on King's B medium.

This assessment of product analysis is based upon adequate quality control as provided at the time of culture (i.e., operation of the BioJect System) of Ps. aureofaciens Tx-1. Quality control measures considered as required for assurance of a safe product include, but are not limited to:

1. Scheduled maintenance and replacement, as per BULB manufacturer's instructions, must be followed to ensure adequate (UV) BACTERICIDAL intensity.

2. the injection system providing for the introduction of Ps. aureofaciens Tx-1 into the fermentation tank must be working in an accurate fashion to assure delivery of inoculum.

3. only potable water is to be used as a source for MEDIA PREPARATION and DISINFECTION of the BioJect System.

4. a disinfecting wash (e.g., peracetic acid) of the fermentation tank and associated lines must be included immediately prior to every fermentation cycle.

If any of the above procedures are not followed, the resulting batch must be aborted and appropriately disposed.

Table 1. Product Characterization

Guideline	Study	Result	MIR
151A-10-12 151A-13, 15 151A-21, 22	Ping Fungicide: Product Identity and Composition; Analysis and Certification of Product Ingredients; Ping Fungicide: Physical and Chemical Properties; Response of EPA Letter of December 17, 1997: Manufacturing Process; Discussion of Impurities; Growth of Pseudomonas aureofaciens strain Tx-1 Using the BioJect Automated Microbial Fermentation and Injection System.	Ps. aureofaciens Tx-1 was isolated from turfgrass. soil in 1956 at Michigan State University; this species occurs naturally in soil and water. Tx-1 is resistant to rifampicin, grows from 4 - 37 C, produces phenazine 1-carboxylic acid and green fluorescent pigment on King's B medium. Substrate utilization, fatty acid methyl ester analysis, and biochemical characteristics were supportive of the finding that Tx-1 belongs to the specific epithet 'aureofaciens'. Growth inhibition of several fungal phytopathogens was demonstrated by in vitro plate assays. Spot-Less®, previously Ping®, Fungicide, is produced at 2.9 x 10 10 to 5.8 x 1011 cfu/fl. oz.	44337 44337 44337 44461 44437 44461
151-20	Supplemental Product Identification data to Support Registration (EPA File Symbol 70688-R)	ATCC Identification: Gram negative, aerobic, motile rods, polar flagella (multiple); translucent, convex, smooth, circular colonies; orange pigment on TSA and BHIA, fluorescent pigment on King's B. DNA sequence of the 16S rRNA gene presented.	44422

B. Human Health Assessment

There is a reasonable certainty that no harm will result from non-dietary exposure to Pseudomonas aureofaciens strain Tx-1.

0. Toxicity/Pathogenicity Assessment

Certain acute toxicity studies for the technical grade active ingredient, Ps. aureofaciens Tx-1, have been submitted and adequately satisfy the requirements as set forth in 40 CFR 158.740 for non-food/feed use of microbial control agents.

Table 2. Acute Toxicity Studies

Guideline	Study	Result	Toxicity Categor
81-1 870.1100 (PDF) (10 pp, 26 K)	Acute Oral Tox.	Test Material: *Ps. aureofaciens* Tx-1, lot 9730004. One female rat displayed weight loss from day 7 to 14; dilated pupils were noted in three female rats days 1 to 4; no abnormalities noted at necropsy; LD50 was greater than 5000 mg/kg of body weight.	IV
81-5 870.2500 (PDF) (8 pp, 21 K)	Primary Skin Irritation	Test Material: Ps. aureofaciens Tx-1, lot 9730004. Slight dermal irritation and erythema at 1 h resolved by 72 h; tan discoloration at test site in all rabbits; primary irritation index = 0.63.	IV
152A-10 885.3050 (PDF) (8 pp, 20 K)	Acute Oral Toxicity / Pathogenicity	Test Material: Ps. fluorescens EG-1053-TGAI; No significant effect on mean body weight and no mortality associated with treatment; Ps. fluorescens considered non-pathogenic at 2.6 x 10 9 cfu/kg *	IV
152A-12 885.3150 (PDF) (8 pp, 20 K)	Acute Pulmonary Toxicity / Pathogenicity	Test Material: Ps. fluorescens EG-1053-TGAI; Ps. fluorescens was not infective, toxic or pathogenic for rats when administered at 1.5 x 108 cfu/animal. There was no effect on mean body weight gain, no mortality recorded and no treatment related lesions. *	IV
152A-13 885.3200 (PDF) (8 pp, 20 K)	Acute Intravenous Toxicity / Path.	Test Material: Ps. fluorescens EG-1053-TGAI; Treatment had no significant effect on mean body weight of mice; no mortalities were recorded and no treatment related lesions when administered at 2.2 x 108 cfu/kg.*	IV
81-2 870.1200 (PDF) (10 pp, 25 K)	Acute Dermal Toxicity	Test Material: Ps. fluorescens EG-1053-TGAI; Mean body weights of rats increased during the 14 d study; Ps. fluorescens was considered non-toxic when applied to rabbit skin*	IV
152A-15 885.3400 (PDF) (3 pp, 8	Hyper-sensitivity	None reported	NA

K)

* studies bridged from (Pseudomonas fluorescens)

The oral toxicity study conducted in support of Ps. aureofaciens Tx-1 resulted in the finding that the LD50 was greater than 5000 mg/kg of body weight and, therefore, correlated with toxicity category IV. Of the 10 Sprague-Dawley Rats tested with the TGAI, one female displayed a weight loss over the last half of the study; all others gained weight. No abnormalities were noted in any of the animals upon examination at the end of the study.

The Primary Dermal Irritation Study indicated that Ps. aureofaciens Tx-1 was in toxicity category IV with respect to dermal irritation. The six New Zealand White rabbits all exhibited a slight erythema at 1 h post treatment with the TGAI. However, all resolved this inflammation by 72 h post-dosing.

The acute oral toxicity and dermal irritation studies submitted for the active ingredient Ps. aureofaciens Tx-1 demonstrate a lack of toxicity or adverse effects following dosage with amounts (cfu) surpassing those present following application of this bacterium through irrigation lines and sprinklers on golf courses. Of course, this determination is for the active ingredient as applied through the BioJect Automated Microbial Fermentation and Injection System as currently designed; any changes in the identity of the active ingredient or significant alterations to the BioJect System will require a full assessment for potential adverse effects.

1. Exposure and Risk Assessment

Based upon the intended application of the product to non-food and non-feed uses, a tolerance exemption or limit is not needed. Due to the method of application (i.e., through irrigation sprinklers), a route for dermal, eye and oral contact exists for this organism. Due in part to the nocturnal application of this product after golf courses would normally be closed to golfers, exposure to non-applicators is greatly reduced. This organism, Ps. aureofaciens, is known to be susceptible to the detrimental effects of UV light, and therefore, would decrease significantly into daylight hours when golfers and turf maintenance staff would be present. Additionally, the volume of water applied as part of the irrigation and application process would not only dilute the concentration of the active

ingredient, but also wash many of the cells into the thatch and soil profile where contact with humans and animals is greatly reduced.

With the BioJect Automated Microbial Fermentation and Injection System being a closed system in that the inoculum and medium are directly injected into the fermentation tank and distribution to the site of application is through sealed irrigation lines, personal protective equipment as often used in the application of microbial biopesticides is not considered relevant. It is the Agency's opinion that Ps. aureofaciens Tx-1 as applied through the BioJect System will not result in a significant risk to human health.

Review of the available toxicology data and literature submitted in support of registration indicates that sufficient information is available to allow for characterization of the risks to humans. Products which contain Ps. aureofaciens Tx-1 are not likely to produce adverse effects on humans and Ps. aureofaciens is generally considered as non-pathogenic to humans. This organism has been used for two years under non-pesticidal claims in the BioJect System with no reports of incidents of adverse effects or reactions. There are no reports of Ps. aureofaciens causing adverse effects in (or under) clinical situations and this organism is not considered a threat to human health.

The use patterns of this biofungicide does not include food or feed uses and, therefore, the establishment of an exemption from tolerance or the establishment of a tolerance limit is not required under the provisions of FFDCA section 408.

C. Environmental Assessment

According to Eco Soil Systems, Ps. aureofaciens strain Tx-1 is currently being applied through approximately 200 Bioject® units, distributed within the U.S., reportedly on a maximum of 19,700 acres of commercial golf course turf. Some of these units have been in use since 1997. To date, "[n]o adverse effects to human health or to the environment have been reported" as a result of the Tx-1 applications.

Exposures of non-target organisms to Ps. aureofaciens strain Tx-1 will increase as a result of continuous applications via the BioJect® System through sprinkler

irrigation and it cannot be determined if such exposures would lead to hazardous effects, based on the submitted information to date. An antibiotic, phenazine-1-carboxylic acid is produced by Pseudomonas aureofaciens strain Tx-1. The average amount that is produced during fermentation of the strain and its spectrum of activity has not been sufficiently addressed to allay nontarget organism toxicity testing. Other secondary metabolites may be produced as well. Since the intended use pattern involves continuous application(s) of Ps. aureofaciens strain Tx-1, there is a concern for increased exposure of the intended strain over naturally-occurring populations of Ps. aureofaciens, other phenazine-producing pseudomonads, and an increased runoff to the aquatic environment. Pseudomonas species are efficient saprophytic chemo-organotrophs (nutritionally versatile) and thrive under moist conditions in soil, in sewage sediments and aquatic environments; capable of survival and multiplication for months[(1)].

However, in consideration of the open literature regarding the biology of Pseudomonas aureofaciens and current golf course management recommendations, it would appear that continuous applications of Ps. aureofaciens strain Tx-1 when applied through the Bioject® system under similar rates, frequencies and conditions as previously applied on the specified golf courses would not lead to irreversible ecological hazards for the duration of a limited acreage conditional golf course registration.

No definitive risk assessments to aquatic and terrestrial wildlife can be made without appropriate pathogenicity and toxicity testing. Therefore, the following data are necessary to more fully characterize the non-target organism risk.

Study and Guideline Test Substance

Guideline	Study
Avian Oral Toxicity/Pathogenicity Bobwhite Quail Harmonized Test Guideline 885.4050 (PDF) (6 pp, 15 K)	Undiluted BioJect® Fermen *Pseudomonas aureofaciens* strain Tx-1 Fermentation B and Solids
Avian Oral Toxicity/ Pathogenicity Mallard Duck Harmonized Test Guideline 885.4050 (PDF) (6 pp, 15 K)	Undiluted BioJect® Fermen *Pseudomonas aureofaciens* strain Tx-1 Fermentation B and Solids
Freshwater Fish Toxicity/Pathogenicity Rainbow Trout Harmonized Test Guideline 885.4200 (PDF) (6 pp, 15 K)	Undiluted BioJect® Fermen Pseudomonas aureofaciens strain Tx-1 Fermentation B and Solids

Freshwater Fish Toxicity/ Pathogenicity Bluegill Sunfish Harmonized Test Guideline 885.4200 (PDF) (6 pp, 15 K)	Undiluted BioJect® Fermen Pseudomonas aureofaciens strain Tx-1 Fermentation B and Solids
Freshwater Aquatic Invertebrate Toxicity/ Pathogenicity/ *Daphnia magna*/ Harmonized Test Guideline 885.4240 (PDF) (7 pp, 16 K)	Undiluted BioJect® Fermen Pseudomonas aureofaciens strain Tx-1 Fermentation B and Solids
Non-Target Insect Toxicity/ Pathogenicity/ Green Lacewing Harmonized Test Guideline 885.4340 (PDF) (6 pp, 16 K)	Undiluted BioJect® Fermen Pseudomonas aureofaciens strain Tx-1 Fermentation B and Solids
Non-Target Insect Toxicity/ Pathogenicity/ Parasitic Wasp Harmonized Test Guideline 885.4340 (PDF) (6 pp, 16 K)	Undiluted BioJect® Fermen Pseudomonas aureofaciens strain Tx-1 Fermentation B and Solids
Non-Target Insect Toxicity/ Pathogenicity/ Ladybird Beetle Harmonized Test Guideline 885.4340 (PDF) (6 pp, 16 K)	Undiluted BioJect® Fermen Pseudomonas aureofaciens strain Tx-1 Fermentation B and Solids
Honey Bee Toxicity/Pathogenicity Harmonized Test Guideline 885.4380 (4 pp, 10 K)	Undiluted BioJect® Fermen Pseudomonas aureofaciens strain Tx-1 Fermentation B and Solids
Quantitative analysis of 5 batches for phenazine-1-carboxylic acid and other Pseudomonas antibiotics along with a complete literature search and copies of relevant publications regarding antibiotic(including 1-phenazine-carboxylic acid) production in Pseudomonas.	Undiluted BioJect® Fermen Pseudomonas aureofaciens strain Tx-1 Fermentation B and Solids

D. Efficay Data

The Eco Soil Systems' submissions contain several efficacy studies which compared Pseudomonas aureofaciens strain Tx-1 (hereafter abbreviated, "Tx-1") application to propiconazole and/or chlorothalonil, iprodione, mycobutanil or triadimefon to control fungal diseases on turf. Abbreviated summaries and results are provided for each study; followed by BPPD evaluation of all submitted studies.

Biological Control of Dollar Spot 1998 - P.J. Dwyer & J.M. Vargas, Jr., Michigan State University: A 10-wk small plot (9 sq. ft) field trial comparing multiple and frequent applications of Tx-1 with or without a single application of

propiconazole (Banner®) was conducted at an on-site university turfgrass research station. Results showed:

0. a significant level of control of dollar spot (*Sclerotinia homeocarpa*) was achieved and sustained by weekly applications (5x per wk) of Tx-1 (=total of 50 applications, by normal irrigation) as compared to field plots which did not receive Tx-1 treatment;

1. application of Tx-1 at weekly intervals (one application/week = 10 total) provided significant levels of control for some weeks, (compared to untreated plots), but not as acceptable as 5 times per week;

2. a single application of propiconazole can reduce the number of dollar spots compared to multiple and frequent applications of Tx-1 (5x per week); both Tx-1 and propiconazole maintained a similar level of control until the 6^{th} week when propiconazole lost its efficacy and Tx-1 provided significantly greater levels of control; and,

3. continued significant control of dollar spot was achieved with a single application of propiconazole in conjunction with multiple and frequent applications of Tx-1 (total of 50 applications).

Evaluation of the BioJect System for the Control of Dollar Spot , 1998 - J. Bresnahan, Univ. of Massachusetts: Comparative efficacy experiments for suppression of dollar spot of turfgrass were evaluated on field plots (3 x 6 ft) in a completely randomized design, replicated 4 times at four western Massachusetts locations (3 golf courses and 1 university research station-abbreviated as U/Mass). Treatment areas were maintained under normal maintenance schedules; no fungicides were applied by golf course superintendents. Five fungicide treatments included nightly applications of Tx-1 every night at 1 x 10^{-8} cfu/ml singly, or in combination with Daconil® (chlorothalonil) applied at 14-day intervals @ 1.5 g/1000 sq.ft; or in combination with Banner® (propiconazole) applied at 21-day intervals @ 1.25 oz/1000 when an average of 5 dollar spots per plot was present; and chemical treatments without nightly application of Tx-1. Results of tests conducted at one golf course ("The Orchards GC") and at U/Mass research station basically showed that all fungicide treatments significantly controlled dollar spot; however, control (or suppression) of dollar spot by the four treatments with the chemical pesticides was significantly greater than the plot receiving only BioJect® application of Tx-1 in 3 of 4 reported evaluations at the U/Mass site. Chemical fungicides were not applied at the Orchards GC, because dollar spot was not severe to justify chemical control. Results of two other golf course sites were not reported.

Evaluation of P. aureofaciens Applied by Irrigation for Disease Control in Fairways -

G. Hardebeck & Z. Reicher, Purdue Univ., IN*:* The control (or suppression) of dollar spot, brown patch and Pythium diseases with Tx-1 applied through an irrigation system was investigated in a split block design with 3 replications at the university research station. The size of the plots was not provided; however, the main plots differentiated Tx-1 application and the subplots were comprised of combinations of chemical fungicide strategies and fertility rates. Results showed:

d. Tx-1 applications reduced the mean number of dollar spots per plot consistently on all 12 observation dates; significant differences were reported for 50% of the test dates. The higher N fertility rate also reduced dollar spot severity. Dollar spot pressure throughout the season required preventative, as well as curative chemical applications to Tx-1 treated and untreated plots.

e. Tx-1 applications did not reduce brown patch (Rhizoctonia solani) severity; in fact, there was a statistically significant increase in brown patch due to bacterial application on Aug. 31. Nitrogen fertility had no significant effect on brown patch disease severity.

f. There was no Pythium disease incidence during the test. Although it is too early to draw definite conclusions from the study, nightly application of Tx-1 through an irrigation system appears to significantly reduce the incidence of dollar spot on fairway height creeping bentgrass; it also appears that Tx-1 does not reduce the incidence of brown patch on fairway height colonial bentgrass.

Pseudomonas aureofaciens TX-1 alone appeared to give control of dollar spot disease of turf (Sclerotinia homeocarpa) similar to or slightly worse than the typical turf fungicides in these tests at university turf farms in Illinois and Maryland. TX-1 gave better dollar spot control than the fungicides at the first two disease determinations in Maryland and lower dollar spot disease incidence than Daconil plus Prostar or Thalonil in Illinois. The results were difficult to interpret due to the lack of statistical analysis in both tests and the low incidence of dollar spot disease in the Illinois location. Brown spot (Rhizoctonia solani) was also controlled by TX-1 similar to the fungicides examined. It appears that TX-1 in combination with fungicides improves the turf disease control activity of the fungicide.

EPA Evaluation

Based on these small plot field trials conducted in one season, it appears that Tx-1 applied through the Bioject® continuous fermentation system and routine irrigation has some efficacy for short term control of dollar spot on turf, provided that: disease pressure is not intense, and multiple and frequent (daily, in some cases) applications of Tx-1 are utilized in the disease management program. Collectively, results of these tests also show that this microbial product may be

integrated with chemical fungicides; and in certain circumstances, may reduce the number of chemical applications. A chemical reduction strategy, coordinated with fertility management and biopesticide application is at the discretion of the user. Application of Tx-1 did not control the "other" turf diseases; in fact, on one day in one study (Hardebeck and Reicher), there was a significant increase in brown patch due to bacterial application; and results regarding Pythium control were inconclusive.

Eco Soil Systems, Inc. did not provide any analysis, but noted that "[t]he data demonstrates that Tx-1 provides, in most situations, fungal control similar to chemical fungicides." Based upon the data submitted and the BPPD's evaluation, this conclusion appears overstated. Furthermore, as noted by Bresnahan (as cited in Nelson[(2)]), efforts to apply microorganisms through irrigation systems have resulted in inconsistent performance. Obviously, the use of this technique may prove to be problematic during periods of rainfall when irrigation could prove to be detrimental, unless sufficient doses of the microbe are demonstrated to be delivered with small amounts of irrigation water.

E. Public Interest Finding

Information was submitted to substantiate a public interest finding for the intended uses of Pseudomonas aureofaciens Strain Tx-1 (Spotless®) on golf courses.

Tx-1 has been demonstrated to be at least somewhat efficacious in early season and/or low disease pressure situations for the control of dollar spot on high value ornamental turf. Therefore this product may potentially be used to replace some applications of certain registered chemical alternatives, some of which are considered higher risk than the candidate naturally occurring microbe. Furthermore, the use of Tx-1 may be of value to golf course superintendents who wish to rotate compounds to prevent the development of resistance.

Additionally, the proposed use as an injection into existing irrigation systems eliminates or reduces certain exposures related to mixer/loader/applicators, and offers advantages regarding fuel-savings and equipment maintenance (tractors and sprayers). Also, the method of application reduces potential problems as a result of sprayer cleanup and the disposal of equipment wash-waters normally associated with turf disease control.

Therefore, Tx-1 is considered to be in the public interest due to a) reduced toxicity (from certain registered alternatives), b) reduced applicator exposure to more toxic alternatives , and c) provide indirect savings to users.

IV.

V. Risk Management/Registration Eligibility

Pursuant to FIFRA section 3(c)(7)(C), EPA may conditionally register a new pesticide active ingredient if: 1) insufficient time has elapsed since the imposition of the data requirement for those data to be developed and all other required data have been submitted, 2) the use of the pesticide product during the period of the conditional registration will not cause any unreasonable adverse effect on the environment, and 3) the registration and use of the pesticide during the conditional registration is in the public interest. BPPD believes that all these criteria have been fulfilled.

The first criteria under FIFRA section 3(c)(7)(C) mentioned above has been met since insufficient time has elapsed since the imposition of the data requirements. BPPD indicated that the application for registration failed the new active ingredient screen on December 17, 1997. However, we indicated in that letter that "[o]ur ongoing review of your bridging rationale will determine the adequacy of the toxicity/pathogenicity data cited. You must also address the honeybee toxicity/pathogenicity requirement via submission, citation, or adequate waiver request." It was not until BPPD's August 18, 1998 letter and accompanying reviews that Eco Soil Systems was made fully aware of the need of Tier I non-target organism studies using BioJect® output as the test substance.

The applicant has submitted or cited data to satisfy the second criterion for conditional registration under FIFRA 3(c)(7)(C) as mentioned above. The human health effects data and nontarget organism effects data are considered sufficient for the period of the conditional registration. These data demonstrate that no foreseeable human health hazards are likely to arise from the use of the product and that ecological effects during the limited two-year registration are not expected to be significant. In view of these minimal risks and the benefits, BPPD believes that the use of the product during the limited period of the conditional registration will not cause any unreasonable adverse effects.

Although the data with respect to this particular new active ingredient is satisfactory, it is not sufficient to support an unconditional registration under FIFRA 3(c)(5). Additional data is necessary to evaluate the risk posed by the continued use of this product. BPPD recommends imposing the data requirements specified earlier in this Registration Eligibility Document in the ecological effects section.

BPPD also believes that the third criterion for a FIFRA 3(c)(7)(C) conditional registration has been fulfilled because the use under this registration would be in the public interest.

This registration will automatically expire on midnight February 9, 2001. EPA will evaluate the required non-target organism data before February 9, 2001 and decide on whether to convert the registration to a non-expiring registration.

VI. Actions Required by Registrants

Submission of outstanding non-target organism data.

Eco Soil Systems must submit a report to the Microbial Pesticides Branch of BPPD for the fiscal year in which this product is conditionally registered which reports the acreage treated during that fiscal year. The fiscal year begins October 1 and ends September 30. Treatment information will be submitted to the Agency no later than November 15, following the end of the preceding fiscal year.

Eco Soil Systems will submit production information for this product to Mr. Owen Beeder of Registration Division (7505C) for the fiscal year in which this product is conditionally registered, in accordance with FIFRA § 29. The fiscal year begins October 1 and ends September 30. Production information will be submitted to the Agency no later than November 15, following the end of the preceding fiscal year. We note that the production data required would be for the amount of product produced by your supplier, not the amount of material produced by the on-site BioJect® fermentor.

Reports of incidences of adverse effects to humans or domestic animals and target pest resistance under FIFRA, Section 6(a)2.

[1]. Organization for Economic Cooperation and Development. 1997. Consensus Document on Information Used in the Assessment of Environmental Applications Involving Pseudomonas. Series on Harmonization of Regulatory Oversight in Biotechnology No. 6. p 110.

[2.] Nelson, M. 1998. The Microbial World. USGA Green Section RECORD 36(4):1-5.