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Pest Management Regulatory Agency

Santé Canada
Agence de réglementation de la lutte antiparasitaire

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PROPOSED REGISTRATION DECISION

Bacillus subtilis strain MBI 600

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FOREWORD

Proposed Decision for *Bacillus subtilis* strain MBI 600

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act*, is proposing full registration for the sale and use of technical grade active ingredient *Bacillus subtilis* MBI 600 Technical containing the microbial pest control agent *Bacillus subtilis* strain MBI 600, the manufacturing-use product Subtilex™ Biological Fungicide and the end-use products Pro-Mix (HP, BX, PGX and TA) with Biogfungicide to suppress damping-off and root-rot diseases caused by *Pythium* spp. on greenhouse vegetables, including transplants and greenhouse ornamentals.

Bacillus subtilis strain MBI 600 in Pro-Mix with Biofungicide is a biological antagonist that colonizes developing root systems and suppresses *Pythium* spp. by competing against it for space and resources and by producing an antibiotic protein with broad fungicidal activity. This microbial pest control agent is naturally occurring and has not been genetically modified.

Microbial pest control agents are increasingly investigated for use as alternatives to conventional pesticides because they are thought to pose a lower potential risk to human health and the environment. Pro-Mix with Biofungicide represents a potential biological replacement for chemical pesticides.

Current scientific data from the applicant, scientific reports and information from other regulatory agencies were evaluated to determine if, under the proposed conditions of use, the end-use products have value and do not present an unacceptable risk to human health or the environment.

This Proposed Registration Decision document summarizes the information that was evaluated, provides the results of the evaluation, describes the conditions that are required to ensure that the health and environmental risks as well as the value of these pest control products are acceptable for their intended use and provides the reasons for the registration decision.

The information in this Proposed Registration Decision document is presented in two parts. The Overview describes the regulatory process and key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessment of *B. subtilis* strain MBI 600.

The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (please see contact information on the cover page of this document).

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OVERVIEW

Proposed Registration Decision for Pro-Mix With Biofungicide

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act*, is proposing full registration for the sale and use of *Bacillus subtilis* MBI 600 Technical containing the microbial pest control agent (MPCA) *Bacillus subtilis* strain MBI 600, the manufacturing-use product Subtilex™ Biological Fungicide and the end-use products Pro-Mix (HP, BX, PGX and TA) with Biofungicide for suppression of damping-off and root-rot diseases caused by *Pythium* spp. on greenhouse vegetables, including transplants and greenhouse ornamentals.

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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 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 conditions or proposed conditions of registration[1]. The Act also requires that products have value[2] when used according to 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* 2002.

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act* 2002: "...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".

Rigorous and modern hazard and risk assessment methods and policies are applied to reach decisions. These methods consider the unique characteristics of sensitive subpopulations in both humans (e.g., children) and organisms in the environment (e.g., those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties present 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 PMRA's website at www.pmra-arla.gc.ca.

What Is Pro-Mix With Biofungicide?

The four Pro-Mix with Biofungicide end-use products (HP, BX, PGX and TA) are peat-based soilless growing media that contain the biological pesticide *B. subtilis* strain MBI 600. *Bacillus subtilis* strain MBI 600 is a naturally occurring bacteria that rapidly colonizes the roots of growing plants and produces an antibiotic protein that suppresses the ability of *Pythium* spp. to grow and reach levels necessary to trigger damping-off or root-rot diseases. The four products are formulated for different uses by greenhouse growers.

Pro-Mix BX with Biofungicide is a general purpose, peat-based professional growing medium designed for the cultivation of a wide variety of horticultural plants including vegetable transplants.

Pro-Mix HP with Biofungicide is a highly porous, peat-based professional growing medium designed for the cultivation of a wide variety of horticultural plants including water sensitive crops, the propagation of plant cuttings and/or use in low-light conditions.

Pro-Mix PGX with Biofungicide is a peat-based professional growing medium designed for the germination of ornamental and vegetable seeds with plug systems.

Pro-Mix TA with Biofungicide is a general purpose, peat-based professional growing medium designed for the germination and growth of tobacco, as well as other vegetable and ornamental transplants.

❖ Health Considerations

♦ Can Approved Uses of Pro-Mix With Biofungicide Affect Human Health?

***Bacillus subtilis* strain MBI 600 is unlikely to affect your health when Pro-Mix with Biofungicide is used according to label directions.**

Exposure to *B. subtilis* strain MBI 600 may occur during handling of Pro-Mix with Biofungicide (soilless growing media). When assessing health risks, several key factors are considered: the microorganism's biological properties (e.g., production of toxic byproducts); reports of any adverse incidents; its potential to cause disease or toxicity as determined in toxicological studies; and the likely levels to which people may be exposed relative to exposures already encountered in nature to other strains of the microorganism. Toxicology studies in laboratory animals describe potential health effects from large

doses in hopes of identifying any potential to cause disease or toxicity. No significant toxicity and no signs of causing diseases were observed when *B. subtilis* strain MBI 600 was tested on laboratory animals.

♦ Residues in Water and Food

Dietary risks from food and water are not of concern.

Bacillus subtilis strains are common in nature, and the use of Pro-Mix with Biofungicide as growing medium for plants is not expected to significantly increase the natural environmental background levels of this microorganism. Few, if any, bacteria are expected to remain as residues on plants at harvest because *B. subtilis* strain MBI 600 is present in the plant's growing medium. Some strains of *B. subtilis* have been isolated from food samples implicated in food poisoning; however, these strains demonstrated the ability to produce a highly heat-stable toxin that may be similar to a toxin produced by *Bacillus cereus*, a known food-borne pathogenic microorganism. *Bacillus subtilis* strain MBI 600 is not reported to produce this toxin. Also, no such effects were reported for this microorganism in the United States where it has been registered since 1994. Furthermore, there was no significant toxicity, and no signs of causing diseases were observed when *B. subtilis* strain MBI 600 was administered orally to rats. The establishment of a maximum residue limit (MRL) is therefore not required for *B. subtilis* strain MBI 600.

The *Food and Drugs Act* prohibits the sale of food containing a pesticide residue that exceeds the established maximum residue limit (MRL). Pesticide MRLs are established for the *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act.* Each MRL value defines the maximum concentration in parts per million (ppm) of a pesticide allowed in/on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk. Furthermore, the likelihood of residues of *B. subtilis* strain MBI 600 contaminating drinking water supplies is negligible to non-existent. Consequently, dietary exposure and risk are minimal to non-existent.

♦ Occupational Risks From Handling Pro-Mix With Biofungicide

Occupational risks are not of concern when Pro-Mix with Biofungicide is used according to label directions, which include protective measures.

Growers handling Pro-Mix with Biofungicide can come into direct contact with *B. subtilis* strain MBI 600 on the skin, in the eyes or by inhalation. For this reason, the label will specify that growers exposed to Pro-Mix with Biofungicide must wear waterproof gloves, a long-sleeved shirt, long pants, shoes and socks. Handlers must also wear a dust filtering mask and eye goggles when opening bags of product and/or filling potting machines. Furthermore, early-entry workers will be restricted from entering areas where dry Pro-Mix with Biofungicide was handled for a period of up to four hours unless wearing the appropriate personal protective equipment.

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

❖ Environmental Considerations

♦ What Happens When Pro-Mix With Biofungicide Is Introduced Into the Environment?

Environmental risks are not of concern.

In published literature, other strains of *B. subtilis* have been reported to cause infections in mammals, terrestrial insects and plants. However, these reports were few in number despite the large amount of published literature on this microorganism and involved either unusual strains or the ability of the select strain of *B. subtilis* to cause disease was not thoroughly investigated. There are no published reports of disease associated with *B. subtilis* in birds, earthworms, bees, aquatic invertebrates, fish, algae and aquatic plants. *Bacillus subtilis* is not generally considered to be a disease-causing agent. Therefore, Pro-Mix with Biofungicide is expected to present a negligible risk to non-target organisms.

❖ Value Considerations

♦ What Is the Value of Pro-Mix With Biofungicide?

The four Pro-Mix with Biofungicide end-use products suppress damping-off and root-rot diseases caused by *Pythium* spp. on greenhouse crops. The use of these products as seeding or planting media will replace the first preventative fungicide application; thus, it may reduce the number of applications of chemical fungicides. This reduction in the number of applications could decrease the possibility of pathogens developing resistance to traditional chemical-based fungicides. These four end-use products can also potentially enhance the adoption of reduced-risk technologies by producers because they are ready for immediate use, have a long shelf-life (up to 24 months) and offer few risks to human health and the environment.

Measures to Minimize Risk

Registered pesticide product labels include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions are required by law to be followed.

The key risk-reduction measures on the label of Pro-Mix with Biofungicide to address the potential risks are as follows:

Key Risk-Reduction Measures

- **Human Health**

Because of concerns with toxicity via the pulmonary route and with users developing allergic reactions through repeated high exposures to *B. subtilis* strain MBI 600, anyone handling Pro-Mix with Biofungicide must wear waterproof gloves, a long-sleeved shirt, long pants, shoes and socks. A dust/mist filtering mask must also be worn when opening bags and/or filling potting machines. Furthermore, early-entry workers will be restricted from entering areas where dry Pro-Mix with Biofungicide was handled for a period of up to four hours unless wearing the appropriate personal protective equipment.

- **Environment**

As a general precaution, handlers are asked to not contaminate irrigation or drinking water or aquatic habitats by cleaning of equipment or by disposing of wastes. In addition, growers must not allow effluent or runoff from greenhouses containing this product to enter lakes, streams, ponds or other water bodies.

Other Information

At the time the PMRA makes its registration decision, the PMRA will publish an Evaluation Report on *B.* subtilis strain MBI 600 (based on the Science Evaluation section of this consultation document). In addition, the test data on which the decision is based will also be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

SCIENCE EVALUATION

Bacillus subtilis strain MBI 600

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Bacillus subtilis* strain MBI 600
Function	Suppress damping-off and root-rot diseases by *Pythium* spp. on greenhouse vegetables, including transplants and greenhouse ornamentals.
Binomial name	*Bacillus subtilis* strain MBI 600
Taxonomic designation	
Kingdom	Eubacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Bacillaceae
Genus	*Bacillus*
Species	*subtilis*
Strain	MBI 600
Patent status information	Canadian Patents: i. 1324099 Issued: 9 November 1993 Expiration: 9 November 2010 ii. 1337935 Issued: 16 January 1996 Expiration: 16 January 2013
Minimum purity of active ingredient	5.5×10^{11} spores/g

Active microorganism	*Bacillus subtilis* strain MBI 600
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. In public scientific literature, *B. subtilis* has been reported to produce small antibiotic peptides and peptidolipids that are predominantly active against Gram-positive bacteria, but also against Gram-negative bacteria, yeast and fungi. These include intracellular peptidolipids (mycosubtilin), extracellular cyclic peptidolipids (*Aspergillus* factor, bacillomycin A/B/D/F/L/R/S, eumycin, fengycin, iturin A and toximycin) and extracellular cyclic peptides (chaetomacin, fungistatin, mycobacillin and *Rhizoctonia* factor). Hemolytic activity has been reported for some peptidolipids. Strain MBI 600 also produces a 63 kDa antibiotic protein with demonstrated activities against Gram-positive bacteria and fungi, but no mammalian toxicity has been reported.

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

Technical Product—*Bacillus subtilis* MBI 600 Technical

Property	Result
Colour	Beige-white
Odour	Musty
Physical state	Powder
Formulation type	Wettable powder
Guarantee	5.5×10^{11} spores/g
Container material and description	25 kg polypropylene container
Density	0.44 g/cm^3
pH of 1% dispersion in water	4.8
Oxidizing or reducing action	None
Storage stability	N/A
Explodability	N/A

Manufacturing-Use Product—Subtilex™ Biological Fungicide

Property	Result
Colour	Beige-white
Odour	Musty
Physical state	Powder
Formulation type	Wettable powder
Guarantee	5.5×10^{10} spores/g
Container material and description	400 g polypropylene vacuum pak
Density	0.700 g/cm^3
pH of 1% dispersion in water	4.8
Oxidizing or reducing action	None
Storage stability	16 months at temperatures up to 25°C
Explodability	N/A

End-Use Products—Pro-Mix (BX, HP, PGX and TA) With Biofungicide

Property	Result
Colour	Not reported
Odour	Not reported
Physical state	Solid-granular (peat-based growing material)
Formulation type	Solid
Guarantee	0.001% *Bacillus subtilis* strain MBI 600 (not less than 1×10^7 colony forming units [CFU] per litre of growing medium)
Container material and description	107 L loose fill bag and 85 L compressed bag
Density	0.13–0.16 g/cm^3
pH of 1% dispersion in water	5.0–6.2
Oxidizing or reducing action	None

Property	Result
Storage stability	24 months at temperatures up to 25°C
Explodability	N/A

1.3 Details of Uses and Further Information

For effective suppression of Pythium damping-off and root-rot diseases, greenhouse growers should sow or transplant vegetable and ornamental crops directly into the Pro-Mix with Biofungicide growing medium. Prior to seeding or transplanting crops into trays, flats or pots, water must be added to Pro-Mix with Biofungicide until slightly moist to the touch. Pro-Mix with Biofungicide should not be reused. Spent media must be disposed in a manner that will not contaminate irrigation or drinking water or aquatic habitats. Growers should follow other use directions specifically mentioned on the labels.

1.4 Mode of Action

Bacillus subtilis strain MBI 600 is a rapid root coloniser, establishing itself quickly on the seed and root surface and thereby outcompeting potential fungal root pathogens. Additionally, a new antifungal protein is produced *in situ* by the bacterium that suppresses the ability of *Pythium* spp. to grow and trigger damping-off or root-rot diseases.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

Three methods are employed to establish the identity of the microbial pest control agent (MPCA). This first method uses a commercial test kit called API 50 CHB (bioMérieux Industry, France) that can identify the microorganism to the species level *B. subtilis*. This test is conducted on each batch of concentrate by an independent laboratory, MicroBioTest Inc., Chantilly, Virginia. The second method is a simple plating test on agar media to confirm the colony morphology of the MCPA and is performed by the manufacturer. The third method uses column chromatography (Sephadex) to determine the molecular weight of a secreted protein. *Bacillus subtilis* strain MBI 600 produces a unique antibiotic protein with a molecular weight that is approximately three times greater than conventional isolates of *B. subtilis*.

2.2 Methods for Establishment of Purity of Seed Stock

The original source of *B. subtilis* strain MBI 600 is stored and maintained at Nottingham University, United Kingdom (original supplier), and the American Type Culture Collection, United States. Mother cultures are also maintained at the manufacturing site as frozen cultures in 3050 ME4 medium (i.e., a standard growth medium for *B. subtilis*).

When mother cultures are replenished, each batch is subject to biochemical testing (API 50 CHB; see Section 2.1) as well as microbial contaminant analysis by streaking onto trypticase

soya agar (TSA), plate count agar (PCA), non-fat milk (NFM) plates and spreading 0.1 mL onto MacConkey's agar (MAC) and TSA phage plates. Only culture showing typical API test results, typical colony morphologies on TSA, PCA, NFM and TSA phage plates and no growth on MAC plates are considered acceptable for use as mother cultures.

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

The concentration of the MPCA in *B. subtilis* MBI 600 Technical and Subtilex™ Biological Biofungicide is routinely assayed by plating serially diluted samples onto nutrient agar (NA) then counting the number of colonies following incubation at 28–29°C for 18–24 hours. This assay method detects both vegetative cells and endospores of *B. subtilis*. The concentration of the MPCA in the Pro-Mix line of end-use products is assayed using a separate method to exclude naturally occurring microorganisms in the peat-based growing medium. A known quantity of end-use product is first homogenized in sterile demineralized water maintained at 80°C then incubated at 80°C for an additional 30-minute period. Following this incubation period, a 5-mL sample is serially diluted in sterile demineralized water then plated onto NA using a spiral system. The plates are then counted following incubation at 20–27°C for 3–5 days. This assay method will only detect endospores of *B. subtilis*.

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

Bacillus subtilis is a ubiquitous microorganism in nature and has been isolated from a wide variety of environments. According to the United States Food and Drug Administration, some strains of *B. subtilis* have been isolated from food samples implicated in food poisoning. These strains, however, demonstrated the ability to produce a highly heat-stable toxin that may be similar to the vomiting type toxin produced by *B. cereus*, a known food-borne pathogenic microorganism. *Bacillus subtilis* strain MBI 600 is not reported to produce this toxin. Also, no such effects were reported for this microorganism in the United States where it has been registered since 1994. Furthermore, CD rats treated with a 1% *B. subtilis* strain MBI 600 suspension in distilled water by gavage in a single oral dose of 20 mL/kg body weight displayed no significant toxicity and no pathogenicity. Spores of *B. subtilis* were recovered from feces, urine and blood of treated animals following treatment, but these completely cleared from all treated animals by study termination on day 22.

Based on the above information, the establishment of a maximum residue limit (MRL) is not required for *B. subtilis* strain MBI 600 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drug Regulations.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

During manufacturing, several approaches are used to limit microbial contamination in *B. subtillis* MBI 600 Technical and Subtilex™ Biological Fungicide. These approaches include large quantities of inocula, inoculation at elevated temperatures, frequent purity checks on agar

media, sterilization of all equipment and media, reducing the pH where relevant and sanitization of recovery equipment. Different approaches are used to limit microbial contamination in Pro-Mix with Biofungicide. These differences reflect the unique manufacturing requirements for producing soilless growing media. These approaches include purity checks on agar media, the sanitization of tanks and lines used to apply *B. subtilis* strain MBI 600 onto the peat medium as well as purges with pure peat moss to "clean" the manufacturing systems and packaging equipment. The peat moss is then collected and recycled in future batches. It is impractical to attempt to sanitize or sterilize the entire production line.

To ensure that the above quality control procedures are limiting contaminating microorganisms, samples of product are tested using standard microbiological procedures. For *B. subtilis* MBI 600 Technical and Subtilex™ Biological Fungicide, samples are streaked onto TSA, PCA and NFM plates and spread onto MAC and TSA phage plates to detect any unusual colonies and verify colony morphology. Samples are also routinely tested via API 50 CHB, and Subtilex™ Biological Fungicide is assayed for *Salmonella* spp. and *Shigella* spp. For Pro-Mix with Biofungicide, samples of the end-use product are assayed for *Trichoderma* spp., *Fusarium* spp., *Pythium* spp., actinomycetes, spores of *B. subtilis* as well as vegetative and/or spore cells of *B. subtilis* and other applicable bacteria at each hour of production (continuous manufacturing process).

2.6 Methods to Show Absence of Any Human and Mammalian Pathogens

As noted in Section 2.5, several approaches are used to limit microbial contamination in *B. subtilis* MBI 600 Technical, Subtilex™ Biological Fungicide and Pro-Mix with Biofungicide. These procedures include frequent purity checks on various agar media to detect any unusual colonies and to verify colony morphology as well as assays designed to detect *Salmonella* spp., *Shigella* spp., *Trichoderma* spp., *Fusarium* spp. and *Pythium* spp. Most of these methods, with the exception of the *Salmonella-Shigella* assay, do not distinguish human and mammalian pathogens from other contaminating microorganisms. However, additional microbial-specific testing will not be required to support registration because detailed microbial screens found no detectable levels of contaminating microorganism in 12 lots of Subtilex™ Biological Fungicide and found reasonable levels of mould, yeast and coliforms in 20 lots of Pro-Mix BX with Biofungicide. Please note that certain levels of contaminating microorganisms are expected in Pro-Mix with Biofungicide based on the type of product, i.e., a peat-based soilless growing medium.

2.7 Methods to Determine Storage Stability, Shelf-Life of the Microorganism

The storage stability of 5 lots of Subtilex™ Biological Fungicide was evaluated after 16 months in a laboratory maintained at approximately 25°C. Assay results showed that Subtilex™ Biological Fungicide was stable for a period of up to 16 months. Similarly, the storage stability of 5 lots of Pro-Mix BX with Biofungicide and single lots of 5 different substrates (BX, PGX, HP, VFT and BRK formulations) were evaluated outdoors over periods of up to 24 months. Assay results showed that Pro-Mix with Biofungicide was stable for a period of up to 24 months at outdoor temperatures when the product was kept dry.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

The PMRA conducted a detailed review of the toxicological database for *B. subtilis* strain MBI 600. The database is complete, consisting of laboratory animal (in vivo) toxicity studies (acute oral toxicity/pathogenicity, acute pulmonary toxicity/pathogenicity, acute intravenous, acute dermal toxicity/irritation, dermal sensitization and eye irritation studies) currently required for health hazard assessment purposes that were carried out in accordance with currently accepted international testing protocols and Good Laboratory Practice. Waiver requests were deemed acceptable to address the acute dermal toxicity and dermal irritation of Pro-Mix with Biofungicide. The scientific quality of the data is high, and the database is considered sufficient to characterize the toxicity and infectivity of this pest control agent and product.

A survey of published literature has revealed a number of instances where other *B. subtilis* strains had been implicated in infections in humans as well as the causal agent in food poisonings. Postoperative cellulitis, septicemia, respiratory disease, endocarditis and pneumonia have been reported in humans. In many instances, the association of *B. subtilis* is not sufficiently rigid for it to be regarded unequivocally as the causative agent. Also, the number of putative infections are extremely low considering the total number of reports of bacterial infections. Many cases of those cases involved drug abuses or severely debilitated patients. *Bacillus subtilis* is virtually ubiquitous in the environment; therefore, it is expected that *B. subtilis* may sometimes be found in association with other microorganisms in infections. Only individuals treated with immunosuppressive drugs appear to be susceptible to infection from *B. subtilis*. In food-borne illnesses, the United States Food and Drug Administration has noted that some strains of *B. subtilis* have been isolated from food implicated in food poisoning. These strains, however, demonstrated the ability to produce a highly heat-stable toxin that may be similar to the vomiting type toxin produced by *B. cereus*, a known food-borne pathogenic microorganism. *Bacillus subtilis* strain MBI 600 is not reported to produce this toxin. Also, no such illnesses were reported for this microorganism in the United States where it has been registered for use on crops since 1994.

In other mammals, *B. subtilis* has been implicated in cases of bovine mastitis and reproductive disorders in goats. In the cases of bovine mastitis, *B. subtilis* could not be excluded as the causative agent. In goats exhibiting reproductive problems, high bacterial loads in infected vaginas were found to correlate with clinical symptoms. However, *B. subtilis* isolated from infected tissue was not pathogenic to Swiss white mice.

Bacillus subtilis has been reported to produce small antibiotic peptides and peptidolipids that are predominantly active against Gram-positive bacteria but also against Gram-negative bacteria, yeast and fungi. These include intracellular peptidolipids (mycosubtilin), extracellular cyclic peptidolipids (*Aspergillus* factor, bacillomycin A/B/D/F/L/R/S, eumycin, fengycin, iturin A and toximycin) and extracellular cyclic peptides (chaetomacin, fungistatin, mycobacillin and *Rhizoctonia* factor). Hemolytic activity has been reported for some peptidolipids.

For strain MBI 600, the following studies were submitted to support registration:

- acute oral toxicity/pathogenicity;
- acute pulmonary toxicity/pathogenicity;
- acute intravenous infectivity;
- acute dermal toxicity/irritation; and
- eye irritation study.

In the oral toxicity/pathogenicity study, no significant toxicity was observed in CD rats following oral gavage with 2×10^8 spores of *B. subtilis* strain MBI 600. The MPCA was initially detected in the feces, urine and gastrointestinal contents of treated rats as well as in blood of treated rats on day 7, but was completely cleared from all organs and fluids by day 22. In the pulmonary toxicity/pathogenicity study, significant mortality was observed following intratracheal treatment with strain MBI 600 at doses ranging from 3.3×10^8 to 3.7×10^8 viable spores per animal. Clinical symptoms included piloerection, hunched posture, abnormal gait, lethargy, pallor of extremities, increased respiration, gasping, collapse, body-weight loss, low body-weight gain and lower body temperatures. In surviving rats, viable spores of the MPCA were initially recovered from the cecum, feces, urine, blood and various other organs. By day 7, viable spores were mainly recovered from the spleen heart and liver of treated rats and, by day 21, were only recovered from feces and cecum of treated animals. Although clearance was not achieved, a definitive pattern of clearance was established. The toxicity observed in this study is consistent with results of other acute pulmonary studies conducted with other *Bacillus* spp. and may possibly be attributed to hemolytic activities of some metabolites produced by *B. subtilis*. To mitigate the risk of pulmonary exposure to *B. subtilis* strain MBI 600, handlers will be required to wear a dust mask when they are most likely to be exposed via the inhalation, i.e., while opening bags and/or filling potting machines. In the intravenous infectivity study, no mortalities and no significant clinical signs of toxicity were observed in CD rats following injection with at least 10^7 spores of *B. subtilis* strain MBI 600 in physiological saline. The MPCA was initially recovered from feces, urine, blood and various organs of all treated rats following treatment. Counts generally decreased as the study progressed, and spores were only recovered from lungs and spleen of treated rats by day 21. A definitive pattern of clearance was established. In the acute dermal toxicity study, no treatment-related mortalities or clinical signs of toxicity other than very slight erythema and/or very slight edema were observed in rabbits treated with an aqueous suspension of *B. subtilis* strain MBI 600 at a dose of 2 mL/kg body weight over approximately 10% of the body surface. In the eye irritation study, only slight conjunctival irritation was observed after 0.1 mL of a suspension of *B. subtilis* strain MBI 600 was instilled into the conjunctival sac of the right eye of New Zealand white rabbits. In the dermal sensitization study, *B. subtilis* strain MBI 600 was found to induce a sensitization reaction in albino guinea pigs following two induction treatments of the MPCA in water and in a 50:50 mixture of Freund's complete adjuvant and water. Please note that all microbial based end-use products are considered to be potential sensitizers regardless of results in dermal sensitization studies. Label statements indicating that Pro-Mix with Biofungicide is a potential sensitizer as well as label precautions requiring personal protective equipment and judicious handling to minimize exposure to workers will be required.

Requests to waive acute dermal toxicity and acute dermal irritation studies were accepted for Subtilex™ Biological Fungicide and all Pro-Mix with Biofungicide end-use products based on the nature and the concentrations of each formulation ingredient. However, Pro-Mix with Biofungicide will be considered to be a mild ocular irritant based on the nature of the end-use product, i.e., a peat-based soilless growing medium, and will require appropriate label statements.

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

Within the available scientific literature, there are no reports that suggest *B. subtilis* has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *B. subtilis* strain MBI 600.

3.2 Occupational/Bystander Exposure and Risk Assessment

3.2.1 Occupational

When handled according to the label instructions, the potential routes of handler exposure to *B. subtilis* strain MBI 600 are pulmonary, dermal and to some extent ocular.

The potential for dermal, eye and inhalation exposure for mixer/loaders, handlers and early-entry workers exists, with the major source of exposure to workers being dermal. Because 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 absorbed dermally. *Bacillus subtilis* has not been identified as a wound pathogen, and there is no indication that it could penetrate intact skin of healthy individuals.

The risk of toxicity exists in individuals exposed to large quantities of spores of *B. subtilis* strain MBI 600. In addition, respiratory hypersensitivity could be expected to develop upon repeated exposure to the product. Specific label wording to minimize exposure to dusts generated while handling dry product is required. Exposure in handlers and early-entry workers will be mitigated by a restricted-entry interval and a label requirement for personal protective equipment, including a dust mask.

Although no dermal toxicity and little dermal irritation are expected based on toxicological studies and the formulation ingredients present in end-use products, all MPCAs are considered potential sensitizers. Label restrictions and risk mitigation measures are required to protect populations that are likely to be primarily exposed to the products. Such exposure to handlers and early-entry workers can be minimized if they wear gloves, a long-sleeved shirt, long pants, shoes and socks. The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions. In addition, a dermal sensitization study found that *B. subtilis* strain MBI 600 was a sensitizing agent.

No eye irritation studies were submitted for any of the end-use products. A study on an aqueous suspension of *B. subtilis* strain MBI 600 showed that the MPCA was minimally irritating. However, the end-use product was considered to be mildly irritating based on the nature of its formulation, i.e., a peat-based growing medium. Consequently, some label restrictions are required to protect populations that are likely to be primarily exposed to the products. Such exposure can be minimized if handlers and early-entry workers wear eye goggles.

3.2.2 Bystander

Overall the PMRA does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for *B. subtilis* strain MBI 600 and the assumption that precautionary label statements will be followed in the use of Pro-Mix with Biofungicide.

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

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

Because the MPCA is present in the soilless growing medium and is not sprayed directly to greenhouse crops, the proposed food use pattern is unlikely to result in significant quantities of residues on treated fruits and vegetables at the time of harvest. While the proposed use pattern may result in some dietary exposure with possible residues in or on agricultural commodities, negligible to no risk is expected for the general population, including infants and children, or animals because *B. subtilis* strain MBI 600 demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity/infectivity study. Dietary exposure to secondary metabolites and antibiotic peptides produced by *B. subtilis* strain MBI 600 is also not expected, given the proposed use pattern of Pro-Mix with Biofungicide and their likely degradation rates in soilless medium. Furthermore, higher-tier subchronic and chronic dietary exposure studies were not required because of the low toxicity of the MPCA and

of the absence of infectivity, toxicity or pathogenicity indications in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity studies. Therefore, there is no concern for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.3.2 Drinking Water

Although *B. subtilis* strain MBI 600 could potentially enter neighbouring aquatic environments via surface-water runoff and can potentially survive in water, no risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and *B. subtilis* strain MBI 600 showed no harmful effects on animals that were exposed orally in Tier I acute oral toxicity and infectivity testing. Specific product labelling will be required to limit surface water runoff. The Pro-Mix with Biofungicide label instructs users not to allow the product to enter bodies of water during use or disposal. Furthermore, municipal treatment of drinking water will likely reduce the transfer of residues to drinking water. Therefore, potential exposure to *B. subtilis* strain MBI 600 in surface and drinking water is negligible.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculation of acute reference doses and acceptable daily intakes is 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 (i.e., 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 *B. subtilis* strain MBI 600 is of low toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCA 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 intraspecies 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 prenatal or postnatal exposures, and cumulative effects on infants and children of the MPCA and other registered microorganisms that have a common mechanism of toxicity, do not apply to this MPCA. As a result, the Agency has not used a margin of exposure (safety) approach to assess the risks of *B. subtilis* strain MBI 600 to human health.

3.4 Maximum Residue Limits

Although the United States Food and Drug Administration has noted that some strains of *B. 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 *B. subtilis* strain MBI 600. Also, no such illnesses were reported for this MPCA in the United States where it has been registered for use on crops since 1994. Furthermore, there were no significant toxicity and no signs of pathogenicity observed when *B. subtilis* strain MBI 600 was administered orally to rats. The establishment of an MRL is therefore not required for *B. subtilis* strain MBI 600 under

Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulation. The Act prohibits the sale of adulterated food, that is, food containing a pesticide residue that exceeds the established MRL. Pesticide MRLs are established for the *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act*. Each MRL value defines the maximum concentration in parts per million (ppm) of a pesticide allowed in/on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

3.5 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the Agency's files, there is reasonable certainty no harm will result from aggregate exposure of residues of *B. subtilis* strain MBI 600 to the general Canadian population, including infants and children, when the microbial pest control product is used according to label directions. 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. The product is to be used in greenhouse sites and is not allowed for use on turf, residential or recreational areas; therefore, dermal and inhalation exposure to the general public will be very low. Furthermore, few adverse effects from exposure to other strains of *B. subtilis* encountered in the environment have been reported. Even if there is an increase in exposure to this microorganism from the use of Pro-Mix with Biofungicide, there should not be any increase in potential human health risk.

3.6 Cumulative Effects

The PMRA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Besides naturally occurring strains of *B. subtilis* in the environment, the Agency is not aware of any other microorganisms or other substances that share a common mechanism of toxicity with this active ingredient. No cumulative effects are anticipated if the residues of *B. subtilis* strain MBI 600 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

No studies were submitted to address the environmental fate and behaviour of *B. subtilis* strain MBI 600. Environmental fate data (Tier II/III) are not required due to the absence of significant toxicological effects in non-target organisms in Tier I testing. Environmental fate testing is intended to demonstrate whether an MPCA is capable of surviving or replicating in the environment to which it is applied. These results could provide an indication of which non-target organisms may be exposed to the MPCA as well as provide an indication of the extent of exposure. Some information on the environmental fate of *B. subtilis* was submitted in support of requests to waive environmental toxicology data requirements and as part of a literature review of *B. subtilis*.

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. Other habitats such as fresh water, polluted sea water, deep-sea sediments, foods, milk, pharmaceuticals, etc. may have acquired bacilli from soil by runoff, from dust and from colonized plant materials.

The primary habitat of the majority of *Bacillus* species is considered to be soil. The isolation of *B. subtilis* from soils have been reported in various regions and soil types, including Australia, United States, the United Kingdom, Egypt, India and Germany. Isolations of *B. subtilis* have also been made from field soils in which crops such as potato, vines and rice were being grown. In a United Kingdom pine forest, *B. subtilis* was found to occur mainly as vegetative cells in the acid, mineral horizon of the soil, whereas it was present mainly as spores in the alkaline, mineral horizon. The total populations of *B. subtilis* in the acid and alkaline, mineral horizons were about 7×10^4 CFU per gram dry weight of soil for both horizons. These concentrations represent approximately 22% and 13% of the total bacterial population for the acid and alkaline horizons, respectively. The proportion of *B. subtilis* cells present as spores in the acid and alkaline horizons was 32% and 81% respectively.

Bacillus subtilis is also frequently found on and in plants. In a survey of bacteria inhabiting the surface-sterilised seeds, bacilli could be found in four broadleaf weed species (*Abutilon theophrasti*, *Datura stramonium*, *Ipomoea hederacea* and *Xanthium strumarium*) and soybean (*Glycine max*). A direct relationship did not exist between the percentage of seed with bacteria and the proportion of the seed determined to be viable for a particular species. Other examples of plant species from whose seeds *B. subtilis* has been isolated include the pepper plant (*Capsicum annuum*) in Tunisia, the soybean (*Glycine max*) in the United States and Ethiopia as well as the chickpea (*Cicer arietinum*) in Ethiopia.

Bacillus subtilis strains have been also been isolated from the rhizosphere and aerial parts of plants parts as well. Strains of *B. subtilis* were isolated from the rhizospheres of carnations in the United States, coffee (*Coffea arabica*) seedlings in India, the rhizosphere of oilseed rape in Germany and in the United Kingdom as well as from the surface of apple roots in the United States. *Bacillus subtilis* was also isolated from geranium leaves in the United States, from the phylloplane of avocado (*Persea americana*) in South Africa, from leaf scars of apple trees in the United Kingdom, from the wood of grapevine (*Vitis vinifera*) in South Africa, from red maple (*Acer rubrum*) and silver maple (*Acer saccharinum*) in the United States and from fruits of citrus trees (*Citrus reticulutu* and *Citrus sinensis*) in Australia.

Several studies on the persistence of *B. subtilis* in soil have showed that, in general, the population sizes of introduced cultures decline more or less rapidly following introduction into natural soils. When introduced into soils already containing a natural microbial flora, populations of the introduced *B. subtilis* strain are likely to decline to 1% or less of the population of indigenous *B. subtilis* strains that the soil can support. Sterile growing media, such as those used in horticulture, may support higher levels of introduced *B. subtilis* strains but, when such media are disposed of into a terrestrial environment, the population of the introduced *B. subtilis* strain is likely to decline to the levels observed in natural soils.

In non-sterile lake water, *B. subtilis* added at an initial level of 4.1×10^5 vegetative cells/mL, quickly fell to 8 vegetative cells/mL following incubation at 30°C for 48 hours. During this period, the spore density remained stable at 5–8 spores/mL, indicating that spores of *B. subtilis* remained stable in lake water; however, vegetative cells were not able to produce spores under these conditions. Similar results occurred when *B. subtilis* was tested in sterilized lake water.

Persistence studies in marine/estuarine environments are not available; however, there are various reports of *B. subtilis* being isolated from marine environment, indicating that *B. subtilis* occurs naturally in marine environments. *Bacillus subtilis* has been isolated from sea water as well as sediment samples and has also found to live in association with sponges, ascidians and corals with no adverse effect.

4.2 Effects on Non-Target Species

4.2.1 Effects on Terrestrial Organisms

Two studies were submitted to address the risks of *B. subtilis* strain MBI 600 to terrestrial organisms, an avian oral study and a plant study. Therefore, the potential risk of *B. subtilis* to terrestrial organisms was largely assessed based on reports in the published scientific literature.

In an acute avian oral toxicity and pathogenicity study, 3 groups of apparently healthy 21-day-old northern Bobwhite quails (*Colinus virginianus*) were administered daily in the crop or proventriculus for 5 days with either *B. subtilis* strain MBI 600 (GUS 378 Concentrate, 30 birds, 4000 mg/kg bw/day), water-soluble metabolites of *B. subtilis* strain MBI 600 (10 birds, 240 mg/kg bw/day) or washed spores of *B. subtilis* strain MBI 600 (10 birds, 3680 mg/kg bw/day) in deionized water and observed over a total period of 30 days. A fourth group (10 birds) was administered daily for 5 days with deionized water at 10 mL/kg bw/day and served as a negative control group. In addition, each pen also included an untreated bird that served as an infectivity control. No mortalities or treatment-related signs of toxicity or pathogenicity were noted throughout the study period. At necropsy, one female administered the water-soluble metabolites and one male administered *B. subtilis* strain MBI 600 were noted with enlarged and/or pale spleens. These spleen effects may be attributed to a normal immunological reaction to foreign materials, but may also be attributed to the reported hemolytic effects of some water-soluble metabolites produced by *B. subtilis* (see Section 3.1). The lack of spleen effects in birds treated with washed spores of *B. subtilis* strain MBI 600 supports the possibility of hemolytic activity. However, only one bird was affected in each of the groups treated with *B. subtilis* strain MBI 600 and water-soluble metabolites of *B. subtilis* MBI 600; therefore, the exact cause of these effects is still equivocal. Irrespective of the underlying cause, the spleen effects were very limited even under maximum hazard conditions. Consequently, *B. subtilis* strain MBI 600, water-soluble metabolites of *B. subtilis* MBI 600 and washed spores of *B. subtilis* MBI 600 are considered to be of low toxicity and not pathogenic to northern Bobwhite quails.

In the plant toxicity and pathogenicity study, seeds of *Glycine max* cv. Asgrow A-3427 were treated with *B. subtilis* strain MBI 600 at rates corresponding to 10^7 and 10^5 viable spores per seed. Treated seeds (50) were dried then incubated for 8 days at 30°C, 35°C or 40°C in

germinator kimpac box systems to determine the effect of *B. subtilis* strain MBI 600 on the appearance of seedlings using a grading key developed by the United States Department of Agriculture. Untreated seeds and seeds treated with water were similarly treated then allowed to germinate. Treatment with *B. subtilis* strain MBI 600 had no effect on the number of normal seedlings under the conditions of this study. The only effect noted was a temperature-dependent effect where a reduction in normal seedlings at 35°C compared to 30°C and no normal seedlings produced at 40°C in any of the treatments. References were provided in the study report that implicated *B. subtilis* as the causal agent for *Bacillus* seed decay on soybean. These reports were all made from one laboratory that no longer had the *B. subtilis* cultures referred to in the original publications. A representative of the laboratory has indicated that the identity of the cultures involved was probably *Bacillus megaterium* rather than *Bacillus subtilis*. This study demonstrated that *B. subtilis* strain MBI 600 was not pathogenic or toxic to seeds of *G. max* cv. Asgrow cultivar A-3427.

No other studies were submitted to address the risks of *B. subtilis* strain MBI 600 to non-target mammals, terrestrial insects and non-arthropod invertebrates. Therefore, the potential risk of *B. subtilis* strain MBI 600 to these terrestrial non-target organisms was assessed based on reports in the published scientific literature.

In mammals, few reports of adverse effects were reported in published scientific literature. *Bacillus subtilis* has been implicated in cases of bovine mastitis and reproductive disorders in goats. No other reports of adverse effects were reported despite this microorganism's ubiquitous nature in the environment. Furthermore, the laboratory animal studies on the rat submitted in support of this registration and reviewed in Section 3.1 indicate that there is no pathogenicity to rodents and little toxicity from most routes of exposure at maximum hazard dose levels with the exception of the pulmonary route of exposure. The toxicity observed in this study is consistent with results of other acute pulmonary studies conducted with other *Bacillus* spp. and may possibly be attributed to hemolytic activities of some metabolites produced by *B. subtilis*. However, such adverse effects in non-target mammals are not anticipated based on the exposure scenario from the proposed use of Pro-Mix with Biofungicide in greenhouses or glasshouses.

For terrestrial arthropods (including honeybees), a published study in which honeybees were directly inoculated with *B. subtilis* as a vector to deliver the biological control agent to open blueberry flowers reported no adverse effects. In this study, however, toxicity was not the experimental outcome of interest in the studies, so only effects severe enough to compromise the results of the study would likely have been reported. In another published study, four strains of *B. subtilis* were isolated from diseased larvae of mosquitoes, *Culex* and *Anopheles culicifacies*, and all strains were found to be pathogenic to *Anopheles culicifacies* larvae with a lethal concentration 50% (LC_{50}) value as low as 1.1×10^3 spores per mL. Similarly, reports indicated that six lepidopteran pests of rice fed on the relevant parts of rice plants that had been inoculated with a bacterial suspension of *B. subtilis*.

For earthworms and other soil macro organisms, a published study was submitted in which a nematode, *Caenorhabditis elegans*, displayed no toxicity or pathogenicity to *B. subtilis* after 20–30 *Caenorhabditis elegans* L4 or young adult hermaphrodites were transferred from lawns of *Escherichia coli* strain OP50 to lawns of *B. subtilis* strain PY79, incubated on brain heart infusion agar at 25°C. In contrast, *B. subtilis* strain VM 132 was reported as a potential biological control agent of the parasitic root-knot nematode (*Meloidogyne incognita*) of tomato. Plants grown from inoculated seed displayed significantly fewer nematode galls; however, the mode of action was not elucidated, and the pathogenic relationship was not established. *Bacillus subtilis* is generally not considered to be a pathogen of non-arthropod invertebrates.

For other soil microorganisms, no study was submitted to address the risks of *B. subtilis* strain MBI 600 to soil microorganisms. Effects data are not required although the product is intended to control pest microorganisms, as *B. subtilis* is a normal component of the soil, and the organism is not expected to affect environmentally or economically important microbial species or microbiologically mediated biogeochemical processes.

Based on all the available information on the effects of *B. subtilis* to terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, arthropods, non-arthropod invertebrates, plants or to other non-target microorganisms from the proposed use of Pro-Mix with Biofungicide as a soilless growing medium in greenhouses and glasshouses.

4.2.2 Effects on Aquatic Organisms

Only one study was submitted to address the risks of *B. subtilis* strain MBI 600 to aquatic organisms, a freshwater fish study. The potential risk of *B. subtilis* to aquatic organisms was, therefore, largely assessed based on reports in the published scientific literature.

In a freshwater fish toxicity and infectivity study, no treatment-related toxicity or pathogenicity was observed when groups of carp (*Cyprinus carpio*) were exposed to *B. subtilis* strain MBI 600 in dilution water at nominal concentrations of 2.0×10^6 CFU/mL, 2.0×10^7 CFU/mL and 2.0×10^8 CFU/mL under static-renewal conditions. Microbiological assays conducted on test water showed that the MPCA remained stable between test water renewals (probably as spores; see Section 4.1).

The use of Pro-Mix with Biofungicide will be limited to greenhouse and glasshouse use as a peat-based soilless growing medium. This intended use pattern minimizes direct exposure to non-target aquatic organisms. Although the product is not intended for direct application to water, surface water runoff from spent growing medium and treated plants may result in contamination of aquatic ecosystems. The biological properties of this microorganisms suggest that spores of this MPCA could survive in aquatic ecosystems (see Section 4.1). However, no harm to aquatic organisms are expected based on the absence of disease or other adverse effects in fish or other aquatic organisms related to *B. subtilis* in published literature despite this microorganism's ubiquitous nature. Instead, *B. subtilis* was studied as a possible probiotic in numerous animals, including the gilthead seabream (*Sparus aurata* L.) and tiger shrimp (*Panaeus monodon*). Although these studies were clearly not designed to assess potential toxicity and pathogenicity of *B. subtilis*, the results showed no obvious adverse effects.

5.0 Value

The efficacy data submitted in support of the claims for suppression of Pythium damping-off and root-rot diseases on greenhouse vegetables and ornamentals consisted of 36 greenhouse trials conducted with Pro-Mix BX with Biofungicide only. No data were submitted for any of the three other Pro-Mix with Biofungicide products, namely PGX, HP and TA.

5.1 Effectiveness Against Pests

5.1.1 Acceptable Efficacy Claims

5.1.1.1 Suppression of Pythium Damping-Off and Root-Rot Diseases

To support the proposed label claims of suppressing Pythium damping-off and root-rot diseases, three greenhouse trials with Pro-Mix BX with Biofungicide were conducted for each of the following crops:

- sweet pepper;
- tomato;
- cucumber;
- garden balsam;
- celosia; and
- geranium.

Pro-Mix BX with Biofungicide inoculated with the target pathogen, *Pythium ultimum*, provided 30–42% control of root rot on sweet pepper under high disease pressure, which was similar to or better than the level of control provided by the commercial standard, RootShield Drench WP.

Pythium damping-off caused by *Pythium ultimum* on tomato was evaluated in terms of percent germination of seedlings. Percent germination of seeds increased by 48 to 100% compared to negative control (Pro-Mix BX plus *Pythium ultimum*). Pro-Mix BX with Biofungicide was equally efficacious as Pro-Mix BX plus RootShield Drench in controlling damping-off on tomato.

Pro-Mix BX with Biofungicide reduced root rot on cucumber caused by *Pythium ultimum* by 16–20% compared to root rot in Pro-Mix BX alone.

Damping-off and root rot on garden balsam caused by *Pythium aphanidermatum* were also evaluated. No significant effect on the percentage of germination was observed between Pro-Mix BX with Biofungicide inoculated with the target pathogen, *Pythium aphanidermatum*, and the control treatments (Pro-Mix BX plus *Pythium aphanidermatum* and Pro-Mix BX only). Root rot of garden balsam was reduced by 28–68% in presence of Pro-Mix BX with Biofungicide compared to Pro-Mix BX only. Pro-Mix BX with Biofungicide was equally efficacious as Pro-Mix BX with RootShield Drench in reducing the visible symptoms of root rot.

Pro-Mix BX with Biofungicide inoculated with the target pathogen *Pythium ultimum* reduced the root rot on celosia by 18–52% compared to Pro-Mix BX only. Pro-Mix BX with Biofungicide was equally or more efficacious than the Pro-Mix BX with RootShield Drench in suppressing root-rot symptoms.

In two out of three trials, blackleg (stem rot) on geranium caused by *Pythium ultimum* was reduced by 33–43% in presence of Pro-Mix BX with Biofungicide compared to Pro-Mix BX alone. Pro-Mix BX with Biofungicide had a positive significant effect on germination of geranium seedlings in only one trial.

Because all four Pro-Mix with Biofungicide end-use products contain the same active ingredient, *B. subtilis* strain MBI 600, and only minor differences in formulations are reported, label claims for the suppression of Pythium damping-off and root-rot diseases on greenhouse crops are supported for all four products.

5.1.1.2 Tank-Mix Combinations

Not applicable.

5.2 Phytotoxicity to Target Plants

5.2.1 Acceptable Claims for Host Plants

Pro-Mix BX with Biofungicide was tested at different concentrations of the active ingredient, *B. subtilis* MBI 600, such as 1× (standard concentration), 2×, 10× and 100× to determine their negative effect on growth in terms of percent germination, shoot dry weight and plant height as well as the development of any visual phytotoxicity and phytopathogenicity effects on sweet pepper, tomato, cucumber, garden balsam, celosia and geranium. Pro-Mix BX with Biofungicide had no significant effect on growth of the test plants in any of the test concentrations when compared to control (Pro-Mix BX only), and no visible sign of phytotoxicity or phytopathogenicity effect was observed. Based on all the submitted data, no phytotoxicity or phytopathogenic effect or any other negative effect on the growth of greenhouse crops are expected following the use of any of the four Pro-Mix with Biofungicide end-use products.

5.3 Impact on Succeeding Crops

Data on the impact of Pro-Mix with Biofungicide on succeeding crops were not submitted for review because the substrate will not be reused.

5.4 Economics

No market analysis was provided for any of the end-use products. Canadian greenhouse production suffers 15–20% losses annually from root and foliar diseases. Pythium diseases alone are estimated to be responsible for billion dollar losses. Pythium damping-off occurs early in the production, which causes seed decay or killing of seedlings before they emerge from soils, whereas root rots affect mature plants. Pythium infections result in poor crop stand, stunting of growth and reduction of yield on a wide range of greenhouse crops.

5.5 Sustainability

5.5.1 Survey of Alternatives

Pythium damping-off and root-rot diseases on greenhouse vegetables and ornamentals are currently managed by sanitation and hygiene, cultural practices such as nutrition and pruning, greenhouse environmental management, growing relatively tolerant cultivars and limited use of chemical and biological fungicides. Products such as No-Damp, Previcur N, Maestro 80 DF, Ridomil Gold 480EC, Subdue-MAXX, Aliette WDG, Truban,Captan 80W, RootShield Drench WP, RootShield Granules and Mycostop are registered in Canada for control or suppression of Pythium damping-off and root-rot diseases on greenhouse vegetables and ornamentals (see Appendix I, Table 3).

5.5.2 Compatibility With Current Management Practices Including Integrated Pest Management

In Canada, there are currently few registered products to control Pythium diseases on greenhouse vegetables and ornamentals. Even with the availability of these registered chemical products, the control of Pythium diseases remains difficult. Growers are urged to rotate the use of available fungicide to reduce the development of resistance in pathogen populations. The limited choice of fungicides available to greenhouse growers, however, may enhance the development of resistance in pathogen populations. Resistance to propamocarb and mefenoxam fungicides has already been reported in species of Pythium in greenhouses. Thus, alternative products are needed for the management of Pythium diseases in greenhouses.

Two biofungicides, RootShield and Mycostop, are currently registered in Canada for control of Pythium diseases in greenhouses. The preventative application of these biological products reduces the possibility of pathogens developing resistance to traditional chemical fungicides. As part of an IPM program, the use of Pro-Mix with Biofungicide at the beginning of crop culture would help to avoid the establishment of damping-off and root rot caused by *Pythium* spp., therefore reducing the number of chemical fungicide applications.

Pro-Mix with Biofungicide will replace the first preventative fungicide application; therefore, it could reduce the possibility of pathogens developing resistance to traditional chemical-based fungicides. The four Pro-Mix with Biofungicide end-use products can also potentially enhance the adoption of reduced-risk technologies by producers because they are ready for immediate use, have a long shelf-life (up to 24 months) and offer few risks to human health and the environment.

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

No information is available on the development of resistance to *B. subtilis* strain MBI 600 present in Pro-Mix with Biofungicide. Resistance to *B. subtilis* strain MBI 600 in target populations is not expected due to its mode of action. The MBI 600 strain was first isolated from the natural microflora on faba leaves (*Vicia faba*). MBI 600 is a rapid root coloniser, establishing itself quickly on the surface of the roots, thereby outcompeting the potential fungal root pathogens. An antifungal protein produced *in situ* by this bacterium is also responsible for the suppression of root pathogens. This strain of *B. subtilis* was isolated from the natural micro flora on faba leaves and the mode of action against *Pythium* spp. appears to be competitive expulsion; therefore, it can be assumed this type of mode of action has a very low risk of developing resistant strains among Pythium populations.

5.5.4 Contribution to Risk Reduction and Sustainability

The four Pro-Mix with Biofungicide end-use products are microbial pest control products whose mode of action is based on competitive inhibition and exclusion of *Pythium* spp. on seeds and roots of greenhouse vegetables, including transplants and greenhouse ornamentals. It is a non-chemical product intended as an alternative to conventional fungicides to suppress damping-off and root-rot diseases caused by *Pythium* spp. As a microbial biopesticide, the PMRA considers it to be a reduced-risk pesticide that has a low potential to harm the health of Canadians and their environment.

6.0 Toxic Substances Management Policy Considerations

The management of toxic substances is guided by the federal government's *Toxic Substances Management Policy*, which puts forward a preventive and precautionary approach to deal with substances that enter the environment and could harm the environment or human health. The policy provides decision makers with direction and sets out a science-based management framework to ensure that federal programs are consistent with its objectives. One of the key management objectives is virtual elimination from the environment of toxic substances that result predominantly from human activity and that are persistent and bioaccumulative. These substances are referred to in the policy as Track 1 substances.

While reviewing *B. subtilis* strain MBI 600, the PMRA took into account the federal Toxic Substances Management Policy and followed its Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*. Substances associated with its use were also considered, including microcontaminants in the technical product *B. subtilis* MBI 600 Technical as well as formulants in the manufacturing-use product Subtilex™ Biological Fungicide and the end-use products Pro-Mix with Biofungicide. The PMRA has reached the following conclusions:

- *Bacillus subtilis* strain MBI 600 does not meet the Track 1 criteria because the active ingredient is a biological organism; therefore, it 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.

- *Bacillus subtilis* strain MBI 600 does not contain any contaminants of health or environmental concern identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern*.

- The manufacturing-use product Subtilex™ Biological Fungicide and end-use products Pro-Mix With Biofungicide do not contain any formulants of health or environmental concern identified in the *Canada Gazette,* Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern*.

Therefore, the use of Pro-Mix with Biofungicide is not expected to result in the entry of Track 1 substances into the environment.

7.0 Summary

7.1 Methods for Analysis of the Microorganism as Manufactured

The product characterization data for *B. subtilis* strain MBI 600 (*Bacillus subtilis* MBI 600 Technical), Subtilex™ Biological Fungicide and Pro-Mix with Biofungicide are adequate to assess their safety to human health. The technical material was fully characterized and the specifications were supported by the analysis of a sufficient number of batches. Although quality control lacked microbe-specific screens for specific pathogens, the absence of contaminating microorganisms in a large number of representative batches indicates that the manufacturer's quality assurance program is successful at limiting contaminating microorganisms. No additional microbe-specific testing is required to assure safety.

Storage stability data were sufficient to support an expiration date of 16 months for Subtilex™ Biological Fungicide when the products are stored at temperatures up to 25°C and an expiration of 24 months for Pro-Mix with Biofungicide when stored at outdoor temperatures and kept dry.

7.2 Human Health and Safety

Acute toxicity and infectivity studies submitted in support of *B. subtilis* strain MBI 600 were determined to be sufficiently complete to permit a decision on registration. *Bacillus subtilis* strain MBI 600 was of low toxicity in the rat when administered via the oral and dermal routes. Significant toxicity was observed when the MPCA was administered via the pulmonary, but no toxicity was observed via the intravenous route. *Bacillus subtilis* strain MBI 600 was not pathogenic or infective via the oral, pulmonary and intravenous routes. Slight dermal irritation was observed in the dermal toxicity study, and slight ocular irritation was observed in the eye irritation study. Waiver requests were submitted to address all toxicology requirements (acute dermal toxicity and dermal irritation) for Subtilex™ Biological Fungicide and Pro-Mix with Biofungicide. These waivers were accepted based on the nature and concentration of the formulation ingredients.

Bacillus subtilis was determined to be a sensitizing agent in a dermal sensitization. All microbial pesticides are considered to be potential sensitizers. Exposure to allergens, including *B. subtilis* strain MBI 600 may cause allergies following repeated exposures. As a result, the signal words POTENTIAL SENSITIZER are required on the principal display panels of all labels for *B. subtilis* MBI 600 Technical, Subtilex™ Biological Fungicide and Pro-Mix with Biofungicide. No eye irritation studies were submitted for Subtilex™ Biological Fungicide or Pro-Mix with Biofungicide was submitted. Because its formulation is similar to *B. subtilis* MBI 600 Technical, no signal words were required for Subtilex™ Biological Fungicide. However, the signal words **CAUTION EYE IRRITANT** are required on the principal display panels of all formulations of Pro-Mix with Biofungicide because their formulations, i.e., peat-based growing media, were considered to be mildly irritating to the eye.

When handled according to the label instructions, the pulmonary, dermal and ocular routes are potential routes of exposure to mixer/loaders, handlers and early-entry workers. While submitted studies on *B. subtilis* strain MBI 600 indicated a potential for toxicity via the pulmonary route, inhalation exposure is not a concern if the required dust mask is worn by mixer/loaders, handlers and early-entry workers. To minimize risk to workers, use of appropriate personal protective equipment will be stipulated on the end-use product labels, as well a restricted-entry interval of four hours. The label does not allow applications to turf, residential or recreational areas. Because the use sites are agricultural, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

Although some strains of *B. subtilis* have been isolated from food implicated in food poisoning, no such illnesses were reported for this MPCA in the United States where it has been registered for use on crops since 1994. Furthermore, there were no significant toxicity and no signs of pathogenicity observed when *B. subtilis* strain MBI 600 was administered orally to rats. The establishment of an MRL is therefore not required for *B. subtilis* strain MBI 600 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drug Regulations.

7.3 Environmental Risk

The non-target studies and published scientific literature submitted in support of *B. subtilis* strain MBI 600 were determined to be sufficiently complete to permit a decision on registration.

No studies were submitted to address the environmental fate and behaviour of *B. subtillis* strain MBI 600. Environmental fate data (Tier II/III) were not required due to the absence of significant toxicological effects in non-target organisms in Tier I testing.

Three environmental effects studies were submitted to address risks of *B. subtilis* strain MBI 600 to non-target organisms. Those studies showed that *B. subtilis* strain MBI 600 was not toxic or pathogenic to birds, freshwater fish and seeds of terrestrial plants. The remaining groups of non-target organisms, mammals, terrestrial insects, non-arthropod invertebrates, estuarine/marine fish and aquatic plants, were assessed based on studies and reports in the published scientific literature. In published scientific literature, few adverse effects attributed to *B. subtilis* were reported in mammals, terrestrial insects and non-arthropod invertebrates. These reports, however, were few in numbers despite this microorganism's ubiquitous nature in the environment, and, in most cases, the implication of select strains of *B. subtilis* was not thoroughly investigated. Furthermore, no adverse effects were reported in aquatic organisms.

The use of Pro-Mix with Biofungicide will be limited to greenhouse and glasshouse use as a peat-based soilless growing medium. This intended use pattern minimizes direct exposure to non-target organisms. Although the product is not intended for direct application to water, surface water runoff from spent growing medium and treated plants may result in contamination of aquatic ecosystems. The biological properties of this species suggest that spores of this MPCA could survive in aquatic ecosystems. However, no harm to aquatic organisms is expected based on the absence of disease or other adverse effects in aquatic organisms. Similarly, no harm to terrestrial organisms is expected based on the number of adverse effects reported in published literature and the limited potential for exposure following its proposed use pattern.

7.4 Value

Suppression of damping-off and root-rot diseases caused by *Pythium* spp. on tobacco and greenhouse vegetables, including transplants and greenhouse ornamentals grown on Pro-Mix products with Biofungicide, is accepted. Pro-Mix products with Biofungicide should be used in an integrated Pythium damping-off and root-rot control program with other registered products.

8.0 Regulatory Decision

Health Canada's Pest Management Regulatory Agency, under the authority of the *Pest Control Products Act*, proposes full registration for the sale and use of the technical grade active ingredient *Bacillus subtilis* strain MBI 600 Technical, the manufacturing-use product Subtilex™ Biological Fungicide and the end-use products Pro-Mix with Biofungicide to suppress damping-off and root-rot diseases caused by *Pythium* spp. on greenhouse vegetables, including transplants and greenhouse ornamentals. An evaluation of current scientific data from the registrant, scientific reports and information from other regulatory agencies has resulted in the determination that, under the approved conditions of use, the end-use products have value and do not present an unacceptable risk to human health or the environment.

List of Abbreviations

bw	body weight
CFU	colony forming units
cm^3	cubic centimetre
EC_{50}	effect concentration 50%
g	gram
h	hour
HP	high porosity
kg	kilogram
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
MAC	MacConkey's agar
MAS	maximum average score
MIS	maximum irritation score
mL	millilitre
MPCA	microbial pest control agent
MRL	maximum residue limit
N/A	not applicable
NA	nutrient agar
NFM	non-fat milk agar
NOEC	no observed effect concentration
PCA	plate count agar
PMRA	Pest Management Regulatory Agency
TSA	trypticase soya agar
TSMP	Toxic Substances Management Policy
USEPA	United States Environmental Protection Agency

Appendix I

Table 1 Toxicity and Infectivity of *Bacillus subtilis* strain MBI 600 and Its Associated Products (Subtilex™ Biological Fungicide and Pro-Mix with Biofungicide)

Study Type	Species, Strain and Doses	Result	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Bacillus subtilis* MBI 600 Technical				
Acute oral toxicity and infectivity	Rat— CD 11/sex treated with MPCA , 1% suspension in sterile distilled water, 20 mL/kg bw (equivalent to 1.9×10^8–2.7×10^8 CFU/animal), interim sacrifices (2/sex) on days 2, 8 and 15 5/sex treated with autoclaved MPCA, 1% suspensions in sterile distilled water, 20 mL/kg bw	$LD_{50} > \sim 2 \times 10^8$ CFU/animal	No mortalities and no abnormalities on necropsy. Slightly low body-weight gains were observed for two MPCA-treated ♂ on day 15, and one ♀ treated with autoclaved MPCA on day 22. The MPCA was initially recovered from the feces, urine and gastrointestinal contents (stomach, small intestines and cecum) of treated rats, and from the blood of treated rats on day 7, but was completely cleared from all organs and fluids by day 22. **LOW TOXICITY, NOT INFECTIVE**	PMRA 1099781

Study Type	Species, Strain and Doses	Result	Significant Effects and Comments	Reference(s)
Acute pulmonary toxicity and infectivity	Rat—CD 16 ♂ and 15 ♀ treated with the MPCA, 1% suspension in sterile distilled water, 1.2 mL/kg bw (equivalent to 3.3×10^8–3.7×10^8 CFU/animal), interim sacrifices (2/sex) on days 1, 2, 8 and 15 5/sex treated with autoclaved MPCA, 1% suspensions in sterile distilled water, 1.2 mL/kg bw	$LC_{50} > \sim 3.5 \times 10^8$ CFU/animal	3 animals died on day 2, 2 animals died on day 3, and 1 animal died on day 4. Piloerection, hunched posture, abnormal gait, lethargy, pallor of the extremities, increased respiration, collapse and gasping were noted in the many of rats treated with the MPCA and autoclaved MPCA. Body-weight loss was observed in all animals that died. Body-weight losses or low body-weight gains were observed in animals treated with the MPCA and autoclaved MPCA. The MPCA was initially recovered from the cecum, feces, urine, blood and various organs of treated animals. By day 7, viable spores were mainly recovered from the spleen, heart and liver of treated rats, and by day 21, spores were only recovered from feces and cecum of treated animals. A 3–4°C drop in body temperature was recorded for all rats dosed with the MPCA or autoclaved MPCA. After 24 hours, the body temperatures returned to normal levels. At necropsy, no macrospic findings were observed. **TOXIC, NOT INFECTIVE**	PMRA 1099782

Study Type	Species, Strain and Doses	Result	Significant Effects and Comments	Reference(s)
Intravenous infectivity	Rat—CD 16/sex treated with the MPCA, 1% suspension in physiological saline, 3 mL/kg bw (equivalent to 3.3×10^8–3.7×10^8 CFU/animal), interim sacrifices (2/sex) on days 1, 2, 8 and 15 5/sex treated with autoclaved MPCA, 1% suspension in physiological saline, 3 mL/kg bw		No treatment-related clinical signs of toxicity, no mortalities and no abnormalities on necropsy. Body temperatures were ~3°C lower in the MPCA-treated rats after 24 hours. The MPCA was recovered from the feces, urine, blood and various organs (predominantly in liver and spleen) following treatment. Counts decreased throughout the study period. By day 22, the MPCA was only recovered from liver and spleen. **NOT INFECTIVE**	PMRA 1099726
Acute dermal toxicity / irritation	Rabbit—New Zealand white 5/sex treated with suspension of the MPCA, undiluted, 2 mL/kg bw (equivalent to 2.0×10^{10} CFU/kg bw) to approximately 10% of the body surface, 24 hours	LD_{50} > 2 mL/kg bw	One ♂ died, but this mortality was not related to treatment. Very slight erythema (grade 1) and/or very slight edema (grade 1) was noted at the treated sites of surviving animals at patch removal. All irritation cleared by day 3, i.e., 24 hours after patch removal. **MINIMALLY IRRITATING, LOW TOXICITY**	PMRA 1099727

Study Type	Species, Strain and Doses	Result	Significant Effects and Comments	Reference(s)
Dermal sensitization	Guninea Pig—albino **Induction** 10/sex, intradermal injections with 0.1 mL a) Freund's complete adjuvant diluted with an equal volume of water; b) 5% MPCA in water; and c) 5% MPCA in a 50:50 mixture of Freund's complete adjuvant and water. After 1 week, topical application with 0.4 mL of the MPCA suspension for 48 hours. **Challenge** Topical applications of 0.2 mL undiluted MPCA and 50:50 mixture of the MPCA and distilled water were applied at naive sites.	SENSITIZER	24 hours after challenge, most animals exhibited well-defined erythema and edema (grade 2) (challenged with 100% MPCA) and 14/20 animals challenged with 50:50 MPCA in water had similar reactions. Moderate to severe erythema (grade 3), in 7/20 and 8/20 animals were noted at 48- and 72-hour timepoints, respectively. Moderate (grade 3) to severe edema (grade 4) were exhibited by more than half of the test animals at 48 and 72 hours. Necrosis, necrotic edge/patch were observed in 12/20 animals at 48 hours and in 6/20 animals at 72 hours.	PMRA 1099729
Primary eye irritation	Rabbit—New Zealand white 6 ♀ instilled with 0.1 mL of the MPCA suspension (equivalent to 1×10^9 CFU/eye), conjunctival sac of right eye.	MAS[1] = 4.6/110 (24, 48 and 72 h) MIS[2] = 6/110 (24 h)	After 1 hour, slight conjunctival redness and discharge (grade 1) were noted. After 24 hours, chemosis (grade 1) was noted. Irritation cleared by day 4. **MINIMALLY IRRITATING**	PMRA 1099731

Study Type	Species, Strain and Doses	Result	Significant Effects and Comments	Reference(s)
Acute Toxicity/Irritation of Pro-Mix (HP, BX, PGX and TA) With Biofungicide				
All acute toxicity testing			Based on the nature and the concentration of each formulation ingredient, data waiver requests were found to be acceptable to fully assess the risks associated with all four end-use products. **WAIVERS ACCEPTED**	PMRA 1098428, 1098429, 1098431, 1098432, 1098434, 1098435, 1098483, 1098484, 1098486, 1098487, 1098489, 1098490, 1098529, 1098530, 1098532, 1098533, 1098535, 1098536, 1098574, 1098575, 1098577, 1098578, 1098580, 1098581

[1] MAS = maximum average score
[2] MIS = maximum irritation score

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Test Substance(s)	Endpoint Value	Significant Effects, Comments	Reference
Terrestrial Organisms					
Vertebrates					
Birds	Oral (*Colinus virginianus*)	*Bacillus subtilis* strain MBI 600	LD_{50} > 4000 mg/kg bw/day for 5 days	No mortalities or clinical signs of toxicity. At necropsy, 1 ♀ administered the water-soluble metabolites and 1 ♂ administered *Bacillus subtilis* MBI 600 were noted with enlarged and/or pale spleens. **LOW TOXICITY, NOT INFECTIVE**	PMRA 1099733, 1099750
		Water solube metabolites from *Bacillus subtilis* strain MBI 600	LD_{50} > 240 mg/kg bw/day for 5 days		
		Washed spores of *Bacillus subtilis* strain MBI 600	LD_{50} > 3680 mg/kg bw/day for 5 days		
Wild Mammals	No study or waiver submitted. Few reports of adverse effects were reported in published scientific literature; *B. subtilis* has been implicated in cases of bovine mastitis and reproductive disorders in goats. No other reports of adverse effects were reported despite this microorganism's ubiquitous nature in the environment. Acute toxicity and infectivity studies (oral, pulmonary, intravenous and dermal) with rats treated with the MPCA showed completely clearance or a clear pattern of clearance of the MPCA in mammals. Toxicity was only observed in pulmonary route of exposure and was consistent with other *Bacillus* spp. However, such adverse effects in non-target mammals are not anticipated based on the exposure scenario from the proposed use of Pro-Mix with Biofungicide in greenhouses or glasshouses. **WAIVER ACCEPTED**				PMRA 1099768, 1099771, 1099726, 1099727, 1099781, 1099782
Invertebrates					
Honeybees	Oral (dietary)	No study was submitted. In a waiver request, a published study in which honeybees were directly inoculated with *B. subtilis* as a vector to deliver the biological control agent to open blueberry flowers reported no adverse effects. **WAIVER ACCEPTED**			PMRA 1099737, 1099738
	Contact brood or hive				

Organism	Exposure	Test Substance(s)	Endpoint Value	Significant Effects, Comments	Reference
Other arthropods	Dietary	No study was submitted. In a waiver request, few adverse effects were reported in literature despite its ubiquitous nature. Four strains of *B. subtilis* were isolated from diseased larvae of mosquitoes, *Culex* and *Anopheles culicifacies*. Similar reports were reported for 6 lepidopteran pests of rice fed on the relevant parts of rice plants that had been inoculated with a bacterial suspension of *B. subtilis*. *Bacillus subtilis* is generally not considered to be a pathogen of insects. **WAIVER ACCEPTED**			PMRA 1099737, 1099738
Earthworms	Acute	No study was submitted. In a waiver request, a published study was submitted in which *Caenorhabditis elegans* (a nematode) demonstrated no toxic or pathogenic effects following dietary exposure to *B. subtilis*. In other published scientific literature, another strain of *B. subtilis* was reported as a potential biological control agent of the parasitic root-knot nematode (*Meloidogyne incognita*) of tomato; however, the mode of action was not elucidated and the pathogenic relationship was not established. *Bacillus subtilis* is generally not considered to be a pathogen of non-arthropod invertebrates. **WAIVER ACCEPTED**			PMRA 1099745, 1099746, 1099747
Soil microbes	Acute	No study or waiver submitted. Effects data are not required although the product is intended to control pest microorganisms, as *B. subtilis* is a normal component of the soil, and the organism is not expected to affect environmentally or economically important microbial species or microbiologically mediated biogeochemical processes.			
Vascular Plants					
Vascular plants	Acute (*Glycine max* cv. Asgrow A-3427)	*B. subtilis* strain MBI 600	$EC_{50} > 10^7$ spores/seed NOEC = 10^7 spores/seed	No treatment-related effects were noted. **NOT TOXIC, NOT PATHOGENIC**	PMRA 1099748, 1099750
Aquatic Organisms					
Vertebrates					
Freshwater fish	Acute (*Cyprinus carpio*)	*B. subtilis* strain MBI 600	$EC_{50} > 2.0 \times 10^8$ CFU/mL NOEC = 2.0×10^8 CFU/mL	No treatment-related effects were noted. **NOT TOXIC, NOT PATHOGENIC**	PMRA 1099734

Organism	Exposure	Test Substance(s)	Endpoint Value	Significant Effects, Comments	Reference
Estuarine/ Marine fish	Acute	No study was submitted. In a waiver request, a published study was submitted in which *Sparus aurata* L. (gilthead seabream) demonstrated no toxic or pathogenic effects following dietary exposure to *B. subtilis* as a potential probiotic. In published scientific literature, no reports of adverse effects were found despite its ubiquitous nature. Also, minimal exposure to *B. subtilis* strain MBI 600 is expected based on the proposed use of Pro-Mix with Biofungicide in greenhouses or glasshouses. **WAIVER ACCEPTED**			PMRA 1099735, 1099736, 1099739, 1099741, 1099743
Invertebrates					
Freshwater arthropods	Acute	No study was submitted. In a waiver request, a published study was submitted in which *Panaeus monodon* (tiger shrimp) demonstrated no toxic or pathogenic effects following aqueous exposure to *B. subtilis*. In published scientific literature, no reports of adverse effects were found despite its ubiquitous nature. Also, minimal exposure to *B. subtilis* strain MBI 600 is expected based on the proposed use of Pro-Mix with Biofungicide in greenhouses or glasshouses. **WAIVER ACCEPTED**			PMRA 1099739, 1099740, 1099741, 1099742, 1099743, 1099744
Estuarine/ Marine arthropods	Acute				
Non-arthropod invertebrates	Acute	No study or waiver submitted. Literature indicated that while *B. subtilis* has been isolated from aquatic environments, there have been no reports of adverse effects on aquatic non-arthropods invertebrates. Also, minimal exposure to *B. subtilis* strain MBI 600 is expected based on the proposed use of Pro-Mix with Biofungicide in greenhouses or glasshouses. **WAIVER ACCEPTED**			PMRA 1099768, 1099771
Plants					
Algae	Acute	No study was submitted. In a terrestrial plant study, no treatment-related effects were noted in seeds of *Glycine max* treated with *B. subtilis* strain MBI 600. In published scientific literature, no reports of adverse effects were found despite its ubiquitous nature. Also, minimal exposure to *B. subtilis* strain MBI 600 is expected based on the proposed use of Pro-Mix with Biofungicide in greenhouses or glasshouses. **WAIVER ACCEPTED**			PMRA 1099749, 1316478, 1316471
Freshwater plants					

Table 3 Alternative Fungicides/Biopesticides for Control/Suppression of Pythium Diseases on Greenhouse Vegetables and Ornamentals

Technical Grade Active Ingredient	End-Use Product(s)	Disease Claim	Fungicide Classification	
			Group	Mode of Action
Oxine Benzoate	No-Damp	Damping-off	M2	Multisite
Captan	Maestro 80 DF	Damping-off	M4	Multisite
Metalaxyl-M and S-isomer	Ridomil Gold 480EC	Damping-off	4	Nucleic acid synthesis
Metalaxyl-M and S-isomer	Subdue-MAXX	Damping-off and root rot	4	Nucleic acid synthesis
Propamocarb hydrochloride	Previcur-N	Root diseases	28	Cell membrane permeability
Fosetyl-Al	Aliette WDG	Daming-off	33	Unknown
Etridiazole	Truban	Damping-off and root rot	14	Lipids and membrane synthesis
Trichoderma harzianum Rifai strain KRL-AG2	RootShield Drench	Damping-off and root rot	—	Multiple
Trichoderma harzianum Rifai strain KRL-AG2	RootShield Granules	Damping-off and root rot	—	Multiple
Streptomyces griseoviridis strain K6	Mycostop	Damping-off, root and crown rot	—	Multiple

References

List of Studies / Information Submitted by Registrant

1.0 The Active Ingredient, Its Properties and Uses

PMRA 1099752 Product profile and proposed use pattern *Bacillus subtilis* MBI 600 Technical. Premier Horticulture. 2005. DACO: M1.2

PMRA 1099753 International Regulatory Status of the *Bacillus subtilis* MBI 600 Technical. Premier Horticulture. 2005. DACO: M1.3

PMRA 1098379 Product profile and proposed use pattern Subtilex™ Biological Fungicide. Premier Horticulture. 2005. DACO: M1.2

PMRA 1098380 International Regulatory Status Subtilex™ Biological Fungicide. Premier Horticulture. 2005. DACO: M1.3

PMRA 1098404 Product profile and proposed use pattern PRO-MIX HP with Biofungicide. Premier Horticulture. 2005. DACO: M1.2

PMRA 1098405 International Regulatory Status PRO-MIX HP with Biofungicide. Premier Horticulture. 2005. DACO: M1.3

PMRA 1098448 Product profile and proposed use pattern PRO-MIX BX with Biofungicide. Premier Horticulture. 2005. DACO: M1.2

PMRA 1098449 International Regulatory Status PRO-MIX BX with Biofungicide. Premier Horticulture. 2005. DACO: M1.3

PMRA 1098505 Product profile and proposed use pattern PRO-MIX PGX with Biofungicide. Premier Horticulture. 2005. DACO: M1.2

PMRA 1098506 International Regulatory Status PRO-MIX PGX with Biofungicide. Premier Horticulture. 2005. DACO: M1.3

PMRA 1098550 Product profile and proposed use pattern PRO-MIX TA with Biofungicide. Premier Horticulture. 2005. DACO: M1.2

PMRA 1098551 International Regulatory Status PRO-MIX TA with Biofungicide. Premier Horticulture. 2005. DACO: M1.3

2.0 Methods of Analysis

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PMRA 1099758 Analysis for Microbial Contaminants. Premier Horticulture. 2005. DACO: M2.10.2

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PMRA 1099762 Canadian Patent Database Patent: CA 1324099 and 1337935, Canadian Intellectual Property Office. 1993. DACO: M2.6

PMRA 1099763 Results of API test Kit. Becker Underwood Inc. 2004. DACO: M2.7.1

PMRA 1099764 Reconnaître la souche de *Bacillus subtilis* MBI 600 parmi différentes souches de *Bacillus* sp. Premier Horticulture. 2005. DACO: M2.7.1

PMRA 1099765 Origin, derivation and identification MPCA *Bacillus subtilis* MBI 600 Technical. Premier Horticulture. 2005. DACO: M2.7.1

PMRA 1099766 Confirmation of Strain Discovery Letter. The University of Nottingham. 2004. DACO: M2.7.1

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PMRA 1099768 Review of litterature on occurrence and persistence of the bacterium Bacillus subtilis (1999-2005). Premier Horticulture. 2005. DACO: M2.7.2

PMRA 1099769 USEPA. 1997. *Bacillus subtilis* Final risk assessment, United States Environmental Protection Agency. DACO: M2.7.2

PMRA 1099770 USEPA. Summary of EPA findings with commercial *Bacillus subtilis* strains, United States Environmental Protection Agency. DACO: M2.7.2

PMRA 1099771 Review of litterature on occurrence and persistence of the bacterium *Bacillus subtilis*. The MicroBio Group Ltd. 1999. DACO: M2.7.2

PMRA 1099773	Manufacturing methods and quality assurance. Premier Horticulture. 2005. DACO: M2.8,M2.9.2
PMRA 1099774	Product Chemistry Data, GUS 376 Concentrate Biological Fungicide. Gustafson. 1993. DACO: M2.8,M2.9.2
PMRA 1099775	Product specifications. Premier Horticulture. 2005. DACO: M2.9.1
PMRA 1099777	Disclosure of Ingredients. Premier Horticulture. 2005. DACO: M2.9.3
PMRA 1100204	Standard Operating procedures for the QC and QA of Becker Underwood's *Bacillus subtilis* (MBI 600) Products. Gustafson. DACO: M2.8
PMRA 1290506	Centre d'expertise en analyse environnementale du Québec. Method ILMA-004: Méthode d'analyse Recherche et dénombrement de *Staphylococcus aureus*: méthode par filtration sur membrane. DACO: M2.10
PMRA 1290520	Methods ILME-8, ILME-4, ILME-31, ILME-28, USP 27 and ILCE-030. DACO: M2.10
PMRA 1290521	Additional Information for DACO M2.11 - Storage Stability. 2006. DACO: M2.10
PMRA 1303381	Préparation d'un milieu de culture, PHL-LA-WI-090. 2002. DACO: M2.8
PMRA 1303387	Centre d'Expertise en Analyse Environnementale du Québec. Méthode d'analyse. Recherche et dénombrement de Straphylococcus aureus méthode par filtration sur membrane, MA.700-STA 1.0. 2005. DACO: M2.8
PMRA 1290484	Cover Letter, A. Bourassa to D. Rochon. August 14, 2006. DACO: 0.8
PMRA 1098381	Product Characterization and Analysis. Premier Horticulture. 2005. DACO: M2.1,M2.2,M2.3,M2.4,M2.5,M2.6
PMRA 1098382	Analysis for Other Unintentional Ingredients. Premier Horticulture. 2005. DACO: M2.10.3
PMRA 1098383	Stability/Shelf-Life Evaluation of Subtilex. Becker Underwood. 2005. DACO: M2.11

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PMRA 1098386 Product Specification Form. Premier Horticulture. 2005. DACO: M2.9.1

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PMRA 1098406 Product Characterization and Analysis Table. Premier Horticulture. 2005. DACO: M2.1,M2.2,M2.3,M2.4,M2.5,M2.6

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PMRA 1098409 Product specifications form and formulants MSDS, Premier Horticulture. 2005. DACO: M2.9.1

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PMRA 1098455 Manufacturing methods/process and quality assurance PRO-MIX BX with Biofungicide. Premier Horticulture. 2005. DACO: M2.8

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PMRA 1098451 Contaminants screening results for PRO-MIX BX with Biofungicide. Premier Horticulture. 2005. DACO: M2.10.2

PMRA 1098452	Analysis for Other Unintentional Ingredients for Pro-Mix BX with Biofungicide. Premier Horticulture. 2005. DACO: M2.10.3
PMRA 1098453	Storage stability results for PRO-MIX BX with Biofungicide. Premier Horticulture. 2005. DACO: M2.11
PMRA 1098454	Summary of Physical and Chemical Properties. Premier Horticulture. 2005. DACO: M2.12
PMRA 1098456	Product specifications form and formulants MSDS. Premier Horticulture. 2005. DACO: M2.9.1
PMRA 1098507	Product Characterization and Analysis Table. Premier Horticulture. 2005. DACO: M2.1,M2.2,M2.3,M2.4,M2.5,M2.6
PMRA 1098508	Analysis for Other Unintentional Ingredients for Pro-Mix PGX with Biofungicide. Premier Horticulture. 2005. DACO: M2.10.3
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PMRA 1098510	Waiver requested for summary of Physical and Chemical Properties. Premier Horticulture. 2005. DACO: M2.12
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PMRA 1098553	Analysis for Other Unintentional Ingredients for Pro-Mix TA with Biofungicide. Premier Horticulture. 2005. DACO: M2.10.3
PMRA 1098554	Storage stability for PRO-MIX TA with Biofungicide. Premier Horticulture. 2005. DACO: M2.11
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PMRA 1098556	Product specifications form and formulants MSDS. Premier Horticulture. 2005. DACO: M2.9.1
PMRA 1099904	Analysis for Other Unintentional Ingredients for Pro-Mix TA with Biofungicide. Premier Horticulture. 2005. DACO: M2.10.2
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3.0 Impact on Human and Animal Health

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PMRA 1098429 Acute pulmonary infectivity/pathogenicity and toxicity for PRO-MIX HP with Biofungicide. Technology Sciences Group. 2005. DACO: M4.2.3

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PMRA 1098434 Acute dermal irritation for PRO-MIX HP with Biofungicide. Technology Sciences Group. 2005. DACO: M4.5.2

PMRA 1098435 Reporting of hypersensitivity incidence for PRO-MIX HP with Biofungicide. Technology Sciences Group. 2005. DACO: M4.6

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PMRA 1098574 Acute oral infectivity/pathogenicity and toxicity for PRO-MIX TA with Biofungicide. Technology Sciences Group. 2005. DACO: M4.2.2

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PMRA 1098577 Intravenous infectivity for PRO-MIX TA with Biofungicide. Technology Sciences Group. 2005. DACO: M4.3.2

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4.0 Impact on the Environment

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5.0 Value

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PMRA 1098473 Letter from Becker Underwood regarding tank-mixing of Subtilex (*Bacillus subtilis* strain MBI 600) with chemical fungicides. p. 6.

PMRA 1098476 Contribution to integrated pest management strategies and practices. p. 4.

PMRA 1098411 Waiver requested for Pro-Mix HP with Biofungicide efficacy results. Premier Horticulture. p. 47.

PMRA 1098513 Waiver requested for Pro-Mix PGX with Biofungicide efficacy results. Premier Horticulture. p. 54.

PMRA 1098558 Waiver requested for Pro-Mix TA with Biofungicide efficacy results. Premier Horticulture. p. 54.

Additional Information Considered

a) Published Information

2.0 Methods of Analysis

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