Proposed Registration Decision PRD2018-16

Bacillus subtilis strain BU1814 and Related End-Use Products

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Overview

Proposed Registration Decision for *Bacillus subtilis* strain BU1814

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing registration for the sale and use of *Bacillus subtilis* strain BU1814 Technical, BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra, containing the technical grade active ingredient *Bacillus subtilis* strain BU1814, to be applied as seed treatments to corn, soybean and wheat for the suppression or partial suppression of soil-borne pathogens that cause seed and seedling diseases.

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

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of *Bacillus subtilis* strain BU1814, BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra.

What Does Health Canada Consider When Making a Registration Decision?

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

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment. These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides section of Canada.ca.

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

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

Before making a final registration decision on *Bacillus subtilis* strain BU1814, BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra, the PMRA will consider any comments received from the public in response to this consultation document.[3] The PMRA will then publish a Registration Decision[4] on *Bacillus subtilis* strain BU1814 and Related End-Use Products, which will include the decision, the reasons for it, a summary of comments received on the proposed registration decision and the PMRA's response to these comments.

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

What Is *Bacillus subtilis* strain BU1814?

Bacillus subtilis strain BU1814 is a naturally occurring bacterium. It acts by rapidly colonizing germinating seeds and the developing root system. It produces a physical barrier to pathogenic fungi and produces antimicrobial components promoting Induced Systemic Resistance within the plant. It is used preventatively for the suppression or partial suppression of soil-borne pathogens that cause seedling diseases in corn, soybean and wheat.

Health Considerations

Can Approved Uses of *Bacillus subtilis* strain BU1814 Affect Human Health?

Bacillus subtilis strain BU1814 is unlikely to affect your health when BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra are used according to the label directions.

Potential exposure to *Bacillus subtilis* strain BU1814 may occur when handling and applying BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra. When assessing health risks, several key factors are considered:

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

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

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

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

Studies in laboratory animals describe potential health effects from large doses of exposure to a microorganism and identify any pathogenicity, infectivity and toxicity concerns. When *Bacillus subtilis* strain BU1814 was tested in laboratory animals, there was no sign that it caused any significant toxicity or disease.

Residues in Water and Food

Dietary risks from food and water are not of concern.

A health risk to the general population, including infants and children, as a result of dietary exposure (food and drinking water), is not expected based on the use pattern and conditions of use.

Risks in Residential and Other Non-Occupational Environments

Estimated risk for non-occupational exposure is not of concern.

BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra are proposed as commercial seed treatments. Consequently, it is unlikely that adults, youths and toddlers will be exposed to *Bacillus subtilis* strain BU1814. Even in the event of exposure, risk to the general population is not a concern since there were no signs that it caused any significant toxicity or disease in studies with laboratory animals.

Occupational Risks From Handling BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra

Occupational risks are not of concern when BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra is used according to label directions, which include protective measures.

Workers handling BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra can come into direct contact with *Bacillus subtilis* strain BU1814 on the skin, in the eyes, or by inhalation. For this reason, the product label will specify that workers must wear personal protective equipment, including waterproof gloves, long-sleeved shirts, long pants, a mist filtering mask or respirator, and socks with shoes.

Environmental Considerations

What Happens When *Bacillus subtilis* strain BU1814 is Introduced Into the Environment?

Environmental risks are not of concern.

Bacillus subtilis is a common microorganism that is widely distributed in the natural environment. Its habitat is predominantly soil, including soils in water columns and bottom deposits aquatic environments. Under adverse conditions, this microorganism produces a resilient endospore that allows it to readily survive in soils, dusts and aerosols. If protected from sunlight, endospores may survive for very long periods.

BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra are for use as seed treatments on corn (all types), soybean and wheat. The end-use products are not intended for aquatic uses. The use of these end-use products as seed treatments is not expected to significantly increase the levels of this microorganism in soil. Exposure to aquatic environments is also expected to be low and limited to leaching and runoff after the seeds are sowed in fields. Published scientific literature on the environmental fate of this species suggests that strain BU1814 will survive in soils and sediment under various environmental conditions. Over time, however, the populations of *Bacillus subtilis* strain BU1814 in soil and sediment are expected to return to naturally sustainable levels.

Based on a critical review of studies and information from public sources, no significant effects to birds, wild mammals, fish, terrestrial and aquatic non-target arthropods, and plants are expected when BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra are applied according to directions on the label.

Value Considerations

What Is the Value of BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra?

For the claims on wheat, and for the *Pythium* claims on soybean, these products will provide the first non-conventional solution and would potentially be of value to the organic industry.

Bacillus subtilis stain BU1814 represents a new mode of action for seed and seedling diseases in wheat caused by *Fusarium*, *Rhizoctonia solani* and *Cochliobolus sativus* and soybean for diseases caused by *Pythium ultimum*.

Measures to Minimize Risk

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

The key risk-reduction measures being proposed on the labels of *Bacillus subtilis* strain BU1814 Technical, BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

All microorganisms, including *Bacillus subtilis* strain BU1814, contain substances that are potential sensitizers and thus, respiratory and dermal sensitivity may possibly develop in individuals exposed to potentially large quantities of *Bacillus subtilis* strain BU1814. In turn, workers handling or applying BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and

Velondis Extra must wear waterproof gloves, a long-sleeved shirt, long pants, a mist filtering mask or respirator, and shoes with socks.

Environment

End-use product labels will include environmental precaution statements to prohibit aerial applications and to reduce contamination of aquatic systems from the use of BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra.

Next Steps

Before making a final registration decision on *Bacillus subtilis* strain BU1814, BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra, the PMRA will consider any comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed decision and the PMRA's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Bacillus subtilis* strain BU1814 and related end-use products (based on the Science Evaluation of this consultation document). In addition, any confidential test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Bacillus subtilis strain BU1814, BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active mircoorganism	*Bacillus subtilis* strain BU1814
Function	Biofungicide–For the suppression or partial suppression of certain seed and seedling diseases in corn, soybean and wheat.
Binomial name	*Bacillus subtilis* strain BU1814
Taxonomic designation[5]	
Kingdom	Eubacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillalaes
Family	Bacillaceae
Genus	*Bacillus*
Species	*subtilis*
Strain	BU1814
Patent Status information	Canadian Patent No. 2791478
Nominal purity of active	Technical grade active ingredient: minimum of 5×10^{10} viable spores/g End-use products: BAS 154 U ST and Velondis Plus contain *Bacillus subtilis* strain BU1814, 1.4×10^9 CFU/mL;[6] BAS 100 U ST and Velondis Flex contain *Bacillus subtilis* strain BU1814, 1.4×10^9 CFU/mL; Velondis Extra contains *Bacillus subtilis* strain BU1814, 1.76×10^8 CFU/mL.[7]

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

6 These end-use products also contain *Bacillus amyloliquefaciens* strain MBI 600, 1.4×10^{10} CFU/mL. The use of *Bacillus amyloliquefaciens* strain MBI 600 as a seed treatment was previously assessed and approved by PMRA (see PRD2009-17).

7 This end-use product also contains *Bacillus amyloliquefaciens* strain MBI 600, 1.76×10^{10} CFU/mL. The use of *Bacillus amyloliquefaciens* strain MBI 600 as a seed treatment was previously assessed and approved by PMRA (see PRD2009-17).

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 Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminant release standards. In addition, there are no known mammalian toxins or other toxic metabolites present in the technical grade active ingredient or end-use products.

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

Technical Product - *Bacillus subtilis* strain BU1814 Technical

Property	Result
Colour	Light brown
Physical State	Powder
Odour	Mould and yeast
Corrosion Characteristics	Non-corrosive to plastic containers
pH (1%)	6.36 at 20.5°C
Relative Density	0.4269 g/mL

End-Use Product - BAS 154 U ST and Velondis Plus

Property	Result
Colour	Opaque light brown
Physical State	Thick liquid
Odour	Mould and yeast
Viscosity	Thixotropic at 20°C
pH (1%)	5.98
Relative Density	1.166 g/cm^3

End-Use Product – BAS 100 U ST and Velondis Flex

Property	Result
Colour	Opaque brown
Physical State	Thick liquid
Odour	Mould and yeast
Viscosity	Thixotropic at 20°C
pH (1%)	5.89
Relative Density	1.160 g/cm^3

End-Use Product –Velondis Extra

Property	Result
Colour	Opaque light brown
Physical State	Thick liquid
Odour	Mould and yeast
Viscosity	Thixotropic at 20°C

Property	Result
pH (1%)	6.12
Relative Density	1.165 g/cm^3

1.3 Directions for Use

BAS 100 U ST, containing *Bacillus subtilis* strain BU1814, is to be applied to corn, soybean and wheat. Velondis Extra, a co-formulation containing *Bacillus subtilis* strain BU1814 and *Bacillus amyloliquefaciens* strain MBI 600, is to be applied to corn. BAS 154 U ST, a co-formulation containing *Bacillus subtilis* strain BU1814 and *Bacillus amyloliquefaciens* strain MBI 600, is to be applied to soybean. Velondis Flex, containing *Bacillus subtilis* strain BU1814, is to be applied on wheat. Velondis Plus, a co-formulation containing *Bacillus subtilis* strain BU1814 and *Bacillus amyloliquefaciens* strain MBI 600, is to be applied on soybean. These products are applied as seed treatments for the suppression or partial suppression of soil-borne pathogens that cause seed and seedling diseases. Products are applied at a rate 2.5–22 mL/100 kg seed.

1.4 Mode of Action

Bacillus subtilis strain BU1814 is a plant growth promoting rhizobacterium that colonizes the root and shoot thereby suppressing the establishment of disease-causing fungi by competitive exclusion and displacement. *Bacillus subtilis* strain BU1814 is a naturally occurring bacterium. It acts by rapidly colonizing the germinating seeds and the developing root system. It produces a biofilm resulting in a physical barrier to pathogenic fungi and produces antimicrobial components promoting Induced Systemic Resistance within the plant. The resistance risk for this active ingredient is considered low given the mode of action.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganisms

Acceptable methodologies for detection, isolation and enumeration of the active ingredient, *Bacillus subtilis* strain BU1814, were submitted by the applicant. The MPCA has been fully characterized with respect to its origin of strain, natural occurrence and biological properties. *Bacillus subtilis* strain BU1814 can be distinguished from other biocontrol agents based on unique colony morphology. Methods for strain-specific identification by sequencing several housekeeping genes are also under development for *Bacillus subtilis* strain BU1814.

2.2 Methods for Establishment of Purity of Seed Stock

The strain has been deposited in the American Type Culture Collection (ATCC) under ATCC No. PTA11847. Stock cultures are kept frozen at -70°C. A master cell bank is also properly maintained at the manufacturer. A separate mother culture is prepared from which a working cell bank is made for manufacturing purposes.

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

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

The guarantee of the technical grade active ingredient is expressed in units of viable spores per gram (g) and the guarantees of the end-use products are expressed in units of colony forming units (CFU) per mL. Representative data on five batches of technical grade active ingredient and each end-use product were submitted. The methods for determining viable spore counts and CFU counts were adequately described.

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

As noted above, acceptable methods are available to enumerate the microorganism and to distinguish this MPCA from other *Bacillus* species.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures used to limit contaminating microorganisms during the manufacture of *Bacillus subtilis* strain BU1814 Technical and the end-use products, BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra are acceptable. These procedures include sterilization of all equipment and media as well as frequent sampling of the stock culture and production batches for purity and contamination.

The absence of human pathogens and below-threshold levels of contaminating microorganisms were shown in the microbial screening of batches of *Bacillus subtilis* strain BU1814 Technical and of the end-use products using standard methods for detecting and enumerating microbial contaminants of concern. All batches of *Bacillus subtilis* strain BU1814 Technical and all batches of BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra conform to the limits set out in the Organisation for Economic Co-operation and Development (OECD) issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43].

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

Acceptable storage stability data were not provided for *Bacillus subtilis* strain BU1814 Technical or for the end-use products, BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra. In lieu of data, default storage statements will be applied to the label to specify that the technical grade active ingredient and end-use products must be stored in sealed containers at $\leq 4°C$ for no more than 6 months.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

3.1.1 Testing

The PMRA conducted a detailed review of the toxicological studies submitted in support of *Bacillus subtilis* strain BU1814 Technical, and related end-use products, BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra.

In addition to *Bacillus subtilis* strain BU1814, three of the end-use products (BAS 154 U ST, Velondis Plus and Velondis Extra) also contain the registered technical grade active ingredient *Bacillus amyloliquefaciens* strain MBI 600 Technical (PCP# 29452) as a co-active ingredient. The use of *Bacillus amyloliquefaciens* strain MBI 600 as a seed treatment was previously assessed and approved by PMRA as *Bacillus subtilis* strain MBI 600. Refer to the Proposed Regulatory Decision PRD2009-17 for details on the review.

***Bacillus subtilis* strain BU1814 Technical**

The studies submitted to fulfil the requirements for the health hazard assessment of *Bacillus subtilis* strain BU1814 Technical included an acute oral infectivity/toxicity, an acute pulmonary infectivity/toxicity, and an acute intravenous injection infectivity study. The substances used for testing were BAS 100 U and *Bacillus subtilis* UD1022 BU1814, which are considered toxicologically equivalent to *Bacillus subtilis* strain BU1814 Technical.

In the acute oral infectivity/toxicity study, groups of young adult Sprague Dawley rats (12/sex) were exposed by oral gavage to BAS 100 U (1.37×10^{11} CFU/g) in sterile phosphate buffered peptone water at a dose of 2.5×10^{8} CFU/animal. Animals were observed for 21 days with interim sacrifices on Days -1, 0, 7, 14 and 21 to evaluate microbial clearance. There were no mortalities, no abnormal findings upon gross necropsy and all animals gained weight and appeared normal for the duration of the study. The test organism was not recovered in the blood nor in any tissues of treated animals at any time point. The test organism cleared the cecum contents by Day 7. The technical grade active ingredient was predominantly unabsorbed and eliminated in the feces following a single oral high dose. Any test substance that was absorbed was excreted in the urine within 24 hours after dosing.

In the acute pulmonary infectivity/toxicity study, groups of young adult Sprague Dawley rats (15/sex) were exposed by the intratracheal route to BAS 100 U (1.37×10^{11} CFU/g) in sterile peptone phosphate buffer at a dose of 1.4×10^{8} CFU/animal. Animals were observed for 21 days, with interim sacrifices on Days 0, 7, 14 and 21 to evaluate microbial clearance. There were no mortalities, and no treatment-related effects observed upon gross necropsy and all animals gained weight and appeared normal for the duration of the study. The test substance cleared from all tissues and organs, including the lungs, of test animals by Day 14. A pattern of clearance was established in the cecum contents by Day 21.

In the acute intravenous infectivity study, groups of Sprague Dawley rats (13/sex) were administered *Bacillus subtilis* UD1022 BU1814 (3.3×10^8 CFU/mL) in sterile phosphate buffered saline (PBS) at a dose of 3.3×10^7 CFU/rat by intravenous injection. Animals were observed for 42 days, with interim sacrifices on Days 0, 7, 14, 21, 28, 35 and 42 to evaluate microbial clearance. There were no mortalities and all animals appeared normal for the duration of the study. While some statistically significant differences in body weight gain and relative organs weights for female animals (mesenteric lymph nodes, liver, kidney) and combined (male plus females; kidney) were noted between treatment groups, the findings are not toxicologically significant. The test substance cleared from the blood, cecum contents, brain, mesenteric lymph nodes, lungs and kidneys by Day 28 or sooner. A pattern of clearance was established in the liver and spleen by Day 42.

An acute oral toxicity study, a dermal toxicity study, a dermal irritation study, and an eye irritation study with *Bacillus subtilis* UD1022 BU1814 were also submitted in addition to the required studies to support the registration of the technical grade active ingredient.

In the acute oral toxicity study, three young adult, fasted, female Sprague-Dawley rats were administered a single oral dose of *Bacillus subtilis* UD1022 BU1814 (40% w/v in water; purity not stated) at 5000 mg/kg bw following the Up-and-Down procedure. The animals were observed for 14 days. There were no mortalities, no clinical findings or abnormal findings upon necropsy and all animals gained weight during the study.

In the acute dermal toxicity study, a group of young adult Sprague Dawley rats (5/sex) was dermally exposed to *Bacillus subtilis* UD1022 BU1814 (technical grade active ingredient; purity not provided) moistened with water at 5050 mg/kg bw, applied to an area equal to approximately 10% of the total body surface area, for 24 hours. Following the 24-hour exposure period, the treated area was gently washed and animals were observed for 14 days for signs of toxicity and dermal irritation. There were no mortalities, no clinical signs of toxicity or dermal irritation, no observable abnormalities noted upon necropsy, and all animals gained weight during the study.

In the primary dermal irritation study, three young adult New Zealand white rabbits (1♂, 2♀) were dermally exposed to 500 mg of *Bacillus subtilis* UD1022 BU1814 (technical grade active ingredient; purity not stated) in 0.5 mL deionized water for 4 hours to an area 8×8 cm^2. The test area was covered with a gauze patch and semi-permeable dressing during the exposure period. After 4 hours, the test site was washed. Animals were observed for 72 hours and scored for irritation by the method of Draize. No erythema and no edema were observed at any time throughout the study. In this study, *Bacillus subtilis* UD1022 BU1814 was not a dermal irritant.

In the primary eye irritation study, 100 mg of *Bacillus subtilis* UD1022 BU1814 (purity not stated) was directly instilled into the conjunctival sac of the right eye of three young adult New Zealand white rabbits (1 ♂; 2♀) for 24 hours. After recording the 24-hour observation, all treated eyes were washed with room temperature deionized water for one minute. Animals were observed for 3 days and scored for ocular irritation by the method of Draize. Redness and discharge was visible in all treated eyes at the 1-hour timepoint. All irritation cleared by 24 hours. Based on the Maximum Irritation Score (MIS) of 6.0/110 at 1 hour, *Bacillus subtilis* UD1022 BU1814 was classified as “minimally irritating” to the eyes.

BAS 154 U ST and Velondis Plus
Studies submitted to fulfil the requirements for the health hazard assessment of the end-use products, BAS 154 U ST and Velondis Plus, included dermal toxicity, dermal irritation and eye irritation studies. Although not required, acute oral toxicity and acute inhalation studies were also conducted. The substance used for testing, BAS 154 01 U, is considered toxicologically equivalent to BAS 154 U ST and Velondis Plus.

In the acute dermal toxicity study, groups of young adult Sprague Dawley rats (5/sex) were dermally exposed to undiluted BAS 154 01 U (*Bacillus subtilis* strain BU1814 at 3.33×10^9 CFU/mL and *Bacillus amyloliquefaciens* strain MBI 600 at 3.62×10^{10} CFU/mL) at 5000 mg/kg bw, over an area of approximately 10% of body surface area, for 24 hours. Following exposure, the dose area was washed and the animals were observed for 14 days. There were no mortalities. Other than very slight erythema in one male animal (Day 1), there were no other clinical findings or abnormal behaviour noted. There were no abnormalities noted upon necropsy, and all animals gained weight throughout the study period.

In a primary dermal irritation study, three young adult female New Zealand albino rabbits were dermally exposed to 0.5 mL of undiluted BAS 154 01 U (*Bacillus subtilis* strain BU1814 at 3.33×10^9 CFU/mL and *Bacillus amyloliquefaciens* strain MBI 600 at 3.62×10^{10} CFU/mL) over a test area equal to 10% body surface area, for 4 hours. The test site was wrapped with a semi-occlusive dressing. After the 4-hour exposure period, the dressing was removed and the test site was wiped cleaned. Animals were observed for 3 days and irritation was scored by the method of Draize. Very slight erythema was observed in one of the three animals at the 30–60 minute timepoint. The MIS (at 1 h) was 0.33/8. All irritation was resolved by 24 hours. The Maximum Average Score (MAS) was 0/8. In this study, BAS 154 01 U was minimally irritating to the skin.

In the primary eye irritation study, 0.1 mL of undiluted BAS 154 01 U (*Bacillus subtilis* strain BU1814: 3.33×10^9 CFU/mL and *Bacillus amyloliquefaciens* strain MBI 600: 3.62×10^{10} CFU/mL) was instilled into the conjunctival sac of the right eye of three young adult female, New Zealand White rabbits for 24 hours. Eyes were washed prior to scoring at 24-hours. Animals were observed for 3 days and irritation was scored by the method of Draize. One hour after test substance instillation, conjunctival redness, chemosis, and conjunctival discharge was observed. All signs of ocular irritation cleared by 24 hours. The MAS was 0/110 and the MIS (at 1 h) was 4.67/110. In this study, BAS 154 01 U was minimally irritating to the eye.

In the acute oral toxicity study, three young adult, female, fasted Sprague Dawley rats were given a single oral dose of BAS 154 01 U (*Bacillus subtilis* strain BU1814 at 3.33×10^9 CFU/mL and *Bacillus amyloliquefaciens* strain MBI 600 at 3.62×10^{10} CFU/mL) at 5000 mg/kg bw following the Up and Down Method. The animals were observed for 14 days. There were no mortalities, no observable abnormalities upon gross necropsy, and all animals gained weight during the study.

In the acute inhalation toxicity study, a group of young adult Sprague-Dawley rats (5/sex) was exposed by the inhalation route to the BAS 154 01 U (containing *Bacillus subtilis* strain BU1814 at 3.33×10^9 CFU/mL and *Bacillus amyloliquefaciens* strain MBI 600 at 3.62×10^{10} CFU/mL) for 4 hours by nose-only exposure at a concentration of 2.13 mg/L. Animals were observed for

14 days. There were no mortalities, no observable abnormalities upon gross necropsy, and all animals gained weight during the study. All rats exhibited irregular respiration following exposure but all recovered after Day 1 and appeared healthy for the remainder of the 14-Day study period.

BAS 100 U ST and Velondis Flex

Studies submitted to fulfil the requirements for the health hazard assessment of the end-use products, BAS 100 U ST and Velondis Flex, included dermal toxicity, dermal irritation and eye irritation studies. Although not required for registration, acute oral toxicity and acute inhalation studies were also conducted. The substance used for testing, BAS 100 02 U, is considered toxicologically equivalent to BAS 100 U ST and Velondis Flex.

In the acute dermal toxicity study, groups of young adult Sprague Dawley rats (5/sex) were dermally exposed to BAS 100 02 U (*Bacillus subtilis* strain BU1814 at 4.03×10^9 CFU/mL) at 5000 mg/kg bw over an area of approximately 10% of body surface area, for 24 hours. Following the 24-hour exposure period, the test area was washed and animals were observed for 14 days. There were no mortalities, no clinical findings or abnormal behaviour reported for any animal, and no abnormalities noted upon necropsy. All animals gained weight throughout the study period.

In the primary dermal irritation study, three young adult female New Zealand white rabbits were dermally exposed to 0.5 mL of undiluted BAS 100 02 U (*Bacillus subtilis* strain BU1814 at 4.03×10^9 CFU/mL) for 4 hours over an area of approximately 10% body surface area. The test site was wrapped with a semi-occlusive dressing. Irritation was scored at 30–60 minutes and at 24, 48 and 72 hours, according to the method of Draize. After the 4-hour exposure period, the dressing was removed and the test site was gently cleansed with a 3% soap solution followed. Very slight erythema and edema was observed in one of the three animals at the 24-hour timepoint. All irritation was resolved by 48 hours. All animals appeared active and healthy and gained body weight during the study. There were no other signs of gross toxicity, no adverse clinical effects and or abnormal behaviour noted throughout the study. The MIS (at 24 h) was 0.67/8 and the MAS was 0.22/8. In this study, BAS 100 02 U was minimally irritating to the skin.

In the primary eye irritation study, 0.1 mL of undiluted BAS 100 02 U (*Bacillus subtilis* strain BU1814: 4.03×10^9 CFU/mL) was instilled into the conjunctival sac of the right eye of three young adult female New Zealand albino rabbits, for 24 hours. The treated eye of each rabbit was rinsed with physiological saline prior to the 24 hour fluorescein procedure for scoring. Animals were observed for three days and irritation was scored by the method of Draize. One hour after test substance instillation, one eye showed conjunctival redness (grade 1), and another eye showed chemosis (grade 1) with conjunctival discharge (grades 1–2). All signs of ocular irritation cleared by 24 hours. In this study, BAS 100 02 U was minimally irritating to the eye. The MIS (at 1 h) was 2/110 and the MAS was 0/110.

In the acute oral toxicity study, three fasted, female Sprague-Dawley rats were given a single oral dose of undiluted BAS 100 02 U (*Bacillus subtilis* strain BU1814 at 4.03×10^9 CFU/mL) at 5000 mg/kg bw following the Up and Down Method. Afterwards, the animals were observed for 14 days. There were no mortalities, no signs of gross toxicity or abnormal behaviour and no abnormalities noted upon necropsy. All animals gained weight during the study.

In the acute inhalation toxicity study, groups of young adult rats (5/sex) were exposed by the inhalation route to undiluted BAS 100 02 U (*Bacillus subtilis* strain BU1814: 4.03×10^9 CFU/mL) for 4 hours by nose only at a concentration of 2.19 mg/L. Animals were then observed for 14 days. There were no mortalities. Some animals exhibited irregular respiration following exposure but recovered by Day 1. All animals appeared normal for reminder of the study. There were no abnormal necropsy findings at the end of the study period and all animals gained weight during the study.

Velondis Extra

Studies submitted to fulfil the requirements for the health hazard assessment of the EP, Velondis Extra, included dermal toxicity, dermal irritation and eye irritation studies. Although not required for registration, acute oral toxicity and acute inhalation studies were also conducted. The substance used for testing, BAS 154 00 U, is considered toxicologically equivalent to Velondis Extra.

In the acute dermal toxicity study, groups of young adult Sprague Dawley rats (5/sex) were dermally exposed to BAS 154 00 U (*Bacillus subtilis* strain BU1814 at 1.33×10^9 CFU/mL and *Bacillus amyloliquefaciens* strain MBI 600 at 3.77×10^{10} CFU/mL) at 5000 mg/kg bw, over an area of approximately 10% of body surface area, for 24 hours. Following exposure, the dose area was washed and the animals were observed for a period of 14 days. There were no mortalities, no signs of dermal toxicity or irritation, no abnormal behaviour, and no abnormalities noted upon necropsy.

In the primary dermal irritation study, three female young adult New Zealand albino rabbits were dermally exposed to 0.5 mL of undiluted BAS 154 00 U (*Bacillus subtilis* strain BU1814 at 1.33×10^9 CFU/mL and *Bacillus amyloliquefaciens* strain MBI 600 at 3.77×10^{10} CFU/mL) for 4 hours over an area of approximately 10% body surface area. The test site was wrapped with a semi-occlusive dressing. Irritation was scored at 30–60 minutes and at 24, 48 and 72 hours, according to the method of Draize. After the 4-hour exposure period, the dressing was removed and then the test site was gently cleansed with a 3% soap solution. At the 24-hour timepoint, one treated site exhibited very slight erythema and very slight edema which cleared by 48 hours. There was no skin irritation observed for the other two animals during the study and no other signs of gross toxicity, adverse clinical effects, or abnormal behaviour. The MIS (at 24 h) was 0.67/8 and the MAS was 0.22/8. BAS 154 00 U was minimally irritating to the skin.

In the primary eye irritation study, 0.1 mL of undiluted BAS 154 00 U (*Bacillus subtilis*, strain BU1814 at 1.33×10^9 CFU/mL and *Bacillus amyloliquefaciens* strain MBI 600 at 3.77×10^{10} CFU/mL) was instilled into the conjunctival sac of the right eye of three young adult female New Zealand albino rabbits, for 24 hours. The treated eye of each rabbit was rinsed with physiological saline prior to the 24 hour fluorescein procedure for scoring. Animals then were observed for

three days and scored for eye irritation according to the method of Draize. One hour after test substance instillation, all three treated eyes exhibited conjunctival redness and conjunctival chemosis, and two treated eyes showed discharge. All signs of eye irritation cleared by 24 hours. There were no other signs of gross toxicity, no adverse clinical effects, and no abnormal behaviour. The MIS (at 1 h) was 5.33/110 and the MAS was 0/110. In this study, BAS 154 00 U was minimally irritating to the eye.

In the acute oral toxicity study, three young adult female fasted Sprague Dawley rats were given a single oral dose of BAS 154 00 U (containing *Bacillus subtilis* strain BU1814 at 1.33×10^9 CFU/mL and *Bacillus amyloliquefaciens* strain MBI 600 at 3.77×10^{10} CFU/mL) at 5000 mg/kg bw following the Up and Down Method. The animals were observed for 14 days. There were no mortalities, no clinical signs of toxicity, no observable abnormalities upon gross necropsy, and all animals gained weight during the study.

In the acute inhalation toxicity study, young adult Charles River Wistar Hanover albino rats (5/sex) were exposed by the inhalation route to undiluted BAS 154 00 U (containing *Bacillus subtilis* strain BU1814 at 1.33×10^9 CFU/mL and *Bacillus amyloliquefaciens* strain MBI 600 at 3.77×10^{10} CFU/mL) for 4 hours by nose only, at a concentration of 2.32 mg/L. Animals were then observed for 14 days. There were no mortalities. Four males and three females exhibited irregular respiration but recovered by Day 1. All animals appeared active and healthy for the remainder of the study. All animals gained weight by Day 3.

Test results are summarized in Appendix I, Tables 1.1–1.4.

3.1.2 Additional Information

A survey of published literature uncovered no reports of adverse effects for *Bacillus subtilis* strain BU1814. There has been rare cases *Bacillus subtilis*-related endocarditis, bacteremia in immunocompromised patients. In some cases, the organism was introduced into sensitive tissues via intravenous catheters or lumbar puncture surgery. Other cases were related to drug abuse, as narcotics are often contaminated with bacilli. The routine use of *Bacillus subtilis* cultures as a non-specific support for a stable gastrointestinal flora has also been suspected as a source. Single cases of meningitis, an eye infection and a shin-bone infection have also been reported for *Bacillus subtilis*.

Rope spoilage in bread is also associated with *Bacillus subtilis* and foodborne illness has occasionally been reported. Other food poisoning incidents related to *Bacillus subtilis* are rare, and the implicated strains produce a highly heat stable toxin (possibly similar to the *Bacillus cereus*-enterotoxin). *Bacillus subtilis* strain BU1814 is not reported to produce this toxin, and no such illnesses have been reported for this microorganism. Furthermore, when *Bacillus subtilis* strain BU1814 was administered orally to rats, no signs of toxicity or disease were observed. On rare occasions, *Bacillus subtilis* was attributed to foodborne illness where no toxin production was detected.

In veterinary medicine, bovine mastitis, as well as reproductive disorders in goats and canine endocarditis have been related to *Bacillus subtilis.*

Hypersensitivity pneumonitis was reported from exposure to *Bacillus subtilis* and *Bacillus lichenformis* spores and vegetative cells released from wood dust in domestic and industrial settings. Production of *Bacillus subtilis* strain BU1814 Technical is not aimed at enzyme enrichment and there have been no adverse health effects reported in workers at the production site where *Bacillus subtilis* strain BU1814 is fermented or formulated.

3.1.3 Incident Reports Related to Human and Animal Health

A search of incident reports was conducted for registered strains of *Bacillus amyloliquefaciens* and *Bacillus subtilis*. As of 4 May 2018, the PMRA received one human incident involving the active *Bacillus subtilis*. In this incident, a person reported minor symptoms of rash and cough following application of an American product containing *Bacillus subtilis*. Given that it was a minor incident involving an American product that occurred in Canada, no additional risk mitigation measures are recommended. The incident information was incorporated into the evaluation of *Bacillus subtilis* strain BU1814.

3.1.4 Hazard Analysis

The database submitted in support of registering *Bacillus subtilis* strain BU1814 Technical, and the end-use products BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra was reviewed from the viewpoint of human health and safety and was determined to be acceptable.

Based on all the available information, the technical grade active ingredient, *Bacillus subtilis* strain BU1814 Technical is of low toxicity and is not infective by the oral and pulmonary routes, and is not pathogenic or infective by the intravenous route. The technical grade active ingredient is also of low toxicity by the dermal route, minimally irritating to the eye and not a dermal irritant. The MPCA is considered to be a potential sensitizer. Consequently, the hazard statement "POTENTIAL SENSITIZER" will appear on the principal display panel of the technical grade active ingredient. The statement, "May cause sensitization." is also required on the secondary panel of the label under the "PRECAUTIONS" section.

The end-use products, BAS 154 U ST and Velondis Plus, are of low toxicity by the oral, inhalation, and dermal routes. BAS 154 U ST and Velondis Plus are minimally irritating to the skin and eyes. As the formulations contain a MPCA, the hazard statement "POTENTIAL SENSITIZER" will appear on the principal display panels of each end-use product label. The statement, "May cause sensitization. Avoid contact with eyes, skin and clothing. Avoid inhaling/breathing mist." is also required on the secondary panels of each label under the "PRECAUTIONS" section.

The end-use products, BAS 100 U ST and Velondis Flex, are of low toxicity by the oral, inhalation, and dermal routes. BAS 100 U ST and Velondis Flex are minimally irritating to the skin and eyes. As the formulations contain a MPCA, the hazard statement "POTENTIAL SENSITIZER" will appear on the principal display panels of each end-use product label.

The statement, “May cause sensitization. Avoid contact with eyes, skin and clothing. Avoid inhaling/breathing mist.” is also required on the secondary panels of each label under the “PRECAUTIONS” section.

The end-use product, Velondis Extra, is of low toxicity by the oral, inhalation, and dermal routes. Velondis Extra is minimally irritating to the skin and eyes. As the formulation contains a MPCA, the hazard statement “POTENTIAL SENSITIZER” will appear on the principal display panel of the end-use product label. The statement, “May cause sensitization. Avoid contact with eyes, skin and clothing. Avoid inhaling/breathing mist.” is also required on the secondary panel of the label under the “PRECAUTIONS” section.

Higher tier subchronic and chronic toxicity studies were not required because the technical grade active ingredient was not acutely toxic by the oral, dermal or pulmonary (intratracheal instillation) route of administration. Furthermore, there were no indications of any infectivity or pathogenicity in any test animals tested with the MPCA at Tier I.

Within the available scientific literature, there are no reports that suggest *Bacillus subtilis* strain BU1814 has the potential to cause adverse effects on the endocrine system of animals. Based on the weight-of-evidence of available data, no adverse effect to the endocrine system is anticipated for this MPCA.

3.2 Occupational, Residential and Bystander Risk Assessment

3.2.1 Occupational Exposure and Risk

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with primary exposure routes being dermal. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe was a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Bacillus subtilis* has not frequently been identified as a dermal wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Furthermore, toxicity testing with the technical grade active ingredient, *Bacillus subtilis* strain BU1814 Technical showed no toxicity and no infectivity via the oral, pulmonary, intravenous and dermal routes, and it was not a dermal irritant. The technical grade active ingredient was minimally irritating to the eye. Toxicity testing of the end-use products also showed no toxicity via the oral, inhalation, and dermal routes, and the formulations were minimally irritating to the skin and eyes. However, the PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing.

Risk mitigation measures, such as personal protective equipment, including waterproof gloves, long-sleeved shirts, long pants, a NIOSH-approved mist filtering respirator or NIOSH-approved mist filtering mask, and shoes with socks are required to minimize exposure and protect applicators, mixer/loaders, and handlers that are likely to be exposed.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra and no significant occupational risks are anticipated for this product.

3.2.2 Residential and Bystander Exposure and Risk

Overall, the PMRA does not expect that residential and bystander exposures will pose a health risk of concern on the basis of the low toxicity profile for BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra, the low infectivity/pathogenicity profile for *Bacillus subtilis* strain BU1814 Technical and the expectation that that the labels will be followed by commercial applicators in the use of the end-use products. As well, *Bacillus subtilis* is a species that is common in the environment and the use of BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra, as seed treatments is not expected to cause sustained increases in exposure to bystanders beyond natural levels. Consequently, a health risk to infants and children is not expected.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

The proposed use pattern (seed treatment) is not expected to result in dietary exposure, and thus, risk is expected to be of no concern for the general population, including infants and children, or animals. The product will not be applied to the edible portions of crops and, as indicated in Section 1.4, the seed treatment applications of *Bacillus subtilis* strain BU1814 are not expected to yield any growth on the edible portions of the crops. Also, *Bacillus subtilis* strain BU1814 demonstrated no pathogenicity or infectivity in Tier I acute oral, pulmonary (intratracheal) and intravenous injection studies; and no oral toxicity in the acute toxicity study. Furthermore, no metabolites of toxicological significance have been shown to be produced by this strain.

3.3.2 Drinking Water

Health risks are not expected from exposure to *Bacillus subtilis* strain BU1814 via drinking water because exposure will be low from operational applications as a seed treatment and there were no harmful effects observed in Tier I acute oral toxicity testing. The labels for BAS 154 U ST, BAS 100 U ST, Velondis Extra, Velondis Plus and Velondis Flex instruct users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Furthermore, municipal treatment of drinking water is expected to reduce the transfer of residues to drinking water.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARfDs) and acceptable daily intakes (ADIs) are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (in other words, no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests.

Based on all the available information and hazard data, the PMRA concludes that *Bacillus subtilis* strain BU1814 is of low oral toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCAs than the general population. Thus there are no threshold effects of concern and, as a result, there is no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCA and other registered microorganisms that have a common mechanism of toxicity, does not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *Bacillus subtilis* strain BU1814 to human health.

3.3.4 Aggregate Exposure and Risk

Based on the toxicity and infectivity test data and other relevant information in the PMRA's files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *Bacillus subtilis* strain BU1814 to the general Canadian population, including infants and children, when the end-use products are 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. Dermal and inhalation exposure to the general public will be low since the product is not allowed for use on turf, residential or recreational areas. Furthermore, the label will only include seed treatments and few adverse effects from exposure to other strains of *B. subtilis* encountered in the environment have been reported in the public literature. Even if there is an increase in exposure to *Bacillus subtilis* strain BU1814 from the use of BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra, there should not be any increase in potential human health risk.

3.3.5 Maximum Residue Limits

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

Residues of *Bacillus subtilis* strain BU1814 on food crops grown from treated seeds, at the time of harvest, are not anticipated following seed treatment. Consequently, the PMRA has applied an exposure-based approach for determining whether an MRL is required for this microorganism. Therefore, the PMRA has determined that specification of an MRL under the *Pest Control Products Act* is not required for *Bacillus subtilis* strain BU1814.

3.4 Cumulative Effects

The *Pest Control Products Act* requires that the PMRA consider the cumulative exposure to pesticides with a common mechanism of toxicity. In its assessment of common mechanism of toxicity, PMRA considers both the taxonomy of MPCAs and the production of any potentially toxic metabolites. For the current evaluation, the PMRA has determined that *Bacillus subtilis* strain BU1814 shares a common mechanism of toxicity with the registered MPCAs, *Bacillus amyloliquefaciens* strain MBI 600, *Bacillus amyloliquefaciens* strain D747, *Bacillus subtilis* strain QST 713, *Bacillus subtilis* strain GB03, and *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24. The potential health risks from cumulative exposure of *Bacillus subtilis* strain BU1814 and these other registered MPCAs are not of concern when used as labelled given their low toxicity and pathogenicity.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

No studies were submitted to address the environmental fate and behaviour of *Bacillus subtilis* strain BU1814; however, environmental fate data (Tier II/III) are not normally required at Tier I, and are only triggered if significant toxicological effects in non-target organisms are noted in Tier I testing.

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

The seed treatment applications of BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra are expected to result in slight increases of *Bacillus* species in the rhizosphere of treated plants. These localized increases in soil are not expected to significantly increase the overall environmental levels of this species above naturally occurring levels. Also, the localized elevated populations of *Bacillus subtilis* strain BU1814 in the rhizosphere of plants are expected to return to naturally sustainable levels over time.

The end use products are not intended to be applied directly to water. As result, exposure to aquatic environments should be low and limited to runoff after the seeds are sowed in fields. While *Bacillus subtilis* is not considered an aquatic species and is not expected to grow in this environment, the endospores of this microorganism are likely to persist in sediment. The seed

treatment applications of BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra are not expected to significantly increase the overall environmental levels of this species in sediment above naturally occurring levels. As noted previously, any localized increases of *Bacillus subtilis* strain BU1814 in aquatic environments are expected to return to naturally sustainable levels over time.

4.2 Effects on Non-Target Species

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

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

The screening level risk assessment uses simple methods, conservative exposure scenarios (for example, direct application at a maximum application rate) and sensitive toxicity endpoints. A risk quotient (RQ) is calculated by dividing the exposure estimate by an appropriate toxicity value (RQ = exposure/toxicity), and the risk quotient is then compared to the level of concern (LOC).

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

4.2.1 Effects on Terrestrial Organisms

Two studies were submitted to address the hazards of *Bacillus subtilis* strain BU1814 to birds and honey bees. Scientific rationales were also submitted in support of requests to waive testing on remaining terrestrial Tier I requirements. Data submitted under human and animal health toxicity testing were considered to assess the risk of harm to wild mammals.

In the avian oral study, the acute oral toxicity of the technical grade active ingredient ($\geq 4.85 \times 10^{11}$ spores of *Bacillus subtilis* strain BU1814/g) to 16-day-old Bobwhite quail (*Colinus virginianus*) was assessed over a period of 30 days. A suspension of the technical grade active ingredient in deionized water (~40% w/v) was administered to the birds (30) by oral gavage at a dose of 5 mL/kg body weight (bw)/day (equivalent to 9.4×10^{10} CFU/kg bw/day) for five consecutive days then observed for a total of 30 days. No treatment-related effects on mortality, body weight gain and behaviour were observed; and no treatment-related findings were observed at necropsy. The 30-day ED_{50} and NOEL values were determined to be greater than 9.4×10^{10} CFU/kg bw/day and 9.4×10^{10} CFU/kg bw/day, respectively.

In the honey bee study, honey bees (*Apis mellifera*; 50 bees/treatment) were exposed to a formulated end use product, BAS 100 AF U (containing 4.13×10^9 viable spores of *Bacillus subtilis* strain BU1814/mL), at a nominal concentration of 4×10^7 CFU/mL via the dietary and contact routes of exposure. This study was carried out a total of seven times with each test ending when the validity threshold of 20% control mortality was exceeded. At study termination, mortality in the test groups was less than 50%. Over the duration of the tests, ranging from 5 to 17 days, BAS 100 AF U exhibited no infectivity and no significant toxicity to honeybees via the contact or oral routes. The mean LT_{50} was greater than 8 days for the oral test and greater than 9 days for the contact test (see Table below).

Test No.	Oral Tests		Contact Tests	
	LT_{50} (days)	**LD_{50} (CFU/mL)**	**LT_{50} (days)**	**LD_{50} (CFU/mL)**
1	> 7	$> 4.3 \times 10^7$	> 7	$> 4.3 \times 10^7$
2	> 10	$> 4.0 \times 10^7$	> 9	$> 4.0 \times 10^7$
3	> 6	$> 6.3 \times 10^8$	> 5	$> 6.3 \times 10^8$
4	> 9	$> 9.8 \times 10^8$	> 17	$> 9.8 \times 10^8$
5	> 9	$> 3.8 \times 10^7$	> 9	$> 3.8 \times 10^7$
6	> 14	$> 3.6 \times 10^7$	> 14	$> 3.6 \times 10^7$
7	> 7	$> 3.9 \times 10^7$	> 7	$> 3.9 \times 10^7$

The scientific rationales provided by the applicant were based on the lack of adverse effects noted in the above environmental toxicology studies and in the mammalian studies described under Section 3.1.1, a long history of exposure to naturally occurring *Bacillus subtilis* in the environment and the low potential for exposure from the use of strain BU1814 as a seed treatment. *Bacillus subtilis* occurs naturally in soils and in association with plants, and organic/inorganic materials. This species is ubiquitous in the environment. While some *Bacillus* species are opportunistic or obligate pathogens of animals, including mammals (*Bacillus*

anthracis), and insects (for example, *Bacillus thuringiensis*), *Bacillus subtilis* is not generally considered to be a pathogen. Furthermore, the use of strain BU1814 is only expected to result in minimal increases of *Bacillus* species in the rhizosphere of treated plants (Section 4.1). These minimal localized increases in soil are not expected to significantly increase the overall environmental levels of this species above naturally occurring levels. Also, the localized elevated populations of *Bacillus subtilis* strain BU1814 in the rhizosphere of plants are expected to return to naturally sustainable levels over time.

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

Based on all the available information on the biological properties of *Bacillus subtilis*, the lack of documented effects in non-target terrestrial organisms and the anticipated minimal environmental exposure resulting from the use of strain BU1814 as a seed treatment, there is reasonable certainty that no harm will be caused to birds, wild mammals, terrestrial non-target arthropod invertebrates, non-arthropod invertebrates, and terrestrial plants from the proposed uses of BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra as seed treatments on corn (all types) and/or soybean and/or wheat. Furthermore, the formulants are not expected to contribute to potential toxicity of the products.

4.2.2 Effects on Aquatic Organisms

No studies were submitted to address the hazards of strain BU1814 to aquatic non-target organisms. Instead, scientific rationales were submitted to waive all aquatic Tier I testing requirements. As described in the rationales provided for terrestrial non-target organisms, *Bacillus subtilis* is a ubiquitous microorganism that occurs naturally in soils and in association with plants, and organic/inorganic materials. Consequently, these microorganisms naturally migrate into aquatic habitats through runoff and, despite this natural exposure, *Bacillus subtilis* is not considered to be a pathogen of aquatic species. Rather, *Bacillus subtilis* is often studied for use as a probiotic in feed for fish and shrimp. In published scientific studies provided by the applicant, no adverse effects were reported in koi carp (*Cyprinus carpio*), Tilapia nilotica (*Oreochromis niloticus*), gilthead seabream (*Sparus aurata*), tiger shrimp (*Penaeus monodon*) and giant freshwater prawn (*Macrobrachium rosenbergii*). Also, the use of strain BU1814 is not expected to significantly increase the overall environmental levels of this species in soils above naturally occurring levels (see Section 4.1). As a result, the overall environmental levels of this species in sediment are not expected to increase above naturally occurring levels.

The search in PubMed using the keywords “bacillus subtilis pathogen” yielded no reports of pathogenicity to aquatic non-target organisms. As noted in Section 4.2.1, the majority of the scientific literature consisted of reports on: i) the ability of *Bacillus subtilis* to promote growth and/or to induce systemic resistance in host crops; ii) the biological control of various plant pathogenic fungi; and iii) the use of *Bacillus subtilis* as a probiotic in animal feed, including fish feed.

Based on all the available information on the effects of *Bacillus subtilis* to non-target aquatic organisms, there is reasonable certainty that no harm will be caused to fish, aquatic arthropod and non-arthropod invertebrates, and aquatic plants from the proposed uses of BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra as seed treatments on corn (all types) and/or soybean and/or wheat. Furthermore, the formulants are not expected to contribute to potential toxicity of the products. As a general precaution, no aerial application is permitted. The label will also direct handlers to not contaminate surface water by disposal of equipment wash waters.

4.3 Incident Reports related to the Environment

A search of incident reports was conducted for registered strains of *Bacillus subtilis* and *Bacillus amyloliquefaciens*. As of 4 May 2018, the PMRA did not receive any incident reports related to the environment. No additional risk mitigation measures are recommended for *Bacillus subtilis* strain BU1814.

5.0 Value

Efficacy data from a total of 32 trials were provided in support of the 19 claims on corn, soybean and wheat. Most use-claims were supported as proposed. Products containing *Bacillus subtilis* strain BU1814 have been demonstrated to have value when used against the listed diseases on the crops specified on these product labels. Supported claims are shown in Appendix I, Table 3.

There are several conventional alternatives for all crop-pathogen combinations. In the case of all claims on wheat and for the *Pythium* claims on soybean, these products will provide the first non-conventional solution and would potentially be of value to the organic industry. In addition, the risk for resistance development for the active ingredient is low with these types of products.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

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

Bacillus subtilis strain BU1814 Technical, BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra were assessed in accordance with the PMRA Regulatory Directive DIR99-03.[8]

- *Bacillus subtilis* strain BU1814 Technical does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.

- There are also no formulants, contaminants or impurities present in the end-use products that would meet the TSMP Track 1 criteria.

6.2 Formulants and Contaminants of Health Concern

During the review process, contaminants in the technical and formulants and contaminants in the end-use products are compared against the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* maintained in the *Canada Gazette.*[9] The list is used as described in the PMRA Notice of Intent NOI2005-01[10] and is based on existing policies and regulations including: DIR99-03; and DIR2006-02[11] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

- The technical grade active ingredient, *Bacillus subtilis* strain BU1814 Technical, does not contain formulants of health or environmental concern as identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern*.

- The end-use products, BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra, do not contain formulants of health or environmental concern as identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641-2643: *List of Pest Control Product Formulants of Health or Environmental Concern*.

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

9 *Canada Gazette*, Part II, Volume 139, Number 24, SI/2005-11-30) pages 2641-2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* and in the order amending this list in the *Canada Gazette*, Part II, Volume 142, Number 13, SI/2008-67 (2008-06-25) pages 1611-1613: *Part I Formulants of Health or Environmental Concern, Part 2 Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions and Part 3 Contaminants of Health or Environmental Concern.*

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

11 Regulatory Directive DIR2006-02, *Formulants Policy and Implementation Guidance Document.*

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

7.0 Summary

7.1 Methods for Analysis of the Microorganism as Manufactured

The product characterization data for *Bacillus subtilis* strain BU1814 Technical and BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra were judged to be adequate to assess their potential human health and environmental risks. The technical grade active ingredient was characterized and the specifications of the technical grade active ingredient and end-use product were supported by the analyses of a sufficient number of batches. All batches of *Bacillus subtilis* strain BU1814 Technical must conform to the limits set out in the OECD issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43].

In lieu of data, default storage statements will be applied to the *Bacillus subtilis* strain BU1814 Technical label and to the BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra labels. The technical grade active ingredient and end-use products must be stored in sealed containers at ≤ 4°C for no more than 6 months.

7.2 Human Health and Safety

The acute toxicity and infectivity studies and other relevant information submitted in support of *Bacillus subtilis* strain BU1814 and BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra were determined to be acceptable. Based on all the available information, the technical grade active ingredient, *Bacillus subtilis* strain BU1814 Technical, is of low toxicity by the oral, pulmonary, and dermal routes, and was not pathogenic or infective by the oral, pulmonary, or intravenous routes. The technical grade active ingredient is not irritating to the skin and was minimally irritating to the eyes. Also, the MPCA is considered to be a potential sensitizer. Each of the end-use product formulations, BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra were of low toxicity by the oral, inhalation, and dermal routes, and were minimally irritating to the skin and eyes. The signal words, “POTENTIAL SENSITIZER” are required on the principal display panel of the technical grade active ingredient and the end-use products; and the precautionary statements: “May cause sensitization.”, “Avoid contact with skin and clothing.”, “Avoid inhaling/breathing mists.”.

When handled according to prescribed label instructions, the potential for dermal, eye and inhalation exposure for mixer/loaders, applicators, and handlers exists, with the primary source of exposure to workers being dermal. Respiratory and dermal sensitivity could possibly develop upon repeated exposure to the product since all microorganisms, including this MPCA, contain substances that are potential sensitizers. Therefore, users handling or applying BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra must wear waterproof gloves, long-sleeved shirts, long pants, a NIOSH-approved mist filtering mask or respirator, and shoes with socks.

A health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is not expected since the products are commercial seed treatments. The specification of an MRL under the *Pest Control Products Act* is not required for *Bacillus subtilis* strain BU1814.

The use of the co-active ingredient, *Bacillus amyloliquefaciens* strain MBI 600, as a seed treatment was previously assessed and approved by PMRA as *Bacillus subtilis* strain MBI 600 (see Proposed Registration Decision PRD2009-17 for details on this review).

7.3 Environmental Risk

The non-target organism tests, scientific rationales and supporting published scientific literature submitted in support of *Bacillus subtilis* strain BU1814 and related end-use products (BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra) were determined to be acceptable. The use of BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex, and Velondis Extra as seed treatments are not expected to pose a risk to non-target organisms when the directions for use on the label are followed.

As a general precaution, the product labels will prohibit aerial application and instruct handlers to not contaminate surface water by disposal of equipment wash.

The use of the co-active ingredient, *Bacillus amyloliquefaciens* strain MBI 600, as a seed treatment was previously assessed and approved by the PMRA as *Bacillus subtilis* strain MBI 600 (see Proposed Registration Decision PRD2009-17 for details on this review).

7.4 Value

Bacillus subtilis stain BU1814 is a new active ingredient to be used as a seed treatment in BAS 100 U ST, Velondis Extra, BAS 154 U ST, Velondis Flex and Velondis Plus. It has been shown to suppress or partially suppress the listed soil-borne pathogens that cause certain seed and seedling diseases in corn, soybean and wheat.

These products will provide non-conventional alternatives to conventional products and are potential products for organic growers. In the case of all claims on wheat and for the *Pythium* claims on soybean, these products will provide the first non-conventional solution and would potentially be of value to the organic industry.

Bacillus subtilis stain BU1814 represents a new mode of action for seed and seedling diseases in wheat caused by *Fusarium, Rhizoctonia solani* and *Cochliobolus sativus* and soybean for diseases caused by *Pythium ultimum.*

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Bacillus subtilis* strain BU1814 Technical, BAS 154 U ST, BAS 100 U ST, Velondis Plus, Velondis Flex and Velondis Extra, containing the technical grade active ingredient *Bacillus subtilis* strain BU1814, to be applied as seed treatments to corn, soybean and wheat for the suppression or partial suppression of soil-borne pathogens that cause seed and seedling diseases.

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

List of Abbreviations

°C	degree(s) Celsius
a.i.	active ingredient
ADI	acceptable daily intake
ARfD	acute reference dose
ATCC	American Type Culture Collection
bw	body weight
CFU	colony forming units
cm	centimetres
DNA	deoxyribonucleic acid
ED_{50}	median effective dose
g	gram
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
K_d	soil-water partition coefficient
K_F	Freundlich adsorption coefficient
km	kilometre
K_{oc}	organic-carbon partition coefficient
K_{ow}	*n*–octanol-water partition coefficient
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LOC	level of concern
LT_{50}	median lethal time
mg	milligram
mL	millilitre
MAS	maximum average score
MCC	maximum challenge concentration
MIS	Maximum Irritation Score
MPCA	microbial pest control agent
MRL	maximum residue limit
MS	mass spectrometry
N/A	not applicable
NOEL	no observed effect level
OECD	Organisation for Economic Co-operation and Development
PBI	plantback interval
PBS	phosphate buffered saline
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
RQ	risk quotient
TSMP	Toxic Substances Management Policy
USEPA	United States Environmental Protection Agency

Appendix I Tables and Figures

Table 1.1 Toxicity Profile of *Bacillus subtilis* strain BU1814 Technical

Study Type/Animal/PMRA#	Study Results
21-day Acute Oral Infectivity and Toxicity[1] Sprague Dawley rat PMRA No. 2748260	There were no mortalities. There were no treatment related clinical signs, no abnormal necropsy findings and no differences in body weight gain between groups. The test organism was not recovered in the blood nor tissues. The test organism cleared the cecum contents by Day 7. The technical grade active ingredient was of low toxicity and not infective when instilled at $LD_{50} > 2.5 \times 10^8$ CFU /rat.
21-day acute pulmonary Infectivity and Toxicity[1] Sprague Dawley rat PMRA No. 2748262	There were no mortalities. There were no treatment related clinical signs, no abnormal necropsy findings and no differences in body weight gain between groups. The test substance cleared from all tissues and organs by Day 14. A pattern of clearance was established in the cecum contents by Day 21. The technical grade active ingredient was not infective and not pathogenic via intratracheal instillation at 1.4×10^8 CFU/rat.
42-day acute intravenous injection Infectivity[2] Sprague Dawley rat PMRA No. 2748264	There were no mortalities and all animals appeared normal during the study. There were no observable abnormalities upon gross necropsy. On Day 42, weight gain in female treated animals was significantly lower compared to untreated female animals. Statistically significant differences in the relative weight of mesenteric lymph nodes, liver and kidneys of female animals in the test substance group were also noted compared to the organ weights of females in the inactivated test substance group. Relative kidney weight of male and females (combined) was also statistically significantly greater than that of the inactive test group. The test substance cleared from the blood, cecum contents, brain, mesenteric lymph nodes, lungs and kidneys by Day 28 or sooner. A pattern of clearance of the test substance was established in the liver and spleen by Day 42. The technical grade active ingredient was not pathogenic when injected at 3.3×10^7 CFU/rat.
14-day acute oral toxicity[3] Sprague Dawley rat, female PMRA No. 2748259	There were no mortalities. There were no clinical signs of toxicity and all animals gained weight during the study. At necropsy, there were no observable abnormalities noted. The acute oral LD_{50} was greater than 5000 mg/kg bw in female rats.
14-day acute dermal toxicity[3]	There were no mortalities.

Study Type/Animal/PMRA#	Study Results
Sprague Dawley rat PMRA No. 2748265	There were no clinical signs of toxicity or irritation, and all animals gained weight during the study. At necropsy, there were no observable abnormalities noted. The acute dermal LD_{50} was greater than 5050 mg/kg bw in male and female rats.
72-hour dermal irritation[3] New Zealand white PMRA No. 2748267	No erythema and no edema was observed at any time throughout the study. The calculated MIS was 0/8. The MAS was 0/8 at 24, 48 and 72 hours. The technical grade active ingredient was not irritating to skin.
7-day eye irritation[3] New Zealand white PMRA No. 2748268	Redness and discharge was visible in the eyes of all three animals at the 1-hour timepoint. All irritation cleared by 24 hours. The calculated MIS was 6.0/110 at 1 hour. The MAS was 0/110 at 24, 48 and 72 hours. The technical grade active ingredient was minimally irritating to the eyes.
[1] The test substance was BAS 100 U containing *Bacillus subtilis* strain BU1814 at 1.37×10^{11} CFU/g which is considered toxicologically equivalent *to Bacillus subtilis* strain BU1814 Technical. [2] The test substance was *Bacillus subtilis* UD1022 BU1814 containing *Bacillus subtilis* strain BU1814 at 3.3×10^{10} CFU/mL which is considered toxicologically equivalent *to Bacillus subtilis* strain BU1814 Technical. [3] The test substance was *Bacillus subtilis* UD1022 BU1814. A Certificate of Analysis was not provided.	

Table 1.2 Toxicity Profile of BAS 154 U ST and Velondis Plus

Study Type/Animal/PMRA#	Study Results
14-day acute oral toxicity[1] Sprague Dawley rat, female PMRA No. 2748116	There were no mortalities. There were no treatment related clinical signs, no abnormal necropsy findings and no differences in body weight gain between groups. The acute oral LD_{50} was greater than 5000 mg/kg bw in female animals.
14-day acute inhalation toxicity[1] Sprague Dawley rat PMRA No. 2748117	There were no mortalities. All animals exhibited irregular respiration following exposure but recovered by Day 1. All animals appeared normal for reminder of the study. There were no other treatment related clinical signs, no abnormal necropsy findings and no differences in body weight gain between groups. The acute inhalation LC_{50} was greater than 2.13 mg/L in male and female animals.
14-day acute dermal toxicity[1] Sprague Dawley rat PMRA No. 2748118	There were no mortalities. Very slight erythema was observed in one male animal on Day 1. All animals gained weight during the study. At necropsy, there were no observable abnormalities noted. The acute dermal LD_{50} was greater than 5000 mg/kg bw in male and female rats.

Study Type/Animal/PMRA#	Study Results
72-hour dermal irritation[1] New Zealand white PMRA No. 2748119	Very slight erythema was observed in one animal 30–60 minutes following exposure. All irritation cleared by 24 hours. The calculated MIS was 0.33/8 at 1 hour. The MAS was 0/8. BAS 154 U ST was minimally irritating to skin.
72-hour eye irritation[1] New Zealand white, female PMRA No. 2748120	One hour after instillation, two of the treated eyes showed conjunctival redness (grade 1) and one treated eye showed chemosis (grade 1). Conjunctival discharge (grade 1–2) was noted in all treated eyes. All irritation cleared by 24 hours. The calculated MIS was 4.67/110 at 1 hour. The MAS was 0/110 at 24, 48 and 72 hours. BAS 154 U ST was minimally irritating to the eyes.
[1]The test substance was BAS 154 01 U (*Bacillus subtilis* strain BU1814 at 3.33×10^9 CFU/mL and *Bacillus amyloliquefaciens* strain MBI 600 at 3.62×10^{10} CFU/mL. The test substance is considered toxicologically equivalent to BAS 154 U ST.	

Table 1.3 Toxicity Profile of BAS 100 U ST and Velondis Flex

Study Type/Animal/PMRA#	Study Results
14-day acute oral toxicity[1] Sprague Dawley rat, female PMRA No. 2748116	There were no mortalities. There were no treatment related clinical signs, no abnormal necropsy findings and no differences in body weight gain between groups. The acute oral LD_{50} was greater than 5000 mg/kg bw in female animals.
14-day acute inhalation toxicity[1] Sprague Dawley rat PMRA No. 2748175	There were no mortalities. All male animals and four female animals exhibited irregular respiration following exposure but recovered by Day 1. All animals appeared normal for reminder of the study. There were no other treatment related clinical signs, no abnormal necropsy findings and no differences in body weight gain between groups. The acute inhalation LC_{50} was greater than 2.19 mg/L in male and female animals.
14-day acute dermal toxicity[1] Sprague Dawley rat PMRA No. 2748177	There were no mortalities. There were no clinical findings and no abnormal behaviour observed, and all animals gained weight during the study. At necropsy, there were no observable abnormalities noted. The acute dermal LD_{50} was greater than 5000 mg/kg bw in male and female rats.
72-hour dermal irritation[1] New Zealand white PMRA No. 2748179	Very slight erythema and edema was observed in one animal at the 24-hour timepoint. All irritation cleared by 48 hours. The calculated MIS was 0.67/8 at 24 hours. The MAS was 0.22/8 at 24, 48 and 72 hours. BAS 100 U ST was minimally irritating to skin.

Study Type/Animal/PMRA#	Study Results
72-hour eye irritation[1] New Zealand white, female PMRA No. 2748181	One hour after test substance instillation, one eye showed conjunctival redness (grade 1), and another eye showed chemosis (grade 1) with conjunctival discharge (grade 1–2). All signs of ocular irritation cleared by 24 hours. The calculated MIS was 2.07/110 at 1 hour. The MAS was 0/110 at 24, 48 and 72 hours. BAS 100 U ST was minimally irritating to the eyes.
[1]The test substance was BAS 100 02 U containing *Bacillus subtilis* strain BU1814 at 4.03×10^9 CFU/mL. The test substance is considered toxicologically equivalent to BAS 100 U ST.	

Table 1.4 Toxicity Profile of Velondis Extra

Study Type/Animal/PMRA#	Study Results
14-day acute oral toxicity[1] Sprague Dawley rat, female PMRA No. 2748002	There were no mortalities. There were no treatment related clinical signs, no abnormal necropsy findings and no differences in body weight gain between groups. The acute oral LD_{50} was greater than 5000 mg/kg bw in female animals.
14-day acute inhalation toxicity[1] Charles River Wistar rat PMRA No. 2748003	There were no mortalities. Four males and three females exhibited irregular respiration but recovered by Day 1. All animals appeared active and healthy for the remainder of the study. All animals gained weight by Day 3. The acute inhalation LC_{50} was greater than 2.32 mg/L in male and female animals.
14-day acute dermal toxicity[1] Sprague Dawley rat PMRA No. 2748004	There were no mortalities. There were no clinical findings and no abnormal behaviour observed, and all animals gained weight during the study. At necropsy, there were no observable abnormalities noted. The acute dermal LC_{50} was greater than 5000 mg/kg bw in male and female rats.
72-hour dermal irritation[1] New Zealand white PMRA No. 2748005	Very slight erythema and very slight edema was observed at one treated site at the 24-hour timepoint. All irritation cleared by 48 hours. The calculated MIS was 0.67/8 at 24 hours. The MAS was 0.22/8 at 24, 48 and 72 hours. Velondis Extra is minimally irritating to skin.
72-hour eye irritation[1] New Zealand white, female PMRA No. 2748006	One hour after test substance instillation, all three treated eyes exhibited conjunctival redness (grade 1) and conjunctival chemosis (grade 1), and two treated eyes showed discharge (grade 1). All signs of eye irritation cleared by 24 hours. The calculated MIS was 5.33/110 at 1 hour. The MAS was 0/110 at 24, 48 and 72 hours. Velondis Extra was minimally irritating to the eyes.

Study Type/Animal/PMRA#	Study Results
[1]The test substance was BAS 154 00 U containing *Bacillus subtilis* strain BU1814 at 1.33×10^9 CFU/mL and *Bacillus amyloliquefaciens* strain MBI 600 at 3.77×10^{10} CFU/mL. The test substance is considered toxicologically equivalent to Velondis Extra.	

Table 2 Toxicity of *Bacillus subtilis* strain BU1814 to Non-Target Species

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Organisms				
Vertebrates				
Bobwhite quail (*Colinus virginianus*), 16 days old	30 days – Oral Technical grade active ingredient: 9.4×10^{10} CFU/kg bw/day for 5 consecutive days (measured)	There were no treatment related effects noted in the test group. The ED_{50} was > 9.4×10^{10} CFU/kg bw/day and the NOEL was 9.4×10^{10} CFU/kg bw/day **LOW TOXICITY** **NOT PATHOGENIC**		PMRA# 2748270
Invertebrates				
Arthropods				
Honeybees (*Apis mellifera*)	Up to 17 days – Contact and Dietary Tests were conducted 7 times with BAS 100 AF U at nominal concentration of 4×10^7 CFU/mL	Mortality in the test group was less than 50% at study termination. Over the duration of the tests, no infectivity and no significant toxicity to honeybees was noted via the contact or oral routes. **LOW TOXICITY** **NOT PATHOGENIC**		PMRA# 2748283

Table 3 List of Supported Uses

Product	Crop	Supported Label Claim
BAS 100 U ST	Corn	Partial suppression of seed rot or pre-emergence damping off caused by *Fusarium graminearum* at 2.5 mL/100 kg seed.
		Partial suppression of seed rot or pre-emergence damping off caused by *Rhizoctonia solani* at 2.5 mL/100 kg seed.
		Partial suppression of post-emergence damping off caused by *Rhizoctonia solani* at 2.5 mL/100 kg seed.
	Soybean	Partial suppression of seed rot or pre-emergence damping off caused by *Pythium ultimum* at 4.4 mL/100 kg seed.
		Partial suppression of post-emergence damping off caused by *Pythium ultimum* at 4.4 mL/100 kg seed.

Product	Crop	Supported Label Claim
		Partial suppression of seedling blight caused by *Pythium ultimum* at 4.4 mL/100 kg seed.
		Partial suppression of seed rot or pre-emergence damping off caused by *Rhizoctonia solani* at 4.4 mL/100 kg seed.
		Partial suppression of post-emergence damping off caused by *Rhizoctonia solani* at 4.4 mL/100 kg seed.
		Suppression of seedling blight caused by *Rhizoctonia solani* at 4.4 mL/100 kg seed.
		Partial suppression of seedling root rot caused by *Rhizoctonia solani* at 4.4 mL/100 kg seed.
BAS 100 U ST & Velondis Flex	Wheat	Partial suppression of seed rot or pre-emergence damping off caused by *Cochliobolus sativus* at 21.1 mL/100 kg seed.
		Partial suppression of post-emergence damping off caused by *Cochliobolus sativus* at 21.1 mL/100 kg seed.
		Partial suppression of seed rot or pre-emergence damping off caused by *Fusarium culmorum* and *Fusarium graminearum* at 21.1 mL/100 kg seed.
		Partial suppression of post-emergence damping off caused by *Fusarium culmorum* and *Fusarium. graminearum* at 21.1 mL/100 kg seed.
		Suppression of seed rot or pre-emergence damping off caused by *Rhizoctonia solani* at 21.1 mL/100 kg seed.
		Suppression of post-emergence damping off caused by *Rhizoctonia solani* at 21.1 mL/100 kg seed.
		Partial suppression of seedling blight caused by *Rhizoctonia solani* at 21.1 mL/100 kg seed.
BAS 154 U ST & Velondis Plus	Soybean	Partial suppression of seed rot or pre-emergence damping off caused by *Pythium ultimum* at 4.4 mL/100 kg seed.
		Partial suppression of post-emergence damping off caused by *Pythium ultimum* at 4.4 mL/100 kg seed.
		Partial suppression of seedling blight caused by *Pythium ultimum* at 4.4 mL/100 kg seed.
		Partial suppression of seed rot or pre-emergence damping off caused by *Rhizoctonia solani* at 4.4 mL/100 kg seed.
		Partial suppression of post-emergence damping off caused by *Rhizoctonia solani* at 4.4 mL/100 kg seed.
		Suppression of seedling blight caused by *Rhizoctonia solani* at 4.4 mL/100 kg seed.
		Partial suppression of seedling root rot caused by *Rhizoctonia solani* at 4.4 mL/100 kg seed.
Velondis Extra	Corn	Partial suppression of seed rot or pre-emergence damping off caused by *Fusarium* spp. at 20 mL/100 kg seed.

Product	Crop	Supported Label Claim
		Partial suppression of post-emergence damping off caused by *Fusarium* spp. at 20 mL/100 kg seed.
		Partial suppression of seed rot or pre-emergence damping off caused by *Rhizoctonia solani* at 20 mL/100 kg seed.
		Partial suppression of post-emergence damping off caused by *Rhizoctonia solani* at 20 mL/100 kg seed.

References

A. List of Studies/Information Submitted by Registrant

PMRA Document Number | References

1.0 Product Characterization and Analysis

2748252 2017, Product Characterization Table Bacillus subtilis BU1814 Technical Grade, DACO: M2.1, M2.2, M2.3, M2.4, M2.5, M2.6

2748253 2016, *Bacillus subtilis* BU1814 Technical Grade Group A - Product Identity, Composition and Analysis, DACO: M2.10.1, M2.10.3, M2.7.1, M2.7.2, M2.8, M2.9.1, M2.9.2, M2.9.3 CBI

2748254 2016, 5-batch Analysis - Enumeration of Active Ingredient and Microbial Impurities Profile According to SANCO/12116/2012 on BAS 100 U (Including Amendment No. 1), DACO: M2.10.2, M2.9.2

2748255 2016, Stability to Elevated Temperatures According to OPPTS 830.6313 on BAS 100 U (Including Amendment No. 1), DACO: M2.11

2748256 2016, Chemical Physical Characterization on Test Item BAS 100 U TGAI (Including Amendment No. 1), DACO: M2.12

2816993 2014, Macromolecule analysis in axenic cultures of the strain BAS100 ABU, DACO: M2.7.2 CBI

2821667 2015, Draft Genome Sequence of a Natural Root Isolate, *Bacillus subtilis* UD1022, a Potential Plant Growth-Promoting Biocontrol Agent, DACO: M2.7.1

2748111 2017, Product Characterization Table BAS 154 U ST, DACO: M2.1, M2.2, M2.3, M2.4, M2.5

2748112 2016, Chemical Physical Characterization on Test Item BAS 154 01 U (Including Amendment No. 1), DACO: M2.12

2748113 2016, Velondis(TM) Plus Biofungicide Group A - Product Identity, Composition and Analysis, DACO: M2.10.1, M2.10.2, M2.10.3, M2.11, M2.8, M2.9.1, M2.9.2, M2.9.3 CBI

2748114 2016, 5-batch Analysis: Enumeration of Active Ingredient and Microbial Impurities Profile according to SANCO/12116/2012 on BAS 154 01 U (Including Amendment No. 1 and No. 2), DACO: M2.10.2, M2.9.2

2748164 2017, Product Characterization Table BAS 100 U ST, DACO: M2.1, M2.2, M2.3, M2.4, M2.5

2748165 2016, Velondis(TM) Flex Biofungicide: Group A - Product Identity, Composition and Analysis, DACO: M2.10.1, M2.10.2, M2.10.3, M2.11, M2.8, M2.9.1, M2.9.2, M2.9.3 CBI

2748167 2016, 5-batch Analysis - Enumeration of Active Ingredient and Microbial Impurities Profile According to SANCO/12116/2012 on BAS 100 02 U (Including Amendment No. 1), DACO: M2.10.2, M2.9.2

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B. Additional Information Considered

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