Proposed Registration Decision PRD2020-14

Lecanicillium muscarium strain Ve6 and Mycotal Biological Insecticide

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Overview

Proposed registration decision for *Lecanicillium muscarium* strain Ve6

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act*, is proposing registration for the sale and use of Mycotal Technical Biological Insecticide and Mycotal Biological Insecticide, containing the technical grade active ingredient *Lecanicillium muscarium* strain Ve6, for suppression of whiteflies on greenhouse tomato.

An evaluation of available scientific information found that, under the approved conditions of use, the health and environmental risks and the value of the pest control products are acceptable.

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 *L. muscarium* strain Ve6 and Mycotal Biological Insecticide.

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 Health Canada regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides section of Canada.ca.

Before making a final registration decision on *L. muscarium* strain Ve6 and Mycotal Biological Insecticide, Health Canada's PMRA will consider any comments received from the public in response to this consultation document.[3] Health Canada will then publish a Registration

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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."

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

Decision[4] on *L. muscarium* strain Ve6 and Mycotal Biological Insecticide, which will include the decision, the reasons for it, a summary of comments received on the proposed registration decision and Health Canada'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 *Lecanicillium muscarium* strain ve6?

Lecanicillium muscarium strain Ve6 is the active ingredient in the commercial class microbial product Mycotal Biological Insecticide, which suppresses whiteflies on greenhouse tomato. *Lecanicillium muscarium* strain Ve6 is a fungus that kills insects by infection and growth of hyphal bodies. It is active by contact.

Health considerations

Can approved uses of *Lecanicillium muscarium* strain Ve6 affect human health?

***Lecanicillium muscarium* strain Ve6 is unlikely to affect your health when Mycotal Biological Insecticide is used according to the label directions.**

Potential exposure to *L. muscarium* strain Ve6 may occur when handling and applying Mycotal Biological Insecticide and when ingesting treated produce. When assessing health risks, several key factors are considered:

- the microorganism's biological properties (for example, infection cycle);
- 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.

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 *L. muscarium* strain Ve6 was tested on laboratory animals, there was low toxicity following oral, inhalation and dermal exposures, and no infectivity when injected (intravenous).

Furthermore, there was no sign that the microbial pest control agent (MPCA), *L. muscarium* strain Ve6 caused any disease or genotoxic effects.

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

Residues in water and food

Dietary risks from food and water are acceptable

Residues of *L. muscarium* strain Ve6 on treated tomatoes are possible at the time of harvest. Metabolites of toxicological significance are not known to be produced by *L. muscarium* strain Ve6. Furthermore, no signs of infectivity or toxicity were observed when *L. muscarium* strain Ve6 was tested on laboratory animals. In addition, the likelihood of residues of *L. muscarium* strain Ve6 contaminating drinking water supplies from the proposed applications of Mycotal Biological Insecticide to greenhouse tomatoes is low and, therefore, not a health concern. Consequently, dietary risks are acceptable for all segments of the population, including infants, children, adults and seniors.

Risks in residential and other non-occupational environments

Estimated risk for non-occupational exposure is acceptable.

Mycotal Biological Insecticide is proposed for use on greenhouse tomatoes. The product label includes measures to prevent bystander exposure such as restricting access to the treated area for 4 hours or until sprays have dried. Residential and non-occupational exposure to Mycotal Biological Insecticide is therefore expected to be low when the label directions are observed. Consequently, the risk to residents and the general public is acceptable.

Occupational risks from handling Mycotal Biological Insecticide

Occupational risks are acceptable when Mycotal Biological Insecticide is used according to label directions, which include protective measures.

Workers handling Mycotal Biological Insecticide can come into direct contact with *L. muscarium* strain Ve6 on the skin, in the eyes or by inhalation. To protect workers from exposure to Mycotal Biological Insecticide, the label will specify that mixers, loaders and applicators must wear waterproof gloves, a long-sleeved shirt, long pants, eye goggles, a NIOSH-approved particulate filtering facepiece respirator, socks and shoes. A restricted-entry interval of 4 hours or until sprays are dried is required, and if re-entry into treated areas is necessary during this restricted-entry interval, workers must wear the above personal protective equipment (PPE). The occupational risks are acceptable when the precautionary statements on the label are observed.

Environmental considerations

What happens when *Lecanicillium muscarium* strain Ve6 is introduced into the environment?

Environmental risks are acceptable.

Lecanicillium muscarium is a common microorganism that is widely distributed in the natural terrestrial environment. It has been isolated from numerous species of insects, mites and spiders in the tropics and in temperate regions. It has also been found on decaying food and organic material and is often isolated from soil and wood.

Mycotal Biological Insecticide is a new end-use product that is proposed for use on greenhouse tomatoes and is not intended for outdoor uses. The greenhouse use of Mycotal Biological Insecticide is not expected to result in sustained increases in *L. muscarium* in terrestrial and aquatic environments beyond natural background levels. *Lecanicillium muscarium* strain Ve6 may be introduced to the environment through disposal of treated plant waste and growth media. The spores of *L. muscarium* strain Ve6 are not easily dispersed by air and are highly sensitive to UV light and desiccation. Any spores that are transferred to soil by rainwater have limited long-term persistence in this environment and do not leach into ground water. While *L. muscarium* strain Ve6 could enter aquatic environments through run-off from soil or treated plants, this microorganism should not become established in non-aerated or deep waters.

No overt adverse effects to birds, freshwater fish and honey bees were observed during testing. No evidence of significant adverse effects on birds, freshwater fish, non-target terrestrial arthropods, aquatic arthropods and terrestrial and aquatic plants were found in the published scientific literature. Also, *L. muscarium* strain Ve6 was not toxic or pathogenic to laboratory mammals through a variety of exposure routes.

Based on a critical review of studies, scientific rationales and information from public sources, no significant effects to birds, wild mammals, fish, non-target terrestrial and aquatic arthropods, and plants are expected when Mycotal Biological Insecticide is applied according to directions on the label.

Value considerations

What is the value of Mycotal Biological Insecticide?

Mycotal Biological Insecticide is a new commercial class product that provides suppression of whiteflies on greenhouse tomatoes.

Applications of Mycotal Biological Insecticide target the nymph stage of whiteflies. *Lecanicillium muscarium* strain Ve6 is a new organism for use against whiteflies on greenhouse tomatoes. Mycotal Biological Insecticide will be a valuable part of an integrated pest management (IPM) program for greenhouse tomatoes.

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 label of Mycotal Technical Biological Insecticide and Mycotal Biological Insecticide to address the potential risks identified in this assessment are as follows:

Key risk-reduction measures

Human health

All microorganisms, including *L. muscarium* strain Ve6, contain substances that are potential sensitizers and thus, sensitivity may possibly develop in individuals exposed to potentially large quantities of *L. muscarium* strain Ve6. In turn, workers handling or applying Mycotal Biological Insecticide must wear a long-sleeved shirt, long pants, protective eyewear (goggles), waterproof gloves, a NIOSH-approved particulate filtering facepiece respirator, socks and shoes. Furthermore, all unprotected workers are restricted from entering treated areas during application and for 4 hours following application or until sprays have dried.

Environment

The end-use product label will include environmental precaution statements that reduce contamination of aquatic systems from the use of Mycotal Biological Insecticide. The label for the end-use product will also include an environmental precaution statement to minimize the risk to beneficial insects and pollinators used in greenhouse IPM programs.

Next steps

Before making a final registration decision on *L. muscarium* strain Ve6 and Mycotal Biological Insecticide, Health Canada's PMRA will consider any comments received from the public in response to this consultation document. Health Canada 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). Health Canada will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed decision and Health Canada's response to these comments.

Other information

When the Health Canada makes its registration decision, it will publish a Registration Decision on *L. muscarium* strain Ve6 and Mycotal Biological Insecticide (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

Lecanicillium muscarium strain Ve6 and Mycotal Biological Insecticide

1.0 The active ingredient, its properties and uses

1.1 Identity of the active ingredient

Active mircoorganism		*Lecanicillium muscarium* strain Ve6
Function		Insecticide–For the suppression of whiteflies on greenhouse tomatoes
Binomial		*Lecanicillium muscarium* strain Ve6
Taxonomic designation[5]		
	Kingdom	Fungi
	Phylum	Ascomycota
	Class	Sordariomycetes
	Order	Hypocreales
	Family	Cordyciptaceae
	Genus	*Lecanicillium*
	Species	*Muscarium*
	Strain	Ve6
Patent Status information		None
Minimum purity of active		Technical grade active ingredient: minimum of 2×10^{10} spores/g End-use product: minimum of 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 microcontaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminant release standards. In addition, there are no known mammalian toxins or other toxic metabolites present in the technical grade active ingredient or end-use product.

[5] National Center for Biotechnology Information - Taxonomy Browser (https://www.ncbi.nlm.nih.gov/taxonomy)

1.2 Physical and chemical properties of the active ingredient and end-use product

Technical product—Mycotal Technical Biological Insecticide

Property	Result
Colour	Beige
Physical State	Solid (frozen paste)
Odour	Odourless
Viscosity	34.1 mPa
pH	4–5
Relative Density	1.011 kg/L

End-use product—Mycotal Biological Insecticide

Property	Result
Colour	Ivory
Physical State	Solid (granules)
Odour	Odourless
Viscosity	Not provided
Suspendibility (0.1% solution)	90–100%
pH (1%)	7–7.2
Relative Density	1.011 kg/L

1.3 Directions for use

Mycotal Biological Insecticide contains the entomopathogenic fungus *L. muscarium* strain Ve6 at a guarantee of 1×10^{10} spores per gram for the suppression of whiteflies on greenhouse tomatoes. It can be applied up to a maximum of 24 applications per year (12 applications per crop cycle) at a concentration of 1 g product/L with a minimum reapplication interval of 7 days. A non-ionic surfactant can be used with Mycotal Biological Insecticide at a rate of 0.02% (v/v).

1.4 Mode of action

Lecanicillium muscarium strain Ve6 is an entomopathogenic fungus, which causes disease in insects. When spores of the fungus come into contact with the cuticle of a host, they germinate, enter the body of the host and grow hyphae, eventually killing the host. It is active by contact.

2.0 Methods of analysis

2.1 Methods for identification of the microorganisms

Acceptable methodologies for detection, isolation and enumeration of the active ingredient, *L. muscarium* strain Ve6, were submitted by the applicant. The MPCA has been fully characterized with respect to its origin of strain, natural occurrence and biological properties.

Lecanicillium muscarium strain Ve6 can be identified to the species level using a combination of phenotypic and biochemical methodologies, as well as phylogenetic analysis. The identity of the MPCA to the strain level may also be confirmed by analysis using specific DNA primers.

2.2 Methods for establishment of purity of seed stock

The strain has been deposited into the CABI Genetic Resource Collection (Surrey, UK), in the Centraal Bureau Schimmelcultures (CBS), Baarn (The Netherlands) and in the United States Department of Agriculture (USDA; Ithaca, United States) Agricultural Research Service Collection of Entomopathogenic Fungi (ARSEF) under the strain identification numbers IMI 268317, and ARSEF 5128, respectively. The strain is maintained by the manufacturer in a manner sufficient to maintain purity and stability.

Acceptable methods for the establishment of the purity, viability and genetic stability of the banks were described.

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 colony-forming units (CFU) per mL. Representative data on five batches of the technical grade active ingredient and seven batches of the end-use product were submitted. The method for determining CFU counts 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, acceptable methods are available to enumerate the microorganism and to distinguish this MPCA from other *Lecanicillium* species.

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 Mycotal Technical Biological Insecticide and Mycotal Biological Insecticide 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 Mycotal Technical Biological Insecticide and Mycotal Biological Insecticide using standard methods for detecting and enumerating microbial contaminants of concern. All batches of Mycotal Technical Biological Insecticide and Mycotal Biological Insecticide conform to the limits set out in the Organisation 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

Storage stability data were provided for Mycotal Biological Insecticide. Results support a storage period of 6 months when refrigerated at 4°C.

3.0 Impact on human and animal health

3.1 Toxicity and infectivity summary

3.1.1 Testing

A detailed review of the toxicity/infectivity/pathogenicity studies was conducted in support of Mycotal Technical Biological Insecticide, and the associated end-use product, Mycotal Biological Insecticide.

The data package consisted of an acute oral toxicity/pathogenicity study, inhalation toxicity studies (acute and sub-acute), an acute intravenous (iv) injection infectivity study, an acute dermal toxicity study, and a micronucleus study. The oral and injection studies were conducted with Mycotal Technical Biological Insecticide. The test substance used for inhalation and dermal studies was a wettable powder formulation equivalent to Mycotal Biological Insecticide. The formulants contained in the wettable powder were not expected to contribute to the toxicity. The rat micronucleus study was conducted on a suspension of *L. muscarium* strain Ve6 (identified as synonym *V. lecanii* strain VE-6).

In the acute oral toxicity/pathogenicity study, groups of fasted, 5-week old Crj:CD (SD) IGS rats (14/sex) were given a single oral dose of Mycotal Technical Biological Insecticide, in phosphate buffered saline (PBS) at 1.2×10^8 spores/animal. The animals were observed for a period of up to 21 days with interim scheduled sacrifices on Days 3, 7, 14 and 21. There was no mortality in any group during the study. No treatment related clinical signs or abnormal necropsy findings were reported and all animals gained weight during the study. The test substance was confirmed to be viable and the testing facility provided acceptable methods for enumeration of the MPCA from tissues. However, it was unusual that the MPCA was never recovered from treated rats at any time point during the study. A pattern of clearance was therefore not established, and a conclusion on infectivity could not be made.

In the acute inhalation toxicity study, groups of 8–9 week old Crl:WI(WU)BR rats (5/sex), were exposed by the inhalation route to Mycotal wettable powder (1.08×10^{10} spores/g) in water for 4 hours to nose only, at a concentration of 0.893 mg/L. Animals were then observed for 14 days. Mortality occurred in one male on Day 6. The macroscopic examinations revealed red and or pale discolouration of the lungs in all rats including the male that died. In addition, incompletely collapsed lungs were noted which may be indicative of a pathological change leading to air trapped in parts of the lung.

In the sub-acute inhalation toxicity study, groups of 8–9 weeks old Crl:WI(WU)BR rats (5/sex), were exposed by the inhalation route (nose only) to Mycotal wettable powder (1.08×10^{10} spores/g in water) for 6 hours per day, 5 days a week over the course of 28 days (20 exposure days), at concentrations of 1, 10 and 100 mg/m^3 (0.001, 0.01, and 0.1 mg/L). Animals were

observed daily for 28 days. No mortality or treatment related clinical signs were noted. The necropsy examination revealed grey discoloured, spongy and or swollen lungs, and enlarged, white discoloured and or/firm mediastinal lymph nodes in several male and female animals in the 0.01 and 0.1 mg/L dose groups. The microscopic examination showed treatment related changes indicative of an inflammatory response in the respiratory tract and mediastinal lymph nodes in all treatment groups; the changes were most pronounced in the 0.01 and 0.1 mg/L dose groups. However since the changes at the low concentration were only slight, this was considered a minimum effect level.

In the acute intravenous infectivity study, groups of young adult Crj:CD (SD) IGS rats, (3 and 5/sex/group) were injected with Mycotal technical grade active ingredient (9.95×10^{10} spores/g) in PBS at a dose of 1.2×10^{7} spores per animal. Animals were then observed for up to 21 days. There was no mortality and all animals appeared normal for the duration of the study. A pattern of clearance for the MPCA was established by Day 7 for the organs and tissues sampled.

In an acute dermal toxicity study, a group of young adult New Zealand White rabbits (5/sex) were dermally exposed to Mycotal wettable powder (10^{8} spores/animal) diluted in sterile physiological saline for 24 hours. The dose was applied to a 6 cm^2 area of the body surface. Following exposure, the animals were observed for a period of 14 days for signs of toxicity and dermal irritation. No skin reactions were observed and body weight gain for test animals was not affected by the dermal administration of the test substance.

In the micronucleus study, a suspension of the MPCA (equivalent to 2.4×10^{9} spores/mL was administered (in 0.5% carboxymethylcellulose) in two consecutive daily doses by gavage, to groups of female and male Sprague Dawley rats (5/sex) at doses of 5, 10 and 20 mL/kg/day. Negative control animals were dosed in the same way with the vehicle carboxymethylcellulose. A similar group of animals were dosed by intraperitoneal injection with cycolphosamide at 25 mg/kg b.w., served as the positive controls. Animals were sacrificed 24 hours following the last administration. At study termination femurs were removed and bone marrow extracted with fetal calf serum. Cell suspensions were centrifuged for 5 minutes at 1000 rpm and the smears prepared from the centrifugate. Smears were stained using a Giemsa technique (Schimd, 1975) and slides examined for polychromatic erythrocytes, and the ratio to normochromatic erythrocytes reported. No toxicity was reported, and the MPCA was not clastogenic or aneugenic under the conditions of study.

Test results are summarized in Appendix I, Table 1.

3.1.2 Additional information

A literature search of PubMed, MEDLINE, and TOXFILE databases up to 2018 was conducted. *Lecanicillium* and other phylogenetically close species/strains in the genus *Verticillium* were used as the search words. No human pathogens are known in the genus *Lecanicillium*. However, under certain conditions naturally occurring spores of *L. muscarium* may cause hypersensitivity responses.

The results of the search uncovered no reports of adverse effects for *L. muscarium* strain Ve6. *Lecanicillium* species have, however, been implicated in human infections where patients may have had underlying health issues. All patients recovered after receiving antifungal therapy.

There was a report of hypersensitivity pneumonitis of a patient exposed to humidifier water from a humidification, ventilation and air conditioning system contaminated with *Lecanicillium* species.

Metabolite production by entomopathogens, including *Lecanicillium,* has been well documented in the scientific literature. Fungal metabolite production appears to be very dependent on the culture conditions and strain. Mammalian toxin production by *L. muscarium* strain Ve6 has not been demonstrated and is not known to be part of its pathogenicity.

3.1.3 Incident reports related to human and animal health

Lecanicillium muscarium strain Ve6 is a new active ingredient pending registration for use in Canada, and as of 1 May 2020, no incident reports have been submitted to the PMRA.

Metarhizium anisopliae strain F52 is considered to be related to *L. muscarium* strain Ve6, based on similarities in taxonomy and mode of action. Three human incidents (affecting a total of nine individuals) involving *M. anisopliae* strain F52 were reported to the PMRA. Adverse effects (for example, coughing, difficulty breathing, headache, fatigue) were reported following respiratory exposures and were determined to be at least possibly related to the pesticide. Overall, based on the low number of human incidents and the fact that exposures occurred because of improper use of PPE as required on the product label, no additional mitigation is proposed based on the incident report review.

3.1.4 Hazard analysis

The data package submitted in support of registering *L. muscarium* strain Ve6 Technical and Mycotal Biological Insecticide was reviewed from the viewpoint of human health and safety and was determined to be acceptable.

Based on all the available information, the technical grade active ingredient, Mycotal Technical Biological Insecticide, is of low toxicity by the oral route and is not pathogenic or infective by the intravenous route. There is no evidence of genotoxic effects in the micronucleus study. The end-use product, Mycotal Biological Insecticide, is of low toxicity by the inhalation and dermal routes, but may cause inflammation of respiratory tissues from repeated pulmonary exposure. The end-use product is also not irritating to the skin. In lieu of acceptable eye irritancy data, the technical grade active ingredient and end-use product label will bear the standard PPE eye protection and the hazard statements “CAUTION: EYE IRRITANT”. The MPCA is considered to be a potential sensitizer. Consequently, the hazard statement “POTENTIAL SENSITIZER” will appear on the principal display panel of the technical grade active ingredient and end-use product. The statements, “May cause sensitization. Avoid contact with skin and clothing. Avoid inhaling/breathing dusts and spray mist” and “May irritate eyes. Avoid contact with eyes” are also required on the secondary display panel of the end-use product label under the “PRECAUTIONS” section.

Higher tier subchronic and chronic toxicity studies were not required because the technical grade active ingredient was not considered to be acutely toxic by the oral 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 *L. muscarium* strain Ve6 has the potential to cause adverse effects on the endocrine system of animals. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for this MPCA.

3.2 Occupational, residential and bystander risk assessment

3.2.1 Occupational and postapplication exposure and risk

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and other handlers exists, with the primary exposure route being dermal. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Lecanicillium muscarium* has not been identified as a dermal wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Furthermore, testing with Mycotal Technical Biological Insecticide showed no toxicity via the oral route or infectivity via intravenous injection and the end-use product was not a dermal irritant. In lieu of acceptable data, the PMRA considers all microorganisms as ocular irritants, therefore the end-use product may cause eye irritation.

Although the end-use product was of low toxicity via the inhalation and dermal routes, inflammation limited to respiratory tissues was noted in the sub-acute study. Furthermore, the PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Consequently, risk mitigation measures, such as PPE, including waterproof gloves, a long-sleeved shirt, long pants, eye goggles or a face shield, a NIOSH-approved particulate filtering facepiece respirator with any N, R, or P filter, socks and shoes are required to minimize exposure and protect 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 Mycotal Biological Insecticide 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 Mycotal Biological Insecticide. Overall, occupational risks to workers are acceptable when the precautionary statements on the label are followed which include PPE.

3.2.2 Residential and bystander exposure and risk

Mycotal Biological Insecticide is proposed for use only in greenhouses. This use is not expected to result in significant residential and bystander exposure due to drift. Bystander exposure will be mitigated by the inclusion of a statement on the label, requiring all unprotected workers to remain out of treated areas until sprays have dried. Also, the end-use product is of low toxicity

via the dermal and inhalation routes and there were no signs that the MPCA, *L. muscarium* strain Ve6, caused any disease in studies on laboratory animals. Consequently, the health risks to bystanders and individuals in residential areas are acceptable.

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, the risks from consuming tomato crops treated with Mycotal Biological Insecticide are acceptable because *L. muscarium* strain Ve6 demonstrated no toxicity, pathogenicity or infectivity in Tier I studies. Furthermore, no metabolites of toxicological significance have been shown to be produced by this MPCA.

3.3.2 Drinking water

Dietary exposure from drinking water is expected to be low as the label has the necessary mitigative measures to limit contamination of drinking water from the proposed greenhouse use of *L. muscarium* strain Ve6. Also, municipal treatment of drinking water is expected to reduce the transfer of residues to drinking water and there were no harmful effects observed in Tier I acute oral toxicity testing with Mycotal Technical Biological Insecticide. Consequently, the health risks from residues of *L. muscarium* strain Ve6 in drinking water are acceptable.

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 *L. muscarium* strain Ve6 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 MPCA, including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCA and other registered microorganisms that have a common mechanism of toxicity, does not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *L. muscarium* strain Ve6 to human health.

3.3.4 Aggregate exposure and risk

Based on the toxicity and infectivity test data and other relevant information in the PMRA's files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *L. muscarium* strain Ve6 to the general Canadian population, including infants and children, when the end-use product is used as labelled. 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 low since the product is for greenhouse use only. Furthermore, few adverse effects from exposure to other strains of *L. muscarium* encountered in the environment have been reported in the public literature. Even if there is an increase in exposure to *L. muscarium* strain Ve6 from the use of Mycotal Biological Insecticide, 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.

Residues of *L. muscarium* strain Ve6 on treated greenhouse tomatoes are possible at the time of harvest. Dietary risk to humans from the proposed use of Mycotal Biological Insecticide is acceptable due to the low toxicity profile of *L. muscarium* strain Ve6 and that metabolites of toxicological significance are not known to be produced by this MPCA. In addition, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Therefore, the PMRA has determined that specification of an MRL under the *Pest Control Products Act* is not required for *L. muscarium* strain Ve6.

3.4 Cumulative assessment

The *Pest Control Products Act* requires that the PMRA consider the cumulative exposure to pesticides with a common mechanism of toxicity. In its assessment, the PMRA considers both the taxonomy of MPCAs and their production of any potentially toxic metabolites. For the current evaluation, the PMRA has determined that *L. muscarium* strain Ve6 is closely related to another species that is registered for use as an MPCA in Canada, *Metarhizium anisopliae* strain F52. These MPCAs do not produce a common toxic metabolite, and given their low toxicity and pathogenicity, the potential health risks from cumulative exposure of *L. muscarium* strain Ve6 and *M. anisopliae* strain F52 are acceptable when used as labelled.

4.0 Impact on the environment

4.1 Fate and behaviour in the environment

Although environmental fate data (Tier II/III) are not normally required at Tier I, information and data on the fate and behaviour of *L. muscarium* strain Ve6 and other closely related microorganisms were submitted as a basis of scientific rationales to waive environmental toxicology testing in non-target organisms.

Lecanicillium muscarium is a ubiquitous microorganism with a worldwide distribution on a variety of substrates in the terrestrial environment. The fungus has been isolated from numerous species of insects, mites and spiders in the tropics and in temperate regions. It also occurs as a saprophyte on foodstuffs and organic material and is commonly isolated from soil and wood. The species is a hyperparasite of phytopathogenic fungi (mostly rusts and powdery mildews), agarics and occasionally on entomogenous fungi. The greenhouse use of Mycotal Biological Insecticide on tomato plants is not expected to significantly increase the level of *L. muscarium* strain Ve6 beyond background levels. Exposure of *L. muscarium* to the outdoor environment due to the proposed greenhouse use of Mycotal Biological Fungicide will be limited to disposal of treated plant waste and growth media.

The long-term persistence of *L. muscarium* strain Ve6 introduced into the environment on treated plant waste is expected to be low based on an experimental field trial in the United States. Aerial applications of Mycotal were made to hemlock stands in Tennessee to control hemlock woolly adelgid in 2009 and 2010. The application rate was equivalent to 1×10^{12} spores/ha. An oil adjuvant, sticker, and in some cases whey protein (an additional food source before fungus came into contact with insect host), were applied in conjunction with Mycotal to increase spore longevity and survival. No *L. muscarium* strain Ve6 was isolated from hemlock needles and branches in 2015 indicating a lack of long-term environmental persistence even under idealized application conditions.

Lecanicillium muscarium is highly sensitive to UV light. Conidial suspensions of *L. muscarium* LSMA 1.08.023 were prepared ($5 \pm 2 \times 10^6$ conidia/mL) from 7 and 14-day old cultures and triplicate 200 µL droplets were placed in a Petri dish. The dishes were placed under a 312 nm UV lamp for 30 minutes corresponding to a total dose of 7.2 kJ/m^2 of UV-B light (for comparison, the UV-B in Logan, Utah in mid-summer is 25 kJ/m^2/day). At the end of UV exposure, 100 µL was taken from each droplet, placed on a piece of cellophane on water agar and incubated at 25°C. After 10–12 hours of incubation, conidial germination, in the absence of UV-B exposure, resulted in approximately 85–95% conidial germination. UV-B exposure reduced conidial germination to less than 35%. This finding is consistent with a study on the effects of UV-B on the germination rate of the closely related *L. longisporum* in which exposure to 120 minutes of UV-B light (irradiance of 452 mW/m^2), equivalent to a dose of 3.26 kJ/m^2, was lethal for the conidia.

The conidia of *L. muscarium* introduced into the environment on treated plant waste and growth media are unlikely to disperse by becoming airborne as they are clustered at the end of phialides that are covered by a slime layer. The spores are not released from conidiophores without water contact for example by rain or splashing. Released conidia would likely either dry or be transferred to the soil.

The dried conidia of the related fungus, *V. lecanii* (whether in slime-heads that become separated from their parent mycelium or washed), are inactivated in less than 24 hours at 58% relative humidity.

In soil, the half-life of *L. muscarium* strain Ve6 was found to be 4–5 days. After 4 days of incubation at 22 ± 2°C and 20–24% maximum water holding capacity of the soil, *L. muscarium* strain Ve6 counts fell rapidly to 30–40% of the applied level and then stabilized. In another study on persistence and viability, soil was artificially inoculated with 10^5 or 10^7 CFU/g of the related *L. lecanii* isolate FZ9906. Soil samples, taken to a depth of 20 cm, were taken at 10-day intervals in the first two months and monthly for the following 12 months for determination of dry weight of the soil and CFU count. Within the first 30–50 days, a rapid decrease in CFU count of approximately 90% was observed in the soil samples. The CFU count then remained relatively stable at 2–4 × 10^4 CFU/g for the following 10 months. Isolates of *L. lecanii* recovered from treated soil 14 months after inoculation remained viable in vitro and in vivo exhibiting similar colony growth, conidial yield and germination, and LC_{50} and LT_{50} values against cotton aphids as the original isolate. The CFU count then declined further to undetectable levels by 16 months after inoculation. Based on these studies, long-term persistence of *L. muscarium* strain Ve6 in soil is not expected.

In a soil percolation study, 1 × 10^7 CFU of *L. muscarium* strain Ve6 was added to the top of 30-cm deep soil columns varying in humus, sand, loam and moisture content. Water flow was adjusted to mimic the level of leaching expected by 200 mm of rainfall/day. *Lecanicillium muscarium* strain Ve6 was not recovered from the filtrate collected on Days 4, 7, 14 and 21 indicating that it will not leach into ground water.

Lecanicillium muscarium strain Ve6 may enter surface water through runoff from discarded treated waste. The available data on the viability of *L. muscarium* strain Ve6 spores in water showed that conidia in non-aerated water remain viable for up to 5 days only. In aerated water, however, more than 95% of conidia remained viable after 7 days. Although filamentous fungi such as *L. muscarium* fill a specific niche in terrestrial environments as the main decomposers and producers of humic matter and play a dominant role in the remineralization of nitrogen, they are not well suited for colonization of aquatic systems.

Overall, it is not expected that the greenhouse use of Mycotal Biological Insecticide will result in a sustained increase of *L. muscarium* strain Ve6 in outdoor terrestrial or aquatic environments beyond naturally occurring background levels.

4.2 Effects on non-target species

The 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 (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 the 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

Three studies were submitted to address the hazards of *L. muscarium* strain Ve6 to birds and honey bees. Scientific rationales were also submitted in support of waiving further testing on these non-target organisms and in lieu of testing on terrestrial arthropods and terrestrial plants. The scientific rationales were largely based on environmental fate and behaviour of *L. muscarium* as a result of the greenhouse use of Mycotal Biological Fungicide (see Section 4.1) and searches of the published literature that were supplemented with host-range or efficacy testing conducted by the applicant. A literature search of the AGRICOLA, AGRIS, BIOSIS

Previews, CAB ABSTRACTS, EMBASE, MEDLINE, SCISEARCH and TOXFILE databases up to 2018 was conducted. *Lecanicillium* and other phylogenetically close species/strains in the genus *Verticillium* were used as the search words. Data submitted under human and animal health toxicity testing were considered to assess the risk of harm to wild mammals.

The acute oral toxicity and infectivity/pathogenicity of Mycotal technical grade active ingredient (6.5×10^{10} spores/g) to 28-day old Japanese quail (*Coturnix japonica*) was assessed over 30 days. Mycotal technical grade active ingredient was administered to a total of 30 birds using a gastric catheter at a nominal dose of 10^8 spores/bird/day for 5 days. There were no mortalities or adverse effects noted throughout the 30-day observation period and all animals gained weight. The MPCA was not recovered from the tissues (kidney, brain, liver, lung, spleen, cecum on Day 30), blood (Day 30) or feces (Days 1, 2, 5, 7, 14 or 28). No abnormalities were noted at necropsy on Day 30. Significant deficiencies, however, were noted for this study. The study was conducted in 1998 and it could not be confirmed whether the test substance was produced using the same manufacturing method as that being currently proposed for the registration of Mycotal Technical Biological Insecticide. Also, the dose administered to Mycotal-treated birds did not meet the MCC (calculated to be at least 7.7×10^9 spores/day for the smallest quail in study) and the viability of the MPCA was not confirmed by the test facility nor was a certificate of analysis provided. Furthermore, the method for recovery of the MPCA from tissues, blood and feces was not validated and the lack of recovery from the feces at any timepoint is unusual. At the dose administered, the test substance does not appear to be toxic to birds via the oral route. No conclusions can be made on the infectivity potential of *L. muscarium* strain Ve6 in birds.

In a 72-hour oral toxicity study, honey bees (*Apis mellifera carnica*) were exposed to Mycotal technical spore powder at measured doses of 1.30, 2.80, 6.02, 13.44, 28.17 and 112.32 µg a.i./bee. Observations were made for mortality and abnormal behaviours. The study was terminated when mortality in the control group exceeded 10% after 96 hours. At 72 hours, the control-corrected mortalities in the 1.30, 2.80, 6.02, 13.44, 28.17 and 112.32 µg a.i./bee treatment groups were 4.3, 0.0, 8.5, 8.5, 0.0 and 8.5 %, respectively. The relatively low (less than or equal to 8.5%) and inconsistent corrected mortalities at the 72-hour timepoint indicate that they were not treatment-related but rather a result of test variability amongst the treatment groups. A no observed effect concentration (NOEC) was not established due to the variable nature of the mortality data.

In a 72-hour contact toxicity study, honey bees (*A. mellifera carnica*) were exposed to a suspension of Mycotal technical spore powder (100.00 µg a.i./bee). Observations were made for mortality and abnormal behaviours. The study was terminated at the 96-hour timepoint when mortality in the control group exceeded 10%. No treatment-related mortality was observed after 72 hours.

Although the honey bee oral toxicity and contact toxicity studies were conducted according to guidelines for assessing toxicity, they are of limited utility in the assessment of risk to honey bees. The oral and contact toxicity studies were conducted in 2000 and it could not be confirmed whether the manufacturing method used to produce the test substance is the same as that being currently proposed for Mycotal Technical Biological Insecticide. Furthermore, the pathogenicity potential of the active ingredient could not be determined as viability of the test substance was

not determined by the Sponsor or the test facility and the short study duration did not allow for infectivity to be assessed. Moreover, for unspecified periods of time, the relative humidity in the oral and contact toxicity studies was below the 70% relative humidity required for germination.

To waive additional testing on honey bees, two bee assays were submitted. In the first bee assay, Mycotal (10^{10} spores/g) was either dusted immediately onto a bumblebee (*Bombus terrestris*) brood (1 g/colony) or mixed with pollen (1 g in 80 g of pollen which is enough to support about 2.5 weeks of colony growth). Three colonies each with approximately 20 emerged workers were used in each of the dust and pollen exposure treatments. The bumblebee colonies were maintained in the laboratory at 28°C and 65% relative humidity (relative humidity in nestboxes was reported to be approximately 10% higher although this value did not seem to have been directly measured). Mycotal did not cause any mortality in the adult bumblebees, larvae or pupae over a period of 2.5 weeks. There was no obvious impact on colony development except that dusted and pollen-exposed colonies showed some tendency for earlier production of young queens. Both the adults and brood in the colonies were covered with a visible layer of powder after treatment that was later cleaned away by the bees and became integrated into the wax.

Nevertheless, no fungal growth was observed in the nestbox, brood or on the adults. The relative humidity, however, may have been too low for the spores to germinate. There was no control included in the study to demonstrate germination of the MPCA under the conditions of the test.

In the second bee assay (unspecified species) where the potential of using Mycotal for the control of varroa mites was investigated, a 50-mL Mycotal suspension (1.5×10^6 spores/mL) was sprayed over the frames and bees of a colony. A total of 7 colonies were treated. Temperature in the hives was approximately 35°C and slightly lower outside the breeding nest. Humidity was not recorded but was inferred to be low. The study was conducted over a period of 49 days. Mycotal had no effect on bee mortality in the treated colonies. The mortalities among bees in the Mycotal treated colonies were, in fact, lower than in the control colonies. These results were likely due to the low humidity levels and the high temperatures in the hives. Given that Mycotal previously demonstrated good efficacy (98% mortality) against varroa mites in laboratory studies when temperature was maintained at 25°C and high relative humidity, the lack of effects on varroa mites is indicative that the environmental conditions in the hives were not conducive for germination of spores.

In contrast to the findings in the bee assays, a German review paper included a report of a laboratory study in which honey bees were exposed to Mycotal at 10× the recommended application rate either through direct spraying or by incorporation into food (no other details provided). Significant treatment-related bee mortality of 15% was observed for both exposure routes compared with the control.

A number of studies on the compatibility of *V. lecanii* and beneficial arthropods were submitted to waive testing on other non-target arthropods. In one study, 3 strains of commercially manufactured and formulated *V. lecanii*, including strain CBS456.82 (synonym for *L. muscarium* strain Ve6) and strain CBS455.82 (synonym for strain VE2) were suspended in tap water at 2.5 g/L (concentration of MPCA not indicated). The suspensions were applied by fine mist to *Blatella germanica* (German cockroach; adult), *Encarsia formosa* (hymenopterous parasite of

greenhouse whitefly; adult), *Phytoseiulus persimilis* (predatory mite of two-spotted spider mite; adult) and *Pieris brassicae* (cabbage butterfly; 3rd instar). Treated insects were maintained at 18–22°C and at either 50–70% or greater than 90% relative humidity. Observations for mortality were made 5 days and 28 days after application of the test solutions. No mortalities were noted among the *B. germanica* or *P. brassicae*. At 90% relative humidity, mortalities in the range of 6–15% were observed on Day 5 among *E. formosa* and *P. persimilis* (12.5% and 6% for strain CBS456.82 specifically). Reproduction of *E. formosa* and *P. persimilis* between Days 5 and 28 resulted in lower and unreliable mortality rates being recorded on Day 28. No mortalities occurred for any of the treated insects at 50–70% humidity.

A second study found that *V. lecanii* (3.6×10^7 spores/mL) and *E. formosa* can co-exist on the same crop and the combination provided better control of whiteflies than *V. lecanii* alone. Low toxicity (less than 10% mortality) was observed among the *E. formosa*. This finding is consistent with a report in which a synergistic effect was observed when Mycotal was used in conjunction with the thrips predator *Amblyseus cucumeris* for the control of western flower thrips on greenhouse chrysanthemums. A review paper also noted that, in a greenhouse setting, the dispersal of conidia by air movement is unlikely. Instead, conidia are dispersed by live insects and mites including predatory mites and parasitic wasps used in IPM programs. The paper notes that *V. lecanii* can occasionally be found infecting adult *E. formosa*, but the impact on the wasp population is very limited.

In another study, two strains of *V. lecanii* were used in bioassays against 20 non-target terrestrial arthropod species in the orders Coleoptera (larvae and adults), Collembola (adults), Hymenoptera (adults), Diptera (adults and larvae), Neuroptera (larvae), Dermaptera (adults), Lepidoptera (larvae) and Isoptera (adults) as well as a spider *Erigone* species and a woodlouse *Oniscus* species. The non-target arthropods were placed in Petri dishes on wet filter paper in groups of 10–20 and sprayed with 3 mL of spore suspension (10^7 spores/mL). The non-target arthropods were then individually placed into perspex containers with damp filter paper and feed supply before being incubated at 25°C and 16:8 light:dark cycle. Observations were made daily for 7–14 days. Mortalities were low (less than10%) in all bioassays conducted on non-target arthropods while concurrent bioassays on six positive control aphid species resulted in 100% mortality by Day 7.

A number of laboratory tests were conducted in which beneficial organisms (larvae and/or adults) including *Trichogramma cacoeciae*, *E. formosa*, *Aphidius matricariae*, *P. persimilis*, *Typhlodromus pyri*, *Chrysoperla carnea*, *Forficula auricularia* and *Semiadalia 11-notata* were exposed to Micro Germin Plus at the highest recommended application rate (equivalent of 4 kg/ha). Micro Germin Plus contains two *Lecanicillium* isolates: 1-72 (synonym for *L. longisporum* VE2) and VT1 which is closely related to *L. muscarium*. Mortalities were all less than 25% in these laboratory tests. Semi-field trials on *C. carnea* larvae and adult *P. persimilis* also resulted in less than 25% mortality when Micro Germin Plus was applied at 4 kg/ha. Field trials on *T. pyri*, however, resulted in 25–50% mortality.

Effects of various isolates of *L. muscarium* on whitefly *Bemisia tabaci* (Gennedius), a predatory beetle *Serangium japonicum*, and a parasitic wasp *Eretmocerus* sp. nr *furuhashii* were evaluated. The four isolates of *L. muscarium* showed significant pathogenicity against third instar

whiteflies. The susceptibility of *S. japonicum* to infection by *L. muscarium* was not significant, but infection may have been an important factor in predator mortality. Results suggest that both *L. muscarium* and *S. japonicum* can be combined for IPM of the whitefly *B. tabaci*, but the fungal spores should be timed to coincide with the less susceptible later developmental stages of the predators as much as possible. *Lecanicillium muscarium* showed some effect on emergence and survivorship of the parasitoid wasp particularly with increasing concentrations of the fungal spores.

Finally, *V. lecanii* blastospores were applied to a severe whitefly infestation in greenhouses. A second application was made 19 days later. Releases of *E. formosa* 2, 3 and 4 weeks after the second spray achieved progressive whitefly control. *Encarsia formosa* emerged from over 90% of the parasitized whitefly larvae demonstrating that it was unaffected by *V. lecanii*. In laboratory conditions, under almost saturated conditions unobtainable in greenhouses, however, emergence of *E. formosa* from young whitefly larvae sprayed with blastospores was reduced to only 10–20%.

The rate of parasitoid emergence from older larvae and unsprayed larvae was greater than 50%. A high level of mortality (82%) was also observed when beneficial *Nabis alternatus* (damsel bug) were dipped in a spore suspension of *V. lecanii* and, therefore, caution was advised in combining the use of both biocontrol methods in an IPM program.

Taken together, the cited terrestrial arthropod studies indicate that beneficial insects within greenhouses, where relative humidity is maintained at high levels and temperature is optimized, may be impacted by *L. muscarium* strain Ve6. Although the hazards to non-target arthropods may not be significant, under certain conditions, they cannot be excluded. Therefore, the label for Mycotal Biological Insecticide must include a statement alerting users of the potential hazard to beneficial insects used in greenhouse IPM programs and to avoid direct applications to beneficial insects or when bees are actively foraging in the treatment area.

As part of another rationale to waive non-target plant testing, five greenhouse trials on tomato and cucumber plants were conducted to determine the efficacy of Mycotal Biological Insecticide (end-use product being proposed for registration) or Mycotal WP Insecticide (a previous end-use product formulation). Observations for phytotoxicity/phytopathogenicity were included in each trial. No adverse effects or signs of phytotoxicity were reported amongst treated plants. Mycotal has been registered in Denmark, Finland, the Netherlands, Italy, Spain and the United Kingdom with no reports of adverse effects to plants. In addition to the efficacy trials, a study examining the antagonistic effect of *V. lecanii* on cucumber powdery mildew showed that cells of *V. lecanii* were unable to penetrate the plant epidermis despite evidence of a series of changes ranging from increased vaculoation to complete necrotization of the haustorial lobes formed by the powdery mildew.

In mammalian studies conducted to satisfy the human health and safety requirements, it was determined that *L. muscarium* strain Ve6 is of low toxicity following oral, inhalation, and dermal routes of exposure, and not pathogenic when injected intravenously. Furthermore, metabolites of toxicological significance are not known to be produced by *L. muscarium* strain Ve6.

Based on all the available information on the biological properties of *L. muscarium*, the lack of or minimal documented effects in non-target terrestrial organisms and the anticipated minimal environmental exposure resulting from the use of Mycotal Biological Insecticide in a greenhouse setting, the risks to birds, wild mammals and terrestrial plants are acceptable when the product is used according to the label directions. While adverse effects to non-target beneficial arthropods present in greenhouse could occur under certain circumstances, the label statement alerting users of the potential hazard to beneficial insects used in greenhouse IPM programs should limit these effects. Furthermore, the formulants are not expected to contribute to potential toxicity of the products.

Test results are summarized in Appendix 1, Table 2.

4.2.2 Effects on aquatic organisms

One study was submitted to address the hazards of *L. muscarium* strain Ve6 to freshwater fish. Scientific rationales were also submitted in support of waiving further testing on these non-target aquatic organisms and in lieu of testing on aquatic plants. The scientific rationales were largely based on environmental fate and behaviour of *L. muscarium* as a result of the greenhouse use of Mycotal Biological Insecticide (see Section 4.1) and searches of the published literature that were supplemented with host-range or efficacy testing conducted. A literature search of the AGRICOLA, AGRIS, BIOSIS Previews, CAB ABSTRACTS, EMBASE, MEDLINE, SCISEARCH and TOXFILE databases up to 2018 was conducted. *Lecanicillium* and other phylogenetically close species/strains in the genus *Verticillium* were used as the search words.

In a 96-hour toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to water containing VE6-58 SSP, a technical grade product containing *L. muscarium* strain Ve6, at measured starting concentrations of 6.5–7.9 $\times$ 10^6 (20 fish), 1.6 $\times$ 10^5 (10 fish) and 3.6 $\times$ 10^4 CFU/mL (10 fish) of water, respectively, under static conditions. There were no mortalities or signs of adverse effects in any of the fish tested. The initial measured concentration of active substance for the fish exposed to the highest dose met the 1000-fold estimated environmental concentration (EEC) requirement based on the minimum application rate but did not meet the requirement when using the maximum application rate. Under the conditions of the test there was an issue with poor miscibility of the test suspension in aquaria water. Moreover, the actual concentration of active substance that fish were exposed to may have been lower than the measured concentrations as each sample was thoroughly mixed to break up cell aggregates before plating. Although this study was scientifically valid, it did not fully address the requirements for freshwater fish testing. The study was conducted in 1983 and it could not be confirmed that the manufacturing process used to produce the test substance is the same as that being currently proposed for registration of Mycotal Technical Biological Insecticide. Furthermore, the study did not include dietary exposure of the test substance through incorporation into feed. Finally, the short duration of the study did not allow for an assessment of infectivity or pathogenicity.

In a scientific rationale to waive additional fish testing, there were no reports in the scientific literature of adverse toxicological, infective or pathogenic effects in freshwater fish following exposure to *L. muscarium* or *V. lecanii*. Reference was made to a German review paper which

reported a case of swimming bladder infection in Atlantic salmon (*Salmo salar*) in a Finnish fish farm that was attributed to a species of *Lecanicillium*. The death rate among salmon was 0.1%. The review paper noted, however, that pathogenicity was not confirmed following Koch's postulate.

Host-range testing was submitted to waive testing on aquatic arthropods. Three strains of commercially manufactured and formulated *V. lecanii*, including strain CBS456.82 (synonym for *L. muscarium* strain Ve6) and strain CBS455.82 (synonym for strain VE2) were suspended in tap water at 0.25 g/L (concentration of MPCA not indicated). Twenty *Aedes aegypti* (yellow fever mosquito) 3rd instar larvae were added to each of the solutions. Solutions prepared with autoclaved *V. lecanii* strain CBS455.82 served as the controls. Post-emergence, treated insects were maintained at 18–22°C and at either 50–70% or >90% relative humidity. Observations for mortality were made 5 days and 28 days after application of the test solutions. No signs of infection or mortalities were noted among the *A. aegypti* at either humidity level. All larvae pupated and adults emerged by Day 28. No infection or mortality was seen in the autoclaved control.

The scientific rationale to waive testing for aquatic plants relied on the same efficacy trials and study that were used to waive testing on terrestrial plants.

Based on all the available information on *L. muscarium* strain Ve6, the lack of documented effects in non-target aquatic organisms and the anticipated minimal environmental exposure resulting from the greenhouse use of Mycotal Biological Insecticide, the risks to aquatic organisms are acceptable. Furthermore, the formulants are not expected to contribute to potential toxicity of the end-use product.

Test results are summarized in Appendix 1, Table 2.

4.3 Incident reports related to the environment

Lecanicillium muscarium strain Ve6 is a new active ingredient pending registration for use in Canada. As of 1 May 2020, no incident reports were submitted to the PMRA.

5.0 Value

The results from two trials demonstrated suppression of whiteflies on greenhouse tomatoes. Two scientific articles also demonstrated a reduction in numbers of whiteflies on greenhouse tomatoes when Mycotal Biological Insecticide was used with a non-ionic surfactant. Mycotal Biological Insecticide is a new non-conventional product containing a novel organism as an active ingredient to suppress whiteflies on greenhouse tomatoes. A concentration of 1 g product/L was supported for use on greenhouse tomatoes.

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, in other words, those that meet all four criteria outlined in the policy: 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*. The *Pest Control Products Act* requires that the TSMP be given effect in evaluating the risks of a product.

During the review process, Mycotal Technical Biological Insecticide and Mycotal Biological Insecticide were assessed in accordance with the PMRA Regulatory Directive DIR99-03[6] and evaluated against the Track 1 criteria. Mycotal Technical Biological Insecticide and Mycotal Biological Insecticide do not meet the Track 1 criteria because the active ingredients are biological organisms and hence are not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.

6.2 Formulants and contaminants of health or environmental concern

During the review process, contaminants in the technical as well as 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.*[7] The list is used as described in the PMRA Notice of Intent NOI2005-01[8] and is based on existing policies and regulations including: DIR99-03; and DIR2006-02[9] 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:

- Technical grade Mycotal Technical Biological Insecticide does not contain any formulants or contaminants identified in the *List of Pest Control Product Formulants of Health or Environmental Concern*. The end-use product, Mycotal Biological Insecticide, contains the allergens, milk and soy, which are on the *List of Pest Control Product Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions*.

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

7 SI/2005-114

8 NOI2005-01, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.*

9 DIR2006-02, *Formulants Policy and Implementation Guidance Document.*

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

7.0 Summary

7.1 Methods for analysis of the microorganism as manufactured

The product characterization data for Mycotal Technical Biological Insecticide and Mycotal Biological Insecticide were adequate to assess their potential human health and environmental risks. The technical grade active ingredient was fully characterized and the specifications of the technical grade active ingredient and end-use product were supported by the analyses of a sufficient number of batches. All batches of Mycotal Technical Biological Insecticide 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 6 months for Mycotal Biological Insecticide.

7.2 Human health and safety

The acute toxicity and pathogenicity/infectivity studies and other relevant information submitted in support of Mycotal Technical Biological Insecticide and Mycotal Biological Insecticide were determined to be acceptable. Based on all the available information, *L. muscarium* strain Ve6 is of low toxicity by the oral, dermal and inhalation routes and is not infective via intravenous injection. There is also no evidence of genotoxic effects in the micronucleus study. The end-use product, Mycotal Biological Insecticide, is not irritating to skin. The MPCA is considered to be a potential sensitizer. The signal words, "POTENTIAL SENSITIZER" are required on the principal display panel of the technical grade active ingredient as well as the precautionary statements: "May cause sensitization" and "Avoid contact with skin and clothing. Avoid inhaling/breathing dusts and spray mist".

In the absence of data, the PMRA considers all microorganisms as mild ocular irritants. The signal words "AUTION-EYE IRRITANT" are required on the principal display panel of the end-use product, as well as the precautionary statements: "May irritate eyes. Avoid contact with eyes".

When handled according to 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. 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 Mycotal Biological Insecticide must wear waterproof gloves, a long-sleeved shirt, long pants, eye goggles or a face shield, a NIOSH-approved particulate filtering facepiece respirator with any N, R, or P filter, socks and shoes are required to minimize exposure and protect 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 Mycotal Biological Insecticide has been applied for 4 hours or until the sprays have dried. Precautionary statements (for example, wearing of PPE) on the end-use product label aimed at mitigating exposure are considered adequate to protect individuals from risk due to occupational exposure.

The health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is not expected due to the low toxicity/pathogenicity profile for Mycotal Technical Biological Insecticide and Mycotal Biological Insecticide. The specification of an MRL under the *Pest Control Products Act* is not required for *L. muscarium* strain Ve6.

7.3 Environmental risk

The non-target organism tests and scientific rationales based on environmental fate and behaviour data along with supporting scientific literature submitted in support of *L. muscarium* strain Ve6 were determined to be acceptable. The greenhouse use of Mycotal Biological Insecticide containing *L. muscarium* strain Ve6 is not expected to pose a risk to non-target organisms when the directions for use on the label are followed. The greenhouse use of Mycotal Biological Insecticide on tomato plants is not expected to result in sustained increases of *L. muscarium* strain Ve6 in terrestrial and aquatic environments.

As a general precaution, the end-use product label will include environmental precaution statements to reduce contamination of aquatic systems from the use of Mycotal Biological Insecticide. The label for the end-use product will also include an environmental precaution statement to minimize the risk to beneficial insects and pollinators used in greenhouse IPM programs.

7.3 Value

The submitted value information supports the use of Mycotal Biological Insecticide for the suppression of whiteflies on greenhouse tomatoes when applied at a concentration of 1 g product/L. Mycotal Biological Insecticide would provide a new organism which provides a new non-conventional active ingredient for use on greenhouse tomatoes.

8.0 Proposed regulatory decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act*, is proposing registration for the sale and use of Mycotal Technical and Mycotal Biological Insecticide, containing the technical grade active ingredient *L. muscarium* strain Ve6, for suppression of whiteflies on greenhouse tomato.

An evaluation of available scientific information found that, under the approved conditions of use, the health and environmental risks and the value of the pest control products are acceptable.

List of abbreviations

°C	degree(s) Celsius
µg	micrograms
a.i.	active ingredient
ADI	acceptable daily intake
ARfD	acute reference dose
ARSEF	Agricultural Research Service Collection of Entomopathogenic Fungi
CAS	Chemical Abstracts Service
CBS	Centraal Bureau Schimmelcultures
CFU	colony-forming units
cm	centimetres
DNA	deoxyribonucleic acid
EC_{50}	effective concentration on 50% of the population
EEC	estimated environmental concentration
g	gram
ha	hectare(s)
IPM	Intergrated Pest Management
IUPAC	International Union of Pure and Applied Chemistry
iv	intravenous
kg	kilogram
kJ	kilojoule
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LOC	level of concern
m^2	square metre
mg	milligram
mL	millilitre
mm	millimetre
MCC	maximum challenge concentration
MPCA	microbial pest control agent
MRL	maximum residue limit
mW	milliwatt
NOEC	no observed effect concentration
NOEL	no observed effect level
OECD	Organisation for Economic Co-operation and Development
PBS	phosphate buffered saline
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
RQ	risk quotient
TSMP	Toxic Substances Management Policy
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
UV	ultraviolet
UV-B	ultraviolet-B
v/v	volume per volume dilution

Appendix I Tables and figures

Table 1 Toxicity profile of Mycotal Technical and Mycotal Biological Insecticide

Study Type/Animal/PMRA#	Study Results
21-day Acute Oral Toxicity/Pathogenicity Sprague Dawley rat PMRA# 2868465	Oral LD_{50} (males and females) greater than 1.2×10^8 spores of *L. muscarium* strain Ve6/rat **LOW Toxicity**
21-day Acute Intravenous Injection Infectivity Sprague Dawley albino rat PMRA# 3089729	**Not pathogenic/infective when injected at 3.0×10^9 CFU /rat**
14-day Acute Inhalation Toxicity[1] Sprague Dawley rat PMRA# 2868466	LC_{50} greater than 0.893 mg/L **LOW Toxicity**
28-day Sub-Acute Inhalation Toxicity[1] Sprague Dawley rat PMRA# 2868467	No mortality occurred. Grey discoloured, spongy and or swollen lungs, and enlarged, white discoloured and or/firm mediastinal lymph nodes were noted in several male and female animals in the 0.01 and 0.1 mg/L dose groups. There were treatment related changes indicative of an inflammatory response in the respiratory tract and mediastinal lymph nodes in all treatment groups. **NOEL= 0.001 mg/L**
14-day Acute Dermal Toxicity 24 h exposure[1] New Zealand Rabbit PMRA# 2942115	The acute dermal LD_{50} was greater than 1×10^8 spores/animal in male and female rats. **LOW Toxicity**
Micronucleus study Sprague Dawley rat PMRA# 2908573	No toxicity or increases in revertant numbers were observed. **Not Genotoxic**

Table 2 Toxicity/Pathogenicity of Mycotal to Non-Target Species

Organism	Exposure	Significant Effect, Comments
Terrestrial Organisms		
Vertebrates		
Birds		
Japanese Quail (*Coturnix japonica*), 28-day-old	10^8 spores/bird/day for 5 days (nominal) – Oral exposure Mycotal technical grade active ingredient containing *L. muscarium* strain Ve6	There were no mortalities or adverse effects. There was no recovery of the MPCA from tissues, blood or feces. No abnormalities at necropsy. The 30-day acute oral LD_{50} of Mycotal technical grade active ingredient to the quail was greater than 10^8 spores/bird/day for 5 days. The 30-day NOEL of Mycotal technical grade active ingredient was also greater than 10^8 spores/bird/day for 5 days. **LOW TOXICITY** at the dose administered. No conclusions can be made on infectivity or pathogenicity. **SUPPLEMENTAL**
Invertebrates		
Arthropods		
Honey bee (*Apis mellifera* L.), 22–32-day-old	Measured doses of 1.30, 2.80, 6.02, 13.44, 28.17 and 112.32 µg a.i./bee in 50% sucrose/water solution – Dietary exposure Mycotal technical spore powder	Study was terminated at 72 hours as the mortality in control group at 96 hours exceeded 10%. Corrected mortalities at 72 hours ranged from 0–8.5% but no dose-response relationship observed. The 72-hour acute oral LD_{50} of Mycotal technical spore powder to honey bees was greater than 112.32 µg a.i./bee. NOEC could not be established. **LOW TOXICITY** under the conditions of the study. No conclusions can be made on pathogenicity. **SUPPLEMENTAL**
Honey bee (*Apis mellifera* L.), 22–32-day-old	100 µg a.i./bee in 4µL water – Contact exposure Mycotal technical spore powder	Study was terminated at 72 hours as the mortality in control group at 96 hours exceeded 10%. At 72 hours, mortality in the treatment groups (6%) did not exceed the mortality in the control group (8%). No treatment-related mortality. The 72-hour acute contact LD_{50} of Mycotal technical spore powder to honey bees was greater than100 µg a.i./bee. **LOW TOXICITY** under the conditions of the study. No conclusions can be made on pathogenicity. **SUPPLEMENTAL**

Organism	Exposure	Significant Effect, Comments
Aquatic Organisms		
Vertebrates		
Fish		
Rainbow Trout (*Oncorhynchus mykiss*), <1 year	Aquatic exposure: 3.6×10^4, 1.6×10^5 and $6.5–7.9 \times 10^6$ CFU/mL (measured) Static renewal 96 hours VE6-58 SSP (synonym of Mycotal technical grade active ingredient)	There were no mortalities or adverse effects observed in fish at any dose. The 96-hour aquatic exposure LC_{50} and EC_{50} values of VE6-58 SSP to fish were greater than $6.5–7.9 \times 10^6$ CFU/mL. The NOEC value was $6.5–7.9 \times 10^6$ CFU/mL. **LOW TOXICITY** under the conditions of this study. No conclusions can be made on infectivity or pathogenicity. **SUPPLEMENTAL**

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B. Additional information considered

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