Proposed Registration Decision PRD2022-13

Bacillus amyloliquefaciens strain F727 and Stargus Biofungicide

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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*, is proposing registration for the sale and use of MBI-110 TGAI and Stargus Biofungicide (previously known as MBI-110 EP Biofungicide), containing the technical grade active ingredient *Bacillus amyloliquefaciens* strain F727, to partially suppress grey mould on indoor-grown cannabis and to suppress or partially suppress certain economically important diseases on field-grown hemp, grape, tomato, potato, red (garden) beet, sugar beet, cabbage and Brussels sprouts.

Bacillus amyloliquefaciens strain F727 is currently registered for suppression or partial suppression of listed diseases on various terrestrial food crops. For details, see Proposed Registration Decision PRD2018-17, *Bacillus amyloliquefaciens strain F727 and MBI-110 EP Biofungicide* and Registration Decision RD2018-20, *Bacillus amyloliquefaciens strain F727 and MBI-110 EP Biofungicide*.

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 *B. amyloliquefaciens* strain F727 and Stargus Fungicide.

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.

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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."

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 the Canada.ca website.

Before making a final registration decision on *B. amyloliquefaciens* strain F727 and Stargus Fungicide, 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 Decision[4] on *B. amyloliquefaciens* strain F727 and Stargus Fungicide, 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 used to manage certain plant diseases by reducing the establishment of causal pathogens, 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 Stargus Biofungicide is used according to the label directions.**

Potential exposure to *Bacillus amyloliquefaciens* strain F727 may occur when handling and applying Stargus Biofungicide and when ingesting treated produce. When assessing health risks, several key factors are considered:

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

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

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

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

Studies in laboratory animals describe potential health effects from large doses of exposure to a microorganism and identify any pathogenicity, infectivity and toxicity concerns.

When MBI-110 TGAI was tested on laboratory animals, there was low toxicity following oral, inhalation and dermal exposures, and no infectivity when injected (intravenous). MBI-110 TGAI was not irritating to the skin or eyes. Stargus Biofungicide is not expected to be irritating to the skin but may be irritating to the eyes.

Furthermore, there was no sign that the microbial pest control agent *Bacillus amyloliquefaciens* strain F727 caused any disease.

The end-use product Stargus Biofungicide and technical grade active ingredient MBI-110 TGAI are considered potential sensitizers.

Residues in water and food

Dietary risks from food and water are acceptable.

Stargus Biofungicide is already approved for use on various food crops, and residues of *Bacillus amyloliquefaciens* strain F727 on 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 and since 2018 in Canada, 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.

Occupational risks from handling Stargus Biofungicide

Occupational risks are acceptable when Stargus Biofungicide is used according to label directions, which include protective measures.

Workers handling Stargus Biofungicide can come into direct contact with *Bacillus amyloliquefaciens* strain F727 on the skin, by inhalation, or in the eyes. To protect workers from exposure to Stargus Biofungicide, the label states that workers must wear personal protective equipment, including a long-sleeved shirt, long pants, protective eyewear (goggles), waterproof gloves, socks and shoes, and a NIOSH-approved particulate filtering facepiece respirator with any N, R or P filter.

The product label includes measures to restrict access to the treated area for four hours or until sprays have settled. The occupational risks are acceptable when the precautionary statements on the label are observed.

Risks in residential and other non-occupational environments

Estimated risk for residential and other non-occupational exposure is acceptable.

Stargus Biofungicide is currently registered for use as a commercial fungicide on various outdoor food crops. Stargus Biofungicide is being proposed for use on additional terrestrial food crops and industrial hemp. There are no residential uses. The product label includes mitigation measures to prevent bystander exposure such as reducing spray drift. Stargus Biofungicide is also being proposed for use on cannabis grown in greenhouses and enclosed structures. This use is not expected to result in significant residential and bystander exposure due to drift. Residential and non-occupational exposure to Stargus Biofungicide is therefore expected to be low when label directions are observed. Consequently, the risk to residents and the general public is acceptable.

Environmental considerations

What happens when *Bacillus amyloliquefaciens* strain F727 is introduced into the environment?

Environmental risks are acceptable.

Stargus Biofungicide is approved for use on various terrestrial food crops. 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 Stargus Biofungicide to agricultural field crops, but that over time its population should return to naturally sustainable levels.

Based on a critical review of previously submitted studies and information from public sources, the risks to birds, wild mammals, fish, terrestrial and aquatic arthropods, terrestrial or aquatic non-arthropod invertebrates and plants expected from the use expansion of Stargus Biofungicide are acceptable when Stargus Biofungicide is applied according to directions on the label.

Value considerations

What is the value of Stargus Biofungicide?

Stargus Biofungicide is applied to indoor-grown cannabis to partially suppress grey mould and to suppress or partially suppress certain economically important diseases on field-grown hemp, grape, tomato, potato, red (garden) beet, sugar beet, cabbage and Brussels sprouts.

Stargus Biofungicide is a biological fungicide that will serve as an alternative disease management product option for use on these crops. This product may reduce the risk of resistance development to conventional fungicides as well as provide organic growers with an additional tool for disease management.

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.

Key risk-reduction measures

Based on the revision to the product guarantee, and on the proposed use expansion to additional outdoor crops and on cannabis grown in greenhouses and enclosed structures, no additional risk-reduction measures are necessary. Risk-reduction measures already present on the registered labels of MBI-110 TGAI and Stargus Biofungicide are described below.

Key risk-reduction measures - Human health

The principal display panel of MBI-110 TGAI and Stargus Biofungicide includes the signal words signal “POTENTIAL SENSITIZER”. The signal words “WARNING - EYE IRRITANT” appears on the principal display panel for Stargus Biofungicide.

The end-use product and technical grade active ingredient are considered potential sensitizers. In turn, workers handling or applying Stargus Biofungicide as a foliar application outdoors or in greenhouses and enclosed structures must wear a long-sleeved shirt, long pants, protective eyewear (goggles), waterproof gloves, socks and shoes, and a NIOSH-approved particulate filtering facepiece respirator with any N, R or P filter. Furthermore, when applied as a foliar treatment, all unprotected workers are restricted from entering treated areas during application and for four hours following application or until sprays have dried.

Key risk-reduction measures - Environment

The end-use product label includes standard environmental precaution statements to prohibit aerial application, limit drift and reduce contamination of aquatic systems from the use of Stargus Biofungicide.

Next steps

Before making a final registration decision on *B. amyloliquefaciens* strain F727 and Stargus Fungicide, 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 *B. amyloliquefaciens* strain F727 and Stargus Fungicide (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. For more information, please contact the PMRA's Pest Management Information Service.

Science evaluation

Bacillus amyloliquefaciens strain F727 and Stargus Biofungicide

1.0 The active ingredient, its properties and uses

1.1 Identity of the active ingredient

MBI-110 TGAI (Reg. No. 33245) and Stargus Biofungicide (Reg. No. 33246) contain the microbial pest control agent (MPCA) *Bacillus amyloliquefaciens* strain F727. The use of *Bacillus amyloliquefaciens* strain F727 as a fungicide for terrestrial food crops was previously assessed and approved by the PMRA.

Refer to PRD2018-17, Bacillus amyloliquefaciens strain F727 and MBI-110 EP Biofungicide for details.

1.2 Directions for use

Stargus Biofungicide is applied to:

- cannabis grown indoors at 5–15 mL product/L water to partially suppress grey mould;
- field hemp at 4–8 L product/ha using ground application equipment to partially suppress grey mould and white mould;
- grape at 5 L product/ha using ground application equipment to partially suppress botrytis bunch rot;
- field tomato at 5–10 L product/ha using ground application equipment to partially suppress early blight;
- field tomato at 7–10 L product/ha using ground application equipment to partially suppress late blight (suppression at 10 L product/ha);
- potato at 8–10 L product/ha using ground application equipment to partially suppress early blight and late blight (suppression of late blight at 10 L product/ha);
- garden beet and sugar beet at 5 L product/ha using ground application equipment to partially suppress cercospora leaf spot; and
- cabbage and Brussels sprouts at 6–8 L product/ha in combination with a non-ionic surfactant at 0.125–0.25% v/v of the spray volume to partially suppress bacterial black rot.

1.3 Mode of action

Bacillus amyloliquefaciens strain F727 is classified as a Group BM 02 fungicide by the Fungicide Resistance Action Committee (FRAC). *Bacillus amyloliquefaciens* strain F727 is a bacterium that reduces the establishment of certain plant disease-causing pathogens by means of

colonizing root hairs, leaves and other plant surfaces. It also produces compounds such as lipoproteins that inhibit mycelial growth and spore germination.

2.0 Methods of analysis

2.1 Methods for identification of the microorganisms

Refer to PRD2018-17, *Bacillus amyloliquefaciens* strain F727 and MBI-110 EP Biofungicide for details.

2.2 Methods for establishment of purity of seed stock

Refer to PRD2018-17, *Bacillus amyloliquefaciens* strain F727 and MBI-110 EP Biofungicide for details.

2.3 Methods to define the content of the microorganism in the manufactured material used for the production of formulated products

The guarantee of the technical grade active ingredient and the end-use product are expressed in units of colony forming units (CFU) per mL. The method for determining the guarantee has been previously reviewed. Refer to PRD2018-17, *Bacillus amyloliquefaciens strain F727 and MB-110 EP Biofungicide* for details.

Representative data on five batches of the technical grade active ingredient from the new manufacturing site were submitted. Potency data for batches of technical grade active ingredient from the new manufacturing site and potency data previously submitted for batches of technical grade active ingredient from the original manufacturing site support a revision to the guarantee of the technical grade active ingredient from $\geq 1 \times 10^8$ CFU/mL to $\geq 1 \times 10^9$ CFU/mL.

Representative data on five batches of the end-use product from the new formulating site were submitted. Potency data for batches of end-use product from the new formulating site and potency data previously submitted for batches of end-use product from the original formulating site support the proposed revision to the guarantee of the end-use product from $\geq 1 \times 10^8$ CFU/mL to $\geq 1 \times 10^9$ CFU/mL.

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

Refer to PRD2018-17, *Bacillus amyloliquefaciens* strain F727 and MBI-110 EP Biofungicide for details.

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 Stargus Biofungicide at the new manufacturing and formulating site 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 Stargus Biofungicide from the new site using standard methods for detecting and enumerating microbial contaminants of concern. All batches of MBI-110 TGAI and Stargus Biofungicide 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].

The methods in place to limit contaminating microorganisms at the final re-packaging sites are also acceptable and are supported by batch data.

2.6 Methods to determine storage stability, shelf-life of the microorganism

The storage stability of MBI-110 TGAI was previously assessed. Refer to PRD2018-17, *Bacillus amyloliquefaciens strain F727 and MBI-110 EP Biofungicide* for details.

The storage stability of Stargus Biofungicide was assessed and the results supported a storage period of 24 months when stored at 4–30°C.

3.0 Impact on human and animal health

3.1 Toxicity and infectivity summary

3.1.1 Testing

No new human health and safety studies were required to address the toxicity and infectivity of *B. amyloliquefaciens* strain F727, MBI 110 TGAI and Stargus Biofungicide. Refer to PRD2018-17, *Bacillus amyloliquefaciens strain F727 and MBI-110 EP Biofungicide* for details.

3.1.2 Health incident reports

Bacillus amyloliquefaciens strain F727 is an active ingredient that has been registered for use in Canada since 2018. As of 17 June 2022, no human or domestic animal incident reports involving *B. amyloliquefaciens* strain F727 had been submitted to the PMRA.

A query for incidents was also conducted on strains related to *B. amyloliquefaciens*, in other words, *B. licheniformis, B. velezensis* and *B. subtilis*. As of 17 June 2022, there was one human incident involving a related strain, *B. subtilis* QST 713. In this incident, a person reported minor symptoms of rash and cough when applying a product containing *B. subtilis* strain QST 713. The

label of the product, Stargus Biofungicide, contains appropriate hazard signal words, precaution statements and personal protective equipment aimed at reducing pesticide exposure when mixing, loading or applying the product. Hence, no additional mitigation measures are proposed based on the incident report review.

3.1 Hazard analysis

There is no change to the hazard analysis as a result of the revision to the product guarantees of the technical grade active ingredient or end-use product. Refer to PRD2018-17, *Bacillus amyloliquefaciens strain F727 and MBI-110 EP Biofungicide* for details.

3.2 Occupational, residential and bystander risk assessment

3.2.1 Occupational and postapplication exposure and risk

Stargus Biofungicide is currently registered as a commercial fungicide for terrestrial food crops. The proposed use expansion includes cannabis grown in greenhouses and enclosed structures, industrial hemp, as well as additional outdoor crops.

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for handlers, mixer/loaders and applicators 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 was 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, *B. amyloliquefaciens* strain F727 is of low toxicity via the dermal and inhalation routes and is not infective via the intravenous route. Hazard testing with the technical grade active ingredient showed that it is not irritating to the skin or eyes. The PMRA assumes that all microbes contain substances that can elicit positive hypersensitivity reactions.

Risk mitigation measures, such as personal protective equipment (PPE), including a long-sleeved shirt, long pants, protective eyewear (goggles), waterproof gloves, socks and shoes, and a NIOSH-approved particulate filtering facepiece respirator with any N, R or P filter are required to minimize exposure and protect applicators, mixer/loaders, and handlers that are likely to be exposed. Furthermore, all unprotected workers are prohibited from entering treated areas where Stargus Biofungicide has been applied for four hours or until sprays have settled.

The label warnings, restrictions and risk mitigation measures apply to the proposed new uses and are adequate to protect users of Stargus Biofungicide. Overall, occupational risks to workers are acceptable when the precautionary statements on the labels are followed which include PPE.

3.2.2 Residential and bystander exposure and risk

Stargus Biofungicide is currently registered for use as a commercial fungicide on various outdoor food crops. Stargus Biofungicide is being proposed for use on additional terrestrial food crops and industrial hemp. There are no residential uses. The product label includes mitigation measures to prevent bystander exposure such as reducing spray drift.

Stargus Biofungicide is also being proposed for use on cannabis grown in greenhouses and enclosed structures. 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 and technical grade active ingredient are of low toxicity and there were no signs that the MPCA, *B. amyloliquefaciens* strain F727, caused any disease in studies on laboratory animals. Consequently, the health risks to bystanders and individuals in residential areas are acceptable.

As well, *B. amyloliquefaciens* is a species that is common in the environment, and the use of Stargus Biofungicide is not expected to cause sustained increases in exposure to bystanders beyond natural levels.

Consequently, the health risks to bystanders and individuals in residential areas from Stargus Biofungicide are considered 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 crops treated with Stargus Biofungicide are acceptable because *B. amyloliquefaciens* strain F727 demonstrated low toxicity and no pathogenicity or infectivity in Tier I studies. Furthermore, no metabolites of toxicological significance have been shown to be produced by this strain.

When the end-use product is applied to cannabis grown in greenhouses and enclosed structures and to industrial hemp, the health risk from consumer exposure is acceptable.

Consequently, there is no health risk for the general population, including infants and children, or animals.

3.3.2 Drinking water

Dietary exposure from drinking water is expected to be low as the label has the necessary mitigation measures to limit contamination of drinking water from the proposed uses of Stargus Biofungicide. The end-use product is applied as a foliar application to terrestrial crops and on cannabis grown in greenhouses and enclosed structures, and the labels will instruct users not to

contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal, and to not allow effluent or runoff from greenhouses and enclosed structures containing this product to enter lakes, streams, ponds or other waters. Municipal treatment of drinking water is also expected to further reduce the transfer of residues to drinking water. Furthermore, *B. amyloliquefaciens* strain F727 demonstrated low toxicity and no pathogenicity or infectivity in Tier I studies.

Health risks from residues of *B. amyloliquefaciens* strain F727 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 *B. amyloliquefaciens* strain F727 is of low oral toxicity and 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 *B. amyloliquefaciens* strain F727 to human health.

3.3.4 Aggregate exposure and risk

Aggregate exposure is the total exposure to a single pesticide that may occur from food, drinking water, residential and other non-occupational sources, and from all known or plausible exposure routes (oral, dermal and inhalation).

In an aggregate risk assessment, the combined potential risk associated with food, drinking water and various residential exposure pathways is assessed. A major consideration is the likelihood of co-occurrence of exposures. Additionally, only exposures from routes that share common toxicological endpoints can be aggregated.

Stargus Biofungicide is considered to be of low toxicity by the oral, dermal, and inhalation routes and the end-use product will not be applied near or to drinking water. Furthermore, non-occupational exposure will be low when Stargus Biofungicide is used as directed on the label.

When the end-use product is used as labelled, there is reasonable certainty that no harm will result from aggregate exposure of residues of *B. amyloliquefaciens* strain F727.

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 in or 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.

Since Stargus Biofungicide is already approved for use on food crops, residues of *B. amyloliquefaciens* strain F727 on treated food crops are possible at the time of harvest. In addition, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Dietary risk to humans from the current uses is acceptable due to the low toxicity profile of *B. amyloliquefaciens* strain F727.

The expanded uses are not expected to result in greater residue levels than those from currently registered uses.

The PMRA has determined that specification of an MRL under the *Pest Control Products Act* is not required for *B. 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 *B. amyloliquefaciens* strain F727 shares a common mechanism of toxicity with other strains of *B. amyloliquefaciens, B. subtilis*, *B. licheniformis, B. velezensis* that are used as MPCAs; *B. amyloliquefaciens* strain MBI 600, *B. amyloliquefaciens* strain D747, *B. amyloliquefaciens* strain PTA-4838, *B. subtilis* strain QST 713, *B. subtilis* strain GB03, *B. subtilis* strain FMCH 001*, B. subtilis* var. *amyloliquefaciens* strain FZB24*, B. licheniformis* FMCH 002, *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477.

The potential health risks from cumulative exposure of *B. amyloliquefaciens* strain F727 and these other MPCAs are acceptable when used as labelled given their low toxicity and lack of pathogenicity.

4.0 Impact on the environment

4.1 Fate and behaviour in the environment

No new studies were required to address the environmental fate and behaviour of *B. amyloliquefaciens* strain F727. Refer to PRD2018-17, *Bacillus amyloliquefaciens strain F727 and MBI-110 EP Biofungicide* for details.

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 (in other words, 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 estimated environmental concentrations. The absence of adverse effects in Tier I studies is 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 LOC, 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 LOC, 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

No new studies were required to address the potential for effects on terrestrial organisms from exposure to *B. amyloliquefaciens* strain F727. Refer to PRD2018-17, *Bacillus amyloliquefaciens strain F727 and MB-110 EP Biofungicide* for details.

The existing database was reconsidered based on the revision to the product guarantee, and the proposed use expansion to cannabis grown in greenhouses and enclosed structures, industrial hemp and additional terrestrial food crops.

Effects were seen in the 13-day acute dietary toxicity/pathogenicity study with Rove beetles (*Dalotia coriaria*). The 13-day dietary LC_{50} was > 1.402 g/mL. A screening level risk assessment was conducted using a revised estimated environmental concentration (EEC) for soil based on the revised product guarantee of Stargus Biofungicide (see Appendix I). Because the LC_{50} is several orders of magnitude greater than the revised EEC in soil, the risk quotient is below the LOC.

Based on all the available information on the effects of *B. amyloliquefaciens* strain F727 to non-target terrestrial organisms, the risks to birds, wild mammals, terrestrial arthropods, non-arthropod invertebrates, plants or to other non-target microorganisms from the proposed expanded uses and increased product guarantee are acceptable.

4.2.2 Effects on aquatic organisms

No new studies were required to address the potential for effects on aquatic organisms from exposure to *B. amyloliquefaciens* strain F727. Refer to PRD2018-17, *Bacillus amyloliquefaciens strain F727 and MBI-110 EP Biofungicide* for details.

The existing database was reconsidered based on the revision to the product guarantee, and the proposed use expansion to cannabis grown in greenhouses and enclosed structures, industrial hemp and additional terrestrial food crops.

Effects were seen in the 30-day toxicity/pathogenicity study in Rainbow trout (*Onchorhyncus mykiss*; LC_{50}: 2.5×10^7 CFU/mL) and in the 21-day toxicity/pathogenicity study in daphnids (*Daphnia magna*; EC_{50}: 1.3×10^7 CFU/mL).

Screening level risk assessments were conducted using the revised EEC for water based on the revised product guarantee (see Appendix I). Because the LC_{50} and the EC_{50} are several orders of magnitude greater than the revised EEC in water, the risk quotients are below the LOC.

Based on all the available data and information on the effects of *B. amyloliquefaciens* strain F727 to non-target aquatic organisms, and the anticipated increased environmental exposure resulting from the increase in product guarantee and the use expansion to cannabis grown in greenhouses and enclosed structures, industrial hemp and additional terrestrial food crops, the risks to fish, aquatic arthropods, plants and algae are acceptable.

4.3 Environment incident reports

Bacillus amyloliquefaciens strain F727 is an active ingredient that has been registered for use in Canada since 2018, and as of 17 June 2022, the PMRA did not receive any incident reports related to the environment.

5.0 Value

The availability of Stargus Biofungicide will serve as a new non-conventional product option to manage economically important diseases in both conventional and organic crop production systems. Its use can be expected to help mitigate the risk of resistance development to conventional fungicides.

Value information was in the form of rationales and multiple efficacy trials in which Stargus Biofungicide or a similar product was applied at one or more rates. Rationales were based on extrapolations between crops affected by the same disease.

A claim of partial suppression of grey mould on indoor-grown cannabis is supported based on the results of one greenhouse cannabis trial and extrapolation from supported partial suppression claims against this disease on field-grown hemp and the similar disease of botrytis bunch rot on grape.

A review of the value information supports a claim of partial suppression for Stargus Biofungicide for each of the following diseases by crop: grey mould on field-grown hemp, white mould on field-grown hemp, botrytis bunch rot on grape, early blight on field tomato and potato, late blight on field tomato and potato, cercospora leaf spot on sugar beet and red (garden) beet, and black rot on cabbage and Brussels sprouts. The data also collectively support a claim of suppression of late blight on both tomato and potato for Stargus Biofungicide when applied at 10 L product/ha.

No injury was observed to any of the crops treated with Stargus Biofungicide or similar products at supported use rates.

Details of the supported uses can be found in Appendix II, Table 1.

6.0 Pest control product policy considerations

6.1 Toxic substances management policy considerations

Refer to PRD2018-17, Bacillus amyloliquefaciens strain F727 and MBI-110 EP Biofungicide for details.

6.2 Formulants and contaminants of health concern

Refer to PRD2018-17, Bacillus amyloliquefaciens strain F727 and MBI-110 EP Biofungicide for details.

7.0 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 MBI-110 TGAI and Stargus Biofungicide, containing the technical grade active ingredient *B. amyloliquefaciens* strain F727, to partially suppress grey mould on indoor-grown cannabis and to suppress or partially suppress certain economically important diseases on field-grown hemp, grape, tomato, potato, red (garden) beet, sugar beet, cabbage and Brussels sprouts.

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

µg	micrograms
ADI	acceptable daily intake
ARfD	acute reference dose
CFU	colony forming units
EC_{50}	effective concentration on 50% of the population
EEC	Estimated Environmental Concentration
EP	end-use product
FRAC	Fungicide Resistance Action Committee
g	Gram
ha	hectare(s)
L	Litres
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LOC	level of concern
MCC	maximum challenge concentration
mL	millilitres
MPCA	microbial pest control agent
MRL	maximum residue limit
OECD	Organisation for Economic Co-operation and Development
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
RQ	risk quotient
TGAI	technical grade of the active ingredient
v/v	volume per volume dilution

Appendix I Estimated environmental concentration

Aquatic

The maximum proposed application rate of Stargus Biofungicide is 10 L/ha or, based on the revised product guarantee, 1×10^{13} CFU/ha. Therefore, assuming that the maximum application rate was applied to surface water, the aquatic exposure estimate is 6.7×10^{3} CFU/mL.

Soil

The maximum proposed application rate of Stargus Biofungicide is 10 L/ha or based on the revised product guarantee, 1×10^{13} 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^{3} CFU/g (4.4 µg Stargus Biofungicide/g).

Appendix II Tables and figures

Table 1 List of supported uses

Supported use claim
Disease claim: Partial suppression of grey mould (*Botrytis cinerea*) Crop: Cannabis (indoor, including greenhouses and other enclosed facilities) Rate: 5–15 mL/L water Adjuvant: None Application method: to foliage using indoor spraying equipment Spray volume: sufficient to achieve full coverage without runoff Application timing: preventatively Maximum number of applications: no limit Application interval: 7 days
Disease claim: Partial suppression of grey mould (*Botrytis cinerea*); partial suppression of white mould (*Sclerotinia sclerotiorum*) Crop: Hemp (field) Rate: 4–8 L product/ha Adjuvant: None Application method: to foliage using ground application equipment Spray volume: 300–500 L water/ha Application timing: begin applications when conditions are conducive for disease development Maximum number of applications: no limit Application interval: 7 days
Disease claim: Partial suppression of botrytis bunch rot (*Botrytis cinerea*) Crop: Grape Rate: 5 L product/ha Adjuvant: None Application method: to foliage using ground application equipment Spray volume: 500–1500 L water/ha Application timing: first application at bloom with subsequent applications at bunch closure, veraison, and preharvest Maximum number of applications: 4 Application interval: in accordance with phenological stage

Disease claim: Partial suppression of early blight (*Alternaria solani*) Crop: Tomato Rate: 5–10 L product/ha Adjuvant: None Application method: to foliage using ground application equipment Spray volume: 300–500 L water/ha Application timing: begin applications when conditions are conducive for disease development Maximum number of applications: no limit Application interval: 7 days
Disease claim: Late blight (*Phytophthora infestans*): partial suppression at 7 L product/ha; suppression at 10 L product/ha Crop: Tomato Rate: 7–10 L product/ha Adjuvant: None Application method: to foliage using ground application equipment Spray volume: 300–500 L water/ha Application timing: begin applications when conditions are conducive for disease development Maximum number of applications: no limit Application interval: 7 days
Disease claim: Partial suppression of early blight (*Alternaria solani*) Crop: Potato Rate: 8–10 L product/ha Adjuvant: None Application method: to foliage using ground application equipment Spray volume: 300–500 L water/ha Application timing: begin applications when conditions are conducive for disease development Maximum number of applications: no limit Application interval: 7 days
Disease claim: Late blight (*Phytophthora infestans*): partial suppression at 8 L product/ha; suppression at 10 L product/ha Crop: Potato Rate: 8–10 L product/ha

Adjuvant: None Application method: to foliage using ground application equipment Spray volume: 300–500 L water/ha Application timing: begin applications when conditions are conducive for disease development Maximum number of applications: no limit Application interval: 7 days
Disease claim: Partial suppression of cercospora leaf spot (*Cercospora beticola*) Crop: Red (garden) beet Rate: 5 L product/ha Adjuvant: None Application method: to foliage using ground application equipment Spray volume: 175–250 L water/ha Application timing: begin applications when conditions are conducive for disease development Maximum number of applications: no limit Application interval: 7 days
Disease claim: Partial suppression of cercospora leaf spot (*Cercospora beticola*) Crop: Sugar beet Rate: 5 L product/ha Adjuvant: None Application method: to foliage using ground application equipment Spray volume: 175–250 L water/ha Application timing: begin applications when conditions are conducive for disease development Maximum number of applications: no limit Application interval: 7 days
Disease claim: Partial suppression of black rot (*Xanthomonas campestris*) Crop: Cabbage Rate: 6–8 L product/ha Adjuvant: addition of 0.125–0.25% v/v non-ionic surfactant Application method: to foliage using ground application equipment Spray volume: 300–500 L water/ha Application timing: begin applications when conditions are conducive for disease

development Maximum number of applications: no limit Application interval: 7 days
Disease claim: Partial suppression of black rot (*Xanthomonas campestris*) Crop: Brussels sprouts Rate: 6–8 L product/ha Adjuvant: addition of 0.125–0.25% v/v non-ionic surfactant Application method: to foliage using ground application equipment Spray volume: 300–500 L water/ha Application timing: begin applications when conditions are conducive for disease development Maximum number of applications: no limit Application interval: 7 days

References

A. List of studies/Information submitted by registrant

1.0 Chemistry

PMRA document number	Reference
3194828	2021, M2.8 and M 2.9, DACO: M2.8,M2.9
3195107	2021, M2.8 and M 2.9, DACO: M2.8,M2.9
3183512	2020, Use Description, DACO: 5.2

2.0 Human and animal health

PMRA document number	Reference
3183512	2020, Use Description, DACO: 5.2

3.0 Value

PMRA document number	Reference
3300462	2019, Foliar Application of MBI-110 EP against Botrytis Bunch Rot (*Botrytis cinerea*) on Grape (*Vitis* sp.), DACO: M10.2.2
3300463	2019, Foliar Application of MBI-110 EP against Botrytis Bunch Rot (*Botrytis cinerea*) on Grape (*Vitis* sp.), DACO: M10.2.2
3300464	2019, Data Summary for Study 19-003INTL: Stargus (MBI-110) for Control of *Botrytis fukeliana* on Grape, DACO: M10.2.2
3300466	2014, Data Summary for Study 14-069EDC: Stargus for Control of *Cercospora beticola*, Leaf Spot of Sugar Beet (*Beta vulgaris*), DACO: M10.2.2
3300467	2015, Determination of Efficacy of MBI 110 against Leaf Spot (*Cercospora beticola*) on sugar beet, 1 Site in Romania 2014, DACO: M10.2.2
3300468	2014, Efficacy of M-110 (MBI-110 EP) against *Cercospora beticola* on sugarbeet, DACO: M10.2.2
3300471	2018, Data Summary for Study 18-018DDM: Stargus for Control of Late Blight (*Phytophthera infestans*) on Potato, DACO: M10.2.2
3300472	2019, Foliar Application of MBI-110 EP against Late Blight (Alternaria solani) on Potatoes (*Solanum tuberosum* 'Ranger Russet'), DACO: M10.2.2
3300473	2020, Foliar Application of MBI-110 EP against Botrytis (*Botrytis cinerea*) on Greenhouse Cannabis (*Cannabis sativa*), DACO: M10.2.2,M10.3

3300474	2018, To determine the efficacy of MBI110 for the control of late blight in Solanaceae, DACO: M10.2.2,M10.3
3300475	2018, To determine the efficacy of MBI110 for the control of late blight in Solanaceae, DACO: M10.2.2,M10.3
3300478	2018, Amplitude *Cercospora* 2, DACO: M10.2.2,M10.3
3300479	2020, Foliar Application of MBI-110 EP against Fungal Diseases on Hemp (*Cannabis sativa*), DACO: M10.2.2,M10.3
3300480	2019, Data Summary for Study 19-018MS: Stargus for Control of Botrytis (*Botrytis cinerea*) on Hemp (*Cannabis* sp.), DACO: M10.2.2,M10.3
3300483	2020, Data Summary for Study 20-001MS-INT: Stargus for Control of Botrytis Bud Rot (*Botrytis cinerea*) on Cannabis, DACO: M10.2.2,M10.3
3300484	2014, Data Summary for Study 14-003EDC: Stargus for Control of *Phytophthora infestans*, Late Blight of Potato (*Solanum tuberosum*), DACO: M10.2.2,M10.3
3300485	2018, Data Summary for Study POT-4 Stargus for Control of Early Blight (*Alternaria solani*) on Potato (*Solanum tuberosum*), DACO: M10.2.2,M10.3
3300486	2014, Data Summary for Study 14-004EDC: Stargus for Control of *Phytophthora infestans*, Late Blight of Potato (*Solanum tuberosum*), DACO: M10.2.2,M10.3
3300487	2014, Efficacy of M110 (MBI-110-EP) applied against *Phytophthora infestans* in potato, DACO: M10.2.2,M10.3
3300488	2014, Data Summary for Study 14-009EDC: Stargus for Control of *Phytophthora infestans*, Late Blight of Potato (*Solanum tuberosum*), DACO: M10.2.2,M10.3
3300489	2014, Data Summary for Study 14-010EDC: Stargus for Control of *Phytophthora infestans*, Late Blight of Potato (*Solanum tuberosum*), DACO: M10.2.2,M10.3
3300492	2020, Foliar Application of MBI-110 EP against Black Rot (*Xanthomonas campestris* pv. *campestris*) and Alternaria leaf spot (*Alternaria brassicicola*) on Brussel sprouts (*Brassica oleracea* 'Hestia'), DACO: M10.2.2,M10.3
3300493	2020, Foliar Application of MBI-110 EP against Black Rot (*Xanthomonas campestris* pv. *campestris*) on Cabbage (*Brassica oleracea* var. *capitata* 'Cheers'), DACO: M10.2.2,M10.3
3300494	2017, Evaluation of fungicides for the control of foliar and fruit diseases of processing tomatoes, 2017, DACO: M10.2.2,M10.3
3357767	2022, Value Summary for Stargus Biofungicide , DACO: 10.1
3357770	2020, Foliar Application of MBI-110 EP (Stargus) against Cercospora leaf spot (*Cercospora beticola*) in Table Beet (*Beta vulgaris* subsp. *vulgaris* L), DACO: 10.2.3.3(C)