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Evaluation Report

Bacillus subtilis strain QST 713

Serenade MAX
Serenade ASO
Rhapsody ASO
Serenade Garden Concentrate
Serenade Garden Ready To Use

(publié aussi en français)

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Overview

Registration Decision for *Bacillus subtilis* strain QST 713

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act*[1] and in accordance with the Pest Control Products Regulations, has granted conditional registration for the sale and use of *Bacillus subtilis* QST 713 Technical Powder and the following end-use products containing the active ingredient *Bacillus subtilis* strain QST 713: Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use to suppress various fungal diseases in asparagus, bushberries, caneberries, bulb vegetables, brassica (cole) crops, cucurbit vegetables, fruiting vegetables, grapes, legume vegetables, leafy vegetables, mint, pome fruits, rutabaga, turnip, strawberries as well as ornamentals.

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

This report summarizes the information evaluated and provides the results of the evaluation as well as the reasons for the conditional registration, with an outline of the additional scientific information required from the applicant. It also describes the conditions of registration that the applicant must meet to ensure that the health and environmental risks as well as the value of these pest control products are acceptable for their intended use.

This overview describes the key points of the evaluation, while the Science Evaluation section provides detailed technical information on human health, environmental and value assessments of *Bacillus subtilis* QST 713 Technical Powder as well as Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks[2] to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its conditions or proposed conditions of registration. The Act also requires that products have value[3]

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1 As per subsection 28(1) of the *Pest Control Products Act*.

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

3 "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".

when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (e.g. children) as well as organisms in the environment (e.g. those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties present when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the PMRA's website at www.pmra-arla.gc.ca.

What Is *Bacillus subtilis* strain QST 713?

Bacillus subtilis strain QST 713 is a microbial pest control agent used to suppress a number of bacterial and fungal plant pathogens.

The five end-use products Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready to Use are preventative biofungicides that contain *Bacillus subtilis* strain QST 713 as the active ingredient. The five products are intended for different target markets. Serenade MAX and Serenade ASO are for agricultural use, particularly for organic growers. Rhapsody ASO is a product that can be used for organic production of ornamentals. Serenade Garden Concentrate and Serenade Garden Ready to Use are for home and garden use on ornamentals, fruits and vegetables.

Health Considerations

Can Approved Uses of *Bacillus subtilis* strain QST 713 Affect Human Health?

Bacillus subtilis strain QST 713 is unlikely to affect your health when Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate or Serenade Garden Ready To Use are used according to the label directions.

People could be exposed to *B. subtilis* strain QST 713 during handling of Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate or Serenade Garden Ready To Use. When assessing health risks, the PMRA considers several key factors such as:

- the microorganism's biological properties (e.g. production of toxic byproducts);
- reports of any adverse incidents;
- its potential to cause disease or toxicity as determined in toxicological studies; as well as
- the levels to which people may be exposed relative to exposures already encountered in nature to other strains of the microorganism.

Toxicology studies in laboratory animals describe potential health effects from large doses in order to determine the potential of this organism to cause disease or toxicity. No significant toxicity and no signs of causing disease were observed when *B. subtilis* strain QST 713 was tested on laboratory animals.

Residues in Water and Food

Dietary risks from food and water are not of concern.

The *Food and Drugs Act* prohibits the sale of food containing a pesticide residue that exceeds the established maximum residue limit (MRL). Pesticide MRLs are established for the *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act*. Each MRL value determines the maximum concentration in parts per million (ppm) of a pesticide allowed in or on certain foods. Pesticide MRLs are established for the *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act.* Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

Bacillus subtilis strains are common in nature, and the use of Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use is not expected to significantly increase the natural environmental background levels of this microorganism. Some strains of *B. subtilis* have been isolated from food samples implicated in food poisoning. However, these strains demonstrated the ability to produce a highly heat-stable toxin that may be similar to a toxin produced by *B. cereus*, a known food-borne pathogenic microorganism. *Bacillus subtilis* strain QST 713 is not reported to produce this toxin. Also, no such effects were reported for this microorganism in the United States where it has been registered since 2000. Furthermore, there was no significant toxicity, and no signs of causing diseases were observed when *B. subtilis* strain QST 713 was administered orally to rats. Therefore, the establishment of an MRL is not required for *B. subtilis* strain QST 713. In addition, the likelihood of residues of *B. subtilis* strain QST 713 contaminating drinking water supplies is negligible to non-existent. Consequently, dietary exposure and risk are minimal to non-existent.

Occupational Risks From Handling Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate or Serenade Garden Ready To Use

Occupational risks are not of concern when Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate or Serenade Garden Ready To Use are used according to the label directions, which include protective measures.

Growers handling Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate or Serenade Garden Ready To Use can come into direct contact with *B. subtilis* strain QST 713 on the skin, in the eyes or by inhalation. For this reason, the labels specify that growers exposed to these end-use products must wear waterproof gloves, a long-sleeved shirt, long pants and shoes plus socks. Commercial users must wear a NIOSH-approved respirator (with any –95, P-95, R-95 or HE filter for biological

products), and domestic users should avoid breathing spray mists. Eye goggles are not required as the eye irritation studies submitted indicated minimal eye irritation potential.

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

Environmental Considerations

What Happens When *Bacillus subtilis* strain QST 713 Is Introduced Into the Environment?

Environmental risks are not of concern.

Studies designed to examine the effects of *B. subtilis* strain QST 713 on various non-target organisms were evaluated. No significant adverse effects were observed in birds, freshwater fish, terrestrial arthropods (including honeybees), aquatic invertebrates, marine animals or algae.

Bacillus subtilis is not generally considered to be a disease-causing agent. Therefore, Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use are expected to present a negligible risk to non-target organisms.

Value Considerations

What Is the Value of Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate, Serenade Garden Ready To Use?

These end-use products contain the active ingredient *B. subtilis* strain QST 713 and are reduced-risk biofungicides that suppress various bacterial and fungal diseases on a number of agricultural crops as well as ornamentals grown indoors, outdoors, in greenhouses, homes and gardens. These products can be used as resistance-management tools because the active ingredient, *B. subtilis* strain QST 713, has a multiple-site mode of action. By rotating their use with other registered chemical fungicides in an integrated pest management program, growers can reduce the possibility of developing resistance among pathogen populations to chemical fungicides as well as potentially reducing the number of applications of chemical fungicides. Serenade MAX, Serenade ASO and Rhapsody ASO can be used in organic vegetable and ornamental production.

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 on the label of Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use to address the potential risks are as follows.

Key Risk-Reduction Measures

Human Health

As with all microbial pest control products, there are concerns with skin irritation and with users developing allergic reactions through repeated high exposures to *B. subtilis* strain QST 713. Therefore, anyone handling Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use must wear waterproof gloves, a long-sleeved shirt, long pants and shoes plus socks. Commercial users must wear a NIOSH-approved respirator (with any –95, P-95, R-95 or HE filter for biological products), and domestic users are directed to avoid breathing spray mists. Eye goggles are not required as the eye irritation studies submitted indicated minimal eye irritation potential.

Environment

As a general precaution, handlers are advised not to contaminate irrigation or drinking water or aquatic habitats by cleaning of equipment or by disposing of wastes.

What Additional Scientific Information Is Required?

Although the risks and value have been found acceptable when all risk-reduction measures are followed, the applicant must submit additional scientific information as a condition of registration. More details are presented in the Science Evaluation section of this Evaluation Report or in the Section 12 Notice associated with these conditional registrations. The applicant is required to submit this information no later than 1 December 2008.

Product Characterization and Analysis

- Results of ribotyping to distinguish *B. subtilis* strain QST 713 from other strains of *B. subtilis* are required.

- Additional storage stability data for Serenade MAX, Serenade ASO/Rhapsody ASO/Serenade Garden Concentrate and Serenade Garden Ready To Use on pilot-scale or production-scale batches are required to ensure product performance and safety.

Value

- Two additional efficacy trials on tomato early blight under adequate disease pressures are required for Serenade MAX.

Other Information

As these conditional registrations relate to a decision on which the public must be consulted[4], the PMRA will publish a consultation document when there is a proposed decision on the applications to convert the conditional registrations to full registrations or on the applications to renew the conditional registrations, whichever occurs first.

The test data cited in this Evaluation Report (i.e. the test data relevant in supporting the registration decision) will be made available for public inspection when the decision is made to convert the conditional registrations to full registrations or to renew the conditional registrations (following public consultation). If more information is required, please contact the PMRA's Pest Management Information Service by phone (1-800-267-6315) or by e-mail (pmra_infoserv@hc-sc.gc.ca).

[4] As per subsection 28(1) of the *Pest Control Products Act*.

Science Evaluation

Bacillus subtilis strain QST 713

1.0 The Active Substance, its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Bacillus subtilis* strain QST 713
Function	Suppresses many fungal and bacterial plant pathogens including powdery mildew, gray mould, downy mildew, early blight, late blight, Botrytis blight, neck rot, pod rot and leaf blight, white mould, pink rot, fire blight, scab
Binomial name	*Bacillus subtilis* strain QST 713
Taxonomic designation	
Kingdom	Eubacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Bacillaceae
Genus	*Bacillus*
Species	*subtilis*
Strain	QST 713
Patent Status information	A Canadian patent application was submitted in May of 1998. The patent is pending.
Minimum purity of active	7.3×10^9 colony forming units (CFU)/g

Identity of relevant impurities of toxicological, environmental and/or significance.	The technical grade active ingredient does not contain any impurities or micro contaminants known to be TSMP Track 1 substances. Secondary metabolites produced by *B. subtilis* strain QST 713 were isolated and identified. A total of 6 iturins, 14 pliplastatins, 4 surfactins and 2 agrastatins were identified. These secondary metabolites work together to destroy germ tubes and mycelia of targeted plant pathogenic fungi. Literature searches failed to uncover any reports regarding in vivo mammalian toxicity associated with these metabolites. Furthermore, the results of human health and toxicity studies did not indicate any significant adverse effects.

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

Technical Product—*Bacillus subtilis* QST 713 Technical Powder

Property	Result
Colour	Light brown
Odour	Earthlike, sweet
Physical state	Powder
Guarantee	7.3×10^9 CFU/g
Density	0.48 g/cm^3
Storage stability	One year at ambient temperatures
Flammability	Non-flammable
Explodability	Non-explosive

End-Use Product—Serenade MAX

Property	Result
Colour	Medium gray-brown
Odour	Sweet, earthy
Physical state	Powder
Formulation type	Wettable powder
Guarantee	14.6% *B. subtilis* strain QST 713 minimum of 7.3×10^9 CFU/g

Property	Result
Density	0.45 g/cm^3
Storage stability	1 year at ambient temperatures
Corrosion characteristics	Non-corrosive
Flammability	Non-flammable
Explodability	Non-explosive

End-Use Products—Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use

Property	Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate	Serenade Garden Ready To Use
Colour	Medium brown	light tan
Odour	Sweet, earthy	earthy
Physical state	Liquid, aqueous suspension	liquid, aqueous suspension
pH	5.36	5.0–5.5
Guarantee	1.34% *B. subtilis* strain QST 713 minimum of 1×10^9 CFU/g	0.074% *B. subtilis* strain QST 713 minimum of 1×10^8 CFU/g
Density	1.023 g/mL	1.0 g/mL
Boiling point	> 100°C	> 100°C
Storage stability	nine months at room temperature	nine months at room temperature
Corrosion characteristics	non-corrosive	non-corrosive
Flammability	non-flammable	non-flammable
Explodability	non-explosive	non-explosive

1.3 Directions for Use

Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use may be applied as a preventative foliar spray alone or in alternating spray programs with other registered chemical fungicides or bactericides with equipment commonly used for ground application. Serenade MAX, Serenade ASO and Rhapsody ASO can be used for organic production. Growers should carefully read and follow the use directions and restrictions specifically mentioned on the products labels.

1.4 Mode of Action

Bacillus subtilis strain QST 713, is antagonistic toward many fungal and bacterial plant pathogens. This antagonism may be achieved in several ways including nutrient competition, site exclusion, colonization and attachment of the bacteria to the fungal pathogen. In addition, the applicant claims that the QST 713 strain is capable of inducing plants' natural systemic resistance or systemic acquired resistance against bacterial pathogens to stop plant pathogen spores from germinating, disrupting germ tube growth and inhibiting attachment of the plant pathogen to the leaf.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

The genus *Bacillus* consists of a large, diverse group of bacteria that includes species such as *thuringiensis, licheniformis, pumilis, cereus* and *anthracis*. Some strains of *B. anthracis* and *B. cereus* are known to be pathogenic to humans and animals. Therefore, methods to distinguish between species of *Bacillus* are important. *Bacillus subtilis* strain QST 713 can be distinguished from *B. anthracis, B. cereus*, and *B. thuringiensis* by a number of morphological or physiological features (e.g. size, motility, spore location, maximum growth temperature) and biochemical tests (API identification system by bioMerieux Vitek).

Strain QST 713 can be distinguished from other *B. subtilis* strains by a ribotyping classification method. Results of ribotyping of strain QST 713, however, were not submitted and are required as a condition of registration.

2.2 Method for Establishment of Purity of Seed Stock

Bacillus subtilis strain QST 713 is stored and maintained at the American Type Culture Collection (ATCC) as strain AQ713.

Practices for ensuring the purity of the seed stock were adequately described in the summary of the method of manufacture and quality assurance program.

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

The potency (CFU/g) of the end-use products is checked at the end of the manufacturing process using the plate count method on nutrient agar plates. Serial dilutions of the test material are plated and incubated and then counted for colony formation.

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

Bacillus subtilis is a ubiquitous microorganism in nature and has been isolated from a wide variety of environments. According to the U.S. Food and Drug Administration, some strains of *B. subtilis* have been isolated from food samples implicated in food poisoning. These strains, however, demonstrated the ability to produce a highly heat-stable toxin which may be similar to the vomiting type toxin produced by *B. cereus*, a known food borne pathogenic microorganism. *Bacillus subtilis* strain QST 713 is not reported to produce this toxin. Also, no such effects were reported for this microorganism in the United States where it has been registered since 2000. Furthermore, CD rats administered an oral dose of 1.13×10^8 CFU of QST 713 Technical Powder displayed no signs of toxicity or pathogenicity.

Based on the above information, the establishment of a maximum residue limit (MRL) is not required for *B. subtilis* strain QST 713 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulations.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures used to limit contaminating microorganisms during manufacture of Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use are acceptable. In the event that the microbial contaminants in a batch exceed the acceptable limits, the contaminated lot is sterilized by chemical or heat treatment or discarded.

Secondary metabolites produced by *B. subtilis* strain QST 713 were isolated and identified using acceptable methods. Literature searches failed to produce any reports of in vivo mammalian toxicity associated with these metabolites. Furthermore, the results of human health and toxicity studies did not indicate any significant adverse effects.

2.6 Methods to Show Absence of Any Human and Mammalian Pathogens

As noted in Section 2.5, several approaches are used to limit microbial contamination in *Bacillus subtilis* QST Technical Powder and Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use. These procedures include frequent purity checks to detect any unusual colonies and to verify colony morphology, as well as standard assays designed to detect *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, *Shigella*, *Vibrio cholerae*, yeasts, moulds, and coliform bacteria.

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

The storage stability of *Bacillus subtilis* QST 713 Technical Powder was assessed by determining the titres initially and after three, six and twelve months of storage under warehouse conditions (18–32°C and 14–80% relative humidity). Assay results indicated that QST 713 Technical Powder was stable for up to one year when stored at warehouse conditions.

Storage stability data were not submitted for Serenade MAX. Without this data, a maximum storage period of up to one year when stored at 25°C is required.

Storage stability data were submitted for one batch of Serenade ASO/Rhapsody ASO/Serenade Garden Concentrate indicating that the product appears to be stable when stored at room temperature for nine months.

Storage stability data were not submitted for Serenade Garden Ready To Use. Based on storage stability data for the end-use products, Serenade ASO/Rhapsody ASO/Serenade Garden Concentrate and in the absence of actual storage data for Serenade Garden Ready To Use, a maximum storage period of up to nine months when stored at room temperature is required.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

The database for *Bacillus subtilis* QST 713 Technical Powder is complete, consisting of laboratory animal (in vivo) toxicity and pathogenicity studies currently required for health hazard assessment purposes which were carried out in accordance with currently accepted international testing protocols and Good Laboratory Practices. The scientific quality of the data is high and the database is considered sufficient to characterize the toxicity and infectivity of this pest control agent and its associated end-use product.

In addition to the studies on the technical grade active ingredient (TGAI), a complete database for the end-use product, Serenade MAX was also submitted. Acute oral toxicity, acute pulmonary toxicity/pathogenicity, acute dermal toxicity/irritation, and eye irritation studies were submitted for Serenade MAX. These studies were conducted with Serenade WP, an alternate formulation, and were considered an acceptable substitute.

For the end-use products, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use, acute oral toxicity, acute pulmonary toxicity, acute dermal toxicity, acute dermal irritation, hypersensitivity and eye irritation studies were conducted using Serenade AS and submitted to support registration. Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use meet organic certification requirements in the United States. The potential for toxicity or irritation for Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use is, therefore, expected to be less than or equivalent to that of Serenade AS. The scientific quality of the data is high and the database is considered sufficient to characterize the toxicity and irritancy of these end-use products.

3.1.1 *Bacillus subtilis* QST 713 Technical Powder

A survey of published literature has revealed a number of instances where other *B. subtilis* strains had been implicated in infections in humans as well as the causal agent in food poisonings. Postoperative cellulitis, septicaemia, respiratory disease, endocarditis, and pneumonia have been reported in humans. In many instances, the association of *B. subtilis* is not

sufficiently rigid for it to be regarded unequivocally as the causative agent. Also, the number of putative infections are extremely low considering the total number of reports of bacterial infections. Many of those cases involved drug abuse or severely debilitated patients. *Bacillus subtilis* is virtually ubiquitous in the environment and therefore it is expected that *B. subtilis* may sometimes be found in association with other microorganisms in infections. Only individuals treated with immunosuppressive drugs appear to be susceptible to infection from *B. subtilis*. In food borne illnesses, the U.S. Food and Drug Administration has noted that some strains of *B. subtilis* have been isolated from food implicated in food poisoning. These strains, however, demonstrated the ability to produce a highly heat-stable toxin which may be similar to the vomiting type toxin produced by *B. cereus*, a known food borne pathogenic microorganism. *Bacillus subtilis* strain QST 713 is not reported to produce this toxin. Also, no such illnesses were reported for this microorganism in the United States where it has been registered for use on crops since 2000.

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

Bacillus subtilis has been reported to produce small antibiotic peptides and peptidolipids that are predominantly active against Gram-positive bacteria but also Gram-negative bacteria, yeast and fungi. Secondary metabolites produced by *B. subtilis* strain QST 713 were isolated and identified. A total of 6 iturins, 14 pliplastins, 4 surfactins and 2 agrastatins were identified. Literature searches failed to produce any reports of in vivo mammalian toxicity associated with any of these secondary metabolites.

For *Bacillus subtilis* QST 713 Technical Powder, acute oral toxicity/pathogenicity, acute pulmonary toxicity/pathogenicity, acute intravenous, acute dermal toxicity/irritation, and eye irritation studies were submitted to support registration.

In the oral toxicity/pathogenicity study, no significant signs of toxicity were observed in CD rats following oral gavage with 1.13×10^8 CFU of *B. subtilis* QST 713 Technical Powder. The test microbe was recovered from the stomach/small intestines, caecum, and faeces of treated rats from days 0–7 but clearance was observed by the day 13 observation, indicating a lack of pathogenicity. The oral LD_{50} was determined to be $>1.13 \times 10^8$ CFU/animal and *Bacillus subtilis* QST 713 Technical Powder is considered to be non-toxic and non-pathogenic to rats via the oral route.

In the pulmonary infectivity and toxicity study, CD rats were each dosed with 1.2×10^8 CFU of *Bacillus subtilis* QST 713 Technical Powder via the intratracheal route. There were no mortalities or statistically significant effects on bodyweight. Differences in weight gain and relative organ weights were not considered biologically significant. Adverse clinical observations consisted only of rough hair coat observed in one male treatment rat. The only necropsy findings were mottled lung parenchyma in all treatment animals sacrificed on day 0. The test substance was detected in the lungs and associated lymph nodes of male and female

treated rats from day 0 to day 35 with a gradually declining trend. Based on the rate of clearance, the estimated time to microbial clearance in the lungs and associated lymph nodes was estimated to be 108 days. The organism was also detected in the spleen, liver and kidneys but was cleared from these organs by the day 21 sacrifice. The pulmonary LD_{50} was determined to be $> 1.2 \times 10^8$ CFU/animal and *Bacillus subtilis* QST 713 Technical Powder is considered to be non-toxic and non-pathogenic when administered via the intratracheal route.

In the intravenous infectivity study, no mortalities and no significant clinical signs of toxicity were observed in CD rats following injection of 9.4×10^6 CFU of *Bacillus subtilis* QST 713 Technical Powder in water. The test microbe was recovered in the blood, lungs, spleen, kidneys, and liver of all treated rats. The test microbe was cleared from most organs and tissues by day 35, but remained at low levels in the spleen and liver. Clearance from all tissues was estimated to occur within approximately 80 days. It was concluded that *B. subtilis* QST 713 is non-infective in rats when administered via the intravenous route.

Acute dermal toxicity, dermal irritation, and eye irritation studies were also submitted for the Technical Product. *Bacillus subtilis* QST 713 was considered to be of low toxicity via the dermal route, slightly irritating to the skin, and minimally irritating to the eye. (Appendix I, Table 1)

3.1.2 Serenade MAX

In an acute oral study, no mortalities, signs of adverse clinical effects or abnormalities were observed in Sprague-Daley rats administered 5000 mg/kg bodyweight (bw) of Serenade WP suspended in water by oral gavage. The LD_{50} was determined to be > 5000 mg/kg.

In a whole body inhalation toxicity study, Sprague-Dawley rats were exposed to a time-weighted average aerosol concentration of 0.63 g/L of Serenade WP (25% w/w in deionized water) for four hours. Some clinical signs (e.g. salivation, shallow/laboured/congested breathing, wobbly gait, decreased activity, hunched posture, dark material around the eyes and/or nose, decreased temperature, misshapen corneas, corneal opacity, few faeces, soft stools, slight urine stains, decreased food consumption and slight faecal stains) and weight loss were noted but no mortality resulted from the dosing. A gross necropsy did not reveal any abnormalities. However, the test substance was performed with material diluted in water at a 25% w/w concentration, and therefore, an actual concentration was not determined. Despite this shortcoming, the aerodynamic particle size data for the dry aerosol indicated that approximately 64% of the particles are larger than 8.8 µm in diameter and approximately 94% of the particles are larger than 5.1 µm in diameter, with a Mass Median Aerodynamic Diameter (MMAD) of 10.5 µm (GSD = 1.6). Normally, particles of this size pose a low risk of inhalation exposure.

In a dermal toxicity study, no mortalities were observed among New Zealand white rabbits dosed with 2000 mg/kg bw of Serenade WP. Clinical observations consisted only of abnormal stance in one animal. Erythema, edema, necrosis, fissuring and/or sloughing of the skin at the application site was noted in all animals. All animals gained weight and visible lesions were not observed upon necropsy. The acute dermal LD_{50} of Serenade WP was determined to be > 2000 mg/kg bw and Serenade WP was considered to be of low dermal toxicity.

In the dermal irritation study, six rabbits were each administered a dermal dose of approximately 500 mg of Serenade WP for four hours. All animals exhibited very slight erythema at the 30–60 minute evaluations, with these signs resolving in each animal by the 72-hour observation. In addition, two animals showed very slight edema at the 30–60 minute and at the 24- and 48-hour observations. No other clinical signs were noted. The maximum individual irritation score was 2 (mildly irritating) occurring at the 30–60 minute and 24-hour timepoints. Based on the mean irritation score (MIS) of 1.333, Serenade WP was considered to be slightly irritating to the skin.

In an eye irritation study, slight to moderate irritation of the conjunctivae (redness, chemosis and/or discharge) was observed in the eyes of all animals dosed with 0.1 mL of Serenade WP. All signs of irritation were resolved by the 72 hour observation. Based on the MIS of 3.33 at the 1-hour observation point and the maximum average score (MAS) for 24-, 48- and 72-hours of 0.78, Serenade WP was to be non- to minimally/slightly irritating to the eye.

In a hypersensitivity study, a very mild delayed contact hypersensitivity response was observed in guinea pigs which were challenged and rechallenged with Serenade WP.

Label statements indicating that *Bacillus subtilis* QST 713 Technical Powder and Serenade MAX are potential sensitizers, and label precautions requiring personal protective equipment and judicious handling to minimize exposure in workers are required.

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

Within the available scientific literature, there are no reports that suggest *B. subtilis* has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *B. subtilis* strain QST 713 (Appendix I, Table 1).

3.1.3 Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use

In an acute oral study, no mortalities or significant signs of adverse clinical effects or abnormalities were observed in Sprague-Dawley rats administered 5000 mg/kg body weight (bw) of Serenade AS by oral gavage. The LD_{50} was determined to be > 5000 mg/kg.

In an acute pulmonary study, Sprague-Dawley rats were exposed for four hours to a time-weighted mean gravimetric concentration of 1.45 mg/L of Serenade AS using a nose-only exposure tube. There were no mortalities. All of the test animals exhibited some clinical signs including laboured/congested breathing, rales, few feces, small feces, slight to moderate urine staining, slight fecal staining, unkempt appearance, rough coat, dark material around the

eyes/nose/mouth and decreased food consumption. All evidence of adverse clinical signs were cleared by day 8. Slight body weight loss was observed in two male and two female rats between days 0 to 7 and in one female rat between days 7 to 14. The observed clinical signs and slight weight loss were not considered significant. Examination of the cranial, thoracic, abdominal and pelvic cavities upon necropsy did not reveal any abnormalities. The 4-hour inhalation LC_{50} was >1.45 mg/L in the rat.

In a dermal toxicity study, no mortalities were observed among Sprague-Dawley rats administered a dermal dose of 5000 mg/kg bw of Serenade AS. Clinical signs observed included coloured materials around the nose and eyes, dehydration, emaciation, few or no feces, desquamation and anogenital staining. Weight loss was observed in 2/10 rats between days 1 and 7 but normal weight gains were noted in these animals by day 14. There were no abnormal findings upon necropsy. The dermal LD_{50} was determined to be >5000 mg/kg bw.

In the dermal irritation study, six rabbits were each administered a dermal dose of 0.5 mL of undiluted Serenade AS for four hours. Very slight erythema/eschar formation was observed in four of the test animals at the 1 hour timepoint leading to well-defined erythema eschar formation by the 24-hour timepoint. At the 48-hour timepoint, erythema/eschar formation in one of these animals had subsided to very slight erythema. At the 72-hour timepoint, one animal continued to exhibit well-defined erythema/eschar and a second animal exhibited very slight erythema. All observations of erythema were reversed by day 7. The maximum individual irritation score was 2 (mildly irritating) at the 24-hour timepoint. The maximum irritation score (MIS) was 1.333 (slightly irritating) at the 24-hour timepoint. The maximum average score (MAS) over the 24-, 48- and 72-hour timepoints was 1 (slightly irritating). Based on the MIS and MAS, Serenade AS is considered to be slightly irritating to the skin.

In an eye irritation study, slight irritation of the conjunctivae (redness, discharge) was observed in the eyes of all animals dosed with 0.1 mL of Serenade AS. Redness persisted in all animals at the 24-hour timepoint but was absent by the 48 hour timepoint. The maximum individual irritation score was 4, occurring at the 1-hour observation timepoint. The maximum irritation score (MIS) was 3 at the 1-hour observation point. The maximum average score (MAS) over 24-, 48- and 72-hours was 0.67. Based on the MIS and MAS, Serenade AS is considered to be non- to minimally/slightly irritating to the eye.

In a hypersensitivity study, Serenade AS was not found to be a dermal sensitizer. Sensitization, however, may occur via other routes of exposure. Label statements indicating that Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use are potential sensitizers and label precautions requiring personal protective equipment and judicious handling to minimize exposure in workers and users are required (Appendix I, Table 2).

3.2 Occupational and Bystander Exposure and Risk Assessment

3.2.1 Occupational

When handled according to the label instructions, the potential routes of handler exposure to *B. subtilis* strain QST 713 are pulmonary, dermal and to some extent ocular.

The potential for dermal, eye and inhalation exposure for mixer/loaders, handlers and early-entry workers exists, with the major source of exposure to workers being dermal. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin or if metabolites were produced that could be dermally absorbed. *Bacillus subtilis* has not been identified as a wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals.

The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions. Label statements (i.e. Potential Sensitizer) and risk mitigation measures are required to protect populations that are likely to be primarily exposed to the products. Such exposure to handlers and early-entry workers is minimized if they wear gloves, long-sleeved shirts, long pants and, shoes and socks. In addition, commercial users are directed to wear a NIOSH approved respirator (with any N–95, P-95, R-95 or HE filter for biological products) and domestic users are directed to avoid breathing spray mists.

Eye irritation studies submitted for *Bacillus subtilis* QST 713 Technical Powder and Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use were found to be minimally irritating to the eye and, therefore, cautionary label statements and eye protection are not required.

3.2.2 Bystander

Overall the PMRA does not expect that bystander exposures pose an undue risk on the basis of the low toxicity/pathogenicity profile for *B. subtilis* strain QST 713 and the assumption that precautionary label statements are followed in the use of Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use.

In the case of Serenade MAX, Serenade ASO and Rhapsody ASO, the labels do not allow applications to turf, residential or recreational areas. Therefore, non-occupational dermal exposure and risk to adults, infants and children are low. As the use sites are agricultural, exposure of infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

In the case of Serenade Garden Concentrate and Serenade Garden Ready To Use in which domestic uses are permitted, the toxicology data are sufficient to conclude that the risks associated with these end-use products due to non-occupational exposure are negligible. No significant adverse effects were noted when Serenade AS, an acceptably similar end-use product, was subjected to toxicology testing at high doses via various routes of exposure.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

While the proposed use pattern may result in dietary exposure with possible residues in or on agricultural commodities, negligible to no risk is expected for the general population, including infants and children, or animals because *B. subtilis* strain QST 713 demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity/infectivity study. Furthermore, higher tier subchronic and chronic dietary exposure studies were not required because of the low toxicity of the MPCA and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity studies. Therefore, there is no concern for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.3.2 Drinking Water

Although *B. subtilis* strain QST 713 could potentially enter neighbouring aquatic environments via surface-water runoff and can potentially survive in water, no risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and it showed no harmful effects on animals that were exposed orally in Tier I acute oral toxicity and infectivity testing. The Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use labels instruct users not to allow the product to enter bodies of water during use or disposal. Furthermore, municipal treatment of drinking water will likely reduce the transfer of residues to drinking water. Therefore, potential exposure to *B. subtilis* strain QST 713 in surface and drinking water is negligible.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculation of acute reference doses (ARfDs) and acceptable daily intakes (ADIs) is not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e. no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that *B. subtilis* strain QST 713 is of low toxicity, is not pathogenic or infective to mammals and that infants and children are likely to be no more sensitive to the MPCA than the general population. Thus there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCA and other registered microorganisms that have a common mechanism of toxicity, do not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *B. subtilis* strain QST 713 to human health.

3.4 Maximum Residue Limits

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

Although the U.S. Food and Drug Administration has noted that some strains of *B. subtilis* have been isolated from food implicated in food poisoning, these strains demonstrated the ability to produce a highly heat-stable toxin that was not reported in *B. subtilis* strain QST 713. In addition, no such illnesses were reported for this MPCA in the United States where it has been registered for use on crops since 2000. Furthermore, there was no significant toxicity and no signs of pathogenicity observed when *B. subtilis* strain QST 713 was administered orally to rats. The establishment of a maximum residue limit (MRL) is therefore not required for *B. subtilis* strain QST 713 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulation.

3.5 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA's files, there is reasonable certainty no harm will result from aggregate exposure of residues of *B. subtilis* strain QST 713 to the general Canadian population, including infants and children, when the microbial pest control product is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Furthermore, few adverse effects from exposure to other strains of *B. subtilis* encountered in the environment have been reported. Even if there is an increase in exposure to this microorganism from the use of Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use, there should not be any increase in potential human health risk.

3.6 Cumulative Effects

The PMRA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Besides naturally occurring strains of *B. subtilis* in the environment, the PMRA is not aware of any other microorganisms or other substances that share a common mechanism of toxicity with this active ingredient. No cumulative effects are anticipated if the residues of *B. subtilis* strain QST 713 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

No studies were submitted to address the environmental fate and behaviour of *B. subtilis* strain QST 713. Environmental fate data (Tier II/III) are not required due to the absence of significant toxicological effects in non-target organisms in Tier I testing.

4.2 Effects on Non-Target Species (Appendix I, Table 3)

4.2.1 Effects on Terrestrial Organisms

A complete ecotoxicology package was submitted to address the risks of *B. subtilis* strain QST 713 to terrestrial organisms.

In an acute avian oral toxicity and pathogenicity study, *Bacillus subtilis* QST 713 Technical Powder was suspended in reverse-osmosis water and administered to 30 Northern Bobwhite quails (*Colinus virginianus*) by oral gavage at a dose of 1×10^8 CFU/g of body weight per day (equivalent to 5000 mg QST 713 Technical Powder/kg body weight/day) for five days. A negative control group consisting of 10 birds were similarly dosed with reverse-osmosis water. Approximately 2.5 hours after dosing on day 0, six birds in the treatment group exhibited signs of toxicity including depression, inability to stand, loss of coordination and a ruffled appearance. One of these birds was later found dead on the morning of day 1. A necropsy of this bird revealed a pale heart, pale spleen, mottled and pale kidneys and autolysis throughout. These findings were considered non-specific and the autolysis observed was attributed to an overnight death and subsequent holding in the brooder unit (38°C) until morning observation. Loss of coordination, reduced reaction to external stimuli, loss of coordination, slight wing droop, and shallow and rapid respiration were also observed in some of the birds on days 1 and 2. There were no significant differences in body weight, body weight gain or feed consumption between the negative control and the treatment group. Upon necropsy, no treatment-related findings were observed for any of the birds in the negative control group. There was no evidence of test substance replication, lesions, plaques or other abnormalities in the treatment group. The avian oral LD_{50} was determined to be >5000 mg/kg bw. *Bacillus subtilis* QST 713 Technical Powder was found to be practically non-toxic to bobwhite quail.

In mammals, few reports of adverse effects were reported in published scientific literature. *B. subtilis* has been implicated in cases of bovine mastitis and reproductive disorders in goats. No other reports of adverse effects were reported despite this microorganism's ubiquitous nature in the environment. Furthermore, the laboratory animal studies on the rat submitted in support of this registration and reviewed in Section 3.1, indicate that there is no pathogenicity and little toxicity from most routes of exposure at maximum hazard dose levels.

A number of terrestrial arthropod studies were submitted. The test species included the aphid parasitoid (*Aphidius rhopalosiphi*), parasitic hymenoptera (*Nasonia vitripennis*), green lacewing (*Chrisoperla carnea*), ladybird beetle (*Hippodamia convergens*) and predatory mite (*Typhlodromus pyri*).

In a 2-day contact toxicity study, *A. rhopalosiphi* were exposed to a freshly applied dry layer of *Bacillus subtilis* QST 713 Technical Powder on glass plates. The dose of the test substance corresponded to an application rate of 16 kg/ha (in 200 L water/ha). A negative, de-ionized water, and positive/toxic, dimethoate, control group were included. The mean mortality of *A. rhopalosiphi* after 48 hours exposure to QST 713 Technical Powder treated glass plates was 7.50% compared to 2.50% mortality in the control group. The mortality in the toxic standard group was 100%. The reproduction rate of the control organisms resulted in 11.10 mummies per female and in the group treated with *Bacillus subtilis* QST 713 Technical Powder, 8.29 mummies per female. The reproduction factor was calculated as 0.75. The reproduction value and the effects compared to the control for the mortality calculations were not significant. The 2-day LC_{50} and the NOEL values were both determined to be >16 kg/ha. There was no significant effect on *A. rhopalosiphi* when tested by contact exposure at a rate of 16 kg/ha of *B. subtilis* QST 713 Technical Powder on glass plates after 48 hours. This study was considered acceptable but supplemental as the test dose did not conform to guideline requirements for contact toxicity studies in terrestrial arthropods.

In a study on parasitic hymenoptera, adult wasps were exposed to 295, 1730, 10 200 or 60 000 ppm of *Bacillus subtilis* QST 713 Technical Powder via the diet (equivalent to 1.5×10^7, 9.1×10^7, 5.4×10^8, and 3.2×10^9 CFU/mL). Negative control, attenuated control and sterile filtrate groups were maintained concurrently. At study termination, the mortality in the negative control group was 28%. The attenuated control and sterile filtrate group mortalities were 45% and 37%, respectively. Unadjusted mortality in the 295, 1730, 10 200 and 60 000 ppm treatment groups was 29%, 25%, 47% and 81%, respectively. Abbott's formula was used to correct for the mortalities in the negative control group to yield adjusted mortalities of 24.1, 13, 1.9, 0, 25.9 and 74.1% for the attenuated control, sterile filtrate, and the 295, 1730, 10 200, and 60 000 ppm treatment groups, respectively. Based on the adjusted values, the LC_{50} of *Bacillus subtilis* QST 713 Technical Powder to parasitic hymenoptera was estimated to be 24 739 ppm. Clinical signs included immobility and lethargy but were not dose-dependent and were not considered treatment-related. It was concluded that *Bacillus subtilis* QST 713 Technical Powder was not pathogenic in parasitic hymenoptera.

In the green lacewing study, larvae were exposed to 600, 6000 or 60 000 ppm of *Bacillus subtilis* QST 713 Technical Powder via the diet. A concurrent negative control group was maintained. No signs of toxicity were noted among the larvae in the treatment groups. At test termination, the % mortality in the 600, 6000 and 60 000 ppm test groups was 13%, 7% and 10%, respectively. The pupation rates in these groups were 67%, 33% and 48%, respectively. Mortality in the negative control group was 7% with 63% pupation. Mortality in the treatment groups was comparable to the negative control group and was not dose-dependent. Pupation rates, however, were significantly lower at the 6000 and 60 000 ppm treatment levels, as compared to the negative control. The LC_{50} was greater than 60 000 ppm (100-times the maximum labelled rate) and the no observed effect concentration (NOEC) was 600 ppm. Significant adverse effects to green lacewing are not expected due to the proposed uses for QST 713 Technical Powder and its associated end-use products.

Adult ladybird beetles were exposed to 600, 6000, and 60 000 ppm of *Bacillus subtilis* QST 713 Technical Powder via the diet (equivalent to 1.2×10^7, 1.2×10^8, and 1.2×10^9 CFU/mL of diet).

A concurrent negative control group was included in the study. Clinical observations included lethargy and immobility in a small number of beetles in both control and treatment groups. At the end of the 30-day study, cumulative mortality in the control, 600 ppm, 6000 ppm and 60 000 ppm treatment groups was 15%, 25%, 19% and 17% , respectively. The relatively high mortality associated with the 600 ppm treatment group was observed in mainly one replicate (44% cumulative mortality) suggesting non-treatment related effects. The mortalities and clinical signs observed in the 6000 and 60 000 ppm treatment groups were similar to those observed in the control group. The mortalities were not occurring in a dose-dependent manner indicating that they were not treatment-related. The 30-day dietary LC_{50} and NOEC of *Bacillus subtilis* QST 713 Technical Powder in ladybird beetles is > 60 000 ppm.

In a 14-day contact toxicity study, predatory mites (*T. pyri)* were exposed to a freshly applied dry layer of *Bacillus subtilis* QST 713 Technical Powder on glass plates. The dose of the test substance corresponded to an application rate of 16 kg/ha (in 200 L water/ha). A negative, de-ionized water, and positive/toxic Dimethoate, control group were included. The mean mortality (including the number of dead and missing mites) of *T. pyri* was 39% compared to 12% in the negative control group and 72% in the toxic control group. The corrected mortality for *T. pyri* was 30.7%. The NOEL was ≤ 16 kg/ha (1.97 mg/cm^2, 1.97 x 10^6 CFU/cm^2). The LD_{50} was ≥16 kg/ha (1. 97 mg/cm^2, 1.97 x 10^6 CFU/cm^2). The glass plates treated with *Bacillus subtilis* QST 713 Technical Powder showed a greasy layer on their surfaces. This layer remained visible after 3 days of exposure. It cannot be excluded that the mortality of *T. pyri* was not affected by this physical property. No significant effects on the reproduction ability of *T. pyri* were observed in the group exposed to *Bacillus subtilis* QST 713 Technical Powder. There was no significant effect on *T. pyri* when tested by contact exposure at a rate of 16 kg/ha of *Bacillus subtilis* QST 713 Technical Powder on glass plates after 14 days. This study was considered acceptable but supplemental as the test dose and the period of observation did not conform to guideline requirements for contact toxicity studies in terrestrial arthropods.

Studies on honey bees were submitted. In the initial study, larvae of the honey bee (*Apis mellifera* L.) were exposed to target concentrations of 1000, 10 000, and 100 000 ppm of *Bacillus subtilis* QST 713 Technical Powder in larval bee diet. The test substance was administered via a single 10 µL droplet of the appropriate dilution to each cell. A negative control cell received 10 µL of larval bee diet and a positive control cell received 10 µL of a 5 ppm Dimethoate (known to have a detrimental effect to maturing larvae) solution in larval bee diet. No behavioural or morphological abnormalities were observed in any of the treatment groups. Mean percentage survival to capping (day 6) and to emergence (day 24) ranged from 67.5–81.25% and from 65–81.25%, respectively, for the treatment groups. No statistically significant differences in survival to capping and survival to emergence were observed between any of the treatment and control groups indicating that the 5 ppm positive control dose was either too low or that it had not been taken up by the larvae. A second study was carried out using a higher concentration of the positive control substance. In this second study, a single treatment group received 10 µL of 100 000 ppm of *Bacillus subtilis* QST 713 Technical Powder in larval bee diet. Concurrent control groups received 10 µL of 5 ppm or 100 ppm Dimethoate in larval bee diet, or 10 µL of larval bee diet. No behavioural or morphological abnormalities were observed in any of the treatment groups in the second study. Ineffectiveness of the 5 ppm dimethoate treatment was confirmed (91.25% survival to capping and emergence) while the

response to the 100 ppm dimethoate treatment was appropriate (8.75% survival to capping and to emergence). Survival of the 100 000 ppm treatment group was significantly lower than that of the negative control group (52.5% vs. 97.5% survival to both capping and emergence). The results of the first and second were pooled and analyzed using ANOVA and Duncan's multiple range test. The estimated survival % for the 1000, 10 000 and 100 000 ppm treatment groups was 84%, 71% and 61.67%, respectively. The negative control group had a survival rate of 96.88% while the 5 ppm and 100 ppm positive control groups had survival rates of 93.75% and 8.75%, respectively. The survival rate of larvae at the highest concentration of 100 000 ppm of *Bacillus subtilis* QST 713 Technical Powder was significantly lower than that of the negative control group. The LD_{50} of *Bacillus subtilis* QST 713 Technical Powder to honey bees was determined to be > 100 000 ppm (400- to 800-times the environmental effects concentration (EEC)). Significant adverse effects to the honey bee are not expected due to the proposed uses for *Bacillus subtilis* QST 713 Technical Powder and its associated end-use products.

The potential toxicity and pathogenicity of Serenade WP, an alternate formulation of *B. subtilis* strain QST 713, to honey bees pollinating on treated alfalfa crops (*Medicago sativa*) was assessed over a 30-day period which included six applications of Serenade WP (1.35 kg/ha). No consistent significant differences in adult or immature bee mortality, foraging activity or in the number of frames of adult honey bees and brood were observed between the Serenade WP-treated plots and the negative control plots. This study was considered as acceptable but supplemental as the viability of the test substance was not assessed.

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

For other soil microorganisms, no study was submitted to address the risks of *B. subtilis* strain QST 713 to soil microorganisms. Effects data are not required although the product is intended to control pest microorganisms, as *B. subtilis* is a normal component of the soil, and the organism is not expected to affect environmentally or economically important microbial species or microbiologically-mediated biogeochemical processes.

Studies on terrestrial plants were not submitted, however, no signs of phytotoxicity were noted in the efficacy trials (see Section 5.3). Furthermore, there have been no reports in the published literature of *B. subtilis* being a plant pathogen.

4.2.2 Effects on Aquatic Organisms

A complete ecotoxicology package was submitted to address the potential risk of *B. subtilis* strain QST 713 to aquatic organisms.

In a freshwater fish study, juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to five test concentrations of *Bacillus subtilis* QST 713 Technical Powder (1.7×10^6, 4.0×10^6, 7.7×10^6, 1.7×10^7, and 3.5×10^7 CFU/mL), a negative control (dilution water), a sterile filtrate control, an attenuated control (autoclaved QST 713 Technical Powder equivalent to 3.5×10^7 CFU/mL) or a broth concentrate control (5.3×10^6 CFU/mL) under static-renewal conditions for 30 days. Fish in the *B. Subtilis* QST 713 Technical Powder treatment groups were also given a diet of salmon started containing 3.68×10^6 CFU/g. Rainbow trout in the negative control, sterile filtrate control and 1.7×10^6 CFU/mL test substance groups appeared normal and healthy throughout the test. After 30 days of exposure, cumulative mortality in the 4.0×10^6, 7.7×10^6, 1.7×10^7, and 3.5×10^7 CFU/mL test substance groups was 10, 40, 40, 40 and 90%, respectively. Mortality in the attenuated control group was 100% by day 8 and 10% in the broth concentrate control group, indicating that mortalities were not due to pathogenicity, but to toxicity or fouling of the test solution with high concentrations of cellular material (present in the test system at >10X the maximum hazard test dose or 1000X the application rate). Probit analysis of the mortality data determined that the 30-day LC50 for aqueous exposure of rainbow trout to *B. Subtilis* QST 713 Technical Powder was 1.4×10^7 CFU/mL with 95% confidence limits of 9.0×10^6 to 2.4×10^7 CFU/mL. No treatment-related signs of infections were observed in any of the fish necropsied. No hazard to freshwater fish populations is expected from terrestrial uses of *B. subtilis* strain QST 713.

Two aquatic invertebrate studies were submitted on *Daphnia magna*. In the first study, neonate daphnids were exposed to nominal test concentrations of 13, 25, 50, 100 or 200 mg/L of *Bacillus subtilis* QST 713 Technical Powder prepared in well water for 48 hours. Actual concentrations were not determined and, consequently, this study was considered acceptable but supplemental. In the second study, daphnids were exposed to 7.9×10^5, 1.8×10^6, 3.4×10^6, 7.3×10^6, or 2.0×10^7 CFU/mL of *Bacillus subtilis* QST 713 Technical Powder. Viability of the test substance was established. Concurrent negative control, sterile filtrate control, attenuated control and broth concentrate control (2.4×10^6 CFU/mL) groups were maintained. After 21 days of exposure, significant reductions in survival were observed between all groups, with the exception of the sterile filtrate control and the 7.9×10^5 CFU/mL treatment group, and the negative control. The 21-day LC50 was calculated to be 1.6×10^6CFU/mL. The NOEC, based on reproduction and growth, was determined to be 7.9×10^5 CFU/mL. Since the EC% was above the maximum hazard test dose of 10^6 CFU/mL, no hazard to aquatic invertebrate populations is expected from terrestrial application of *B. subtilis* strain QST 713 at the label use rates.

Adult grass shrimp (*Palaemonetes pugio*) were exposed to *Bacillus subtilis* QST 713 Technical Powder at a dose of 4.0×10^6 CFU/g of diet for 30 days. Negative control, heat-attenuated control, sterile filtrate control and whole broth (2.8×10^6 CFU/g diet) groups were also maintained. There were no mortalities, abnormal physical appearance or behaviour observed in any of the shrimp in the treatment or control groups. Grass shrimp in all control and treatment groups molted. Measurements of growth established that the growth of the test organism was not adversely affected by the dietary exposure to the test substance. No evidence of inflammatory response or necrosis were observed upon necropsy. The LC_{50} of *Bacillus subtilis* QST 713 Technical Powder to grass shrimp via the diet was, therefore, determined to be $>4.0 \times 10^6$ CFU/g. No significant adverse effects are expected for grass shrimp due to the proposed uses for *Bacillus subtilis* QST 713 Technical Powder and its associated end-use products.

The effect of *B. subtilis* strain QST 713 on single cell green alga (*Scenedesmum subspicatus*), was studied at measured average concentrations of 0, 38, 3.9×10^2, 5.2×10^3, 3.5×10^4, and 5.1×10^5 CFU/mL under static conditions for 72 hours. No significant inhibitory effects were observed at the highest dose tested.

5.0 Value

5.1 Effectiveness Against Pests

5.1.1 Acceptable Efficacy Claims for Serenade MAX

Various formulations of Serenade including Serenade MAX, Serenade WP and Serenade WPO were assessed in the efficacy trials.

5.1.1.1 Crop Group 3 (Bulb vegetables): onion, garlic, shallots, and leeks.

Neck rot (*Botrytis allii*) on onions
One US trial with low disease pressure was reviewed. Serenade WP at 4.4-6.7 kg/ha (3.0–4.6 kg of Serenade MAX/ha) provided good control (69–87%) of neck rot on onions. Increasing the rate to 6.7 and 8.9 kg/ha provided 100% control. Suppression of neck rot on onions with Serenade MAX at 3.0–4.5 kg/ha is therefore supported. This claim is extended to include other Crop Group 3 Crops (Bulb Vegetables) which are susceptible to this disease.

Leaf blight (*Botrytis squamosa*) on onions
Two US trials of Serenade WP were assessed. Results showed that under low disease pressure, when applied 4.4–6.7 kg/ha (3.0–4.6 kg of Serenade MAX/ha) provided moderate control of Botrytis leaf blight on onions. Suppression of leaf blight on onions with Serenade MAX at 3.0–4.5 kg/ha is supported. This claim is extended to include other Crop Group 3 Crops (Bulb Vegetables) which are susceptible to this disease.

Downy mildew (*Peronospora destructor*) on onions
One US trial was assessed for this claim. Under high disease pressure, when applied at 4.5, 6.7 and 8.9 kg /ha, Serenade WP (3.0, 4.6 and 6.1 kg/ha of Serenade MAX) provided 25, 38 and 50% control of downy mildew on onion. The commercial standard, Bravo, provided a level of control equivalent to that obtained with Serenade WP. Suppression of downy mildew on onions with Serenade MAX at 3.0–6.0 kg/ha is supported. This claim is extended to include other Crop Group 3 Crops (Bulb Vegetables) which are susceptible to this disease.

5.1.1.2 Crop Group 9 (Cucurbits): cucumber, cantaloupe, melon, muskmelon, squash, watermelon and pumpkin

Powdery mildew (*Erysiphe cichoracearum, Sphaerotheca fuliginea*) on cucurbits
Eight trials conducted in the US were reviewed on the following crops: zucchini, cantaloupe, honeydew melon and pumpkin. Under moderate-high disease pressure, when applied at 2.2, 4.4, and 8.9 kg/ha, Serenade WP (1.5, 3.0 and 6.1 kg/ha of Serenade MAX) provided 66–83% control

of disease severity on the leaves. The commercial standard gave 99% control. Suppression of powdery mildew on cucurbits with Serenade MAX at 3.0–6.0 kg/ha is supported.

5.1.1.3 Crop Group 8 (Fruiting Vegetables): pepper, tomato, eggplant, ground cherry, tomatillo and other fruiting vegetables

Early blight (*Alternaria solani*) on tomatoes
One tomato trial and one potato trial conducted in US were assessed. At the rate of 4.4 kg of Serenade MAX/ha, 42% control of early blight on tomato was obtained. The commercial standard provided 95–98% control. The claim that Serenade MAX suppresses tomato early blight is supported at 4.5 kg/ha but additional trials are required to demonstrate consistency. The claim is extended to include other fruiting vegetables which are susceptible to this disease.

Powdery mildew (*Leveillula taurica*) on pepper
Two US trials on bell pepper with high disease pressure were reviewed. The results showed that with Serenade WP at 4.4, 6.7 and 9 kg/ha, (3.0, 4.6 and 6.1kg/ha of Serenade MAX) percent control of powdery mildew on pepper ranged from 12 to 60%. The claim that Serenade MAX suppresses powdery mildew on pepper only is supported at 3.0–6.0 kg/ha. This pathogen has not been reported to cause powdery mildew on other fruiting vegetables in Canada.

Powdery mildew (*Erysiphe orontii*) on tomatoes
One US trial was provided in support of this claim. Serenade WP at the rate of 2.2 and 4.4 kg/ha (1.5 and 3.0 kg of Serenade MAX/ha) provided 34 and 62% control of powdery mildew on tomato. The claim that Serenade MAX suppresses powdery mildew on tomato is supported at 3.0–6.0 kg/ha. The claim is not extended to other fruiting vegetables since *E. orontii* is host-specific to tomato.

5.1.1.4 Grapes

Powdery mildew (*Uncinula necator*) on grapes
Three US trials with moderate to high disease pressures were submitted and considered for review. When applied at 4.4 and 8.9 kg/ha, Serenade WP (3.0 and 6.1 kg of Serenade MAX/ha) significantly reduced both disease incidence and disease severity compared to the untreated control. The claim that Serenade MAX suppresses grape powdery mildew is supported at 3.0–6.0 kg/ha.

Botrytis rot (*Botrytis cinerea*) on grapes
Three US trials with moderate disease pressure were reviewed. When Serenade WP was applied at 4.4, 6.7 and 8.9 kg/ha (3.0, 4.6 and 6.1 kg of Serenade MAX/ha), disease severity reduced by 32.5, 50 and 64%, respectively. The claim that Serenade MAX suppresses botrytis rot on grapes is supported at 3.0–6.0 kg/ha.

Sour rot (complex of pathogens) on grapes
Two efficacy trials conducted in the US were assessed. Serenade WP at 4.4 and 8.9 kg/ha (3.0–6.1 kg/ha Serenade MAX) provided 25–29% and 58–64%, respectively. The claim that Serenade MAX suppresses sour rot on grapes is supported at 3.0–6.0 kg/ha.

5.1.1.5 Leafy Vegetables: lettuce, celery

Downy mildew (*Bremia lactucae*) on lettuce
Three lettuce trials with high disease pressure conducted in the US were submitted. Serenade WP at 4.6 and 8.9 kg/ha (3.0 and 6.1 kg/ha of Serenade MAX) significantly reduced the downy mildew on lettuce compared to control treatment. The claim that Serenade MAX suppresses lettuce downy mildew is supported at 3.0–6.0 kg/ha. The claim is not extended to other leafy vegetables since *B. lactucae* is host-specific to lettuce.

Powdery mildew (*Erysiphe cichoracearum*) on lettuce
One lettuce trial was reviewed. Under low disease pressure Serenade WP at 2.2 and 4.4 kg/ha (1.5 and 3.0 kg/ha of Serenade MAX) provided 79–92% and 92–95% control of powdery mildew on lettuce. The claim that Serenade MAX suppresses lettuce powdery mildew is supported at 3.0–6.0 kg/ha. The claim is not extended to other leafy vegetables since *E. cichoracearum* is host-specific to lettuce.

Sclerotinia leaf drop (*Sclerotinia minor*) on lettuce
Two US efficacy trials were submitted. Under low disease pressure, Serenade WP at 4.5 and 6.7 kg/ha (3.0 and 4.6 kg/ha of Serenade MAX) provided 38–41% and 54–55% control of Sclerotinia leaf drop. Under moderate-high disease pressure, 6.7 kg/ha provided 60% control of Sclerotinia leaf drop. The claim that Serenade MAX suppresses head and leaf drop of lettuce is supported at 3.0–6.0 kg/ha.

Pink rot (*Sclerotinia sclerotiorum*) on celery
One US trial on celery with low disease pressure was reviewed. Serenade WP at 4.4 and 6.7 kg/ha (3.0 and 4.6 kg/ha of Serenade MAX) provided 24% and 51% control of pink rot on celery. Based on the submitted data, the claim that Serenade MAX suppresses pink rot on celery is supported at 3.0–6.0 kg/ha.

5.1.1.6 Legume vegetables: beans, peas and lentils

White Mould (*Sclerotinia sclerotiorum*) on beans
One trial on lima bean with moderate disease pressure and another trial on snap bean with low disease pressure were assessed. Based on disease index (DI) where 1=healthy plants and 4=dead plants, when applied on lima bean for control of white mould, Serenade WP at 6.7 kg/ha had DI=1.91 vs 2.33 for the untreated control. However, when applied at 4.4 and 8.9 kg/ha on snap bean, Serenade WP provided 33.7 and 29.7% control of white mould, respectively. The claim that Serenade MAX suppresses white mould (*Sclerotinia sclerotiorum*) on beans, peas and lentils is supported at the rate of 3.0–6.0 kg/ha.

5.1.1.7 Crop Group 11 (Pome fruit): apple, crabapple, pear, Oriental pear, quince, mayhaw and loquat

Apple and pear fire blight (*Erwinia amylovora*)
Five apple and two pear trials were reviewed. All were conducted in the US except for 1 apple trial which was conducted in BC. Under moderate to high disease pressures, Serenade WP at

6.7 kg/ha (4.6 kg/ha of Serenade MAX) resulted in 36–46% control of apple fire blight. In the pear trial with moderate disease pressure, when applied at 4.4–8.9 kg/ha, Serenade WP (3–6.1 kg/ha of Serenade MAX) gave 26.2–65% control. Serenade WP at 8.9 kg/ha provided the similar level of fireblight control as the commercial standard. Therefore, the claim that Serenade MAX suppresses fire blight on apple and pear is supported at 3.0–6.0 kg/ha.

Apple and pear scab (*Venturia inaequalis; V. pirina*)
Nine efficacy trials conducted in the US (six apple and three pear trials) were reviewed. When applied at 6.7, 8.9 and 11.1 kg/ha, Serenade WP (4.6, 6.1 and 7.6 kg/ha of Serenade MAX) provided 16–50% control of apple scab under moderate and high disease pressure. The studies with pear demonstrated that under high disease pressure, 8 kg/ha of Serenade WP (5.5 kg/ha Serenade MAX) provided up to 92% control of disease severity on fruits. The claim that Serenade MAX suppresses apple and pear scab is supported at 3.0–6.0 kg/ha.

Apple powdery mildew (*Podosphaera leucotricha*)
Eight apple trials were reviewed to support this claim. Under high disease pressure, Serenade WP at 4.4, 6.7 and 8.9 kg/ha (3.0, 4.6 and 6.1 kg/ha Serenade Max) gave up to 77% control of apple powdery mildew. The claim that Serenade MAX suppresses apple powdery mildew is supported at 3.0–6.0 kg/ha.

5.1.1.8 Tank-Mix Claims

The applicant proposed the use of Serenade MAX in tank-mix with other registered fungicides for control of different diseases on several crops. However, although in the US and in other countries the Serenade product lines are commonly tank-mixed with different chemical fungicides, no specific information about the tank-mix partners of Serenade MAX was provided. Therefore, the tank-mix claims are not supported.

5.1.2 Acceptable Efficacy Claims for Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready to Use

For registration of the four Serenade suspension products, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready to Use, data from trials conducted with Serenade AS and Rhapsody AS were submitted. The efficacy data submitted for Serenade AS and Rhapsody AS were accepted for review to support the claims of Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready to Use. Although Serenade Garden Ready to Use formulation has a higher amount of active ingredient compared to the Serenade Garden Concentrate once it is diluted with water, this is acceptable due to the wide variation in the number of colony forming units/g present in each batch that is manufactured. This variation in the colony forming units/g due to the manufacturing process is much larger than the rate difference between the two products. Therefore, the rates applied using either the concentrate or the ready to use formulation are essentially comparable. In addition, Serenade end use products are reduced-risk pesticides that do not pose an unacceptable risk to human health or the environment. Serenade Garden Ready To Use also poses a lower risk to users compared to the concentrate product since the mixing/dilution step is avoided.

The efficacy of Serenade AS and Rhapsody AS was not consistent across the trials, particularly under moderate to high disease pressure. Therefore, the level of disease control was accepted as suppression and not control for the four products.

5.1.2.1 Lettuce, celery, chicory and endive

Control of Downy mildew (*Bremia lactucae*)on lettuce
Two trials with low to moderate disease pressure were reviewed. Serenade AS at 6.75, 8.97, 11.21 and 22.42 kg/ha provided 48.6%, 70.4%, 72.1% and 40.0% control of downy mildew (*Bremia lettuce)* on lettuce. Suppression of downy mildew on lettuce with Serenade ASO at 7.0–15.0 L/ha is supported. This support was extended to Serenade Garden Concentrate and Serenade Garden Ready to Use for home and garden grown lettuce. This claim was not extended to other leafy vegetables because *Bremia lactucae* is a host specific pathogen.

Control of Sclerotinia head and leaf drop (*Sclerotinia sclerotiorum* and *Sclerotinia minor*) on lettuce
Three trials were reviewed. Under high disease pressure, Serenade AS at 9.3 L/ha provided 27.7% control of lettuce head and leaf drop caused by *Sclerotinia minor* and 9.6% control of head and leaf drop caused by *S. sclerotiorum*. Under low to moderate disease pressure, Serenade AS at 4.7, 9.3 and 18.7 L/ha provided 37.6%, 50.2% and 54.7% control of lettuce leaf drop caused by *S. minor*. Suppression of Sclerotinia head and leaf drop on lettuce, chicory and endive with Serenade ASO at 5.0 to 15.0 L/ha is supported. This claim was extended to Serenade Garden Concentrate and Serenade Garden Ready to Use for home and garden grown lettuce and chicory.

Control of pink rot (*Sclerotinia sclerotiorum*) on celery
One trial on celery with moderate disease pressure was reviewed. Serenade AS at 4.7, 9.3 and 18.7 L/ha provided 37.5%, 54.4% and 52.3% control of Sclerotinia pink rot on celery. Suppression of Sclerotinia pink rot on celery with Serenade ASO at 5.0 to 15.0 L/ha is supported. This claim was extended to Serenade Garden Concentrate and Serenade Garden Ready to Use for home and garden grown celery. Other proposed crops are not supported.

5.1.2.2 Crop Group 5 (Brassica vegetables): broccoli, cabbage, cauliflower, brussels sprouts, collards, kale, kohlrabi, mustard greens, mustard spinach and other brassica crops

Control of downy mildew (*Peronospora parasitica*)
Two trials on broccoli and cauliflower with moderate disease pressure were reviewed. Serenade AS at 9.3 and 18.7 L/ha provided 18.4 -21.1% control of leaf lesions on broccoli and at 9.3–37.34 L/ha provided 38.1–65.5% control of leaf lesion on cauliflower. Suppression of downy mildew on broccoli, brussels sprouts, cauliflower, cabbage, kale and kohlrabi with Serenade ASO at 10.0 L/ha is supported. This claim was extended to other brassica crops as well as rutabaga, radish and turnip of crop group 1 (root and tuber vegetables) as they are susceptible to downy mildew (*Peronospora parasitica*). This claim was also extended to Serenade Garden Concentrate and Serenade Garden Ready to Use for home and garden use on brassica crops mentioned above.

5.1.2.3 Crop group 8 (Fruiting vegetables): pepper, tomato, eggplant, ground cherry, tomatillo and pepino

Suppression of powdery mildew (*Leveillula taurica*) on pepper
Three trials on bell peppers with moderate disease pressure were reviewed. Serenade AS at 4.7, 9.4 and 18.7 L/ha provided 10.9% (range: 0–77.1%), 11.0% (range: 0–40.0%) and 19.0% (range: 0–57.1%) control of powdery mildew incidence and 38.5% (range: 0-97.1%), 51.0% (range: 0–88.2%) and 30.3% (range: 0–64.9%) control of powdery mildew severity on bell pepper. Suppression of powdery mildew on pepper with Serenade ASO at 5.0 to 15.0 L/ha is supported. This claim is extended to Serenade Garden Concentrate and Serenade Garden Ready to Use for home and garden and includes all types of peppers (bell pepper, chili pepper, pimento and sweet pepper).

5.1.2.4 Crop group 9 (Cucurbits vegetables): cucumber, cantaloupe, melon, muskmelon, squash, watermelon and pumpkin

Control of Powdery mildew (*Sphaerotheca fuliginea*) on zucchini and cantaloupe
Four trials with high disease pressure were reviewed. Serenade AS at 4.48, 9.3 and 18.7 L/ha provided 63.8%, 54.2% and 67.8% control of powdery mildew severity on lower leaves and 92.5%, 84.7% and 76.3% control of powdery mildew severity on upper leaves of zucchini. Serenade AS at 9.3 L/ha provided 30.5% and 89.4% control of powdery mildew on lower and upper leaves of cantaloupe. Serenade AS also provided good control (72.7–82.4%) of powdery mildew on zucchini stalks and reduced (68.1%) the sporulation on leaves. Suppression of powdery mildew on cucurbits with Serenade ASO at 5.0 to 15.0 L/ha is supported. This claim is extended to Serenade Garden Concentrate and Serenade Garden Ready to Use for home and garden use on cucurbit crops. The following crops are supported: cucumber, cantaloupe, melon, muskmelon, squash, watermelon and pumpkin.

5.1.2.5 Crop Group 11 (Pome fruits): apple, crabapple, quince, mayhaw, loquat, pear and Oriental pear

Control of Fire blight (*Erwinia amylovora*) on apple
Three trials were reviewed. A single application of Serenade AS at 37.3 L/ha controlled blossom blight incidence of apple by 43.0% under moderate disease pressure. Serenade AS at 4.7, 9.4 and 18.7 L/ha provided 63%, 56% and 61% control of blossom blight and 43%, 49% and 41% control of shoot blight incidence on apple under low to moderate disease pressure. Serenade AS 14.0 L/ha was tank-mixed with an adjuvant, Biotune at 1.75L/ha and provided 61% control of blossom blight and 56% control of shoot blight under high disease pressure. Suppression of fire blight (blossom blight and shoot blight) on pome fruits with Serenade ASO at 5.0 to 15.0 L/ha is supported.

Control of Powdery mildew (*Podosphaera leucotricha*) on apple
Two trials with low to moderate disease pressure were reviewed. Serenade AS at 9.3 and 18.7 L/ha provided 68% and 65.5% control of leaf powdery mildew incidence and 87.0% and 72.0% control of leaf powdery mildew severity on apples. Suppression of powdery mildew on pome fruits with Serenade ASO at 10.0 to 15.0 L/ha is supported.

5.1.2.6 Grape

Control of gray mould (bunch rot) (*Botrytis cinerea*)
Three trials with moderate disease pressure were reviewed. Serenade AS at 9.3 L and 18.7 L/ha provided 12.4% and 25.9% control of disease incidence and 28.8% and 49.6% control of disease severity. Serenade AS at 3.58 and 10.76 kg/ha provided 32.7% and 25.8% control of disease incidence and 76.5% and 68.5% control of disease severity. Suppression of Botrytis gray mould on grape with Serenade ASO at 4.0–15.0 L/ha is supported.

Control of powdery mildew (*Uncinula necator*)
Three trials with moderate to high disease pressure were reviewed. Serenade AS at 9.3 and 18.7 L/ha provided 44.2% and 45.6% control of powdery mildew incidence and 47.15% and 59.0% control of powdery mildew severity on fruits. Suppression of powdery mildew on grapes with Serenade ASO at 9.0–15.0 L/ha is supported.

Control of Sour rot (a complex of pathogens such as *Aspergillus niger, Alternaria tenuis, Botrytis cinerea, Cladosporium herbarum, Rhizopus arrhizus, Penicillium* spp. and others)
Two trials with moderate disease pressure were reviewed. Serenade AS at 3.58, 7.18, 10.76 and 21.52 L/ha provided 25.0%, 35.2% 30.2% and 26.7% control of sour rot incidence and 41.2%, 61.5%, 69.5% and 42.2% control of sour rot severity on grapes. Suppression of sour rot on grapes with Serenade ASO at 5.0–15.0 L/ha is supported.

5.1.2.7 Ornamentals

Poinsettia

Control of powdery mildew (*Oidium* spp.)
One trial was submitted. Serenade AS at 3.78 and 7.56 L in 378 L of water provided 99.5% and 99.1% control of powdery mildew colonies on leaves and 91.0% and 82.1% control of powdery mildew colonies on bracts of poinsettia. Suppression of powdery mildew on poinsettia with Rhapsody ASO at 1.0 to 2.0 L in 100 L of water is supported. This claim is extended to Serenade Garden Concentrate and Serenade Garden Ready to Use.

Control of Botrytis blight (*Botrytis cinerea*)
One trial with low disease pressure was reviewed. Serenade AS at 3.78 and 7.56 L in 378 L of water provided 69.5% and 59.2% control of Botrytis leaf lesion incidence. Suppression of Botrytis blight on poinsettia with Rhapsody ASO at 1.0 to 2.0 L in 100 L of water is supported. This claim is extended to Serenade Garden Concentrate and Serenade Garden Ready to Use.

Garden phlox and dwarf bee balm

Powdery mildew (*Erysiphe cichoracearum*)
One trial was reviewed. Serenade AS at 3.78 and 7.56 L in 378 L of water provided 29.2% and 83.3% control of powdery mildew on phlox leaves under low disease pressure and 69.3% and 52.0% control of powdery mildew on dwarf bee balm leaves under moderate disease pressure. Suppression of Powdery mildew on garden phlox and dwarf bee balm with Rhapsody ASO at

1.0 to 2.0 L in 100 L of water is supported. This claim is extended to Serenade Garden Concentrate and Serenade Garden Ready to Use.

Geranium

Botrytis leaf blight (*Botrytis cinerea*)
Two trials with moderate disease pressure were reviewed. Serenade AS at 3.78 and 7.56 L in 378 L of water provided 43.6% and 41.1% control of Botrytis leaf lesions on geranium. Suppression of Botrytis leaf blight on geranium with Rhapsody ASO at 1.0 to 2.0 L in 100 L of water is supported. This claim is extended to Serenade Garden Concentrate and Serenade Garden Ready to Use.

Leaf spot (*Xanthomonas campestris* pv. *pelargonii*)
One trial with moderate disease pressure was reviewed. Serenade AS at 1.89, 3.78 and 7.56 L in 378 L of water provided 38.3%, 27.1% and 81.6% control of Xanthomonas leaf spot on zonal geranium. Suppression of leaf spot (*Xanthomonas campestris* pv. *pelargonii*) on geranium with Rhapsody ASO at 1.0 to 2.0 L in 100 L of water is supported. This claim is extended to Serenade Garden Concentrate and Serenade Garden Ready to Use.

Delphinium

Leaf spot (*Pseudomonas delphinii*)
One trial with low to moderate disease pressure was reviewed. Serenade AS at 3.78 L and 7.56 L in 378 L of water provided 84.2% and 5.0% control of leaf spot on delphinium. The higher rate of Serenade AS (7.56 L) caused phytotoxicity. Suppression of leaf spot (*Pseudomonas delphini*) on delphinium with Rhapsody ASO at 1.0 L in 100 L of water is supported. This claim is extended to Serenade Garden Concentrate and Serenade Garden Ready to Use.

Aglaonema

Leaf spot (*Xanthomonas* spp.)
One trial with low to moderate disease pressure was reviewed. Rhapsody AS at 3.78, 5.67 and 7.56 L in 378 litres of water provided 52.8%, 76.8% and 100.0% control of leaf spot on aglaonema. Suppression of leaf spot (*Xanthomonas* spp.) on aglaonema with Rhapsody ASO at 1.0 to 2.0 L in100 L of water is supported. This claim is extended to Serenade Garden Concentrate and Serenade Garden Ready to Use.

Roses

Powdery mildew (*Sphaerotheca* spp.)
Two trials with low to high disease pressure were reviewed. Serenade AS at 3.78 L in 378 L of water applied at 7 day intervals provided an average of 73.6% (range: 29.2–95.7%) control of powdery mildew incidence but gave poor control (3.2–12.1%) at 14 day application intervals. Suppression of powdery mildew on roses with Rhapsody ASO at 1.0 to 2.0 L in 100 L of water applied at 7 day intervals is supported. This claim is extended to Serenade Garden Concentrate and Serenade Garden Ready to Use.

Leatherleaf fern

Anthracnose (*Colletotrichum acutatum*)
One trial with moderate disease pressure was reviewed. Serenade AS at 3.78 and 7.56 L in 378 L of water provided 83.1% and 64.1% control of anthracnose on leatherleaf fern. Suppression of Anthracnose on Leatherleaf fern with Rhapsody ASO at 1.0 to 2.0 L in 100 L of water is supported. This claim is extended to Serenade Garden Concentrate and Serenade Garden Ready to Use.

White Petunia

Botrytis lesion (*Botrytis* cinerea)
One trial with low disease pressure was reviewed. Serenade AS at 3.78 and 7.56 L in 378 L of water provided 21.0% and 26.3% control of Botrytis incidence. Suppression of Botrytis lesion on petunia with Rhapsody ASO at 1.0 to 2.0 L in 100 L of water is supported. This claim is extended to Serenade Garden Concentrate and Serenade Garden Ready to Use.

5.1.2.10 Tank-Mix

The applicant has proposed that Serenade ASO may be tank mixed with other registered fungicides to enhance the control of different diseases on several crops. However, no specific information about the tank-mix partners of Serenade ASO was provided. Therefore, the tank-mix claims are not supported.

5.2 Extrapolations

The data reviewed for *Botrytis cinerea* and *Sclerotinia sclerotiorum*, in conjunction with low phytotoxicity concerns of Serenade MAX, were sufficient to extrapolate the claims of control of diseases caused by these pathogens to other crops: (i) the claim that Serenade MAX suppresses *B. cinerea* when applied at 3.0–6.0 kg product/ha, can be extrapolated from grapes to include asparagus, Crop Group 13 Crops (bushberries and caneberries), field beans (all varieties, dried or succulent), fruiting vegetables (eggplant, ground cherry, pepino, all varieties of peppers, tomatillo and tomatoes) and strawberry. This claim cannot be extrapolated to include any orchard fruits (pome fruits, stone fruits), since there are differences in the volumes of water used, coverage of foliage based on application equipment and demonstration of efficacy on stored fruits which should be directly assessed in efficacy trials; (ii) the claim that Serenade MAX suppresses *S. sclerotiorum* when applied at 3.0–6.0 kg/ha can be extrapolated from celery and field beans to Crop Group 5 (cole) crops (watery soft rot).

Serenade AS at 4.0 to 15.0 L/ha suppressed gray mould (*Botrytis cinerea*) on grapes. Based on these results, the claims of suppression of gray mould on: asparagus, bushberry, caneberry and other berry crops (except strawberry), legume vegetables, fruiting vegetables and strawberry at 4.0 to 15.0 L/ha is supported. Since it was shown that Serenade AS at 5.0–15.0 L/ha suppressed Sclerotinia diseases of lettuce and celery, it can be expected that Serenade AS, and therefore Serenade ASO, Serenade Garden Concentrate and Serenade Garden Ready to Use, will also

suppress Sclerotinia white rot (*Sclerotinia sclerotiorum*) on legumes. Extrapolations were possible because these two pathogens are non-host specific and attack a large number of crops.

5.3 Phytotoxicity

Serenade MAX and other Serenade products were tested at different concentrations on various crops. For the majority of the trials submitted, there were no reports of adverse effects or phytotoxicity to the host plant as a result of applying Serenade MAX. Of the trials that did report some adverse effects, these effects were not repeated in other trials for that crop, and therefore it may have been caused by agents present in the tank of the sprayer before that application was made. In general, there are no concerns of phytotoxicity of Serenade MAX when applied as directed.

Serenade AS and Rhapsody AS were tested at different concentrations on a number of crops and ornamentals. Phytotoxicity or any other adverse effects of Serenade AS and Rhapsody AS on tested crops and ornamentals were not observed except when a higher concentration of Serenade AS (2.0% solution) was applied on delphinium. In general, there are no concerns of phytotoxicity of Serenade ASO, Rhapsody AS, Serenade Garden Concentrate and Serenade Garden Ready to Use when applied as directed on the label.

5.4 Impact on Succeeding Crops

Data on the impact of Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready to Use on succeeding crops were not submitted for review.

5.5 Economics

No market analysis was provided for Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready to Use.

5.6 Sustainability

5.6.1 Survey of Alternatives

The alternative active ingredients registered for control or suppression of claims on the Serenade MAX label are presented in Appendix I, Table 4. The alternative active ingredients registered for control or suppression of claims on the Serenade ASO and Rhapsody ASO labels are presented in Appendix I, Table 5.

No alternatives are available for the following pest/crop combinations: Botrytis blight on asparagus, watery soft rot on cole crops, sour rot on grapes, pink rot on celery, rust on mint, leaf spot on delphinium, leaf spot on aglaonema and anthracnose on leatherleaf fern.

5.6.2 Compatibility with Current Management Practices Including Integrated Pest Management

The applicant stated that Serenade MAX is compatible with registered products such as copper, sulfur and other foliar-applied micronutrients, insecticides, fungicides and non-penetrating, non-ionic products such as silicon surfactants. However, no data were provided to confirm the compatibility claims. The applicant has also claimed that Serenade ASO and Rhapsody ASO are compatible with many commonly used pesticides, fertilizers, adjuvants and surfactants but no data was provided to confirm these compatibility claims. Therefore, all references to tank mixing with other pest control products, fertilizers, adjuvants and surfactants are removed from the labels. Serenade ASO and Rhapsody ASO can be used as companion products in an integrated disease suppression program.

5.6.3 Resistance Management

The active ingredient in Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready to Use, *Bacillus subtilis* strain QST 713, has a multiple site mode of action which will make it difficult for pathogens to develop resistance against these products. In addition, the use of microbial pesticides in rotation with conventional pesticides will enable growers to manage resistance development more effectively.

5.6.4 Contribution to Risk Reduction and Sustainability

Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready to Use are microbial pest control products and intended as an alternative to conventional fungicides to suppress many diseases on crops and ornamentals. These products are designated as reduced-risk pesticides in both Canada and the U.S.

6.0 Formulants and Microcontaminants of Health or Environmental Concern

6.1 Toxic Substances Management Policy Considerations

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

While reviewing *B. subtilis* strain QST 713, the PMRA took into account the federal Toxic Substances Management Policy and followed its Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*. Substances associated with its use were also considered, including microcontaminants in

the technical product, *Bacillus subtilis* QST 713 Technical Powder and formulants in the end-use products, Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use. The PMRA has reached the following conclusions:

- *Bacillus subtilis* strain QST 713 does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. There are also no formulants, contaminants or impurities present in the end-use products that would meet the TSMP Track-1 criteria.

Therefore, the use of Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use is not expected to result in the entry of Track-1 substances into the environment.

6.2 Formulants of Health Concern

Bacillus subtilis strain QST 713 does not contain any contaminants of health or environmental concern identified in Canada Gazette Part II, Volume 139, Number 24, pages 2641-2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.*

The end-use products, Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use, do not contain any formulants of health or environmental concern identified in Canada Gazette Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.*

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for *B. subtilis* strain QST 713 (*Bacillus subtilis* QST 713 Technical Powder), Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use are adequate to assess their safety to human health and the environment. The technical material was adequately characterized and the specifications were supported by the analysis of a sufficient number of batches. A method to identify the strain of the active ingredient was proposed but results were not submitted.

Storage stability data were sufficient to support an expiration date of one year for *Bacillus subtilis* QST 713 Technical Powder when stored at ambient temperatures. Storage stability data were not submitted for the end-use product, Serenade MAX. Until such data are submitted, an expiration date of one year when stored at 25°C is required.

Storage stability data for Serenade ASO, Rhapsody ASO and Serenade Garden Concentrate were sufficient to support an expiration date of nine months when stored at room temperature. Storage stability data were not submitted for Serenade Garden Ready To Use. Until such data are submitted, an expiration date of nine months when stored at room temperature is required.

7.2 Human Health and Safety

The acute toxicity and infectivity studies submitted in support of *B. subtilis* strain QST 713 and the acute toxicity studies submitted in support of its associated end-use products were determined to be sufficiently complete to permit a decision on registration. Infectivity testing is not required for end-use products.

Bacillis subtilis QST 713 Technical Powder was of low toxicity in the rat when administered via the oral, pulmonary, intravenous and dermal routes and was not pathogenic or infective via the oral, pulmonary and intravenous routes. The estimated clearance time from the lung and associated lymph nodes after pulmonary exposure was 108 days. Slight dermal irritation and minimal eye irritation were observed with the technical product.

Human health and safety studies submitted in support of Serenade MAX were conducted with Serenade WP, which was considered an acceptable substitute. Serenade MAX was found to be non-toxic via the oral and dermal routes. An acute inhalation study was submitted for Serenade MAX, however, the actual concentration of the product in the test was not measured and this study was, therefore, considered acceptable but supplemental. A replacement study was waived as the particle size of the wettable powder poses a low risk of inhalation exposure and the nature of the formulants in the product are not of concern. In addition, any risk is mitigated by the use of standard personal protective equipment. Serenade MAX was found to be slightly irritating via the dermal route and non- to minimally irritating to the eye.

Human health and safety studies submitted in support of the end-use products, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate, and Serenade Garden Ready To Use were conducted with Serenade AS which was considered an acceptable substitute. Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use were found to be non-toxic via the oral, pulmonary and dermal routes. These end-use products are expected to be slightly irritating to the skin and non- to minimally irritating to the eyes.

Serenade MAX was determined to be a sensitizing agent in a hypersensitivity study while Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use were not found to be dermal sensitizers. Sensitization, however, may occur via other routes of exposure and all microbial pesticides are considered to be potential sensitizers. Exposure to allergens, including *B. subtilis* strain QST 713 may cause allergies following repeated exposures. As a result, the signal words “POTENTIAL SENSITIZER” are required on the principal display panels of all technical and end-use product labels.

When handled according to the label instructions, the pulmonary, dermal and ocular routes are potential routes of exposure to mixer/loaders, handlers and early-entry workers. While submitted studies on *B. subtilis* strain QST 713 indicated persistence in the lungs and associated lymph nodes after exposure via the pulmonary route, inhalation exposure is not a concern as commercial users (e.g. mixer/loaders, handlers, early-entry workers) are required to wear NIOSH respirators and domestic users are directed to avoid breathing spray mists. To minimize risk to workers, use of appropriate personal protective equipment (PPE) are stipulated on the end-use product labels.

The Serenade MAX, Serenade ASO, and Rhapsody ASO labels do not allow application to turf, residential or recreational areas. As the use sites are agricultural, exposure of infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children from the use of these products is expected to be negligible.

In the case of the domestic use products, Serenade Garden Concentrate and Serenade Garden Ready To Use, the single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (e.g. no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in the acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that *B. subtilis* strain QST 713 and its associated end-use products are of low toxicity, are not pathogenic or infective to mammals and that infants and children are likely to be no more sensitive to the MPCA than the general population.

Although some strains of *B. subtilis* have been isolated from food implicated in food poisoning, no such illnesses were reported for this MPCA in the United States where it has been registered for use on crops since 2000. Furthermore, there was no significant toxicity and no signs of pathogenicity observed when *B. subtilis* strain QST 713 or its associated end-use products were administered orally to rats. The establishment of a maximum residue limit (MRL) is therefore not required for *B. subtilis* strain QST 713 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulations.

7.3 Environmental Risk

The non-target studies and published scientific literature submitted in support of *B. subtilis* strain QST 713 were determined to be sufficiently complete to permit a decision on registration.

No studies were submitted to address the environmental fate and behaviour of *B. subtilis* strain QST 713. Environmental fate data (Tier II/III) were not required, due to the absence of significant toxicological effects in non-target organisms in Tier I testing.

Environmental effects studies were submitted to address risks of *B. subtilis* strain QST 713 to non-target organisms. Those studies showed that the proposed uses of products containing *B. subtilis* strain QST 713 would not pose a significant hazard to birds, terrestrial arthropods (including honey bees), freshwater fish, aquatic invertebrates and algae. The remaining groups of non-target organisms, mammals, earthworms and other soil macroorganisms, microogranisms and terrestrial plants, were assessed based on studies and reports in the published literature or studies submitted for human health and safety testing or efficacy testing. In published scientific literature, few adverse effects attributed to *B. subtilis* were reported in mammals, terrestrial insects and non-arthropod invertebrates. These reports, however, were few in numbers despite this microorganism's ubiquitous nature in the environment and, in most cases, the implication of select strains of *B. subtilis* was not thoroughly investigated.

Although the product is not intended for direct application to water, surface water runoff may result in contamination of aquatic ecosystems. The biological properties of this microorganism suggest that spores of this MPCA could survive in aquatic ecosystems. However, no harm to aquatic organisms are expected based on the absence of disease or other adverse effects in aquatic organisms. Similarly, no harm to terrestrial organisms are expected based on the number of adverse effects reported in published literature and the results of the ecotoxicology tests submitted.

7.4 Value

Various formulations of Serenade including Serenade MAX, Serenade WP and Serenade WPO were assessed in the efficacy trials. Very few trials with Serenade MAX were submitted and the level of disease control under moderate to high disease pressures could not be determined to be consistent. Therefore, in general, Serenade MAX was considered to provide "suppression" and not "control" of certain plant diseases.

Efficacy data with Serenade AS and Rhapsody AS were assessed in support of the registration of the four Serenade suspension products, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready to Use. Based on the lack of consistency of the efficacy data, the level of disease control was accepted as suppression and not control for a number of bacterial and fungal diseases on various agricultural and ornamental crops.

8.0 Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and in accordance with the Pest Control Products Regulations, has granted conditional registration for the sale and use of the technical grade active ingredient *Bacillus subtilis* QST 713 Technical Powder and the end-use products Serenade MAX, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready To Use to suppress various fungal and bacterial diseases in asparagus, bushberries and caneberries, bulb vegetables, brassica (cole) crops, celery, cucurbit vegetables, fruiting vegetables, grapes, legume vegetables, leafy vegetables, mint, pome fruits, root/tuber and corm vegetables, strawberries and ornamentals.

An evaluation of the current scientific data from the applicant, scientific reports and information from other regulatory agencies indicated that, under the approved conditions of use, the products have value and do not present an unacceptable risk to human health or the environment.

Although the risks and value have been determined to be acceptable when all risk reduction measures are followed, as a condition of these registrations, additional scientific information is being requested from the applicant as a result of this evaluation (see below).

- **Product Characterization and Analysis**

    - Results of ribotyping to distinguish *B. subtilis* strain QST 713 from other strains of *B. subtilis* are required.

    - Additional storage stability data on pilot-scale or production-scale batches are required to ensure product performance and safety.

- **Value**

    - Two additional efficacy trials on tomato early blight under adequate disease pressures are required for Serenade MAX.

List of Abbreviations

µg	micrograms
a.i.	active ingredient
ADI	acceptable daily intake
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
ARfD	acute reference dose
ASO	aqueous suspension organic
ATCC	American Type Culture Collection
BAM	*Bacteriological Analytical Manual*
bw	body weight
CFU	colony forming units
cm	centimetre(s)
DACO	data code
DER	Data Evaluation Report
DI	disease index
EEC	environmental effects concentration
g	gram
GSD	geometric standard deviation
ha	hectare(s)
kg	kilogram
KTG	killed test substance group
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LOEC	lowest observed effect concentration
mg	milligram
mL	millilitre
MAS	maximum average score
MIS	maximum irritation score
MMAD	mass median aerodynamic diameter
MPCA	microbial pest control agent
MRL	maximum residue limit
NC	naive control
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
NZW	New Zealand white
PCPA	*Pest Control Products Act*
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
ppm	parts per million
RTU	Ready To Use
SC	shelf control
TG	test substance group
TGAI	technical grade active ingredient

TRR	total radioactive residue
TSMP	Toxic Substances Management Policy
WP	wettable powder
w/w	weight/weight

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *Bacillus subtilis* QST 713 Technical Powder and Serenade MAX

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Bacillus subtilis* QST 713 Technical Powder				
Acute Oral Toxicity and Infectivity	Rat— CD - TG[1] group: 15/sex treated with TGAI, 1.13×10^8 CFU/animal in a 1 mL volume of water by oral gavage - KTG[2] group: 15/sex treated with autoclaved TGAI, equivalent to 1.13×10^8 CFU/animal in a 1 mL volume of water by oral gavage - NC group: 15/sex naive control -SC group: 3/sex shelf control -Interim sacrifices from the TG, KTG and NC groups (3/sex/group) on days 0, 3, 7 and 14	$LD_{50} > 1.13 \times 10^8$ CFU/animal	- No mortalities and no abnormalities upon necropsy. -No statistically significant differences in body weight or body weight gain. -Clinical signs of adverse effects not observed in any of the groups. -No statistically significant differences in organ weights. -MPCA recovered from the stomach, intestines, caecum and faeces of male and female TG rats. Clearance was observed in these organs prior to or by day 14. -MPCA also detected in the lungs and liver of one female TG rat and in the mesenteric lymph nodes of two female TG rats on day 0, however, clearance was established by day 3. **NON- TOXIC, NOT INFECTIVE**	PMRA 1116023

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Acute Pulmonary Toxicity and Infectivity	Rat—CD - TG group: 20/sex treated with TGAI, 1.2×10^8 CFU/animal in a 0.1 mL of purified water via the intratracheal route - KTG group: 20/sex treated with autoclaved MPCA, equivalent to 1.2×10^8 CFU/animal in a 0.1 mL of purified water via the intratracheal route -NC group: 20/sex naive control -SC group: 5/sex shelf control -Interim sacrifices from the TG, KTG and NC groups (5/sex/group) on days 0, 7, 21, and 35.	$LD_{50} > 1.2 \times 10^8$ CFU/animal	-No mortalities. -No statistically significant effects on body weight. -Reduced body weight gains in male and female TG rats from days 0–7 and in female TG rats between days 14–21. Male TG and SC rats had higher weight gains between days 21–28. Total weight gain between days 0–35 did not vary significantly between groups. -The only necropsy findings were mottled lung parenchyma in all TG-dosed animals sacrificed on day 0. -Relative lung and associated lymph node weights higher in male and female TG rats on day 0, as compared to NC group. Liver weights lower in male TG rats on day 7, as compared to NC group. Higher spleen weights in female TG and KTG rats on day 7, as compared to NC group. Differences in weight gain and relative organ weights not considered biologically significant. -Test substance detected in lungs and associated lymph nodes of male and female TG rats from days 0–35. Based on the rate of clearance from the lungs and associated lymph nodes, the time for microbial clearance estimated to be 108 days. -Test substance also detected in spleen, liver and kidney of some animals on days 0–7 with clearance from these organs established by day 21. **NON-TOXIC, NOT INFECTIVE**	PMRA 1116087

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Intravenous Infectivity	Rat—CD - TG group: 12/sex treated with TGAI, 9.4×10^8 CFU/animal in 0.5 mL of purified water - KTG group: 12/sex treated with autoclaved TGAI, equivalent to 9.4×10^8 CFU/animal in 0.5 mL of purified water -NC group: 12/sex naive control -SC group: 3/sex shelf control -Interim sacrifice from the TG, KTG and NC groups (3/sex/group) on days 0, 7, 32 and 35		-No statistically significant differences in body weight among groups. -No adverse clinical signs observed in any of the animals. -No gross lesions noted upon necropsy. -Test substance detected in blood, lungs, spleen, liver, and kidneys of male and female TG animals on day 0. -With the exception of the spleen and liver, microbial clearance was demonstrated in all other organs by day 21. -Based on the clearance rates from the spleen and liver, microbial clearance in these organs was estimated to be 80 days and 70 days, respectively. **NOT INFECTIVE**	PMRA 1116024

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Acute Dermal Toxicity	Rabbit—New Zealand white -5/sex treated with TGAI, 2 g/kg bw (equivalent to 2.30×10^{11}–2.73×10^{11} CFU/animal) mixed into a paste with 3 mL of purified water, 24-hours	LD_{50} > 2 g/kg bw	-No mortalities or overt signs of toxicity. -All animals gained weight. -Slight to moderate edema and severe erythema observed in all animals upon unwrapping. Erythema and edema began to subside on days 6 and 8, respectively, and continued to do so until the end of the study (day 14), at which time only one rabbit exhibited severe erythema and very slight edema. -Eschar formation persisted until days 5–13, with the exception of one animal in which eschar formation observed at study termination. -Superficial flaking of the skin seen in nine animals midway through the observation period and persisting in three of the animals at study termination. -Cracked skin observed in four animals but this was resolved by day 8. -New or repaired skin seen in all animals beginning on days 5–7. **LOW TOXICITY**	PMRA 1116025
Dermal Irritation	Rabbit - New Zealand -3/sex treated with TGAI, 500 mg (equivalent to ~ 2.4×10^{10} CFU/animal) moistened with 0.3 mL of saline, 4 hours		-Very slight erythema observed in 4 animals 30-60 minutes after unwrapping and in 3 animals at the 24 hour timepoint. -Maximum individual irritation score = 1 at 30-60 minute timepoint. -Maximum irritation score (MIS) = 0.667 (slightly irritating) at 30-60 minute timepoint -Maximum average score (MAS; 24, 48, 72 hours) = 0.167 (minimally irritating) **SLIGHTLY IRRITATING**	PMRA 1116027

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Eye Irritation	Rabbit - New Zealand -3/sex treated with 0.1 mL (packed volume equivalent to ~ 4.7×10^9 CFU/animal)		-Redness (score = 2), chemosis (scores ranging from 1 in two animals to 3 in one animal), and discharge (score = 1) observed in all animals 1 hour post-dosing. -Iritis (score = 1) seen in two animals at 1 hour post-dosing and in 2 additional animals at the 24 hour timepoint. -All signs of eye irritation absent by 96 hour timepoint. -MIS = 11.3 at 1 hour timepoint. -MAS (24, 48, 72) hours = 3.22 **MINIMALLY IRRITATING**	PMRA 1116032, 1116031
Acute Toxicity/Irritation Serenade MAX				
Acute Oral Toxicity	Rat - Sprague Dawley -5/sex, treated with Serenade WP, 5000 mg/kg bw in distilled water (equivalent to ~ 3.3×10^{10} CFU–3.6×10^{10} CFU per male and 2.2×10^{10} CFU–2.4×10^{10} CFU per female) by oral gavage	LD_{50} > 5000 mg/kg bw.	-No mortalities, signs of adverse clinical effects or abnormalities upon necropsy. -All animals gained weight. **NON-TOXIC**	PMRA 1116592

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Acute Pulmonary Toxicity	Rat - Sprague Dawley -5/sex exposed to Serenade WP (25% w/w in deionized water), time-weighted aerosol concentration of 0.63 g/L		-No mortalities. -All animals exhibited signs of clinical abnormalities on the day of dosing including salivation, shallow/laboured/congested breathing, wobbly gait, decreased activity, hunched posture, dark material around the eyes and/or nose, decreased temperature, and misshaped corneas. -By day 3, 7/10 animals appeared normal; some animals exhibited additional clinical abnormalities including corneal opacity, few faeces, soft stools, slight urine stains, decreased food consumption and slight faecal stains that persisted until euthanasia on day 14. -All male rats gained weight. Among female rats, slight weight loss observed in four animals but this was not considered biologically significant. -No significant gross internal findings upon necropsy. -Actual test substance concentration was not determined. -Aerodynamic particle size of Serenade WP poses a low risk of inhalation exposure. **ACCEPTABLE but SUPPLEMENTAL**	PMRA 1116593

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Acute Dermal Toxicity	Rabbit - New Zealand White -5/sex treated with Serenade WP, 2000 mg/kg bw moistened with water, 24-hours	LD_{50} > 2000 mg/kg bw	-No mortalities. -Clinical observations included abnormal stance in one animal on days 3–6. -Erythema, edema, necrosis, fissuring and/or sloughing of the skin observed in all animals. -All animals gained weight. -No visible lesions observed upon necropsy. **LOW TOXICITY**	PMRA 1116595
Dermal Irritation	Rabbit - New Zealand White -3/sex treated with Serenade WP, 500 mg/animal moistened with 0.2 mL of saline; 4-hour		-All animals exhibited very slight erythema (score = 1) and 2/6 animals exhibited very slight edema (score = 1) at the 30–60 minute timepoint. Absence of all signs of dermal irritation by the 72-hour timepoint. -Maximum individual irritation score = 2 (mildly irritating). -MIS = 1.333 (slightly irritating) at the 30–60 minute timepoint. -MAS (24, 48, and 72 hours) = 0.444 (minimally irritating). **SLIGHTLY IRRITATING**	PMRA 1116597

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Hypersensitivity	Guinea Pigs - Hartley -TG group: 10/sex treated with Serenade WP -induction phase: Serenade WP applied to a 25 mm Hilltop Chamber®, moistened with 0.3 mL of distilled water, three six-hour occluded applications seven days apart -challenge phase: Serenade WP applied to a 25 mm Hilltop Chamber®, moistened with 0.3 mL of distilled water on left flank, distilled water on right flank, 14 days after last induction, 18–24 hour exposure, naive site -rechallenge: naive site -VC group: 5/sex vehicle control group, same induction phase as above with distilled water, same challenge phase as above -PC group: 3/sex positive control group, treated with 1-chloro-2,4-dinitrobenzene (DNCB)		-Slight or moderate erythema after inductions on weeks 1, 2, and 3 in 8/20, 7/20, and 3/20 animals, respectively. -Upon challenge, average severity scores on the left flanks were 0.3, 0.45 and 2.5 for the VC, TG, and PC groups, respectively, 24 hours after challenge. Average scores at 48 hours after challenge for these groups were 0.1, 0.8, and 1.8, respectively. -Upon rechallenge, average severity in TG group at 24 hours was 0.6, and at 48 hours was 0.3. -No signs of systemic toxicity. -All animals gained weight. **SLIGHT SENSITIZER**	PMRA 1116599

Study Type	Species, Strain, and Doses	Result	Significant Effects and Comments	Reference(s)
Eye Irritation	Rabbit - New Zealand White -3/sex treated with Serenade WP, 0.1 mL packed volume/animal		-All animals exhibited redness (score = 1 to 2) at 1 hour timepoint. Chemosis (score = 1) seen in 2/6 animals, discharge (score = 1) seen in 1/6 animals at this timepoint. -At the 24 hour timepoint, 4/6 animals continued to exhibit redness (score = 1). -All signs of ocular irritation cleared by 72 hour timepoint. -MIS = 3.33 -Maximum individual irritation score = 8 at 1 hour timepoint. -MAS (24, 48, 72 hours) = 0.78. **NON- TO MINIMALLY IRRITATING**	PMRA 1116598

[1] TG = Test Substance Group
[2] KTG = Killed Test Group

Table 2 Toxicity and Infectivity of Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden RTU

Study Type	Species, Strain and Doses	Result	Significant Effects and Comments	Reference(s)
Acute Oral Toxicity	Rat - Sprague Dawley -5/sex, treated with Serenade AS, 5000 mg/kg bw (equivalent to ~ 2.9×10^9 CFU–3.4×10^9 CFU per male and 2.4×10^9 CFU–2.6×10^9 CFU per female) by oral gavage	LD_{50} > 5000 mg/kg bw.	-No mortalities. -Hair loss in the middle to lower abdominal area of one rat from days 1 to 14. -All animals gained weight. -No abnormal findings upon necropsy. **NON-TOXIC**	1120456

Study Type	Species, Strain and Doses	Result	Significant Effects and Comments	Reference(s)
Acute Pulmonary Toxicity	Rat - Sprague Dawley -5/sex exposed to Serenade AS, time-weighted mean gravimetric concentration of 1.45 mg/L		-No mortalities. -All animals exhibited signs of clinical abnormalities including laboured/congested breathing, rales, few faeces, small faeces, slight to moderate urine staining, slight faecal staining, unkempt appearance, rough coat, dark material around the eyes/nose/mouth and decreased food consumption. All evidence of adverse clinical signs cleared by day 8. -Slight body weight loss in two male and two female rats between days 0 to 7. One female rate continued to lose a small amount of weight from days 7 to 14. Slight weight loss not considered significant. -No abnormalities noted at necropsy. **NON-TOXIC**	1120455
Acute Dermal Toxicity	Rat - Sprague-Dawley -5/sex treated with Serenade AS, 5000 mg/kg bw, 24 hours	LD_{50} > 5000 mg/kg bw	-No mortalities. -Clinical observations included coloured materials around nose and eyes, dehydration, emaciation, few/no faeces, desquamation, anogenital staining. All clinical signs resolved by day 10. -Two females lost weight between days 1 to 7 but weight gain by day 14 was normal in these animals. All other animals gained weight. -No abnormal findings upon necropsy. **LOW TOXICITY**	1120453

Study Type	Species, Strain and Doses	Result	Significant Effects and Comments	Reference(s)
Dermal Irritation	Rabbit - New Zealand White -6 female treated with Serenade AS, 0.5 mL/animal; 4 hour		-Very slight erythema/eschar formation in 4/6 animals at the 1 hour timepoint leading to well-defined erythema/eschar formation by the 24 hour timepoint. At the 48 hour timepoint, erythema/eschar formation in one of the animals had subsided to very slight erythema. At the 72 hour timepoint, one animal continued to exhibit well-defined erythema/eschar and a second animal exhibited very slight erythema. All observations of erythema were reversed by day 7. -Maximum individual irritation score = 2 (mildly irritating) at the 24 hour timepoint. -MIS = 1.333 (slightly irritating) at the 24 hour timepoint. -MAS (24, 48 and 72 hours) =1 (slightly irritating). **SLIGHTLY IRRITATING**	1120451
Hypersensitivity	Guinea Pigs - Hartley -20 females -induction phase: 0.4 mL Serenade AS applied to a Hilltop Chamber®, three six-hour applications seven days apart -challenge phase: 0.4 mL Serenade AS applied to a Hilltop Chamber®, 14 days after last induction, 6 hour exposure, naive site		-Dermal irritation not observed after first induction exposure. Faint erythema observed in 2 animals 24 hours after the second induction which subsided to very faint erythema in one animal by the 48 hour timepoint. Twenty-four hours after the third induction phase, one animal exhibited very faint erythema. -No reactions observed upon challenge in any of the animals. -No mortalities. -No clinical signs of adverse effects. -All animals gained weight. **NOT A DERMAL SENSITIZER**	1120447

Study Type	Species, Strain and Doses	Result	Significant Effects and Comments	Reference(s)
Eye Irritation	Rabbit - New Zealand White -6 females treated with Serenade AS, 0.1 mL/animal		-At the 1 hour timepoint, all animals exhibited redness (score = 1) and discharge (score = 1) was noted in three animals. -At the 24 hour timepoint, redness (score = 1) persisted in all six animals but was absent by the 48 hour timepoint. -MIS = 3 at 1 hour observation timepoint -Maximum individual irritation score = 4 at 1 hour timepoint. -MAS (24, 48, 72 hours) = 0.67. **NON- TO MINIMALLY IRRITATING**	1120448

Table 3 Toxicity to Non-Target Species

Organism	Exposure	Test Substance(s)	End Point Value	Significant Effects, Comments	Reference
Terrestrial Organisms					
Vertebrates					
Birds (*Colinus virginianus*; Northern Bobwhite quail)	Oral	i. *Bacillus subtilis* QST 713 Technical Powder ii. reverse-osmosis water	LD_{50} > 5000 mg/kg bw/day for 5 days	-Signs of toxicity in 6/30 birds in the treatment group included depression, inability to stand, loss of coordination, and a ruffled appearance shortly after dosing. Over the following two days, 1/30 birds displayed loss of coordination, 2/30 birds exhibited reduced reaction to external stimuli, loss of coordination, slight wing droop, and shallow and rapid respiration. -One bird exhibiting clinical signs of toxicity was found dead on the morning of day 1. -No differences in body weight, body weight gain or feed consumption. -No treatment-related gross lesions or abnormalities noted at necropsy. **LOW TOXICITY; NOT INFECTIVE**	PMRA 1116043
Wild Mammals	No study or waiver submitted. Few reports of adverse effects were reported in published scientific literature; *B. subtilis* has been implicated in cases of bovine mastitis and reproductive disorders in goats. No other reports of adverse effects were reported despite this microorganism's ubiquitous nature in the environment. Acute toxicity and infectivity studies (oral, pulmonary, intravenous and dermal) with rats treated with the MPCA showed complete clearance or a clear pattern of clearance of the MPCA in mammals. **WAIVER ACCEPTED**				PMRA 1106087, 1116023, 1116024, 1116025, 1116033, 1116034, 1116035, 1116036

Organism	Exposure	Test Substance(s)	End Point Value	Significant Effects, Comments	Reference
Invertebrates					
Honey bees (*Apil mellifera* L.)	Oral (Dietary)	i. *Bacillus subtilis* QST 713 Technical Powder ii. larval bee diet iii. 5 ppm or 100 ppm dimethoate (positive control)	LD_{50} > 100 000 ppm	-No behavioural or morphological abnormalities in any groups. -Estimated % survival for 1000, 10000, and 100000 ppm treatment groups was 84%, 71%, and 61.67%, respectively. The negative control group had a survival rate of 96.88% while the 5 ppm and 100 ppm positive control groups had survival rates of 93.75% and 8.75%, respectively. Statistically significant reduction in survival rate between 100 000 ppm treatment group and negative control group. **ACCEPTABLE**	PMRA 1116048

Organism	Exposure	Test Substance(s)	End Point Value	Significant Effects, Comments	Reference
Honey Bees (*Apil mellifera* L.)	Field Pollinator	i. alfalfa field sprayed with Serenade WP, 6 sprays of 1.35 kg/ha over ~ 1 month ii. alfalfa field sprayed with water iii. alfalfa field sprayed with dimethoate 4E (positive control)		-No consistent statistically significant differences in the number of dead adult honey bees or immature bees between the Serenade WP-treated and negative control plots. -No statistical differences in the number of foraging honey bees or in the number of frames of adult honey bees and brood in the Serenade WP-treated plots and the negative control. -Mortality in the dimethoate 4E-treated plots was significantly greater than that observed in the Serenade WP and negative control plots as early as one day after the first treatment. -Viability of Serenade WP not assessed. **ACCEPTABLE but SUPPLEMENTAL**	PMRA 1116610

Organism	Exposure	Test Substance(s)	End Point Value	Significant Effects, Comments	Reference
Aphid Parasitoid (*Aphidus rhopalosiphi*)	Contact	i. *Bacillus subtilis* strain QST 713 Technical Powder ii. de-ionized water iii. dimethoate (positive control)	2-day LC_{50} > 16 kg/ha NOEL > 16 kg/ha	-Mean mortality of treatment group was 7.50% compared to 2.50% in the negative control (water) group and 100% in the dimethoate group. -Reproduction rate of 8.29 mummies/female in the treatment group compared to 11.10 mummies/female in the negative control group. -Differences in mortality and reproduction between treatment and negative control groups not statistically significant. -Dose was too low. **ACCEPTABLE but SUPPLEMENTAL**	PMRA 1116051
Parasitic Hymenopteran (*Nasonia vitripenis*)	Oral (diet)	i. *Bacillus subtilis* QST 713 Technical Powder ii. negative control iii. attenuated control iv. sterile filtrate control	LC_{50} ~ 24 739 ppm	-Immobility and lethargy observed in all groups and at all treatment levels tested. These clinical signs were not dose-dependent and were not considered treatment-related. -Mortality in negative control group was 28%. Adjusted mortalities (to correct for mortalities in the negative control group) for the attenuated control, sterile filtrate control, and the 295, 1730, 10 200, and 60 000 ppm treatment groups were 24.1, 13, 1.9, 0, 25.9, and 74.1%, respectively. **ACCEPTABLE**	PMRA 1116052

Organism	Exposure	Test Substance(s)	End Point Value	Significant Effects, Comments	Reference
Green Lacewing (*Chrisoperla carnea*)	Oral (diet)	i. *Bacillus subtilis* QST 713 Technical Powder ii. negative control	LC_{50} > 60 000 ppm	-No signs of toxicity in treatment or negative control groups. -In the negative control group, mortality was 7% and pupation rate was 63%. -In the 600, 6000 and 60 000 ppm test groups, mortality was 13, 7, and 10%, respectively. The pupation rates in these groups were 67, 33, and 48%, respectively. -No significant differences in mortality. -Pupation rates significantly lower at the 6000 and 60 000 ppm treatment levels as compared to the negative control. **ACCEPTABLE**	PMRA 1116053
Ladybird Beetle (*Hippodamia convergens*)	Oral (diet)	i. *Bacillus subtilis* QST 713 Technical Powder ii. negative control	LC_{50} > 60 000 ppm NOEC > 60 000 ppm	-Clinical observations included lethargy and immobility in a small number of beetles in both control and treatment groups. -Mortality in the control group was 15%. Mortalities in the 600, 6000, and 60 000 ppm treatment groups were 15, 25, 19 and 17%, respectively. (High mortality in 600 ppm treatment group observed in mainly one replicate.) -Differences in mortality not considered significant. **ACCEPTABLE**	PMRA 1116049

Organism	Exposure	Test Substance(s)	End Point Value	Significant Effects, Comments	Reference
Predatory Mite (*Typhlodromus pyri)*	Contact	i. *Bacillus subtilis* strain QST 713 Technical Powder ii. de-ionized water iii. dimethoate (positive control)		-Mean mortality in the treatment group was 39% compared to 12% in the negative control group and 72% in the positive control group. The corrected mortality for *T. pyri* was 30.7%. -Number of offspring per female in the treatment group was calculated as 10.0 compared to 11.5 in the control group. No significant effects on the reproduction ability of *T. pyri* were observed. -Dose was too low. **ACCEPTABLE but SUPPLEMENTAL**	PMRA 1116050
Earthworms	Acute	No study was submitted. Published study demonstrated no toxic or pathogenic effects following dietary exposure to *Caenorhabditis elegans* (a nematode) of *B. subtilis*. In other published scientific literature, another strain of *B. subtilis* was reported as a potential biological control agent of the parasitic root-knot nematode (*Meloidogyne incognita*) of tomato, however, the mode of action was not elucidated and the pathogenic relationship was not established. *Bacillus subtilis* is generally not considered to be a pathogen of non-arthropod invertebrates. **RATIONALE ACCEPTED**			PMRA 1099746, 1099747
Soil microbes	Acute	No study or waiver submitted. Effects data are not required although the product is intended to control pest microorganisms, as *B. subtilis* is a normal component of the soil, and the organism is not expected to affect environmentally or economically important microbial species or microbiologically-mediated biogeochemical processes.			
Vascular Plants					
Vascular Plants	Acute	No study or waiver submitted. Effects data are not required as *B. subtilis* is not generally considered to be a pathogen of vascular plants. Furthermore, no phytotoxicity was observed in the efficacy trials.			

Organism	Exposure	Test Substance(s)	End Point Value	Significant Effects, Comments	Reference
Aquatic Organisms					
Vertebrates					
Freshwater fish (*Oncorhynchus mykiss*; Rainbow trout)	Aqueous and oral (diet)	i. *Bacillus subtilis* QST 713 Technical Powder ii. dilution water (negative control) iii. sterile filtrate control iv. attenuated control v. broth concentrate control	30-day LC_{50} ~ 1.4×10^7 CFU/mL	-Fish in the negative control, sterile filtrate control and 1.7×10^6 CFU/mL treatment groups appeared normal and healthy. There were no mortalities in these groups. -Mortality in the 4.0×10^6, 7.7×10^6, 1.7×10^7, and 3.5×10^7 CFU/mL treatment groups was 10, 40, 40, 40, and 90%, respectively. -Mortality in the attenuated control group was 100%, suggesting that the deaths were the result of the physical nature of the test substance rather than the active microbial agent, and in the broth concentrate control group was 10%. -No treatment-related signs of infection in any fish upon necropsy. **ACCEPTABLE**	PMRA 1116046
Estuarine/ marine fish	Acute	No study was submitted. Effects data not required as minimal exposure is expected due to the proposed terrestrial uses.			

Organism	Exposure	Test Substance(s)	End Point Value	Significant Effects, Comments	Reference
Invertebrates					
Daphnia magna	48-hour aqueous	i. *Bacillus subtilis* QST 713 Technical Powder ii. dilution water (negative control) iii. spray-dried filtrate		-Daphnids in the negative control, spray-dried filtrate control and 13 mg/L treatment groups were healthy and normal throughout the test. -After 48 hours of exposure, mortality in the 25, 50, 100 and 200 mg/L treatment groups was 15, 15, 45 and 85%, respectively. -Two of the surviving daphnids in the 100 mg/L treatment group exhibited lethargy at the 48-hour timepoint. -Verification of test substance concentrations, stability and homogeneity of the test substance in well water were not determined. **ACCEPTABLE but SUPPLEMENTAL**	PMRA 1116055

Organism	Exposure	Test Substance(s)	End Point Value	Significant Effects, Comments	Reference
Daphnia magna	21-day aqueous	i. *Bacillus subtilis* QST 713 Technical Powder. Viability of the test substance was established. ii. negative control iii. sterile filtrate control iv. attenuated control v. broth concentrate control	21-day LC_{50} ~ 1.6×10^6CFU/mL NOEC 7.9×10^5 CFU/mL.	-Survival in the negative control group was 85% and all surviving daphnids appeared normal and healthy. -Survival in the sterile filtrate control, attenuated control, broth concentrate control, 7.9×10^5, 1.8×10^6, 3.4×10^6, 7.3×10^6 and 2.0×10^7 CFU/mL treatment groups was 65, 0, 30, 95, 25, 15, 0, and 0, respectively. -Survival in all groups, with the exception of the sterile filtrate control and the 7.9×10^5 treatment groups, was significantly different from the negative control group. -Mean number of young produced per reproductive day in the negative control, attenuated control and 7.9×10^5 CFU/mL groups was 5.22, 1.96 and 6.53, respectively. A statistically significant difference in reproduction was only noted between the negative control and sterile filtrate control groups. A similar pattern was observed between the negative control, sterile filtrate control and 7.9×10^5 CFU/mL treatment groups for mean length and mean dry weight. **ACCEPTABLE**	PMRA 1116056

Organism	Exposure	Test Substance(s)	End Point Value	Significant Effects, Comments	Reference
Grass Shrimp (*Palaemonetes pugio*)	Oral (diet)	i. *Bacillus subtilis* QST 713 Technical Powder. Viability of the test substance was established. ii. negative control iii. sterile filtrate control iv. attenuated control v. broth concentrate control	LC_{50} >4.0 × 10^6 CFU/g.	-No mortalities, abnormal physical appearance or behaviour observed in any of the shrimp in the treatment or control groups. -Grass shrimp in all control and treatment groups molted. -Growth not adversely affected by the dietary exposure to the test substance. -No evidence of an inflammatory response or necrosis observed upon necropsy. **ACCEPTABLE**	PMRA 1115976
Plants					
Single Cell Green Alga (*Scenedesmus subspicatus*)	Aqueous	i. *Bacillus subtilis* QST 713 Technical Powder	NOEC ≥ 100 mg/L LOEC > 100 mg/L	-No significant inhibitory effects observed at concentrations of 0 to 100 mg/L for three days. **ACCEPTABLE**	PMRA 1115978

Table 4 Alternative active ingredients registered for control or suppression of claimed diseases on the Serenade MAX accepted label

Crop	Disease Claim	Active Ingredient
Apple and pear	Scab (*V. inaqualis* and *V. pirina*)	Thiram, captan, folpet, metiram, myclobutanil, cyprodinil, pyrimethanil, ferbam, mancozeb, trifloxystrobin, pyraclostrobin, kresoxim-methyl
	Powdery mildew (*Podosphaera leucotricha*)	Kresoxim-Methyl, sulfur
Beans: Succulent and dried beans	White mould (*Sclerotinia sclerotiorum*)	Iprodione, boscalid, dicloran, vinclozolin
	Botrytis pod rot (*Botrytis cinerea*)	Iprodione, vinclozolin, boscalid, dicloran
Crop Group 3 Crops (Bulb vegetables)	Downy mildew (*Peronospora destructor*)	Sulfur
	Leaf blight (*Botrytis squamosa*)	Boscalid
Crop Group 13 Crops (bushberries and caneberries)	Botrytis blight (*Botrytis cinerea*)	Iprodione, boscalid, fenhexamid
Cucumber (Greenhouse)	Powdery mildew (*Erysiphe cichoracearum*, *Sphaerotheca fuliginea*)	Sulfur, myclobutanil, potassium bicarbonate
Cucumber (field)	Powdery mildew (*E. cichoracearum*, *S. fuliginea*)	Potassium bicarbonate, pyraclostrobin, chlorothalonil, folpet
Fruiting vegetables: pepper, tomato, eggplant, tomatillo, ground cherry	Early blight (*Alternaria solani*)	Zineb, pyraclostrobin, copper, boscalid
Fruiting vegetables: pepper, tomato, eggplant, tomatillo, ground cherry	Gray mould (*Botrytis cinerea*)	Boscalid
Grapes	Gray mould (*Botrytis cinerea*)	*Pantoea agglomerans* strain E325, iprodione, cyprodinil, fenhexamid
Grapes	Powdery mildew (*Uncinula necator*)	Sulfur, boscalid, mancozeb, folpet, myclobutanil, azoxystrobin, trifloxystrobin, potassium bicarbonate
Lettuce	Lettuce drop (*Sclerotinia minor*)	Dicloran, vinclozolin, boscalid
	Lettuce downy mildew (*Bremia lactucae*)	Mancozeb, metalaxyl-M
		Fosetyl-Al

Crop	Disease Claim	Active Ingredient
Onion	Onion neck rot blight (*Botrytis allii*)	Mancozeb
	Downy mildew (*Peronospora destructor*)	Mancozeb, copper, fosetyl-Al, zineb
	Onion leaf blight (*Botrytis squamosa*)	Iprodione, maneb, zineb, chlorothalonil, mancozeb
Peppers (Greenhouse)	Powdery mildew (*Leveillula taurica*)	Potassium bicarbonate
Strawberry	Botrytis gray mould (*Botrytis cinerea*)	Boscalid, vinclozolin, thiophanate-methyl
Tomato	Early blight (*Alternaria solani*)	Azoxystrobin, mancozeb, copper, captan, ziram, chlorothalonil, metiram, maneb, zineb
Tomato (Greenhouse)	Powdery mildew (*Erysiphe orontii)*	Potassium bicarbonate
	Gray mould (*Botrytis cinerea)*	Fenhexamid

Table 5 Alternative active ingredients registered for control or suppression of diseases present on, Serenade ASO, Rhapsody ASO, Serenade Garden Concentrate and Serenade Garden Ready to Use accepted labels

Crop	Disease Claim	Technical Grade Active Ingredient
Asparagus	Botrytis blight (*Botrytis cineria*)	Iprodione, boscalid
Bushberry, caneberry and other berry crops	Botrytis blight (*Botrytis cinerea*)	Ferbam, iprodione, fenhexamid, boscalid
Brassica vegetables: broccoli, brussels sprouts, cauliflower, cabbage, Chinese cabbage, kale and kohlrabi	Downy mildew (*Peronospora parasitica*)	Fosetyl Al, chlorothalonil, tribasic copper sulphate
Bulb vegetables	Botrytis leaf blight (*Botrytis squamosa*)	Boscalid, boscalid + pyraclostrobin, cyprodinil + fludioxonil
Carrot	Altenaria leaf blight (*Altenaria dauci)*	Boscalid, boscalid + pyraclostrobin, cyprodinil + fludioxonil
Cucurbits	Powdery mildew (*Sphaerotheca fuliginea*)	Potassium bicarbonate (greenhouse), pyraclostrobin, chlorothalonil, folpet, myclobutanil (greenhouse)
Fruiting vegetables: pepper	Powdery mildew (*Leveillula taurica, Oidiopsis taurica*)	Myclobutanil (greenhouse), potassium bicarbonate (greenhouse)
Fruiting vegetables	Gray mould (*Botrytis cinerea*)	Chlorothalonil, fenhexamid, boscalid
Grape	Gray mould / bunch rot (*Botrytis cinerea*)	Iprodione, cyprodinil, fenhexamid, boscalid
Grape	Powdery mildew (*Uncinula necator*)	Boscalid, azoxystrobin, trifloxystrobin, kresoxim methyl, potassium bicarbonate, sulfur, mancozeb, folpet, myclobutanil
Lettuce and spinach	Downy mildew (*Bremia lactucae, Perenospora farinosa*)	Carbaryl + copper, tribasic copper sulphide, azoxystrobin, Fosetyl-Al, metalaxyl-m + mancozeb, zineb
Lettuce	Sclerotinia head and leaf drop (*Sclerotinia sclerotiorum, Sclerotinia minor*)	Iprodione, vinclozolin, boscalid, dicloran
Celery	Pink rot (*Sclerotinia sclerotiorum*	Not found
Legume vegetables	White mould (*Sclerotinia sclerotiorum*)	Iprodione, vinclozolin, boscalid, dicloran
	Gray mould (*Botrytis cinerea*)	Iprodione, vinclozolin, boscalid, dicloran
Mint	Rust (*Puccinia menthae*)	Not found

Crop	Disease Claim	Technical Grade Active Ingredient
Pome fruits	Fire blight (*Erwinia amylovora*)	sulfate, *Pantoea agglomerans* C9-1, *Pantoea agglomerans* strain E325, Prohexadione calcium, Cupric sulfate pentahydrate, Copper oxychloride, tribasic copper sulfate
	Powdery mildew (*Podosphaera leucotricha*)	Kresoxim-Methyl, sulphide sulfur, metiram + myclobutanil. Mancozeb + myclobutanil, calcium polysulphide, fulsilazole, cyprodinil + myclobutanil, trifloxystrobin, triforine,.
Strawberry	Gray mould (*Botrytis cinerea*)	Fenhexamid, thiram, captan, chlorothalonil, vinclozolin, boscalid
Poinsettia	Powdery mildew (*Oidium* spp.)	Trifloxystrobin, potassium bicarbonate
	Botrytis blight (*Botrytis cinerea*)	Fenhexamid, Iprodione, copper
Garden phlox and Dwarf bee balm	Powdery mildew (*Erysiphe cichoracearum*)	Boscalid
Geranium	Botrytis leaf blight (*Botrytis cinerea*)	Dicloran, chlorothalonil, fenhexamid, iprodione, copper
	leaf spot (*Xanthomonas campestris* pv. *pelargon*i)	Elemental copper
Petunia	Botrytis lesion (*Botrytis cinerea*)	Chlorothalonil, fenhexamid
Roses	Powdery mildew (*Sphaerotheca* spp.)	Myclobutanil, elemental copper, tribasic copper sulfate, folpet, thiophenate methyl

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A. LIST OF STUDIES/INFORMATION SUBMITTED BY REGISTRANT

1.0 The Active Ingredient, Its Properties And Uses

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2.0 Methods Of Analysis

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3.0 Impact On Human And Animal Health

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