Proposed Registration Decision PRD2021-06

Bacillus velezensis strain RTI301, *Bacillus subtilis* strain RTI477, Ataplan Biological Fungicide, and Arolist Biological Fungicide

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Overview

Proposed registration decision for *Bacillus velezensis* strain RTI301 and *Bacillus subtilis* strain RTI477

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act*, is proposing registration for the sale and use of Bacillus velezensis strain RTI301 Technical, Bacillus subtilis strain RTI477 Technical, Ataplan Biological Fungicide and Arolist Biological Fungicide, containing the technical grade active ingredients *Bacillus velezensis* strain RTI301 and *Bacillus subtilis* strain RTI477 for the suppression of seed rot and seedling blight in corn (field, sweet, pop and corn grown for seed), soybean and sunflower, and for suppression of sudden death syndrome of soybean.

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

This Overview describes the key points of the evaluation, while the Science evaluation provides detailed technical information on the human health, environmental and value assessments of *Bacillus velezensis* strain RTI301, *Bacillus subtilis* strain RTI477 and Ataplan Biological Fungicide and Arolist 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 (for example, children) as well as organisms in the environment. These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the Health Canada regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides section of Canada.ca.

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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 velezensis* strain RTI301, *Bacillus subtilis* strain RTI477 and Ataplan Biological Fungicide and Arolist Biological Fungicide, Health Canada's PMRA will consider any comments received from the public in response to this consultation document.[3] Health Canada will then publish a Registration Decision[4] on *Bacillus velezensis* strain RTI301, *Bacillus subtilis* strain RTI477 and Ataplan Biological Fungicide and Arolist Biological Fungicide, which will include the decision, the reasons for it, a summary of comments received on the proposed registration decision and Health Canada's response to these comments.

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

What Are *Bacillus velezensis* strain RTI301 and *Bacillus subtilis* strain RTI477?

Both *Bacillus velezensis* strain RTI 301 and *Bacillus subtilis* strain RTI 477 are new active ingredients for disease management in Canada. Both *B. velezensis* and *B. subtilis* may result in induced systemic resistance (ISR) and systemic acquired resistance (SAR) responses through the reactions to the host plant's immune system. Combination of these two plant-associated bacteria makes them compete with the target plant pathogens in the rhizosphere, inhibit fungal growth and enhance plant growth when applied for seed treatment in corn, soybean and sunflower.

Health considerations

Can approved uses of *Bacillus velezensis* strain RTI301 and *Bacillus subtilis* strain RTI477 affect human health?

Bacillus velezensis strain RTI301 and _Bacillus subtilis_ strain RTI477 are unlikely to affect your health when Ataplan Biological Fungicide and Arolist Biological Fungicide are used according to the label directions.

Potential exposure to *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 may occur when handling and applying Ataplan Biological Fungicide and Arolist Biological Fungicide. When assessing health risks, several key factors are considered:

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

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

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

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

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

When Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical were tested on laboratory animals, there was low toxicity following oral, pulmonary instillation and dermal exposures. Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical are irritating to the eyes and skin. Furthermore, there was no sign that the microbial pest control agents, *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477, caused any disease.

Residues in water and food

Dietary risks from food and water are acceptable

Residues of *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 are not likely at the time of harvest. Ataplan Biological Fungicide and Arolist Biological Fungicide will not be applied to the edible portions of crops and the seed treatment applications are not expected to result any growth on the edible portions of the crops. In addition, the likelihood of residues of *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 contaminating drinking water supplies from the proposed seed treatments is low and therefore not a health concern. Consequently, dietary risks are acceptable for all segments of the population, including infants, children, adults and seniors.

Occupational Risks From Handling Ataplan Biological Fungicide and Arolist Biological Fungicide

Occupational risks are acceptable when Ataplan Biological Fungicide and Arolist Biological Fungicide are used according to label directions, which include protective measures

Workers handling Ataplan Biological Fungicide and Arolist Biological Fungicide can come into direct contact with *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 on the skin, by inhalation, or in the eyes. To protect workers from exposure to Ataplan Biological Fungicide and Arolist Biological Fungicide, the labels states that workers must wear personal protective equipment, including waterproof gloves, long-sleeved shirt, long pants, a NIOSH-approved particulate filtering facepiece respirator, socks and shoes.

Risks in residential and other non-occupational environments

Estimated risk for non-occupational exposure is acceptable.

Ataplan Biological Fungicide and Arolist Biological Fungicide are proposed for use as commercial seed treatments on-farm and in commercial facilities. Residential and non-occupational exposure to Ataplan Biological Fungicide and Arolist Biological Fungicide is expected to be low when label directions are observed. Consequently, the risk to residents and the general public is acceptable.

Environmental considerations

What happens when *Bacillus velezensis* strain RTI301 and *Bacillus subtilis* strain RTI477 are introduced into the environment?

Environmental risks are acceptable.

Bacillus velezensis and *B. subtilis* are common microorganisms that are widely distributed in the natural environment. Habitats are predominantly soils, including soils in water columns and bottom deposits of aquatic environments. Under adverse conditions, these microorganisms produce resilient endospores that allow them to readily survive in soils, dusts and aerosols. If protected from sunlight, endospores may survive for very long periods.

Ataplan Biological Fungicide and Arolist Biological Fungicide are intended for use as seed treatments on corn (all types), soybean, and sunflower. No adverse effects to birds or fish were observed during testing. Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical were toxic to honey bees, springtails, and predatory mites at high concentrations, however, exposure to honey bees and other terrestrial invertebrates is expected to be low and not of concern since the end-use products are only used as seed treatments. The end-use products are not intended for aquatic uses and their use as a seed treatment is not expected to significantly increase the levels of these microorganisms in soil. Exposure to aquatic environments is also expected to be low and limited to leaching and run-off after the seeds are sown in fields. While published scientific literature on the environmental fate of these species suggests that *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 will survive in soils and sediment under various environmental conditions, the populations of *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 in soil and sediment are expected to return to naturally sustainable levels.

Based on a critical review of studies, scientific rationales and information from public sources, the risks to birds, wild mammals, fish, terrestrial and aquatic invertebrates, and plants are acceptable when Ataplan Biological Fungicide and Arolist Biological Fungicide are applied according to directions on the labels.

Value considerations

What is the value of Ataplan Biological Fungicide and Arolist Biological Fungicide?

***Bacillus velezensis* strain RTI 301 and *Bacillus subtilis* strain RTI 477 are the active ingredients in Ataplan Biological Fungicide, and *B. velezensis* strain RTI 301 alone is the active ingredient in Arolist Biological Fungicide. The registrations of Ataplan Biological Fungicide and Arolist Biological Fungicide will provide Canadian users with two new products to manage these important diseases in corn, soybean and sunflower.**

Ataplan Biological Fungicide and Arolist Biological Fungicide are applied as seed treatments to protect corn, soybean and sunflower against seed rot and seedling blight caused by certain soil-borne fungal pathogens, and to protect soybean against sudden death syndrome (SDS).

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 labels of Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical and the end-use products, Ataplan Biological Fungicide and Arolist Biological Fungicide to address the potential risks identified in this assessment are as follows.

Key risk-reduction measures

Human health

All microorganisms, including *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477, contain substances that are potential sensitizers and thus, sensitivity may possibly develop in individuals exposed to potentially large quantities of *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477. In turn, workers handling or applying Ataplan Biological Fungicide and Arolist Biological Fungicide must wear waterproof gloves, a long-sleeved shirt, long pants, a NIOSH-approved particulate filtering facepiece respirator, socks and shoes.

Environment

The end-use product labels will include environmental precaution statements to reduce contamination of aquatic systems from the use of Ataplan Biological Fungicide and Arolist Biological Fungicide.

Next steps

Before making a final registration decision on *Bacillus velezensis* strain RTI301, *Bacillus subtilis* strain RTI477, Ataplan Biological Fungicide and Arolist Biological Fungicide, Health Canada's PMRA will consider any comments received from the public in response to this consultation document. Health Canada will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). Health Canada will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed decision and Health Canada's response to these comments.

Other information

When the Health Canada makes its registration decision, it will publish a Registration Decision on *Bacillus velezensis* strain RTI301, *Bacillus subtilis* strain RTI477, Ataplan Biological Fungicide and Arolist 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

Bacillus velezensis strain RTI301, *Bacillus subtilis* strain RTI477, Ataplan Biological Fungicide, and Arolist Biological Fungicide

1.0 The active ingredients, their properties and uses

1.1 Identity of the active ingredients

Active microorganism	***Bacillus velezensis* strain RTI301**	***Bacillus subtilis* strain RTI477**
Function	Fungicide –For the partial suppression of certain fungal diseases on seeds of corn, soybean, and sunflower	
Binomial	*Bacillus velezensis* strain RTI301	*Bacillus subtilis* strain RTI477
Taxonomic designation[5]		
Kingdom	Bacteria	
Phylum	Firmicutes	
Class	Bacilli	
Order	Bacillales	
Family	Bacillaceae	
Genus	*Bacillus*	
Species Group	*Bacillus subtilis* group	*Bacillus subtilis* group
Species Subgroup	*Bacillus amyloliquefaciens* subgroup	-
Species	*Velezensis*	*subtilis*
Strain	RTI301	RTI477
Patent Status information	None	
Minimum purity of active	Technical grade active ingredient: minimum of 2 × 10^{11} colony forming units (CFU)/g	Technical grade active ingredient: minimum of 2 × 10^{11} CFU/g
	Ataplan Biological Fungicide End-use product: minimum of 1.5 × 10^{10} CFU/mL *B. velezensis* strain RTI301, and minimum of 1.15 × 10^{9} CFU/mL *B. subtilis* strain RTI477.	
	Arolist Biological Fungicide End-use product: minimum of 1.1 × 10^{10} CFU/mL *B. velezensis* strain RTI301	-
Identity of relevant impurities of	The technical grade active ingredients do not contain any impurities or microcontaminants known to be Toxic Substances Management	

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

toxicological, environmental and/or significance.	Policy (TSMP) Track 1 substances. The products must meet microbiological contaminant release standards. In addition, there are no known mammalian toxins or other toxic metabolites present in the technical grade active ingredients or end-use products. Some species of the *Bacillus subtilis* species complex can produce the heat-stable peptide toxin amylosin. Both *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 were tested and determined to be negative for the production of amylosin.

1.2 Physical and chemical properties of the active ingredients and end-use products

Technical Product—Bacillus velezensis strain RTI301 Technical

Property	Result
Colour	Brownish beige
Physical State	Solid powder
Odour	Sweet, yeast-like
pH	8.01
Relative Density	0.34–0.44 g/mL

Technical Product—Bacillus subtilis strain RTI477 Technical

Property	Result
Colour	Brownish beige
Physical State	Solid powder
Odour	Slightly sweet, musty
pH	8.09
Relative Density	0.60–0.63 g/mL

End-Use Product—Ataplan Biological Fungicide

Property	Result
Colour	Brownish beige
Physical State	Liquid suspension
Odour	Slightly sweet, musty
Viscosity (mPa at 20°C)	604.0 (30 rpm), 446.3 (50 rpm), 397.1 (60 rpm)
pH (1%)	6.81
Relative Density	1.16 g/mL

End-Use Product—Arolist Biological Fungicide

Property	Result
Colour	Brownish beige
Physical State	Liquid suspension
Odour	Sweet, musty
Viscosity (mPa at 20°C)	609.6 (30 rpm), 451.3 (50 rpm), 408.6 (60 rpm)
pH (1%)	7.98
Relative Density	1.14 g/mL

1.3 Directions for use

Ataplan Biological Fungicide is applied as a seed treatment for partial suppression of certain seed rot and seedling blight on corn at 3 to 6.4 mL/unit (80 000 seeds), on soybean at 5 to 11.2 mL/unit (140 000 seeds), or on sunflower at 4 to 8 mL/unit (100 000 seeds). Ataplan Biological Fungicide may also partially suppress sudden death syndrome (SDS) of soybean when applied as a seed treatment at 11.2 mL/unit. Ataplan Biological Fungicide may be mixed with other registered fungicide seed treatment products.

Arolist Biological Fungicide is applied as a seed treatment for partial suppression of certain seed rot and seedling blight on corn at 4 to 8.7 mL/unit (80 000 seeds), on soybean at 7 to 15 mL/unit (140 000 seeds), or on sunflower at 5 to 11 mL/unit (100 000 seeds). Arolist Biological Fungicide may also partially suppress sudden death syndrome (SDS) of soybean when applied as a seed treatment at 15 mL/unit. Arolist Biological Fungicide may be mixed with other registered fungicide seed treatment products.

1.4 Mode of action

The bacteria *Bacillus subtilis* and *Bacillus amyloliquefaciens* (a heterotypic synonym of *Bacillus velezensis*) are classified as Group BM 02 fungicides by the Fungicide Resistance Action Committee (FRAC). The bacteria in this group have multiple effects, including competition, mycoparasitism, antibiosis, and membrane disruption resulted in induced systemic resistance (ISR) and systemic acquired resistance (SAR) responses through the reactions to the host plant's immune system. Combination of these two plant-associated bacteria makes them compete with the target plant pathogens in the rhizosphere, inhibit fungal growth and enhance plant growth when applied for seed treatment in corn, soybean and sunflower.

2.0 Methods of analysis

2.1 Methods for Identification of the Microorganisms

Acceptable methodologies for detection, isolation and enumeration of the active ingredients, *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477, were submitted by the applicant. The microbial pest control agents (MPCAs) have been fully characterized with respect to the origin of strain, natural occurrence and biological properties. *Bacillus velezensis* strain RTI301 and

B. subtilis strain RTI477 can be identified to the species level using a combination of phenotypic and biochemical methodologies, as well as phylogenetic analysis. The identity of the MPCAs to the strain level may also be confirmed by multi-locus sequence analysis using groEL, gyrA, polC, purH and rpoB genes for *B. velezensis* strain RTI301, and 16S rDNA and rpoB genes for *B. subtilis* strain RTI477.

2.2 Methods for establishment of purity of seed stock

Bacillus velezensis strain RTI301 has been deposited into the German Collection of Microorganisms and Cell Cultures (Leibniz Institute DSMZ) under the strain identification number CHCC19594 – DSM 32473. It has also been deposited into the American Type Culture Collection (ATCC), under the Patent Deposit Designation PTA-121165. *Bacillus subtilis* strain RTI477 has been deposited into the ATCC under the Patent Deposit Designation PTA-121167. The strains are maintained by the manufacturer in a manner sufficient to maintain purity and stability.

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

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

The guarantees of the technical grade active ingredients are expressed in units of CFU/g. The guarantees of the end-use products are expressed in units of CFU/mL. Representative data on five batches of each technical grade active ingredient and end-use product were submitted. The method for determining CFU counts was adequately described.

2.4 Methods to determine and quantify residues (viable or non-viable) of the active microorganism and relevant metabolites

As noted above, acceptable methods are available to enumerate the microorganisms and to distinguish these MPCAs from other *Bacillus* species.

2.5 Methods for determination of relevant impurities in the manufactured material

The quality assurance procedures used to limit contaminating microorganisms during the manufacture of Bacillus velezensis strain RTI301 Technical, Bacillus subtilis strain RTI477 Technical, Ataplan Biological Fungicide, and Arolist Biological Fungicide are acceptable. These procedures include sterilization of all equipment and media as well as frequent sampling of the stock culture and production batches for purity and contamination.

The absence of human pathogens and below-threshold levels of contaminating microorganisms were shown in the microbial screening of batches of Bacillus velezensis strain RTI301 Technical, Bacillus subtilis strain RTI477 Technical, Ataplan Biological Fungicide, and Arolist Biological Fungicide using standard microbiological methods as well as quantitative polymerase chain reaction for detecting and enumerating microbial contaminants of concern. All batches

of Bacillus velezensis strain RTI301 Technical, Bacillus subtilis strain RTI477 Technical, Ataplan Biological Fungicide, and Arolist Biological Fungicide conform to the limits set out in the Organisation for Economic Co-operation and Development (OECD) issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43].

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

Storage stability data were provided for Bacillus velezensis strain RTI301 Technical, Bacillus subtilis strain RTI477 Technical, Ataplan Biological Fungicide, and Arolist Biological Fungicide. Results support a storage period of 24 months for the technical grade active ingredients and end-use products when stored at 25°C.

3.0 Impact on human and animal health

3.1 Toxicity and infectivity summary

3.1.1 Testing

A detailed review of the toxicological studies was conducted in support of the two technical grade active ingredients, Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical, and the associated end-use products, Ataplan Biological Fungicide and Arolist Biological Fungicide.

Bacillus velezensis strain RTI301 Technical

To address the health hazard requirements for Bacillus velezensis strain RTI301 Technical, the applicant submitted acute pulmonary infectivity/toxicity, acute oral infectivity/toxicity, acute dermal toxicity, and dermal and eye irritation studies. These studies were performed with *B. velezensis* strain RTI301 spores which was considered equivalent to Bacillus velezensis strain RTI301 Technical.

In the acute oral infectivity and toxicity study, young Sprague Dawley CD IGS rats (19/sex) were given a single oral dose of 2.1×10^8 CFU of *B. velezensis* strain RTI301 in phosphate buffered saline. Animals were observed for a period of up to 21 days with interim scheduled sacrifices on Days 3, 7, 14, and 21. There were no mortalities, no treatment related clinical signs, necropsy findings or changes in body weight. The test organism was below the detection limit for blood, brain, lungs, liver, spleen, kidneys, mesenteric lymph nodes at interim sacrifices and study termination, and a pattern of clearance was established for the treated animals' feces by Day 14.

In the acute pulmonary infectivity and toxicity study, young Sprague Dawley CD IGS rats (26/sex) were given a single dose of 5.27×10^8 viable spores of *B. velezensis* strain RTI301 by intratracheal instillation. Another group of rats (3/sex) was exposed to a similar suspension of inactivated spores. Animals were then observed for up to 86 days.

There were no mortalities, no treatment related clinical signs, or necropsy findings. Although still detectable by Day 86, a pattern of clearance was established for the MPCA in lung tissue, and the spores had cleared from all other tissues by Day 57.

In the acute dermal toxicity study, young Sprague Dawley, albino rats (5/sex) were dermally exposed to 5000 mg/kg body weight (bw) of *B. velezensis* strain RTI301 spores (3.76×10^{11} CFU/g) for 24 hours to an area of approximately 10% of body surface area. Following exposure, the animals were observed for a period of 14 days. There were no mortalities, no treatment related clinical signs, necropsy findings or changes in body weight.

In the primary eye irritation study, 44 mg of *B. velezensis* strain RTI301 spores (3.76×10^{11} spores/g) was instilled neat into the conjunctival sac of the right eye of three young female New Zealand White albino rabbits for 72 hours. The treated eye was not rinsed. Animals then were observed for 3 days and irritation was scored. One hour after test substance instillation, minimal conjunctivitis was noted for all three treated eyes. There was no corneal opacity or iritis observed in any treated eye during this study. The maximum irritation score (MIS) was 4.7/110 (at 1 h). Based on the Maximum Average Score (MAS; at 24, 48, 72 h) of 1.55/110, Bacillus velezensis strain RTI301 Technical is slightly irritating to eyes.

In the primary dermal irritation study, three young New Zealand White albino, female rabbits were dermally exposed to 500 mg of *B. velezensis* strain RTI301 spores (3.76×10^{11} spores/g) in distilled water to a 6-cm^2 site. The test area was covered with a gauze patch and semi-permeable dressing during the exposure period. Following exposure, the animals were observed for a period of 10 days and irritation was scored. Within 30–60 minutes of patch removal, all three treated sites exhibited very slight erythema and one treated site exhibited very slight edema. By 24 hours, very slight erythema and very slight edema were reported for two rabbits. All animals were free of erythema and edema by Day 10 (study termination). The MIS was 1.33/8 (at 72 h). In this study, Bacillus velezensis strain RTI301 Technical is slightly irritating to skin based on the MAS of 1.33/8 (at 24, 48, 72 h).

Bacillus subtilis strain RTI477 Technical

To address the health hazard requirements for Bacillus subtilis strain RTI477 Technical, the applicant submitted acute pulmonary infectivity/toxicity, acute oral toxicity, acute dermal toxicity and dermal irritation studies. These studies were performed with *B. subtilis* strain RTI477 spores which was considered equivalent to Bacillus subtilis strain RTI477 Technical.

In the acute oral up-and-down toxicity study, three female Sprague Dawley, albino rats were given a single oral dose of a suspension of *B. subtilis* strain RTI477 spores (5.40×10^{11} spores/g) in distilled water at a dose of 5000 mg/kg bw. The animals were then observed for a period of up to 14 days. There was no mortality and no treatment related clinical signs, necropsy findings or changes in body weight.

In the acute pulmonary infectivity and toxicity study, young adult Sprague Dawley CD IGS rats (26/sex) were given a single dose of 3.08×10^8 *B. subtilis* strain RTI477 viable spores by intratracheal instillation. Another group of rats (3/sex) was exposed to a similar suspension of inactivated spores. Animals were then observed for up to 86 days. There were no mortalities, no treatment related clinical signs, or necropsy findings. At study termination, the MPCA was still detectable in lung tissue at levels close to the detection limit for this assay. However, a pattern of clearance was established for this tissue and the spores had cleared from all other treated tissues by Day 57.

In the acute dermal toxicity study, a group of young (9-week-old) Sprague Dawley, albino rats (5/sex) were dermally exposed to 5000 mg/kg bw of *B. subtilis* strain RTI477 spores (5.40×10^{11} spores/g) for 24 hours to an area of approximately 10% of body surface area. Following exposure, the animals were observed for a period of 14 days. There was no mortality and no treatment related clinical signs, necropsy findings or changes in body weight.

In the primary dermal irritation study, three young female New Zealand White albino rabbits were dermally exposed to 500 mg of *B. subtilis* strain RTI477 spores (5.40×10^{11} spores/g) in distilled water to a 6-cm^2 site. The test area was covered with a gauze patch and semi-permeable dressing during the exposure period. After 4 hours the dressings were removed and the test site was washed with 3% soap solution. Following exposure, the animals were observed for a period of 3 days. Irritation was scored by the method of Draize. Within 30–60 minutes of patch removal, all three treated sites exhibited very slight erythema and two treated sites exhibited very slight edema. All animals were free of dermal irritation by 48 hours. The MIS was 1.6/8 (at 1 h). In this study, Bacillus subtilis strain RTI477 Technical is slightly irritating to skin based on the MAS of 0.33/8 (at 24, 48, 72 h).

Ataplan Biological Fungicide

To address the health hazard requirements for the end-use product, Ataplan Biological Fungicide, the applicant submitted an eye irritation study in which the test substance was F4034-5, which was considered equivalent to Ataplan Biological Fungicide.

In the primary eye irritation study, 44 mg of F4034-5 containing *B. subtilis* strain RTI477 (1.9×10^{10} CFU/g) and *B. velezensis* strain RTI301 (2.74×10^{10} CFU/g) was instilled neat into the conjunctival sac of right eyes of three young female, New Zealand White albino rabbits for 72 hours. The treated eye was not rinsed, and the left eye remained untreated and served as a control. Animals then were observed for three days. Irritation was scored by the method of Draize. One hour after test substance instillation, all three treated eyes exhibited positive conjunctivitis. There was no corneal opacity or iritis observed in any treated eye during this study. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation by 48 hours. The MIS was 6.67/110 (at 1 h). Based on the MAS of 0.89/110 (at 24, 48, and 72 h), F4034-5 is slightly irritating to eyes.

Arolist Biological Fungicide

To address the health hazard requirements for the end-use product, Arolist Biological Fungicide, the applicant submitted dermal irritation and eye irritation studies. In these studies the test substance was F4007-9, which is considered equivalent to Arolist Biological Fungicide.

In the primary eye irritation study, 0.1 mL of F4007-9 containing *B. velezensis* strain RTI301 (2.80×10^{10} CFU/g) was instilled neat into the conjunctival sac of right eyes of three young adult female, New Zealand White albino rabbits for 72 hours. Prior to dosing, a systemic analgesic (Buprenophine) was administered by injection, and both eyes of each animal were anesthetized with Teracaine Ophthalmic Solution. Animals then were observed for three days. Irritation was scored by the method of Draize. One hour after test substance instillation, all three treated eyes exhibited minimal conjunctivitis. There was no corneal opacity or iritis observed in any treated eye during this study. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation by 48 hours.The MIS was 4/110 (at 24 h). Based on the MAS of 0.67/110 (at 24, 48, 72 h), F4007-9 is slightly irritating to eyes.

In the primary dermal irritation study, three young female New Zealand White albino rabbits were dermally exposed to 0.5 mL of F4007-9 containing *B. velezensis* strain RTI301 (2.80×10^{10} CFU/g) to a 6-cm^2 site. The test area was covered with a gauze patch and semi-permeable dressing during the exposure period. After 4 hours the dressings were removed and the test site was washed with 3% soap solution. Following exposure, the animals were observed for a period of 7 days. Irritation was scored by the method of Draize, within 30–60 minutes of patch removal, all three treated sites exhibited erythema and edema. The overall incidence and severity of irritation decreased with time. Desquamation was noted at one dose site at 72 hours. All animals were free of dermal irritation by Day 7. The MIS was 3/8 (at 24 h). Based on the MAS of 0.78/8 (at 24, 48, 72 h), F4007-9 is slightly irritating to skin.

Test results are summarized in Appendix I, Tables 1, 2, 3 and 4.

3.1.2 Additional information

Scientific rationales were provided to waive the technical grade active ingredient requirements for acute oral toxicity and injection testing on Bacillus velezensis strain RTI301 Technical. Scientific rationales were also provided to waive the technical grade active ingredient requirements for acute oral toxicity/pathogenicity and injection studies on Bacillus subtilis strain RTI477 Technical. A scientific rationale was not provided to waive the requirement for irritation testing for Ataplan Biological Fungicide, however it is considered to be irritating to the skin. Additional scientific rationales were also provided to waive studies that are not on the PMRA's list of requirements for MPCAs, including acute inhalation testing for the technical grade active ingredients and end-use products, as well as an acute oral toxicity study for the end-use products.

Scientific rationales were accepted based on the low toxicity/pathogenicity seen in testing with these MPCAs and a search of the peer-reviewed scientific literature which returned no cases of *B. subtilis* or *B. velezensis* having been identified as an infective or pathogenic agent. A survey of published literature uncovered no reports of adverse effects for *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477.

The applicant indicated that, since *B. velezensis* was previously classified as *B. amyloliquefaciens* (within the broader *B. subtilis* species complex), there is a paucity of published data on *B. velezensis* compared to other members of the group, and the accuracy with which these species have been identified and distinguished in the public data is uncertain. Therefore, public domain data relating to the wider *B. subtilis* group have also been relied on in determining the relationships of these MPCAs to known pathogens.

As a strict aerobe, *B. subtilis* is unable to grow or metabolize extensively in the bloodstream. A search of the peer-reviewed scientific open literature found a few cases of *B. subtilis* having been associated with bacteraemia, endocarditis, pneumonia, and septicaemia in drug users and severely debilitated patients, but both immunosuppression and inoculation with high numbers of *B. subtilis* were prerequisites for infection.

In one study, clinical cases investigated over a 6-year period revealed *Bacillus* species in twelve patients, two of which were receiving intensive chemotherapy for acute leukemia; in the remaining ten patients, the infections were restricted to surgical wounds from which other pathogenic bacteria were also isolated in some cases, and which did not appear to affect wound healing. In the absence of any invasive growth the authors concluded that the ubiquitous distribution of *B. subtilis* makes it inevitable that it will sometimes be found in association with other bacteria in infected humans.

Seventy-five *Bacillus* or *Paenibacillus* strains were isolated from clinical samples over a two-year period, of which 11 were identified as *B. subtilis* by MALDI-TOF MS. However, species of the genus *Bacillus* are common laboratory contaminants, and consequently the isolation of these organisms from blood or tissue cultures does not always indicate infection. Moreover, with the exception of *B. anthracis* and *B. cereus*, most species of the genus are not considered human pathogens, especially in immunocompetent individuals. To the contrary, there is evidence to suggest that *B. subtilis* may be a natural constituent of the human gastrointestinal tract.

A case of simultaneous coinfection with *B. licheniformis* and *B. subtilis* was reported in a patient with esophageal perforation and underlying tuberculosis and chronic obstructive pulmonary disease. This case was specifically cited by the European Food Safety Authority (EFSA) in their 2013 review of the ongoing applicability of the Qualified Presumption of Safety (QPS) approach to *B. subtilis* in which they stated that “These cases of infections in humans are linked to specific predisposing factors and do not suggest a risk for the consumer via exposure through the food and feed chain.” Accordingly, *B. subtilis* is still considered suitable for the QPS approach when used as a direct feed additive.

The safety of *B. subtilis* as a food supplement was investigated by oral exposure in rabbits and Guinea pigs, concluding safety on the basis of the lack of observed toxicity or virulence. In another report, it was concluded that *B. subtilis* may be considered as non-pathogenic and safe for human consumption.

In consideration of the toxigenic potential of *Bacillus* species, EFSA concluded that species outside of the *B. cereus* group are very rare causes of food poisoning, and that any toxigenic potential is far more likely to arise from the production of surfactins than from *B. cereus*-like enterotoxins. More recent information suggesting that the production of such surfactin-like cyclic peptides is universally present in *B. subtilis* and related species led EFSA to conclude that their existing position that any indication of a capacity to produce such compounds represented a hazard that should be avoided was disproportionate to the risk posed, and consequently their guidance was revised. Furthermore, they concluded that food poisoning from strains outside the *B. cereus* group required food to be contaminated at very high levels, and that the majority of cases are likely to have resulted from a misidentification of the strain involved.

Due to the absence of toxigenic potential *B. subtilis* is included on the EFSA list of microorganisms considered suitable for the QPS approach when used as direct feed additives. Evidence also suggests that *B. subtilis* is adapted to life in the human gastrointestinal tract, having been isolated at levels in excess of those attributable to the consumption of contaminated food, and can therefore be considered as a commensal organism in the human gut.

Some strains of *B. amyloliquefaciens* or *B. subtilis* can produce the heat-stable peptide toxin amylosin, which has immunotoxic effects in mammals. Nuclear magnetic resonance (NMR) spectroscopy analysis of samples of *B. velezensis* RTI301 and *B. subtilis* strain RTI477 detected no amylosin-specific signals, indicating that it is unlikely to be produced by these strains.

3.1.3 Incident reports related to human and animal health

Bacillus velezensis strain RT1301 and *B. subtilis* strain RT1477 are two new active ingredients pending registration for use in Canada, and as of 2 February 2021, no incident reports had been submitted to the PMRA.

There was one human incident involving a related strain, *B. subtilis* strain QST 713. In this incident, a person reported minor symptoms of rash and cough after applying a product containing *B. subtilis* strain QST 713.

The labels of the two proposed products, Ataplan Biological Fungicide and Arolist Biological Fungicide, contains appropriate hazard signal words, precaution statements and personal protective equipment aimed at reducing pesticide exposure when mixing, loading or applying the product. Hence, no additional mitigation measures are recommended based on the incident report review.

3.1.4 Hazard analysis

The data package submitted in support of registering Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical, and the associated end-use products, Ataplan Biological Fungicide and Arolist Biological Fungicide, was reviewed from the viewpoint of human health and safety and was determined to be acceptable.

Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical are of low toxicity by the dermal, pulmonary and oral routes and not pathogenic or infective by the pulmonary route. Bacillus velezensis strain RTI301 Technical was also not pathogenic or infective by the oral route. Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical are slightly irritating to the skin and Bacillus velezensis strain RTI301 Technical is slightly irritating to the eyes. These MPCAs are considered to be potential sensitizers. Consequently, the hazard statements "POTENTIAL SENSITIZER" will appear on the principal display panel of the technical grade active ingredients. The statement, "May cause sensitization. Avoid contact with skin, eyes, and clothing. Avoid inhaling/breathing mist." is also required on the secondary panel of the labels under the "PRECAUTIONS" section.

The end-use products, Ataplan Biological Fungicide and Arolist Biological Fungicide are slightly irritating to the eyes. Also, Arolist Biological Fungicide is slightly irritating to the skin and, while no dermal irritation study was provided for Ataplan Biological Fungicide, this end-use product was also considered to be slightly irritating to the skin. The hazard statements "POTENTIAL SENSITIZER" will also appear on the principal display panel of the end-use product labels. The statement, "May cause sensitization. Avoid contact with skin, eyes, and clothing. Avoid inhaling/breathing spray mist." is also required on the secondary panel of the labels under the "PRECAUTIONS" section.

Higher tier subchronic and chronic toxicity studies were not required because the Tier I studies: a) did not indicate the technical grade active ingredients to be acutely toxic by the oral, pulmonary or dermal routes of administrations; and b) there were no indications of any infectivity or pathogenicity in any test animals tested with these MPCAs.

Within the available scientific literature, there are no reports that suggest *B. subtilis* and *B. velezensis* have the potential to cause adverse effects on the endocrine system of animals. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for this MPCA.

3.2 Occupational, residential and bystander risk assessment

3.2.1 Occupational and postapplication exposure and risk

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with primary exposure routes being dermal. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe was a pathogen equipped with

mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Bacillus subtilis* and *B. velezensis* have not frequently been identified as a dermal wound pathogens and there is no indication that they could penetrate intact skin of healthy individuals. Furthermore, technical grade active ingredient testing with *B. subtilis* strain RTI477 and *B. velezensis* strain RTI301 showed low toxicity and no infectivity via the pulmonary route. Technical grade active ingredient testing with *B. velezensis* strain RTI301 showed low toxicity and no infectivity via the oral route. Hazard testing with the end-use products also showed that Arolist is slightly irritating to the skin and eyes, and Ataplan is slightly irritating to eyes. In the absence of a study, Ataplan Biological Fungicide was considered slightly irritating to the skin.

Although Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical were of low toxicity via the oral, pulmonary and dermal routes, and the formulants in the end-use products are not expected to contribute additional toxicity, the PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Consequently, risk mitigation measures, such as personal protective equipment (PPE), including waterproof gloves, a long-sleeved shirt, long pants, a NIOSH-approved particulate filtering facepiece respirator, socks and shoes are required to minimize exposure and protect applicators, mixer/loaders, and handlers that are likely to be exposed.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of Ataplan Biological Fungicide and Arolist Biological Fungicide. Overall, occupational risks to workers are acceptable when the precautionary statements on the labels are followed which include PPE.

3.2.2 Residential and bystander exposure and risk

The use of Ataplan Biological Fungicide and Arolist Biological Fungicide as seed treatments on-farm and in commercial facilities as described on the labels is not anticipated to result in any significant residential and bystander exposure. Also, Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical were of low toxicity and there were no signs that these MPCAs, *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477, caused any disease in studies on laboratory animals. Consequently, the health risks to bystanders and individuals in residential areas are acceptable.

3.3 Dietary exposure and risk assessment

3.3.1 Food

The proposed use pattern (seed treatment) is not expected to result in dietary exposure from food, since the product will not be applied to the edible portions of crops and the seed treatment applications of *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 are not expected to yield any growth on the edible portions of the crops.

Furthermore, *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 demonstrated no pathogenicity or infectivity in Tier I studies. In addition, no metabolites of toxicological significance have been shown to be produced by this strain. Consequently, there is no health risk for the general population, including infants and children, or domestic animals.

3.3.2 Drinking water

Dietary exposure from drinking water is expected to be low as the labels have the necessary mitigation measures to limit contamination of drinking water from the proposed uses of Ataplan Biological Fungicide and Arolist Biological Fungicide. The end-use products are used to treat seeds on-farm and in commercial facilities, and the labels will instruct users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Municipal treatment of drinking water is also expected to further reduce the transfer of residues to drinking water. Furthermore, *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 demonstrated no pathogenicity or infectivity in Tier I studies. Health risks from residues of *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 in drinking water are acceptable due to the low toxicity/pathogenicity profiles of Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical, and their limited exposure following application of the end-use products.

3.3.3 Acute and chronic dietary risks for sensitive subpopulations

Calculations of acute reference doses and acceptable daily intakes are not usually possible for predicting acute and long-term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (in other words, no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 are of low oral toxicity, are not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to these MPCAs than the general population. Thus there are no threshold effects of concern and, as a result, there is no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of these MPCAs, including neurological effects from pre- or postnatal exposures, and cumulative effects on infants and children of the MPCAs and other registered microorganisms that have a common mechanism of toxicity, does not apply to these MPCAs. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 to human health.

3.3.4 Aggregate exposure and risk

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

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

Ataplan Biological Fungicide and Arolist Biological Fungicide are considered to be of low toxicity by the oral, pulmonary and dermal routes and end-use products will not be applied near or to drinking water. When the end-use products are used as labelled, there is reasonable certainty that no harm will result from aggregate exposure of residues of *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477.

3.3.5 Maximum residue limits

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

Dietary risk to humans from the proposed use of *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 as seed treatments is acceptable since residues are not anticipated on food crops grown from treated seeds. Therefore, the PMRA has determined that specification of an MRL under the PCPA is not required for *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477.

3.4 Cumulative assessment

The *Pest Control Products Act* requires that the PMRA consider the cumulative exposure to pesticides with a common mechanism of toxicity. In its assessment of common mechanism of toxicity, the PMRA considers both the taxonomy of MPCAs and the production of any potentially toxic metabolites. For the current evaluation, the PMRA has determined that *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 share a common mechanism of toxicity with other strains of *B. amyloliquefaciens*, *B. subtilis* and *B. licheniformis* that are used as MPCAs; *B. amyloliquefaciens* strain F727, *B. amyloliquefaciens* strain MBI 600, *B. amyloliquefaciens* strain D747, *B. amyloliquefaciens* strain PTA-4838, *B. subtilis* strain QST 713, *B. subtilis* strain GB03, *B. subtilis* strain FMCH 001, *B. subtilis* var. *amyloliquefaciens* strain FZB24 and *B. licheniformis* FMCH 002.

The potential health risks from cumulative exposure of *B. velezensis* strain RTI301, *B. subtilis* strain RTI477 and these other MPCAs are acceptable when used as labelled given their low toxicity and pathogenicity.

4.0 Impact on the environment

4.1 Fate and behaviour in the environment

No studies were submitted to address the environmental fate and behaviour of *B. velezensis* strain RTI301 or *B. subtilis* strain RTI477; however, environmental fate data are only triggered at Tier II/III if significant toxicological effects in non-target organisms are noted in Tier I testing.

According to published information, *Bacillus* species are saprophytes that are widely distributed in the natural environment. The habitats of most species are soils of all kinds (for example, temperate, acidic, neutral, alkaline), including soils in water columns and bottom deposits of fresh and marine waters. Their endospores are very durable and they readily survive in soils, dusts and aerosols. If protected from solar radiation, endospores may survive for very long periods. The presence of spores in a particular environment, however, does not necessarily indicate that the organism is metabolically active in this environment. Most species of *Bacillus* are heterotrophic organisms that have been isolated on complex organic media. Some species will degrade biopolymers such as leather and feathers, with versatilities varying according to species. It is, therefore, postulated that these species have important roles in the biological cycling of carbon and nitrogen.

The seed treatment applications of Ataplan Biological Fungicide and Arolist Biological Fungicide are expected to result in slight increases of *Bacillus* species in the rhizosphere of treated plants. These localized increases in soil are not expected to significantly increase the overall environmental levels of these species above naturally occurring levels. Also, the localized elevated populations of *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 in the rhizosphere of plants are expected to return to naturally sustainable levels over time.

The end-use products are for on-farm and commercial use in seed treating facilities, and are not intended to be applied directly to water. As result, exposure to aquatic environments should be low and limited to run-off after the seeds are sowed in fields. While *B. velezensis* and *B. subtilis* are not considered aquatic species and are not expected to grow in this environment, the endospores of this microorganism are likely to persist in sediment. The seed treatment applications of Ataplan Biological Fungicide and Arolist Biological Fungicide are not expected to significantly increase the overall environmental levels of these species in sediment above naturally occurring levels. As noted previously, any localized increases of *B. velezensis* strain RTI301 or *B. subtilis* strain RTI477 in aquatic environments are expected to return to naturally sustainable levels over time.

4.2 Effects on non-target species

The PMRA has a four-tiered approach to environmental testing of microbial pesticides. Tier I studies consist of acute studies on up to seven broad taxonomic groups of non-target organisms exposed to a maximum hazard or Maximum Challenge Concentration of the MPCA. The Maximum Challenge Concentration is generally derived from the amount of the MPCA, or its toxin, expected to be available following application at the maximum recommended label rate multiplied by a safety factor. Tier II studies consist of environmental fate (persistence and dispersal) studies as well as additional acute toxicity testing of MPCAs. Tier III studies consist of chronic toxicity studies (life cycle studies), as well as definitive toxicity testing (for example, LC_{50}, LD_{50}). Tier IV studies consist of experimental field studies on toxicity and fate, and are required to determine whether adverse effects are realized under actual use conditions.

The type of environmental risk assessment conducted on MPCAs varies depending on the tier level that was triggered during testing. For many MPCAs, Tier I studies are sufficient to conduct environmental risk assessments. Tier I studies are designed to represent "worst-case" scenarios where the exposure conditions greatly exceed the expected environmental concentrations. The absence of adverse effects in Tier I studies are interpreted as minimal risk to the group of non-target organisms. However, higher tiered studies will be triggered if significant adverse effects on non-target organisms are identified in Tier I studies. These studies provide additional information that allows the PMRA to refine the environmental risk assessments. In the absence of adequate environmental fate and/or field studies, a screening level risk assessment can be performed to determine if the MPCA is likely to pose a risk to a group of non-target organisms.

The screening level risk assessment uses simple methods, conservative exposure scenarios (for example, direct application at a maximum application rate) and sensitive toxicity endpoints. A risk quotient (RQ) is calculated by dividing the exposure estimate by an appropriate toxicity value (RQ = exposure/toxicity), and the risk quotient is then compared to the level of concern (level of concern = 1 for most species, 0.4 for acute risk to pollinators, and 2 for glass plate studies using the standard beneficial arthropod test species, *Typhlodromus pyri* and *Aphidius rhopalosiphi*; level of concern = 1 is used for higher tier tests of the standard arthropod test species and for other arthropod test species).

If the screening level risk quotient is below the level of concern, the risk is considered negligible and no further risk characterization is necessary. If the screening level risk quotient is equal to or greater than the level of concern, then a refined risk assessment is performed to further characterize the risk. A refined assessment takes into consideration more realistic exposure scenarios (environmental fate and/or field testing results). Refinements may include further characterization of risk based on exposure modelling, monitoring data, results from field or mesocosm studies, and probabilistic risk assessment methods. Refinements to the risk assessment may continue until the risk is adequately characterized or no further refinements are possible.

4.2.1 Effects on terrestrial organisms

A detailed review of the terrestrial non-target studies and other supporting information was conducted in support of the two technical grade active ingredients, Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical, and the associated end-use products, Ataplan Biological Fungicide and Arolist Biological Fungicide.

Bacillus velezensis strain RTI301 Technical

Four studies were submitted to address the hazards of *B. velezensis* strain RTI301 to birds, honey bees, springtails, and predatory mites. These studies were performed with Bacillus velezensis strain RTI301 Technical. Acceptable scientific rationales were also provided in support of requests to waive further testing on birds, mammals, terrestrial arthropods, and plants. Data submitted under human and animal health toxicity testing were considered to assess the risk of harm to wild mammals.

The acute oral toxicity/pathogenicity of Bacillus velezensis strain RTI301 Technical (4.18×10^{11} CFU/g) to 16-day-old bobwhite quail (*Colinus virginianus*) was assessed over 30 days. Bacillus velezensis strain RTI301 Technical was administered to birds (3 replicates of 10), as a suspension in deionized water, by oral gavage at 1.2×10^{11} CFU/kg bw daily for five consecutive days. There were no signs of toxicity or pathogenicity in any treated animal for the duration of the study. One test group animal was found dead on Day 27. The deceased animal appeared normal and gained weight throughout the study; no abnormalities were observed upon necropsy.

In a 19-day dietary toxicity study, honey bees (*Apis mellifera*) were exposed to Bacillus velezensis strain RTI301 Technical in the diet (50% w/w sucrose solution) for four days at nominal concentrations of 1.0×10^7, 1.0×10^8, and 1.0×10^9 CFU/mL. Percent mortality in the untreated control, inactivated test substance, and the 1.0×10^7, and 1.0×10^8 CFU/mL groups on Day 19 was 25%, 97.5%, 18% and 53%, respectively. In the 1.0×10^9 CFU/mL group, there was 100% mortality by Day 8. While consumption of dosing suspensions were confirmed, it is noted that the inactivated test substance, 1.0×10^7, 1.0×10^8, and 1.0×10^9 CFU/mL test groups consumed significantly less than the untreated control group on various days.

In a 28-day contact toxicity study, 80 (female; 8 replicates of 10 insects) springtails (*Folsomia candida*) were exposed to Bacillus velezensis strain RTI301 Technical in artificial soil substrate at a measured concentration of 1.90×10^7 CFU/g of soil (test group). In another test group, 80 (female; 8 replicates of 10 insects) springtails were exposed to Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical at a combined measured concentration of 5.07×10^7 CFU/g of soil (combined test group). Mean juvenile production per replicate in the test, combined test, and untreated control groups was 78.63, 37.50, and 166.71 respectively. Mean adult survival per replicate in the test, combined test, and untreated control groups was 5.13, 2.75, and 8.71 respectively.

In a 14-day contact toxicity study, 80 (female; 8 replicates of 10 mites) predatory mites (*Hypoaspis aculeifer*) were exposed to Bacillus velezensis strain RTI301 Technical in artificial soil substrate at a measured concentration of 1.87×10^7 CFU/g of soil. Mean juvenile production per replicate in the test, attenuated test substance, and untreated control groups was 3.25, 11.50, and 53.125 respectively. Mean adult survival per replicate in the test, attenuated test substance, and untreated control groups was 4.125, 6.250, and 9.125 respectively.

Test results are summarized in Appendix I, Table 5.

The applicant also supplied scientific rationales to address avian, mammalian, arthropod and plant testing requirements for Bacillus velezensis strain RTI301 Technical. *Bacillus velezensis* is a natural soil organism that is ubiquitous in various terrestrial environments. The peer-reviewed scientific open literature contains no cases of *B. velezensis* having been associated with any toxicity, pathogenicity or infectivity in birds, mammals, arthropods or plants. In testing, Bacillus velezensis strain RTI301 Technical was shown to be neither toxic nor pathogenic to birds by oral administration and the proposed use pattern makes inhalation an unlikely route of systemic exposure. Bacillus velezensis strain RTI301 Technical was also shown to be non-toxic and non-pathogenic to rats by oral, and pulmonary exposure, and non-toxic to rats by dermal exposure. *Bacillus velezensis* is a natural and ubiquitous soil bacterium that is widely recognized as a pathogen of plant diseases. No activity against invertebrates has been reported for the species, and the proposed use pattern is restricted to seed treatment which results in minimal exposure to foliar invertebrates. Also, *B. velezensis* is not known to be pathogenic to plants, and is not listed as a plant pathogen by the United States Department of Agriculture (*Federal Plant Pest Act* Regulations, 7CFR Part 330) or by the European and Mediterranean Plant Protection Organization (EPPO Global Database). *Bacillus velezensis* is a natural and ubiquitous component of the rhizosphere. Furthermore, a search of the scientific peer reviewed open literature by the applicant revealed no records of the species being associated with any detrimental effects in any plant species.

A search in PubMed by the PMRA using the keywords "*Bacillus velezensis* pathogenicity" yielded no reports of pathogenicity to terrestrial non-target organisms. The majority of the scientific literature consisted of reports on a) the ability of *B. velezensis* to promote growth and/or to induce systemic resistance in host crops; b) the biological control of various plant pathogenic fungi; and c) the use of *B. subtilis* as a probiotic in animal feed (for example, chickens, fish).

Bacillus subtilis strain RTI477 Technical

Four studies were submitted to address the hazards of *B. subtilis* strain RTI477 to birds, honey bees, and predatory mites. These studies were performed with Bacillus subtilis strain RTI477 Technical. Acceptable scientific rationales were also submitted in support of requests to waive further testing on birds, mammals, terrestrial arthropods, and plants. Data submitted under human and animal health toxicity testing were considered to assess the risk of harm to wild mammals.

The acute oral toxicity/pathogenicity of Bacillus subtilis strain RTI477 Technical (5.02×10^{11} CFU/g) to 16-day-old bobwhite quail (*C. virginianus*) was assessed over 30 days. Bacillus subtilis strain RTI477 Technical was administered to birds (3 replicates of 10), as a suspension in deionized water, by oral gavage at 3.2×10^{11} CFU/kg bw daily for five consecutive days. Following treatment, animals, on average, had a weight gain of 107.5 g, 103.8 g, 103.3 g and 100.2 g in the untreated, sterile filtrate, heat inactivated, and test group, respectively. Overall, the test group gained statistically significantly less weight than the control group. There were no other treatment related effects or pathogenicity in any treated animal for the duration of the study.

The following three honey bee trials were part of the same range-finding study. Even though the study was scientifically valid, none of the three range-finding trials fully address the PMRA's guideline requirements since toxicity and pathogenicity could not be distinguished because inactivated test substance controls were not used.

In a 20-day dietary toxicity trial, four groups of 20 honey bees (*A. mellifera*) were continuously exposed to Bacillus subtilis strain RTI477 Technical via the diet (50% w/w sucrose solution) at concentrations of 5.0×10^6, 5.0×10^7, 5.0×10^8, and 5.0×10^9 CFU/mL. Percent mortality in the control, 5.0×10^6, 5.0×10^7, 5.0×10^8, and 5.0×10^9 CFU/mL groups on Day 20 was 35%, 55%, 40%, 65% and 100%, respectively. Increased mortality in the 5.0×10^8 and 5.0×10^9 CFU/mL groups could be due to toxicity and/or pathogenicity.

In a 6-day dietary toxicity trial, two groups consisting of three replicates of 20 honey bees each were continuously exposed to Bacillus subtilis strain RTI477 Technical via the diet (50% w/w sucrose solution) at concentrations of 1×10^8 and 1×10^9 CFU/mL. Feed consumption was measured. Percent mortality was 37.5%, 36.7% and 63.3% for the control, 1×10^8 CFU/mL and 1×10^9 CFU/mL groups, respectively. Increased mortality in the 1×10^9 CFU/mL group could be due to toxicity and/or pathogenicity.

In a 6-day dietary toxicity trial, one group consisting of three replicates of 20 honey bees was exposed to Bacillus subtilis strain RTI477 Technical via the diet (50% w/w sucrose solution) for a 4-hour period at a concentration of 1×10^9 CFU/mL. Feed consumption was measured. Percent mortality was 21.7% and 37.5% for the control and 1.0×10^9 CFU/mL groups respectively. Increased mortality in the 1×10^9 CFU/mL group could be due to toxicity and/or pathogenicity.

In a 9-day dietary toxicity study, honey bees (*A. mellifera*) were exposed to Bacillus subtilis strain RTI477 Technical via the diet (50% w/w sucrose solution) for four days at nominal concentrations of 1.0×10^6, 1.0×10^7, and 1.0×10^8 CFU/mL. Percent mortality in the control, inactive, sterile filtrate, 1.0×10,^{6} 1.0×10^7 and 1.0×10^8 CFU/mL groups was 23%, 34%, 31%, 45%, 22% and 30%, respectively. There were no significant differences in feed consumption among treatment groups. There were no toxic or pathogenic effects observed. Even though the study was scientifically valid, this study did not entirely meet PMRA requirements for a Tier I or Tier III study since a dose sufficient to cause 50% mortality was not used despite the results of a range-finding study.

That study determined a dose in the order of 10^9 CFU/mL would have likely been sufficient to achieve this goal. Therefore, this study neither qualifies as a definitive or maximum hazard toxicity test.

In a 14-day contact toxicity study, 80 (female; 8 replicates of 10 mites) predatory mites (*Hypoaspis aculeifer*) were exposed to Bacillus subtilis strain RTI477 Technical in artificial soil substrate at a measured concentration of 3.2×10^7 CFU/g of soil. Mean juvenile production per replicate in the test, attenuated test substance, and untreated control groups was 25.63, 31.00, and 52.71 respectively. Mean adult survival per replicate in the test, attenuated test substance, and untreated control groups was 10.0, 8.75, and 10.0 respectively.

Test results are summarized in Appendix I, Table 6.

The applicant also supplied scientific rationales to address the avian, mammalian, arthropod, and plant testing requirements for Bacillus subtilis strain RTI477 Technical. *Bacillus subtilis* is a natural soil organism that is ubiquitous in various terrestrial environments. It has a long history of safe use in avian health preparations in the United States and Europe and as a direct feed additive in live stock production. In testing, Bacillus subtilis strain RTI477 Technical was neither toxic nor pathogenic to birds by oral administration and the proposed use pattern makes inhalation an unlikely route of systemic exposure. Bacillus subtilis strain RTI477 Technical was also shown to be non-toxic and non-pathogenic to rats by pulmonary exposure, and non-toxic to rats by oral and dermal exposure. *Bacillus subtilis* is a natural and ubiquitous soil bacterium that is widely recognized as a pathogen of plant diseases. Little to no activity against invertebrates has been reported for the species, either in the scientific literature or in the regulatory assessments of plant protection products conducted in the United States or Europe. The proposed use patterns are restricted to seed treatment application, which results in minimal exposure to foliar invertebrates. Also, *B. subtilis* is a natural constituent of the rhizosphere where it contributes to nitrogen fixation. It is not known to be pathogenic to plants, and is not listed as a plant pathogen by the United States Department of Agriculture or by the European and Mediterranean Plant Protection Organization. Furthermore, a search of the scientific peer reviewed open literature by the applicant revealed no records of the species being associated with any detrimental effects in any plant species.

A search in PubMed by the PMRA using the keywords "*Bacillus subtilis* pathogenicity" yielded very few reports of pathogenicity. The reports of pathogenicity consisted mostly of reports of infections in humans with potentially compromised immune systems. The majority of the scientific literature consisted of reports on: a) the ability of *B. subtilis* to promote growth and/or to induce systemic resistance in host crops; b) the biological control of various plant pathogenic fungi; and c) the use of *B. subtilis* as a probiotic in animal feed (for example, chickens).

No information was provided to address potential hazards to non-target non-arthropod invertebrates. *Bacillus velezensis*, *B. subtilis* and closely related *B. amyloliquefaciens*, occur naturally in soils and in association with plants, particularly in the plant rhizosphere. While *B. velezensis*, *B. subtilis* and *B. amyloliquefaciens* are not generally considered to be pathogens, there are recent publications implicating *B. amyloliquefaciens* in nematicidal effects. According

to these publications, these nematicidal effects are attributed to secondary metabolites and/or enzymes. While the use of Ataplan Biological Fungicide and Arolist Biological Fungicide as seed treatments may potentially affect non-arthropod invertebrates, these adverse effects would be limited since this microorganism is only expected to result in minimal increases of *Bacillus* species in the rhizosphere of treated plants (see Section 4.1). These minimal localized increases in soil are not expected to significantly increase the overall environmental levels of this species above naturally occurring levels. Also, the localized elevated populations of *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 in the rhizosphere of plants are expected to return to naturally sustainable levels over time.

Based on all the available information on the biological properties of *B. velezensis* and *B. subtilis*, the absence of documented effects in non-target terrestrial organisms, and the anticipated minimal environmental exposure resulting from the use of *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 as seed treatments, the risks to birds, wild mammals, terrestrial non-target arthropod invertebrates, non-arthropod invertebrates, and terrestrial plants are acceptable. While dietary toxicity to honey bees, and contact toxicity to springtails and predatory mites was observed in laboratory testing at high concentrations from both Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical, due to the low exposure expected from the proposed seed treatment use, risks to honey bees and terrestrial arthropods are acceptable. Furthermore, the formulants contained in Arolist Biological Fungicide and Ataplan Biological Fungicide are not expected to contribute to potential toxicity of the products.

4.2.2 Effects on aquatic organisms

A detailed review of the aquatic non-target studies and other supporting information was conducted in support of the two technical grade active ingredients, Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical, and the associated end-use products, Ataplan Biological Fungicide and Arolist Biological Fungicide.

Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical

No studies were submitted to address the hazards of *B. velezensis* strain RTI301 or *B. subtilis* strain RTI477 to aquatic non-target organisms. Scientific rationales were submitted in support of requests to waive further testing on freshwater fish, estuarine and marine fish, and aquatic arthropods.

The applicant supplied scientific data waiver rationales to address the toxicity and pathogenicity of Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical to freshwater, estuarine, and marine fish; and aquatic arthropod and non-arthropod invertebrates. A search of the peer-reviewed scientific literature by the applicant returned no cases of *B. velezensis* or *B. subtilis* having been identified as an infective or pathogenic agent in freshwater, estuarine, and marine fish; or freshwater aquatic invertebrates. The primary habitat of *Bacillus* species is soil, from where they may naturally and continuously enter water bodies via run-off, airborne dust deposition and infected plant material. However, they are not regarded as

autochthonous inhabitants of freshwater environments, which, being poor in organic carbon, do not provide optimal conditions for growth. While bacterial cells, particularly endospores, may be able to survive in water, they will be subject to natural competition, and proliferation is unlikely to occur. Furthermore, the exclusive seed treatment use proposed for *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 significantly reduces the potential for exposure to watercourses, and any increase in *B. subtilis* in water is likely to be minimal.

Searches in PubMed by the PMRA using the keywords "*Bacillus velezensis* pathogenicity" and "*Bacillus subtilis* pathogenicity" yielded no reports of pathogenicity to aquatic non-target organisms. The reports of pathogenicity consisted mostly of reports of infections in humans with potentially compromised immune systems. The majority of the scientific literature consisted of reports on: a) the ability of *B. velezensis* and *B. subtilis* to promote growth and/or to induce systemic resistance in host crops; b) the biological control of various plant pathogenic fungi; and c) the use of *B. velezensis* and *B. subtilis* as a probiotic in animal feed (for example, chickens, fish).

Ataplan Biological Fungicide

One study was submitted to address the hazards of Ataplan Biological Fungicide to freshwater fish.

In a 30-day toxicity/pathogenicity study, 10 rainbow trout (*Oncorhynchus mykiss*) were aquatically exposed to Arolist Biological Fungicide at nominal concentrations of 4.0×10^6 CFU/mL *B. subtilis* strain RTI477 and 4.0×10^6 CFU/mL *B. velezensis* strain RTI301 under semi-static renewal test conditions. The diet contained Bacillus subtilis strain RTI477 Technical and Bacillus velezensis strain RTI301 Technical each at a nominal concentration of 0.9×10^6 CFU/mL. One, non-treatment related mortality occurred in the attenuated test substance group on Day 23. There were no significant treatment related toxicity or pathogenicity effects observed. Even though the study was scientifically valid, this study did not entirely meet PMRA requirements and was of limited use in the risk assessment due to an inadequate number of test fish.

Test results are summarized in Appendix I, Table 7.

Based on all the available information on the biological properties of *B. velezensis* and *B. subtilis*, the absence of documented effects in non-target aquatic organisms, and the anticipated minimal environmental exposure resulting from the use of *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 as seed treatments, the risks to aquatic organisms are acceptable. Furthermore, the formulants contained in Ataplan Biological Fungicide and Arolist Biological Fungicide are not expected to contribute to potential toxicity of the end-use product.

4.3 Incident reports related to the environment

As of 2 February 2021, there was one environment incident involving *B. subtilis* (strain QST 713). Mortality was reported in spinach plants. The incident was assigned a certainty index of unlikely. Since the causality of the incident does not meet the criteria of highly probable, probable and possible, the incident was not considered in this review. No additional risk mitigation measures are recommended for *B. velezensis* strain RTI301 or *B. subtilis* strain RTI477.

5.0 Value

Ataplan Biological Fungicide and Arolist Biological Fungicide contain species of *Bacillus* that can grow around the treated seeds and roots of the crops in soil, and provide protection against the target soil-borne pathogens. Various fungicide products are registered in Canada against listed soil-borne pathogens on corn, soybean and sunflower on the Ataplan Biological Fungicide or Arolist Biological Fungicide labels. Registration of these new biological seed treatments will give Canadian growers additional tools to combat these important diseases.

Scientific rationales and efficacy data from field and greenhouse trials demonstrated that Ataplan Biological Fungicide and Arolist Biological Fungicide are expected to partially suppress *Rhizoctonia* infection on corn, sunflower and soybean, partially suppress *Fusarium* infection on corn, or partially suppress sudden death syndrome (SDS) infection on soybean when applied as seed treatments. Both Ataplan Biological Fungicide and Arolist Biological Fungicide are effective in improving seedling establishment and yield in corn, soybean and sunflower. As no phytotoxicity or other adverse effects were observed on corn, soybean or sunflower in the trial studies, application of Ataplan Biological Fungicide or Arolist Biological Fungicide is not expected to result in injury to corn, soybean or sunflower when used according to label directions.

Since Ataplan Biological Fungicide and Arolist Biological Fungicide target certain specific seed or seedling diseases on corn, soybean or sunflower, tank-mixing with other registered fungicide seed treatments would broaden the disease control spectrum of the seed treatments. These tank-mixing combinations were tested and there was no adverse effect observed in field trials.

Details of the supported uses are provided in Appendix I, Table 8.

6.0 Pest control product policy considerations

6.1 Toxic Substances Management Policy considerations

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

Canadian Environmental Protection Act. The *Pest Control Products Act* requires that the TSMP is given effect in evaluating the risks of a product.

During the review process, Bacillus velezensis strain RTI301 Technical, Bacillus subtilis strain RTI477 Technical, Ataplan Biological Fungicide, and Arolist Biological Fungicide were assessed in accordance with the PMRA Regulatory Directive DIR99-03[6] and evaluated against the Track 1 criteria. The PMRA has reached the conclusion that Bacillus velezensis strain RTI301 Technical, Bacillus subtilis strain RTI477 Technical, Ataplan Biological Fungicide, and Arolist Biological Fungicide do not meet the Track 1 criteria because the active ingredients are biological organisms and hence are not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.

6.2 Formulants and contaminants of health or environmental concern

During the review process, contaminants in the technical as well as formulants and contaminants in the end-use products are compared against Parts 1 and 3 of the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.*[7] The list is used as described in the PMRA Notice of Intent NOI2005-01[8] and is based on existing policies and regulations, including the Toxic Substances Management Policy[1] and Formulants Policy[9] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

- Technical grade products Bacillus velezensis strain RTI301 Technical and Bacillus subtilis strain RTI477 Technical, and their end-use products, Ataplan Biological Fungicide and Arolist Biological Fungicide, do not contain any formulants or contaminants identified in the *List of Pest Control Product Formulants of Health or Environmental Concern*.

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

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

7 SI/2005-114, last amended on 25 June 2008. See Justice Laws website, Consolidated Regulations, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.*

8 PMRA's Notice of Intent NOI2005-01, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern under the New Pest Control Product Act*.

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

7.0 Proposed regulatory decision

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act*, is proposing registration for the sale and use of Bacillus velezensis strain RTI301 Technical, Bacillus subtilis strain RTI477 Technical, Ataplan Biological Fungicide and Arolist Biological Fungicide, containing the technical grade active ingredients *Bacillus velezensis* strain RTI301 and *Bacillus subtilis* strain RTI477 for the suppression of seed rot and seedling blight in corn (field, sweet, pop and corn grown for seed), soybean and sunflower, and for suppression of sudden death syndrome of soybean.

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

List of abbreviations

°C	degree(s) Celsius
ATCC	American Type Culture Collection
bw	body weight
cm	centimetre
CFU	colony forming units
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures)
EFSA	European Food Safety Authority
FRAC	Fungicide Resistance Action Committee
g	gram
h	hour(s)
ISR	induce systemic resistance
kg	kilogram
LD_{50}	median lethal dose
LC_{50}	median lethal concentration
LOC	level of concern
mL	millilitre
MAS	Maximum Average Score
mg	milligram
mPa	milliPascal
MIS	Maximum Irritation Score
MPCA	microbial pest control agent
MRL	maximum residue limit
NMR	nuclear magnetic resonance
NOEC	no observed effect concentration
OECD	Organisation for Economic Co-operation and Development
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
QPS	qualified presumption of safety
RQ	risk quotient
rpm	revolutions per minute
rDNA	ribosomal deoxyribonucleic acid
SAR	systemic acquired resistance
SDS	sudden death syndrome
TSMP	Toxic Substances Management Policy

Appendix I Tables and figures

Table 1 Toxicity profile of Bacillus velezensis strain RTI301 Technical

(Effects are known or assumed to occur in both sexes unless otherwise noted; in such cases, sex-specific effects are separated by semi-colons)

Study Type/Animal/PMRA#	Study Results
21-day Acute Oral Infectivity and Toxicity[1] Sprague Dawley rat PMRA# 2927129	Oral $LD_{50} > 2.1 \times 10^8$ CFU/rat (Limit Test) **Low Toxicity** and not infective via oral gavage.
86-day Acute Pulmonary Instillation Infectivity[1] Sprague Dawley rat PMRA# 3040049	Acute pulmonary LD_{50} was greater than 5.27×10^8 CFU/rat (Limit Test) **Low Toxicity** and not infective via pulmonary instillation.
14-day acute dermal toxicity[1] Sprague Dawley rat PMRA# 3040067	Acute dermal LD_{50} was greater than 5000 mg/kg bw.
72-hour dermal irritation[1] New Zealand white, female PMRA# 2937136	Slightly irritating to the skin (MIS, 72h. = 1.33/8; MAS=1.33/8).
72-hour eye irritation[1] New Zealand white, female PMRA# 2927139	Slightly irritating to the eyes (MIS, 48h. = 4.7/110; MAS=1.55/110).

[1] The test substance was *B. velezensis* strain RTI301 spores, which is considered equivalent to Bacillus velezensis strain RTI301 Technical.

Table 2 Toxicity profile of Bacillus subtilis strain RTI477 Technical

(Effects are known or assumed to occur in both sexes unless otherwise noted; in such cases, sex-specific effects are separated by semi-colons)

Study Type/Animal/PMRA#	Study Results
14-day acute oral toxicity[1] Sprague Dawley rat, female PMRA# 2927130	Up and Down method female Acute oral LD_{50} was greater than 5000 mg/kg bw.
86-day Acute Pulmonary Instillation Infectivity[1] Sprague Dawley rat PMRA# 2927131	Acute pulmonary LD_{50} was greater than 3.08×10^8 CFU/rat (Limit Test) **Low Toxicity** and not infective via pulmonary instillation.
14-day acute dermal toxicity[1] Sprague Dawley rat PMRA# 2927135	Acute dermal LD_{50} was greater than 5000 mg/kg bw.
72-hour dermal irritation[1] New Zealand white PMRA# 2927138	Minimally irritating to the skin. (MIS, 1h = 1.67/8; MAS = 0.33/8).
72-hour eye irritation[1] New Zealand white, female PMRA# 2927141	Slightly irritating to the eyes (MIS, 24h = 6/110; MAS = 0/110).

[1] The test substance was *B. subtilis* strain RTI477 spores, which is considered equivalent to Bacillus subtilis strain RTI477 Technical.

Table 3 Toxicity profile of Ataplan Biological Fungicide

Study Type/Animal/PMRA#	Study Results
72-hour eye irritation[1] New Zealand white, female PMRA# 2927140	Slightly irritating to the eyes (MIS, 1h = 6.67/110; MAS = 0.89/110).

[1] The test substance was F4034-5 containing *B. subtilis* strain RT1477 at 1.9×10^{10} CFU/g and *B. velezensis* strain RTI301 at 2.74×10^{10} CFU/g, which was considered equivalent to Ataplan Biological Fungicide.

Table 4 Arolist Biological Fungicide

Study Type/Animal/PMRA#	Study Results
72-hour dermal irritation[1] New Zealand white, female PMRA# 2927174	Slightly irritating to the skin (MIS, 24h = 3/8; MAS=0.78/8).
72-hour eye irritation[1] New Zealand white, female PMRA# 2927176	Slightly irritating to the eyes (MIS, 24h = 4 /110; MAS = 0.67/110).

[1] The test substance was F4007-9 containing 2.80×10^{10} CFU of *B. velezensis* strain RTI301/g, which was considered equivalent to Arolist Biological Fungicide.

Table 5 Toxicity of Bacillus velezensis strain RTI301 Technical to non-target species

Organism	Exposure	Significant Effect, Comments	Reference
Terrestrial Organisms			
Vertebrates			
Birds			
Bobwhite Quail (*Colinus virginianus*), 16-day-old	5-day – Dietary exposure	One test group animal was found dead on Day 27; the deceased animal appeared normal and gained weight throughout the study; no abnormalities were observed upon necropsy.	PMRA# 2953938

Organism	Exposure	Significant Effect, Comments	Reference
		There were no treatment related toxicity effects or signs of pathogenicity observed. The 30-day acute oral LD_{50} of Bacillus velezensis strain RTI301 Technical to the quail was greater than 1.2×10^{11} CFU/kg bw. **LOW TOXICITY** **NOT PATHOGENIC**	
Invertebrates			
Arthropods			
Honey Bee (*Apis mellifera*), young adult	Dietary – 1.0×10^7, 1.0×10^8, and 1.0×10^9 CFU/mL in 50% sucrose/water	Study was terminated on Day 19 when mortality in control group exceeded 20%. There were significant increases in mortality in the inactivated test substance, 1.0×10^8, and 1.0×10^9 CFU/mL test groups. The inactivated test substance, 1.0×10^7, 1.0×10^8, and 1.0×10^9 CFU/mL test groups consumed significantly less diet. The 19-day LC_{50} of Bacillus velezensis strain RTI301 Technical to the honey bee was 5.8×10^7 CFU/mL. **TOXIC** **NOT PATHOGENIC**	PMRA# 2927151
Springtail (*Folsomia candida*), female	Soil contact – 1.90×10^7 CFU/g *B. velezensis* strain RTI301; and 5.07×10^7 CFU/g *B. velezensis* strain RTI301 and *B. subtilis* strain RTI477 (combined)	Study ended on Day 28 There were significant reductions in juvenile production and adult survival in the active and combined active test groups. **TOXIC**	PMRA# 3040064

Organism	Exposure	Significant Effect, Comments	Reference
Predatory mite (*Hypoaspis aculeifer*), female	Soil contact – 1.87×10^7 CFU/g	Study ended on Day 14 There were significant reductions in juvenile production and adult survival in the test and attenuated test groups. **TOXIC**	PMRA# 3040065

Table 6 Toxicity of Bacillus subtilis strain RTI477 Technical to Non-Target Species

Organism	Exposure	Significant Effect, Comments	Reference
Terrestrial Organisms			
Vertebrates			
Birds			
Bobwhite Quail (*Colinus virginianus*), 16-day-old	5-day – Dietary exposure	The test group gained a statistically significantly less weight than the control group. There were no treatment related toxicity effects or signs of pathogenicity observed. The 30-day acute oral LD_{50} of Bacillus subtilis strain RTI477 Technical to the quail was greater than 3.2×10^{11} CFU/kg bw. **LOW TOXICITY** **NOT PATHOGENIC**	PMRA# 2927145
Invertebrates			
Arthropods			
Honey Bee (*Apis mellifera*), young adult	Dietary – Trial 1: 5.0×10^6, 5.0×10^7, 5.0×10^8, and 5.0×10^9 CFU/mL Trial 2: 1×10^8 and 1×10^9 CFU/mL Trial 3: 1×10^9 CFU/mL	Trial 1: Study was terminated on Day 20 when mortality in control group exceeded 20%. There were significant increases in mortality in the 5.0×10^8 and 5.0×10^9 CFU/mL groups. Trial 2: Study was terminated on Day 6 when mortality in control group exceeded 20%. There was a significant increase in mortality in the 1.0×10^9 CFU/mL group. Trial 3: Study was terminated on Day 6 when mortality in control group exceeded 20%. There was a significant increase in	PMRA# 2927148

Organism	Exposure	Significant Effect, Comments	Reference
	All trials were dietary in 50% sucrose/water	mortality in the 1.0×10^9 CFU/mL group. **TOXIC AND/OR PATHOGENIC**	
Honey Bee (*Apis mellifera*), young adult	Dietary – 1.0×10^7, 1.0×10^8, and 1.0×10^9 CFU/mL in 50% sucrose/water	Study was terminated on Day 9 when mortality in control group exceeded 20%. There were no significant differences in mortality or in food consumption among treatment groups. The 9-day LC_{50} of Bacillus subtilis strain RTI477 Technical to the honey bee was greater than 1.0×10^8 CFU/mL. **TOXIC** **NOT PATHOGENIC**	PMRA# 2927150
Predatory mite (*Hypoaspis aculeifer*), female	Soil contact – 3.2×10^7 CFU/g	Study ended on Day 14 There was significant reduction in juvenile production in the test and attenuated test groups. There was significant reduction in adult survival in the attenuated test group. **TOXIC**	PMRA# 3040759

Table 7 Toxicity of Ataplan Biological Fungicide to non-target species

Organism	Exposure	Significant Effect, Comments	Reference
Aquatic Organisms			
Vertebrates			
Fish			
Rainbow Trout (*Oncorhynchus mykiss*), juvenile	Aquatic exposure: 4.0×10^6 CFU/mL *B. subtilis* strain RTI477 and 4.0×10^6 CFU/mL *B. velezensis* strain RTI301	One, non-treatment related mortality occurred in the attenuated test substance group on Day 23. There were no significant treatment related toxicity or pathogenicity effects observed. This study is of limited utility due to an inadequate number of test subjects.	PMRA# 3040259

Organism	Exposure	Significant Effect, Comments	Reference
	(nominal) Dietary exposure: 0.9 × 10^6 CFU/mL of *B. subtilis* strain RTI477 and 0.9 × 10^6 CFU/mL of *B. velezensis* strain RTI301 (nominal)	**SUPPLEMENTAL**	

Table 8 List of supported uses

Supported use claims for Ataplan Biological Fungicide
1. Partial suppression of seed rot and seedling blight caused by *Rhizoctonia solani* and *Fusarium graminearum* on corn (field, sweet, pop and corn grown for seed) at 3–6.4 mL/unit (80 000 seeds). Apply as a seed treatment prior to planting.
2. Partial suppression of seed rot and seedling blight caused by *Rhizoctonia solani* on soybean at 5–11.2 mL/unit (140 000 seeds), and suppression of Sudden Death Syndrome (SDS) of soybean (*Fusarium virguliforme*) at 11.2 mL/unit (140,000 seeds). Apply as a seed treatment prior to planting.
3. Partial suppression of seed rot and seedling blight caused by *Rhizoctonia solani* on sunflower at 4–8 mL/unit (100 000 seeds). Apply as a seed treatment prior to planting.
4. Ataplan Biological Fungicide may be applied in mixture with other registered seed treatment products, including HELIX, Maxim 480FS, Allegiance FL, Apron XL LS and Apron MAXX RFC.
Supported use claims for Arolist Biological Fungicide
1. Partial suppression of seed rot and seedling blight caused by *Rhizoctonia solani* and *Fusarium graminearum* on corn (field, sweet, pop and corn grown for seed) at 4–8.7 mL/unit (80 000 seeds). Apply as a seed treatment prior to planting.
2. Partial suppression of seed rot and seedling blight caused by *Rhizoctonia solani* on soybean at 7–15 mL/unit (140 000 seeds), and suppression of Sudden Death Syndrome (SDS) of soybean (*Fusarium virguliforme*) at 15 mL/unit (140 000 seeds). Apply as a seed treatment prior to planting.
3. Partial suppression of seed rot and seedling blight caused by *Rhizoctonia solani* on sunflower at 5–11 mL/unit (100 000 seeds). Apply as a seed treatment prior to planting.
4. Arolist Biological Fungicide may be applied in mixture with other registered seed treatment products, including HELIX, Maxim 480FS, Allegiance FL, Apron XL LS and Apron MAXX RFC.

References

A. List of studies/Information submitted by registrant

1.0 The active substance, its properties and uses

PMRA document number	Reference
2927119	2018, Characterization and Analysis for *Bacillus velezensis* strain RTI301 and *Bacillus subtilis* RTI477, DACO: M2.7.1,M2.7.2 CBI
2927123	2017, Determination of Physical Chemical Properties of the Microbial Pest control Agent F4007 (*Bacillus velezensis* strain RTI301), DACO: M2.12 CBI
3012116	2018, Product Identity for Bacillus velezensis strain RTI301 Technical, DACO: M2.7.1,M2.7.2 CBI
3040044	2017, Detection and enumeration of *Bacillus velezensis* strain RTI301 and microbial contaminants in five batches of the Microbial Pest Control Agent F4007, DACO: M2.10.1,M2.10.2 CBI
3040047	2017, Method validation for the enumeration of *Bacillus velezensis* strain RTI301 (F4007), DACO: M2.9.2 CBI
3040048	2019, Results of the Amylosin Production Analysis, DACO: M2.9.3
3042250	2019, Revised Product Chemistry for Bacillus velezensis strain RTI301 Technical, DACO: M2.12, M2.2, M2.3, M2.4, M2.5, M2.7.2, M2.8, M2.9.1, M2.9.2, M2.9.3 CBI
3129055	2020, Method validation of the sensitivity of pathogen detection via qPCR, DACO: M2.10.2 CBI
3173881	2020, Long-term storage stability test on the Microbial Pest Control Agent F4007 (*Bacillus velezensis* strain RTI301), DACO: M2.11
3186731	2020, Analysis of microbial contaminants in five batches of the Microbial Pest Control Agent F4007, DACO: M2.11 CBI
3012115	2018, Product Identity for Bacillus subtilis strain RTI 477 Technical, DACO: M2.7.1 CBI
3040720	2017, Detection and enumeration of *Bacillus subtilis* strain RTI477 and microbial contaminants in five batches of the Microbial Pest Control Agent F4009, DACO: M2.10.1, M2.10.2 CBI
3040723	2017, Method validation for the enumeration of *Bacillus subtilis* strain RTI477 (F4009), DACO: M2.9.2 CBI
3042255	2019, Revised Product Chemistry for Bacillus subtilis strain RTI477 Technical, DACO: M2.12, M2.2, M2.3, M2.4, M2.5, M2.7.2, M2.8, M2.9.1, M2.9.2, M2.9.3 CBI
3173874	2020, Long-term storage stability test on the Microbial Pest Control Agent F4009 (*Bacillus subtilis* strain RTI477), DACO: M2.11
3186735	2020, Analysis of microbial contaminants in five batches of the Microbial Pest Control Agent F4009, DACO: M2.11 CBI

PMRA document number	Reference
2927120	2017, Determination of Physical Chemical Properties of the Microbial Pest Control Product F4034-5 (*Bacillus subtilis* strain RTI477 and *Bacillus velezensis* strain RTI301), DACO: M2.12, M2.7.2 CBI
3040254	2017, Product Identity, Manufacturing Process, and Certification of Limits for the Microbial Pest Control Product F4034-5, DACO: M2.1, M2.2, M2.3, M2.8 CBI
3040256	2017, Detection and enumeration of *Bacillus subtilis* strain RTI477, *Bacillus velezensis* strain RTI301 and microbial contaminants in five batches of the Microbial Pest Control Product F4034-5, DACO: M2.10.1, M2.10.2 CBI
3125675	2020, Long-term storage stability test on the Microbial Pest Control Product F4034-5, DACO: M2.11 CBI
2927123	2017, Determination of Physical Chemical Properties of the Microbial Pest control Agent F4007 (*Bacillus velezensis* strain RTI301), DACO: M2.12, M2.7.2 CBI
3040343	2017, Determination of Product Chemistry, Composition and Certified Limits of the Microbial Pest Control Product F4007-9 (*Bacillus velezensis* strain RTI301). DACO: M2.1, M2.2, M2.3, M2.8 CBI
3040345	2017, Detection and enumeration of *Bacillus velezensis* strain RTI301 and microbial contaminants in five batches of the Microbial Pest Control Product F4007-9, DACO: M2.10.1, M2.10.2 CBI
3040346	2019, Long-term storage stability test on the Microbial Pest Control Product F4007-9 (*Bacillus velezensis* strain RTI301), DACO: M2.11 CBI
3125682	2020, Long-term storage stability test on the Microbial Pest Control Product F4007-9, DACO: M2.11 CBI
2927127	2018, Response to Tier I Microbial Pesticide Mammalian Toxicology Data Requirements for *Bacillus velezensis* strain RTI301 Technical Grade Active Ingredient, DACO: M4.1
2927126	2018, Response to Tier I Microbial Pesticide Mammalian Toxicology Data Requirements for *Bacillus subtilis* strain RTI477 Technical Active Ingredient, DACO: M4.1

2.0 Human and Animal Health

PMRA document number	Reference
2927125	2018, Response to Tier I Microbial Pesticide Mammalian Toxicology Data Requirements for F4034-5 (*Bacillus velezensis* strain RTI301 + *Bacillus subtilis* strain RTI477) SC End-Use Product, DACO: M4.1, M4.2.1, M4.2.2, M4.3.1, M4.3.2, M4.4, and M4.5.1
2927126	2018, Response to Tier I Microbial Pesticide Mammalian Toxicology Data Requirements for *Bacillus subtilis* strain RTI477 Technical Active Ingredient, DACO: M4.1, M4.2.1, M4.3.1, M4.3.2, M4.5.1, M4.6
2927127	2018, DACO: M4.1,M4.2.1,M4.3.1,M4.3.2,M4.5.1,M4.6

PMRA document number	Reference
2927129	2017, *Bacillus velezensis* strain RTI301: Acute Oral Toxicity/Pathogenicity In Rats, DACO: M4.2.2
2927130	2017, *Bacillus subtilis* strain RTI477: Acute Oral Toxicity - Up-And-Down Procedure in Rats, DACO: M4.2.2
2927131	2017, *Bacillus subtilis* strain RTI477: acute pulmonary toxicity/pathogenicity in rats, DACO: M4.2.3
2927132	2017, *Bacillus velezensis* strain RTI301: acute pulmonary toxicity/pathogenicity in rats, DACO: M4.2.3
2927135	2017, *Bacillus subtilis* strain RTI477: Acute Dermal Toxicity in Rats, DACO: M4.4, M4.9
2927136	2017, *Bacillus velezensis* strain RTI301: Primary Skin Irritation in Rabbits, DACO: M4.5.2
2927138	2017, *Bacillus subtilis* strain RTI477: Primary Skin Irritation in Rabbits, DACO: M4.5.2
2927139	2017, *Bacillus velezensis* strain RTI301: Primary Eye Irritation in Rabbits, DACO: M4.5.2, M4.9
2927140	2017, F4034-5: Primary Eye Irritation in Rabbits, DACO: M4.9
2927141	2017, *Bacillus subtilis* strain RTI477: Primary Eye Irritation in Rabbits, DACO: M4.9
2927174	2017, F4007-9: Primary Skin Irritation in Rabbits, DACO: M4.5.2
2927176	2017, F4007-9: Primary Eye Irritation in Rabbits, DACO: M4.9
3012120	2018, Response to Tier I Microbial Pesticide Mammalian Toxicity Data Requirements for F4007-9 (*Bacillus velezensis* strain RTI301) SC End-Use Product, DACO: M4.1, M4.2.1, M4.2.2, M4.3.1, M4.3.2, M4.4, M4.5.1
3040049	2017, Method Validation for Viable CFU Detection and Enumeration in Rat Matrices Inoculated with *Bacillus velezensis* strain RTI301, DACO: M4.2.3
3040050	2016, Identification and Pathogenic Potential of Clinical *Bacillus* and *Paenibacillus* Isolates, DACO: M4.3.2
3040051	1973, Clinical Spectrum of Infection Due to *Bacillus* Species, DACO: M4.3.2
3040052	2012, Combined *Bacillus licheniformis* and *Bacillus subtilis* infection in a patient with oesophageal perforation, DACO: M4.3.2
3040053	2016, Effects of *Bacillus velezensis* strain BAC03 in promoting plant growth, DACO: M4.3.2
3040054	2012, *Bacillus velezensis* sp. nov., a surfactant producing bacterium isolated from the river Velez in Malaga, southern Spain, DACO: M4.3.2
3040055	2007, The Safety of Two *Bacillus* Probiotic Strains for Human Use, DACO: M4.3.2
3040056	1991, On the safety of *Bacillus subtilis* and *B. amyloliquefaciens*: a review, DACO: M4.3.2
3040057	2016, *Bacillus velezensis* is not a later heterotypic synonym of *Bacillus amyloliquefaciens; Bacillus methylotrophicus, Bacillus amyloliquefaciens* subsp. plantarum and *Bacillus oryzicola* are later heterotypic synonyms of *Bacillus velezensis* based on phylogenomics, DACO: M4.3.2

PMRA document number	Reference
3040058	2013, Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update), DACO: M4.3.2
3040059	2013, The need to revise the Technical Guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition, DACO: M4.3.2
3040060	2014, Guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition, DACO: M4.3.2
3040061	2017, *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* Form an Operational Group *B. amyloliquefaciens* within the *B. subtilis* Species Complex, DACO: M4.3.2
3040062	2008, The safety of *Bacillus subtilis* and *Bacillus indicus* as food probiotics, DACO: M4.3.2
3040063	2008, *Bacillus subtilis* isolated from the human gastrointestinal tract, DACO: M4.3.2
3040067	2017, *Bacillus velezensis* strain RTI301: Acute Dermal Toxicity in Rats, DACO: M4.4, M4.9
3040772	2016, Identification and Pathogenic Potential of Clinical *Bacillus* and *Paenibacillus* Isolates, DACO: M4.3.2
3040777	2007, The Safety of Two *Bacillus* Probiotic Strains for Human Use, DACO: M4.3.2
3040784	2008, The safety of *Bacillus subtilis* and *Bacillus indicus* as food probiotics, DACO: M4.3.2

3.0 Environment

PMRA document number	Reference
2927129	2017, *Bacillus velezensis* Strain RTI301: Acute Oral Toxicity/Pathogenicity In Rats, DACO: M4.2.2
2927130	2017, *Bacillus subtilis* Strain RTI477: Acute Oral Toxicity - Up-And-Down Procedure in Rats, DACO: M4.2.2
2927131	2017, *Bacillus subtilis* Strain RTI477: Acute Pulmonary Toxicity/Pathogenicity In Rats, DACO: M4.2.3
2927132	2017, *Bacillus velezensis* Strain RTI301: Acute Pulmonary Toxicity/Pathogenicity In Rats, DACO: M4.2.3
2927142	2018, Response to Tier I Microbial Pesticide Ecotoxicology Data Requirements for Bacillus subtilis strain RTI477 Technical, DACO: M9.1, M9.2.2, M9.3, M9.4.1, M9.4.2, M9.5.1, M9.5.2, M9.8.1, M9.8.2
2927143	2018, Response to Tier I Microbial Pesticide Ecotoxicology Data Requirements for Bacillus velezensis strain RTI301 Technical, DACO: M9.1, M9.2.2, M9.3, M9.4.1, M9.4.2, M9.5.1, M9.5.2, M9.8.1, M9.8.2
2927145	2017, *Bacillus subtilis* strain RTI477: Acute Oral Toxicity Study in Bobwhite Quail, DACO: M9.2.1
2927148	2017, Bacillus subtilis strain RTI477: Honey Bee, *Apis mellifera*, Oral Microbial Toxicity Limit Test, DACO: M9.5.1

PMRA document number	Reference
2927150	2017, *Bacillus subtilis* strain RTI477: Honey Bee, *Apis mellifera*, Oral Definitive Toxicity Test, DACO: M9.5.1
2927151	2018, *Bacillus velezensis* strain RTI301: Honey Bee, *Apis mellifera*, Oral Microbial Definitive Toxicity Test, DACO: M9.5.1
2953938	2017, Bacillus velezensis strain RTI301: Acute Oral Toxicity Study in Bobwhite Quail, DACO: M9.2.1
3040048	2019, Results of the Amylosin Production Analysis, DACO: M2.9.3
3040053	2016, Effects of *Bacillus velezensis* strain BAC03 in promoting plant growth, DACO: M4.3.2
3040054	2012, *Bacillus velezensis* sp. nov., a surfactant producing bacterium isolated from the river Velez in Malaga, southern Spain, DACO: M4.3.2
3040057	2016, *Bacillus velezensis* is not a later heterotypic synonym of *Bacillus amyloliquefaciens; Bacillus methylotrophicus, Bacillus amyloliquefaciens* subsp. plantarum and *Bacillus oryzicola* are later heterotypic synonyms of *Bacillus velezensis* based on phylogenomics, DACO: M4.3.2
3040061	2017, *Bacillus amyloliquefaciens, Bacillus velezensis,* and *Bacillus siamensis* Form an Operational Group *B. amyloliquefaciens* within the *B. subtilis* Species Complex, DACO: M4.3.2
3040063	2008, *Bacillus subtilis* isolated from the human gastrointestinal tract, DACO: M4.3.2
3040064	2019, Effects of the Microbial Pest Control Agent F4007 (*Bacillus velezensis* strain RTI301) on reproduction and mortality of the Collembolan, *Folsomia candida*, in an artificial soil substrate, DACO: M9.5.1
3040065	2019, Effects of the Microbial Pest Control Agent F4007 (*Bacillus velezensis* strain RTI301) on the predatory mite, *Hypoaspis (Geolaelaps) aculeifer*, in a reproduction test in an artificial soil substrate, DACO: M9.5.1
3040066	2019, 90-day Preliminary Technical Screening Results, DACO: M9.5.1
3040067	2017, *Bacillus velezensis* strain RTI301: Acute Dermal Toxicity in Rats, DACO: M4.4, M4.9
3040259	2018, Toxicity and pathogenicity of Microbial Pest Control Product F4034-5 (*Bacillus subtilis* strain RTI477 and *Bacillus velezensis* strain RTI301) to Rainbow trout (*Oncorhynchus mykiss*) during a 30 day test, DACO: M9.4.1
3040724	1967, Application of the Fluorescent-Antibody Techlnique to an Ecological Study of Bacteria in Soil, DACO: M9.1
3040725	2014, *Bacillus subtilis* A Potential Probiotic Bacterium to Formulate Functional Feeds for Aquaculture, DACO: M9.1
3040726	1991, Body temperature in birds, DACO: M9.1
3040727	2005, Inhibition of *Clostridium perfringens* by a Novel Strain of *Bacillus subtilis* Isolated from the Gastrointestinal Tracts of Healthy Chickens, DACO: M9.1
3040728	2015, Logan, NA and P. De Vos. Bacillus, Bergey's Manual of Systematics of Archaea and Bacteria Online. John Wiley and Sons Inc. DACO: M9.1
3040729	1998, Normal Body Temperature of Rats: The Set-point Controversy, DACO: M9.1
3040730	2018, 2018 AAFCO Annual Meeting Committee Reports, DACO: M9.1

PMRA document number	Reference
3040731	2003, Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on a request from the Commission on micro-organism product BioPlus 2B, authorised as feed additive, DACO: M9.1
3040732	2005, Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the modification of terms of authorisation of the micro-organism, DACO: M9.1
3040733	2006, Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the modification of terms of authorisation of the micro-organism, DACO: M9.1
3040734	2007, Compatibility of the microbial preparation of *Bacillus licheniformis* and *Bacillus subtilis* (BioPlus 2B) with the coccidiostat lasalocid A sodium in feed for turkeys, DACO: M9.1
3040735	2008, Compatibility of the microbial product BioPlus 2B (*Bacillus licheniformis* and *Bacillus subtilis*) with lasalocid sodium, DACO: M9.1
3040736	2010, Scientific Opinion on the safety and efficacy of Calsporin (*Bacillus subtilis*) for turkeys for fattening, ducks, geese, pigeons and other game birds, DACO: M9.1
3040737	2011, Scientific Opinion on the compatibility of BioPlus 2B (*Bacillus licheniformis* and *Bacillus subtilis*) with semduramycin and formic acid in turkeys for fattening, DACO: M9.1
3040738	2016, Safety and efficacy of BioPlus 2B (*Bacillus subtilis* DSM 5750 and *Bacillus licheniformis* DSM 5749) as a feed additive for sows, piglets, pigs for fattening, turkeys for fattening and calves, DACO: M9.1
3040739	2017, Effect of *Bacillus subtilis* and *Bacillus licheniformis* supplementation in diets with low- and high-protein content on ileal crude protein and amino acid digestibility and intestinal microbiota composition of growing pigs, DACO: M9.3
3040740	2004, European Union Commission Regulations, DACO: M9.3
3040741	2004, European Union Commission Regulations, DACO: M9.3
3040742	2005, European Union Commission Regulations, DACO: M9.3
3040743	2017, Safety and efficacy of *Bacillus subtilis* PB6 (*Bacillus subtilis* ATCC PTA-6737) as a feed additive for sows, DACO: M9.3
3040744	2008, Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to challenge infections, DACO: M9.4.1
3040745	2011, Evaluation of probiotic strain *Bacillus subtilis* C-3102 as a feed supplement for koi carp (*Cyprinus carpio*), DACO: M9.4.1
3040746	2013, Studies on *Bacillus subtilis*, as Potential Probiotics, on the Biochemical Parameters of Rainbow trout, *Oncorhynchus mykiss* (Walbaum) to Challenge Infections, DACO: M9.4.1
3040747	2002, Thermostable a-1,4- and a-1,6-glucosidase enzymes from *Bacillus* sp. isolated from a marine environment, DACO: M9.4.2
3040748	2001, *Bacillus subtilis* strain QST 713, DACO: M9.4.2
3040749	2014, A marine sponge associated strain of *Bacillus subtilis* and other marine bacteria can produce anticholinesterase compounds, DACO: M9.4.2

PMRA document number	Reference
3040750	2004, Screening of bacterial associates of marine sponges for single cell oil and PUFA, DACO: M9.4.2
3040751	1998, Characterization of *Bacillus* strains of marine origin, DACO: M9.4.2
3040752	2002, Control of pathogenic *Vibrio* spp. by *Bacillus subtilis* BT23, a possible probiotic treatment for black tiger shrimp *Penaeus monodon*, DACO: M9.4.2
3040753	2013, Diversity and functional significance of cellulolytic microbes living in termite, pill-bug and stem-borer guts, DACO: M9.5.1
3040754	2004, Effectiveness of honey bees in delivering the biocontrol agent *Bacillus subtilis* to blueberry flowers to suppress mummy berry disease, DACO: M9.5.1
3040755	2013, Diverse Microbiota Identified in Whole Intact Nest Chambers of the Red Mason Bee *Osmia bicornis* (Linnaeus 1758), DACO: M9.5.1
3040756	2013, Microbial Gut Diversity of Africanized and European Honey Bee Larval Instars, DACO: M9.5.1
3040758	2015, *Bacillus* in the guts of honey bees (*Apis mellifera*; Hymenoptera: Apidae) mediate changes in amylase values, DACO: M9.5.1
3040759	2019, Effects of the Microbial Pest Control Agent F4009 (*Bacillus subtilis* strain RTI477) on the predatory mite, *Hypoaspis (Geolaelaps) aculeifer*, in a reproduction test in an artificial soil substrate, DACO: M9.5.1
3040760	2019, Effects of Probiotic *Bacillus* Species in Aquaculture – An Overview, DACO: M9.5.2
3040761	2011, Probiotic activity of *Bacillus subtilis* in juvenile freshwater prawn, *Macrobrachium rosenbergii* (de Man) at different methods of administration to the feed, DACO: M9.5.2
3040762	2012, Potential *Bacillus* probiotics enhance bacterial numbers, water quality and growth during early development of white shrimp (*Litopenaeus vannamei*), DACO: M9.5.2
3040763	2012, Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*, DACO: M9.5.2
3040764	2014, Isolation and Identification of *Bacillus* Species from Soil and Evaluation of Their Antibacterial Properties, DACO: M9.8.2
3040765	2000, Integrated Biological and Chemical Control of Damping-Off Caused by *Rhizoctonia solani* using *Bacillus subtilis* lXB14-C and Flutolanil, DACO: M9.8.2
3040766	2012, Isolation and Characterization of Nitrogen Fixing *Bacillus subtilis* Strain AS-4 from Agricultural Soil, DACO: M9.8.2
3040767	1997, Improvement of Arbuscular Mycorrhiza Development by Inoculation of Soil with Phosphate-Solubilizing Rhizobacteria To Improve Rock Phosphate Bioavailability (32P) and Nutrient Cycling, DACO: M9.8.2
3040768	1977, Chapter 2, Bacteria, Source unknown. DACO: M9.8.2
3081905	Liu, X, et. al., 2010, Production and characterization of a group of bioemulsifiers from the marine *Bacillus velezensis* strain H3, DACO: M9.8.1. Appl. Microbiol. Biotech. 87(5):1881-93

PMRA document number	Reference
3081907	Bacon, C.W. etc., 2001, Biological Control of *Fusarium moniliforme* in Maize, DACO: M9.8.1. Environ. Health Perspect. 109(suppl 2):325-332.
3081908	Salinas, I. etc., 2005, Dietary administration of *Lactobacillus delbrüeckii* and *Bacillus subtilis*, single or combined, on gilthead seabream cellular innate immune responses, DACO: M9.4.2. Fish and Shellfish Immunology 19:67-77

4.0 Value

PMRA document number	Reference
3012103	2019, Pre-submission Consultation: Preliminary Value Summary for F4034-5, containing *Bacillus velezensis* RTI 301 and *B. subtilis* RTI 477 and F4007-9, Containing *B. velezensis* RTI 301, DACO: 10.1
3040199	2017, RTI301 Spot Plate Assay Summary, DACO: M10.2.1
3040200	2017, RTI301 and RTI477 Spot Plate Assay Summary, DACO: M10.2.1
3040201	2015, Biological IP Spectrum Testing RTI301: Spot Plate Inhibition, DACO: M10.2.1
3040202	2015, FB 4175 FF128 Spot Plate Screening, DACO: M10.2.1
3040203	2016, Efficacy of soil treatment against *Rhizoctonia solani* on corn seedlings. Greenhouse experiment (nursery). France 2016-2017., DACO: M10.2.2
3040204	2016, Efficacy of different strains/combination of strains applied as seed treatment on corn against *Rhizoctonia solani*, soil born disease, greenhouse screening pp1/125(4), 2016, DACO: M10.2.2
3040205	2017, Efficacy of different strains/combination of strains applied as seed treatment on corn against *Rhizoctonia solani*, soil born disease, greenhouse screening pp1/125(4), 2017, DACO: M10.2.2
3040206	2016, Efficacy of different strains/combination of strains applied as seed treatment on corn against *Rhizoctonia solani*, soil born disease, greenhouse screening pp1/125(4), 2016, DACO: M10.2.2
3040207	2016, Efficacy of different strains/combination of strains applied as seed treatment on sunflower against *Rhizoctonia*, soil born disease, greenhouse screeningpp1/125(4), 2016, DACO: M10.2.2
3040208	2016, Efficacy of different strains/combination of strains applied as seed treatment on sunflower against *Rhizoctonia*, soil born disease, greenhouse screening pp1/125(4), 2016, DACO: M10.2.2
3040209	2017, Efficacy of different strains/combination of strains applied as seed treatment on sunflower against *Rhizoctonia*, soil born disease, greenhouse screening pp1/125(4), 2017, DACO: M10.2.2
3040210	2018, Control of *Rhizoctonia* of soybeans with biological seed treatments, DACO: M10.2.2
3040211	2017, *Rhizoctonia* protection provided by biological seed treatments applied to soybeans, DACO: M10.2.2

PMRA document number	Reference
3040212	2017, *Rhizoctonia* protection provided by biological seed treatments applied to soybeans, DACO: M10.2.2
3040213	2016, Impact of biological seed treatments on growth and yield of soybean inoculated with *Rhizoctonia*, DACO: M10.2.2
3040214	2015, Effectiveness of different biofungicides applied to soybeans (natural soil-*Rhizoctonia*), DACO: M10.2.2
3040215	2015, Effectiveness of different biofungicides applied to soybeans (*Rhizoctonia* inoculated), DACO: M10.2.2
3040216	2015, Stand and yield from FMC soybean seed treatment products compared to commercial standard (*Rhizoctonia* inoculated), DACO: M10.2.2
3040217	2015, Stand and yield from FMC soybean seed treatment products compared to commercial standard (*Rhizoctonia* inoculated), DACO: M10.2.2
3040218	2015, Biological seed treatment impact on corn growth and yield, DACO: M10.2.2
3040219	2015, Biological seed treatment impact on corn growth and yield, DACO: M10.2.2
3040220	2016, Impact of biological seed treatments on growth and yield of corn (*Rhizoctonia*), DACO: M10.2.2
3040221	2015, Biological seed treatment impact on corn growth and yield, DACO: M10.2.2
3040222	2016, Impact of biological seed treatments on growth and yield of corn (*Fusarium*), DACO: M10.2.2
3040223	2017, Fusarium protection provided by biological seed treatments applied to corn, DACO: M10.2.2
3040224	2017, Fusarium protection provided by biological seed treatments applied to corn, DACO: M10.2.2
3040225	2017, SDS protection provided by biological seed treatments applied to soybeans, DACO: M10.2.2
3040226	2017, SDS protection provided by biological seed treatments applied to soybeans, DACO: M10.2.2
3040227	2017, SDS protection provided by biological seed treatments applied to soybeans, DACO: M10.2.2
3040228	2016, Impact of biological seed treatments on growth and yield of soybean inoculated *Fusarium virguliforme*, DACO: M10.2.2
3040229	2016, Impact of biological seed treatments on growth and yield of soybean inoculated *Fusarium virguliforme*, DACO: M10.2.2
3040230	2016, Impact of biological seed treatments on growth and yield of soybean inoculated *Fusarium virguliforme*, DACO: M10.2.2
3040231	2016, Impact of biological seed treatments on growth and yield of soybean inoculated *Fusarium virguliforme*, DACO: M10.2.2
3040232	2016, Impact of seed treatment fungicides and biologicals applied to soybeans inoculated with SDS (*Fusarium*) in IA, DACO: M10.2.2
3040233	2018, Control of SDS of soybeans with biological seed treatments, DACO: M10.2.2
3040234	2018, Control of SDS of soybeans with biological seed treatments, DACO: M10.2.2
3040235	2017, Germination of soybean seed treated with F4034 and F4007 seed treatments, DACO: M10.3.1

PMRA document number	Reference
3040236	2017, Germination of soybean seed treated with F4034 and F4007 seed treatments, DACO: M10.3.1
3040237	2017, Germination of soybean seed treated with F4034 and F4007 seed treatments, DACO: M10.3.1
3040238	2017, Germination of corn seed treated with F4034 and F4007 seed treatments (Iowa Cold Test), DACO: M10.3.1
3040239	2017, Germination of corn seed treated with F4034 and F4007 seed treatments, DACO: M10.3.1
3040240	2017, Germination of corn seed treated with F4034 and F4007 seed treatments (Iowa Cold Test), DACO: M10.3.1
3040241	2017, Germination of corn seed treated with F4034 and F4007 seed treatments, DACO: M10.3.1
3040242	2017, Germination of corn seed treated with F4034 and F4007 seed treatments, DACO: M10.3.1
3040243	2017, Germination of corn seed treated with F4034 and F4007 seed treatments, DACO: M10.3.1
3040244	2017, Section M10.3.1 Crop Tolerance (Seed Safety), DACO: M10.3

B. Additional Information Considered

None