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Proposed Registration Document

PRD2014-06

Beauveria bassiana **strain ANT-03**

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Overview

Proposed Registration Decision for *Beauveria bassiana* strain ANT-03

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* strain ANT-03 and the end-use products Bio-Ceres G WP and Bio-Ceres G WB, containing the technical grade active ingredient *Beauveria bassiana* strain ANT-03, to reduce numbers of whiteflies, thrips and aphids on greenhouse grown tomatoes, cucumbers and ornamentals.

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

This Overview describes the key points of the evaluation, while the Science Evaluation section provides detailed technical information on the human health, environmental and value assessments of *Beauveria bassiana* strain ANT-03 and the end-use products Bio-Ceres G WP and Bio-Ceres G WB.

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 acceptable¹ 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 value² 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 (for example, those most sensitive to environmental contaminants). 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 Health Canada's website at healthcanada.gc.ca/pmra.

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 ANT-03, the PMRA will consider all comments received from the public in response to this consultation document³. The PMRA will then publish a Registration Decision⁴ on *Beauveria bassiana* strain ANT-03, 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 section of this consultation document.

What Is *Beauveria bassiana* strain ANT-03?

Beauveria bassiana strain ANT 03 is a microbial pest control agent in the end-use products, Bio-Ceres G WP and Bio-Ceres G WB. These end-use products reduce numbers of whiteflies, aphids, and thrips on greenhouse tomato, cucumber and ornamentals.

Beauveria bassiana is a fungus that grows naturally in soils throughout the world. It is a generalist entomopathogenic fungus that causes a disease in many types of insect 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 most other insects. The ANT 03 strain of *B. bassiana* was isolated in 2001 from a tarnished plant bug found dead in Île Perrot, Quebec.

Health Considerations

Can Approved Uses of *Beauveria bassiana* strain ANT-03 Affect Human Health?

***Beauveria bassiana* strain ANT-03 is unlikely to affect your health when Bioceres G WP and Bioceres G WB are used according to the label directions.**

People could be exposed to *Beauveria bassiana* strain ANT-03 when handling and applying Bioceres G WP and Bioceres G WB. 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.

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*.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns. When the technical grade active ingredient (TGAI), *Beauveria bassiana* strain ANT-03, was tested on laboratory animals, there were no signs that it caused any significant toxicity or disease.

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 established 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 sets science-based MRLs to ensure that the food Canadians eat is safe.

Beauveria bassiana is encountered in nature as it is a ubiquitous soil microorganism that causes disease in insects. When the technical grade active ingredient was administered orally to rats, no signs of toxicity or disease were observed, and no metabolites of toxicological significance have been shown to be produced by this strain of *B. bassiana*. Therefore, the establishment of an MRL is not required for *B. bassiana* strain ANT-03. As well, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently, dietary risks are minimal to non-existent.

Occupational Risks from Handling Bioceres G WP and Bioceres G WB

Occupational risks are not of concern when Bioceres G WP and Bioceres G WB are used according to label directions, which include protective measures.

Workers handling Bioceres G WP and Bioceres G WB can come into direct contact with *B. bassiana* strain ANT-03 on the skin or by inhalation. For this reason, the product label will specify that workers exposed to the end-use product (EP) must wear waterproof gloves, long-sleeved shirts, long pants, eye goggles, a dust/mist filtering respirator/mask (NIOSH approval number prefix TC-21) or NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter), and shoes plus socks.

For the bystander, exposure is expected to be much less than that of handlers and mixer/loaders and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When Bioceres G WP and Bioceres G WB Are Introduced Into the Environment?

Environmental risks are not of concern

Bioceres G WP and Bioceres G WB contain *Beauveria bassiana* strain ANT-03, a fungus that infects insects. *Beauveria bassiana* occurs naturally in soils. The microorganism's ability to infect insects is achieved through the germination of a fungal spore on the insect cuticle, which then grows throughout the body and kills the insect.

Minimal environmental exposure to *B. bassiana* strain ANT-03 is expected following the proposed greenhouse use of Bioceres G WP and Bioceres G WB because the applications are made indoors.

Studies were conducted to determine the effects of the technical grade active ingredient, *Beauveria bassiana* strain ANT-03, on birds, and terrestrial and aquatic arthropods. These studies showed that the technical grade active ingredient was not pathogenic to birds; however, it may be pathogenic/toxic to insects and daphnids. As a result, the EP labels will contain statements identifying the potential for harm to beneficial insects and bees and that applications avoid direct contact to foraging bees. The EP labels will also contain standard statements restricting users from activities that may release *B. bassiana* strain ANT-03 into aquatic environments from its use in the greenhouse.

Although no other studies were considered, exposure to outdoor environments is expected to be minimal since the use is restricted to greenhouses, therefore, significant adverse effects to non-target organisms is not expected.

Value Considerations

What Is the Value of Bio-Ceres G WP and Bio-Ceres G WB?

Bio-Ceres G WP and Bio-Ceres G WB can be used to reduce numbers of whiteflies, aphids, and thrips on greenhouse tomato, cucumber and ornamentals.

Bio-Ceres G WP and Bio-Ceres G WB may be used on greenhouse tomato, cucumber and ornamentals as part of an integrated pest management program. The data reviewed demonstrated that Bio-Ceres G WP and Bio-Ceres G WB reduce numbers of whiteflies, aphids, and thrips on greenhouse tomato, cucumber and ornamentals. However, depending on humidity and temperature, efficacy can be variable. Multiple applications may be required for optimum performance of the products. This is not unexpected given the mode of action of these products (a fungus that causes a disease).

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 Bioceres G WP and Bioceres G WB to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

In individuals exposed to large quantities of Bioceres G WP or Bioceres G WB, respiratory and dermal sensitivity could possibly develop upon repeated exposure to the product since all microorganisms, including *B. bassiana* strain ANT-03, contain substances that are potential sensitizers. Therefore, anyone handling or applying Bioceres G WP or Bioceres G WB must wear appropriate waterproof gloves, a long-sleeved shirt, long pants, eye goggles, a dust/mist filtering respirator/mask (NIOSH approval number prefix TC-21) or NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter), and shoes plus socks. Also, the signal words, “POTENTIAL SENSITIZER” are required on the principal display panel of the technical grade active ingredient, Bioceres G WP and Bioceres G WB; and the precautionary statements: “Avoid contact with eyes, skin and clothing.”, “Avoid inhaling/breathing mists.” and “May cause sensitization.” are required on the secondary display panel of the label for Bioceres G WP and Bioceres G WB.

Environment

The end-use product label will include environmental precaution statements that prevent the contamination of aquatic systems from the use of Bioceres G WP and Bioceres G WB, as well as statements identifying the potential for harm to beneficial insects and bees.

Next Steps

Before making a final registration decision on *Beauveria bassiana* strain ANT-03, the PMRA will consider all 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 *Beauveria bassiana* strain ANT-03 (based on the Science Evaluation section 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 ANT-03

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	<i>Beauveria bassiana</i> strain ANT-03
Function	To reduce numbers of whiteflies, aphids and thrips in greenhouse grown tomatoes, cucumbers and ornamental crops.
Binomial name	<i>Beauveria bassiana</i> strain ANT-03
Taxonomic designation	
Kingdom	Fungi
Phylum	Ascomycota
Class	Sordariomycetes
Order	Hyprocreales
Family	Cordycipitacea
Genus	<i>Beauveria</i>
Species	<i>bassiana</i>
Strain	ANT-03
Patent Status information	Not provided
Minimum purity of active	<i>Beauveria bassiana</i> strain ANT-03 (technical grade active ingredient): 100% at 1.0×10^{10} spores/g Bioceres G WP (end-use product): 20% at 1.0×10^{10} spores/g Bioceres G WB (end-use product): 20% at 1.0×10^{10} spores/g
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 Track 1 substances. The product must meet microbiological contaminants release standards. <i>Beauveria bassiana</i> strains are known to produce toxic secondary metabolites but the technical grade active ingredient is not expected to contain any of these metabolites.

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

Technical Product—*Beauveria bassiana* strain ANT-03

End-Use Products—Bioceres G WP and Bioceres G WB

Properties	<i>Beauveria bassiana</i> strain ANT-03
Physical state	Powder
Colour	Cream-white
Odour	odourless
Specific gravity/bulk density (g/cm ³) at 22°C	0.280 ± 0.003 loose 0.454± 0.009 (300 times tapped)
Moisture content	5.3± 0.81
Guarantee	1.0 × 10 ¹⁰ spores/g
Corrosion Character	None
Viscosity	Not applicable

Properties	Bioceres G WP
Physical state	Wettable powder
Colour	White
Odour	odourless
Specific gravity/bulk density (g/cm ³) at 22°C	0.280 ± 0.016 loose 0.372± 0.007 (300 times tapped)
Moisture content	6.8± 0.42
Guarantee	1.0 × 10 ¹⁰ spores/g
Corrosion Character	None
Viscosity	Not applicable

Properties	Bioceres G WB
Physical state	Water dispersible briquette
Colour	White
Odour	odourless
Specific gravity/bulk density (g/cm ³) at 22°C	0.880 ± 0.016 (dry compression at 500 kg/cm)
Moisture content	6.4± 0.51
Guarantee	1.0 × 10 ¹⁰ spores/g
Corrosion Character	None
Viscosity	Not applicable

1.3 Directions for Use

For use on greenhouse tomato, cucumber and ornamentals for reduction in numbers of whiteflies, aphids and thrips. The application rate is 2-4 g product/L of water (2×10^{10} – 4×10^{10} spores/L of water), in a spray volume of 500-1000 L/ha. Applications should begin at the first appearance of the pest. These products are most effective when used early before high pest populations develop. Repeat applications may be made at 3-7 day intervals, if monitoring indicates that they are necessary. As many applications as required may be made. The pre-harvest interval is 0 days.

1.4 Mode of Action

The mode of action of *B. bassiana* is as a generalist entomopathogenic fungus, which causes white muscardine disease in insects. When spores of the fungus come into contact with an insect host, they germinate, enter the body of the insect and grow hyphae, eventually killing the insect. After death, a white mould grows on the cadaver and produces new spores. Most insects living in or near the soil have evolved natural defences against this fungus as it is common in the natural environment.

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 ANT-03, were submitted by the applicant. The MPCA has been fully characterized with respect to its origin of strain, natural occurrence and biological properties. *Beauveria bassiana* strain ANT-03 can be identified to the species level using a combination of colony morphologies on agar media and biochemical tests and to the strain level using the latest DNA-based methodologies.

2.2 Methods for Establishment of Purity of Seed Stock

The strain has been deposited into the international depository authority in Illinois USA under NRRL # 50797. A stock culture of *B. bassiana* strain ANT-03 is maintained in the National Mycological Herbarium (DAOM) of Agriculture and Agri-Food Canada, and the private collection of Anatis Bioprotection Inc. Stock cultures are kept frozen at -80°C.

Practices for ensuring the purity of the seed stock were adequately described in the method of manufacture and quality assurance program.

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

The potency (spores/g) of the technical grade active ingredient, based on the percentage of viable spores is determined using hemacytometry. The end-use products are bioassayed through an insect host to confirm biological activity.

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

As noted in Section 2.1, the MPCA can be identified to the species level using a combination of colony morphologies on agar media and biochemical tests. No methods are required to quantify viable or non-viable residues of *B. bassiana* strain ANT-03. *Beauveria bassiana* is a ubiquitous microorganism in nature and has been isolated from a wide variety of environments. *Beauveria bassiana* strains are known to produce the toxic metabolite beauvericin, however, *B. bassiana* strain ANT-03 is not reported to produce this toxin. Furthermore, when *B. bassiana* strain ANT-03 was administered orally to rats, no signs of toxicity or disease were observed.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures that will be used to limit contaminating microorganisms during manufacture of the technical grade active ingredient, and the associated end-use products, Bio-Ceres G WP and Bio-Ceres G WB are acceptable.

During manufacturing, several approaches will be used to monitor microbial contamination in the technical grade active ingredient and associated end-use products. These approaches will include frequent purity checks on agar media, sterilization of all equipment and media, and sanitization of recovery equipment.

The absence of human pathogens and below-threshold levels of contaminants was demonstrated in representative batches using pathogen-specific growth media. Microbe-specific screening methods for enteric bacteria/total coliforms, yeasts/moulds, *Salmonella* spp., *Shigella* spp. and *Staphylococcus aureus* are adequate for detecting and enumerating microbial contaminants of concern. Release standards for microbial contaminants in the production batches comply with those permitted by the PMRA and are adequate to ensure that the end-use products do not contain unacceptable levels of human and animal disease-causing microorganisms.

No known toxic metabolites or hazardous substances are present in Bio-Ceres G WB or Bio-Ceres G WB.

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

Results from storage stability testing of Bio-Ceres G WP and Bio-Ceres G WB showed that these products can be stored unopened for up to 12 months in the refrigerator at $4\pm 2^{\circ}\text{C}$ or up to 6 months at $21\text{--}23^{\circ}\text{C}$. No storage stability data are required for the technical grade active ingredient since it is not stored as a separate product.

3.0 Impact on Human and Animal Health

3.1 Toxicology

The PMRA conducted a detailed review of the toxicological database for *B. bassiana* strain ANT-03. The database is complete, consisting of laboratory animal (in vivo) toxicity studies (acute oral toxicity/pathogenicity, acute pulmonary toxicity/pathogenicity, acute intraperitoneal infectivity, and acute dermal toxicity/irritation) currently required for health hazard assessment purposes which were carried out in accordance with currently accepted international testing protocols and Good Laboratory Practices. The scientific quality of the data is high and the database is considered sufficient to characterize the toxicity and infectivity of this pest control agent and product.

In an acute oral toxicity study, one group of 8 week old Sprague-Dawley rats (12/sex) were given a single oral dose of technical grade active ingredient (1.3×10^{10} colony forming units [CFU]/mL) in sterile phosphate buffered saline (PBS) and Tween 20 at a dose of 1.3×10^9 CFU/animal (0.1 mL/animal). The animals were then observed for a period of up to 21 days with interim scheduled sacrifices on Days 3, 7, and 14. An untreated control and shelf control were used as well as an inactivated test substance control. The oral LD_{50} was $> 1.3 \times 10^9$ CFU/animal. There were no treatment related clinical signs, necropsy findings or changes in body weight. A pattern of clearance was achieved by Day 14. Based on the results of this study, *B. bassiana* strain ANT-03 is of low toxicity and is not infective or pathogenic to the rat.

In an acute pulmonary infectivity and toxicity study, one group of 8 week old Sprague-Dawley rats (15/sex) were given a single intratracheal dose of technical grade active ingredient (1.1×10^{10} CFU/mL) in sterile PBS and Tween 20 at a dose of 1.1×10^9 CFU/animal (0.1 mL/animal). The animals were then observed for a period of up to 21 days with interim scheduled sacrifices on Days 3, 7, and 14. An untreated control and shelf control were used as well as an inactivated test substance control. The pulmonary LD_{50} was $> 1.1 \times 10^9$ CFU/animal. There were no treatment related clinical signs, necropsy findings or changes in body weight. A pattern of clearance was achieved by Day 3. Based on these results, *B. bassiana* strain ANT-03 is of low toxicity and is not infective or pathogenic in the rat.

In an acute intraperitoneal infectivity study, one group of 8 week old, Sprague-Dawley rats (15/sex) were given a single intraperitoneal injection of technical grade active ingredient (1.4×10^{10} CFU/mL) in sterile PBS at doses of 1.4×10^9 CFU/animal (0.1 mL/animal). The animals were then observed for a period of up to 21 days with an interim scheduled sacrifice on Day 0. An untreated control and inactivated test substance control were used. There were no treatment related clinical signs, necropsy findings or changes in body weight. Based on the results of this study, *B. bassiana* strain ANT-03 is not pathogenic in the rat.

In an acute dermal toxicity/irritation study, one group of nine-week-old, Sprague-Dawley rats (5/sex) were dermally exposed to 5050 mg/kg body weight (bw) technical grade active ingredient (1.2×10^{11} conidia/g) for 24 hours. The test substance was applied evenly in a thin uniform layer. Following exposure, the animals were observed for a period of 14 days. The dermal LD₅₀ was > 5050 mg/kg body weight. There were no treatment related effects or signs of irritation observed throughout the study. Based on the results of this study, *B. bassiana* strain ANT-03 is of low toxicity and is non-irritating to the skin of the rat.

An eye irritation study was not submitted and as such it is assumed that the technical grade active ingredient and end-use products may cause eye irritation, therefore, the hazard statement 'CAUTION – EYE IRRITANT' will appear on the principal display panel of the technical grade active ingredient and EP labels.

A survey of published literature has revealed that *Beauveria* species have been rarely identified as agents of human infections, and in an overview on the emergence of less common, but medically important fungal pathogens, the genus *Beauveria* is not mentioned.

Beauveria bassiana has the potential to act as an opportunistic pathogen, but as the scientific literature demonstrates, *Beauveria* infections are extremely rare events. A detailed analysis of case reports allegedly involving *B. bassiana* reveals that extraordinary circumstances, such as a severely compromised immune system or a history of surgery/injury, are required for a *B. bassiana* infection to occur.

Several cases of allergic reactions in humans caused by this species have been documented, especially during the production process. Generally, headache, weakness and fever were noticed. Researchers working with conidia of *B. bassiana* reported high fever and a reaction similar to an anaphylactic shock. In contrast to these findings, no incidents of human hypersensitivity reaction were reported by workers of a manufacturer of a different strain of *B. bassiana* during many years of mass production. Furthermore, it has been used in biocontrol for over 100 years with no confirmed reports of illness related to exposure to *B. bassiana* strains used in biocontrol.

Although information on reporting of hypersensitivity and a dermal sensitization study were not submitted, PMRA considers all MPCAs to have the potential to cause pulmonary and/or dermal sensitization and as such the hazard statement 'POTENTIAL SENSITIZER' will appear on the principal display panel of the technical grade active ingredient and end-use product labels.

There are other strains of *B. bassiana* that are known to produce the secondary metabolites beauvericin, bassianolide, oosporein, bassiacridin, bassianin and tenellin. *Beauveria bassiana* strain ANT-03 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 the associated end-use products, Bio-Ceres G WP and Bio-Ceres G WB, do not contain beauvericin.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the MPCA, and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity tests.

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 ANT-03.

3.2 Occupational / Bystander Exposure and Risk Assessment

3.2.1 Occupational

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/or 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, dermal toxicity studies in animals demonstrated no signs of systemic toxicity to the technical grade active ingredient.

The toxicity testing with the technical grade active ingredient showed no toxicity or infectivity via the oral, dermal, pulmonary, or intraperitoneal routes of exposure. The submitted dermal irritation studies using the MPCA showed no dermal irritation and the formulants contained in Bio-Ceres G WP and Bio-Ceres G WB are not dermal irritants. No eye irritation studies were submitted for the end-use products. Ocular exposure can be minimized if applicators, mixer/loaders, handlers and early-entry workers wear eye goggles. Therefore, precautionary label statements instruct workers to avoid contact of the EP with the eyes and to wear protective eye-wear are required to mitigate occupational exposure concerns.

Although dermal toxicity or toxicity from inhalation exposure is considered minimal from the proposed EP use, 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, eye goggles, a dust/mist filtering respirator/mask (NIOSH approval number prefix TC-21) or NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter), and shoes plus socks are required to minimize exposure and protect applicators, mixer/loaders, and handlers that are likely to be primarily exposed.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of Bio-Ceres G WP and Bio-Ceres G WB, and no significant occupational risks are anticipated for this product.

3.2.2 Bystander

Overall, the PMRA does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for the MPCA and the assumption that precautionary label statements will be followed by commercial applicators in the use of Bio-Ceres G WP and Bio-Ceres G WB.

The label does not allow applications outdoors including: turf, residential or recreational areas; therefore, non-occupational dermal exposure and risk to adults, infants and children are low. Because the use site is limited to the greenhouse, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

3.3 Incident Reports Related to Human and Animal Health

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website www.healthcanada.gc.ca/pesticideincident. Incidents from Canada and the United States were searched and reviewed for *B. bassiana* strain ANT-03.

As of 6 November 2013, there have been no incidents related to health or the environment reported to the PMRA, nor summarized by the USEPA or the California Department of Pesticide regulation, for products containing *B. bassiana* strain ANT-03.

3.4 Dietary Exposure and Risk Assessment

3.4.1 Food

While the proposed use pattern may result in some 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 ANT-03 demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity/infectivity study. After application, *B. bassiana* strain ANT-03 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, *B. bassiana* strain ANT-03 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 and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary 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.

3.4.2 Drinking Water

The likelihood of *B. bassiana* strain ANT-03 entering neighbouring aquatic environments or surface water run-off from greenhouse use of Bio-Ceres G WP or Bio-Ceres G WB is considered very low.

No risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and because there were no harmful effects observed in Tier I acute oral toxicity testing and infectivity testing. The EP label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Users are also requested not to allow effluent from greenhouses or runoff 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. Therefore, potential exposure to *B. bassiana* strain ANT-03 in surface and drinking water is negligible.

3.4.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARDs) 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 ANT-03 is of low 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 micro-organisms 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 ANT-03 to human health.

3.4.4 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 established 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 sets 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 ANT-03 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 ANT-03 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 (particularly the oral study) and the fact that *Beauveria bassiana* strain ANT-03 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 technical grade active ingredient or end-use products. 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 established for *B. bassiana* strain ANT-03.

3.4.5 Aggregate Exposure

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 ANT-03 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 agricultural sites 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 Bio-Ceres G WP and Bio-Ceres G WB, there should not be any increase in potential human health risk.

3.4.6 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 ANT-03. No cumulative effects are anticipated if the residues of *B. bassiana* strain ANT-03 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

The proposed use of Bio-Ceres G WP and Bio-Ceres G WB 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 ANT-03 through human activity, such as composting of plant waste and water management practices. Afterwards, the dispersal of *B. bassiana* strain ANT-03 should be limited to runoff and natural vectors (for example, insects). Based on these considerations, the amount of *B. bassiana* strain ANT-03 transferring to outdoor environments from the use of Bio-Ceres G WP and Bio-Ceres G WB in greenhouses is expected to be minimal.

In the event that *B. bassiana* strain ANT-03 did 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 ANT-03 could survive in the soil under field conditions, given that environmental conditions (in other words, moisture, pH) were suitable, but that over time the populations of *B. bassiana* strain ANT-03 should return to naturally occurring levels.

Overall, it is not expected that the greenhouse use of Bio-Ceres G WP and Bio-Ceres G WB would significantly increase natural populations of *B. bassiana* in outdoor terrestrial or aquatic environments and therefore hazards to non-target terrestrial and aquatic organisms are expected to be minimal to non-existent.

4.2 Effects on Non-Target Species

PMRA has a four-level 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 of the MPCA. The maximum challenge concentration 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 some 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, (in other words, life cycle studies) as well as definitive toxicity testing (for example, LC₅₀, LD₅₀). 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 ($RQ = \text{exposure/toxicity}$), 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 assess the hazards of the technical grade active ingredient to terrestrial non-target organisms. These studies included avian and arthropod species. Data waiver requests for terrestrial plants, and wild mammals were also submitted. Effects on non-target plants were addressed via efficacy studies and effects to wild mammals were addressed via human health studies submitted with this application.

The acute oral pathogenicity of the technical grade active ingredient to 24-day-old bobwhite quail (*Colinus virginianus*) was assessed over 30 days. The technical grade active ingredient was administered to the birds (3 groups; 10/group) by oral gavage at 10 mg/kg bw/day for 5 days (1.2×10^9 CFU/kg bw/day for 5 days). There were no treatment related effects. This pathogenicity study is classified as acceptable.

In a 25-day dietary toxicity/pathogenicity study, green lacewing (*Chrysoperla rufilabris*) were exposed to the technical grade active ingredient in the diet by spraying a concentration of 4.8×10^{11} CFU/L directly onto corn earworm eggs. An inactivated test substance and untreated control were also used. The test group and inactivated test substance group consumed significantly less food through Day 7. There were no other treatment related effects. The 25-day dietary LC50 for the technical grade active ingredient for the green lacewing is $> 4.8 \times 10^{11}$ CFU/L. This study is classified acceptable and satisfies the guideline requirement for a dietary toxicity/pathogenicity study for terrestrial arthropods.

In an 11-day dietary toxicity/pathogenicity study, 150 ladybird beetles (*Hippodamia convergens*) were exposed to the TGA1 that were sprayed onto corn earworm eggs at a concentration of 4.8×10^{11} CFU/L. An untreated control group and inactivated test substance group were used. Food consumption data from Days 7 – 11 showed a significant reduction in consumption in the treatment group compared to the untreated control. No mortality data were reported for Day 11. In the absence of a known cause, it is assumed that the reduced feeding may have been due to the pathogenicity of *B. bassiana* strain ANT-03, a known insect pathogen, to ladybird beetles. Therefore, *B. bassiana* strain ANT-03 may be pathogenic to ladybird beetles. This study is classified as acceptable and satisfies the guideline requirement for a dietary toxicity/pathogenicity study for terrestrial arthropods.

No test data were submitted to address the potential impact of *B. bassiana* strain ANT-03 on pollinators. As a broad-spectrum mycoinsecticide this MPCA is therefore assumed to be potentially harmful to greenhouse pollinators. While no data were submitted to assess the hazard to pollinators, bumble bees, the pollinators most often used in greenhouses, are purchased from various commercial sources and possess a limited lifetime of 10 to 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 are necessary for pollinating plants in greenhouses and can therefore suffer mortalities without any ill effect 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 treated greenhouses, precautionary measures are required on the Bio-Ceres G WP and Bio-Ceres G WB labels to alert operators of the potential hazard to beneficial insects that may be used in greenhouse Integrated Pest Management programs. Users are also to be advised to avoid direct contact to beneficial insects and to not make applications while bees are actively foraging (if employed in the greenhouse).

A waiver request was submitted to address the risk of the use of *B. bassiana* strain ANT-03 to terrestrial plants. The lack of toxic or pathogenic effects noted in efficacy testing of *B. bassiana* strain ANT-03 on field lettuce, strawberry, field strawberry, greenhouse tomato and cucumber, field pepper, and field celery was cited. As well, there are no reports of phytotoxicity or plant pathogenicity in the published scientific literature from *B. bassiana* (from a search of PubMed using keywords ‘Beauveria and phytotoxicity’ and ‘Beauveria and plant and pathogenicity’). Therefore, based on a weight of evidence approach, the risk to terrestrial plants from the use of *B. bassiana* strain ANT-03 as an MPCA is minimal.

Based on all the available data and information on the effects of *B. bassiana* strain ANT-03 to non-target terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, arthropods (including honey bees), non-arthropod invertebrates plants and other microorganisms from the proposed use of Bio-Ceres G WP and Bio-Ceres G WB in the greenhouse since exposure to outdoor environments is expected to be minimal.

4.2.2 Effects on Aquatic Organisms

An aquatic arthropod study was submitted to address the hazards of *B. bassiana* strain ANT-03 to aquatic non-target organisms. A data waiver request for aquatic plants was also submitted.

In a 21-day toxicity/pathogenicity study 2 groups of 20 daphnids (*Daphnia magna*) were exposed under static/renewal conditions to the technical grade active ingredient at concentrations of 1.0×10^3 and 1.0×10^6 CFU/mL. A negative control, sterile filtrate control and vehicle control were also tested. Immobility in the 1.0×10^6 CFU/mL test group and vehicle control was 100% and 45%, respectively. There were no statistically significant differences of effects between the 1.0×10^3 CFU/mL group and the negative control. The 21-day EC₅₀ for immobility was 4.07×10^5 CFU/mL. The 21-day EC₅₀ for reproductivity was 4.58×10^5 CFU/mL; and the EC₅₀ for biomass was 3.30×10^5 CFU/mL. The vehicle used to administer the test substance may have confounded the observed effects in the test group. *Beauveria bassiana* strain ANT-03 is toxic and/or pathogenic to daphnia. This study is classified as acceptable and satisfies the guideline requirement for a toxicity/pathogenicity study for aquatic arthropods.

The applicant submitted a waiver request to address the risk of the use of *B. bassiana* strain ANT-03 to aquatic plants and algae. The lack of toxic or pathogenic effects noted in efficacy testing of *B. bassiana* strain ANT-03 on field lettuce, strawberry, field strawberry, greenhouse tomato and cucumber, field pepper, and field celery was cited. As well, there are no reports of phytotoxicity or plant pathogenicity reported in the published scientific literature from *B. bassiana* (from a search of PubMed using keywords 'Beauveria and phytotoxicity' and 'Beauveria and plant and pathogenicity'). Therefore, the risk to aquatic plants and from the use of *B. bassiana* strain ANT-03 as an MPCA is minimal.

Based on all the available data and information on the effects of *B. bassiana* strain ANT-03 to non-target aquatic organisms, there is reasonable certainty that no harm will be caused to fish, aquatic arthropods, aquatic non-arthropod invertebrates, and aquatic plants from the proposed use of Bio-Ceres G WP and Bio-Ceres G WB in the greenhouse since exposure to outdoor environments is expected to be minimal. As a precaution, standard label statements will prohibit handlers from contaminating aquatic habitats.

4.3 Incident Reports Related to the Environment

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website: <http://www.hc-sc.gc.ca/cps-spc/pest/part/protect-proteger/incident/indexeng.php>. Only incidents in which the pesticide is determined to be linked to the effects (Canadian causality of highly probable, probable and possible; U.S. causality of highly probable, probable and possible) are considered in the reviews.

As of 7 November 2013, there were no environmental incidents reported in the PMRA Incident reporting database nor in the USEPA's Ecological Incident Information System (EIS) for products containing *B. bassiana* strain ANT-03 for use as pesticides.

5.0 Value

5.1 Effectiveness Against Pests

The application rate for both products is 2-4 g product/L of water (2×10^{10} - 4×10^{10} spores/L of water) in a spray volume of 500-1000 L/ha, with applications repeated at weekly intervals, or every 3 to 5 days under high insect pressure. The effectiveness of this use pattern against each pest is discussed below. All percent control data are relative to the untreated control (UTC) at each rating date. UTC treatments were conducted using water only.

5.1.1 Whiteflies

Three trials on greenhouse tomatoes were reviewed to support a claim against whiteflies. Application rates of 1, 2 and 4 g product/L were used in the first trial; 4, 6 and 8 g product/L in the second and 2, 4 and 6 g product/L in the third. Applications were made weekly for up to six weeks. Each treatment block consisted of six plants and treatment blocks were placed directly adjacent to each other. Re-infestation of treated plants by adult whiteflies from untreated blocks may have been a problem.

Bio-Ceres at the concentrations of 2-4 g product/L reduced numbers of whiteflies, albeit after multiple applications. In the first trial, the 4 g product/L rate reduced whitefly density by >90% after the second and third applications, which was a significant reduction compared to the control. However, no significant reduction in whitefly numbers was observed after any of the other applications. This was the only trial that demonstrated a consistent rate effect. In the second trial, reduction in whitefly density ranged from 61-83% after all applications. However, significant difference from the control was observed after the second, third and fifth applications only. In the third trial, reduction in whitefly density was significantly different from the control only after the fifth application, with all rates providing 71-81% reduction in whitefly density at this time. All rates provided 48-60% reduction in whitefly density after each application, although this difference was not significant compared to the control after the first four applications.

5.1.2 Thrips

Three trials on greenhouse cucumber and two on greenhouse ornamentals were reviewed to support a claim against thrips. The greenhouse cucumber trials used application rates of 1, 2 and 4 g product/L in the first trial; 4, 6 and 8 g product/L in the second and 2, 4 and 6 g product/L in the third. Applications were made weekly for up to six weeks. The level of efficacy against thrips was variable in these trials. In general, the level of efficacy tended to increase with subsequent applications, however there was little difference between the application rates. Efficacy generally ranged from lows of around 20% up to highs of around 75% after six weeks of application.

Efficacy was sometimes significant and sometimes not after the first five applications. Significant efficacy was demonstrated after the sixth application in all three trials. No significant difference was observed between the tested rates of Bio-Ceres at any time. The submitted efficacy review explained that all trials took place at suboptimal air temperatures, but claims that this was compensated for by sufficient moisture levels.

Two trials on greenhouse ornamentals were conducted. One used Bio-Ceres at the rates of 2 and 4 g/L and the other used Bio-Ceres at the rate of 4 g/L. Efficacy against thrips was demonstrated after multiple applications. While the level of efficacy did increase with subsequent applications, in one trial it reached a maximum of only 51% after five applications and was significant only after the fifth application, with no significant differences between rates. In the other trial, the 2 g/L rate provided a maximum of 90% reduction of thrips after six applications, however the UTC pest pressure was extremely low at this rating date (0.21 thrips/leaf). Significant differences from the UTC were demonstrated after the fourth and sixth applications. Pest pressure was low during the entire course of this trial, and no consistent rate effect was demonstrated.

5.1.3 Aphids

Trials on greenhouse lettuce, field strawberry and field pepper, as well as a laboratory trial were provided to support a claim against aphids. None of these trials demonstrated an acceptable level of aphid control. In addition, the researcher noted that use of *Beauveria bassiana* ANT-03 during colder months in greenhouses may not provide acceptable control due to cold air conditions.

Two trials on greenhouse ornamentals were also provided. One used five applications of Bio-Ceres treatments at the rates of 2 and 4 g/L; and the other used six applications of Bio-Ceres at the rate of 4 g/L. Applications were made at 6-8 day intervals. In both trials, efficacy against aphids was demonstrated only after multiple applications. Statistically significant efficacy (71-86% reduction of aphids) was demonstrated after five applications in one trial and six applications in the other, with no significant difference between rates. Pest pressure was low in both trials, rarely exceeding 1 insect/leaf.

5.1.4 Efficacy Conclusions

Sufficient efficacy data were provided to support claims of reduction in numbers of whiteflies, thrips and aphids on greenhouse tomato, cucumber and ornamentals. Pest pressure was low in trials against thrips and aphids, and the level of efficacy observed against all three pests was variable. Based on the submitted information, a claim of “control” of whiteflies, thrips or aphids cannot be supported. However, a claim that the product “reduces numbers” of whiteflies, thrips and aphids is supported, at the concentration range of 2-4 g product/L of water. The product requires certain conditions of temperature and humidity for optimum function which can likely be achieved in a greenhouse environment. Therefore, based on the data provided and on the likelihood of optimum conditions being achieved in a greenhouse environment, it is expected that the product will provide reduction in numbers of whiteflies, thrips and aphids on greenhouse tomato, cucumber and ornamentals. A label statement is required stating that multiple applications may be required to reduce numbers of whiteflies, thrips and aphids.

5.2 Economics

Not assessed.

5.3 Sustainability

5.3.1 Survey of Alternatives

Table 5.3.1 Active Ingredients Currently Registered on Greenhouse Cucumber, Tomato and/or Ornamentals for Aphids, Whiteflies and Thrips

Pest	Insecticide Active Ingredients
Whiteflies	<i>Beauveria bassiana</i> strain GHA, chlorpyrifos, dichlorvos, imidacloprid, s-kinoprene, acetamiprid, pyrethrins, permethrin, potassium salts of fatty acids, pyridaben, pymetrozine, spiromesifen
Thrips	Malathion, nicotine, chlorpyrifos, spinosad, <i>Metarhizium anisopliae</i> strain F52, <i>Beauveria bassiana</i> strain GHA
Aphids	Malathion, chlorpyrifos, pyrethrins, dichlorvos, s-kinoprene, imidacloprid, potassium salts of fatty acids, acetamiprid, pymetrozine, <i>Beauveria bassiana</i> strain GHA

5.3.2 Compatibility with Current Management Practices Including Integrated Pest Management

Use of the entomopathogenic fungus *Beauveria bassiana* strain ANT 03 is consistent with standard integrated pest management practices for greenhouse cucumber, tomato and ornamentals. Since this product is a fungus, fungicides may kill the spores. In order to mitigate this occurrence, statements are present on the labels stating not to mix the product with fungicide, and to delay the application of fungicide for four days before or until two days after the application of *Beauveria bassiana* strain ANT 03.

5.3.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

Due to the mode of action of Bio-Ceres G WP and Bio-Ceres G WB (generalist entomopathogenic fungus), resistance to these products is not expected to develop.

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: in other words, 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 strain ANT-03 (technical grade active ingredient), Bio-Ceres G WP and Bio-Ceres G WB were assessed in accordance with the PMRA Regulatory Directive DIR99-03.⁵

- The technical grade active ingredient 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 NOI2005-01⁷ 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:

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 active ingredient, *B. bassiana* strain ANT-03, 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 end-use products, Bio-Ceres G WP and Bio-Ceres G WB, do 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 the technical grade active ingredient and the end-use products, Bio-Ceres G WB and Bio-Ceres G WB, were deemed adequate to assess their potential human health and environmental risks. The technical grade active ingredient was characterized and the specifications of the end-use products were supported by the analyses of a sufficient number of batches. Storage stability data were sufficient to support a shelf life of 1 year when refrigerator at 4°C or up to 6 months at 21–23°C if the package is unopened.

7.2 Human Health and Safety

The acute toxicity and infectivity studies and other relevant information submitted in support of *B. bassiana* strain ANT-03 were determined to be sufficiently complete to permit a decision on registration. Submitted information suggests that the technical grade active ingredient was of low toxicity by the oral, pulmonary, and dermal routes, and *B. bassiana* strain ANT-03 was not pathogenicity or infective by the oral, pulmonary or intraperitoneal routes. The technical grade active ingredient and the end-use products are considered to be potential sensitizers.

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.

In individuals exposed to large quantities of Bio-Ceres G WB or Bio-Ceres G WB, respiratory and dermal sensitivity could possibly develop upon repeated exposure to the product since all microorganisms, including *B. bassiana* strain ANT-03, contain substances that are potential sensitizers. Therefore, anyone handling or applying Bio-Ceres G WB or Bio-Ceres G WB must wear waterproof gloves, long-sleeved shirts, long pants, eye goggles, a dust/mist filtering respirator/mask (NIOSH approval number prefix TC-21) or NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter), and shoes plus socks. Also, the signal words, “POTENTIAL SENSITIZER” are required on the principal display panel of the technical grade active

ingredient, and the end-use products, Bio-Ceres G WP and Bio-Ceres G WB; and the precautionary statements: “Avoid contact with eyes, skin and clothing.”, “Avoid inhaling/breathing mists.” and “May cause sensitization.” are required on the secondary display panel of the labels for Bio-Ceres G WP and Bio-Ceres G WB.

The health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is expected to be minimal.

7.3 Environmental Risk

The non-target organism tests, scientific rationales and supporting published scientific literature submitted in support of the technical grade active ingredient, and the end-use products, Bio-Ceres G WP and Bio-Ceres G WB, were determined to be sufficiently complete to permit a decision on registration. The use of Bio-Ceres G WP and Bio-Ceres G WB containing *B. bassiana* strain ANT-03 in greenhouses 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, standard label statements will prohibit handlers from contaminating aquatic habitats. The product labels also advise users that Bio-Ceres G WP and Bio-Ceres G WB may be harmful to pollinators (including bees) and to some beneficial insects that may be used in greenhouse integrated pest management programs. A statement also instructs users to avoid direct contact to beneficial insects and not to make applications while bees are actively foraging (if employed in the greenhouse).

7.4 Value

Bio-Ceres G WP and Bio-Ceres G WB reduce numbers of whiteflies, aphids, and thrips on greenhouse tomato, cucumber and ornamentals. However, depending on humidity and temperature, efficacy can be variable. Multiple applications may be required for optimum performance of the products.

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* strain ANT-03 and the end-use products Bio-Ceres G WP and Bio-Ceres G WB, containing the technical grade active ingredient *Beauveria bassiana* strain ANT-03, to reduce numbers of whiteflies, thrips and aphids on greenhouse grown tomatoes, cucumbers and ornamentals.

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

ADI	acceptable daily intake
ARD	acute reference dose
bw	body weight
CFU	colony forming unit
cm	centimetres
DACO	data code
EC ₅₀	effective concentration on 50% of the population
EEC	expected environmental concentration
EIIS	Ecological Incident Information System
g	gram
ha	hectare
kg	kilogram
L	litre
LC ₅₀	lethal concentration 50%
LD ₅₀	lethal dose 50%
LOC	level of concern
mL	millilitre
MCC	maximum challenge concentration
MPCA	microbial pest control agent
MRL	maximum residue limit
NIOSH	National Institute of Occupational Safety and Health
PMRA	Pest Management Regulatory Agency
RBC	randomized complete block
RQ	risk quotient
TGAI	technical grade active ingredient
TSMP	Toxic Substances Management Policy
USEPA	United States Environmental Protection Agency
UTC	untreated control
w/v	weight per volume dilution
WB	water-dispersible briquette
WP	wettable powder

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *Beauveria bassiana* strain ANT-03 (Technical Grade Active Ingredient [TGAI])

Study Type	Species, Strain, and Doses	Results	Comments	Reference(s)
Acute Toxicity/Infectivity of <i>B. bassiana</i> strain ANT-03 (TGAI)				
Acute Oral Toxicity/ Infectivity (21-Day study)	<p>Rat- Sprague-Dawley</p> <p>12/sex, single oral dose of 0.1 mL (1.3×10^9 CFU /animal)</p> <p>Inactivated test substance control, 5/sex, single oral dose of 0.1 mL equivalent to 1.3×10^9 CFU/animal</p> <p>Untreated and shelf control, 5/sex each</p> <p>Body weight measured on Days 0, 7, 14, 21</p> <p>Interim and final sacrifices including necropsy, organ weight and microbial enumeration on Days 3, 7, 14, 21</p>	<p>$LD_{50} > 1.3 \times 10^9$ CFU/animal</p> <p>No signs of toxicity or infectivity.</p> <p>Although complete clearance of MPCA was not achieved, a pattern of clearance was achieved by Day 21</p>	<p>LOW TOXICITY NOT PATHOGENIC</p> <p>ACCEPTABLE</p>	PMRA 2153596
Acute Dermal Toxicity/ Irritation (14-Day study)	<p>Rat- Sprague-Dawley</p> <p>5/sex, 24 hour dermal exposure, 5050 mg/kg bw (1.2×10^{11} conidia/g)</p> <p>Body weight measured on Days 0, 7 and 14</p>	<p>$LD_{50} > 5050$ mg/kg bw</p> <p>There were no signs of toxicity or irritation throughout the study.</p>	<p>LOW TOXICITY NON-IRRITATING</p> <p>ACCEPTABLE</p>	PMRA 2153602

Study Type	Species, Strain, and Doses	Results	Comments	Reference(s)
Acute Pulmonary Toxicity/ Infectivity (21-Day study)	<p>Rat- Sprague-Dawley</p> <p>15/sex, single intratracheal instillation of 0.1 mL (1.1×10^9 CFU/animal)</p> <p>Inactivated test substance control, 5/sex, single intratracheal instillation of 0.1 mL equivalent to 1.1×10^9 CFU/animal</p> <p>Untreated control (5/sex); shelf control (4/sex)</p> <p>Body weight measured on Days 0, 7, 14, 21</p> <p>Interim and final sacrifices including necropsy, organ weight and microbial enumeration on Days 3, 7, 14, 21</p>	<p>$LD_{50} > 1.1 \times 10^9$ CFU/animal</p> <p>No signs of toxicity or infectivity.</p> <p>Clearance of MPCA was achieved by Day 3</p>	<p>LOW TOXICITY</p> <p>NOT PATHOGENIC</p> <p>ACCEPTABLE</p>	PMRA 2153597
Acute Intraperitoneal Infectivity (21-Day study)	<p>Rat- Sprague-Dawley</p> <p>15/sex, intraperitoneal injection, 0.1 mL/animal (1.4×10^9 CFU/animal)</p> <p>Inactivated test substance control, 5/sex, single intraperitoneal injection of 0.1 mL equivalent to 1.4×10^9 CFU/animal</p> <p>Untreated control (5/sex)</p> <p>Body weights measured on Days 0, 7, 14 and 21</p> <p>Microbial enumeration of intraperitoneal cavity and necropsy performed on 6 animals on Day 0</p>	<p>No signs of infection or pathogenicity were observed.</p>	<p>NOT PATHOGENIC</p> <p>ACCEPTABLE</p>	PMRA 2153599

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Organisms				
Vertebrates				
Birds	Oral – <i>Colinus virginianus</i> , 14 days old	<p>Three replicates of birds (10/replicate) were gavaged with TGAI at a dose of 10 mg/kg bw (equivalent to 1.2×10^9 CFU/kg/ bw) for 5 consecutive days.</p> <p>One replicate of birds (10 /replicate) was gavaged with sterile production filtrate.</p> <p>One replicate of birds (10 /replicate) was gavaged with inactivated test substance.</p> <p>One replicate of birds (10 /replicate) was gavaged with sterile saline and Tween 20.</p> <p>Birds were observed for 30 days.</p>	<p>There were no treatment-related mortalities or effects on body weight and behaviour.</p> <p>There were no signs of pathogenicity or infectivity.</p> <p>ACCEPTABLE (Pathogenicity only)</p>	PMRA 2153622
Wild Mammals	From the data submitted under the Part M4 Human Health and Safety Testing, it was determined that the TGAI, Bio-Ceres G WP and Bio-Ceres G WB were not toxic to mammals via the oral, pulmonary or dermal routes and not infective or pathogenic via the oral, pulmonary and intraperitoneal routes. No further data are required to assess the risk of harm to non-target wild mammals.			PMRA 2153623

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Invertebrates				
Arthropods				
Terrestrial Arthropods	Dietary – <i>Chrysoperla rufilabris</i> larvae	<p>A group of larvae (30) was fed TGAI on corn earworm eggs sprayed at a concentration of 4.8×10^{11} CFU/L.</p> <p>A separate group of larvae (30) was fed inactivated TGAI on corn earworm eggs sprayed at a concentration equivalent to 4.8×10^{11} CFU/L.</p> <p>Another group of larvae (30) was fed corn earworm eggs only (Negative Control).</p> <p>Larvae were observed over a period of 25 days.</p>	<p>There were no significant differences between the untreated control, inactivated test substance, and active test substance groups for mortality, vestigial wings, non-viable pupae, time to pupation or time from pupation to emergence.</p> <p>Groups 2 and 3 consumed a significantly lower amount of food through Day 7 than the untreated control group.</p> <p>The 25-day dietary LC_{50} for <i>B. bassiana</i> strain ANT-03 for the green lacewing is $> 4.8 \times 10^{11}$ CFU/L applied directly to food.</p> <p>ACCEPTABLE</p>	PMRA 2153630
	Dietary – <i>Hippodamia convergens</i> , adults	<p>Three replicates (50/replicate) were fed TGAI on corn earworm eggs sprayed at a concentration of 4.8×10^{11} CFU/L.</p> <p>Three replicates (50/replicate) were fed inactivated TGAI on corn earworm eggs sprayed at a concentration equivalent to 4.8×10^{11} CFU/L.</p> <p>Another three replicates (50/replicate) were fed corn earworm eggs</p>	<p>The data from Days 7 – 11 clearly showed a large reduction in food consumption in the treatment group compared to the untreated control.</p> <p>Reduced feeding was likely a result of the poor general health of the treated beetles and may have been due to the pathogenicity of the test substance, a known insect pathogen.</p> <p>ACCEPTABLE MAY BE PATHOGENIC</p>	PMRA 2153629

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
		only (Negative Control). Larvae were observed over a period of 11 days.		
Plants				
Plants	The lack of toxic or pathogenic effects noted in efficacy testing of <i>B. bassiana</i> strain ANT-03 on field lettuce, strawberry, field strawberry, greenhouse tomato and cucumber, field pepper, and field celery was cited. As well, there are no reports of phytotoxicity or plant pathogenicity in the published scientific literature from <i>B. bassiana</i> (from a search of PubMed using keywords ‘Beauveria and phytotoxicity’ and ‘Beauveria and plant and pathogenicity’). Therefore, based on a weight of evidence approach, the risk to terrestrial plants from the use of <i>B. bassiana</i> strain ANT-03 as a microbial pest control agent is minimal. ACCEPTABLE			PMRA 2153632
Aquatic Organisms				
Invertebrates				
Aquatic Arthropods	Aqueous – <i>Daphnia magna</i> , <24 hours old, static renewal	Two groups (20/group) of daphnids were exposed to TGAI at concentrations of either 1.0×10^3 or 1.0×10^6 CFU/mL. A group (20) of daphnids was exposed to sterile filtrate at a concentration equivalent to the highest test substance concentration. A group (20) of daphnids was exposed to surfactant at the same rate used in the treatment groups. Negative control with 20 daphnids in untreated test culture. Test suspensions were renewed every 24 hours.	Mortality in the negative control, surfactant control and sterile filtrate control groups was 5, 45 and 17%, respectively. Mortality in the groups treated with <i>B. bassiana</i> strain ANT-03 at 1.0×10^3 and 1.0×10^6 CFU/mL was 20 and 100%, respectively. The EC ₅₀ for immobility was 4.07×10^5 CFU/mL; the EC ₅₀ for reproduction was 4.58×10^5 CFU/mL; and the EC ₅₀ for biomass was 3.30×10^5 CFU/mL. No significant decreases in survival and reproduction were observed in organisms treated with 1×10^3 CFU/mL. Significant decreases in survival, reproduction and biomass were observed in organisms treated with the surfactant, Tween 20. ACCEPTABLE PATHOGENIC AND/OR TOXIC	PMRA 2153631

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
		Observed daily for immobility and reproduction for 21 days.		
Plants				
Aquatic Plants	The lack of toxic or pathogenic effects noted in efficacy testing of <i>B. bassiana</i> strain ANT-03 on field lettuce, strawberry, field strawberry, greenhouse tomato and cucumber, field pepper, and field celery was cited. As well, there are no reports of phytotoxicity or plant pathogenicity in the published scientific literature from <i>B. bassiana</i> (from a search of PubMed using keywords ‘Beauveria and phytotoxicity’ and ‘Beauveria and plant and pathogenicity’). Therefore, the risk to aquatic plants and from the use of <i>B. bassiana</i> strain ANT-03 as an MPCA is minimal.			PMRA 2153635
ACCEPTABLE				

Table 3 Use Claims Proposed by Applicant and Whether Acceptable or Unsupported

Proposed claim	Acceptable or Unsupported?
Control of whiteflies, aphids and thrips in greenhouse cucumber, tomato and ornamentals at a rate range of 2 to 4 grams product per litre, applied at a rate of 500 to 1000 L per hectare, with applications repeated at weekly intervals, or every 3 to 5 days under high insect pressure.	<p><u>Supported as follows:</u></p> <p>Reduction in numbers of whiteflies, aphids and thrips in greenhouse cucumber, tomato and ornamentals at a rate range of 2 to 4 grams product per litre, applied at a spray volume of 500 to 1000 L per hectare, with applications repeated at weekly intervals, or every 3 to 5 days under high insect pressure.</p>

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