PRD2009-17

Proposed Registration Decision

Bacillus subtilis strain MBI 600 Integral ™ Liquid Biological Fungicide

(publié aussi en français)

30 December 2009

This document is published by the Health Canada Pest Management Regulatory Agency. For further information, please contact:

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HC Pub: 091204

ISBN: 978-1-100-14488-7 (978-1-100-14489-4)
Catalogue number: H113-9/2009-17E (H113-9/2009-17E-PDF)

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Overview

Proposed Registration Decision for Integral ™ Liquid Biological Fungicide

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Bacillus subtilis* strain MBI 600 and Integral ™ Liquid Biological Fungicide, containing the technical grade active ingredient *Bacillus subtilis* strain MBI 600, for use as a seed treatment to suppress the seedling disease complex in canola (seed rot, pre and post emergent damping-off, seedling blight and root rot) caused by *Rhizoctonia* spp. and *Fusarium* spp.

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

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of *Bacillus subtilis* strain MBI 600 and Integral ™ Liquid Biological Fungicide.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (e.g. children) as well as 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 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.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act.*

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's *(a)* efficacy; *(b)* effect on host organisms in connection with which it is intended to be used; and *(c)* health, safety and environmental benefits and social and economic impact."

Before making a final registration decision on *Bacillus subtilis* strain MBI 600 and Integral ™ Liquid Biological Fungicide, the PMRA will consider all comments received from the public in response to this consultation document[3]. The PMRA will then publish a Registration Decision[4] on *Bacillus subtilis* strain MBI 600 and Integral ™ Liquid Biological Fungicide, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What Is *Bacillus subtilis* strain MBI 600 and Integral ™ Liquid Biological Fungicide?

Bacillus subtilis strain MBI600 is a microbial pest control product that is antagonistic to fungal pathogens that cause seedling diseases caused by *Rhizoctonia* and *Fusarium* in canola. The protective effect of *Bacillus subtilis* is primarily attributed to competitive exclusion of pathogenic organisms. The end-use product Integral™ Liquid Biological Fungicide is a liquid seed treatment that contains *Bacillus subtilis* strain MBI600. It is to be used primarily in combination with a conventional canola seed treatment fungicide.

Health Considerations

Can Approved Uses of *Bacillus subtilis* strain MBI 600 and Integral ™ Liquid Biological Fungicide Affect Human Health?

Bacillus subtilis strain MBI 600 is unlikely to affect your health when Integral™ Liquid Biological Fungicide is used according to the label directions.

People could be exposed to *B. subtilis* strain MBI 600 when handling and applying the product. When assessing health risks, several key factors are considered: the microorganism's biological properties (e.g., production of toxic by-products), reports of any adverse incidents, its potential to cause disease or toxicity as determined in toxicological studies and the level to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

Toxicological studies in laboratory animals describe potential health effects from large doses for the purpose of identifying any potential pathogenicity, infectivity and toxicity concerns. When *B. subtilis* MBI 600 was tested on laboratory animals, there were no signs that it caused any significant toxicity or disease.

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

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

Residues in Water and Food

Dietary risks from food and water are not of concern

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the Pest Control Products Act (PCPA) for the purposes of the adulteration provision of the Food and Drugs Act (FDA). Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

Strains of *B. subtilis* are common in nature and the use of Integral™ Liquid Biological Fungicide as a seed treatment to control fungal disease in crops is not expected to significantly increase 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 applied as a seed treatment to canola seeds. 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, when *B. subtilis* strain MBI 600 was administered orally to rats, no signs that it caused toxicity or disease were observed.

The establishment of a MRL is therefore not required for *B. subtilis* strain MBI 600. As well, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently, dietary exposure and risks are minimal to non-existent.

Occupational Risks From Handling Integral ™ Liquid Biological Fungicide

Occupational risks are not of concern when Integral™ Liquid Biological Fungicide is used according to label directions, which include protective measures

Workers using Integral™ Liquid Biological Fungicide 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 workers exposed to Integral™ Liquid Biological Fungicide, must wear gloves, long-sleeved shirts, long pants, and shoes plus socks. Furthermore applicators, mixers, and loaders of Integral™ Liquid Biological Fungicide will be required to wear a NIOSH approved respirator (with any N, P, R or HE filter).

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 Integral ™ Liquid Biological Fungicide Is Introduced Into the Environment?

Environmental risks are not of concern

Information on the environmental fate of *Bacillus subtilis* strain MBI 600 suggests that, as a soil microorganism, it is likely that *B. subtilis* strain MBI 600 could survive in outdoor soil under suitable environmental conditions (i.e., type of soil, moisture, acidity levels, and temperature) but that over time the populations of *B. subtilis* strain MBI 600 should return to naturally occurring levels.

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 considering the large amount of published literature on this microorganism. Furthermore, these reports involved either unusual strains, or select strains, of *B. subtilis,* for which their ability to cause disease was not thoroughly investigated. There are no published reports of disease associated with *B. subtilis* strain MBI 600 in birds, earthworms, bees, aquatic invertebrates, fish, algae, and aquatic plants, except for the intended pest. Furthermore, studies designed to examine the effects of *B. subtilis* to birds, wild mammals, terrestrial insects, earthworms, soil microorganisms reported no adverse effects.

Value Considerations

What Is the Value of Integral ™ Liquid Biological Fungicide?

Integral ™ Liquid Biological Fungicide is a reduced-risk biofungicide that may provide suppression of seed rot, pre and post emergent damping off, seedling blight and root rot in canola seedlings when used alone. Control of these diseases is achieved when Integral ™ Liquid Biological Fungicide is used in combination with Helix Liquid Seed Treatment, Helix Xtra Seed Treatment or Prosper FL Flowable Insecticide and Fungicide Seed Treatment.

Integral ™ Liquid Biological Fungicide has been shown to provide an increase of about five to ten percent in final plant stands in canola fields when applied as a stand-alone seed treatment or in combination with other conventional seed treatments commonly used by seed companies in Canada. Because of the microbial nature of its active ingredient, seedling disease suppression of Integral ™ Liquid Biological Fungicide is likely to persist late into the seedling development stages. Integral ™ Liquid Biological Fungicide is the first biological fungicide being registered for use as a canola seed treatment in Canada.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the label of Integral ™ Liquid Biological Fungicide to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

Because of concerns with users developing allergic reactions through repeated high exposures to *B. subtilis* strain MBI 600, anyone formulating, handling, mixing/loading, applying or involved in clean-up/repair activities of Integral™ Liquid Biological Fungicide must wear waterproof gloves, a long-sleeved shirt, long pants, and a dust/mist filtering respirator (MSH/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator with any N-95, R-95, P-95 or HE filter.

Environment

As a general precaution, label statements will be added to the label requiring handlers to not contaminate irrigation or drinking water or aquatic habitats by cleaning of equipment or disposal of wastes.

Next Steps

Before making a final registration decision on *Bacillus subtilis* strain MBI 600 and Integral ™ Liquid Biological Fungicide, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document.

The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the PMRA's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Bacillus subtilis* strain MBI 600 and Integral ™ Liquid Biological Fungicide (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Integral ™ Liquid Biological Fungicide

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Bacillus subtilis* strain MBI 600
Function	Biological Fungicide
Binomial name	*Bacillus subtilis* strain MBI 600
Taxonomic designation	
Kingdom	Eubacteria
Phylum	Firmicutes
Order	Bacillales
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	5.5×10^{11} spores/g

Identity of relevant impurities of toxicological, environmental and/or significance.	The technical product 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 Ingredients and End-Use Product

Technical Product— *Bacillus subtilis* strain MBI 600 and End-Use Product—Integral ™ Liquid Biological Fungicide

Properties	*Bacillus subtilis* strain MBI 600 Technical	Integral ™ Liquid Biological Fungicide
Colour	*Beige-white*	*Beige/white*
Odour	*Musty*	*Musty*
Physical state	*wettable powder*	*Liquid*
Guarantee	*5.5×10^{11} spores/g*	*2.2×10^{10} spores/mL*
Density	*0.46 g/cm^3 ± 0.03 g/cm^3*	*1.10–1.22 g/mL at 20°C*

1.3 Directions for Use

Integral™ Liquid Biological Fungicide is a seed treatment for the partial suppression of the seedling disease complex in canola (seed rot, pre and post emergent damping-off, seedling blight and root rot) caused by *Rhizoctonia* and *Fusarium* spp. Additionally, Integral ™ Liquid Biological Fungicide, when tank mixed with Helix Liquid Seed Treatment, Helix XTra Seed Treatment or Prosper FL Flowable Insecticide and Fungicide Seed Treatment, will provide control of the seedling disease complex in canola caused by *Rhizoctonia* and *Fusarium* spp..

Integral ™ Liquid Biological Fungicide is applied using commercial seed treating equipment with a registered canola seed treatment. The required amount of the chemical seed treatment is added, followed by Integral ™ Liquid Biological Fungicide which is slowly added to the

solution and mixed until uniform. The mixed solutions should not be stored for longer than 72 hours before applying to the seed.

The application rate is 160 mL of Integral ™ Liquid Biological Fungicide per 100 kg of seed alone or tank mixed with Helix Liquid Seed Treatment, Helix XTra Seed Treatment or Prosper FL Flowable Insecticide and Fungicide Seed Treatment at labelled rates. If used in combination, the required amount of the chemical seed treatment should be added first followed by Integral Liquid Biological Fungicide with continued mixing until uniform.

1.4 Mode of Action

Bacillus subtilis strain MBI 600 is a rapid root coloniser, establishing itself quickly on the seed and root surface where it out-competes potential fungal root pathogens. When grown on nutrient-limiting medium, strain MBI 600 also produces a novel anti-fungal metabolite with a broad range of activity against various root pathogens (including *Fusarium* spp., and *Rhizoctonia* spp.) which may also contribute to its mode of action.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

Identification of *B. subtilis* to the species level is achieved using the commercial test kits API 20E and API Rapid CH identification strips. Further identification was carried out by Polymerase Chain Reactions (PCR) and DNA sequencing of the PCR product using 16S rRNA primers followed by a blast search of the sequence which identified the isolate as *B. subtilis*. A simple plating test on agar media is also used to confirm the colony morphology of the MPCA.

To distinguish *B. subtilis* strain MBI 600 from other strains, gel filtration chromatography can be used to determine the molecular weight of an antibiotic protein unique to strain MBI 600. The molecular weight of the antibiotic is approximately three times greater than conventional isolates of *B. subtilis*. Also, an agar diffusion bioassay is performed to check for activity of the strain-specific metabolite.

2.2 Method for Establishment of Purity of Seed Stock

Spores of *B. subtilis* strain MBI 600 are suspended in 10% glycerol/water solution and stored at -80°C in an environmentally controlled room designated for making seed stocks only. The inoculant is sampled and analyzed for bacterial contamination by identifying the colony morphology on standard Nutrient Agar (NA) plates. Multiple aliquots of stock solutions are stored in two separate freezers designated for microbial mother cultures of strain MBI 600 cultures only. Stock solutions are labelled with a lot number and the date the container was first put in the freezer.

2.3 Methods for 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* strain MBI 600 Technical and Integral™ Liquid Biological Fungicide is routinely assayed by plate counts of serial dilutions of samples on NA plates incubated at 37°C for 16–20 hours and again after another overnight incubation. This assay method detects both vegetative cells and endospores of *B. subtilis*.

Secondly, antibiotic properties of the MPCA are confirmed using an agar diffusion bioassay in which *B. subtilis* strain MBI 600 is grown on NA plates overnight at 37°C. The next day Sabouraud medium containing *Botrytis cinerea* is plated over the colony of *B. subtilis* strain MBI 600 and incubated for 4 days at 18°C. Following this incubation period, the radius of the inhibitory circle in the Sabouraud medium is measured; a radius of ≥2 cm confirms the isolate to be strain MBI 600.

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. The seed treatment use of strain MBI 600 is not expected to significantly increase natural environmental background levels of *B. subtilis*. 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, when *B. subtilis* strain MBI 600 was administered orally to rats, no signs of toxicity or disease were observed. Consequently no methods are required to quantify viable or non-viable residues of *B. subtilis* strain MBI 600.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

During manufacturing, several approaches are used to limit microbial contamination in *Bacillus subtilis* strain MBI 600 Technical and Integral™ Liquid Biological Fungicide. These approaches include large quantities of inocula, elevated inoculation temperatures, frequent purity checks on agar media, sterilization of all equipment and media, elevated inoculation temperatures, and sanitization of recovery equipment.

The quality control procedures used to limit contaminating microorganisms during manufacture of *Bacillus subtilis* strain MBI 600 Technical and Integral™ Liquid Biological Fungicide end use product are acceptable. Regular quality checks on the identity of *Bacillus subtilis* are conducted during the production process by plating and by visual inspection to verify colony morphology and to detect any unusual colonies. The final product is also plated onto Trypticase Soy agar (TSA) for detection of contaminating microorganisms. All bacterial and fungal colonies are purified and identified using traditional typing methods.

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 *Bacillus subtilis* strain MBI 600 Technical and Integral™ Liquid Biological Fungicide. These procedures include frequent purity checks on agar media to detect any unusual colonies and to verify colony morphology.

Acceptable microbial contaminant analysis data were submitted for five batches of the Integral™ Liquid Biological Fungicide end-use product. The analysis included standard assays designed to detect contaminating yeasts and coliform bacteria, *Staphylococcus*, and *Salmonella.* Although microbe-specific screens for specific pathogens are not part of the regular quality control program, 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.

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

The viability of *Bacillus subtilis* strain MBI 600 in Integral™ Liquid Biological Fungicide end-use product was assessed by determining the guarantee over a period of time and over a range of storage temperatures. Results showed that *B. subtilis* strain MBI 600 remained viable at the guarantee under warehouse conditions (25–28°C in summer; 10–15°C in winter) in the Technical for a period of up to 27 months and in Integral™ Liquid Biological Fungicide for a period of up to 19 months.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

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 in being 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 of those cases involved drug abuses or severely debilitated patients. As *B. subtilis* is ubiquitous in the environment, 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. No such illnesses were reported for this microorganism in the United States where it has been registered for use on crops since 1994.

Also, in Canada where this microorganism has been registered since 2007, no such illnesses were reported.

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.

A detailed review of the toxicological database for *B. subtilis* strain MBI 600 has been completed. The database for *B. subtilis* strain MBI 600 is complete (see Appendix I) 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 which were carried out in accordance with currently accepted international testing protocols and Good Laboratory Practices. Waiver requests were deemed acceptable to address the acute dermal toxicity and dermal irritation of the end-use product, Integral™ Liquid Biological Fungicide.

The scientific quality of the data is high and the database is considered sufficient to characterize the toxicity and infectivity of this MPCA and end use product.

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. Based on the results of this study strain MBI 600 is of low toxicity in the rat when challenged via the oral route.

In the pulmonary toxicity/pathogenicity study, mortality was observed following intratracheal treatment with strain MBI 600 at doses ranging from 3.2×10^8 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, applicators, mixers and loaders who apply the end-use product to the seed will be required to wear a dust mask when they are most likely to be exposed via the inhalation (i.e., when applying the product to seeds, or handling the treated seeds).

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 the liver and spleen of treated rats by Day 21. A definitive pattern of clearance was established. Based on the results of this study, there was no evidence of pathogenicity observed in rats following intravenous injection with *B. subtilis* strain MBI 600 at approximately 10^7 CFU per test animal.

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 one hour 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. Irritation was completely resolved by Day 4 of the treatment period. *B. subtilis* strain MBI 600 is minimally irritating to the eye based on the maximum average score (MAS) of 4.6/110 (for Days 1, 2 and 3) and the maximum irritation score (MIS) of 6/110 on Days 1 and 2.

In the dermal sensitization study, *B. subtilis* strain MBI 600 induced 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. Like most microorganisms, *B. subtilis* strain MBI 600 contains substances that elicit positive hypersensitivity reactions in humans, and therefore *B. subtilis* strain MBI 600 is considered to be a potential sensitizing agent. Consequently the signal words "POTENTIAL SENSITIZER" are required on the principal display panels of *Bacillus subtilis* strain MBI 600 technical and Integral™ Liquid Biological Fungicide formulation labels, as well as label precautions requiring personal protective equipment and judicious handling to minimize exposure to workers.

A rationale to waive the requirement for a dermal toxicity study and a dermal irritation study with Integral™ Liquid Biological Fungicide was submitted. Based on the lack of toxicity of *B. subtilis* strain MBI 600 observed in the dermal toxicity study in the technical grade active ingredient, in as well as the low toxicity and lack of pathogenicity of *B. subtilis* strain MBI 600 via the acute oral, pulmonary, and intravenous routes of exposure; an absence of adverse effects reported for workers involved in the manufacture and use of the end use product in the United States since 1994; the intended use of application in commercial canola seed treating facilities with closed transfer systems; and the lack of toxicological concern for, and widespread use of, the end-use product formulation ingredients, the waiver request was accepted.

Higher-tier subchronic and chronic toxicity studies were not required based on the toxicity profile demonstrated in the test animals in the Tier I acute oral, pulmonary, toxicity/infectivity and intravenous injection infectivity studies.

The active ingredient, *B. subtilis* strain MBI 600, is not known to be a human pathogen nor an endocrine disruptor. 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 for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with the primary source of exposure to workers being dermal. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Bacillus subtilis* has not been identified as a dermal wound pathogen, and there is no indication that it could penetrate intact skin of healthy individuals. Furthermore, *B. subtilis* strain MBI 600 demonstrated low toxicity and minimal irritation in the dermal toxicity/irritation studies.

The risk of toxicity exists in individuals exposed to large quantities of spores of *B. subtilis* strain MBI 600 by inhalation. 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. To mitigate the risk of pulmonary exposure to *B. subtilis* strain MBI 600, applicators, mixers and loaders who apply the end-use product to the seed will be required to wear a dust mask when they are most likely to be exposed via the inhalation.

The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Label statements (i.e., Potential Sensitizer) and risk mitigation measured such as personal protective equipment, including gloves, long-sleeved shirts, long pants, NIOSH approved respirators (with any N–95, P-95, R-95 or HE filter), shoes and socks, are required to minimize exposure and protect handlers that are likely to be primarily exposed.

3.2.2 Bystander

Inhalation or dermal exposure to the general public is expected to be low based on the proposed seed treatment application of Integral™ Liquid Biological Fungicide indoors on canola. Overall the PMRA does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for *Bacillus subtilis* strain MBI 600 and the related end-use formulation.

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

Based on the proposed indoor use pattern of Integral™ Liquid Biological Fungicide as a seed treatment dietary exposure to food commodities grown from treated seeds are expected to be very low. Furthermore, dietary exposure to the microbial agent is unlikely to result in any undue hazard to consumers because no adverse effects were observed at maximum hazard dose levels in the submitted Tier I acute oral study. Moreover, *B. subtilis* is a ubiquitous organism found in most terrestrial environments.

Higher-tier subchronic and chronic toxicity studies were not required based on the toxicity profile demonstrated in the test animals in the Tier I acute oral, pulmonary, toxicity/infectivity and intravenous injection infectivity studies. Therefore, there are no concerns for chronic risks posed by dietary exposure to the general population and sensitive subpopulations, such as infants and children.

3.3.2 Drinking Water

The likelihood that *B. subtilis* strain MBI 600 could enter neighbouring aquatic environments via leaching through soil from seed treated with Integral™ Liquid Biological Fungicide is considered very low. *Bacillus subtilis* strain MBI 600 is not generally recognized as an aquatic microorganism. Strain MBI 600 therefore is not expected to proliferate in aquatic habitats following direct or indirect (e.g., leaching) application to bodies of water. Moreover, *B. subtilis* is not considered to be a risk to drinking water sources. Accordingly, drinking water is not specifically screened for *B. subtilis* as a potential indicator of microbial contamination or as a direct pathogenic contaminant. Both percolation through soil and municipal treatment of drinking water would reduce the possibility of significant transfer of residues to drinking water.

Also, the labels for Integral™ Liquid Biological Fungicide will instruct users not to contaminate drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes.

Therefore the potential of exposure and risk via drinking water is likely to be minimal to non-existent for this MPCA.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARDs) and acceptable daily intakes (ADIs) are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (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 PMRA 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, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further, factoring in consumption patterns among infants and children, special susceptibility of these subpopulations to the effects of the MPCA, including neurological effects from pre- or post-natal exposures and cumulative effects on infants and children of the MPCA and other registered micro-organisms that have a common mechanism of toxicity, does not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *B. subtilis* strain MBI 600 to human health.

3.4 Maximum Residue Limits

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the Pest Control Products Act (PCPA) for the purposes of the adulteration provision of the Food and Drugs Act (FDA). Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

The seed treatment use of Integral™ Liquid Biological Fungicide is not expected to result in a sustained increase in background levels of this organism. 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. No such illnesses were reported for this MPCA in the United States where it has been registered for use on crops since 1994. Also, in Canada where the MPCA has been registered since 2007, no such illnesses were reported. Therefore the establishment of an MRL is not required for *B. subtilis* strain MBI 600 under the PCPA.

3.5 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA's files, there is reasonable certainty 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 as a seed treatment as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Even if there is an increase in exposure to this microorganism from the use of Integral™ Liquid Biological Fungicide 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, and the biological fungicide products, Subtilex™ Biological Fungicide and ProMix™ With Biofungicide, which also contains *B. subtilis* strain MBI600 as the active ingredient, the PMRA 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

Environmental fate testing is intended to demonstrate whether a microbial pest control agent (MPCA) is capable of surviving or replicating in the environment to which it is applied, and 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. Environmental fate data (Tier II/III) are not normally required at Tier I, and are only triggered if significant toxicological effects in non-target organisms are noted in Tier I testing.

No studies were submitted to address the environmental fate and behaviour of *B. subtilis* strain MBI 600. However, some information on the environmental fate of *B. subtilis* was submitted in support of requests to waive environmental toxicological data requirements 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 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.

In non-sterile lake water, *B. subtilis* added at an initial level of 4.1×10^5 vegetative cells/mL, quickly fell to eight 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. Also, in the 30-day exposure toxicity study with carp (*Cyprinus carpio*; see Section 4.2.2) *B. subtilis* strain MBI 600 survived in dechlorinated tap water between renewal periods of 3 days, most likely as spores.

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.

The proposed indoor application of Integral™ Liquid Biological Fungicide as a seed treatment for canola, and the subsequent outdoor planting of treated seeds into soil is not expected to result in a sustained increase of populations of the MPCA beyond those of naturally occurring soil dwelling *Bacillus* species found in the environment.

4.2 Effects on Non-Target Species

An avian oral toxicity study, terrestrial arthropod toxicity studies (five species tested), and a terrestrial plant phytotoxicity study were submitted to address the risks of *B. subtilis* strain MBI 600 to terrestrial organisms. The risks to non-target aquatic organisms were addressed in a freshwater fish toxicity study. Waiver requests were deemed acceptable to address the remaining environmental toxicological requirements. The rationales were based on the ubiquitous nature of *B. subtilis* in both soil and water whose level in the terrestrial and aquatic environment will not increase as a result of the use of Integral™ Liquid Biological Fungicide as a seed treatment, which is applied indoors under a controlled environment by trained personnel; the toxicity profile of *B. subtilis* strain MBI 600 from laboratory animal studies; and a review of published literature which indicated a few reports of adverse effects to terrestrial organisms, and a lack of adverse effects to aquatic organisms from natural populations of *B. subtilis*.

4.2.1 Effects on Terrestrial Organisms

There is the potential for oral exposure to birds from the proposed seed treatment use, as birds may consume food items that may have been exposed to seeds treated with *B. subtilis* strain MBI 600. The potential for the MPCA to cause acute oral toxicity and pathogenicity in an avian species was assessed in a study with northern Bobwhite quails (*Colinus virginianus)*. Three groups of apparently healthy 21-day-old northern Bobwhite quails were administered daily in the crop or proventriculus for 5 days with either *B. subtilis* strain MBI 600 (GUS 378 Concentrate, 4000 mg/kg bw/day), water-soluble metabolites of *B. subtilis* strain MBI 600 (240 mg/kg bw/day) or washed spores of *B. subtilis* strain MBI 600 (3680 mg/kg bw/day) in deionized water and observed over a total period of 30 days. A fourth group administered deionized water at 10 mL/kg bw/day daily for 5 days, 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 which was 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.

The potential for adverse effects from the MPCA to non-target terrestrial arthropods was assessed in a honeybee toxicity study, a silkworm toxicity study, and a combined non-target insect toxicity study which assessed the effects on the predatory coleoptora *Harmonia axyridis* (Lady beetle), the predatory neuropteran *Chrysoperla carnea* (Green lacewing), and the predatory mite *Phytoseiulus persimilis*. The studies were conducted with IK-1080 WP Technical which contains *Bacillus subtilis* strain NCIM 12376. *Bacillus subtilis* strain NCIM 12376 is the parent strain of *B. subtilis* strain MBI 600, and thus the strains are considered equivalent.

In the 48-hour dietary toxicity study, honeybees *(Apis mellifera)* were exposed to IK-1080 at 1×10^8 CFU/mL and monitored for mortality for 20 days. No effect on bee mortality was observed, as mortality in the untreated control group (26.0%) was higher than that in the treatment group (15.3%). The 48-hour LC_{50} is estimated to be $> 1 \times 10^8$ CFU/mL.

The combined non-target insect study examined the effects of dietary exposure, or contact exposure, of the predatory coleoptora *Harmonia axyridis* (Lady beetle), the predatory neuropteran *Chrysoperla carnea* (Green lacewing), and the predatory mite *Phytoseiulus persimilis* to the MPCA. Although the observation periods were considerably shorter than recommended, the study was acceptable. Briefly, the acute dietary toxicity of the MPCA was assessed with Lady beetles (*Harmonia axyridis*) by exposing larvae and adults to IK-1080 WP Technical in the diet at 1.5×10^5 CFU/mg of food for 24 hours (larvae) or 48 hours (adults). Larvae and adults were examined for mortality and growth (number of adults emerging) for 10 days. No considerable effect on mortality or emergence (growth) of larvae or adult of *Harmonia* sp. was noted. The acute dietary LC_{50} was estimated to be $> 1.5 \times 10^5$ CFU/mg of food.

The acute contact toxicity of the MPCA to the Green lacewing (*Chrysoperla carnea*) was assessed by dipping Green lacewing eggs once in a solution of IK-1080 WP Technical at 1×10^9 CFU/mL, and counting the number of larvae hatched three days after treatment. Dietary exposure was also assessed by feeding larvae IK-1080 WP Technical in the diet at 1.5×10^5 CFU/mg of food for 72 hours. Larvae were examined for mortality and growth (number of adults emerging) for 21 days. No considerable effect on mortality or emergence (growth) of eggs or adult *Chrysoperla sp.* were noted. The acute contact LC_{50} (eggs) was estimated to be $> 1 \times 10^9$ CFU/mL, and the acute dietary LC_{50} (adults) was estimated to be $> 1.5 \times 10^5$ CFU/mg of food.

The acute contact toxicity of the MPCA to predatory mites (*Phytoseiulus persimilis*) was assessed by exposing adults to kidney bean leaves treated with IK-1080 WP Technical (1×10^9 CFU/mL) at a rate of 4 mg/cm^2 for 72 hours (equivalent to 3.2×10^9 CFU/cm^2), and examining for mortality during the 72-hour observation period. Although an increase in mortality was observed in the treatment group (12.5%) versus the control group (2.5%), adverse effects from the use of Integral ^{TM} Liquid Biological Fungicide as a seed treatment are not expected since

these effects were observed from an exposure scenario that is not likely to be encountered based on use pattern of Integral™ Liquid Biological Fungicide as a seed treatment.

In the 48-hour dietary toxicity study with silkworm larvae, a suspension of IK-1080 WP Technical at 1.66×10^{10} CFU/mL (in tween 40) was brushed onto mulberry leaves and offered to silkworm larvae for two days. After the 48-hour exposure period, the silkworm larvae were fed untreated leaves and monitored daily for mortality, signs of toxicity, and for quality of cocoons for approximately 20 days. There were no effects on the mortality or quality of silkworm cocoons and no signs of toxicity from dietary exposure to IK-1080 WP Technical for 48-hours. The acute dietary LC_{50} was estimated to be $> 1.66 \times 10^{10}$ CFU/mL.

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 *B. megaterium* rather than *B. 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.

Requests to waive the requirement for avian pulmonary toxicity testing, wild mammal toxicity testing and non-arthropod invertebrate toxicity testing were accepted based on the following rationale. *Bacillus subtilis* is a ubiquitous microorganism in soil and the use of Integral ™ Liquid Biological Fungicide as a seed treatment on canola is not expected to considerably increase the natural background level of the microorganism in the terrestrial environment. Furthermore, Integral™ Liquid Biological Fungicide is applied indoors under a controlled environment by trained personnel, and treated seeds are subsequently planted outdoors in soil. Therefore the level of exposure of *B. subtilis* strain MBI 600 to non-target terrestrial organisms is expected to be minimal. In particular, exposure to birds by the pulmonary route is expected to be minimal. There were also no reports of adverse effects to avian species from natural populations of *B. subtilis* in the published literature. There were, however, a few reports of adverse effects in mammals. These reports implicated *B. subtilis* in cases of bovine mastitis and reproductive disorders in goats. No other reports of adverse effects were reported to mammals, 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 Integral™ Liquid Biological Fungicide in commercial seed treating facilities with closed transfer systems.

For earthworms and other soil macroorganisms, published literature revealed one report on the use of *B. subtilis* strain VM 132 as a potential biological control agent of the parasitic root-knot nematode (*Meloidogyne incognita*) of tomato. Plants grown from inoculated seed displayed fewer nematode galls; however, the mode of action was not elucidated, and the pathogenic relationship was not established. In contrast, another published study demonstrated that *B. subtilis* displayed no toxicity or pathogenicity to the nematode, *Caenorhabditis elegans*, 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. *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* strain MBI 600 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 Integral™ Liquid Biological Fungicide as a seed treatment.

4.2.2 Effects on Aquatic Organisms

In the 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).

Requests to waive the requirement for toxicity testing of estuarine and marine fish, aquatic arthropods, and aquatic plants were accepted based on the following rationale. The use of Integral™ Liquid Biological Fungicide will be limited to seed treatment application in commercial seed treatment facilities with closed transfer systems. This intended use pattern minimizes direct exposure to non-target aquatic organisms. Given that the product is not intended for direct application to water, indirect contamination of aquatic ecosystems is only expected to occur from leaching from treated seed planted in soil. Given the expected levels of *B. subtilis* strain MBI 600 on the treated seeds and the lack of persistence of non-indigenous strains of *B. subtilis* introduced into soils already containing a natural microbial flora (see

Section 4.1), the amount of *B. subtilis* strain MBI 600 reaching aquatic environments from leaching is expected to be negligible. Nevertheless, the biological properties of this microorganism 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 the ubiquitous nature of the microorganism. 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.

Based on all the available data and information on the effects of *Bacillus subtilis* strain MBI 600 to aquatic organisms, there is reasonable certainty that no harm will be caused to non-target aquatic organisms from the proposed seed treatment of Integral™ Liquid Biological Fungicide. As a general precaution, label statements will be added to the label requiring handlers to not contaminate irrigation or drinking water or aquatic habitats by cleaning of equipment or disposal of wastes.

5.0 Value

5.1 Acceptable Efficacy Claims

5.1.1 Partial Suppression of the Seedling Disease Complex (Seed Rot, Pre and Post Emergent Damping-off, Seedling Blight and Root Rot) Caused by *Rhizoctonia* and *Fusarium* spp. by Integral ™ Liquid Biological Fungicide alone

B. subtilis MBI 600, the active ingredient of Integral ™ Liquid Biological Fungicide, was highly effective in inhibiting growth of *Rhizoctonia* and *Fusarium* in solid and liquid media. In addition, cell-free extracts of *B. subtilis* MBI 600 were also effective in inhibiting fungal growth. This result further demonstrates that the mode of action of Integral ™ Liquid Biological Fungicide depends not only on competition for available resources with pathogenic fungi, but also on direct antibiosis. The majority of the submitted data demonstrated significant, albeit low, levels of Integral ™ Liquid Biological Fungicide efficacy against the canola seedling disease complex (seed rot, pre and post emergent damping-off, seedling blight and root rot). Significant differences were observed in disease incidence and severity assessments. Early emergence was higher in the Integral ™ Liquid Biological Fungicide treatment compared to the untreated inoculated check when the three submitted *Rhizoctonia* trials were pooled and analysed as a multi-site analysis. A 10% improvement in emergence was observed in the Integral ™ Liquid Biological Fungicide treated seed compared to the untreated inoculated check. In greenhouse trials, Integral ™ Liquid Biological Fungicide seed treatments again resulted in increases in emergence compared to the untreated control in each of four submitted trials (two with *Rhizoctonia* challenge and two with *Fusarium* challenge). Increases in emergence ranged from 10 to 48%. Overall, the levels of disease reduction observed for Integral ™ Liquid Biological Fungicide applied alone as a seed treatment are consistent with partial suppression of the diseases in question.

5.1.2 Control of Seedling Disease Complex (Seed rot, Pre and Post Emergent Damping-off, Seedling Blight and Root Rot) Caused by *Rhizoctonia* and *Fusarium* spp. by Integral ™ Liquid Biological Fungicide in a Tank Mix with Helix Liquid Seed Treatment, Helix XTra Seed Treatment or Prosper FL Flowable Insecticide and Fungicide Seed Treatment

In multi-site field trials conducted in 2007 and repeated in 2008, the efficacy of the Integral ™ Liquid Biological Fungicide /Helix Liquid Seed Treatment tank mix was compared with applications of Helix Liquid Seed Treatment alone. An increase by the tank mix treatment over Helix XTra Seed Treatment alone was observed in 2007 in early emergence (8%) and plant stand (4%) when the data from all of the field sites were averaged. This difference resulted in a yield increase of 31 kg/ha in the tank mix treatment compared to the Helix XTra Seed Treatment control. In 2008, the tank mix seed treatment resulted in an increase of approximately 11% in plant stands compared to the treatment with Helix XTra Seed Treatment alone. In addition, visual assessments from both years showed increased plant vigour in the plots where tank mix treated seeds had been sowed.

5.2 Phytotoxicity

Integral ™ Liquid Biological Fungicide treatments did not show any negative effect of concern on the growth and development of canola crops in any of the trials when treated plants were compared to controls (untreated or conventional seed treatments only). No visible sign of phytotoxicity nor phytopathogenicity effect was observed. Based on all the submitted data, no phytotoxicity, phytopathogenic effect, nor any other negative effect on the growth of canola crops are expected following the application of Integral ™ Liquid Biological Fungicide as a seed treatment at the labelled rates.

5.4 Economics

Based on information from the Canola Council of Canada, the applicant provided the following assessment of Integral ™ Liquid Biological Fungicide's forecasted economic contribution. The seedling disease complex often results in reduced or patchy stands or stands with poor vigour and ultimately reduced yield potential causing significant financial losses to producers. Often growers will decide to re-seed their crop at considerable time and expense. Besides the obvious costs associated with re-seeding (cost of the seed, fuel and one's time) one also needs to consider the cost of additional chemical seed treatment since most canola seed is sold pre-treated. In addition to the direct re-seeding costs there will also be the potential cost of a second herbicide application as well as possible loss of income due to reduced yield resulting from later seeding. Based on canola prices at the time of review, the additional 31 kg/ha observed in the field trials would result in an additional $9.88 per hectare. It was estimated that if Integral ™ Liquid Biological Fungicide were used on only 10% of the total canola growing area in Canada, this could potentially translate into $6.4 million extra margin.

5.5 Sustainability

5.5.1 Survey of Alternatives

The current strategy used for managing the seedling disease complex consists of a combination of agronomic management practices and on-seed chemical seed treatments. Management practices include the use of good quality certified seed and planting at a depth of 10 to 20 mm in firm, moist, and adequately fertilized soil when the temperature is above 10°C to ensure rapid and vigorous germination. Rotation of canola with non-cruciferous crops such as cereals (wheat, barley) will also reduce occurrence of the disease. The use of chemical seed treatments containing fungicides is the primary means used by growers to minimize the impact of the seedling disease complex. Treatment intensity is currently estimated to be greater than 90%. Currently, there are several chemical seed treatment products registered for use on canola seed including Helix Liquid Seed Treatment, Helix XTra Seed Treatment, and Prosper FL Flowable Insecticide and Fungicide Seed Treatment while there is no biological product registered for the management of the seedling disease complex in canola.

5.5.2 Compatibility with Current Management Practices Including Integrated Pest Management

Integral ™ Liquid Biological Fungicide has demonstrated good compatibility in combination with Helix Liquid Seed Treatment, Helix XTra Seed Treatment and Prosper FL Flowable Insecticide and Fungicide Seed Treatment chemical seed treatments, excellent seed safety and improved canola emergence and stand establishment. Use of Integral ™ Liquid Biological Fungicide in combination with the chemical seed treatments will provide the canola grower with an additional management tool that has a different mode of action against the seedling disease complex. It fits well into a sound integrated pest management strategy against the seedling disease complex.

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 Integral ™ Liquid Biological Fungicide. Resistance to *B. subtilis* strain MBI 600 in target populations is not expected due to its mode of action. *B. subtilis* strain MBI 600 is a rapid root coloniser, establishing itself quickly on the surface of roots, thereby outcompeting potential fungal root pathogens. Antifungal peptides and proteins produced in situ by the bacterium also likely contribute to the suppression of root pathogens. Given that the primary mode of action against fungal pathogens appears to be competitive exclusion, there appears to be a very low risk of developing resistant strains among *Rhizoctonia* and *Fusarium* populations. This type of mode of action typically has a very low risk of developing resistance in target organisms.

5.5.4 Contribution to Risk Reduction and Sustainability *(Defer to SMC)*

Integral ™ Liquid Biological Fungicide is a microbial pest control products whose mode of action is based on competitive inhibition and exclusion of *Rhizoctonia* and *Fusarium* spp. on seeds and roots of canola. It is a biological product intended to provide additional protection against the seedling disease complex in canola as a microbial biopesticide that has a low potential to harm the health of Canadians and their environment.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The management of toxic substances is guided by the federal government's *Toxic Substances Management Policy* (TSMP), 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.

In reviewing *B. subtilis* MBI 600, the PMRA took into account the federal TSMP 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 micro contaminants in the technical product, *Bacillus subtilis* strain MBI 600 Technical , and formulants in the end-use product Integral™ Liquid Biological Fungicide. The PMRA has reached the following conclusions:

> *Bacillus subtilis* strain MBI 600 Technical does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. There are also no formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track-1 criteria. Therefore, the seed treatment use of Integral™ Liquid Biological Fungicide is not expected to result in the entry of Track 1 substances into the environment.

6.2 Formulants and Contaminants of Health or Environmental Concern

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

The end-use product, Integral™ Liquid Biological Fungicide, does not contain any formulants and contaminants of health or environmental concern identified in Canada Gazette Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern*.

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for the *Bacillus subtilis* strain MBI 600 Technical and End-Use Product (Integral™ Liquid Biological Fungicide) were judged to be adequate to assess their potential human health and environmental risks. The technical material was fully characterized and the specifications were supported by the analyses of a sufficient number of batches. Although microbe-specific screens for specific pathogens are not part of the regular quality control program, 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 submitted were sufficient to support a shelf life for Integral ™ Liquid Biological Fungicide of a minimum of 19 months at 4–30°C.

7.1 Human Health and Safety

The acute toxicity and infectivity studies submitted in support of *B. subtilis* strain MBI 600 and IntegralTM Liquid Biological Fungicide 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 route of exposure, but the MPCA was neither pathogenic nor infective via the pulmonary or intravenous injection routes of administration. The toxicity observed is consistent with results of other acute pulmonary studies conducted with other *Bacillus* species (e.g., *B. thuringiensis*) and may be attributed to either a non-specific toxicological response to the very high dose administered or to the possible presence of hemolytic metabolic by-products contained in the dosing preparation.

Waiver requests to address the requirement for acute dermal toxicity and dermal irritation studies for Integral™ Liquid Biological Fungicide were accepted. *Bacillus subtilis* strain MBI 600 was of low toxicity and minimally irritating to the skin, and was minimally irritating to the eyes.

Bacillus subtilis was determined to be a sensitizing agent in a dermal sensitization study, and all microbial pesticides are assumed to be potential sensitizers. Repeated exposure to high concentrations of *B. subtilis* strain MBI 600 could lead to allergic reactions in certain individuals. As a result, the signal words POTENTIAL SENSITIZER are required on the principal display panels of *Bacillus subtilis* strain MBI 600 Technical and Integral ™ Liquid

Biological Fungicide. Additional mitigative measures to minimize exposure to workers and applicators will include the wearing of appropriate PPE (e.g., long-sleeved shirt, pants, shoes plus socks and gloves) as well as a biological dust/mist filtering respirator/mask.

Given that *B. subtilis* is an indigenous soil microorganism, it is unlikely that the proposed use of Integral™ Liquid Biological Fungicide as a seed treatment will result in residues on treated food/feed stuffs. Although some strains of *B. subtilis* have been isolated from food implicated in food poisoning incidents, no such reports for this MPCA have been made in the United States where it has been registered for use on crops since 1994. Also, *B. subtilis* strain MBI 600 demonstrated no oral toxicity and was not pathogenic/infective via pulmonary or intravenous exposure routes at the maximum hazard dose in the Tier I acute toxicity/infectivity studies. Therefore, negligible to no risk is expected for the general population, including infants and children, or animals from residues in or on agricultural commodities. Consequently, the establishment of a maximum residue limit (MRL) is not required for *Bacillus subtilis* strain MBI600.

7.2 Environmental Risk

Sufficient information and data regarding environmental fate and ecotoxicological effects were submitted to support registration of *Bacillus subtilis* strain MBI 600 Technical containing *B. subtilis* strain MBI 600 and the end-use product Integral™ Liquid Biological Fungicide.

Environmental effects studies submitted to address risks of *B. subtilis* strain MBI 600 to non-target organisms demonstrated that *B. subtilis* strain MBI 600 was not toxic or pathogenic to birds, freshwater fish, terrestrial arthropods and seeds of terrestrial plants. Waiver requests were deemed acceptable to address the remaining groups of non-target organisms, including mammals, non-arthropod invertebrates, estuarine/marine fish, aquatic arthropods, and aquatic plants. The rationales were based on the ubiquitous nature of *B. subtilis* in both soil and water such that its level in the terrestrial and aquatic environment will not significantly increase from the use of Integral™ Liquid Biological Fungicide as a seed treatment which is applied indoors under a controlled environment by trained personnel. A review of published literature revealed no reports of adverse effects to aquatic organisms from natural populations of *B. subtilis*, but revealed a few reports of potential adverse effects to terrestrial organisms (i.e., to a plant pathogenic nematode and to certain livestock) none of which were thoroughly investigated or substantiated. Based on the results of toxicity/pathogenicity studies with *B. subtilis* strain MBI 600 on laboratory mammals, and the ubiquitous nature of *B. subtilis* in the environment, the MBI600 strain is not expected to pose a risk to terrestrial non-target organisms, nor is it expected to pose a risk to aquatic non-target organisms.

As a general precaution, standard label statements will prohibit handlers from contaminating irrigation or drinking water or aquatic habitats with Integral™ Liquid Biological Fungicide.

7.3 Value

The claim for partial suppression of the seedling disease complex in canola by Integral ™ Liquid Biological Fungicide applied alone as a seed treatment is supported. The claim for control of the seedling disease complex in canola by Integral ™ Liquid Biological Fungicide, when tank mixed with Helix Liquid Seed Treatment, Helix XTra Seed Treatment, or Prosper FL Flowable Insecticide and Fungicide Seed Treatment is also supported.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Bacillus subtilis* strain MBI 600 and Integral ™ Liquid Biological Fungicide, containing the technical grade active ingredient *Bacillus subtilis* strain MBI 600, as a seed treatment to suppress the seedling disease complex in canola (seed rot, pre and post emergent damping-off, seedling blight and root rot) caused by *Rhizoctonia* spp. and *Fusarium* spp..

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

List of Abbreviations

µg	micrograms
1/n	exponent for the Freundlich isotherm
a.i.	active ingredient
ADI	acceptable daily intake
ARD	acute reference dose
ALS	acetolactate synthase
ARfD	acute reference dose
atm	atmosphere
bw	body weight
^{o}C	degree(s) Celcius
CAS	Chemical Abstracts Service
CFU	colony forming units
cm	centimetres
cv	cultivar
DF	dry flowable
DNA	deoxyribonucleic acid
DT_{50}	dissipation time 50% (the dose required to observe a 50% decline in the test population)
DT_{75}	dissipation time 75% (the dose required to observe a 75% decline in the test population)
EC_{10}	effective concentration on 10% of the population
EC_{25}	effective concentration on 25% of the population
EP	end-use product
ER_{25}	effective rate for 25% of the population
g	gram
ha	hectare(s)
HDT	highest dose tested
Hg	mercury
HPLC	high performance liquid chromatography
IUPAC	International Union of Pure and Applied Chemistry
kDa	kilodalton
kg	kilogram
K_d	soil-water partition coefficient
K_F	Freundlich adsorption coefficient
km	kilometre
K_{oc}	organic-carbon partition coefficient
K_{ow}	*n*–octanol-water partition coefficient
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LOAEL	lowest observed adverse effect level
LOEC	low observed effect concentration
LOQ	limit of quantitation
LR_{50}	lethal rate 50%
mg	milligram

mL	millilitre
MAS	maximum average score
MPCA	microbial pest control agent
MOE	margin of exposure
MRL	maximum residue limit
MS	mass spectrometry
N/A	not applicable
NA	nutrient agar
NCIM	national collection of industrial microorganisms
NIOSH	National Institute of Occupational Safety and Health
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
NOER	no observed effect rate
N/R	not required
NZW	New Zealand white
OC	organic carbon content
OM	organic matter content
PBI	plantback interval
PCR	Polymerase Chain Reaction
PCPA	*Pest Control Products Act*
PHI	preharvest interval
p*K*a	dissociation constant
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
ppm	parts per million
rRNA	ribosomal ribonucleic acid
RSD	relative standard deviation
SC	soluble concentrate
sp.	Species
spp.	species
$t_{1/2}$	half-life
T3	tri-iodothyronine
T4	thyroxine
TGAI	technical grade of the active ingredient
TM	trademark
TRR	total radioactive residue
TSA	trypticase soy agar
TSMP	Toxic Substances Management Policy
UAN	urea ammonium nitrate
UF	uncertainty factor
USEPA	United States Environmental Protection Agency
UV	ultraviolet
v/v	volume per volume dilution

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *Bacillus subtilis* strain MBI 600 and its associated end-use product (Integral™ Liquid Biological Fungicide)

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Bacillus subtilis* strain MBI 600 Technical				
Acute Oral Toxicity and Infectivity	*Rat- CD, 11/sex, 1% suspension in sterile distilled water 10mL/kg bw (1.9–2.7 × 108 CFU/animal), interim sacrifices on Days 2, 8, and 15* *5/sex treated with autoclaved MPCA, 1% suspensions in sterile distilled water, 20 mL/kg bw*	*LD_{50} > ~2.0 × 10^8 CFU/animal*	*No mortalities. Slightly low body-weight gains were observed for two MPCA-treated ♂ (Day 15), and one ♀ treated with autoclaved MPCA (Day 22). The MPCA was initially recovered from the feces, urine and gastrointestinal contents (stomach, small intestines and cecum) and from the blood Day 7, but was completely cleared from all organs and fluids by Day 22. No significant findings observed at necropsy.* ***LOW TOXICITY AND NO PATHOGENICITY***	*PMRA 1623586*
Acute Pulmonary Toxicity and Infectivity	*Rat-CD* *16 ♂ and 15 ♀: 1% sterile distilled water, 3.2 –3.7 × 10^8 CFU/animal, 1.2 mL/kg bw, interim sacrifices on Days 1, 2, 8, and 15* *5/sex treated with autoclaved MPCA, 1% suspension in sterile distilled water, 1.2 mL/kg bw.*	*LD_{50} > ~ 3.5 x 10^8 CFU/animal*	*Between Day 2 and Day 4 of the observation period, 5 of 16 males died and 3 of 15 females died. Body weight loss was observed in all animals that died, as well as body weight losses or low body weight gains in animals treated with the MPCA and autoclaved MPCA. 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. A 3–4℃ 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 macroscopic findings were observed. The MPCA was initially recovered from the cecum, feces, urine, blood and various organs (brain, heart, liver, kidney, spleen, lymph nodes) of treated animals. By Day 7, viable spores were mainly*	*PMRA 1623587*

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	
			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. ***TOXIC,*** ***NO PATHOGENICITY***	
Intravenous Injection Infectivity	*Rat-CD, 13/sex, 4.2–4.8 × 10^7 CFU/animal* *5/sex treated with autoclaved MPCA, 1% suspension in physiological saline, 3 mL/kg bw*	*LD_{50} > 4.0 x 10^7 CFU/animal*	*No mortalities. No effect on body-weight gain and no apparent signs of treatment-related toxicity or pathogenicity. No abnormalities on necropsy. Body temperatures were approximately 3℃ lower in the treated rats after 24 hours. Following injection, the MPCA was recovered from the feces, urine, blood and various organs (predominantly in liver and spleen). Counts decreased throughout the study period. By Day 22, the test microbe was only recovered from the liver and spleen.* ***NO PATHOGENICITY***	*PMRA 1623589*
Acute Dermal Toxicity and Irritation	*Rabbit- New Zealand white, 5/sex, undiluted, 2 mL/kg bw (equivalent to approximately. 10^{10} CFU/kg bw), administered to 10% of the body surface*	*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 1623590*

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	
Dermal sensitization	*Guinea 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 1623592*
Eye Irritation	*Rabbit-New Zealand white, 6 ♀, 0.1 mL of the technical powder (equivalent to 10^9 CFU/animal), conjunctival sac of right eye.*	*MAS[1]=4.6/110 (24, 48 and 72 hours)* *MIS[2] = 6/110 (24 hours)*	*After 1 hour, slight conjunctivae redness and discharge (grade 1) were noted. After 24 hours, chemosis (garde 1) was noted. Irritation cleared by Day 4.* ***MINIMALLY IRRITATING***	*PMRA 1623593*

Acute Toxicity/Irritation of **Integral™ Liquid Biological Fungicide**				
Acute Dermal Toxicity and Irritation- end-use product	waiver rational submitted in lieu of data for end-use product	**WAIVER ACCEPTED**	Based on the lack of toxicity of *B. subtilis* strain MBI 600 observed in the dermal toxicity study, as well as the low toxicity and lack of pathogenicity of the MPCA via the acute oral, pulmonary, and intravenous routes of exposure; an absence of adverse effects reported for workers involved in the manufacture and use of the end use product in the United States since 1994 and in Canada since 2007; the intended use of application in commercial canola seed treating facilities with closed transfer systems; and the lack of toxicological concern for, and widespread use of, the formulation ingredients, the request to waive dermal toxicity and irritation testing for Integral™ Liquid Biological Fungicide was accepted.	PMRA 1623590, 1623586, 1623587, 1622722, 1622723.

[1] MAS = maximum average score

[2] MIS = maximum irritation score

Table 3 Toxicology Endpoints for Use in Health Risk Assessment for Integral ™ Liquid Biological Fungicide

Table 4 Integrated Food Residue Chemistry Summary

Table 5 Food Residue Chemistry Overview of Metabolism Studies and Risk Assessment

Table 6 Fate and Behaviour in the Environment

Table 7 Toxicity to Non-Target Species

Organism	Exposure	Test Substance(s)	Significant Effects, Comments	Reference(s)
Terrestrial Organisms				
Vertebrates				
Birds (Bobwhite Quail)	Acute Oral	*Bacillus subtilis* strain MBI 600, 30 birds	30-day $LD_{50} > 2.4 \times 10^9$ CFU/kg bw (or 4000 mg a.i./kg bw/day)	PMRA 1623596
		Water soluble metabolites from *B. subtilis* strain MBI 600, 10 birds	$LD_{50} > 240$ mg a.i./kg bw/day	
			$LD_{50} > 2.2 \times 10^9$ CFU/kg bw (or 3680 mg a.i./kg bw/day)	
		Washed spores of *B. subltilis* strain MBI 600, 10 birds	No signs of toxicity or pathogenicity. No mortalities. When compared to controls, there were no apparent effects on body weight or feed consumption. At necropsy, 1 ♀ administered the water-soluble metabolites and 1 ♂ administered *Bacillus subtilis* MBI 600 were noted with enlarged and/or pale spleens.	
		Deionized water, 10 birds	**LOW TOXICITY, NOT INFECTIVE**	
	Pulmonary/ Inhalation/ Injection	A waiver request was submitted based on the ubiquitous nature of *B. subtilis* in the environment whose level will not significantly increase from the seed treatment use, and for which the anticipated exposure to birds by the pulmonary route is expected to be minimal; on the published literature search that did not report any adverse effects from natural populations of *B. subtilis* on avian species; and on the toxicity profile of the MPCA from laboratory animal studies. **WAIVER ACCEPTED**		PMRA 1623597, 1623596

Organism	Exposure	Test Substance(s)	Significant Effects, Comments	Reference(s)
Wild Mammals		No study or waiver submitted. Given the ubiquitous nature of *B. subtilis* in the environment, it is not expected that its level will significantly increase from the seed treatment use. Also, few reports of adverse effects from natural populations of *B. subtilis* 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 Integral™ Liquid Biological Fungicide in seed treatment facilities.		PMRA 1623586, 1623587, 1623589, 1623590
Invertebrates				
Terrestrial Arthropods	Honeybee-Dietary Toxicity	IK-1080 WP Technical, containing *B. subtilis* strain MBI 600 at 1×10^8 CFU/mL, 100 bees	48-hour $LC_{50} > 1 \times 10^8$ CFU/mL No effect on bee mortality was observed over the 20 day monitoring period. **LOW TOXICITY**	PMRA 1623602
	Lady beetle (*Harmonia axyridis*)-Dietary toxicity	IK-1080 WP Technical, containing *B. subtilis* strain MBI 600 at 1×10^9 CFU/mL Larvae (n=30) and adult (n=30) exposed(24-, 48-hours, respectively) in the diet at 1.5×10^5 CFU/mg of food; observed for 10 days	No considerable effect on mortality or emergence (growth) of larvae or adult Lady beetles. Acute dietary $LC_{50} > 1.5 \times 10^5$ CFU/mg of food. Although, the observation period was considerably shorter than the recommended 21–30 days, study is acceptable. **ACCEPTABLE**	PMRA 1623603

Organism	Exposure	Test Substance(s)	Significant Effects, Comments	Reference(s)
Terrestrial Arthropods (cont'd)	Green lacewing (*Chrysoperla carnea)*- Contact toxicity and dietary toxicity	IK-1080 WP Technical, containing *B. subtilis* strain MBI 600 at 1×10^9 CFU/mL Contact exposure- eggs (n=30) dipped once in a solution at 1×10^9 CFU/mL and Dietary exposure- larvae (n=30) exposed (72-hours) in the diet at 1.5×10^5 CFU/mg of food; observed for 21 days	No considerable effect on mortality or emergence (growth) of eggs or adult Green lacewing. Acute contact LC_{50} (eggs) > 1×10^9 CFU/mL, and acute dietary $LC_{50} > 1.5 \times 10^5$ CFU/mg of food. **ACCEPTABLE**	PMRA 1623603
	Predatory mite (*Phytoseiulus persimilis)*- Contact toxcity	IK-1080 WP Technical, containing *B. subtilis* strain MBI 600 at 1×10^9 CFU/mL Adults (n=40) exposed (72 hours) to a diet consisting of kidney bean leaves treated at a rate of 4 mg/cm^2 (equivalent to 3.2×10^9 CFU/cm^2); observed for 72-hours	An increase in mortality observed for predatory mites in the treatment group (12.5% vs 2.5%). Although, the observation period was considerably shorter than the recommended 21–30 days, the study is acceptable. **ACCEPTABLE**	PMRA 1623603

Organism	Exposure	Test Substance(s)	Significant Effects, Comments	Reference(s)
Terrestrial Arthropods (cont'd)	Silkworm larvae-Dietary toxicity	IK-1080 WP Technical, containing *B. subtilis* strain MBI 600 at 8.3×10^{11} CFU/g Larvae (n=100) fed on mulberry leaves brushed with the test substance at 1.66×10^{10} CFU/mL; observed for approximately 20 days Untreated control group: distilled water plus tween 40 Positive control group: Thuricide WP, containing *Bacillus thruingiensis*)	No effect on mortality or quality of cocoons. Acute dietary $LC_{50} > 1.66 \times 10^{10}$ CFU/mL. **ACCEPTABLE**	PMRA 1623604
Non-arthropod Invertebrates	Acute-earthwoms	A waiver request was submitted, based on the ubiquitous nature of *B. subtilis* in the environment whose level will not significantly increase from the seed treatment use; in published literature, one study reported a strain of *B. subtilis* with the potential application as biological control agent of the parasitic root-knot nematode (*Meloidogyne incognita*) of tomato while another reported no toxicity or pathogenicity from *B. subtilis* to the nematode (Caenorhabditis elegans); 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 1623612, 1623613
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.		N/A

Organism	Exposure	Test Substance(s)	Significant Effects, Comments	Reference(s)
Plants				
Terrestrial 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 1623614
Aquatic Organisms				
Vertebrates				
Freshwater Fish	Acute (*Cyprinus carpio)*	*B. subtilis* strain MBI 600, 30 fish	$EC_{50} > 2.0 \times 10^8$ CFU/mL NOEC = 2.0×10^8 CFU/mL No treatment-related effects were noted. **NOT TOXIC, NOT PATHOGENIC**	PMRA 1623598
Estuarine/Marine fish	A waiver request was submitted based on the ubiquitous nature of *B. subtilis* in the environment whose level will not significantly increase from the seed treatment use, and for which the potential for leaching to aquatic environments is expected to be minimal; and on the lack of adverse effects to estuarine or marine fish species from natural populations of *B. subtilis* reported in published literature. In fact, in a published study, *Sparus aurata* L. (gilthead seabream) demonstrated no toxic or pathogenic effects following dietary exposure to *B. subtilis* as a potential probiotic. **WAIVER ACCEPTED**			PMRA 1623600, 1623599, 1623579, 1623605, 1623607, 1623608, 1623609, 1623612, 1623598
Invertebrates				
Aquatic Arthropods	A waiver request was submitted based on the ubiquitous nature of *B. subtilis* in the environment whose level will not significantly increase from the seed treatment use, and for which the potential for leaching to aquatic environments is expected to be minimal; and on the lack of adverse effects to aquatic arthropods from natural populations of *B. subtilis* reported in published literature. **WAIVER ACCEPTED**			PMRA 1623610, 1623605, 1623606, 1623607, 1623608, 1623609, 1623612, 1623598

Organism	Exposure	Test Substance(s)	Significant Effects, Comments	Reference(s)
Plants				
Aquatic Plants	A waiver request was submitted based on the ubiquitous nature of *B. subtilis* in the environment whose level will not significantly increase from the seed treatment use, and for which the potential for leaching to aquatic environments is expected to be minimal; and on the lack of adverse effects to aquatic plant species from natural populations of *B. subtilis* reported in published literature. **WAIVER ACCEPTED**			PMRA 1623615, 1623598, 1623605, 1623607, 1623608, 1623609, 1623614, 1623579

Table 8 Alternative Products Registered for Control/Suppression of *Rhizoctonia* and *Fusarium* on canola seedlings

Active Ingredient	End-Use Product(s)	Disease Claim	Fungicide Classification	
			Group	Mode of Action
Iprodione	Foundation Lite, Nisso Foundation Lite	Damping off and root rot	M	Multisite
Thiram	Foundation Lite, Nisso Foundation Lite	Damping off and root rot	M	Multisite
Azoxystrobin	Dynasty 100FS	Seed decay, damping-off and seedling blight	11	Respiration
Fludioxonil	Maxim 480FS	Seed decay, damping-off and seedling blights	12	Signal transduction
Trifloxystrobin	Trilex FS	Seed decay/ preemergence damping off and post-emergence damping off	11	Respiration
Metalaxyl	Apron FL, Allegiance FL,	Seed rots and seedling blights	4	Nucleic acid synthesis
Carbathiin	Vitavax RS, Gaucho CS FL, Prosper FL, Prosper T 200, Prosper FX	Seed rot, damping-off, seedling blight and early season root rot	7	Respiration
Thiram	Vitavax RS, Gaucho CS FL, Prosper FL, Prosper T 200, Prosper FX	Seed rot, damping-off, seedling blight and early season root rot	M	Multisite
Metalaxyl	Proposer FL, Prosper T 200, Prosper FX	Seed rot, damping-off, seedling blight and early season root rot	4	Nucleic acid synthesis
Difenoconazole	Helix, Helix XTra, Tribune	Seedling disease complex	3	Signal transduction
Metalaxyl-M and S-isomer	Helix, Helix XTra, Tribune	Seedling disease complex	4	Nucleic acid synthesis
Fludioxonil	Helix, Helix XTra, Tribune	Seedling disease complex	12	Signal transduction

Table 9 Use (label) Claims Proposed by Applicant and Whether Acceptable or Unsupported

Proposed label claim	Supported / Unsupported
Proposed as: Suppression of seedling disease complex (seed rot, pre and post emergent damping-off, seedling blight and root rot) in canola caused by *Rhizoctonia* and *Fusarium* spp. applied as a seed treatment at a rate of 160 mL of INTEGRAL per 100 kg of seed.	Supported as: Partial suppression of seedling disease complex (seed rot, pre and post emergent damping-off, seedling blight and root rot) in canola caused by *Rhizoctonia* and *Fusarium* spp. applied as a seed treatment at a rate of 160 mL of Integral ™ Liquid Biological Fungicide per 100 kg of seed.
Proposed as: Suppression of seedling disease complex (seed rot, pre and post emergent damping-off, seedling blight and root rot) in canola caused by *Rhizoctonia* and *Fusarium* spp. applied as a seed treatment at a rate of 160 mL of INTEGRAL per 100 kg of seed tank mixed with Helix, Helix XTra or Prosper FL at labeled rates.	Supported as: Control of seedling disease complex (seed rot, pre and post emergent damping-off, seedling blight and root rot) in canola caused by *Rhizoctonia* and *Fusarium* spp. applied as a seed treatment at a rate of 160 mL of Integral ™ Liquid Biological Fungicide per 100 kg of seed tank mixed with Helix Liquid Seed Treatment, Helix XTra Seed Treatment or Prosper FL Flowable Insecticide and Fungicide Seed Treatment at labeled rates.

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A. List of Studies/Information Submitted by Registrant

1.0 Chemistry

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