Proposed Registration Decision PRD2017-19

Beauveria bassiana strain PPRI 5339 and Velifer

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Overview

Proposed Registration Decision for *Beauveria bassiana* strain PPRI 5339

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Beauveria bassiana* PPRI 5339 Technical and Velifer, containing the technical grade active ingredient *Beauveria bassiana* strain PPRI 5339, to suppress aphids, whiteflies, thrips and twospotted spider mites on greenhouse ornamentals and greenhouse vegetables.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of *Beauveria bassiana* strain PPRI 5339 and Velifer.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment. These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides and Pest Management portion of the Canada.ca website at https://www.canada.ca/en/health-canada/services/consumer-product-safety/pesticides-pest-management.html.

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[1] *"Acceptable risks" as defined by subsection 2(2) of the* Pest Control Products Act.

[2] *"Value" as defined by subsection 2(1) of the* Pest Control Products Act*: "… the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (*a*) efficacy; (*b*) effect on host organisms in connection with which it is intended to be used; and (*c*) health, safety and environmental benefits and social and economic impact."*

Before making a final registration decision on *Beauveria bassiana* strain PPRI 5339 and Velifer, the PMRA will consider any comments received from the public in response to this consultation document.[3] The PMRA will then publish a Registration Decision[4] on *Beauveria bassiana* strain PPRI 5339 and Velifer, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What Is *Beauveria bassiana* strain PPRI 5339?

Beauveria bassiana strain PPRI 5339 is a fungus that kills various insects and mites and it is the active ingredient in the commercial class end-use product, Velifer. Velifer is a biological insecticide applied as a foliar spray in greenhouses to suppress whiteflies, aphids, twospotted spider mites and thrips on ornamentals, and vegetables.

Beauveria bassiana grows naturally in soils throughout the world. It is a generalist entomopathogenic fungus that causes a disease in many types of insects that is often fatal. While insects living in or near the soil have evolved natural defences against this fungus as it is common in their natural environment, it can be used as an insecticide against many other insects. The PPRI 5339 strain of *B. bassiana* was isolated in 1993 from the larvae of a tortoise beetle, collected in South Africa.

Health Considerations

Can Approved Uses of *Beauveria bassiana* strain PPRI 5539 Affect Human Health?

Beauveria bassiana strain PPRI 5339 is unlikely to affect your health when Velifer is used according to the label directions.

Potential exposure to *B. bassiana* strain PPRI 5339 may occur when handling and applying Velifer and when ingesting treated produce. When assessing health risks, several key factors are considered:

- the microorganism's biological properties (for example, production of toxic by-products);
- reports of any adverse incidents;
- its potential to cause disease or toxicity as determined in toxicological studies; and
- the level to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

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[3] "*Consultation statement" as required by subsection 28(2) of the* Pest Control Products Act.

[4] "*Decision statement" as required by subsection 28(5) of the* Pest Control Products Act.

The levels used to assess risks are established to protect the most sensitive human population (for example, children and nursing mothers). As such, sex and gender are taken into account in the risk assessment. Only uses that are determined as having no health risks of concern are considered acceptable for registration.

Studies in laboratory animals describe potential health effects from large doses of exposure to a microorganism and identify any pathogenicity, infectivity and toxicity concerns. When dry spores of *B. bassiana* strain PPRI 5339 were tested on laboratory animals, there was no sign of disease and no sign of toxicity via the oral, dermal and pulmonary (intratracheal) routes of exposure. However, dry spores of *B. bassiana* strain PPRI 5339 were acutely toxic via the inhalation route. No such toxicity was noted in laboratory animals tested with the liquid end-use product, Velifer.

Residues in Water and Food

Dietary risks from food and water are not of concern

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally specified as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada specifies science-based MRLs to ensure that the food Canadians eat is safe.

Residues of *B. bassiana* strain PPRI 5339 on the treated crops, at the time of harvest, are possible following foliar applications to agricultural crops. *Beauveria bassiana* is a naturally occurring fungus in soil that regularly causes infections in susceptible insects. *Beauveria bassiana* strain PPRI 5339 produced no adverse effects (disease or toxicity) when administered orally to rats and no metabolites of toxicological significance have been shown to be produced by this strain of *B. bassiana*. As well, the likelihood of residues of *B. bassiana* strain PPRI 5339 contaminating drinking water supplies resulting from operational applications as a pesticide is considered to be low. Consequently, dietary risks are considered to be low and not of concern. Therefore, the Pest Management Regulatory Agency (PMRA) has determined that the specification of an MRL under the *Pest Control Products Act* is not required for *B. bassiana* strain PPRI 5339.

Risks in Residential and Other Non-Occupational Environments

Estimated risk for non-occupational exposure is not of concern.

When Velifer is used in commercial greenhouses, it is unlikely that adults, youths, and toddlers will be exposed to *B. bassiana* strain PPRI 5339. Even in the event of exposure, risk to the general population is not a concern since there were no signs of disease noted in toxicological studies with the technical grade active ingredient and no toxicity noted in toxicological studies with the end-use product.

Occupational Risks From Handling Velifer

Occupational risks are not of concern when Velifer is used according to label directions, which include protective measures

Commercial workers handling Velifer can come into direct contact with *B. bassiana* strain PPRI 5339 on the skin, in the eyes, or by inhalation. For this reason, the product label will specify that commercial workers must wear waterproof gloves, long-sleeved shirts, long pants, a mist/dust filtering mask or respirator, and shoes plus socks.

Environmental Considerations

What Happens When *Beauveria bassiana* strain PPRI 5539 Is Introduced Into the Environment?

Velifer is not expected to pose risks of concern to the environment when used according to label directions.

Beauveria bassiana is a naturally occurring fungus that is commonly found in soil, as well as on plants. Although *B. bassiana* is not an aquatic microorganism, it likely enters bodies of water through runoff from soil and plants as well as from contact with dead and dying insects infected with *B. bassiana*. Therefore, both terrestrial and aquatic non-target organisms are likely exposed regularly to populations of *B. bassiana.*

Velifer is proposed for use in greenhouses only. No direct outdoor uses are proposed. The amount of *B. bassiana* strain PPRI 5339 transferring to outdoor environments from the use of Velifer in greenhouses is expected to be minimal and is not expected to significantly increase the level of *B. bassiana* in the environment beyond what is naturally occurring- provided that label instructions are followed.

Based on a critical review of information in the published scientific literature, no significant effects to birds, wild mammals, aquatic arthropods, plants or fish are expected when Velifer is applied according to directions on the label. Studies conducted to determine the effects of *B. bassiana* strain PPRI 5339 on terrestrial arthropods (including bumblebees and honeybees) were inconclusive. Consequently, the Velifer label will include statements identifying the potential harm to beneficial insects and bees in greenhouses, and instruct applicators to avoid direct contact of the product with foraging bees.

Value Considerations

What Is the Value of Velifer?

Velifer suppresses aphids, whiteflies, thrips and twospotted spider mites in greenhouse ornamentals and greenhouse vegetables.

Velifer is a new pest management tool for use in greenhouse production of ornamentals and vegetables. It is the first *B. bassiana* product for management of the twospotted spider mite which is a major pest on greenhouse crops, as well as a new *B. bassiana* strain to use against aphids, thrips and whiteflies.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the labels of the technical grade active ingredient, *Beauveria bassiana* PPRI 5339 Technical, and the end-use product, Velifer, to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

All microorganisms, including *B. bassiana* strain PPRI 5339, contain substances that are potential sensitizers and thus, respiratory and dermal sensitivity may possibly develop in individuals exposed to potentially large quantities of *B. bassiana* strain PPRI 5339. In turn, commercial workers handling or applying Velifer must wear appropriate waterproof gloves, a long-sleeved shirt, long pants, a mist/dust filtering mask or respirator, and socks with shoes.

Environment

The Velifer label will include environmental precaution statements that prevent the contamination of aquatic systems from the use of Velifer and from greenhouse effluent. The label will also include statements indicating that Velifer may be harmful to pollinators (including bees) and some beneficial insects used in greenhouse integrated pest management programs. Additionally, the label will instruct users to avoid direct contact to beneficial insects and avoid applications while bees are actively foraging.

Next Steps

Before making a final registration decision on *Beauveria bassiana* strain PPRI 5339 and Velifer, the PMRA will consider any comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact

information on the cover page of this document). The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision, and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *B. bassiana* strain PPRI 5339 and Velifer (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Beauveria bassiana strain PPRI 5339

1.0 The Active Substance, Its Properties And Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Beauveria bassiana* strain PPRI 5339
Function	For the suppression of aphids, twospotted spider mites, thrips and whiteflies in greenhouse vegetables and greenhouse ornamentals.
Binomial name	*Beauveria bassiana* strain PPRI 5339
Taxonomic designation[1]	
Kingdom	Fungi
Phylum	Ascomycota
Class	Sordariomycetes
Order	Hyprocreales
Genus	*Beauveria*
Species	*bassiana*
Strain	PPRI 5339
Patent Status information	None identified by the applicant.
Nominal purity of active	Technical grade active ingredient: minimum of 1×10^{11} viable spores/g Velifer (end-use product): minimum of 8×10^{9} viable spores/mL
Identity of relevant impurities of toxicological, environmental and/or significance.	The technical grade active ingredient does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards. *Beauveria bassiana* strains are known to produce toxic secondary metabolites but the technical grade active ingredient is not expected to contain any of these metabolites.

[1] Taxonomy browser at: http://www.ncbi.nlm.nih.gov/pubmed/

1.2 Physical and Chemical Properties of the Technical Grade Active Ingredient and the End-Use Products

Technical Grade Active Ingredient – *Beauveria bassiana* PPRI 5339 Technical

Property	Result
Colour	Light Cream
Physical State	Fine dry Powder
Odour	Musty odour
Corrosion Characteristics	Non-corrosive to HDPE containers
pH	6 (1% aqueous)
Relative Density	0.28 g/cm^3

End-Use Product – Velifer

Property	Result
Colour	Light Brown Opaque
Physical State	Liquid
Odour	Smoky
Viscosity	122 mPas at 100s^{-1}
Corrosion Characteristics	Non-corrosive to HDPE containers
pH	6.6 (1% in water)
Relative Density	0.949g/cm^3

1.3 Directions for Use

Foliar applications of Velifer at a concentration of 450–900 mL product/1000 L of water suppress aphids, whiteflies, thrips and twospotted spider mites on greenhouse ornamentals and greenhouse vegetables. Applications can be made every 3–10 days in an application volume sufficient for uniform coverage, but not to the point of runoff. Higher concentrations are to be used under high pest pressure along with shorter application intervals.

1.4 Mode of Action

Beauveria bassiana is a generalist entomopathogenic fungus which causes white muscardine disease in various species of insects and mites. When fungal spores come into contact with target pests, the spores germinate into hyphae which penetrate the host cuticle leading to desiccation and death of the target pest. After death, a white mould grows on the cadaver and produces new spores.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganisms

Appropriate methodologies for detection, isolation and enumeration of the active ingredient, *B. bassiana* strain PPRI 5339, were submitted by the applicant. The microbial pest control agent (MPCA) has been fully characterized with respect to its origin of strain, natural occurrence and biological properties. *Beauveria bassiana* strain PPRI 5339 can be identified to the species level using a combination of colony morphologies on agar media and using the latest DNA-based methodologies. *Beauveria bassiana* strain PPRI 5339 can be differentiated from other *Beauveria* species based on sequencing analysis of internal transcribed spacer (ITS) ribosomal region including the 3' end of the 18S region, the ITS1 region, the 5.8S region and the ITS2 region.

2.2 Methods for Establishment of Purity of Seed Stock

The strain has been deposited into the international depository authority of the Agricultural Research Culture Collection (NRRL) Peoria, Illinois under the identification number NRRL 50757. Stock cultures are kept frozen at -80°C.

Master *B. bassiana* strain PPRI 5339 cultures are stored on sterile silica gel. At the start of each production run, *B. bassiana* strain PPRI 5339 working stock cultures are prepared from the master culture stock onto potato dextrose agar (PDA) Schott bottles slants and stored at 4°C. The colony morphology of the working stock is checked to confirm identity.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The guarantees of the technical grade active ingredient and the end-use product are expressed in units of viable spores/g and viable spores/mL, respectively. Representative data on five batches of technical grade active ingredient and end-use product were submitted. The method for determining viable spore concentration was adequately described.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

As noted above, appropriate methods are available to enumerate viable spores and to distinguish this MPCA from other *Beauveria* species and other strains of *B. bassiana.*

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures used to limit contaminating microorganisms during the manufacture of *Beauveria bassiana* PPRI 5339 Technical and Velifer are acceptable. These procedures include sterilization of all equipment and media as well as frequent sampling of the stock culture and production batches for purity and contamination.

The absence of human pathogens and below-threshold levels of contaminating microorganisms were shown in the microbial screening of batches of *Beauveria bassiana* PPRI 5339 Technical using standard methods for detecting and enumerating microbial contaminants of concern as well as by results of mouse toxicity testing. Although limits for microbial contamination were not proposed, all batches of *Beauveria bassiana* PPRI 5339 Technical must conform to the limits set out in the Organization for Economic Co-operation and Development (OECD) issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43].

2.6 Methods to Determine Storage Stability, Shelf-life of the Microorganism

The storage stabilities of *Beauveria bassiana* PPRI 5339 Technical and Velifer have been assessed at 4°C for up to 12 months.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

3.1.1 Testing

The PMRA conducted a detailed review of the toxicological studies submitted in support of *Beauveria bassiana* PPRI 5339 Technical, and Velifer.

The studies submitted to fulfil the requirements for health hazard assessment of the technical grade active ingredient, *Beauveria bassiana* PPRI 5339 Technical, included an acute pulmonary infectivity/toxicity, an acute intraperitoneal injection infectivity, an acute oral toxicity study, four acute inhalation studies, an acute dermal toxicity study, a dermal irritation study and an eye irritation study.

In the acute pulmonary infectivity/toxicity study, a group of young adult Sprague Dawley rats (18/sex) was exposed by the intratracheal route to the technical grade active ingredient (2.06×10^{11} spores/gram) in sterile phosphate buffered saline at a dose of 7.7×10^{7} CFU/animal. All animals were observed for up to 21 days following treatment. Interim sacrifices were also performed on Days 0, 3, 7 and 14 to evaluate microbial clearance. There were no mortalities observed in animals treated with live test material, however, one male rat that was treated with inactivated test material was found dead on Day 19. All animals appeared normal for the duration of the study. The gross necropsy conducted at study termination revealed no observable effects, with the exception of animal 14-M that was found dead on Day 19. Necropsy findings for this animal reported black internal organs, however the examination was not performed in a timely manner. The test substance was considered completely clear in the lungs of test animals by Day 14.

In the acute intraperitoneal infectivity study, a group of Sprague Dawley rats (15/sex) was injected with the technical grade active ingredient in phosphate buffered saline at a dose of 7.8×10^7 CFU/rat. All animals were observed for up to 21 days following treatment. An interim sacrifice was also performed on Day 0 following testing to enumerate the test material in the peritoneal wash. In this study, there was no mortality and all animals appeared normal for the duration of the study. The gross necropsy conducted at termination also revealed no observable abnormalities.

In the acute oral toxicity study, three fasted, female Sprague Dawley rats were given a single oral dose of technical grade active ingredient (2.06×10^{11} spores/gram) in deionized water at a dose of 5000 mg/kg bw using the Up and Down Method. The animals were then observed for a period of 14 days. There were no mortalities and no effects noted on weight gain during the study period. The only clinical sign was a slight activity decrease in one animal on the day of dosing. No observable abnormalities were observed at necropsy.

In three of the acute inhalation studies, the acute inhalation toxicity of the technical grade active ingredient ($1.77–2.27 \times 10^{11}$ CFU/gram) was tested in groups of young adult Sprague Dawley (5/sex/group) for 4 hours to nose only at concentrations of 0.05, 0.52, 1.26, 2.59 and 5.28 mg/L. Animals then were observed for a period of up to 14 days. In these studies, significant mortalities were noted in both male and female animals (>50% mortality). Clinical signs of toxicity included lethargy, piloerection, rapid breathing, body tremors, and hypothermia. Respiratory gurgle was also observed in animals that later died. Surviving animals generally exhibited weight loss in the first week of testing followed by weight gain by Day 14. Abnormal necropsy findings included red crust at the mouth, stained/matted genital fur, and mottled/discoloured lungs, kidneys and liver. In these studies, the acute inhalation toxicity LC_{50} was determined to be less than 0.05 mg/L.

In the fourth acute inhalation toxicity study, two male Sprague Dawley rats were similarly exposed to the technical grade active ingredient (2.06×10^{11} spores/gram) for 4 hours to nose-only at a concentration of 5.39 mg/L. The two animals were then observed until they died on Day 2. The clinical signs and gross necropsy findings observed during this study were consistent with the previous three studies. Histopathologic examination of tissues obtained from these animals indicated that lesions consisted of necrosis of the lining epithelium of secondary bronchi, terminal bronchioles and alveolar ducts. Acute inflammation in the walls of airways and adjacent alveoli was observed in both animals.

Mortality data from the four acute inhalation studies were combined to calculate an LC_{50} value by the method of probit analysis. Specifically, mortality data at the lowest three dose levels studies were used to estimate LC_{50}. This analysis determined that the acute inhalation LC_{50} value for the technical grade active ingredient was 0.02 mg/L with a 95% confidence interval of 0.0109 mg/L to 0.0574 mg/L.

In the acute dermal toxicity study, a group of young adult Sprague Dawley rats (5/sex) was dermally exposed to the technical grade active ingredient (2.06×10^{11} spores/gram) in deionized water for 24 hours to an area not less than 10% of the total body surface area. Following exposure, the animals were observed for a period of 14 days.

There were no mortalities or any clinical signs of toxicity noted throughout the study period. Animals exhibited weekly weight gain during the study, with the exception of one female that lost 4g between Days 0 and 7. At necropsy, there were no observable abnormalities noted.

In the primary dermal irritation study, three young adult New Zealand white rabbits (2♂, 1♀) were dermally exposed to 500 mg of technical grade active ingredient (2.06×10^{11} spores/gram) in 0.5 mL deionized water for 4 hours to an area 8 × 8 cm. Animals then were observed for 72 hours. Irritation was scored by the method of Draize. Very slight erythema was present at each observation through 24 hours. Edema was not observed at any time throughout the study. No other signs of irritation were observed during the study. The calculated Maximum Irritation Score (MIS) was 0.67/8 at 1 hour, and the Maximum Average Score (MAS) was 0.11/8 at 24, 48 and 72 hours.

In the primary eye irritation study, the technical grade active ingredient (100 mg; 2.06×10^{11} spores/gram) was directly instilled into the conjunctival sac of the right eye of three young adult New Zealand white rabbits then were observed for 7 days. Irritation was scored at 1, 24, 48 and 72 hours, and at 4 and 7 days after treatment by the method of Draize. Following treatment, conjunctival redness (grades 1–3), chemosis (grades 1–3) and discharge (grades 1–3) were noted in all three animals. Corneal opacity (grade 1) and positive fluorescein staining was also observed in all three animals. All irritation cleared by Day 7. The calculated MAS and MIS were 18.7/110 at 24, 48 and 72 hours, and 20/110 at 48 hours and at 72 hours.

Studies submitted to fulfil the requirements for the health hazard assessment of the end-use product, Velifer, included oral toxicity, inhalation toxicity, dermal toxicity, dermal irritation, and eye irritation studies.

In the acute oral toxicity study, three fasted, female Sprague Dawley rats were given a single oral dose of undiluted end-use product (1.42×10^{10} spores/mL) at a dose of 5000 mg/kg bw using the Up and Down Method. The animals were then observed for a period of 14 days. There were no mortalities, no clinical signs and no effects noted on weight gain during the study period. The gross necropsy conducted at study termination revealed no observable abnormalities.

In the acute inhalation toxicity study, a group of young adult Sprague Dawley rats (5/sex) was exposed by the inhalation route to the end-use product (1.42×10^{10} spores/mL) for 4 hours to nose only at a concentration of 3.09 mg/L. Animals were then observed for 14 days. There was no mortality during the study and all animals appeared normal for duration of the study. The animals exhibited weight gain during the study with the exception from Day 0 to Day 1, where all animals either lost weight or failed to gain weight. Additionally, one female lost weight from Day 3 to Day 7. No observable abnormalities were observed at necropsy.

In the acute dermal toxicity study, a group of young adult Sprague Dawley rats (5/sex) was dermally exposed to the end-use product (1.42×10^{10} spores/gram) in deionized water for 24 hours to an area not less than 10% of the total body surface area. Following exposure, the animals were observed for a period of 14 days.

There were no mortalities or any clinical signs of toxicity noted throughout the study period. Two male animals had very slight erythema (grade 1) on Day 1. All animals exhibited weekly weight gain during the study. At necropsy, there were no observable abnormalities noted.

In the primary dermal irritation study, three young adult New Zealand white rabbits (2♂, 1♀) were dermally exposed to undiluted end-use product (1.42×10^{10} spores/mL; 0.5 mL) for 4 hours to an area 8 × 8 cm. Animals then were observed for 72 hours. Irritation was scored by the method of Draize. No dermal irritation was observed throughout the study period. The calculated MIS and MAS was 0/8.

In the primary eye irritation study, aliquots (0.1 mL) of end-use product (1.42×10^{10} spores/mL) were directly instilled into the conjunctival sac of the right eye of three young adult New Zealand white rabbits. Animals then were observed for 7 days. Irritation was scored at 1, 24, 48 and 72 hours, and at 7 days after treatment by the method of Draize. Following treatment, conjunctival redness (grades 1–2), chemosis (grade 1) were noted in all three animals. No corneal opacity, positive fluorescein staining or iritic irritation was observed in any of the treated eyes. All irritation cleared by Day 7. The calculated MAS and MIS were 3.1/110 at 24, 48 and 72 hours, and 5.3/110 at 1 hour and at 24 hours.

Test results are summarized in Appendix I, Tables 1 and 2.

3.1.2 Additional Information

Scientific rationales were provided to waive the technical grade active ingredient requirement for an acute oral infectivity and toxicity study, as well as to discuss the findings in the acute inhalation toxicity studies for the technical grade active ingredient.

The request to waive the technical grade active ingredient requirement for an acute oral infectivity and toxicity study was supported by a lack of infectivity and/or toxicity effects for strain PPRI 5339 in acute pulmonary infectivity/toxicity, acute intraperitoneal injection infectivity, and acute oral toxicity testing as well as a lack of infectivity for a surrogate isolate (i.e., strain GK2016) following intramuscular injection. The request to waive acute oral infectivity/toxicity testing was accepted.

The scientific discussion on the acute inhalation findings for the technical grade active ingredient noted that the observed mortality was not uncommon for experimental animals treated with living organisms. Mortality as well as direct toxicity to the lung have been reported extensively in many studies following single exposures of rabbits, rats, mice or guinea pigs to spores of various fungal species. The mode of action leading to mortality in rats following continuous, 4-hr aerosol exposure to pure *B. bassiana* strain PPRI 5339 spores remains unclear. However, organic dusts and bioaerosols do pose a potential respiratory hazard via either an allergic or non-allergic mechanism. Since the measured indoor levels of *B. bassiana* generally tend to be very low, the mode of action is likely a non-allergic inflammatory response. This explanation is supported by the histopathological results from an early acute inhalation toxicity study with the technical grade active ingredient.

Also, mottled or discoloured lungs were observed at necropsy in each of those studies, with the spotted discolourations likely being lesions distributed throughout the lung. However, no such local inflammation, lung toxicity, or mortality was observed when the end-use product, Velifer, a suspension of 8% *B. bassiana* spores, was tested above the limit dose (3.09 mg/L).

It is unclear if single or multiple components of the technical grade active ingredient preparation are responsible for the lung inflammation. Levels of the mycotoxin, beauvericin, were below the limit of quantification (<0.5 ppm) in five tested batches of technical grade active ingredient. Overload of alveolar macrophages (i.e., exceedance of the capacity to phagocytize and clear the spores from the alveoli) was also considered as a contributor to the high mortality in the acute inhalation toxicity studies. Alveolar macrophages are the first line of defence to fungal aerosol exposures in the deep lung, and together with neutrophils, produce pro-inflammatory cytokines as part of an attempt to phagocytize fungal spores in the airway, as well as reactive oxygen and nitrogen species (ROS/RNS) during this process, both of which can damage lung tissue and lead to a breakdown of the alveolar epithelial barrier. In addition, the particle overload concept has historically been associated with poorly soluble particles of low cytotoxicity following chronic administration. The spores of *B. bassiana* possess hydrophobic cell walls and are obviously insoluble, but given the heterogeneous nature of fungal preparations, and the results from the acute inhalation toxicity studies, a cytotoxic effect cannot be ruled out. Testing of other fungal species (e.g., *Trichoderma fertile* strain JM41R and *Alternaria destruens*) and end-use products containing strain PPRI 5339 have previously been tested under an inhalation (aerosol) protocol up to very high concentrations (>2 mg/L) that could have led to particle overload, yet mortality was not observed in those cases. Therefore, lung overload would not entirely explain the observed mortality, especially at low concentrations. Inflammation is a common finding following single exposures of the lung to spores of various fungal species, as well as exposures to poorly soluble particles in general. In addition to this local innate immune response to foreign spores, perhaps exacerbated by particle overload, biochemical or physical irritation of the lining of the lungs by the technical grade active ingredient preparation itself cannot be ruled out. From the acute irritation studies in rabbits, the technical grade active ingredient has been shown to be an ocular irritant. Unlike direct intratracheal (IT) pulmonary exposures, inhalation of aerosol leads to much more extensive exposures, with all major sections of the respiratory tract (upper, tracheobronchial, pulmonary) exposed, rather than primarily the lower airway as in IT. Furthermore, spores under the aerosol protocol are 'naked' particles administered continuously for a 4 hour period, whereas those tested under an IT (pulmonary) toxicity/pathogenicity protocol are suspended as a clump in phosphate-buffered saline (PBS) and administered as a single, bolus dose. An irritant effect may be mitigated in the latter case by a coating of the spores to some degree. Adverse effects of Velifer are almost certainly mitigated by its formulation as well as its lower spore count. Also, no evidence of any adverse health effects have been observed by employees who have produced, processed, packaged, analyzed, handled, applied or otherwise been exposed to *B. bassiana* strain PPRI 5339 or its formulated product.

A survey of published literature performed by PMRA found that, in rare cases, *B. bassiana* has the potential to act as an opportunistic pathogen. These incidents, however, are associated with compromised immune systems or a history of surgery/injury. This survey of published literature also found evidence that some isolates of *B. bassiana* possess IgE reactive proteins and thus could potentially elicit sensitization reactions. In addition, some strains of *B. bassiana* are known

to produce the secondary metabolites beauvericin, bassianolide, oosporein, bassiacridin, bassianin, and tenellin. *Beaveria bassiana* strain PPRI 5339 has not been demonstrated to produce any of these metabolites. Furthermore, a specific analysis was performed to show that the technical grade active ingredient and its associated end-use product do not contain beauvericin.

3.1.3 Incident Reports Related to Human and Animal Health

As of 10 July 2017, no human or domestic animal incident reports involving *Beauveria bassiana* have been submitted to the PMRA.

3.1.4 Hazard Analysis

The database submitted in support of registering *Beauveria bassiana* PPRI 5339 Technical and Velifer was reviewed from the viewpoint of human health and safety and was determined to be sufficiently complete.

Based on all the available information, the technical grade active ingredient, *Beauveria bassiana* PPRI 5339 Technical, is of low toxicity by the oral and dermal routes, and was not pathogenic or infective by the intraperitoneal route. Although the technical grade active ingredient was of low toxicity and not infective or pathogenic when administered directly to the lungs by intratracheal instillation, the technical grade active ingredient is acutely toxic via the inhalation route. In irritation studies, the technical grade active ingredient was minimally irritating to the skin and mildly irritating to the eyes. Also, the MPCA is considered to be a potential sensitizer. Consequently, the hazard statements “DANGER POISON”, “CAUTION EYE IRRITANT” and “POTENTIAL SENSITIZER” will appear on the principal display panel of the technical grade active ingredient. The statements, "Fatal if inhaled. DO NOT inhale/breath dusts. May irritate eyes. May cause sensitization. Avoid contact with eyes, skin and clothing." is also required on the secondary panel of the label under the "PRECAUTIONS" section.

Similarly, the end-use product, Velifer, is of low toxicity by the oral, inhalation, and dermal routes. The end-use product is also minimally irritating to the skin and eyes. Consequently, the hazard statement "POTENTIAL SENSITIZER" will appear on the principal display panel of the end-use product label. The statement, "May cause sensitization. Avoid contact with eyes, skin and clothing. Avoid inhaling/breathing mist." is also required on the secondary panel of the label under the “PRECAUTIONS” section.

Notwithstanding the acute inhalation study results, higher tier subchronic and chronic toxicity studies were not required because the technical grade active ingredient was not acutely toxic by the oral, dermal or pulmonary (intratracheal instillation) route of administration. Furthermore, there were no indications of any infectivity or pathogenicity in any test animals tested with the MPCA at Tier I.

Within the available scientific literature, there are no reports that suggest *B. bassiana* has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *B. bassiana* stain PPRI 5339.

3.2 Occupational, Residential and Bystander Risk Assessment

3.2.1 Occupational Exposure and Risk

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with primary exposure routes being dermal and inhalation. 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 was a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Beauveria bassiana* has not been identified as a dermal wound pathogen, there is no indication that it could penetrate intact skin of healthy individuals, and does not contain any known toxic secondary metabolites. Furthermore, toxicity testing with the end-use product showed no toxicity via the oral, inhalation and dermal routes, and it was minimally irritating to the skin and eyes. Also, testing with the technical grade active ingredient showed no signs of infectivity or pathogenicity via the pulmonary or intraperitoneal injection routes.

Although dermal toxicity or toxicity from inhalation exposure is considered minimal from the proposed end-use product uses, the PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Risk mitigation measures, such as personal protective equipment, including waterproof gloves, long- sleeved shirts, long pants, a NIOSH-approved dust/mist filtering respirator/mask, and shoes plus socks are required to minimize exposure and protect commercial applicators, mixer/loaders, and handlers that are likely to be exposed. In addition, all unprotected workers and users are prohibited from entering treated areas where Velifer has been applied for 4 hours or until the sprays have dried.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of Velifer and no significant occupational risks are anticipated for this product.

3.2.2 Residential and Bystander Exposure and Risk

Overall, the PMRA does not expect that residential and bystander exposures will pose a health risk of concern on the basis of the low toxicity profile for Velifer, the low infectivity/pathogenicity profile for *Beauveria bassiana* PPRI 5339 Technical, and the assumption that precautionary label statements will be followed by commercial applicators in the use of Velifer. As well, *B. bassiana* is a species that is common in the environment and the use of Velifer is not expected to cause sustained increases in exposure to bystanders beyond natural levels. Consequently, the health risk to infants and children is expected to be low.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

While the proposed use pattern may result in dietary exposure with possible residues in or on agricultural commodities, negligible to no risk is expected for the general population, including infants and children because *B. bassiana* strain PPRI 5339 demonstrated no pathogenicity or infectivity in Tier I acute pulmonary and intraperitoneal injection studies; and no oral toxicity in the acute toxicity study. After application, *B. bassiana* strain PPRI 5339 is only expected to grow on susceptible insects. If toxic secondary metabolites are produced by the MPCA in insects, their occurrence in edible food commodities would be negligible due to common hygiene practices and standards that prevent insect parts from being contained in foodstuff. Although there are other strains of *B. bassiana* that are known to produce the secondary metabolites beauvericin, bassianolide, oosporein, bassiacridin, bassianin and tenellin, strain PPRI 5339 is not known to produce any of these metabolites. Higher tier subchronic and chronic dietary exposure studies were not required because of the low toxicity of the MPCA in the acute oral toxicity study and the absence of infectivity, toxicity or pathogenicity in toxicity/infectivity studies. Therefore, there are no concerns for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.3.2 Drinking Water

Health risks are not expected from exposure to *B. bassiana* strain PPRI 5339 via drinking water because exposure will be low from operational applications and because there were no harmful effects observed in Tier I acute oral toxicity testing and infectivity testing. The end-use product label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Users are also to prevent effluent or runoff from greenhouses containing this product to enter lakes, streams, ponds or other waters. Furthermore, municipal treatment of drinking water is expected to reduce the transfer of residues to drinking water.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARfDs) and acceptable daily intakes (ADIs) are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (in other words, no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that *B. bassiana* strain PPRI 5339 is of low oral toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCAs than the general population. Thus, there are no threshold effects of concern and as a result, there is no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects

of the MPCAs (including neurological effects from pre- or post-natal exposures), and cumulative effects on infants and children of the MPCAs and other registered microorganisms that have a common mechanism of toxicity, does not apply to these MPCAs. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *B. bassiana* strain PPRI 5339 to human health.

3.3.4 Aggregate Exposure and Risk

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA's files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *B. bassiana* strain PPRI 5339 to the general Canadian population, including infants and children, when the microbial pest control product is used as labeled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Dermal and inhalation exposure to the general public will be very low since the product is to be used in greenhouses and is not allowed for use on turf, residential or recreational areas. Furthermore, few adverse effects from exposure to *B. bassiana* encountered in the environment have been reported. Even if there is an increase in exposure to this microorganism from the use of Velifer, there should not be any increase in potential human health risk.

3.3.5 Maximum Residue Limits

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally specified as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada specifies science-based MRLs to ensure the food Canadians eat is safe.

Beauveria bassiana is encountered in nature as it is a ubiquitous soil microorganism. Residues of *B. bassiana* strain PPRI 5339 on treated food crops, at the time of harvest, are also anticipated. Consequently, the PMRA has applied a hazard-based approach for determining whether an MRL is required for this microorganism. *Beauveria bassiana* strain PPRI 5339 is only expected to grow on host insects and if secondary metabolites were to be produced in vivo, their occurrence in edible food commodities would be negligible due to common hygiene practices and standards that prevent insect parts from being contained in foodstuff. Based on the lack of toxicity and pathogenicity effects observed in the acute toxicity and infectivity studies and the fact that *B. bassiana* strain PPRI 5339 has not been demonstrated to produce toxic secondary metabolites, the risks anticipated for dietary exposure are considered low. The secondary metabolite beauvericin was not detected in the technical grade active ingredient. In addition, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Therefore, the PMRA has determined that an MRL does not need to be specified for *B. bassiana* strain PPRI 5339.

3.4 Cumulative Effects

The PMRA has considered available information on the cumulative effects of residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity.

Besides naturally occurring strains of *B. bassiana* in the environment, the PMRA is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity with *B. bassiana* strain PPRI 5339. No cumulative effects are anticipated if the residues of *B. bassiana* strain PPRI 5339 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

No studies were submitted to address the environmental fate and behaviour of *B. bassiana* strain PPRI 5339. Environmental fate data (Tier II/III) are not normally required at Tier I, and are only triggered if significant toxicological effects in non-target organisms are noted in Tier I testing.

The proposed use of Velifer is limited to greenhouse use sites. The intended applications are foliar. Although the greenhouse use site precludes any direct exposure to outdoor environments, outside soils may be exposed to *B. bassiana* strain PPRI 5339 through human activity such as composting of plant waste and water management practices. Afterwards, the dispersal of *B. bassiana* strain PPRI 5339 should be limited to the movement of treated plant materials, natural vectors (for example, insects), and to a limited extent runoff. Based on these considerations, the amount of *B. bassiana* strain PPRI 5339 transferring to outdoor environments from the use of Velifer in greenhouses is expected to be minimal.

In the event that *B. bassiana* strain PPRI 5339 does reach outdoor soil environments, the organism is expected to behave as it would in nature. As a ubiquitous soil microorganism, it is likely that *B. bassiana* would settle in the soil where it is commonly found, rather than percolate through soil. Therefore, mobility through the soil is expected to be minimal. Evidence suggests that *B. bassiana* strain PPRI 5339 is able to survive in soil, if certain environmental conditions (for example, moisture, pH) are met, but that over time populations would be expected to return to naturally occurring levels.

Overall, the proposed greenhouse use of Velifer is not expected to significantly increase natural populations of *B. bassiana* in outdoor terrestrial or aquatic environments.

4.2 Effects on Non-Target Species

PMRA has a four-tiered approach to environmental testing of microbial pesticides. Tier I studies consist of acute studies on up to seven broad taxonomic groups of non-target organisms exposed to a maximum hazard or Maximum Challenge Concentration (MCC) of the MPCA. The MCC is generally derived from the amount of the MPCA, or its toxin, expected to be available following application at the maximum recommended label rate multiplied by a safety factor. Tier II studies

consist of environmental fate (persistence and dispersal) studies as well as additional acute toxicity testing of MPCAs. Tier III studies consist of chronic toxicity studies (such as, life cycle studies) as well as definitive toxicity testing (for example, LC_{50}, LD_{50}). Tier IV studies consist of experimental field studies on toxicity and fate, and are required to determine whether adverse effects are realized under actual use conditions.

The type of environmental risk assessment conducted on MPCAs varies depending on the tier level that was triggered during testing. For many MPCAs, Tier I studies are sufficient to conduct environmental risk assessments. Tier I studies are designed to represent "worst-case" scenarios where the exposure conditions greatly exceed the expected environmental concentrations. The absence of adverse effects in Tier I studies are interpreted as minimal risk to the group of non-target organisms. However, higher tiered studies will be triggered if significant adverse effects on non-target organisms are identified in Tier I studies. These studies provide additional information that allows PMRA to refine the environmental risk assessments. In the absence of adequate environmental fate and/or field studies, a screening level risk assessment can be performed to determine if the MPCA is likely to pose a risk to a group of non-target organisms.

The screening level risk assessment uses simple methods, conservative exposure scenarios (for example, direct application at a maximum application rate) and sensitive toxicity endpoints. A risk quotient (RQ) is calculated by dividing the exposure estimate by an appropriate toxicity value (RQ = exposure/toxicity), and the risk quotient is then compared to the level of concern (LOC).

If the screening level risk quotient is below the level of concern, the risk is considered negligible and no further risk characterization is necessary. If the screening level risk quotient is equal to or greater than the level of concern, then a refined risk assessment is performed to further characterize the risk. A refined assessment takes into consideration more realistic exposure scenarios (environmental fate and/or field testing results). Refinements to the risk assessment may continue until the risk is adequately characterized or no further refinements are possible.

4.2.1 Effects on Terrestrial Organisms

Several studies were submitted to address the hazards of *B. bassiana* strain PPRI 5339 to terrestrial non-target arthropods. Scientific rationales were also submitted in support of requests to waive testing on avian species and terrestrial plants. Data submitted under human and animal health toxicity testing were considered to assess the risk of harm to wild mammals.

Increased exposure of *B. bassiana* strain PPRI 5339 to terrestrial non-target species including birds, wild mammals, arthropods and plants is expected to be minimal due to the proposed greenhouse uses and the naturally occurring populations of *B. bassiana* in the environment.

In the event of either oral or inhalation exposure, no infectivity is expected in birds or mammals as *B. bassiana* strain PPRI 5339 does not proliferate at body temperatures. The body temperatures of wild mammals is approximately 37°C and the body temperature of various bird species range from 37.5°C–44.6°C while *B. bassiana* strain PPRI 5339 does not grow at temperatures above 36°C. While some strains of *B. bassiana* are known to produce the

secondary metabolites beauvericin, bassianolide, oosporein, bassiacridin, bassianin and tenellin, *B. bassiana* strain PPRI 5339 has not been demonstrated to produce any of these metabolites. Furthermore, a specific analysis was performed to show that the technical grade active ingredient and its associated end-use product do not contain beauvericin.

In laboratory studies conducted to satisfy the human health and safety requirements, it was determined that *B. bassiana* strain PPRI 5339 was not toxic in rats via the oral, pulmonary (intratracheal), and dermal routes of exposure and not pathogenic in rats following pulmonary exposure or intraperitoneal injection. A published study indicating lack of pathogenicity was also submitted in which viable spores were not recovered from the injection sites of mice following intramuscular injection (up to 2×10^8 spores/animal) of another isolate of *B. bassiana* beyond 3 days post-injection.

To characterize the risk to non-target terrestrial arthropods, six terrestrial arthropod studies were submitted for review. Test species included *Aphidius rhopalosiphi*, *Orius laevigatus*, *Bombus terrestris* and *Apis mellifera*.

In a 14-day contact toxicity and pathogenicity study, 40 adult *A. rhopalosiphi* (parasitic wasps) were exposed to Broadband, another oil-based formulation containing *B. bassiana* strain PPRI 5339, at a concentration of 3.7×10^8 CFU/mL (equivalent to 51× the maximum field concentration for Velifer). The test material was sprayed on to test arenas and allowed to dry before the wasps were added. After 48 hours of exposure, 15 females from each of the treatment groups were removed and confined individually over pots of aphid-infested (*Rhopalosiphum padi*) cereal plants. After 24 hours, the wasps were removed and confined individually in petri dishes. The wasps were observed for a total of 14 days. The aphids were allowed to develop on the plants for a further 10–12 days after which the number of parasitized aphids that developed through to pupae was assessed. There were no significant effects noted in mortality or fecundity and no signs of toxicity or pathogenicity were noted. Infectivity was not assessed. The 10-day LC_{50} was found to be greater than 3.7×10^8 CFU/mL and the LT_{50} was determined to be greater than 10 days. However, this study was of limited utility in the risk assessment as exposure was incidental and was not reflective of the proposed use of Velifer as a foliar spray.

In a 10-day contact toxicity and pathogenicity study, 80 second instar *O. laevigatus* nymphs were exposed to Broadband at a concentration of 4.1×10^8 CFU/mL (equivalent to 57× the maximum field concentration for Velifer). The test material was sprayed on to test arenas and allowed to dry before addition of the *O. laevigatus*. Mortality was assessed following an exposure period of 10 days. After a further 4 days, individual surviving females were transferred to leaf discs of cow pea (*Vigna sinensis*) to assess fecundity. The leaf discs were replaced after two and four days. Following this period, the number of eggs on the leaf discs was counted and hatch success was assessed. There were no significant effects on mortality, fecundity or egg hatching and no signs of toxicity or pathogenicity were noted. Infectivity was not assessed. The 10-day LC_{50} was greater than 4.1×10^8 CFU/mL and the LT_{50} was determined to be greater than 10 days. However, this study was of limited utility in the risk assessment as the exposure was incidental and was not reflective of the proposed use of Velifer as a foliar spray.

In a 10-day contact pathogenicity/infectivity study, 30 bumblebees (*B. terrestris*) were exposed to *B. bassiana* strain PPRI 5339. A 100 µL droplet of a *B. bassiana* strain PPRI 5339 suspension (3.1×10^6 CFU/mL corresponding to a dose of 3.1×10^5 CFU/bee) was dosed onto the dorsal surface of the thorax of each bee. Bees that died during the study were removed from the test chambers and incubated to allow for the growth of any infective organisms which, if present, were plated to confirm their identity. At the end of the study, three bees from each test item chamber were subjected to necropsy consisting of an external examination for signs of infection and internal examinations of the head and abdomen. No signs of toxicity or infectivity were observed. This study was of limited utility in the risk assessment. The large volume of 100 µL administered to each bee, was not appropriate as it was likely that at least some of the dosing suspension ran off the bees. The study cited OECD Test Guideline 214 as justification for the increase in dose volume. The OECD 214 guideline, however, specifies a dose in a volume of 1 µL/bee at a suitable concentration (i.e., a geometric series covering the range for LD_{50}) or, for a limit test, a dose of 100 µg a.i./bee (in this case, equivalent to 10^7 CFU/bee). Neither of these requirements was met. Therefore, the actual amount of active ingredient administered to each bee is not known and the LD_{50} and LT_{50} could not be determined. Furthermore, the addition of Tween 80 to the dosing suspension decreased the adherence of the spores to the cuticle of bees and thus reduced the potential for germination and infectivity.

In a 10-day contact pathogenicity/infectivity study, 30 honeybees (*A. mellifera*) were exposed to *B. bassiana* strain PPRI 5339. A 100 µL droplet of *B. bassiana* strain PPRI 5339 suspension (2.9×10^6 CFU/mL corresponding to a dose of 2.9×10^5 CFU/bee) was dosed onto the dorsal surface of the thorax of each bee. Bees that died during the study were removed from the test chambers and incubated to allow for the growth of any infective organisms which, if present, were plated to confirm their identity. At the end of the study, three bees from each test item chamber were subjected to necropsy consisting of an external examination for signs of infection and internal examinations of the head and abdomen. No signs of toxicity, pathogenicity or infectivity were observed. This study was of limited utility in the risk assessment for the same reasons outlined in the above bumblebee contact study.

In a 10-day acute oral toxicity/pathogenicity study, 30 bumblebees *(B. terrestris*) were communally fed a suspension of *B. bassiana* strain PPRI 5339 (2.9×10^8 CFU/mL; equivalent to 40× maximum field application rate) over a period of 4–6 hours. Bees that died during the study were removed from the test chambers and incubated to allow for the growth of any infective organisms which, if present, were plated to confirm their identity. At the end of the 10-day observation period, *B. bassiana* strain PPRI 5339 exhibited no signs of toxicity, pathogenicity or infectivity to bumblebees. The 10-day LC_{50} was greater than 2.9×10^8 CFU/mL and the LT_{50} was greater than 10 days. This study was of limited utility in the risk assessment as it was not possible to determine the precise dose administered to each bee. Furthermore, topical exposure is a more relevant route of exposure for *B. bassiana* as its mode of action is via germination upon contact with the body of an insect host.

In a 10-day acute oral toxicity/pathogenicity study, 30 honeybees *(A. mellifera*) were communally fed a suspension of *B. bassiana* strain PPRI 5339 (2.9×10^8 CFU/mL; 40× maximum field application rate) over a period of 4–6 hours. Bees that died during the study were removed from the test chambers and incubated to allow for the growth of any infective

organisms which, if present, were plated to confirm their identity. *Beauveria bassiana* strain PPRI 5339 exhibited no signs of toxicity but did show signs of pathogenicity and infectivity in some bees. However, no statistically significant difference in mortality was observed. The 10-day LC_{50} was greater than 2.9×10^8 CFU/mL and the LT_{50} was greater than 10 days. This study was of limited utility in the risk assessment for the same reasons outlined in the above bumblebee acute oral toxicity/pathogenicity study.

Although the non-target terrestrial arthropods were found to be of limited utility in the risk assessment, no additional data are required to support the proposed use of Velifer in greenhouses. As a broad-spectrum mycoinsecticide, *B. bassiana* strain PPRI 5339 is assumed to be potentially harmful to non-target arthropods and greenhouse pollinators. Bumblebees, the pollinators most often used in greenhouses, are purchased from various commercial sources and possess a limited lifetime of 10–12 weeks. The queens and/or hives from these greenhouses are never returned to their commercial sources for fear of pesticide exposure and/or parasites. Furthermore, these colonies contain far more bees than necessary for pollinating plants in greenhouses and can therefore suffer mortalities without any ill effects to their role in pollination. The potential effects to non-target pollinators and beneficial insects should be limited to the treated areas and to the immediate surroundings around treated plants. However, due to the potential for adverse effects on beneficial arthropods and pollinators in greenhouses, precautionary measures are required on the Velifer label to alert operators of the potential hazard to beneficial insects that may be used in greenhouse Integrated Pest Management programs. Users are also advised to avoid direct contact to beneficial insects and to not make applications while bees are actively foraging (if employed in the greenhouse).

In addition to the general scientific rationale to waive actual testing on non-target terrestrial organisms, *B. bassiana* is not taxonomically related to known plant pathogens and it is not listed as a plant pathogen by the International Society of Plant Pathology. Crop safety was also demonstrated in 31 greenhouse trials on various flower and vegetable crops. Therefore, terrestrial plants are not expected to be adversely affected by exposure to *B. bassiana* strain PPRI 5339.

Based on all the available data and information on the effects of *B. bassiana* strain PPRI 5339 to non-target terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, arthropods, non-arthropod invertebrates, microorganisms and plants from the proposed use of Velifer on greenhouse crops.

4.2.2 Effects on Aquatic Organisms

A scientific rationale was submitted in lieu of testing on freshwater fish, estuarine and marine fish, aquatic arthropods and aquatic plants. Increased exposure of *B. bassiana* strain PPRI 5339 to aquatic organisms is expected to be minimal due to the proposed use of Velifer in greenhouses and application instructions that minimize the potential for the MPCA to reach surface water. In the case of aquatic plants, *B. bassiana* is not taxonomically related to known plant pathogens and it is not listed as a plant pathogen by the International Society of Plant Pathology.

Based on the available information on the ubiquity of the MPCA and the proposed use description for Velifer, there is reasonable certainty that no harm will be caused to fish, aquatic arthropods, non-arthropod invertebrates and aquatic plants from the use of Velifer in greenhouses. As a precaution standard label statements will prohibit handlers from contaminating aquatic habitats by cleaning of equipment, disposal of wastes or effluent from greenhouses.

4.3 Incident Reports related to the Environment

As of 10 July 2017, no environmental incident reports involving *Beauveria bassiana* have been submitted to the PMRA.

5.0 Value

Velifer is a new pest management tool for suppression of aphids, whiteflies, thrips and twospotted spider mites on greenhouse ornamentals and greenhouse vegetables. Several alternative active ingredients are registered for use against these pests in greenhouses, including conventional and non-conventional products. However, Velifer is the first product containing *B. bassiana* for use against twospotted spider mite, a major greenhouse pest. Additionally, numerous pest generations can occur in greenhouses and pesticide resistance can become a problem, but development of resistance to *B. bassiana* strain PPRI 5339, is unlikely because of its complex mode of action.

Product performance and crop safety were demonstrated in 31 greenhouse trials. These trials were conducted in the United States and Europe on various flower and vegetable crops under conditions similar to those in Canadian greenhouses. The data demonstrated suppression of aphids, twospotted spider mites, thrips and whiteflies in greenhouse ornamentals and vegetables at a concentration of 450-900 mL product/1000 L of water with multiple applications. It was also shown that humidity and temperature conditions within the greenhouse must be favourable for development of *B. bassiana* for consistent product performance.

Phytotoxicity was rated in 11 of the submitted efficacy trials and was not observed in any treatments. Host crops evaluated were cucumber, tomato, bell pepper, Gerbera daisy and a variety of Lantana. Ornamentals and vegetables constitute a wide assortment of plant species, each with many cultivars. Therefore, to mitigate the potential risk for phytotoxicity, label statements direct growers to assess the suitability of each cultivar by testing a small portion of the crop under local growing conditions.

The value information supported the use of Velifer for the suppression of aphids, whiteflies, thrips and twospotted spider mites on greenhouse ornamentals and greenhouse vegetables.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The Toxic Substances Management Policy (TSMP) is a federal government policy developed to provide direction on the management of substances of concern that are released into the environment. The TSMP calls for the virtual elimination of Track 1 substances [those that meet all four criteria outlined in the policy, i.e. persistent (in air, soil, water and/or sediment), bio-accumulative, primarily a result of human activity and toxic as defined by the *Canadian Environmental Protection Act*].

Beauveria bassiana PPRI 5339 Technical and Velifer were assessed in accordance with the PMRA Regulatory Directive DIR99-03[5] and evaluated against the Track I criteria. The PMRA has reached the following conclusions:

- *Beauveria bassiana* PPRI 5339 Technical does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.
- There are also no formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track-1 criteria.

6.2 Formulants and Contaminants of Health or Environmental Concern

During the review process, contaminants in the technical and formulants and contaminants in the end-use products are compared against the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* maintained in the *Canada Gazette*[6]. The list is used as described in the PMRA Notice of Intent NOI2005-01[7] and is based on existing policies and regulations including: DIR99-03; and DIR2006-02[8] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

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5 *Regulatory Directive DIR99-03, The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*

6 Canada Gazette, *Part II, Volume 139, Number 24, SI/2005-11-30) pages 2641-2643:* List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern *and in the order amending this list in the* Canada Gazette, *Part II, Volume 142, Number 13, SI/2008-67 (2008-06-25) pages 1611-1613:* Part I Formulants of Health or Environmental Concern, Part 2 Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions and Part 3 Contaminants of Health or Environmental Concern.

7 *Notice of Intent NOI2005-01,* List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern *under the New* Pest Control Products Act

8 *Regulatory Directive DIR2006-02,* Formulants Policy and Implementation Guidance Document.

- The technical grade of the active ingredient, *Beauveria bassiana* PPRI 5339 Technical does not contain any formulants of health or environmental concern identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641–2643:*List of Pest Control Product Formulants of Health or Environmental Concern*.
- The end-use product, Velifer, does not contain formulants of health or environmental concern as identified in the Canada Gazette, Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants of Health or Environmental Concern*.

The use of formulants in registered pest control products is assessed on an ongoing basis through PMRA formulant initiatives and DIR2006-02.

7.0 Summary

7.1 Methods for Analysis of the Microorganism as Manufactured

The product characterization data for *Beauveria bassiana* PPRI 5339 Technical and Velifer were judged to be adequate to assess their potential human health and environmental risks. The technical grade active ingredient was characterized and the specifications of the end-use product were supported by the analyses of a sufficient number of batches. All batches of *Beauveria bassiana* PPRI 5339 Technical must conform to the limits set out in the OECD issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43]. Storage stability data support storage at 4°C for up to 12 months for *Beauveria bassiana* PPRI 5339 Technical and for Velifer.

7.2 Human Health and Safety

The acute toxicity and infectivity studies and other relevant information submitted in support of *B. bassiana* strain PPRI 5339 were determined to be sufficiently complete to permit a decision on the registration of the technical grade active ingredient, *Beauveria bassiana* PPRI 5339 Technical and the end-use product, Velifer. Based on all the available information, the technical grade active ingredient is of low toxicity by the oral, pulmonary, and dermal routes, and was not pathogenic or infective by the pulmonary or intraperitoneal routes. The technical grade active ingredient, however, is acutely toxic via the inhalation route. In irritation studies, the technical grade active ingredient was minimally irritating to the skin and mildly irritating to the eyes. Also, the MPCA is considered to be a potential sensitizer. The end-use product was of low toxicity by the oral, inhalation, and dermal routes. The end-use product was also minimally irritating to the skin and eyes. Consequently, the signal words, “POTENTIAL SENSITIZER” are required on the principal display panel of the technical grade active ingredient and the end-use product; and the precautionary statements: “May cause sensitization.”, “Avoid contact with skin and clothing.”, “Avoid inhaling/breathing mists.” The technical grade active ingredient must also include the signal words, “DANGER POISON” and “CAUTION- EYE IRRITANT” on the principal display panel; and the precautionary statements: “Fatal if inhaled.”, “DO NOT inhale/breath dusts.” and “May irritate eyes.”

When handled according to prescribed label instructions, the potential for dermal, eye and inhalation exposure for mixer/loaders, applicators, and handlers exists, with the primary source of exposure to workers being dermal and to a lesser extent inhalation. Respiratory and dermal sensitivity could possibly develop upon repeated exposure to the product since all microorganisms, including this MPCA, contain substances that are potential sensitizers. Therefore, users handling or applying Velifer must wear waterproof gloves, long-sleeved shirts, long pants, a dust/mist filtering respirator/mask, and shoes plus socks. In addition, all unprotected workers and users are prohibited from entering treated areas where Velifer has been applied for 4 hours or until the sprays have dried.

The health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is low and not of concern due to the low pathogenicity profile for *B. bassiana* strain PPRI 5339 and the low toxicity profile for Velifer. The specification of an MRL under the PCPA is not required for *B. bassiana* strain PPRI 5339.

7.3 Environmental Risk

The non-target organism tests, scientific rationales and supporting published scientific literature submitted in support of *Beauveria bassiana* PPRI 5339 Technical and Velifer were determined to be sufficiently complete to permit a decision on the environmental fate and effects of these products. The use of *Beauveria bassiana* PPRI 5339 Technical and Velifer containing *B. bassiana* strain PPRI 5339 is not expected to pose a risk to non-target organisms when the directions for use on the label are followed.

As a general precaution, the label will prohibit the direct application of Velifer to aquatic habitats, and direct handlers to not contaminate surface water by disposal of equipment wash waters or greenhouse effluent. The label also advises users that Velifer may be harmful to pollinators (including bees) and some beneficial insects that may be used in greenhouse integrated pest management programs. A statement will instruct users to avoid direct contact to beneficial insects and prevent applications while bees are actively foraging (if employed in the greenhouse).

No other environmental fate studies or non-target organism studies are required for the proposed use pattern in greenhouses.

7.4 Value

Velifer suppresses aphids, twospotted spider mites, thrips and whiteflies in greenhouse ornamentals and vegetables at a concentration of 450-900 mL product/1000 L of water with multiple applications at 3-10 day intervals. The higher concentration and shorter application intervals are to be used when pest populations are high. Velifer has value as a new pest management tool in greenhouse production of ornamentals and vegetables. It is the first *B. bassiana* product for management of the twospotted spider mite which is a major pest on greenhouse crops, as well as a new *B. bassiana* strain to use against aphids, thrips and whiteflies.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Beauveria bassiana* PPRI 5339 Technical and Velifer, containing the technical grade active ingredient *Beauveria bassiana* strain PPRI 5339, to suppress aphids, whiteflies, thrips and twospotted spider mites on greenhouse ornamentals and greenhouse vegetables.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

°C	degree(s) Celsius
µg	micrograms
µL	microliter
a.i.	active ingredient
ADI	acceptable daily intake
ARfD	acute reference dose
bw	body weight
CFU	colony forming unit
cm	centimetres
DNA	deoxyribonucleic acid
EP	end-use product
g	gram
HDPE	high density polyethylene
IT	intratracheal
ITS	internal transcribed spacer
kg	kilogram
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LOC	level of concern
LT_{50}	median lethal time
IgE	immunoglobulin E antibodies
MCC	maximum challenge concentration
mg	milligram
mL	millilitre
MAS	maximum average score
MIS	maximum irritation score
MPCA	microbial pest control agent
mPas	milliPascal
MRL	maximum residue limit
NIOSH	National Institute for Occupational Safety and Health
NRRL	Agricultural Research Culture Collection
OECD	Organisation for Economic Co-operation and Development
PBS	phosphate-buffered saline
PDA	potato dextrose agar
PMRA	Pest Management Regulatory Agency
ppm	parts per million
ROS/RNS	reactive oxygen and nitrogen species
RQ	risk quotient
TGAI	technical grade of the active ingredient
TSMP	Toxic Substances Management Policy

Appendix I Tables and Figures

Table 1 Toxicity Profile of *Beauveria bassiana* PPRI 5339 Technical (TGAI)

Study Type/Animal/PMRA#	Study Results
21-day acute pulmonary infectivity and toxicity Sprague Dawley rat PMRA No. 2431227	One male rat that was treated with inactivated test material was found dead on Day 19. All animals appeared normal for the duration of the study. The gross necropsy conducted at study termination revealed no observable effects, with the exception of one male rat that was found dead on Day 19. Necropsy findings for this animal reported black internal organs. The test substance was considered completely clear in the lungs from test animals by Day 14. The technical grade active ingredient was of low toxicity and not infective when instilled at 7.7×10^7 CFU/rat.
21-day acute intraperitoneal injection Infectivity Sprague Dawley rat PMRA No. 2431229	There was no mortality in any group during the study. During observations for clinical signs, all animals appeared normal for the duration of the study. The gross necropsy conducted at termination of the study also revealed no observable abnormalities. The technical grade active ingredient was not pathogenic when injected at 7.8×10^7 CFU/rat.
14-day acute dermal toxicity Sprague Dawley rat PMRA No. 2431230	There were no mortalities or any clinical signs of toxicity noted throughout the study period. Animals exhibited weekly weight gain during the study, with the exception of one female that lost 4g between Days 0 and 7. At necropsy, there were no observable abnormalities noted. The acute dermal LD_{50} was greater than 5050 mg/kg bw in male and female rats.
14-day acute inhalation toxicity Sprague Dawley rat	At 5.28 mg/L, two males and one female died on Day 2, and one male and two females died on Day 3. At 2.59 mg/L, two males and two females died on Day 3, and two males and one female died on Day 4.

Study Type/Animal/PMRA#	Study Results
PMRA No. 2431228	Approximately 4.5 to 6.0 hours after treatment, all treated animals displayed moderate activity decrease and moderate piloerection. The observed effects on activity and piloerection cleared by Day 4. Respiratory gurgle was also observed in animals that later died. Most surviving animals exhibited weekly weight gain during the study except in one male and one female that lost weight between Days 0 and 7. At necropsy, crusted muzzle, stained/matted genital fur, discoloured lungs/liver/kidneys, and empty gastrointestinal tract was noted in animals that died during the study. In surviving animals, there were no observable abnormalities noted in male and female rats treated at 5.28 mg/L; however, mottled lungs were noted in male and female rats treated at 2.59 mg/L. The acute inhalation LC_{50} was less than 2.59 mg/L in male and female animals.
2-day acute inhalation toxicity Sprague Dawley rat PMRA No. 2681070	Both male animals died on Day 2. Clinical signs included activity decrease, body tremors, piloerection and respiratory gurgle. Abnormal necropsy findings included red crust at the mouth, and mottled lungs and liver. Histopathologic examination of tissues indicated that lesions consisted of necrosis of the lining epithelium of secondary bronchi, terminal bronchioles and alveolar ducts. There was acute inflammation in the walls of these airways and adjacent alveoli. The acute inhalation LC_{50} was less than 5.39 mg/L in male animals.
14-day acute inhalation toxicity Sprague Dawley rat PMRA No. 2681071	Four males and all females died during the study. Clinical signs included piloerection and rapid breathing. The only animal surviving to termination exhibited weight loss throughout the study. Gross necropsy revealed discoloured lungs in all animals. The acute inhalation LC_{50} was less than 1.26 mg/L in male and female animals.

Study Type/Animal/PMRA#	Study Results
14-day acute inhalation toxicity Sprague Dawley rat PMRA No. 2681073	At 0.52 mg/L, all males were found dead on Days 2 to 9, and all females died on Days 3 to 4. At 0.05 mg/L, two males died on Day 3 and on Day 4. For females, one animal was found dead on Days 3, 4 and 4. Prominent in-life observations included piloerection, rapid breathing, lethargy and hypothermia. Piloerection and rapid breathing persisted through Day 14 in survivors. All animals surviving to termination exhibited weight loss by Day 1, weight loss in 2 of 3 animals by Day 3, weight gain in 2 of 3 animals by Day 7, and weight gain in all animals by Day 14. At necropsy, crusted facial areas; nasal discharge; small heart; discoloured lungs and portions of gastrointestinal tract; discoloured/enlarged liver; small or enlarged spleen; and empty/gas in intestines was noted in animals that died during the study. In surviving animals at 0.05 mg/L, abnormal necropsy findings included mottled lungs. The acute inhalation LC_{50} was less than 0.05 mg/L in male and female animals.
14-day acute oral toxicity Sprague Dawley rat PMRA No. 2431225	There were no mortalities and no effects noted on weight gain during the study period. The only clinical sign was a slight activity decrease in one animal on the day of dosing. The gross necropsy conducted at study termination revealed no observable abnormalities. The acute oral LD_{50} was greater than 5000 mg/kg bw in female animals.
72-hour dermal irritation New Zealand white PMRA No. 2431231	Very slight erythema was present at each observation through 24 hours. Edema was not observed at any time throughout the study. The calculated Maximum Irritation Score (MIS) was 0.67/8 at 1 hour, and the Maximum Average Score (MAS) was 0.11/8 at 24, 48 and 72 hours. The technical grade active ingredient was minimally irritating to skin.

Study Type/Animal/PMRA#	Study Results
7-day eye irritation New Zealand white PMRA No. 2431232	Conjunctival redness (grades 1–3), chemosis (grades 1–3) and discharge (grades 1–3) was noted in all three animals. Corneal opacity (grade 1) and positive fluorescein staining was also observed in all three animals. All irritation cleared by Day 7. The calculated Maximum Average Score (MAS) and Maximum Irritation Score (MIS) were 18.7/110 at 24, 48 and 72 hours, and 20/110 at 48 hours and at 72 hours. The technical grade active ingredient was mildly irritating to the eyes.

Table 2 Toxicity Profile of Velifer (EP)

Study Type/Animal/PMRA#	Study Results
14-day acute oral toxicity Sprague Dawley rat PMRA No. 2681113	There were no mortalities. There were no clinical signs and no effects noted on weight gain during the study period. The gross necropsy conducted at study termination revealed no observable abnormalities. The acute oral LD_{50} was greater than 5000 mg/kg bw in female animals.
14-day acute inhalation toxicity Sprague Dawley rat PMRA No. 2681114	There was no mortality during the study. All animals appeared normal for duration of the study. Animals exhibited weight gain during the study, except from Day 0 to Day 1, during which time all animals either lost weight or failed to gain weight. Additionally, one female lost weight from Day 3 to Day 7. Gross necropsy revealed no observable abnormalities. The acute inhalation LC_{50} was greater than 3.09 mg/L in male and female animals.

Study Type/Animal/PMRA#	Study Results
14-day acute dermal toxicity Sprague Dawley rat PMRA No. 2681116	There were no mortalities or any clinical signs of toxicity noted throughout the study period. Two male animals had very slight erythema (grade 1) on Day 1. Animals exhibited weekly weight gain during the study. At necropsy, there were no observable abnormalities noted. The acute dermal LD_{50} was greater than 5000 mg/kg bw in male and female rats.
72-hour dermal irritation New Zealand white PMRA No. 2681117	No dermal irritation was observed throughout the study. The calculated Maximum Irritation Score (MIS) and Maximum Average Score (MAS) was 0/8. The technical grade active ingredient was not irritating to skin.
7-day eye irritation New Zealand white PMRA No. 2681120	Conjunctival redness (grades 1–2), chemosis (grade 1) was noted in all three animals. No corneal opacity, positive fluorescein staining or iritic irritation was observed in any of the treated eyes. All irritation cleared by Day 7. The calculated Maximum Average Score (MAS) and Maximum Irritation Score (MIS) were 3.1/110 at 24, 48 and 72 hours, and 5.3/110 at 1 hour and at 24 hours. The technical grade active ingredient was minimally irritating to the eyes.

Table 3 Toxicity of *Beauveria bassiana* PPRI 5339 to Non-Target Species

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Organisms				
Vertebrates				
Birds	A scientific rationale was submitted to waive avian oral toxicity and pathogenicity testing and avian inhalation testing. The rationale was based on the lack of increased exposure to birds due to the proposed greenhouses uses, the already naturally			PMRA 847915 2499034 2499046

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
	occurring populations of *B. bassiana*, the spray nature of the application, the level of active ingredient in Velifer and that *B. bassiana* strain PPRI 5339 does not proliferate at avian body temperatures. A published avian oral infectivity and toxicity study was also reviewed. No further information is required to assess the risk of harm to birds.			2499051 2681075
Wild Mammals	A scientific rationale was submitted to waive testing on wild mammals. The rationale was based on the lack of increased exposure to wild mammals due to the proposed greenhouse uses, the already naturally occurring levels of *B. bassiana* and the level of active ingredient in Velifer. Furthermore, *B. bassiana* strain PPRI 5339 was not toxic in an acute oral study in rats and not pathogenic following exposure by intraperitoneal injection in rats. A published study was also cited in which *B. bassiana* was not isolated three days post-injection after intramuscular injections.			PMRA 2431225 2431229 2499046 2499047 2681075
Invertebrates				
Arthropods				
Terrestrial Arthropods	Contact – *Aphidius rhopalosiphi*, adult	Wasps (40) were exposed for 48 hours to a test arena previously sprayed with a solution of Broadband containing 3.7×10^8 CFU/mL of *B. bassiana* strain PPRI 5339. Three other groups of wasps (40/group) were administered water, inactivated Broadband and Perfekthion (positive control). After the exposure period, 15 females from each of the treatment groups were removed and confined individually over pots of aphid-infested cereal plants for 24 hours. The aphids developed on the plants for 10–12 days after which the number of parasitized aphids that developed to	Cumulative mortality on Day 10 did not differ significantly (p=0.903) between the water control (26/40) and Broadband-treated (17/40) groups. No sublethal effects observed. Mean number of parasitized aphids per wasp in the water control and Broadband-treated groups was 18.2 ± 1.4 and 16.1 ± 1.1, respectively and was not considered significant (p=0.266).	PMRA 2499059

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
		pupae was assessed.	10-day LC_{50} > 3.7 × 10^8 CFU/mL LT_{50} > 10 days **NOT TOXIC**	
Terrestrial Arthropods	Contact – *Orius laevigatus*, 2nd instar nymphs	*Orius laevigatus* (80) were exposed for 10 days to a test arena previously sprayed with a solution of Broadband containing 4.1 × 10^8 CFU/mL of *B. bassiana* strain PPRI 5339. Three other groups of wasps (80/group) were administered water, inactivated Broadband and Perfekthion (positive control). Four days after the end of the exposure period, surviving females were transferred to leaf discs. Numbers of eggs on the leaf discs were counted and hatch success was assessed.	Cumulative mortality on Day 10 in the water, inactivated Broadband and Broadband-treatment groups was 4/80, 4/80 and 5/80, respectively. No sublethal effects observed. Total number of eggs produced by the water, inactivated Broadband and Broadband-treated groups was 222, 206 and 218, respectively. Percent egg hatch in the water group was 81% and 86% in each of the inactivated Broadband and Broadband-treatment groups 10-day LC_{50} > 4.1 × 10^8 CFU/mL LT_{50} > 10 days **NOT TOXIC**	PMRA 24990606

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Arthropods	Contact – *Apis mellifera* (honeybee), young worker bees	A 100 µL droplet of a *B. basianna* strain PPRI 5339 suspension containing 2.9 × 10^6 CFU/mL was administered to the dorsal surface of the thorax of each honeybee (30). Four other groups of honeybees (30/group) were administered water, inactivated *B. bassiana* strain PPRI 5339, adjuvant (Tween 80) and dimethoate (positive control). Bees were monitored for 10 days. Bees that died during the study were removed from the test chamber and incubated. Any resulting infective organisms were plated to confirm their identity. Autopsies were conducted on 3 bees from each test chamber.	Cumulative mortality on Day 10 in the water, adjuvant, inactivated *B. bassiana* strain PPRI 5339 and *B. bassiana* strain PPRI 5339 groups was 4/30, 19/30, 9/30 and 8/30, respectively. No significant difference in cumulative mortality between water control and *B. bassiana* strain PPRI 5339 group (p=0.1667). No sublethal effects observed. No signs of infectivity after incubation. No signs of infection upon necropsy. **NOT TOXIC NO INFECTIVITY**	PMRA 2499057
Terrestrial Arthropods	Contact– *Bombus terrestris* (bumblebee), young worker bees	A 100 µL droplet of a *B. basianna* strain PPRI 5339 suspension containing 3.1 × 10^6 CFU/mL was administered to the dorsal surface of the thorax of each bumblee (30). Four other groups of bumblebees (30/group) were administered water, inactivated *B. bassiana*	Cumulative mortality on Day 10 in the water, adjuvant, inactivated *B. bassiana* strain PPRI 5339 and *B. bassiana* strain PPRI 5339 groups was 2/30, 3/30, 4/30 and 1/30, respectively. No	PMRA 2681076

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
		strain PPRI 5339, adjuvant (Tween 80) and dimethoate (positive control). Bees were monitored for 10 days. Bees that died during the study were removed from the test chamber and incubated. Any resulting infective organisms were plated to confirm their identity. Autopsies were conducted on 3 bees from each test chamber.	sublethal effects observed. No signs of infectivity after incubation. No signs of infection upon necropsy. **NOT TOXIC NO INFECTIVITY**	
Terrestrial Arthropods	Dietary– *Apis mellifera* (honeybee), young worker bees	Bees (30) were communally fed *B. bassiana* strain PPRI 5339 in deionized water at a concentration of 2.9×10^8 CFU/mL over a period of 4–6 hours. Four other groups of bees (30/group) were fed 50% sucrose, adjuvant (Tween 80), inactivated *B. bassiana* strain PPRI 5339 and dimethoate (positive control). Bees were monitored for survival for 10 days. Bees that died during the study were removed from the test chamber and incubated. Any resulting infective organisms were plated to confirm their identity. Autopsies were conducted on 3 bees from each test chamber.	Cumulative mortality on Day 10 in the water, adjuvant, inactivated *B. bassiana* strain PPRI 5339 and *B. bassiana* strain PPRI 5339 groups was 6/30, 10/30, 2/30 and 9/30, respectively. No significant difference in cumulative mortality between the water and *B. bassiana* strain PPRI 5339 groups (p=0.276). No sublethal effects observed. Signs of infectivity observed in 5/9 bees that died in the *B. bassiana*	PMRA 2499058

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
			strain PPRI 5339 group. No signs of infection upon necropsy in surviving bees. 10-day LC_{50} > 2.9 × 10^8 CFU/mL LT_{50} > 10 days **NOT TOXIC INFECTIVITY IN SOME BEES**	
Terrestrial Arthropods	Dietary–*Bombus terrestris* (bumblebee), young worker bees	Bees (30) were communally fed *B. bassiana* strain PPRI 5339 in deionized water at a concentration of 2.9 × 10^8 CFU/mL over a period of 4–6 hours. Four other groups of bees (30/group) were fed 50% sucrose, adjuvant (Tween 80), inactivated *B. bassiana* strain PPRI 5339 and dimethoate (positive control). Bees were monitored for survival for 10 days. Bees that died during the study were removed from the test chamber and incubated. Any resulting infective organisms were plated to confirm their identity. Autopsies were conducted on 3 bees from each test chamber.	Cumulative mortality on Day 10 in the water, adjuvant, inactivated *B. bassiana* strain PPRI 5339 and *B. bassiana* strain PPRI 5339 groups was 3/30, 2/30, 2/30 and 2/30, respectively. No sublethal effects observed. No signs of infectivity observed after incubation. No signs of infection upon necropsy in surviving bees. 10-day LC_{50} > 2.9 × 10^8 CFU/mL LT_{50} > 10 days **NOT TOXIC NO**	PMRA 2681077

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
			INFECTIVITY	
Terrestrial Arthropods	A scientific rationale was submitted to supplement the studies conducted on terrestrial arthropods. The rationale was based on the lack of increased exposure to non-target terrestrial arthropods due to the proposed greenhouse uses, the already naturally occurring populations of *B. bassiana*, and the level of active ingredient in Velifer. No further testing is required in non-target terrestrial arthropods for the proposed greenhouse uses.			PMRA 2499046 2681075
Plants				
Plants	A scientific rationale was submitted to waive testing on terrestrial plants. The rationale was based on the lack of increased exposure to terrestrial plants due to the proposed greenhouse uses and the already naturally occurring levels of *B. bassiana*. Furthermore, *B. bassiana* is not taxonomically related to known plant pathogens nor is it listed as a plant pathogen by the International Society of Plant Pathology.			PMRA 2499046 2681075 2681078
Aquatic Organisms				
Vertebrates				
Freshwater Fish	A scientific rationale was submitted to waive testing in freshwater fish. The rationale was based on the lack of increased exposure to freshwater fish due to the proposed greenhouses uses, the already naturally occurring populations of *B. bassiana*, the level of active ingredient in Velifer and the application instructions which minimize the potential for the MPCA to reach surface water.			PMRA 2499046 2681075
Estuarine and Marine Fish	A scientific rationale was submitted to waive testing in freshwater fish. The rationale was based on the lack of increased exposure to estuarine and marine fish due to the proposed greenhouses uses, the already naturally occurring populations of *B. bassiana*, the level of active ingredient in Velifer and the application instructions which minimize the potential for the MPCA to reach surface water.			PMRA 2499046 2681075
Invertebrates				
Aquatic Arthropods	A scientific rationale was submitted to waive testing in aquatic arthropods. The rationale was based on the lack of increased exposure to aquatic arthropods due to the proposed greenhouses uses, the already naturally occurring populations of *B. bassiana*, the level of active ingredient in Velifer and the application instructions which minimize the potential for the MPCA to reach			PMRA 2499046 2681075

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
	surface water.			
Plants				
Aquatic Plants	A scientific rationale was submitted to waive testing on aquatic plants. The rationale was based on the lack of increased exposure to aquatic plants due to the proposed greenhouse uses and the already naturally occurring levels of *B. bassiana*. Furthermore, *B. bassiana* is not taxonomically related to known plant pathogens nor is it listed as a plant pathogen by the International Society of Plant Pathology.			PMRA 2499046 2681075 2681078

References

A. List of Studies/Information Submitted by Registrant

1.0 Chemistry

PMRA Document Number	Reference
2431214	2014, Product chemistry for *Beauveria bassiana* strain PPRI 5339 technical, DACO: M2.12, M2.9.1 CBI
2431215	2014, Product chemistry for *Beauveria bassiana* strain PPRI 5339 technical, DACO: M2.12, M2.9.1
2431216	2013, Detection and enumeration of *Beauveria bassiana* and microbial contaminants in five production batches of PPRI 5339 Technical Grade Active Ingredient, DACO: M2.10.1, M2.10.3, M2.9.2 CBI
2431222	2013, Assay to determine beauvericin within *Beauveria bassiana* concentrate with associated validation, DACO: M2.10.3, M2.9.3 CBI
2499044	2007, Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*, DACO: M2.7.2
2681061	2016, Product Characterization Table - *Beauveria bassiana* PPRI 5339 Technical, DACO: M2.1, M2.2, M2.3, M2.4, M2.5, M2.6
2681062	2012, Molecular identity of the South African *Beauveria bassiana* isolate, strain PPRI 5339, DACO: M2.7.1 CBI
2681063	2016, *Beauveria bassiana* strain PPRI 5339 Technical Description of the Manufacturing Process Discussion of Formation of Unintentional Ingredients, DACO: M2.8, M2.9.1, M2.9.3 CBI
2681064	2016, Germination Test - Fungi, DACO: M2.10.1, M2.9.2 CBI
2681065	2016, Total Spore Count (Fungi), DACO: M2.10.1, M2.9.2 CBI
2681066	2016, Shelf Life: Conidia Spores of *Beauveria bassiana* PPRI5339, DACO: M2.11
2681109	2016, Product Characterization Table - Velifer(TM), DACO: M2.1, M2.2, M2.3, M2.4, M2.5
2681110	2016, Velifer(TM) Fungal Contact Insecticide Group B - Physical/Chemical Properties, DACO: M2.11, M2.12
2681111	2016, Velifer(TM) Fungal Contact Insecticide Group B - Physical/Chemical Properties, DACO: M2.11, M2.12 CBI
2681112	2016, Velifer(TM) Fungal Contact Insecticide Group A - Product Identity, Composition and Analysis, DACO: M2.10.1, M2.10.2, M2.10.3, M2.8, M2.9.1, M2.9.2, M2.9.3 CBI

2.0 Human and Animal Health

PMRA Document Number	Reference
2431225	2011, *Beauveria bassiana* strain PPRI 5339 - Acute oral toxicity study (UDP) in rats, DACO: M4.2.2, M4.9
2431227	2013, *Beauveria bassiana* strain PPRI 5339 - Acute pulmonary toxicity/pathogenicity study in rats, DACO: M4.2.3
2431228	2012, *Beauveria bassiana* strain PPRI 5339 - Acute inhalation toxicity study in rats, DACO: M4.2.3, M4.9
2431229	2012, *Beauveria bassiana* strain PPRI 5339 spore concentrate - Acute intraperitoneal injection toxicity/pathogenicity study in rats, DACO: M4.3.3
2431230	2011, *Beauveria bassiana* strain PPRI 5339 - Acute dermal toxicity study in rats, DACO: M4.4
2431231	2011, *Beauveria bassiana* strain PPRI 5339 - Acute dermal irritation study in rabbits, DACO: M4.5.2
2431232	2011, *Beauveria bassiana* strain PPRI 5339 - Acute eye irritation study in rabbits, DACO: M4.9
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2435503	2014, Use Description/Scenario, DACO: 5.2
2499030	2010, Cosmetic Ingredient Review Expert Panel, Plant-Derived Edible Oils, and Other Derivatives as Used in Cosmetics, DACO: M4.2.2, M4.4, M4.5.2, M4.9
2499031	2013, Cosmetic Ingredient Review Expert Panel, Amended Safety Assessment of PEGylated Oils as Used in Cosmetics, DACO: M4.2.2, M4.4, M4.5.2, M4.9
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2681072	2015, *Beauveria bassiana* strain PPRI 5339 Amended Final Report Acute Inhalation Toxicity Study in Rats, DACO: M4.2.3
2681073	2016, BAS 480 I Acute Inhalation Toxicity in Rats, DACO: M4.2.3
2681074	2016, Acute Inhalation Toxicity in Rats, DACO: M4.2.3
2681113	2016, BAS 480 03 I Acute Oral Toxicity In Rats - Acute Toxic Class Method, DACO: M4.2.2
2681114	2016, BAS 480 03 I Acute Inhalation Toxicity In Rats, DACO: M4.2.3
2681116	2016, BAS 480 03 I Acute Dermal Toxicity In Rats, DACO: M4.4
2681117	2016, BAS 480 03 I Acute Dermal Irritation In Rabbits, DACO: M4.5.2
2681118	2016, Overall Summary of Toxicity, Pathogenicity, and Infectiveness of BAS 480 I and BAS 480 03 I, DACO: M4.1, M4.6
2681120	2016, BAS 480 03 I Acute Eye Irritation In Rabbits, DACO: M4.9

3.0 Environment

PMRA Document Number	Reference
2431225	2011, *Beauveria bassiana* strain PPRI 5339 - Acute oral toxicity study (UDP) in rats, DACO: M4.2.2, M4.9
2431229	2012, *Beauveria bassiana* strain PPRI 5339 spore concentrate - Acute intraperitoneal injection toxicity/pathogenicity study in rats, DACO: M4.3.3
2499034	2015, Attachment 1 Response to Completeness Check Letter dated November 5, 2014, DACO: 10.1, 10.2.3.3, M2.11, M4.2.2, M4.2.3, M4.4, M4.5.2, M4.9, M9.5.1 CBI
2499046	2010, A fungal pathogen in time and space: the population dynamics of *Beauveria bassiana* in a conifer forest, DACO: M4.2.2, M9.2.1, M9.2.2, M9.3, M9.4.1, M9.4.2, M9.5.1, M9.5.2, M9.8.1, M9.8.2
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2499051	1965, An Analysis of the Body Temperature of Birds, DACO: M9.2.1, M9.2.2
2499056	2015, Supplemental Response to Tier 1 Microbial Pesticide Data Requirements for *Beauveria bassiana* strain PPRI 5339, DACO: M9.5.1
2499057	2013, Effects of *Beauveria bassiana* PPRI 5339 on the honeybee, *Apis mellifera*, in an acute contact toxicity test, DACO: M9.5.1
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2499059	2014, Effects of Broadband (BAS 480 00 l) on the parasitic wasp, *Aphidius rhopalosiphi*, in a glass plate contact toxicity test, DACO: M9.5.1
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2681076	2013, Effects of *Beauveria bassiana* PPRI 5339 on the Bumblebee, *Bombus terrestris*, in an acute contact toxicity test, DACO: M9.5.1
2681077	2013, Effects of *Beauveria bassiana* PPRI 5339 on the Bumblebee, *Bombus terrestris*, in an acute oral toxicity test, DACO: M9.5.1
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4.0 Value

PMRA Document Number	Reference
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2431257	2014, Mode of Action, DACO: 10.2.1
2431266	2014, Efficacy summary tables, DACO: 10.2.3.1
2431267	2014, Efficacy on Aphids, DACO: 10.2.3.3

2431269	2014, Efficacy on Mites, DACO: 10.2.3.3
2431270	2014, Efficacy on Thrips, DACO: 10.2.3.3
2431271	2014, Efficacy on Whitefly, DACO: 10.2.3.3
2431273	2014, BroadBand for Greenhouse Ornamentals and Vegetables, DACO: 10.2.4
2431274	2014, Survey of Alternatives, DACO: 10.5.1
2499024	2015, Efficacy summary tables, DACO: 10.2.3.1
2499025	2015, Additional Efficacy Small Scale Trials Field and Greenhouse Individual Trials On Thrips, Trials 4-9, DACO: 10.2.3.3
2499026	2015, Additional Efficacy Small Scale Trials Field and Greenhouse Individual Trials On Two-Spotted Spider Mites, Trials 4-5, DACO: 10.2.3.3
2499034	2015, Attachment 1 Response to Completeness Check Letter dated November 5, 2014, DACO: 10.1, 10.2.3.3, M2.11, M4.2.2, M4.2.3, M4.4, M4.5.2, M4.9, M9.5.1 CBI
2681100	2016, Velifer for Greenhouse Ornamentals and Vegetables, DACO: 10.1
2681102	2016, Efficacy summary tables - Velifer, DACO: 10.2.3.1
2681103	2016, Efficacy With Velifer Against Thrips In Individual Small Scale Greenhouse Trials 10-14, DACO: 10.2.3.3
2681104	2016, Efficacy With Velifer Against Whitefly In Individual Small Scale Greenhouse Trials 4-9, DACO: 10.2.3.3
2681105	2016, Survey Of Chemical And Non-Chemical Alternatives, DACO: 10.5.1

B. Additional Information Considered

i) Published Information

1.0 Environment

PMRA Document Number	Reference
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