Bacillus subtilis var. *amyloliquefaciens* strain FZB24 (006480) Biopesticide Registration Action Document

I. Abstract

See **Fact Sheet**

II. Overview

A. Overview

- **Microbial Pesticide Name:** Bacillus subtilis var. amyloliquefaciens strain FZB24
- **Trade Name(s):** TAE-022 WDG
- **OPP Chemical Code:** 006480
- **Basic Manufacturer:** Taensa Inc.
- **US Agent:**

  Edgar R. Butts, Ph.D.
  26 Sherman Ct.
  P.O. Box 764
  Fairfield, Ct 06430

B. Use Profile

Type of Pesticide: Microbial Fungicide and Plant Regulator

Mode of action: Bacillus subtilis var. amyloliquefaciens strain FZB24 is a naturally occurring soil microorganism. The mode of action appears to be an enzyme production, which acts as an antifungal agent and plant growth promoter.

Food Clearances/Tolerances: There are no food uses associated with this action, therefore, a tolerance establishment/exemption is not an issue for the proposed uses.

Use Sites: At this time, Bacillus subtilis var. amyloliquefaciens strain FZB24 is only registered for greenhouse and indoor use.

Greenhouse Food: None

Greenhouse and Indoor Non-Food: Ornamentals, shrubs, shade and forest tree seedlings.

Target Pests for Active Ingredient: TAE-022 is used for plant strengthening, enhancing, growth, increasing yields, and for suppressing selected soil-born diseases such as *Rhizoctonia* and *Fusarium*.

Formulation Types Registered:

Form

Technical - 73.3% (5.3 H 1011 cfu/g) Bacillus subtilis var. amyloliquefaciens strain FZB24.

End-use product - water dispersible granule; 24.5% (5.0 H 1010 cfu/g) Bacillus subtilis var. amyloliquefaciens strain FZB24

Method and Rates of Application:

Types of Treatment: Water dispersible granules should be pre-mixed thoroughly with water to assure a properly concentrated suspension. For best results, apply TAE-022 to seedlings or to newly rooted cuttings.

Equipment: None specified

Timing: Apply content of entire suspension within a few hours of mixing to ensure viability of TAE-022. Apply TAE-022 as early as possible in the life cycle of the plant to enhance growth and disease resistance. TAE-022 should be applied to plants every few weeks for up to three to four applications as needed.

Rates of Application:

Drenching: Per 100 gallons of water - by weight use 75 grams or 2.6 ounces; by volume use 3.5 fluid ounces. Per one gallon of water - by weight use 0.75 grams; by volume use 0.2 teaspoon.

Cutting and Root Dips: Per 10 gallons of water - by weight use 40 grams; by volume use 1.8 fluid ounces. Per one gallon of water - by weight use 4 grams; by volume use one teaspoon. Per one liter of water - by weight use one gram.

Transplants, Including Plugs: Dip or Drench until root system is thoroughly soaked

Method of Application: When drenching, apply TAE-022 to seedlings or to newly rooted cuttings. Cutting and root dips should be placed in the suspension for five to ten minutes allowing time for TAE-022 to penetrate the rootzone. Ornamentals should receive at least one follow-up drench treatment two to three weeks following initial treatment. Transplants and plugs root system should be dipped or drenched prior to planting.

C. Regulatory History

Taensa Inc. submitted an application February 8, 1999 for registration of TAE-022 Technical and TAE-022 WDG; active ingredient Bacillus subtilis var. amyloliquefaciens strain FZB24.

III.

IV. Science Assessment

A. Physical and Chemical Properties Assessment

Product Identity:

The agency has classified *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 as a microbial pesticide because it is a naturally occurring soil microorganism. *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 was shown to grow from 30EC to 50EC. *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 is recognized by Deutsche Sammlung von Mikroorganism (DSM): DSM ID 96-2, with an optimal antifungal activity in minimal media (e.g., SNA media) at cooler temperatures (e.g., 17EC), and on a broad spectrum scale.

Product chemistry data which support the registration of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 are summarized in Table 1.

Table 1. Physical and Chemical Properties for *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24.

Guideline No	Study	Results	MRID No.
885.1100	Product Identity and Disclosure of Ingredients	Acceptable	447581-01
885.1200	Manufacturing Process	Acceptable	447581-02
885.1300	Formation of Unintentional Ingredients	Acceptable	447581-03
885.1400	Analysis of Samples	Acceptable	447581-04

885.1500	Certification of Limits	Limits listed in the CSF are adequate	447581-05
30.6302, 830.6303, 830.6304, 830.7000, 885.7300	Product Chemistry	Acceptable. TAE-022 Technical is beige, solid, acidic odor, a bulk density of 0.186 g/mL, and pH 6.57 (20EC). TAE-022 WDG is beige, solid, carbonyl odor, bulk density of 0.739 g/mL, and pH 6.52 (20EC)	447581-06 447581-07
885.1100	Growth Parameters at Various Temperatures	Acceptable. *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 grows from 4.2 H 10^{11} cfu/g at 37EC to 6.0 H 10^{11} cfu/g at 34EC.	447581-19
885.1100	Sensitivity Detection	Acceptable. The detection method for *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 was demonstrated.	447581-20

B. Human Risk Assessment

The *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 acute oral, pulmonary, and intravenous toxicity tests were conducted according to Agency guidelines and demonstrated little to no potential adverse effects. There is, therefore, a reasonable certainty that no harm will result from exposure to *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24. This includes all anticipated dietary exposures and all other exposures for which there is reliable information.

Although *B. subtilis* is not a frank human pathogens, there have been strains isolated from human infections. However, such occurrences are typically limited to patients who are immuno-compromised because of some other condition or disease. In addition, the organism does produce the enzyme subtilisin which has been reported to produce allergenic or hypersensitivity reactions to individuals repeatedly exposed to the enzyme. However, OSHA has established standards for industrial settings (the only likely potential for repeated exposure) for exposure limits to workers.

Overall, *B. subtilis* produces only a very small potential risk to human health. The organism is not a frank pathogen, but is similar to numerous other opportunistic pathogens which are ubiquitous in nature. In all likelihood, individuals who may be sensitive to the organism have already been exposed to it, and risk of increased exposure is likely only to exist for pesticide applicators and manufacturers of the product. These individuals should be equipped with adequate personal protection equipment.

1. Human Toxicity Assessment

a. Acute Toxicity

All mammalian toxicology data requirements have been submitted and adequately satisfy data requirements to support registration.

The acute oral, acute pulmonary toxicity/pathogenicity, and acute eye irritation studies resulted in Toxicity Category III classification and acute dermal irritation studies resulted in Toxicity Category III for the end-use product. The technical or manufacturing product has a Toxicity Category I for eye irritation, Toxicity Category III for dermal irritation, and Toxicity Category III for other exposures.

Table 2. Toxicity Data Requirements

Guideline No	Study	Results	MRID No.
85.3050	Acute Oral Toxicity/ Pathogenicity - Technical	Acceptable. Not toxic/pathogenic to rats dosed at 1.3 H 10^8 cfu.	447581-08
870.1100	Acute Oral Toxicity - End-Use Product	Acceptable, Toxicity Category III. Toxic/limit dose in rats is >2.8 g/kg body weight (6.7 H 10^{10} cfu/kg).	447581-09
870.2500/885.3100	Acute Dermal Toxicity/Pathogenicity - Technical	Acceptable, Toxicity Category III. The LD_{50} in rats is >2000 mg/kg.	447581-10
870.2500/885.3100	Acute Dermal Toxicity/Pathogenicity - End-Use Product	Acceptable, Toxicity Category III. The LD_{50} in rats is >2000 mg/kg.	447581-11
870.2500/885.3100	Acute Dermal Irritation - Technical	Acceptable, Toxicity Category II.	447581-10
870.2500/885.3100	Acute Dermal Irritation - End-Use Product	Acceptable, Toxicity Category II.	447581-11
885.3150	Acute Pulmonary Toxicity/ Pathogenicity - Technical	Acceptable. Not toxic/pathogenic to rats dosed at 1.3 H 10^8 cfu.	447581-12
885.3200	Acute Intravenous Toxicity/Pathogenicity - Technical	Acceptable. Not toxic/pathogenic to rats dosed at 1.7 H 10^8 cfu.	447581-13
870.2400	Primary Eye Irritation - Technical	Acceptable, Toxicity Category I.	447581-14
870.2400	Primary Eye Irritation - End-Use Product	Acceptable, Toxicity Category III	447581-15
885.3400	Hypersensitivity	Acceptable. No	447581

	Incidents - Human Exposure	hypersensitivity incidents reported. If any are found, they must be reported.	-16
870.2600	Hypersensitivity Testing - Technical	Acceptable. Not a sensitizer when dosed at 3.6 H 10^{10} cfu.	447581 -17
885.1100	Potential Health Effects - Review of Literature	Acceptable. No apparent negative effects from *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24	447581 -18

b. **Subchronic Toxicity and Chronic Toxicity**

Subchronic and chronic toxicity were not required because survival, replication, infectivity, toxicity, or persistence of the microbial agent was not observed in the test animals treated in the infectivity tests for oral, pulmonary and injection exposures.

2. **Effects on the Immune and Endocrine Systems**

The Agency is not requiring information on the endocrine effects of this biological pesticide at this time. The Agency has no information to suggest that *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 has an effect on the immune and endocrine systems. No specific tests have been conducted with *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 to determine such effects. However, as is expected from a non-pathogenic microorganism, the submitted toxicity/pathogenicity studies in rodents indicated that following several routes of exposure, the immune system is still intact and able to process and clear the active ingredient. There are no reports indicating that *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 produces any toxins or antibiotics. Therefore, it is unlikely that this organism would have estrogenic or endocrine effects because it is practically non-toxic to mammals.

3. **Dose Response Assessment**

No toxicological endpoints are identified.

4. **Dietary Exposure and Risk Characterization**

The use of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 is not expected to result in any new dietary exposure to this organism. Bacteria such as *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 are ubiquitous in the agricultural environment. It is anticipated that the concentrations of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 on treated plants may be elevated immediately after application but will rapidly decline to environmental background levels. The risks anticipated for dietary exposure are considered low because *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 was not toxic/pathogenic to rats dosed at 1.3 H 10^8 cfu. . (Toxicity Category III)

5. **Occupational, Residential, School and Day care Exposure and Risk Characterization**

a. **Occupational Exposure and Risk Characterization**

Dermal and inhalation exposure would be the primary routes of exposure for mixer/loader applicators. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Bacillus subtilis* is not considered to be a human pathogen nor is it known to produce metabolites that are dermally absorbed. Since the intravenous study demonstrated no adverse effects, it is the Agency's opinion that even cut skin should not pose a risk to health if *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 was absorbed into the body via entry through a cut. The dermal irritation study demonstrated a potential for skin irritation (Toxicity Category II). The acute pulmonary toxicity/pathogenicity study demonstrated that *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 is not toxic/pathogenic to rats dosed at 1.3 H 10^8 cfu. Adverse effects from inhalation exposure are, therefore, considered minimal. The required personal protective equipment for applicators and handlers is expected to adequately reduce dermal or inhalation exposure to this microbial pesticide.

Occupational exposures and subsequent risks are negligible because the organism has been determined not to be pathogenic to humans

and animals. The risks are expected to be minimal based on evaluations of submitted Tier I acute toxicity tests (Table 2).

b. **Residential, School and Day Care Exposure and Risk Characterization**

Bacillus subtilis var. *amyloliquefaciens* strain FZB24 is currently limited to greenhouse and indoor use. The potential risks to humans, including children, are considered negligible based on the lack of significant toxicological concerns, as demonstrated in the Agency's evaluation of the mammalian toxicology studies, low application rates, and low exposure subsequent to application due to volatilization.

The primary toxicological concerns for humans are dermal irritation and/or sensitization from dermal exposure at high concentrations. The potential of significant exposure to children from dermal exposure is anticipated to be considerably less than that used in exposing experimental animals in tests. Therefore, additional requirements for evaluation of exposure over that mitigated by the precautionary labeling statements currently proposed (refer to Section V) are not necessary. In addition, systemic toxicity is not expected to occur from pesticide uses since dermal irritation occurs at high doses and typically results in self-discontinuation of product use.

6. **Drinking Water Exposure and Risk Characterization**

Bacillus subtilis var. *amyloliquefaciens* strain FZB24 is a naturally-occurring microorganism. *Bacillus subtilis* is widespread in the environment throughout the world. Although *Bacillus subtilis* spores may be found in water, it is not known as an aquatic bacterium, and therefore is not expected to proliferate in aquatic habitats. Moreover, the risk from non-occupational exposure is considered minimal as there is no evidence of adverse effects from oral, dermal, or inhalation exposure to this microbial agent is not considered to be a risk to drinking water. Drinking water is accordingly not being screened for *Bacillus subtilis* as a potential indicator of microbial contamination or as a direct pathogenic contaminant. Both percolation through soil and municipal treatment of drinking water would reduce the possibility of exposure to *Bacillus subtilis* through drinking water. Therefore, the potential of significant transfer to drinking water is minimal to nonexistent. In addition, the risk from consumption of drinking water containing *Bacillus subtilis* is considered

minimal as there is no evidence of adverse effects from oral exposure to this microbial agent.

7. **Acute and Chronic Dietary Risks for Sensitive Subpopulations Particularly Infants and Children**

Although there have been reports of *B. subtilis* causing allergic reactions, particularly when added to laundry detergent, no confirmed reports of immediate or delayed allergic reactions to *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 have been reported.

Based on the acute toxicity information discussed above, EPA concludes that there is a reasonable certainty that no harm will result from aggregate exposure to the United States population, including infants and children, to residues of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion because, as discussed in Unit B. Human Risk Assessment, *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 is practically non-toxic to mammals and under reasonably foreseeable circumstances it does not pose a risk.

8. **Aggregate Exposure from Multiple Routes Including Dermal, Oral, and Inhalation**

Bacillus subtilis var. *amyloliquefaciens* strain FZB24 is currently limited to greenhouse and indoor use. The potential risks to humans, including children, are considered negligible based on the lack of significant toxicological concerns, as demonstrated in the Agency's evaluation of the mammalian toxicology studies, low application rates, and low exposure subsequent to application due to volatilization.

The primary toxicological concerns for humans are dermal irritation and/or sensitization from dermal exposure at high concentrations. The potential of significant exposure to children from dermal exposure is anticipated to be considerably less than that used in exposing experimental animals in tests. Therefore, additional requirements for evaluation of exposure over that mitigated by the precautionary labeling statements currently proposed (refer to Section V) are not necessary. In addition, systemic toxicity is not expected to occur from pesticide uses since dermal irritation occurs at high doses and typically results in self-discontinuation of product use.

9. **Cumulative Effects**

Bacillus subtilis var. *amyloliquefaciens* strain FZB24 is practically non-toxic to mammals. No mechanism of toxicity in mammals has been identified for this organism. Therefore, no cumulative effect with other toxins or related organisms is anticipated.

C. **Environmental Assessment**

0. **Environmental Fate**

Environmental fate data were waived because *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 is a naturally occurring microorganism found in soil and plant litter. The ubiquitous nature of the organism suggests that the environmental burden is not likely to increase substantially with the proposed limited indoor and greenhouse use patterns of the microbial pesticide.

1. **Ecological Toxicity**

The intended applications of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 to greenhouse non-food crops including ornamentals, shrubs, shade and forest tree seedlings, and indoor use patterns are generally not expected to pose adverse risks to non-target organisms. Phytotoxicity to grapes and rot to potato tubers may be possible and further testing or information is necessary to quantify these risks. Non-target organism testing data are not required for these use patterns of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24.

D.

V. Risk Management and Re/Registration Decision

A. Determination of Eligibility

Section 3(c)(5) of FIFRA provides for the registration of new active ingredients if it is determined that

. its composition is such as to warrant the proposed claims for it;
A. its labeling and other materials required to be submitted comply with the requirements of FIFRA;
B. it will perform its intended function without unreasonable adverse effects on the environment; and
C. when used in accordance with widespread and commonly recognized practice, it will not generally cause unreasonable adverse effects on the environment.

To satisfy criterion "A" above, *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 has well known properties. The Agency has no knowledge that would contradict the claims made on the label of this product. Criterion "B" is satisfied by the current label and by the data presented in this document. It is believed that this new pesticidal active ingredient will not cause any unreasonable adverse effects, is a broad spectrum microbial fungicide, and does provide protection as claimed satisfying criterion "C". Criterion "D" is satisfied in that *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 is not expected to cause unreasonable adverse effects when used according to label instructions.

Therefore, *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 is eligible for registration. The uses are listed in the Section II, B. Use Profile. These eligible uses are limited to indoor, greenhouse food and greenhouse-non -food sites. There are no ineligible uses for *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24.

B. Regulatory Position

0. **Unconditional Registration:** The data requirements are fulfilled and the Biopesticides and Pollution Prevention Division recommends unconditional registration of products that contain *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 as the sole Active ingredient (TAE-022 Technical and TAE-022 WDG).

1. **Tolerances for Food Uses and /or exemptions:** *Bacillus subtilis*var. *amyloliquefaciens* strain FZB24 is not registered food uses. A tolerance was, therefore, not required.

2. **CODEX Harmonization:** There are no CODEX values for *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24.

3. **Risk Mitigation:** Since there are no risk issues, no risk mitigation measures are required at this time for dietary risk, occupational and residential risk, or ground and surface water contamination for *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24.

4. **Endangered Species Statement:** Based on the use pattern and the results of toxicity and exposure studies, the Agency has determined that this action will have no effect on listed species.

C. Labeling Rational

0. **Human Health Hazard (WPS and non-WPS)**

Bacillus subtilis var. *amyloliquefaciens* strain FZB24 products with commercial use sites are subject to the Worker Protection Standard. Due to the low toxicity of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24, the Re-Entry Interval for uses within the scope of WPS is four hours. Precautionary statements and personal protective equipment as specified below are required based on the acute toxicity categories of this organism.

1. **Environmental Hazard**

Precautionary labeling is required as indicated below.

VI.

VII. Actions Required By Registrants

A. Precautionary Labeling

Bacillus subtilis var. *amyloliquefaciens* strain FZB24 products must state the following under the heading "Precautionary Statements":

Personal Protective Equipment

Applicators and other handlers must wear: Long sleeved shirt and long pants. Waterproof gloves. Shoes plus socks. Dust/mist filtering respirator (MSHA/NIOSH approval number prefix TC-21C), or a NIOSH approved respirator with any N, P, R, or HE filter.

Follow manufacturers instructions for cleaning/maintaining PPE. If no such instructions for washables, use detergent and hot water. Keep and wash PPE separately from other laundry.

WPS labels must state the following under the heading "User Safety Recommendations"

Users should wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet.

Users should remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.

Users should remove PPE immediately after handling this product. If gloves are worn, wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

B. Environmental Hazards Labeling

Provided the following statement is placed into the environmental hazards statement, the risk of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 is minimal to nonexistent to non-target organisms including endangered species.

0. **End-Use Product Environmental Hazards Labeling**

   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 by cleaning of equipment or disposal of equipment washwaters.

1. **Manufacturing-Use Product Environmental Hazards Labeling**

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.

2. **Application Rate**

It is the Agency's position that the labeling for the pesticide products containing *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 as the active ingredient complies with the current pesticide labeling requirements. The Agency has not required a maximum number of applications per a season of this active ingredient.

C. **Labeling**

The attached label for TAE-022 WDG (EPA file number 72098-L) conforms with the labeling requirements for *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 . Some of the essential label requirements are highlighted below.

Signal word is "Warning," based on toxicity category II for dermal irritation. The product shall contain the following information:

- Product Name
- Ingredient Statement
- Registration Number
- "Keep Out of Reach of Children"
- Signal Word (Warning)
- Personal Protective Equipment (PPE) Requirements
- Environmental Hazard Statement
- Storage and Disposal Statement
- Agricultural Use Requirements
- Non-Agricultural Use Requirements
- Directions for Use

Biblography

Citations Considered to be part of the Data Base Supporting the Registration of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24

MIRD	Citation
44758101	Product Identity and Disclosure of Ingredients
44758102	Beginning Materials and Manufacturing Process
44758103	Discussion of Formation of Unintentional Ingredients
44758104	Analysis of Samples

44758105	Certification of Limits
44758106	Physical/Chemical Properties B Technical
44758107	Physical/Chemical Properties B "WDG" End-Use Product
44758108	Acute Oral Toxicity/Pathogenicity B Technical
44758109	Acute Oral Toxicity B "WDG" End-Use Product
44758110	Acute Dermal Toxicity/Pathogenicity B Technical
44758111	Acute Dermal Toxicity/Pathogenicity B "WDG" End-Use Product
44758112	Acute Pulmonary Toxicity/Pathogenicity B Technical
44758113	Acute Intravenous Toxicity/Pathogenicity B Technical
44758114	Primary Eye Irritation B Technical
44758115	Primary Eye Irritation B "WDG" End Use Product
44758116	Hypersensitivity Incident Reporting
44758117	Hypersensitivity Testing B Technical
44758118	Potential Health Effects of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 B Review of the Literature
44758119	Growth Parameters of *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 at Various Temperatures
44758120	Sensitivity Detection