Proposed Registration Decision PRD2018-17

Bacillus amyloliquefaciens strain F727 and MBI-110 EP Biofungicide

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Publications
Pest Management Regulatory Agency
Health Canada
2720 Riverside Drive
A.L. 6607 D
Ottawa, Ontario K1A 0K9

Internet: canada.ca/pesticides
hc.pmra.publications-arla.sc@canada.ca
Facsimile: 613-736-3758
Information Service:
1-800-267-6315 or 613-736-3799
hc.pmra.info-arla.sc@canada.ca

Canada

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Overview

Proposed Registration Decision for *Bacillus amyloliquefaciens* strain F727

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing registration for the sale and use of MBI-110 TGAI and MBI-110 EP Biofungicide, containing the technical grade active ingredient *Bacillus amyloliquefaciens* strain F727, for use on cucurbit crops, grapes, legume vegetables, canola, sunflower, and potatoes to control or suppress various diseases.

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 *Bacillus amyloliquefaciens* strain F727 and MBI-110 EP Biofungicide.

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 *Pest Control Products Act* also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment. These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides section of Canada.ca.

Before making a final registration decision on *Bacillus amyloliquefaciens* strain F727 and MBI-110 EP Biofungicide, 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 *Bacillus amyloliquefaciens* strain F727 and MBI-110 EP Biofungicide, 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 *Bacillus amyloliquefaciens* strain F727?

Bacillus amyloliquefaciens strain F727 is a bacterium that colonizes plant root hairs, leaves and other plant surfaces, preventing the establishment of grey mould and powdery mildew on cucurbit crops, grapes, legume vegetables, canola, sunflower, and potatoes. It also produces compounds such as proteins that inhibit mycelial growth and spore germination.

Health Considerations

Can Approved Uses of *Bacillus amyloliquefaciens* strain F727 Affect Human Health?

***Bacillus amyloliquefaciens* strain F727 is unlikely to affect your health when MBI-110 EP Biofungicide is used according to the label directions.**

Potential exposure to *Bacillus amyloliquefaciens* strain F727 may occur when handling and applying MBI-110 EP Biofungicide 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 *Bacillus amyloliquefaciens* strain F727 was tested on laboratory animals, there was no sign that it caused any significant toxicity or disease.

[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 not of concern

Residues of *Bacillus amyloliquefaciens* strain F727 on the treated crops are possible at the time of harvest. While *Bacillus amyloliquefaciens* and its close relative, *Bacillus subtilis*, are abundant in nature, only a few cases involving foodborne illness in people have been reported and only with isolates that are able to produce a toxin which is not known to be produced by *Bacillus amyloliquefaciens* strain F727. Since its registration on food crops in 2016 in the United States there have been no foodborne illnesses reported for *Bacillus amyloliquefaciens* strain F727, and no signs of infectivity or toxicity were observed when *Bacillus amyloliquefaciens* strain F727 was tested on laboratory animals. In addition, the likelihood of residues of *Bacillus amyloliquefaciens* strain F727 contaminating drinking water supplies from the proposed spray applications of MBI-110 EP Biofungicide on food crops is not expected and therefore not a health concern. Consequently, dietary risks are not expected.

Risks in Residential and Other Non-Occupational Environments

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

MBI-110 EP Biofungicide is only for use on agricultural field crops, and the product label includes mitigation measures to prevent bystander exposure. Consequently, it is unlikely that adults, youths and toddlers will be exposed to *Bacillus amyloliquefaciens* strain F727. Even in the event of exposure, risk to the general population is not a concern since there were no signs that it caused any significant toxicity or disease in studies on laboratory animals.

Occupational Risks From Handling MBI-110 EP Biofungicide

Occupational risks are not of concern when MBI-110 EP Biofungicide is used according to label directions, which include protective measures.

Workers handling MBI-110 EP Biofungicide can come into direct contact with *Bacillus amyloliquefaciens* strain F727 on the skin, in the eyes or by inhalation. For this reason, the product label will specify that workers must wear personal protective equipment, including waterproof gloves, a long-sleeved shirt, long pants, eye goggles, a particulate filtering respirator, and shoes with socks. In addition, all unprotected workers are restricted from entering areas during application and for 4 hours following application or until all sprays have dried.

Environmental Considerations

What Happens When *Bacillus amyloliquefaciens* strain F727 Is Introduced Into the Environment?

Environmental risks are not of concern.

Information on the environmental fate of *Bacillus amyloliquefaciens* strain F727 suggests that, as a soil microorganism, it is likely to readily survive after applications of MBI-110 EP Biofungicide to agricultural field crops, but that over time its population should return to naturally sustainable levels.

There are no published reports of disease associated with natural populations of *Bacillus amyloliquefaciens* in birds, wild mammals, fish, terrestrial and aquatic arthropods, terrestrial and aquatic non-arthropod invertebrates, or terrestrial and aquatic plants. Also, the applicant submitted studies designed to examine the effects of *Bacillus amyloliquefaciens* strain F727 to birds, bees, terrestrial and aquatic arthropods, fish, and terrestrial plants. No adverse effects were observed in birds, bees, or terrestrial plants. There were toxic effects noted in fish and daphnids at the highest concentrations tested and toxicity/pathogenicity to one species of insect; however, these effects occurred at levels that exceed expected exposure levels when MBI-110 EP Biofungicide is used according to the label.

Based on a critical review of registrant-submitted studies and information from public sources, no significant effects to birds, wild mammals, fish, terrestrial and aquatic arthropods, terrestrial and aquatic non-arthropod invertebrates and plants are expected when MBI-110 EP Biofungicide is applied according to directions on the label.

Value Considerations

What Is the Value of MBI-110 EP Biofungicide?

MBI-110 EP Biofungicide is a biological fungicide containing *Bacillus amyloliquefaciens* strain F727 that controls or suppresses downy mildew, black rot, white mould, and pink rot in certain crops.

MBI-110 EP Biofungicide is effective when applied as a foliar spray to control or suppress grey mould and powdery mildew on cucurbit crops, grapes, legume vegetables, canola, sunflower, and potatoes. MBI-110 EP Biofungicide will contribute to an integrated disease management program as it provides an additional option for disease management in these crops, particularly for organic producers.

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 MBI-110 EP Biofungicide to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

All microorganisms, including *Bacillus amyloliquefaciens* strain F727, contain substances that are potential sensitizers and thus, respiratory and dermal sensitivity may possibly develop in individuals exposed to potentially large quantities of *Bacillus amyloliquefaciens* strain F727. In turn, workers handling or applying MBI-110 EP Biofungicide must wear waterproof gloves, a long-sleeved shirt, long pants, eye goggles, a particulate filtering respirator, and shoes with socks. Furthermore, all unprotected workers are restricted from entering treated areas during application and for 4 hours following application or until sprays have dried.

A standard drift statement is also required on the MBI-110 EP Biofungicide label to minimize the potential for drift to areas of human habitation or areas of human activity such as houses, cottages, schools and recreational areas.

Environment

The end-use product label will include standard environmental precaution statements to prohibit aerial application, limit drift and reduce contamination of aquatic systems from the use of MBI-110 EP Biofungicide.

Next Steps

Before making a final registration decision on *Bacillus amyloliquefaciens* strain F727 and MBI-110 EP Biofungicide, 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 Health Canada makes its registration decision, it will publish a Registration Decision on *Bacillus amyloliquefaciens* strain F727 and MBI-110 EP Biofungicide (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

Bacillus amyloliquefaciens strain F727 and MBI-110 EP Biofungicide

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active mircoorganism	*Bacillus amyloliquefaciens* strain F727
Function	For the suppression of various fungal diseases in various outdoor food and feed crops
Binomial	*Bacillus amyloliquefaciens* strain F727
Taxonomic designation[5]	
Kingdom	Eubacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillalaes
Family	Bacillaceae
Genus	*Bacillus*
Species	*amyloliquefaciens*
Strain	F727
Patent Status information	None
Minimum purity of active	Technical grade active ingredient: minimum of 1.0×10^8 CFU/mL End-Use Product: minimum of 1.0×10^8 CFU/mL
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 products.

1.2 Physical and Chemical Properties of the Active Ingredients and End-Use Product

Technical Product—MBI-110 TGAI

Property	Result
Colour	Light brown/ beige
Physical State	Liquid

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

Property	Result
Odour	Musty/sour
Corrosion Characteristics	Not corrosive to packaging materials
pH (1%)	7.15
Relative Density	1.026 g/mL

End-Use Product—MBI-110 EP Biofungicide

Property	Result
Colour	Light tan
Physical State	Thin creamy liquid
Odour	Mild musty odour
Viscosity	627 cP
Corrosion Characteristics	Not corrosive to packaging materials
pH (1%)	7
Relative Density	1.0 g/mL

1.3 Directions for Use

MBI-110 EP Biofungicide is applied preventatively to foliage of cucurbit crops, grapes, soybean, pea, bean, canola, rapeseed and sunflower or to the open furrow at planting for potatoes at rates of 2–8 L/ha. The product may be re-applied at 7–14 day intervals. Higher rates or shorter intervals are to be used under conditions of high disease pressure.

1.4 Mode of Action

Bacillus amyloliquefaciens strain F727 produces lipopeptides that inhibits mycelial growth and spore germination of certain fungal plant pathogens.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganisms

Acceptable methodologies for detection, isolation and enumeration of the active ingredient, *Bacillus amyloliquefaciens* strain F727, were submitted by the applicant. The microbial pest control agent (MPCA) has been fully characterized with respect to its origin of strain, natural occurrence and biological properties. *Bacillus amyloliquefaciens* strain F727 can be identified to the species level using a combination of phenotypic and biochemical methodologies, as well as phylogenetic analysis based on DNA sequence analysis of the DNA gyrase subunit A.

2.2 Methods for Establishment of Purity of Seed Stock

The strain has been deposited into the international depository authority of the Agricultural Research Culture Collection Peoria, IL under the identification number NRRL B-50768. Stock cultures are kept frozen at -80°C.

A master cell bank is properly maintained at the manufacturer. A working cell bank is generated from the master cell bank for manufacturing purposes.

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 technical grade active ingredient and 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 *Bacillus* 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 MBI-110 TGAI and MBI-110 EP Biofungicide 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 MBI-110 TGAI and MBI-110 EP Biofungicide using standard methods for detecting and enumerating microbial contaminants of concern. All batches of MBI-110 TGAI and MBI-110 EP Biofungicide conform to the limits set out in the Organization for Economic Co-operation and Development (OECD) issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43].

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

The storage stability of MBI-110 TGAI has been assessed at 4–25°C for up to 12 months. For MBI-110 EP Biofungicide, the storage stability has been assessed at 4–30°C for up to 24 months.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

3.1.1 Testing

The PMRA conducted a detailed review of the toxicological studies submitted in support of MBI-110 TGAI, and the associated end-use product, MBI-110 EP Biofungicide.

The studies submitted to fulfil the requirements for the health hazard assessment of MBI-110 TGAI included an acute oral toxicity study, an acute inhalation toxicity study, an intravenous injection infectivity study, a dermal toxicity study, a dermal irritation study, and an eye irritation study with technical grade active ingredient.

In the oral toxicity study, three fasted, young adult Sprague-Dawley rats were given a single oral dose of MBI-110 TGAI (1.9–2.4 × 10^9 CFU/mL) by gavage at 5000 mg/kg (Limit test; equivalent to 1.2 × 10^{10} CFU/animal). The animals were observed for 14 days. There were no mortalities, no treatment related clinical signs, no necropsy findings and all animals gained weight during the study.

In the acute inhalation toxicity study, one group of young adult Sprague-Dawley rats (5/sex) were exposed for 4 hours by nose-only to an aerosol generated from undiluted MBI-110 TGAI (1 × 10^9 CFU/mL) at 5.19 mg/L. The animals were observed for 14 days. There were no mortalities, no clinical signs of toxicity, no necropsy findings, and all animals exhibited weight gain during most of the study.

In the acute intravenous infectivity study, young Sprague-Dawley rats (15/sex) were administered a single high dose of MBI-110 TGAI (1.16 × 10^8 CFU/mL) in 0.1 mL of phosphate buffered saline (equivalent to 1.16 × 10^7 CFU/animal) by intravenous injection in the tail vein. Animals were observed for up to 38 days with interim sacrifices on Days 3, 7, 14 and 21 to evaluate microbial clearance. There were no mortalities, no necropsy findings and all animals appeared normal throughout the study period. Weight gain data were compromised due to a faulty water connection for the control group animals. The test organism had cleared from the brain and mesenteric lymph nodes, blood, kidneys, and cecum contents by Day 38 or sooner. Although the test organism had not cleared completely from the lungs, liver and spleen by Day 38, a pattern of clearance was established in these organs.

In the acute dermal toxicity study, a group of young Sprague-Dawley rats (5/sex) were dermally exposed to MBI-110 TGAI (2.4 × 10^9 CFU/mL) for 24 hours at 5050 mg/kg bw. The dose was applied to an area of approximately 10% of body surface area. The animals were observed for 14 days for signs of toxicity and dermal irritation. There were no mortalities, no signs of dermal irritation, no treatment-related clinical signs of toxicity, and no necropsy findings noted during the study period. All animal gained weight throughout the study, except for three animals which failed to gain weight or lost weight between Day 7 and 14.

In the primary dermal irritation study, three young adult albino New Zealand White rabbits (1 ♂, 2♀) were dermally exposed to 0.5 mL of undiluted MBI-110 TGAI ($2.4–3.1 \times 10^9$ spores/mL) for 4 hours. The dose was applied to an 8 × 8 cm body surface area, covered with a semi-permeable dressing. After 4 hours, the dressings were removed and the test site was washed and animals were observed for dermal irritation for 3 days by the method of Draize et al. (1944). No dermal irritation was noted during the study period.

In the primary eye irritation study, 0.1 mL of undiluted MBI-110 TGAI ($2.4–3.1 \times 10^9$ spores/mL) was directly instilled into the conjunctival sac of the right eye of young adult New Zealand White albino rabbits (1♂, 2♀) for 24 hours. After recording the 24-hour observation, all treated eyes were washed with room temperature deionized water for one minute. The animals were observed for 3 days and scored for irritation by the method of Draize at 1, 24, 48 and 72 hours. Redness and discharge was visible in two of the three rabbits at the 1-hour time point. All irritation cleared by 24 hours. The calculated Maximum Irritation Score (at 1 hour) was 2.7/110.

Test results are summarized in Appendix I, Table 1.

3.1.2 Additional Information

Scientific rationales were provided to waive the technical grade active ingredient requirements for acute oral and pulmonary infectivity/toxicity studies, as well as to waive the requirements for acute dermal toxicity and dermal irritation.

The requests to waive the technical grade active ingredient requirements for infectivity/toxicity testing via the oral and pulmonary routes were supported by the lack of infectivity and toxicity for *Bacillus amyloliquefaciens* strain F727 following intravenous injection. Based on the scientific rationale presented in support of the low infectivity potential for the technical grade active ingredient, the requests to waive these requirements were accepted.

The requests to waive the end-use product requirements for dermal toxicity and dermal irritation studies were supported by the lack of toxicity of *Bacillus amyloliquefaciens* strain F727 by the dermal route and an assessment of the formulation ingredients in MBI-110 EP Biofungicide. The requests to waive these requirements were accepted.

A survey of published scientific literature for *Bacillus amyloliquefaciens*, and the closely-related species *Bacillus subtilis* uncovered no reports of adverse effects for *Bacillus amyloliquefaciens* strain F727. There was one report of an outbreak of eosinophilia-myalgia syndrome involving a chemical impurity in a tryptophan supplement produced by a different strain of *Bacillus amyloliquefaciens*. A similar case of a nutritional supplement contaminated with *Bacillus subtilis* was also reported.

In addition, there have been rare cases of *Bacillus subtilis*-related endocarditis, bacteremia in immunocompromised patients. In some cases, the organism was introduced into sensitive tissues via intravenous catheters or lumbar puncture surgery. Other cases were related to drug abuse, as narcotics are often contaminated with bacilli.

The routine use of *Bacillus subtilis* cultures as a non-specific support for a stable gastrointestinal flora has also been suspected as a source. Single cases of meningitis, an eye infection and a shin-bone infection have also been reported for *Bacillus subtilis*.

Rope spoilage in bread is also associated with *Bacillus subtilis* and foodborne illness has occasionally been reported. Other food poisoning incidents related to *Bacillus subtilis* are rare, and the implicated strains produce a highly heat stable toxin (possibly similar to the *Bacillus cereus*-enterotoxin). *Bacillus amyloliquefaciens* strain F727 is not reported to produce this toxin, and no such illnesses have been reported for this microorganism. Furthermore, when *Bacillus amyloliquefaciens* strain F727 was administered orally to rats, no signs of toxicity or disease were observed. On rare occasions, *Bacillus subtilis* was attributed to foodborne illness where no toxin production was detected.

In veterinary medicine, bovine mastitis, as well as reproductive disorders in goats and canine endocarditis have been related to *Bacillus subtilis,*

Hypersensitivity pneumonitis was reported from exposure to *Bacillus subtilis* and *Bacillus licheniformis* spores and vegetative cells released from wood dust in domestic and industrial settings. Production of MBI-110 TGAI is not aimed at enzyme enrichment and there have been no adverse health effects reported in workers at the production site where *Bacillus amyloliquefaciens* strain F727 is fermented or formulated.

3.1.3 Incident Reports Related to Human and Animal Health

A search of incident reports was conducted for registered strains of *Bacillus amyloliquefaciens* and *Bacillus subtilis*. As of 4 May 2018, the PMRA received one human incident involving the active *Bacillus subtilis*. In this incident, a person reported minor symptoms of rash and cough following application of a registered American product containing *Bacillus subtilis*. Given that the incident was minor and occurred in Canada with an American product, no additional risk mitigation measures are recommended. The incident information was incorporated into the evaluation of *Bacillus amyloliquefaciens* strain F727.

3.1.4 Hazard Analysis

The database submitted in support of registering MBI-110 TGAI and MBI-110 EP Biofungicide 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, MBI-110 TGAI, is of low toxicity by the oral, inhalation and dermal routes, and was not pathogenic or infective by the intravenous. The technical grade active ingredient was also not irritating to the skin and was minimally irritating to the eyes. 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. The statement, "May cause sensitization" is also required on the secondary display panel of the label under the "PRECAUTIONS" section.

The end-use product, MBI-110 EP Biofungicide, contains formulation ingredients which may be irritating to the eyes and is an MPCA which is considered a potential sensitizer. Consequently, the hazard statements 'WARNING: EYE IRRITANT' and 'POTENTIAL SENSITIZER' will appear on the principal display panel of the end-use product label. The statements, "May cause eye irritation. DO NOT get in eyes. May cause sensitization. Avoid contact with skin and clothing. Avoid inhaling/breathing spray mist" are also required on the secondary display panel of the label under the "PRECAUTIONS" section.

Higher tier subchronic and chronic toxicity studies were not required because the technical grade active ingredient was not acutely toxic by the oral, dermal or inhalation 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 *Bacillus amyloliquefaciens* strain F727 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 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 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. *Bacillus amyloliquefaciens* has not frequently been identified as a dermal wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Furthermore, toxicity testing with the technical grade active ingredient, MBI-110 TGAI, showed no toxicity via the oral, inhalation and dermal routes, and no signs of infectivity or pathogenicity via the intravenous injection route. While MBI-110 TGAI was not a dermal or eye irritant, MBI-110 EP Biofungicide may cause skin and eye irritation based on a review of the formulants in MBI-110 EP Biofungicide. In addition, the PMRA also 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, a long-sleeved shirt, long pants, eye goggles or a face shield, a NIOSH-approved mist filtering respirator or NIOSH-approved mist filtering mask, and shoes with socks 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 MBI-110 EP Biofungicide 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 MBI-110 EP Biofungicide and no significant occupational risks are anticipated for this product.

3.2.2 Residential and Bystander Exposure and Risk

There is a potential for bystander exposure to spray drift from applications to outdoor field crops. For bystanders, inhalation exposure is expected to be much less than that of handlers and mixer/loaders.

Overall, the PMRA does not expect that residential and bystander exposures will pose a health risk of concern on the basis of the low toxicity profile for MBI-110 EP Biofungicide, the low infectivity/pathogenicity profile for MBI-110 TGAI and the expectation that precautionary label statements will be followed by applicators in the use of the MBI-110 EP Biofungicide. As well, *Bacillus amyloliquefaciens* is a species that is common in the environment and the use of MBI-110 EP Biofungicide is not expected to cause sustained increases in exposure to bystanders beyond natural levels. Consequently, a health risk to infants and children is not expected.

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, a dietary risk to the general population and sensitive subpopulations such as infants and children, or to animals is not expected because *Bacillus amyloliquefaciens* strain F727 demonstrated no pathogenicity or infectivity in Tier I acute intravenous injection study; and no oral toxicity in the acute toxicity study. Furthermore, no metabolites of toxicological significance have been shown to be produced by this strain.

3.3.2 Drinking Water

Health risks are not expected from exposure to *Bacillus amyloliquifaciens* strain F727 via drinking water because exposure will be low from operational applications and because there were no harmful effects observed in Tier I acute oral toxicity testing. The MBI-110 EP Biofungicide label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Furthermore, municipal treatment of drinking water is expected to reduce the transfer of residues to drinking water.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARfDs) and acceptable daily intakes (ADIs) are not usually possible for predicting acute and long-term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (in other words, no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the Agency concludes that *Bacillus amyloliquefaciens* strain F727 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 *Bacillus amyloliquefaciens* strain F727 to human health.

3.3.4 Aggregate Exposure and Risk

Based on the toxicity and infectivity test data previously 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 *Bacillus amyloliquefaciens* strain F727 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 not allowed for use on turf, residential or recreational areas. Furthermore, the label will include mitigation measures to reduce spray drift and few adverse effects from exposure to other strains of *Bacillus amyloliquefaciens* encountered in the environment have been reported in the public literature. Even if there is an increase in exposure to this *Bacillus amyloliquefaciens* strain F727 from the use of MBI-110 EP Biofungicide, 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 *Bacillus amyloliquefaciens* strain F727 on treated food crops, at the time of harvest, are anticipated following foliar applications to crops. Consequently, the PMRA has applied a hazard-based approach for determining whether an MRL is required for this microorganism. Although the United States Food and Drug Administration has noted that some strains of *Bacillus subtilis* have been isolated from food implicated in food poisoning, these strains demonstrated the ability to produce a highly heat-stable toxin that was not reported in *Bacillus amyloliquefaciens* strain F727. No such illnesses were reported for this microorganism in the United States where it has been registered for use on crops since 2016. There are no risks anticipated for dietary exposure based on the low toxicity profile demonstrated in the test animals in the Tier I acute oral and inhalation toxicity studies and the intravenous injection infectivity study.

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 *Bacillus amyloliquefaciens* strain F727.

3.4 Cumulative Effects

The *Pest Control Products Act* requires that the PMRA consider the cumulative exposure to pesticides with a common mechanism of toxicity. In its assessment of common mechanism of toxicity, the PMRA considers both the taxonomy of MPCAs and the production of any potentially toxic metabolites. For the current evaluation, the PMRA has determined that *Bacillus amyloliquefaciens* strain F727 shares a common mechanism of toxicity with other strains of *Bacillus amyloliquefaciens* and *Bacillus subtilis* that are registered for use in Canada; *Bacillus amyloliquefaciens* strain MBI 600, *Bacillus amyloliquefaciens* strain D747, *Bacillus subtilis* strain QST 713, *Bacillus subtilis* strain GB03, and *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24. The potential health risks from cumulative exposure of *Bacillus amyloliquefaciens* strain F727 and these other registered species or strains of this microorganism are not of concern when used as labelled given their low toxicity and pathogenicity.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

No studies were submitted to address the environmental fate and behaviour of *Bacillus amyloliquefaciens* strain F727; however, environmental fate data (Tier II/III) are not normally required at Tier I, and are only triggered if significant toxicological effects in non-target organisms are noted in Tier I testing.

Bacillus amyloliquefaciens strain F727 is a rhizobacterium that colonizes plant root hairs, leaves and other plant surfaces to prevent establishment of fungal and bacterial plant diseases by competitive exclusion. *Bacillus* species are commonly found in soil and in plant litter where they play an important role in the biological cycling of carbon and nitrogen. Under unfavourable growth conditions, this species can undergo sporulation and create a resilient endospore that can endure many adverse environmental conditions.

While the broadcast application to outdoor food crops of MBI-110 EP Biofungicide is expected to temporarily increase natural populations of *Bacillus amyloliquefaciens* in outdoor terrestrial or aquatic environments, the levels of *Bacillus amyloliquefaciens* strain F727 are expected to return to naturally sustainable levels.

The end-use product is not intended to be applied directly to water. As result, exposure to aquatic environments should be low and limited to run-off after application. While *Bacillus amyloliquefaciens* is not considered an aquatic species and is not expected to grow in this environment, the endospores of this microorganism are likely to persist in sediment.

The broadcast application of MBI-110 EP Biofungicide is not expected to significantly increase the overall environmental levels of this species in sediment above naturally occurring levels. As noted previously, any localized increases of *Bacillus amyloliquefaciens* strain F727 in aquatic environments are expected to return to naturally sustainable levels over time.

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 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 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 (i.e., 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.

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

Eight studies were submitted to address the hazards of *Bacillus amyloliquefaciens* strain F727 to birds, terrestrial arthropods, and terrestrial plants. Data submitted under human and animal health toxicity testing were considered to assess the risk of harm to wild mammals.

The acute oral toxicity of MBI-110 TGAI to 25-day-old Bobwhite quail (*Colinus virginianus*) was assessed over 30 days. MBI-110 TGAI was administered once per day for five days to 30 birds by oral gavage at 1182 mg/kg body weight (bw; equivalent to 7.1×10^9 CFU/kg bw). There were no treatment related toxicity effects. The 30-day acute oral LD_{50} was greater than 1182 mg/kg bw. The 30-day NOEL (no observed effect level) of MBI-110 TGAI to the Bobwhite quail, based on mortality, general health, body weight, and feed consumption was greater than 1182 mg/kg bw. The required maximum hazard dose of 5000 mg/kg bw was not achieved.

An additional avian oral toxicity/pathogenicity study was submitted. The acute oral toxicity/pathogenicity of MBI-110 TGAI to 3-week-old Bobwhite quail (*Colinus virginianus*) was assessed over 30 days. MBI-110 TGAI was administered to the birds (30 mixed sex) by oral gavage at 5 mL/kg bw (equivalent to 5.10×10^{10} CFU/kg bw) once per day for five consecutive days. There were no treatment related signs of toxicity or pathogenicity. The 30-day acute oral LD_{50} was greater than 5.10×10^{10} CFU/kg bw. The 30-day NOEL for MBI-110 TGAI, based on mortality, general health, body weight, and feed consumption was greater than 5.10×10^{10} CFU/kg bw.

In a 9-day dietary toxicity/pathogenicity study, newly emerged adult honey bees (*Apis mellifera*) were fed MBI-110 TGAI ad libitum at a concentration of 50 mg/mL (2.5×10^8 CFU/mL) in a 50% w/v sugar solution for 4 hours. There was no effect on mortality on Day 9 when mortality in untreated control group reached 20% or on Day 16 when the study was terminated. The 9-day dietary LC_{50} was greater than 50 mg/mL.

In a 13-day acute dietary toxicity/pathogenicity study, Rove beetles (*Dalotia coriaria*) were exposed to MBI-110 TGAI (containing *Bacillus amyloliquefaciens* strain F727 at 5.0×10^9 CFU/mL) in 50% w/w honey solutions at 0.035 g/mL, 0.140 g/mL, 0.421 g/mL, 0.701 g/mL and 1.402 g/mL for 4 hours. Mortality on Day 13 was 40%, 40%, 43%, 48%, and 50% in the 0.035 g/mL, 0.140 g/mL, 0.421 g/mL, 0.701 g/mL and 1.402 g/mL treatment groups, respectively. The study was terminated on Day 13 when mortality in the untreated control achieved was 20%. The 13-day LC_{50} was greater than 1.402 g/mL. Although feed consumption was not measured, it was assumed that Rove beetles fed on test concentrations due to increased mortalities observed in those groups. Despite increased mortality observed in the test groups, the LC_{50} is several orders of magnitude greater than the exposure estimate and therefore, the risk quotient is below the level of concern.

In a 17-day acute dietary toxicity study, Convergent Ladybird beetles (*Hippodamia convergens*) were exposed to MBI-110 TGAI (containing *Bacillus amyloliquefaciens* strain F727 at 3.0×10^9 CFU/mL) in 50% w/w honey solutions at 0.701 g/mL via the diet for 4 hours. There were no significant differences in mortality between the test group and untreated control. Feed consumption was not measured or otherwise observed; therefore, exposure cannot be confirmed. Although there were no signs of pathogenicity, this end-point cannot be assessed as the choice of carrier (honey) is inappropriate due to its antimicrobial properties.

In a 14-day acute dietary toxicity study, Green Lacewing larvae (*Chrysoperla rufilabris)* were exposed to MBI-110 TGAI (containing *Bacillus amyloliquefaciens* strain F727 at 5.0×10^9 CFU/mL) in 50% w/w honey solutions at 0.701 g/mL via the diet for 4 hours. There were no

significant differences in mortality between the test group and untreated control. Feed consumption was not measured or otherwise observed; therefore, exposure cannot be confirmed. Although there were no signs of pathogenicity, this end-point cannot be assessed as the choice of carrier (honey) is inappropriate due to its antimicrobial properties.

The effect of MBI-110 EP Biofungicide (containing *Bacillus amyloliquefaciens* strain F727 at 5.0×10^9 CFU/mL) on emergence, shoot length, dry weight or seedling survival of onion (*Allium cepa*), purple nutsedge (*Cyperus rotundus*), wheat (*Triticum aestivum*), corn (*Zea mays*), sorghum (*Sorghum bicolor*), sunflower (*Helianthus annuus*), mustard greens (*Brassica juncea*), cucumber (*Cucumis sativus*), soybean (*Glycine max*), tomato (*Lycospersicum esculentum*), pea (*Pisum sativum*), spinach (*Spinacia oleracea*), pumpkin (*Cucurbita pepo*), and radish (*Raphanus sativus*) was studied after a single pre-emergent soil spray/drench application of 9.9 L/ha (1.04×10^{14} CFU/ha). There were no treatment related effects for any of the measured parameters.

The effect of MBI-110 EP Biofungicide (containing *Bacillus amyloliquefaciens* strain F727 at 1.05×10^{10} CFU/mL) on shoot length and dry weight on onion (*Allium cepa*), purple nutsedge (*Cyperus rotundus*), rice (*Oryza sativa*), wheat (*Triticum aestivum*), corn (*Zea mays*), sunflower (*Helianthus annuus*), mustard greens (*Brassica juncea*), cucumber (*Cucumis sativus*), soybean (*Glycine max*), tomato (*Lycopersicum esculentum*), beet (*Beta vulgaris*), and radish (*Raphanus sativus*) was studied after a single post-emergent (1^{st} true leaf stage) foliar spray application of 11.2 L/ha (1.10×10^{14} CFU/ha). Qualitative observations for phytotoxicity were also made. There were no treatment related effects for shoot length and dry weight. No significant signs of phytotoxicity were observed.

In studies conducted to satisfy the human health and safety requirements, it was determined that *Bacillus amyloliquefaciens* strain F727 was not toxic in rats via the oral, pulmonary (inhalation), and dermal routes of exposure and not pathogenic in rats following intravenous injection.

A search in PubMed using the keywords "*Bacillus amyloliquefaciens* toxicity" and "*Bacillus amyloliquefaciens* pathogenicity" found no reports of adverse effects to terrestrial non-target organisms from natural populations of *Bacillus amyloliquefaciens*. One study was found where a mutant strain of *Bacillus amyloliquefaciens* was shown to have nematicidal activity. The keywords "*Bacillus subtilis* pathogen" were also searched and yielded very few reports of pathogenicity. The reports of pathogenicity consisted mostly of reports of infections in humans with potentially compromised immune systems. The majority of the scientific literature consisted of reports on: i. the ability of *Bacillus subtilis* to promote growth and/or to induce systemic resistance in host crops; ii. the biological control of various plant pathogenic fungi; and iii. the use of *Bacillus subtilis* as a probiotic in animal feed (for example, chickens).

Based on all the available information on the effects of *Bacillus amyloliquefaciens* strain F727 to terrestrial non-target organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, terrestrial arthropods, non-arthropod invertebrates, plants or to other non-target microorganisms from the proposed use of MBI-110 EP Biofungicide as a broadcast application on outdoor food crops.

4.2.2 Effects on Aquatic Organisms

Two studies were submitted to address the hazards of *Bacillus amyloliquefaciens* strain F727 to freshwater fish and aquatic arthropods.

In a 30-day toxicity/pathogenicity study, 5 groups of 30 Rainbow trout (*Oncorhyncus mykiss*) were exposed to MBI-110 TGAI (1.3×10^{10} CFU/mL) under static-renewal conditions both by aquatic and via feed. Nominal aquatic concentrations were 5×10^3, 5×10^4, 5×10^5, 5×10^6, and 5×10^7 CFU of *Bacillus amyloliquefaciens* strain F727/mL; and nominal feed concentrations were 5×10^2, 5×10^3, 5×10^4, 5×10^5, and 5×10^6 CFU/mL respectively. The high dose test group resulted in complete mortality by Day 1 which is indicative of acute toxicity. There were no signs of pathogenicity or infectivity in any test group. The 30-day LC_{50} was 2.5×10^7 CFU/mL; the NOEC (no observed effect concentration) was 5.0×10^6 CFU/mL; and the LOEC (lowest observed effect concentration) was 5.0×10^7 CFU/mL. *Bacillus amyloliquefaciens* strain F727 is of low toxicity to the Rainbow trout and is not pathogenic or infective. Despite the complete mortality observed in the highest test group, the LC_{50} is several orders of magnitude greater than the exposure estimate and therefore, the risk quotient is below the level of concern.

In a 21-day toxicity/pathogenicity study, 5 groups of 10 daphnids (*Daphnia magna*) were exposed to MBI-110 TGAI (1.3×10^{10} CFU/mL) under static renewal conditions. Five test concentrations were used; 5.0×10^3, 5.0×10^4, 5.0×10^5, 5.0×10^6 and 5.0×10^7 CFU/mL. Neonate production, biomass and mortality were observed daily. All daphnids in the highest test group died on Day 1. Mortality on Day 21 was 10%, 10%, 10%, and 40% in the 5.0×10^3, 5.0×10^4, 5.0×10^5, and 5.0×10^6 CFU/mL test group respectively. There were no observed effects on biomass of daphnids and, at the three lower concentrations; there were no effects on production of neonates. The production of neonates at 5.0×10^6 CFU/mL was significantly lower. Based on the immediate effect on mortality in the highest test concentration, the observed effects are due to toxicity rather than pathogenicity. The 21-day NOEC for survival is 5.0×10^5 CFU/mL and the 21-day NOEC for reproduction and biomass is 5.0×10^6 CFU/mL. The EC_{50} for survival is 1.3×10^7 CFU/mL. Despite the increased mortality and decreased neonate production observed in the highest test group, the EC_{50} is several orders of magnitude greater than the exposure estimate and therefore, the risk quotient is below the level of concern.

A search in PubMed using the keywords "*Bacillus amyloliquefaciens* toxicity" and "*Bacillus amyloliquefaciens* pathogenicity" found no reports of adverse effects to aquatic non-target organisms. The keywords "*Bacillus subtilis* pathogen" were also searched and yielded no reports of pathogenicity to aquatic non-target organisms. As noted in Section 4.2.1, the majority of the scientific literature consisted of reports on: i.) the ability of *Bacillus subtilis* to promote growth and/or to induce systemic resistance in host crops; ii.) the biological control of various plant pathogenic fungi; and iii.) the use of *Bacillus subtilis* as a probiotic in animal feed, including fish feed.

Based on all the available information on the effects of *Bacillus amyloliquefaciens* strain F727 to aquatic non-target organisms, there is reasonable certainty that no harm will be caused to fish, aquatic arthropods, aquatic non-arthropod invertebrates or aquatic plants from the proposed use of MBI-110 EP Biofungicide as a broadcast application on outdoor food crops.

4.3 Incident Reports related to the Environment

A search of incident reports was conducted for registered strains of *Bacillus amyloliquefaciens* and *Bacillus subtilis*. As of 4 May 2018, the PMRA did not receive any incident reports related to the environment. No additional risk mitigation measures are recommended for *Bacillus amyloliquefaciens* strain F727.

5.0 Value

The applicant provided the results of efficacy trials, scientific rationales, published scientific articles, and benefit information to support the claims. The efficacy trials provided for the various crops were conducted under low to high disease pressures. Based on the weight of evidence, suppression of the following diseases were supported: downy mildew of cucurbits, downy mildew of grape, black rot of grape, and pink rot of potato. Partial suppression of sclerotinia stem rot of canola, white mould of dried beans, soybean and pea, as well as stalk rot of sunflower were also supported. MBI-110 EF Biofungicide has been evaluated for phytotoxicity on a variety of crops, and in a number of growing conditions. No phytotoxicity was observed on the tested crops in any of these trials. There are many alternatives registered for the same uses as those on the label of MBI-110 EP Biofungicide, including non-conventional alternatives. However, the use of MBI-110 EP is beneficial for Canadian growers as it provides an additional option for disease management in these crops, particularly for organic producers.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

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

MBI-100 TGAI and MBI-110 EP Biofungicide were assessed in accordance with the PMRA Regulatory Directive DIR99-03.[6]

- MBI-110 TGAI 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] Regulatory Directive DIR99-03, The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy.

6.2 Formulants and Contaminants of Health 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.*[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:

- The technical grade active ingredient MBI-110 TGAI 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 and Contaminants of Health or Environmental Concern*.

- The end-use product, MBI-110 EP Biofungicide, does not contain formulants of health or environmental concern as identified in the *Canada Gazette,* Part II, Volume 139, Number 24, pages 2641-2643: *List of Pest Control Product Formulants of Health or Environmental Concern*.

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

7.0 Summary

7.1 Methods for Analysis of the Microorganism as Manufactured

The product characterization data for MBI-110 TGAI and MBI-110 EP Biofungicide were adequate to assess their potential human health and environmental risks. The technical grade active ingredient was fully characterized and the specifications of the end-use product were supported by the analyses of a sufficient number of batches.

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7 *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.*

8 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.*

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

All batches of MBI-110 TGAI 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 4–25°C for no more than 12 months for MBI-110 TGAI and for no more than 2 years at 4–30°C for MBI-110 EP Biofungicide.

7.2 Human Health and Safety

The acute toxicity and infectivity studies and other relevant information submitted in support of MBI-110 TGAI and MBI-110 EP Biofungicide were determined to be acceptable. Based on all the available information, the technical grade active ingredient, MBI-110 TGAI, is of low toxicity by the oral, inhalation, and dermal routes, and was not pathogenic or infective by the intravenous route. The technical grade active ingredient is non-irritating to the skin and minimally irritating to the eyes and 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", "Avoid inhaling/breathing spray mist".

The end-use product, MBI-110 EP Biofungicide, is considered to be a skin and eye irritant due to certain formulation ingredients, and a potential sensitizer. The signal words 'WARNING-EYE IRRITANT', and 'POTENTIAL SENSITIZER' are required on the principal display panel of the end-use product, as well as the precautionary statements: "May cause eye irritation. DO NOT get in eyes. May cause sensitization. Avoid contact with skin and clothing. Avoid inhaling/breathing spray mist."

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. 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 MBI-110 EP Biofungicide must wear waterproof gloves, a long-sleeved shirt, long pants, eye goggles or a face shield, a NIOSH-approved mist filtering respirator or NIOSH-approved mist filtering mask, and shoes with socks 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 MBI-110 EP Biofungicide has been applied for 4 hours or until the sprays have dried.

A 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 *Bacillus amyloliquefaciens* strain F727, MBI-110 TGAI, and MBI-110 EP Biofungicide. The specification of an MRL under the *Pest Control Products Act* is not required for *Bacillus amyloliquefaciens* strain F727.

7.3 Environmental Risk

The submitted non-target organism test data and supporting published scientific literature submitted in support of MBI-110 TGAI and its associated EP, MBI-110 EP Biofungicide, were determined to be acceptable. The broadcast application of MBI-110 EP Biofungicide, containing *Bacillus amyloliquefaciens* strain F727, to food crops is not expected to pose a risk to non-target organisms when the directions for use on the label are followed.

As a general precaution, the product label will prohibit aerial application or the direct application of MBI-110 EP Biofungicide to aquatic habitats (such as lakes, rivers, sloughs, ponds, prairie potholes, creeks, marshes, streams, reservoirs, and wetlands), estuaries or marine habitats, and will direct handlers to not contaminate surface water by disposal of equipment wash waters and to limit runoff from treated areas.

7.4 Value

MBI-110 EP Biofungicide has been demonstrated to suppress or partially suppress certain diseases of cucurbits, grape, potato, canola, dried beans, soybean, pea and sunflower. It offers growers a non-conventional alternative for an integrated disease management program.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing registration for the sale and use of MBI-110 TGAI and MBI-110 EP Biofungicide, containing the technical grade active ingredient *Bacillus amyloliquefaciens* strain F727, for use on cucurbit crops, grapes, legume vegetables, canola, sunflower, and potatoes to control or suppress various diseases.

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

♂	male
♀	female
ºC	degree(s) Celsius
µg	micrograms
ADI	acceptable daily intake
ARfD	acute reference dose
bw	body weight
CFU	colony forming units
cm	centimetres
cP	centipoise
DACO	data code
DNA	deoxyribonucleic acid
EC_{50}	effective concentration on 50% of the population
g	gram
ha	hectare(s)
IL	Illinois
kg	kilogram
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LOEC	low observed effect concentration
mg	milligram
mL	millilitre
MPCA	microbial pest control agent
MRL	maximum residue limit
NOEC	no observed effect concentration
NOEL	no observed effect level
OECD	Organization for Economic Co-operation and Development
PMRA	Pest Management Regulatory Agency
ppm	parts per million
RQ	risk quotient
TSMP	Toxic Substances Management Policy
USEPA	United States Environmental Protection Agency
w/v	weight per volume
w/w	weight per weight

Appendix I Tables and Figures

Table 1 Toxicity Profile of MBI-110 TGAI

Study Type/Animal/PMRA#	Study Results
14-day Acute Oral Toxicity Sprague-Dawley rat PMRA# 2711335	There was no mortality in any group during the study. There were no treatment related clinical signs, no abnormal necropsy findings and all animals gained weight during the study. The acute oral LD_{50} was greater than 5000 mg/kg (1.2×10^{10} CFU/rat).
14-day Acute Inhalation Toxicity Sprague-Dawley rat PMRA# 2711332	There were no mortalities in any group during the study. There were no clinical signs of toxicity, no necropsy findings, and all animals exhibited weight gain during all, or most, of the study. The acute inhalation LC_{50} was greater than 5.19 mg/L in male and female animals.
14-day Acute Dermal Toxicity Sprague-Dawley rat PMRA# 2711326	There were no mortalities or any clinical signs of toxicity or irritation noted throughout the study period. Animals exhibited weekly weight gain during the study, with the exception of three of ten animals which failed to gain weight or lost weight between Day 7 and 14. At necropsy, there were no observable abnormalities noted. The acute dermal LD_{50} was greater than 5050 mg/kg bw in male and female rats.
72-hour Dermal Irritation New Zealand white PMRA# 2711329	No dermal irritation was noted during the study period. The calculated Maximum Irritation Score was 0/8 at 1 hour. The technical grade active ingredient was non-irritating to skin.
38-day Acute Intravenous Injection Infectivity Sprague-Dawley rat PMRA# 2871116	There was no mortality in any group during the study. During observations for clinical signs, all animals appeared normal for the duration of the study. Data on weight gain was compromised due to a faulty water connection in the control group animals; gross necropsy findings in the affected control group animals (for example, bloated stomachs with air and liquid substance) were attributed to a lack of water for

Study Type/Animal/PMRA#	Study Results
	3 days. Gross necropsies for all other animals revealed no observable abnormalities at any time point. The test substance cleared from all tissues and organs (except the lungs, liver and spleen) by Day 38 or sooner. A pattern of clearance was established in the lungs, liver and spleen by Day 38. The technical grade active ingredient was not pathogenic when injected at 1.16×10^7 CFU/animal.
3-day Eye Irritation New Zealand White rabbit PMRA# 2711331	Redness and discharge was visible in two of the three rabbits at the 1-hour time point. All irritation cleared by 24 hours. The calculated Maximum Irritation Score was 2.7/110 at 1 hour. The technical grade active ingredient is minimally irritating to eyes.

Table 2 Toxicity/Pathogenicity of MBI-110 TGAI to Non-Target Species

Organism	Exposure	Significant Effect, Comments	Reference
Terrestrial Organisms			
Vertebrates			
Birds			
Bobwhite quail (*Colinus virginianus*), 25-day-old	5-day – Dietary exposure	There were no treatment related toxicity effects. 30-day acute oral LC_{50} was > 1182 mg/kg bw.	PMRA# 2711337
Bobwhite quail (*Colinus virginianus*), 3-week-old	5-day – Dietary exposure	There were no treatment related toxicity or pathogenic effects. 30-day acute oral LC_{50} was > 5.10×10^{10} CFU/kg bw (5 mL/kg bw) **LOW TOXICITY** **NOT PATHOGENIC**	PMRA# 2840848

Organism	Exposure	Significant Effect, Comments	Reference
Invertebrates			
Arthropods			
Honeybees (*Apis mellifera*), young adult worker	4-hour – Dietary exposure	There was no effect on mortality. The 9-day dietary LC_{50} was > 50 mg/mL. **LOW TOXICITY** **NOT PATHOGENIC**	PMRA# 2711345
Rove beetle (*Dalotia coriaria*), nymph	4-hour – Dietary exposure	Mortality on Day 13 was 40%, 40%, 43%, 48%, and 50% in the 0.035 g/mL, 0.140 g/mL, 0.421 g/mL, 0.701 g/mL and 1.402 g/mL treatment groups, respectively. The 13-day acute dietary LC_{50} was > 1.402 g/mL. **TOXIC AND/OR PATHOGENIC**	PMRA# 2711343
Convergent Ladybird beetle (*Hippodamia convergens*), adult	4-hour – Dietary exposure	There was no effect on mortality.	PMRA# 2711342
Green Lacewing (*Chrysoperla rufilabris*), adult	4-hour – Dietary exposure	There was no effect on mortality.	PMRA# 2711341
Aquatic Organisms			
Vertebrates			
Fish			
Rainbow trout (*Onchorhycus mykiss*)	30-day – Aquatic exposure	Complete mortality on Day 1 in 5×10^7 CFU/mL test group. There was no effect on mortality in the other test groups. The 30-day aquatic LC_{50} was 2.5×10^7 CFU/mL. **LOW TOXICITY** **NOT PATHOGENIC**	PMRA# 2711339
Invertebrates			
Arthropod			
Daphnids (*Daphnia*	21-day – Aquatic	Complete mortality on Day 1 in 5×10^7 CFU/mL test group. Significantly increased	PMRA# 2711349

Organism	Exposure	Significant Effect, Comments	Reference
magna)	exposure (static renewal conditions)	mortality and reduced neonate production in 5×10^6 CFU/mL test group. There was no effect on mortality or neonate production in the other test groups. There was no effect on biomass. The 21-day NOEC for survival is 5.0×10^5 CFU/mL. **LOW TOXICITY** **NOT PATHOGENIC**	

Table 3 Toxicity/Pathogenicity of MBI-110 EP Biofungicide to Non-Target Species

Organism	Exposure	Significant Effect, Comments	Reference
Terrestrial Organisms			
Plants			
Various species	Pre-emergent soil exposure	There was no effect on emergence, shoot length, dry weight or seedling survival.	PMRA# 2711964
Various species	Post-emergent foliar exposure	There was no effect on emergence, shoot length, dry weight or seedling survival.	PMRA# 2711967

Appendix II Expected Environmental Concentration

Aquatic

The maximum proposed application rate of MBI-110 EP Biofungicide is 10 L/ha or 1×10^{12} CFU/ha. Therefore, assuming that the maximum application rate was applied to surface water, the aquatic exposure estimate is 6.7×10^{2} CFU/mL.

Soil

The maximum proposed application rate of MBI-110 EP Biofungicide is 10 L/ha or 1×10^{12} CFU/ha. Therefore, assuming that the maximum application rate was applied to the soil surface and the soil has a density of 1.5 g/mL, the soil exposure estimate is 4.4×10^{2} CFU/g (4.4 µg MBI-110 EP Biofungicide /g).

References

A. List of Studies/Information Submitted by Registrant

PMRA Document Number	References
	1.0 Chemistry
2711314	2016, DACO M2.1-2.5 Product Characterization and Analysis, DACO: M2.1,M2.2,M2.3,M2.4,M2.5 CBI
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2711318	2016, Microbial Identification and Characterization for *Bacillus amyloliquefaciens* strain F727, DACO: M2.7.1,M2.7.3.1 CBI
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2711960	2015, Product Identity, Manufacturing Process, Discussion of Formation of Unintentional Ingredients, Analysis of Samples, Certification of Limits, and Physical and Chemical Properties, DACO: M2.14 CBI
2711961	2015, MBI-110 EP Product Chemistry and Composition, DACO: M2.10.1,M2.10.2,M2.10.3,M2.11,M2.12,M2.13,M2.14,M2.8,M2.9.1,M2.9.2,M2.9.3 CBI
	2.0 Human and Animal Health
2711319	2016, DACO M4.1 Human Health and Safety Testing - TGAI, DACO: M4.1,M4.2.1,M4.3.1,M4.5.1
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2711332 2013, 4.9-3_MBI-110 TGAI Acute Inhalation Toxicity in Rats, DACO: M4.9
2711333 2016, DER Acute Inhalation Toxicity in Rats; OCSPP 870.1300; OECD 403, DACO: M4.9
2711334 2016, DER Primary Eye Irritation in Rabbits; OCSPP 870.2400; OECD 405, DACO: M4.9
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3.0 Environment

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2711337 2014, MBI-110 TGAI Acute MPCA Oral Toxicity Study in Bobwhite Quail, DACO: M9.2.1
2711338 2016, Endangered Species Assessment for MBI-110, DACO: M9.3, M9.9
2711339 2015, MBI-110 TGAI Microbial Pest Control Agent (MPCA) Tier 1, Freshwater Fish Test with *Oncorhynchus mykiss* (Rainbow Trout), DACO: M9.4.1
2711341 2015, Effects of MBI-110 TGAI on the Green Lacewing (NEUROPTERA: *Chrysopidae Chrysoperla rufilabirs*) in a Laboratory Study in North Carolina, DACO: M9.5.1
2711342 2015, M9.5.1-1_Effects of MBI-110 TGAI on the Convergent Ladybird Beetle (Coleoptera: *Coccinellidae Hippodamia convergens*) in a Laboratory Study in North Carolina, DACO: M9.5.1
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2711348 2016, Nontarget Insect Testing-the Rove Beetle, *Dalotia coriaria*, Coleoptera: *Staphylinidae* - Data Evaluation Record, DACO: M9.5.1, M9.9
2711349 2016, MBI-110 TGAI Microbial Pest Control Agent (MPCA) Freshwater Aquatic Invertebrate Test with Daphnia magna, DACO: M9.5.2
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4.0 Value

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B. Additional Information Considered

i) Published Information

1.0 Chemistry

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