



BIOPESTICIDES REGISTRATION ACTION DOCUMENT

Pasteuria usgae

PC Code: 006545

**U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division**

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I. EXECUTIVE SUMMARY

Pasteuria spp. are gram-positive, mycelial, endospore-forming bacteria that are endoparasitic to nematodes and water fleas. *Pasteuria usgae*, a recently discovered strain, is host-specific to the sting nematode (*Belonolaimus longicaudatus*), which can be damaging to a wide variety of crops, particularly turf. The active agent of *Pasteuria usgae* is an endospore that attaches and infects the host nematode during all life stages (except eggs). After attachment of the endospore, a germ tube penetrates the nematode cuticle and mycelial microcolonies are formed in the pseudocoelom, leading to eventual death of the host. The endospores formed inside the host are released into the soil when the infected nematode decomposes. The spores are non-motile and stable in the soil environment for several years.

To decide whether to grant a conditional, time-limited registration under Section 3(c)(7)(C) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) to *Pasteuria usgae* – BL1, a proposed manufacturing-use product containing *Pasteuria usgae* as an active ingredient at 0.01% by weight, the Biopesticides and Pollution Prevention Division (BPPD) reviewed microbial pesticide product analysis, toxicology, and non-target organism and environmental fate data requirements [40 Code of Federal Regulations (CFR) §§ 158.2120, 158.2140, and 158.2150, respectively]. It was determined that the data/information submitted adequately satisfy current guideline requirements for the purposes of this type of registration only.

For the purposes of a conditional, time-limited FIFRA section 3(c)(7)(C) registration only, product analysis data requirements (to include product chemistry and composition, analysis and certified limits, and physical and chemical characteristics) for the technical grade of the active ingredient (TGAI)/manufacturing-use product (MP), *Pasteuria usgae* – BL1, were satisfied by acceptable guideline studies and waiver rationales. Additional product analysis data that the Agency is requiring by specific dates falling within two years of registration include the following:

- A written description of quality control measures taken during the manufacturing process to screen for *Pasteuria* species that parasitize saprophytic nematodes.
- A new five-batch analysis with all batches from production level.
- Results from an ongoing 12-month storage stability study.

Lastly, documentation indicating official recognition of *Pasteuria usgae* by the Judicial Commission of the International Committee for Systematic Bacteriology must be submitted to the Agency when it becomes available.

Adequate mammalian toxicology data/information were submitted to support the registration of *Pasteuria usgae* – BL1. Acceptable acute toxicity guideline studies were submitted, and data waivers were granted by the Agency to fulfill the remaining Tier I acute toxicity data requirements based on the lack of toxicity and/or pathogenicity of *Pasteuria usgae*, because the manufacturing-use product is equivalent to the technical grade of the active ingredient, and/or the low potential for worker exposure attributed to

appropriate precautionary statements and requirements for personal protective equipment on the label.

Dietary, drinking water, and non-dietary, non-occupational exposures to *Pasteuria usgae* are unlikely to occur because the use of the proposed product is limited to manufacturing of end-use products intended for eventual application to turf. The associated end-use products are still undergoing Agency review. Despite the low toxicological profile of *Pasteuria usgae*, personal protective equipment (PPE) is required for handlers that may be exposed to the active ingredient, due to their occupation, for prolonged periods. Handlers working with *Pasteuria usgae* in manufacturing facilities must wear a long-sleeved shirt, long pants, socks, shoes, waterproof gloves, and a dust/mist filtering respirator meeting National Institute for Occupational Safety and Health (NIOSH) standards of at least N-95, R-95, or P-95. Overall, a determination has been made that no unreasonable adverse effects to the United States population in general, and to infants and children in particular, will result from the use of *Pasteuria usgae* when used in accordance with Environmental Protection Agency (EPA)-approved labeling.

Data waiver rationales were submitted in response to data requirements for avian, freshwater fish and invertebrate, insect, and honey bee non-target organism testing requirements. The information provided is sufficient to satisfy the Tier I non-target organism data requirements for the technical product and proposed end-use products containing *Pasteuria usgae* as an active ingredient. Further testing of non-target organisms at higher tier levels is not required. Based on the rationales submitted, adverse effects to terrestrial animals and plants or freshwater and marine/estuarine fish, invertebrates, and plants are not expected as a result of exposure to proposed labeled applications of *Pasteuria usgae*. Furthermore, BPPD makes “No Effect” (NE) determinations for direct and indirect effects to listed species and their habitat as a result of the proposed uses of *Pasteuria usgae*.

The Agency determines whether conditional registration of a pesticide is in the public interest in accordance with the criteria set forth in the Federal Register dated March 5, 1986 ([58 Federal Register 7268](#)). There is a presumption that registration of a pesticide is in the public interest if (1) the use is for a minor crop, (2) the use is a replacement for another pesticide that is of continuing concern to the Agency, (3) the use is one for which an emergency exemption under FIFRA section 18 has been granted (i.e., the basis for the exemption was lack of a registered alternative product), or (4) the use is against a pest of public health significance. *Pasteuria usgae* – BL1 will be used to create end-use products, that once reviewed and determined to be eligible for registration under FIFRA, will serve as partial replacements for conventional nematicides of continuing concern to the Agency [e.g., methyl bromide (ozone-depleting substance) and 1,3-dichloropropene (probable human carcinogen)]. Based on this information, this registration is presumed to be in the public interest.

II. ACTIVE INGREDIENT OVERVIEW

Biological Name:	<i>Pasteuria usgae</i>
Culture Deposit:	American Type Culture Collection in Manassas, Virginia under Accession Number SD-5835
Office of Pesticide Programs (OPP) Chemical Code:	006545
Type of Pesticide:	Microbial Pesticide – Nematicide

See [Appendix B](#) for specific information (i.e., use sites, application rate, method of application, formulation type, and target pests) regarding the manufacturing-use product, *Pasteuria usgae* – BL1, containing this active ingredient.

III. REGULATORY BACKGROUND

On May 5, 2008, MacIntosh and Associates, Incorporated (address: 1203 Hartford Avenue, Saint Paul, Minnesota 55116-1622), acting as the authorized agent for Pasteuria Bioscience, Incorporated (address: 12085 Research Drive, Suite 185, Alachua, Florida 32615), submitted an application to register *Pasteuria usgae* – BL1 (EPA File Symbol 85004-R) under Section 3 of the Federal Insecticide, Fungicide, and Rodenticide Act. On August 13, 2008, the EPA announced receipt of this application to register a pesticide product containing a new active ingredient [[73 Federal Register \(FR\) 47166](#)] and opened a 60-day public comment period. No comments were received following this publication.

Pursuant to FIFRA section 3(c)(7)(C), a conditional, time-limited registration was issued for *Pasteuria usgae* – BL1 on June 2, 2009 (EPA Registration Number 85004-1). The Agency announced the approval to conditionally register *Pasteuria usgae* – BL1, which does not contain an active ingredient included in any previously registered product, in the Federal Register of July 1, 2009 ([74 FR 31426](#)).

IV. RISK ASSESSMENT

On October 26, 2007, the Agency issued a Final Rule in the Federal Register on the data requirements to support registration of biochemical and microbial pesticides, and updated the definitions for biochemical and microbial pesticides ([72 FR 61002](#)). The rule became effective on December 26, 2007. The data and information evaluated for this Biopesticides Registration Action Document (BRAD) were considered in light of these requirements.

The classifications that are found for each data submission are assigned by EPA science reviewers and are an indication of the usefulness of the information contained in the documents for risk assessment. A rating of “ACCEPTABLE” indicates the study is scientifically sound and is useful for risk assessment. A “SUPPLEMENTAL” rating indicates the data provide some information that can be useful for risk assessment. The studies may have certain aspects determined not to be scientifically acceptable (“SUPPLEMENTAL: UPGRADABLE”). If a study is rated as “SUPPLEMENTAL: UPGRADABLE,” the Environmental Protection Agency always provides an indication of what is lacking or what can be provided to change the rating to “ACCEPTABLE.” If there is simply a “SUPPLEMENTAL” rating, the reviewer will often state that the study is not required by the current 40 CFR Part 158. Both “ACCEPTABLE” and “SUPPLEMENTAL” studies may be used in the risk assessment process as appropriate. An “UNACCEPTABLE” rating indicates that new data need to be submitted.

For the acute toxicity data requirements, toxicity categories are assigned based on the hazard(s) identified from studies and/or information submitted to the Agency. The active ingredient or particular product is classified into Toxicity Category I, II, III, or IV, where Toxicity Category I indicates the highest toxicity and Toxicity Category IV indicates the lowest toxicity.

A. Product Analysis Assessment ([40 CFR § 158.2120](#))

Although there are some product analysis data that are lacking, a reasonable period of time sufficient for generation of these data has not elapsed since the Agency first imposed the relevant data requirements. Therefore, all product analysis data requirements for a conditional, time-limited FIFRA section 3(c)(7)(C) registration of *Pasteuria usgae* – BL1, containing *Pasteuria usgae* as an active ingredient, have been satisfied by either acceptable guideline studies or waiver rationales. The Agency will allow for generation and submission of the lacking product analysis data within two years of registration as the use of the manufacturing-use *Pasteuria usgae* product will not cause any unreasonable adverse effects on the environment and registration of such a product is presumed to be in the public interest.

For a comprehensive guideline-by-guideline summary of the product analysis data requirements described in sections IV(A)(1), IV(A)(2), and IV(A)(3), refer to [Table 1](#) in Appendix A.

1. Product Chemistry and Composition [Office of Prevention, Pesticides, and Toxic Substances (OPPTS) Guidelines 885.1100, 885.1200, and 885.1300]

Pasteuria spp. are gram-positive, mycelial, endospore-forming bacteria that are endoparasitic to nematodes and water fleas. The endospores of *Pasteuria* spp. can be identified and counted microscopically, but it is difficult to distinguish between species unless high magnification electron microscopy is used so that size and shape of the spores

can be visualized and measured. Molecular techniques such as polymerase chain reaction (PCR) methods have been reported for *Pasteuria penetrans* but have not been developed for the other species. Therefore, species identification *via* host specificity assays, requiring the establishment of nematode cultures and a bioassay, is the most direct and reliable technique for species identification.

Pasteuria usgae, a recently discovered strain, is host-specific to the sting nematode (*Belonolaimus longicaudatus*), which can be damaging to a variety of crops, particularly turf. Currently, full recognition of *Pasteuria usgae* is still pending with the Judicial Commission of the International Committee for Systematic Bacteriology. Once *Pasteuria usgae* has been recognized and removed from the category *Candidatus*, documentation indicating official recognition must be provided to the Agency.

The active agent of *Pasteuria usgae* is an endospore that attaches and infects the host nematode during all life stages (except eggs). Increased moisture, neutral pH, temperatures above 10°C, and sandy soil seem to provide the best environments for spore attachment to the host. In laboratory studies, *Pasteuria* spp. were able to attach to nematodes after exposure to high temperatures and wide ranges of pH; however, at extremes, the number of attached spores per nematode was reduced and, in some cases, no infection occurred after attachment. After attachment of the endospore, a germ tube penetrates the nematode cuticle and mycelial microcolonies are formed in the pseudocoelom, leading to eventual death of the host. The endospores formed inside the host are released into the soil when the infected nematode decomposes. The spores are non-motile and stable in the soil environment for several years.

Submitted data also adequately describe the production process and potential microbial contaminants and these requirements have been satisfied for the purposes of a conditional, time-limited FIFRA section 3(c)(7)(C) registration only. The original manufacturing process did not describe quality control measures taken to confirm that *Pasteuria usgae* was the only *Pasteuria* species present in the master stock. A follow-up explanation, which demonstrated that batches are screened for the presence of other microbes and screened against sting nematodes to confirm the presence of *Pasteuria usgae*, still did not provide sufficient information to indicate that screening for other *Pasteuria* species is conducted during the manufacturing process. Other *Pasteuria* species, specifically those that parasitize saprophytic (non-plant pathogenic) nematodes, must be screened for during the manufacturing process. Pasteuria Bioscience, Incorporated must integrate quality control measures that screen for *Pasteuria* species that parasitize saprophytic nematodes, either immediately after freezing or after making seed stock, into their current manufacturing process and provide a written description of these measures to the Agency on or before October 1, 2009.

2. Analysis and Certified Limits (OPPTS Guidelines 885.1400 and 885.1500)

Results of a preliminary five-batch analysis were provided and the requirement for analysis of samples has been satisfied for purposes of a conditional, time-limited FIFRA section 3(c)(7)(C) registration only. The preliminary analysis of samples provided to the

Agency revealed a wide range of concentrations, with many replicates containing 1–2 orders of magnitude fewer *Pasteuria usgae* spores than the minimum specified on the Confidential Statement of Formula (CSF) and product label (i.e., 1×10^6 spores per milliliter). A follow-up explanation, attempting to address this variability, mentioned several factors that contributed to this inconsistency and discussed how the concentration of spores could be adjusted during the manufacturing process, if necessary. Despite this additional information, a new five-batch analysis, with all batches from production level, must be submitted to the Agency on or before May 1, 2011.

The certified limits for the active and inert ingredients fall within the OPPTS Guideline 830.1750 specified ranges; therefore, the requirement for certified limits has been satisfied.

3. Physical and Chemical Characteristics (OPPTS Guidelines 830.6302, 830.6303, 830.6304, 830.6313, 830.6317, 830.6319, 830.6320, 830.7000, 830.7100, and 830.7300)

Submitted data adequately describe the physical and chemical characteristics and waiver rationales provided are acceptable; however, a 12-month storage stability study is cited to as ongoing and miscibility is not required because *Pasteuria usgae* – BL1 is not an emulsifiable form of microbial pesticide. Therefore, the requirements for color, physical state, odor, stability to normal and elevated temperatures, metals, and metal ions, corrosion characteristics, pH, viscosity, and density/relative density/bulk density (specific gravity) have been satisfied. When the storage stability study has been completed and the results have been compiled, these data must be submitted to the Agency on or before June 1, 2010.

B. Human Health Assessment

1. Toxicology

Adequate mammalian toxicology data/information are available to support the registration of *Pasteuria usgae* – BL1, which contains *Pasteuria usgae*. All Tier I toxicology data requirements for the technical grade of the active ingredient/manufacturing-use product have been satisfied by guideline studies or waiver rationales. Tier II and Tier III studies were not required for *Pasteuria usgae* based on the lack of acute toxicity/pathogenicity in the Tier I studies.

For a comprehensive guideline-by-guideline summary of the toxicology data requirements described in sections IV(B)(1)(a) and IV(B)(1)(b), refer to [Table 2](#) in Appendix A.

a. Acute Toxicity/Pathogenicity – Tier I ([40 CFR § 158.2140](#))

Acute Oral Toxicity and Pathogenicity – Rat [OPPTS Guideline 885.3050; Master Record Identification (MRID) Number (No.) 474267-09]: There were no treatment-

related clinical signs or necropsy findings in rats receiving a single oral dose of 1×10^8 *Pasteuria usgae* spores. Three males in the microbial pest control agent (MPCA)-treated group gained weight through day 14 but lost weight by day 21. All other animals gained weight prior to scheduled sacrifice. *Pasteuria usgae* does not appear to be toxic and/or pathogenic in rats when dosed at 1×10^8 spores/animal. Since microbial enumeration was not performed because the test material would not grow on agar media, the infectivity was uncertain. However, because the spores are highly specific to sting nematode, infectivity is unlikely to be a concern. This study was rated “ACCEPTABLE” and *Pasteuria usgae* was classified as TOXICITY CATEGORY IV.

Acute Dermal Toxicity – Rat (OPPTS Guideline 885.3100; MRID No. 474267-12): There were no treatment-related significant adverse effects seen in the dosed rats. Two males and one female had very slight erythema on day 1 with clearance by day 4. One male lost weight slightly during the second week and one male and two females lost weight during the first week, but all gained weight by the end of the study. All other animals gained weight throughout the study. Based on the results of this study, *Pasteuria usgae* does not appear to be toxic in rats when treated with 2,000 milligrams (mg)/kilogram (kg) at 10^8 spores/milliliter (mL). The acute dermal LD₅₀ is greater than 2,000 mg/kg for 10^8 spores/mL in male and female rats. This study was rated “ACCEPTABLE” and *Pasteuria usgae* was classified as TOXICITY CATEGORY IV.

Acute Pulmonary Toxicity and Pathogenicity – Rat (OPPTS Guideline 885.3150; MRID No. 474267-10): In an acute pulmonary toxicity and pathogenicity assessment, there were no test substance-related significant adverse effects seen in rats receiving a single dose of approximately $1-3 \times 10^8$ spores of *Pasteuria usgae*. One dosed female exhibited pale lungs. Additionally, one untreated control female lost weight by day 21 and another untreated control female lost weight by day 14 but gained weight by day 21. One MPCA-treated male did not gain weight by day 7 but gained weight thereafter. All other animals gained weight throughout the study. Based on these results, *Pasteuria usgae* does not appear to be toxic and/or pathogenic in rats when dosed at approximately $1-3 \times 10^8$ spores/animal. Since microbial enumeration was not performed because the test material would not grow on agar media, the infectivity was uncertain. However, because the spores are highly specific to sting nematode, infectivity is unlikely to be a concern. This study was rated “ACCEPTABLE” and *Pasteuria usgae* was classified as TOXICITY CATEGORY IV.

Acute Injection Toxicity and Pathogenicity – Rat (OPPTS Guideline 885.3200; MRID No. 474267-11): There were no treatment-related significant adverse effects seen in rats receiving a single intravenous dose of 10^8 *Pasteuria usgae* spores. One treated female lost weight by day 7 but gained weight prior to sacrifice on day 14. All other animals gained weight throughout the study. All animals survived and appeared normal during the study. No abnormalities were observed in any animal at necropsy or in harvested organs. No significant variations in organ weight were found between different groups or sexes. The acute intravenous LD₅₀ of *Pasteuria usgae* is greater than 1×10^8 spores/animal in male and female rats. *Pasteuria usgae* does not appear to be toxic and/or pathogenic in rats when dosed at 10^8 spores/animal. Since microbial enumeration was not performed

because the test material would not grow on agar media, the infectivity was uncertain. However, because the spores are highly specific to sting nematode, infectivity is unlikely to be a concern. This study was rated “ACCEPTABLE” and *Pasteuria usgae* was classified as TOXICITY CATEGORY IV.

Hypersensitivity Incidents (OPPTS Guideline 885.3400; MRID No. 474350-02): No hypersensitivity incidents, including immediate-type or delayed-type reactions of humans and domestic animals, occurring during the testing or production of the TGAI/MP were reported by Pasteuria Bioscience, Incorporated. Any future hypersensitivity incidents must be reported per OPPTS Guideline 885.3400.

Cell Culture (OPPTS Guideline 885.3500): This study is not required because *Pasteuria usgae* is not a virus (refer to test note #4 of 40 CFR § 158.2140(d)).

Acute Oral Toxicity (OPPTS Guideline 870.1100; MRID No. 474979-01): Waived based on the results of MRID No. 474267-09, which showed that *Pasteuria usgae* was not toxic and/or pathogenic by oral route of exposure, and because the MP is equivalent to the TGAI. This waiver request was rated “ACCEPTABLE” and *Pasteuria usgae* – BL1 was classified as TOXICITY CATEGORY IV.

Acute Dermal Toxicity (OPPTS Guideline 870.1200; MRID No. 474979-01): Waived based on the results of MRID No. 474267-12, which showed that *Pasteuria usgae* was not toxic by dermal route of exposure, and because the MP is equivalent to the TGAI. This waiver request was rated “ACCEPTABLE” and *Pasteuria usgae* – BL1 was classified as TOXICITY CATEGORY IV.

Acute Inhalation Toxicity (OPPTS Guideline 870.1300; MRID No. 474979-01): Waived based on the results of MRID No. 474267-10, which showed that *Pasteuria usgae* was not toxic and/or pathogenic by pulmonary route of exposure, and because the MP is equivalent to the TGAI. This waiver request was rated “ACCEPTABLE” and *Pasteuria usgae* – BL1 was classified as TOXICITY CATEGORY IV.

Acute Eye Irritation (OPPTS Guideline 870.2400; MRID No. 474350-03): Waived based on *Pasteuria usgae*'s lack of toxicity in the other toxicology studies and precautionary statements on the label that mitigate potential for worker exposure. This waiver request was rated “ACCEPTABLE” and *Pasteuria usgae* – BL1 was classified as TOXICITY CATEGORY III.

Primary Dermal Irritation (OPPTS Guideline 870.2500; MRID No. 474350-03): Waived based on the results of MRID No. 474267-12, which showed that *Pasteuria usgae* was not toxic by dermal route of exposure, and precautionary statements/personal protective equipment on the label that mitigate potential for worker exposure. This waiver request was rated “ACCEPTABLE” and *Pasteuria usgae* – BL1 was classified as TOXICITY CATEGORY III.

b. Acute Toxicology and Subchronic Toxicity/Pathogenicity – Tier II; Reproductive Fertility Effects, Carcinogenicity, Immunotoxicity, and Infectivity/Pathogenicity Analysis – Tier III ([40 CFR § 158.2140](#))

Tier II and Tier III studies were not required for *Pasteuria usgae* based on the lack of acute toxicity/pathogenicity in the Tier I studies.

c. Effects on the Endocrine System

Section 408(p) of the Federal Food, Drug, and Cosmetic Act (FFDCA) requires EPA to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) “may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or such other endocrine effect as the Administrator may designate.” Following the recommendations of its Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), EPA determined that there were scientific bases for including, as part of its program, androgen and thyroid hormone systems, in addition to the estrogen hormone system. The Environmental Protection Agency also adopted EDSTAC’s recommendation that the Program include evaluations of potential effects on wildlife.

The Agency has no knowledge of *Pasteuria usgae* being an endocrine disruptor, nor is this microbe related to any class of known endocrine disruptors. Due to the unique mode of action of *Pasteuria usgae*, the narrow host range, and lack of mammalian toxicity (see [section IV\(A\)\(1\)](#) and [section IV\(B\)\(1\)\(a\)](#)), no effects on the immune or endocrine systems are anticipated. Additional data, specifically on the endocrine effects of this microbial pesticide, are not required at this time. When the appropriate screening and/or testing protocols being considered under the Agency’s Endocrine Disruptor Screening Program (EDSP) have been developed and vetted, *Pasteuria usgae* may be subjected to additional screening and/or testing to better characterize effects related to endocrine disruption.

2. Dietary Exposure and Risk Characterization

Dietary exposure to *Pasteuria usgae* is not expected because there are no approved food uses for this active ingredient at this time.

3. Drinking Water Exposure and Risk Characterization

Exposure of humans to residues of *Pasteuria usgae* in drinking water is unlikely. The current use pattern for *Pasteuria usgae* (i.e., manufacturing into nematicide end-use products, which are still undergoing Agency review) does not include direct application to aquatic environments. In the unlikely event that *Pasteuria usgae* is transferred to surface or ground water intended for eventual human consumption, the microbe would not survive the conditions water is subjected to in a drinking water treatment facility, including chlorination, pH adjustments, and/or filtration. Even if oral exposure should occur through drinking water, the Agency concludes that there is a reasonable certainty

that no harm will result from the exposure to the residues of *Pasteuria usgae* in all the anticipated drinking water exposures because of the lack of acute oral toxicity/pathogenicity to mammals ([see section IV\(B\)\(1\)\(a\)](#)) and the host specific nature of the microbe.

4. Acute and Chronic Dietary Exposure and Risks for Sensitive Subpopulations, Particularly Infants and Children

Acute and chronic dietary exposure to *Pasteuria usgae* is not expected for sensitive subpopulations, particularly infants and children, because there are no approved food uses for this active ingredient at this time.

5. Occupational, Residential, School, and Daycare Exposure and Risk Characterization

Significant additional human exposure to *Pasteuria usgae* from its use as a manufacturing product to produce nematicide end-use products is not expected in occupational, residential, school, or daycare areas.

a. Occupational Exposure and Risk Characterization

In light of the Tier I acute toxicity/pathogenicity studies, which did not show any toxic and/or pathogenic effects to rats *via* oral, pulmonary, dermal, and intravenous routes of exposure ([see section IV\(B\)\(1\)\(a\)](#)), handler exposure to *Pasteuria usgae* is not expected to pose any undue risk. Regardless, appropriate personal protective equipment and precautionary statements are required on the product label to mitigate any potential risks to pesticide handlers due to prolonged exposure. Handlers working with *Pasteuria usgae* in manufacturing facilities must wear a long-sleeved shirt, long pants, socks, shoes, waterproof gloves, and a dust/mist filtering respirator meeting NIOSH standards of at least N-95, R-95, or P-95.

b. Residential, School, and Daycare Exposure and Risk Characterization

According to the label, *Pasteuria usgae* – BL1 is only to be used to manufacture nematicide end-use products. No indoor residential, school, or daycare uses currently appear on the label; thus, human exposure to *Pasteuria usgae* should not occur in these areas.

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

Potential non-occupational dermal, oral, and inhalation exposure to *Pasteuria usgae* is not expected as there are no approved food uses for this active ingredient at this time and the active ingredient is to be used only to manufacture nematicide end-use products, which are still undergoing Agency review.

7. Cumulative Effects

Cumulative effects of exposure to *Pasteuria usgae* and to other substances that might have a common mechanism of toxicity have not been considered because there are no approved food uses for this active ingredient at this time.

8. Risk Characterization

The Agency considered human exposure to *Pasteuria usgae* in light of the standard for registration in FIFRA, as amended by the Food Quality Protection Act (FQPA) of 1996. A determination has been made that no unreasonable adverse effects to the United States population in general, and to infants and children in particular, will result from the use of *Pasteuria usgae* when used in accordance with EPA-approved labeling.

C. Environmental Assessment

Exposure of non-target organisms is possible with application of end-use products containing *Pasteuria usgae* to turf. Pasteuria Bioscience, Incorporated has submitted data waiver rationales containing information cited in published literature to satisfy data requirements for non-target organism testing with the technical grade of the active ingredient (TGAI). The following is a review of the rationales submitted to support the registration of the manufacturing-use product and proposed end-use products, as well as resulting conclusions regarding environmental risks based on the submitted waiver justifications.

For a comprehensive guideline-by-guideline summary of the non-target toxicity data requirements, refer to [Table 3](#) in Appendix A.

1. Summary of Non-Target Organism Waiver Rationales ([40 CFR § 158.2150](#))

Data waiver rationales were submitted in response to data requirements for avian, freshwater fish and invertebrate, insect, and honey bee non-target organism testing requirements. Justification for these waivers is described in sections IV(C)(1)(a), IV(C)(1)(c), and IV(C)(1)(f). Some data requirements were not required based on the test notes described in 40 CFR § 158.2150 (e) and explanations are provided in sections IV(C)(1)(b), IV(C)(1)(d), and IV(C)(1)(e). Overall, the information provided is sufficient to satisfy the Tier I non-target organism data requirements for the manufacturing-use product and proposed end-use products. Further testing of non-target organisms at higher tier levels is not required.

a. Avian Oral Toxicity/Pathogenicity (OPPTS Guideline 885.4050)

The request to waive avian oral toxicity/pathogenicity testing is based on the rationale that exposure of birds to *Pasteuria usgae* as a result of its proposed applications will be minimal, and that no records are available in which toxicity or pathogenicity of *Pasteuria usgae* or other *Pasteuria* spp. to birds is reported. *Pasteuria* spp. are ubiquitous in the

environment and are found in soil environments where plant-parasitic nematodes exist (Chen and Dickson 1998; Hewlett *et al.* 1994), and their population dynamics are strongly linked to parasite-prey interactions with their nematode hosts (Ciancio and Queneherve 2000; Darban *et al.* 2005). *Pasteuria usgae* is very specific to its host (Bekal *et al.* 2001; Giblin-Davis *et al.* 2001, 2003).

Extensive literature searches were performed within *Agricola* and *PubMed* databases, as well as several relevant journals, and no information was found relating exposure to naturally occurring *Pasteuria usgae* or other *Pasteuria* spp. to toxic or pathogenic effects in birds. The searches also did not result in finding any reports of genotoxic, carcinogenic, allergenic, mutagenic, or teratogenic effects.

The rationale that exposure of birds to *Pasteuria usgae* would be minimal is acceptable. An argument could be made that the absence of a record of toxicity/pathogenicity to birds is not evidence that such effects could not occur. However, *Pasteuria usgae* is a naturally occurring and ubiquitous bacterium and would be recorded as an agent of avian disease if it were toxic or pathogenic to birds. Therefore, the rationale presented is acceptable and fulfills the data requirement for avian oral toxicity/pathogenicity testing. Based on this information, adverse effects to avian wildlife resulting from exposure to *Pasteuria usgae* are not anticipated.

b. Avian Inhalation Toxicity/Pathogenicity (OPPTS Guideline 885.4100)

This data requirement is not required as the nature of the microbial pesticide does not indicate potential pathogenicity to birds or relatedness to any known bird pathogens (refer to test note #3 of 40 CFR § 158.2150(e)).

c. Freshwater Fish Toxicity/Pathogenicity (OPPTS Guideline 885.4200) and Freshwater Invertebrate Toxicity/Pathogenicity (OPPTS Guideline 885.4240)

The requests to waive requirements for freshwater fish toxicity/pathogenicity and freshwater invertebrate toxicity/pathogenicity were combined. The rationale is similar to that for birds described above, wherein exposure to freshwater fish and invertebrates is expected to be limited and no records are available in which toxicity or pathogenicity of *Pasteuria usgae* to freshwater fish or invertebrates is reported. Additionally, *Pasteuria usgae* and other *Pasteuria* spp. do not produce crystalline insect toxins as seen in other bacteria that are known to be toxic to insects (e.g., *Bacillus thuringiensis*). *Pasteuria ramosa* is a closely related species to *Pasteuria usgae* that is known to parasitize *Daphnia magna* and other *Daphnia* species. However, *Pasteuria ramosa* does not infect nematode hosts typical to other *Pasteuria* spp. (Ebert *et al.* 1996). *Pasteuria usgae* is host-specific and it is unique in its spore ultrastructural characteristics that contributes to its specificity and separates it from other closely related *Pasteuria* spp. (Ebert *et al.* 1996; Giblin-Davis *et al.* 2001). Furthermore, neither *Pasteuria usgae* nor *Pasteuria ramosa* are included in published fish pathogen listings.

This rationale is acceptable and sufficient to fulfill the requirements for freshwater fish toxicity/pathogenicity and freshwater invertebrate toxicity/pathogenicity testing. Based on this information, adverse effects to freshwater fish or invertebrates resulting from proposed label applications of *Pasteuria usgae* are not expected.

d. Estuarine/Marine Fish Testing and Estuarine/Marine Invertebrate Testing (OPPTS Guideline 885.4280)

This data requirement is not required because *Pasteuria usgae* will not be applied to water and is not expected to enter marine/estuarine environments in amounts that are significantly higher than naturally occurring concentrations (refer to test note #6 of 40 CFR § 158.2150(e)).

e. Non-Target Plant Testing (OPPTS Guideline 885.4300)

This data requirement is not required because *Pasteuria usgae* is not related to known plant pathogens, and adverse effects to plants are not expected (refer to test note #7 of 40 CFR § 158.2150(e)).

f. Non-Target Insect Testing (OPPTS Guideline 885.4340) and Honey Bee Testing (OPPTS Guideline 885.4380)

The requests to waive requirements for non-target insect testing and honey bee testing were combined. The rationale is similar to that given for other taxa above, wherein exposure to non-target insects and honey bees is expected to be limited, and neither *Pasteuria usgae* nor other *Pasteuria* spp. are included in published insect or honey bee pathogen listings. *Pasteuria usgae* and other *Pasteuria* spp. do not produce crystalline insect toxins as seen in other bacteria that are known to be toxic to insects.

This rationale is acceptable and sufficient to fulfill the requirements for non-target insect testing and honey bee testing. Based on this information, adverse effects to non-target insects or honey bees resulting from proposed labeled applications of *Pasteuria usgae* are not expected.

2. Environmental Effects Conclusions

a. Terrestrial Animals and Plants

Data waiver rationales provide sufficient information to conclude that adverse effects are not expected in birds, non-target insects, and honey bees as a result of exposure to *Pasteuria usgae* (MRID No. 474267-13). These rationales address exposure *via* applications of granules containing *Pasteuria usgae* to turf; however, the rationales presented are also applicable to liquid applications to turf. *Pasteuria* spp. are ubiquitous in soils worldwide, and *Pasteuria usgae* is highly host-specific, parasitizing exclusively the plant-parasitic sting nematode (*Belonolaimus longicaudatus*). None of the proposed applications are expected to increase the concentrations of *Pasteuria usgae* in the soil above naturally occurring levels. Soil applications of granules to turf are expected to limit

exposure in the environment. While liquid applications would result in exposure on foliar surfaces of turf, there is no available literature documenting reports of terrestrial animal pathogenicity or toxicity as a result of exposure to *Pasteuria usgae*.

An acute oral toxicity and pathogenicity study with laboratory rats (MRID No. 474267-09) is available with which to determine the potential effects of *Pasteuria usgae* on wild mammals. Laboratory rats were dosed with 10^8 spores/mL, and were observed for 21 days. No signs of toxic or pathogenic effects were observed throughout the test or at necropsy. This study demonstrates that toxicity/pathogenicity to wild mammals is not expected from labeled applications of *Pasteuria usgae*.

Non-target plant testing is not required because *Pasteuria usgae* is not related to any known plant pathogen. Adverse effects on plants are not expected to result from labeled applications of *Pasteuria usgae*.

Based on the above rationales, adverse effects are not expected to occur to terrestrial animals or plants as a result of proposed labeled applications of *Pasteuria usgae*.

b. Aquatic Animals and Plants

Data waiver rationales were submitted to fulfill data requirements for and support effects conclusions for freshwater aquatic organisms (MRID No. 474267-13). These rationales address exposure *via* applications of granules containing *Pasteuria usgae* to turf; however, the rationales presented are also applicable to liquid applications to turf. The rationales provide sufficient information to conclude that adverse effects are not expected in freshwater fish or freshwater invertebrates as a result of exposure to *Pasteuria usgae*. The rationales were similar to those presented for terrestrial organisms in that *Pasteuria* spp. are ubiquitous in soils worldwide, and *Pasteuria usgae* is highly host-specific, parasitizing exclusively the plant-parasitic sting nematode (*Belonolaimus longicaudatus*). Soil applications in turf only are expected to limit exposure in the aquatic environment, and runoff from both soil and liquid applications is not expected to increase the concentrations of *Pasteuria usgae* in freshwater environments above naturally occurring levels. There is no available literature documenting reports of pathogenicity or toxicity to freshwater fish or invertebrates as a result of exposure to *Pasteuria usgae*. *Pasteuria ramosa* is a closely related species to *Pasteuria usgae* that does parasitize *Daphnia magna* and other *Daphnia* spp. However, *Pasteuria ramosa* does not infect nematode hosts typical to other *Pasteuria* spp., and *Pasteuria usgae* is very host-specific and is not known to parasitize aquatic invertebrates.

Because the use of *Pasteuria usgae* is limited to applications to turf and is not intended for application directly to water, exposure is not expected to be significant in aquatic environments, including marine or estuarine systems. Therefore, testing with marine/estuarine animals and testing with aquatic plants is not necessary.

Based on the rationales submitted, adverse effects to freshwater and marine/estuarine fish, invertebrates, and plants are not expected as a result of exposure to proposed labeled applications of *Pasteuria usgae*.

3. Threatened and Endangered Species Assessment

There are no listed endangered or threatened species related to the target pest. Since it is concluded that effects are not anticipated for non-target species exposed to *Pasteuria usgae* as a result of proposed labeled applications, direct and indirect effects to listed species or their habitat are also not expected. Therefore, BPPD makes “No Effect” (NE) determinations for direct and indirect effects to listed species and their habitat as a result of the proposed uses of *Pasteuria usgae*.

V. ENVIRONMENTAL JUSTICE

The Environmental Protection Agency seeks to achieve environmental justice—the fair treatment and meaningful involvement of all people regardless of race, color, national origin, or income—with respect to the development, implementation, and enforcement of environmental laws, regulations, and policies. Fair treatment means that no group of people, including racial, ethnic, or socioeconomic groups, should bear a disproportionate share of the negative environmental consequences resulting from industrial, municipal, and commercial operations or the execution of federal, state, local, and tribal environmental programs and policies. Meaningful involvement means that (1) potentially affected community residents have an appropriate opportunity to participate in decisions about a proposed activity that will affect their environment and/or health; (2) the public’s contribution can influence the regulatory agency’s decision; (3) the concerns of all participants involved will be considered in the decision-making process; and (4) the decision-makers seek out and facilitate the involvement of those potentially affected. EPA has this goal for all communities and persons across the United States.

At this time, the Environmental Protection Agency does not believe the use of *Pasteuria usgae* – BL1, which contains *Pasteuria usgae* as an active ingredient, will cause harm or a disproportionate impact on at-risk communities.

For additional information regarding environmental justice issues, please visit EPA’s web site at <http://www.epa.gov/compliance/environmentaljustice/index.html>.

VI. PUBLIC INTEREST FINDING

EPA determines whether conditional registration of a pesticide is in the public interest in accordance with the criteria set forth in the Federal Register dated March 5, 1986 ([58 FR 7268](#)). There is a presumption that registration of a pesticide is in the public interest if (1) the use is for a minor crop, (2) the use is a replacement for another pesticide that is of continuing concern to the Agency, (3) the use is one for which an emergency exemption

under FIFRA section 18 has been granted (i.e., the basis for the exemption was lack of a registered alternative product), or (4) the use is against a pest of public health significance. *Pasteuria usgae* – BL1 will be used to create end-use products, that once reviewed and determined to be eligible for registration under FIFRA, will serve as partial replacements for conventional nematicides of continuing concern to the Agency [e.g., methyl bromide (ozone-depleting substance) and 1,3-dichloropropene (probable human carcinogen)]. Based on this information, this registration is presumed to be in the public interest.

VII. RISK MANAGEMENT AND REGISTRATION DECISIONS

A. Determination of Eligibility

Section 3(c)(7)(C) of the Federal Insecticide, Fungicide, and Rodenticide Act provides for the conditional registration of a pesticide containing an active ingredient not contained in any currently registered pesticide for a period reasonably sufficient for the generation and submission of required data if the following are determined:

- (1) Use of the pesticide during a defined period will not cause any unreasonable adverse effect on the environment;
- (2) Use of the pesticide is in the public interest; AND
- (3) For data that are lacking, a reasonable period of time sufficient for generation of that data has not elapsed since the Agency first imposed the data requirements.

As discussed in this document, use of the manufacturing-use product containing *Pasteuria usgae* is not likely to result in any unreasonable adverse effects to human health or the environment, fulfilling criterion 1. Furthermore, as mentioned in section VI, the registration of *Pasteuria usgae* – BL1 is presumed to be in the public interest because it will be used to create end-use products that will eventually serve as partial replacements for particular conventional nematicides of continuing concern to the Agency; thus, criterion 2 has been satisfied. To satisfy criterion 3, insufficient time has elapsed since the initial imposition of the data requirements outlined in section VIII(B).

B. Registration Review

The Federal Insecticide, Fungicide, and Rodenticide Act, as amended by the FQPA of 1996, mandated the continuous review of existing pesticides. All pesticides distributed and sold in the United States must generally be registered by the EPA, based on scientific data showing that they will not cause unreasonable risks to human health, workers, or the environment when used as directed in product labeling. The registration review program is intended to make sure that, as the ability to assess risk evolves and as policies and practices change, all registered pesticides continue to meet the statutory standard of no unreasonable adverse effects to human health or the environment. Changes in science, public policy, and pesticide use practices will occur over time. Through the registration

review program, the Agency periodically reevaluates pesticides to make sure that as change occurs, products in the marketplace can be used safely. Information on this program is provided at http://www.epa.gov/oppsrrd1/registration_review/.

The Agency has implemented the registration review program pursuant to FIFRA section 3(g) and will review each registered pesticide every 15 years to determine whether it continues to meet the FIFRA standard for registration. A registration review decision is the Agency's determination whether a pesticide meets, or does not meet, the standard for registration in the Federal Insecticide, Fungicide, and Rodenticide Act. *Pasteuria usgae* will be included in the schedule for registration review at the end of the Fiscal Year when schedules are updated.

C. Regulatory Decision

Based on the data submitted and under FIFRA section 3(c)(7)(C), BPPD recommends conditional, time-limited registration of the manufacturing-use product, *Pasteuria usgae* – BL1, containing *Pasteuria usgae* as a new active ingredient. Although the data are satisfactory with respect to this particular new active ingredient in this pesticide, they are not sufficient to support an unconditional registration under FIFRA section 3(c)(5). Additional data, as outlined in section VIII(B), are necessary for a finding of registrability under FIFRA section 3(c)(5) and are considered terms or conditions for the purposes of this registration.

Handler exposure to *Pasteuria usgae* is not expected to pose any undue risk. Regardless, appropriate personal protective equipment and precautionary statements are required on the product label to mitigate any potential risks to pesticide handlers due to prolonged exposure. Handlers working with *Pasteuria usgae* in manufacturing facilities must wear a long-sleeved shirt, long pants, socks, shoes, waterproof gloves, and a dust/mist filtering respirator meeting NIOSH standards of at least N-95, R-95, or P-95.

D. Labeling

The label for the registered manufacturing-use product containing the active ingredient, *Pasteuria usgae*, is available at <http://oaspub.epa.gov/pestlabl/ppls.home>.

VIII. ACTIONS REQUIRED BY THE REGISTRANT

A. Final Printed Labeling

Before releasing pesticide products containing *Pasteuria usgae* for shipment, the registrant is required to provide appropriate final printed labeling to the Agency.

B. Conditional, Time-Limited Registration Required Data/Information

The Agency evaluated the data submitted in connection with the initial registration of the manufacturing-use product, *Pasteuria usgae* – BL1, containing *Pasteuria usgae* as a new active ingredient. Although it was determined that these data fulfill current guideline requirements for a conditional, time-limited FIFRA section 3(c)(7)(C) registration, the Agency is requiring Pasteuria Bioscience, Incorporated to submit the following additional data and/or information in the time frames listed:

Study Type	Required Data/Information	Due Date
Analysis of Samples (OPPTS Guideline 885.1400)	The preliminary analysis of samples provided to the Agency revealed a wide range of concentrations, with many replicates containing 1–2 orders of magnitude fewer <i>Pasteuria usgae</i> spores than the minimum specified on the Confidential Statement of Formula and product label (i.e., 1×10^6 spores per milliliter). A follow-up explanation, attempting to address this variability, mentioned several factors that contributed to this inconsistency and discussed how the concentration of spores could be adjusted during the manufacturing process, if necessary. Despite this additional information, a new five-batch analysis, with all batches from production level, must be submitted to the Agency.	May 1, 2011
Manufacturing Process (OPPTS Guideline 885.1200)	The original manufacturing process did not describe quality control measures taken to confirm that <i>Pasteuria usgae</i> was the only <i>Pasteuria</i> species present in the master stock. A follow-up explanation, which demonstrated that batches are screened for the presence of other microbes and screened against sting nematodes to confirm the presence of <i>Pasteuria usgae</i> , still did not provide sufficient information to indicate that screening for other <i>Pasteuria</i> species is conducted during the manufacturing process. Other <i>Pasteuria</i> species, specifically those that parasitize saprophytic (non-plant pathogenic) nematodes, must be screened for during the manufacturing process. Pasteuria Bioscience, Incorporated must integrate quality control measures that screen for <i>Pasteuria</i> species that parasitize saprophytic nematodes, either immediately after freezing or after making seed stock, into their current manufacturing process and provide a written description of these measures to the Agency.	October 1, 2009
Product Identity (OPPTS Guideline 885.1100)	Currently, full recognition of <i>Pasteuria usgae</i> is still pending with the Judicial Commission of the International Committee for Systematic Bacteriology. Once <i>Pasteuria usgae</i> has been recognized and removed from the category <i>Candidatus</i> , documentation indicating official recognition must be provided to the Agency.	As soon as the specified information becomes available
Storage Stability (OPPTS Guideline 830.6317)	The storage stability study for the technical grade of the active ingredient (TGAI)/manufacturing-use product (MP) has been cited as ongoing. When the study has been completed and the results have been compiled, these data must be submitted to the Agency. Within the discussion of these results, the test substance must be described to ensure that this data requirement has been fulfilled for both the TGAI and the MP (i.e., reference that the TGAI is equivalent to the MP for <i>Pasteuria usgae</i> – BL1).	June 1, 2010

C. Reporting of Adverse Effects and Hypersensitivity Incidents

Notwithstanding the information stated in the previous section, it should be clearly understood that certain, specific data are required to be reported to the Agency as a requirement for maintaining the federal registration for a pesticide product. A brief summary of these types of data are described below.

Reports of all incidents of adverse effects to the environment must be submitted to the Agency under the provisions stated in FIFRA section 6(a)(2). Additionally, all incidents of hypersensitivity (including both suspected and confirmed incidents) must be reported to the Agency under the provisions of 40 CFR § 158.2140(d).

IX. GLOSSARY OF ACRONYMS AND ABBREVIATIONS

BPPD	Biopesticides and Pollution Prevention Division
BRAD	Biopesticides Registration Action Document
CFR	Code of Federal Regulations
cm ³	cubic centimeter
CSF	Confidential Statement of Formula
°C	degrees Celsius
EDSP	Endocrine Disruptor Screening Program
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EPA	Environmental Protection Agency (the “Agency”)
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act
FR	Federal Register
g	gram
kg	kilogram
LD ₅₀	median lethal dose. A statistically derived single dose that can be expected to cause death in 50% of the test animals when administered by the route indicated (oral, dermal, or inhalation). It is expressed as a weight of substance per unit weight of animal (e.g., mg/kg).
MRID No.	Master Record Identification Number
mg	milligram
mL	milliliter
MP	manufacturing-use product
MPCA	microbial pest control agent
NE	“No Effect”
NIOSH	National Institute for Occupational Safety and Health
OPP	Office of Pesticide Programs
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
PCR	polymerase chain reaction
PPE	personal protective equipment
TGAI	technical grade of the active ingredient

X. BIBLIOGRAPHY

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APPENDIX A – MICROBIAL PESTICIDE DATA REQUIREMENTS

TABLE 1. Product Analysis Data Requirements for the Technical Grade of the Active Ingredient (TGAI)/ Manufacturing-Use Product (MP), *Pasteuria usgae* – BL1 (40 CFR § 158.2120)

Data Requirement (OPPTS Guideline)	Results		MRID Number
	TGAI	MP	
<i>Product Chemistry and Composition</i>			
Product Identity (885.1100)	Not applicable	<p><i>Pasteuria</i> spp. are gram-positive, mycelial, endospore-forming bacteria that are endoparasitic to nematodes and water fleas. The endospores of <i>Pasteuria</i> spp. can be identified and counted microscopically, but it is difficult to distinguish between species unless high magnification electron microscopy is used so that size and shape of the spores can be visualized and measured. Molecular techniques such as PCR methods have been reported for <i>Pasteuria penetrans</i> but have not been developed for the other species. Therefore, species identification <i>via</i> host specificity assays, requiring the establishment of nematode cultures and a bioassay, is the most direct and reliable technique for species identification.</p> <p><i>Pasteuria usgae</i>, a recently discovered strain, is host-specific to the sting nematode (<i>Belonolaimus longicaudatus</i>), which can be damaging to a variety of crops, particularly turf. The active agent of <i>Pasteuria usgae</i> is an endospore that attaches and infects the host nematode during all life stages (except eggs). Increased moisture, neutral pH, temperatures above 10°C, and sandy soil seem to provide the best environments for spore attachment to the host. In laboratory studies, <i>Pasteuria</i> spp. were able to attach to nematodes after exposure to high temperatures and wide ranges of pH; however, at extremes, the number of attached spores per nematode was reduced and, in some cases, no infection occurred after attachment.</p> <p>After attachment of the endospore, a germ tube penetrates the nematode cuticle and mycelial microcolonies are formed in the pseudocoelom, leading to eventual death of the host. The endospores formed inside the host are released into the soil when the infected nematode decomposes. The spores are non-motile and stable in the soil environment for several years.</p> <p>Classification: Acceptable. Currently, full recognition of <i>Pasteuria usgae</i> is still pending with the Judicial Commission of the International Committee for Systematic Bacteriology. Once <i>Pasteuria usgae</i> has been recognized and removed</p>	474267-01

Data Requirement (OPPTS Guideline)	Results		MRID Number
	TGAI	MP	
		from the category <i>Candidatus</i> , documentation indicating official recognition must be provided to the Agency.	
Manufacturing Process (885.1200)	Submitted data satisfy the requirements of manufacturing process for the TGAI/MP for the purposes of a conditional, time-limited FIFRA section 3(c)(7)(C) registration only. Classification: Supplemental: Upgradeable. Pasteuria Bioscience, Incorporated must integrate quality control measures that screen for <i>Pasteuria</i> species that parasitize saprophytic nematodes, either immediately after freezing or after making seed stock, into their current manufacturing process and provide a written description of these measures to the Agency on or before October 1, 2009.		474267-02
Deposition of a Sample in a Nationally Recognized Culture Collection (Not applicable)	<i>Pasteuria usgae</i> is on deposit with the American Type Culture Collection in Manassas, Virginia under Accession Number SD-5835.	Not applicable	Not applicable
Discussion of Formation of Unintentional Ingredients (885.1300)	Submitted data satisfy the requirements of discussion of formation of unintentional ingredients for the TGAI/MP. Classification: Acceptable		474267-03
Analysis and Certified Limits			
Analysis of Samples (885.1400)	Submitted data satisfy the requirements of analysis of samples for the TGAI/MP for the purposes of a conditional, time-limited FIFRA section 3(c)(7)(C) registration only. Classification: Unacceptable. A new five-batch analysis, with all batches from production level, must be submitted to the Agency on or before May 1, 2011.		474267-04
Certification of Limits (885.1500)	Not applicable	The certified limits for the active and inert ingredients fall within the OPPTS Guideline 830.1750 specified ranges. Classification: Acceptable	474267-05
Physical and Chemical Characteristics			
Color (830.6302)	Clear to very faint yellow-brown or cloudy at 24°C (5 batches)	Not applicable	474267-06
Physical State (830.6303)	Liquid at 20°C	Not applicable	474267-06
Odor (830.6304)	No odor	Not applicable	474267-06
Stability to Normal and Elevated Temperatures, Metals, and Metal Ions (830.6313)	Stable after exposure to 54°C for 14 days.	Not applicable	474267-07
Storage Stability (830.6317)	Preliminary results indicate that after 1 month of storage at 4°C, the TGAI/MP had a 5% loss of attachment and a 15% loss of infectivity. A 12-month storage stability study is ongoing and results must be submitted to the Agency upon its completion (on or before June 1, 2010).		474267-08

Data Requirement (OPPTS Guideline)	Results		MRID Number
	TGAI	MP	
Miscibility (830.6319)	Not applicable	Not required because <i>Pasteuria usgae</i> – BL1 is not an emulsifiable form of microbial pesticide (refer to test note #2 of 40 CFR § 158.2120(d)).	Not applicable
Corrosion Characteristics (830.6320)	Not applicable	Waived because <i>Pasteuria usgae</i> – BL1 will be packaged for very short time periods in plastic as it moves from the manufacturing facility to the formulation facility for end-use products. There will be no long-term exposure to the packaging. Classification: Acceptable	474350-01
pH (830.7000)	6.80–6.96 (5 batches)* 6–7.3**	Not applicable	474267-06* CSF**
Viscosity (830.7100)	Not applicable	Waived because <i>Pasteuria usgae</i> – BL1 is not viscous and will not be applied as a product. There will be no environmental exposure. Classification: Acceptable	474350-01
Density/Relative Density/Bulk Density (Specific Gravity) (830.7300)	Specific gravity = 1.006–1.008 gram (g)/cubic centimeter (cm ³)(5 batches)* Specific gravity = 1.0 g/cm ³ **	Not applicable	474267-06* CSF**

TABLE 2. Toxicology Data Requirements for the Technical Grade of the Active Ingredient (TGAI)/ Manufacturing-Use Product (MP), *Pasteuria usgae* – BL1 (40 CFR § 158.2140)

Data Requirement (OPPTS Guideline)	Results		MRID Number
	TGAI	MP	
<i>Tier I</i>			
Acute Oral Toxicity/Pathogenicity (885.3050)	Not toxic and/or pathogenic to rats by oral dose of 1 x 10 ⁸ spores/animal. Since microbial enumeration was not performed because the test material would not grow on agar media, the infectivity was uncertain. However, because the spores are highly specific to sting nematode, infectivity is unlikely to be a concern. Classification: Acceptable TOXICITY CATEGORY IV	Not applicable	474267-09

Data Requirement (OPPTS Guideline)	Results		MRID Number
	TGAI	MP	
Acute Dermal Toxicity (885.3100)	Not toxic to rats when treated with 2,000 mg/kg at 10 ⁸ spores/mL. The acute dermal LD ₅₀ is greater than 2,000 mg/kg for 10 ⁸ spores/mL. Classification: Acceptable TOXICITY CATEGORY IV	Not applicable	474267-12
Acute Pulmonary Toxicity/Pathogenicity (885.3150)	Not toxic and/or pathogenic to rats by pulmonary dose of 1–3 x 10 ⁸ spores/animal. Since microbial enumeration was not performed because the test material would not grow on agar media, the infectivity was uncertain. However, because the spores are highly specific to sting nematode, infectivity is unlikely to be a concern. Classification: Acceptable TOXICITY CATEGORY IV	Not applicable	474267-10
Acute Injection Toxicity/Pathogenicity (885.3200)	Not toxic and/or pathogenic to rats by intravenous dose of 1x 10 ⁸ spores/animal. Since microbial enumeration was not performed because the test material would not grow on agar media, the infectivity was uncertain. However, because the spores are highly specific to sting nematode, infectivity is unlikely to be a concern. Classification: Acceptable TOXICITY CATEGORY IV	Not applicable	474267-11
Hypersensitivity Incidents (885.3400)	No hypersensitivity incidents, including immediate-type or delayed-type reactions of humans and domestic animals, occurring during the testing or production of the TGAI/MP were reported by the registrant. Any future hypersensitivity incidents must be reported per OPPTS Guideline 885.3400.		474350-02
Cell Culture (885.3500)	Not required because <i>Pasteuria usgae</i> is not a virus (refer to test note #4 of 40 CFR § 158.2140(d)).	Not applicable	Not applicable
Acute Oral Toxicity (870.1100)	Not applicable	Waived based on the results of MRID Number 474267-09 and because the MP is equivalent to the TGAI. Classification: Acceptable TOXICITY CATEGORY IV	474979-01
Acute Dermal Toxicity (870.1200)	Not applicable	Waived based on the results of MRID Number 474267-12 and because the MP is equivalent to the TGAI.	474979-01

Data Requirement (OPPTS Guideline)	Results		MRID Number
	TGAI	MP	
		Classification: Acceptable TOXICITY CATEGORY IV	
Acute Inhalation Toxicity (870.1300)	Not applicable	Waived based on the results of MRID Number 474267-10 and because the MP is equivalent to the TGAI. Classification: Acceptable TOXICITY CATEGORY IV	474979-01
Acute Eye Irritation (870.2400)	Not applicable	Waived based on <i>Pasteuria usgae</i> 's lack of toxicity in the other toxicology studies and precautionary statements that mitigate potential for worker exposure. Classification: Acceptable TOXICITY CATEGORY III	474350-03
Primary Dermal Irritation (870.2500)	Not applicable	Waived based on the results of MRID Number 474267-12 and precautionary statements/personal protective equipment that mitigate potential for worker exposure. Classification: Acceptable TOXICITY CATEGORY III	474350-03
Tiers II and III			
Not required for <i>Pasteuria usgae</i> based on the lack of acute toxicity/pathogenicity in the Tier I studies.			

TABLE 3. Non-Target Organisms and Environmental Fate Data Requirements for the Technical Grade of the Active Ingredient (TGAI), *Pasteuria usgae* (40 CFR § 158.2150)

Data Requirement (OPPTS Guideline)	Results	Toxicity Category/Description	MRID Number
Tier I			
Avian Oral Toxicity (885.4050)	Data waiver rationale provides sufficient information to determine that toxicity/pathogenicity to avian wildlife is not expected. Classification: Acceptable	Not applicable	474267-13
Avian Inhalation Toxicity/Pathogenicity (885.4100)	Not required as the nature of the microbial pesticide does not indicate potential pathogenicity to birds or relatedness to any known bird pathogens (refer to test note #3 of 40 CFR § 158.2150(e)).	Not applicable	Not applicable

Data Requirement (OPPTS Guideline)	Results	Toxicity Category/Description	MRID Number
Wild Mammal Toxicity/Pathogenicity (885.4150)	Tests required by 40 CFR § 158.2140 are adequate and appropriate for assessment of hazards to wild mammals (refer to test note #4 of 40 CFR § 158.2150(e)). Classification: Acceptable for wild mammal risk assessment	Testing indicates no adverse effects to laboratory rats at 1 x 10 ⁸ spores/mL when dosed orally.	474267-09
Freshwater Fish Toxicity/Pathogenicity (885.4200)	<i>Pasteuria usgae</i> will not be applied directly to water and is not expected to enter freshwater environments in amounts that are significantly higher than naturally occurring concentrations. Data waiver rationale provides sufficient information to determine that toxicity/pathogenicity or substantial exposure to freshwater fish is not expected. Classification: Acceptable	Not applicable	474267-13
Freshwater Invertebrate Toxicity/Pathogenicity (885.4240)	<i>Pasteuria usgae</i> will not be applied directly to water and is not expected to enter freshwater environments in amounts that are significantly higher than naturally occurring concentrations. Data waiver rationale provides sufficient information to determine that toxicity/pathogenicity or substantial exposure to freshwater invertebrates is not expected. Classification: Acceptable	Not applicable	474267-13
Estuarine/Marine Fish Testing Estuarine and Marine Invertebrate Testing (885.4280)	Not required because <i>Pasteuria usgae</i> will not be applied to water and is not expected to enter marine/estuarine environments in amounts that are significantly higher than naturally occurring concentrations (refer to test note #6 of 40 CFR § 158.2150(e)).	Not applicable	Not applicable
Non-Target Plant Testing (885.4300)	Not required because <i>Pasteuria usgae</i> is not related to known plant pathogens, and adverse effects to plants are not expected (refer to test note #7 of 40 CFR § 158.2150(e)).	Not applicable	Not applicable
Non-Target Insect Testing (885.4340)	Data waiver rationale provides sufficient information to determine that toxicity/pathogenicity to non-target insects is not expected. Classification: Acceptable	Not applicable	474267-13
Honey Bee Testing (885.4380)	Data waiver rationale provides sufficient information to determine that toxicity/pathogenicity to honey bees is not expected. Classification: Acceptable	Not applicable	474267-13

Data Requirement (OPPTS Guideline)	Results	Toxicity Category/Description	MRID Number
<i>Tiers II, III, and IV</i>			
Not required for <i>Pasteuria usgae</i> based on the acceptability of the waiver rationales provided for Tier I.			

APPENDIX B – *PASTEURIA USGAE* MANUFACTURING-USE AND END-USE PRODUCTS

EPA Registration Number	Registration Name	Percentage Active Ingredient	Formulation Type	Use Site	Method of Application	Application Rate	Target Pests
85004-1	<i>Pasteuria usgae</i> – BL1	0.01%	Technical	N/A	N/A	N/A	N/A