

Volume 10 of 10

TITLE

**PETITION FOR A TEMPORARY EXEMPTION FROM THE
REQUIREMENT OF A TOLERANCE FOR *PASTEURIA USGAE* FORMULATIONS
NOTICE OF FILING**

AUTHORS

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TEST GUIDELINE

Not Applicable

COMPLETED ON

November 1, 2008

SPONSOR

Pasteuria Bioscience, Inc.

TESTING FACILITY

Not Applicable

Total Number of Pages: 9

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

Compounds: *Pasteuria usgae* – Clay Granule, *Pasteuria usgae* – Liquid Formulation

Title: Petition for a Temporary Exemption from the Requirement of a Tolerance for *Pasteuria usgae* formulations

Claim of confidentiality under FIFRA section 10(d)(1)(A), (B), or (C).

Information claimed confidential on the basis of its falling within the scope of FIFRA section 10(d)(1)(A), (B), or (C) has been removed to a confidential appendix, and is cited by cross-reference number in the body of the study.

Company: Pasteuria Bioscience, Inc.
12085 Research Drive, Suite 185
Alachua, FL 32615

Company Agent:  (Date: November 1, 2008)

Susan MacIntosh, B.A.
President
MacIntosh & Associates, Inc.
Regulatory Consultant On behalf of Pasteuria Bioscience, Inc.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was not conducted in accordance with the requirements for the U.S. EPA Good Laboratory Practice (GLP) Standards, 40 CFR 160, 1989.

Study Director  31 Oct 2008
Susan MacIntosh, B.A. Date
Regulatory Consultant to PBI

Sponsor  31 Oct 08
Kelly Smith, Ph.D. Date
Chief Technical Officer

Submitter  31 Oct 2008
Susan MacIntosh, B.A. Date
Regulatory Consultant to PBI

APPROVALS PAGE

We, the undersigned, declare that this report accurately represents the results observed during the course of this study.

Study Director/Author  31 Oct 2008
Susan MacIntosh, B.A. Date
President

Co-Author  31 Oct 08
Kelly Smith, Ph.D. Date
Chief Technical Officer



**EPA BIOPESTICIDES AND POLLUTION PREVENTION DIVISION
COMPANY NOTICE OF FILING FOR PESTICIDE PETITIONS PUBLISHED IN
THE FEDERAL REGISTER**

**EPA Biopesticides and Pollution Prevention Division contact: Jeannine Kausch,
(703) 347-8920**



INSTRUCTIONS: Please utilize this outline in preparing the pesticide petition. In cases where the outline element does not apply, please insert "NA-Remove" and maintain the outline. Please do not change the margins, font, or format in your pesticide petition. Simply replace the instructions that appear in green, i.e., "[insert company name]," with the information specific to your action.

SUBMISSION: E-mail the completed template to: hollis.linda@epa.gov.

TEMPLATE:

Pasteuria Bioscience, Inc.
12085 Research Drive, Suite 185
Alachua, FL 32615

[Insert petition number]

EPA has received a pesticide petition ([insert petition number]) from Pasteuria Bioscience, Inc., 12085 Research Drive, Suite 185, Alachua, FL 32615 proposing, pursuant to section 408(d) of the Federal Food, Drug, and Cosmetic Act (FFDCA), 21 U.S.C. 346a(d), to amend 40 CFR part 180.

(Options (pick one))

2. to establish an exemption from the requirement of a tolerance for

(Options (pick one))

1. microbial pesticide *Pasteuria usgae* [AF254387]

Pursuant to section 408(d)(2)(A)(i) of FFDCA, as amended, **Pasteuria Bioscience, Inc.** has submitted the following summary of information, data, and arguments in support of their pesticide petition. This summary was prepared by **Pasteuria Bioscience, Inc.** EPA has not fully evaluated the merits of the pesticide petition. The summary may have been edited by EPA if the terminology used was unclear, the summary contained extraneous material, or the summary unintentionally made the reader conclude that the findings reflected EPA's position and not the position of the petitioner.

I. **Pasteuria Bioscience, Inc. Petition Summary**

[Insert petition number]

A. Product Name and Proposed Use Practices

Pasteuria usgae is a gram-positive, mycelial, endospore-forming bacterial product that is endoparasitic to nematodes. Bacteria of the genus *Pasteuria* have long been recognized as promising biological control agents for plant-parasitic nematodes. Despite extensive testing with a wide range of nematode species, *P. usgae* has an extremely narrow host range, specific to a single nematode species, sting nematodes (*B. longicaudatus*) and poses no foreseeable risks to non-target organisms. Sting nematodes attack a wide range of vegetable and fruit crops, and turf, but the focus of this temporary exemption from the requirement of a tolerance will be for use on strawberries. *Pasteuria* spp. are ubiquitous in nature, found in nearly every location where investigated. *Pasteuria* spores are the infective agent applied to crops for nematode control. This natural product will be an excellent addition to growers' options for nematode control that reduces or eliminates the need for chemical inputs and fits well within an integrated pest management program for organic or conventional production.

B. Product Identity/Chemistry

1. *Identity of the pesticide and corresponding residues.* The bacterial strain, *Pasteuria usgae* [AF254387] was isolated from soil samples collected in Florida USA. The strain is a natural bacterial strain that has not been modified in any way and is identical to *Pasteuria* spp. that are ubiquitous in nature. They are known for their ability to disrupt reproduction in their nematode hosts and have been shown to reduce nematode damage below economic levels leading to improved crop production.

2. *Magnitude of residues at the time of harvest and method used to determine the residue.* [NA remove – see 1. above]

3. *A statement of why an analytical method of detecting and measuring the levels of the pesticide residue are not needed.* No analytical method is included since this petition requests an exemption from the requirement of a tolerance.

C. Mammalian Toxicological Profile

A range of toxicology studies has been conducted on *Pasteuria usgae*. The tissue and blood samples were to be prepared for culture to quantitate the test microbe and determine the infectivity and clearance of microbial pest control agent (MPCA) in tissues; however, the test substance could not be quantified using an agar plating method.

An acute oral toxicity/pathogenicity of *Pasteuria usgae* – Liquid suspension (TGAI/MP), was conducted; the test material was administered to 12 rats (6 female and 6 male) by gavage in a single high dose exposure of 1×10^8 spores/animal (OPPTS 885.3050; MRID 474267-09). Another 6 rats (3 female and 3 male) received the same amount of test material inactivated by autoclaving. There was no mortality during the study. Mean weight gain in males was 74 g and 70 g for the inactivated MPCA and active MPCA, respectively. There were no signs of pharmacologic and/or toxicologic effects observed in any animals in any group during the study. There were no adverse effects produced by *Pasteuria usgae* spores – Liquid suspension dosed orally 10^8 spores/animal.

Acute pulmonary toxicity/pathogenicity and infectivity of *Pasteuria usgae* – Liquid suspension (TGAI/MP), was conducted using a single high dose exposure of 1×10^7 spores/animal administered by the intratracheal route to rats (OPPTS 885.3150; MRID 474267-10). Three treatment groups were included in the study, Group I (untreated, 5 females and 5 males), Group II (inactivated MPCA, 5 females and 5 males) and Group III (MPCA, 6 females and 6 males). There was no mortality during the study. One female in the untreated control group lost 4 g between Days 7 and 14, while another female lost 19 g between Days 14 and 2, and a third female in the active MPCA group failed to gain weight between Days 0 and 7. Mean body weight gain in males was 75 g, 67 g and 42 g for Groups I, II and III, respectively. Mean body weight gain in females was 24 g, 36 g and 38 g for Groups I, II and III, respectively. There were no signs of pharmacologic and/or toxicologic effects observed in any animals in any group during the study. There were no adverse effects produced by *Pasteuria usgae* – Liquid suspension dosed by intratracheal instillation.

The test substance, *Pasteuria usgae* - Liquid suspension (TGAI/MP), was evaluated for its acute intravenous toxicity, infectivity and pathology in albino rats when administered as a single high dose injection of at least 10^8 spores/animal (OPPTS 885.3200; MRID 474267-11). Three treatment groups were included in the study, Group I (untreated, 3 females and 3 males), Group II (inactivated MPCA, 3 females and 3 males) and Group III (MPCA, 6 females and 6 males). No mortality occurred during the study. There were no clinical signs of toxicity during the study. There was no meaningful effect on body weight gain. The gross necropsy conducted on each animal of the study revealed no observable abnormalities. The acute intravenous LD₅₀, as indicated by the data, was determined to be greater than 1×10^8 spores/animal.

The test substance, *Pasteuria usgae* - Liquid suspension (TGAI/MP), was evaluated for its dermal toxicity potential and relative skin irritancy, or other health hazards, when a single undiluted dose of 2000 mg/kg of the MPCA (at least 1×10^8

spores/mL) was applied to the intact skin of albino rats (OPPTS 885.3100; MRID 474267-12). A single treatment group included 5 females and 5 male albino rats. No mortality occurred during the study. There were no clinical signs of toxicity at any time throughout the study. The only sign of dermal irritation was erythema in three animals on Day 1. There was minimal effect on body weight gain; three animals lost weight during the first week, and one animal lost weight during the second week of the study. The gross necropsy conducted at termination of the study revealed no observable abnormalities. The estimated LD₅₀, as indicated by the data, was determined to be greater than 2000 mg/kg.

An acute eye irritation of *Pasteuria usgae* – Clay Granule (EP) was conducted on albino rabbits (OPPTS 870.2400; MRID 475218-08). The MCPA was ground to a fine powder before dosing and 100mg (3.7×10^6 spores/animal) was placed into the conjunctival sac of the right eye of 3 rabbits (1 female and 2 males). “Positive” findings were observed for some measurements at the 1hr post-treatment time point, but cleared completely by the 24hr time point for all eyes. The maximum average irritation score of 37.0, obtained at 1 hr after treatment, was used to rate *Pasteuria usgae* – Clay Granule (EP) moderately irritating. Fluorescein staining did not occur in any of the eyes. As a result, the test substance is assigned to Toxicity Category IV.

A primary dermal irritation of *Pasteuria usgae* – Clay Granule (EP) was conducted on albino rabbits (OPPTS 870.2500; MRID 475218-09). One test site for each of 3 rabbits (2 females and 1 male) was treated with 500mg (1.85×10^6 spores/animal) of test substance moistened with 0.5mL of deionized water and covered with a semi-permeable dressing. The test substance was maintained in contact with the skin for 4hrs. Observations for dermal irritation and defects were recorded at 1, 24, 48 and 72 hrs after removal of the dressings. Based on the PII of 0.1, the test substance is rated slightly irritating. Based on the 72hr observation, the test substance is assigned to Toxicity Category IV.

D. Aggregate Exposure

1. *Dietary exposure.* Since *Pasteuria usgae* is applied to the soil, where nematodes live, there will be negligible to non-existent dietary, dermal or inhalation exposure. Use of this bacterium in pesticide products will not increase the exposure of humans beyond normal background levels.

i. *Food.* There will be no accumulation of the bacteria or this bio-nematicide product in any plant tissues or food.

ii. *Drinking water.* Drinking water is unlikely to be contaminated with *Pasteuria usgae* spores, because *P. usgae* only attaches to plant parasitic nematodes, which are limited to soil habitats and do not live in water environments.

2. *Non-dietary exposure.* Non-dietary exposure of infants, children or the US population in general, to *Pasteuria usgae* are not expected due to the uses of this product within agricultural settings.

E. Cumulative Effects

The unique mode-of-action and narrow host range *P. usgae*, and of *Pasteuria* spp. spores in general, coupled with the lack of mammalian toxicity provides no basis for the expectation of cumulative exposure with other compounds.

F. Safety Determination

1. *U.S. population.* The lack of mammalian toxicity to the bacterium *Pasteuria usgae* provides support for our request of an exemption from the requirement of a tolerance set forth in this petition, including infants and children.

2. *Infants and children.*

G. Effects on the Immune and Endocrine Systems

The unique mode-of-action and narrow host range *P. usgae*, and of *Pasteuria* spp. spores in general, coupled with the lack of mammalian toxicity provides no basis for the expectation of effects on the immune or endocrine systems.

H. Existing Tolerances

No tolerances or tolerance exemptions have been granted for *Pasteuria usgae*. However, EPCOT center in Disney World regularly applies a closely related bacterial strain to *P. usgae*, *Pasteuria penetrans*, in the agriculture show called "The Land". EPA granted an exemption from the requirement of a tolerance for *P. penetrans* (December 28, 1994; 40 CFR 180.1135), which is produced *in vivo* using a vegetable system to grow *P. penetrans* infested nematodes. This agricultural show demonstrates crops from around the world and typically hosts over 14,000 people a day.

I. International Tolerances

No international tolerances or tolerance exemptions have been granted for *Pasteuria usgae*.