Proposed Registration Decision PRD2018-18

Bacillus licheniformis strain FMCH001, Bacillus subtilis strain FMCH002 and F4018-4

(publié aussi en français)

1 November 2018

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

Publications
Pest Management Regulatory Agency
Health Canada
2720 Riverside Drive
A.L. 6607 D
Ottawa, Ontario K1A 0K9

Internet: canada.ca/pesticides
hc.pmra.publications-arla.sc@canada.ca
Facsimile: 613-736-3758
Information Service:
1-800-267-6315 or 613-736-3799
hc.pmra.info-arla.sc@canada.ca

Canada

ISSN: 1925-0878 (print)
1925-0886 (online)

Catalogue number: H113-9/2018-18E (print version)
H113-9/2018-18E-PDF (PDF version)

© Her Majesty the Queen in Right of Canada, represented by the Minister of Health Canada, 2018

All rights reserved. No part of this information (publication or product) may be reproduced or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, or stored in a retrieval system, without prior written permission of the Minister of Public Works and Government Services Canada, Ottawa, Ontario K1A 0S5.

Table of Contents

Overview

Proposed Registration Decision for *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002

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 the technical products FMCH001 Technical (containing *Bacillus licheniformis* strain FMCH001) and FMCH002 Technical (containing *Bacillus subtilis* strain FMCH002) and the end-use product F4018-4 (containing *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002) to suppress certain fungal diseases and nematodes in specific crops.

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 product(s) are acceptable.

This summary describes the key points of the evaluation, while the Science Evaluation section provides detailed technical information on the human health, environmental and value assessments of *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 and the end-use product F4018-4.

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 *Pest Control Products Act* also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

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

---

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 licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 and the end-use product F4018-4, Health Canada's PMRA will consider any comments received from the public in response to this consultation document.[3] Health Canada will then publish a Registration Decision[4] on *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 and the end-use product F4018-4, which will include the decision, the reasons for it, a summary of comments received on the proposed registration decision and Health Canada's response to these comments.

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

What are *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002?

Bacillus licheniformis and *Bacillus subtilis* are soil bacteria whose secondary metabolites are believed to have antagonistic properties against infection by the fungal pathogen *Rhizoctonia solani* and certain types of nematodes that infect roots.

Can Approved Uses of *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 Affect Human Health?

***Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 are unlikely to affect your health when F4018-4 is used according to the label directions.**

Potential exposure to *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 may occur when handling and applying F4018-4. 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.

---

[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 licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 were tested on 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. Health risks to the general population, including infants and children, as a result of dietary exposure (food and drinking water), are 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.

Risk to the general population is not a concern since there were no signs that these microorganisms caused any significant toxicity or disease in studies on laboratory animals. Moreover, F4018-4 is for use as a commercial seed treatment and thus, it is unlikely that adults, youths and toddlers will be exposed to *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002.

Occupational Risks From Handling F4018-4

Occupational risks are not of concern when F4018-4 is used according to label directions, which include protective measures.

Workers handling F4018-4 can come into direct contact with *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 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 particulate filtering respirator, and socks with shoes.

Environmental Considerations

What Happens When *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 are Introduced Into the Environment?

Environmental risks are not of concern.

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

F4018-4 is for use as a seed treatment on a variety of seeds. The end-use product is not intended for aquatic uses. The use of F4018-4 as a seed treatment is not expected to significantly increase the levels of these microorganisms in soil. Exposure to aquatic environments is also expected to be low and limited to leaching and runoff after the seeds are sowed in fields. Published scientific literature on the environmental fate of these species suggests that *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 will survive in soils and sediment under various environmental conditions. Over time, however, the populations of these microorganisms in soil and sediment are expected to return to naturally sustainable levels.

Based on a critical review of registrant-submitted studies and information from public sources, no significant effects to birds, wild mammals, fish, terrestrial and aquatic arthropods, terrestrial and aquatic non-arthropod invertebrates and plants are expected when F4018-4 is applied according to directions on the label.

Value Considerations

What Is the Value of F4018-4?

F4018-4 is a microbial seed treatment that partially suppresses seed rot and seedling blight caused by the soil fungus *Rhizoctonia solani* in corn, soybean and sunflower, as well as destructive nematode species in corn and soybean.

F4018-4 is the first registered biological product that has been shown to be both an effective fungicide and nematicide. F4018-4 constitutes a useful management tool in these crops to reduce plant loss from seed and seedling disease and nematodes, populations of which have been increasing in Canada as a result of changes in agricultural practices.

Measures to Minimize Risk

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

The key risk-reduction measures being proposed on the label of the technical products (FMCH001 Technical and FMCH002 Technical) and the end-use product F4018-4 to address the potential risks identified in this assessment are as follows:

Key Risk-Reduction Measures

Human Health

All microorganisms, including *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002, contain substances that are potential sensitizers and thus, respiratory and dermal sensitivity may possibly develop in individuals exposed to potentially large quantities of *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002. In turn, workers handling or applying F4018-4 must wear waterproof gloves, a long-sleeved shirt, long pants, a particulate filtering respirator, and socks with shoes.

Environment

The end-use product label will include environmental precaution statements to prohibit aerial application, limit drift and reduce contamination of aquatic systems from the use of F4018-4.

Additional Information Being Requested

Since the end-use product was formulated only at pilot-scale prior to registration, guarantee data representing five commercial-scale production batches from each formulating site will be required as post-market information after registration.

Next Steps

Before making a final registration decision on *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 and the end-use product F4018-4, Health Canada's PMRA will consider any comments received from the public in response to this consultation document. Health Canada will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). Health Canada will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed decision and Health Canada's response to these comments.

Other Information

When the Health Canada makes its registration decision, it will publish a Registration Decision on *Bacillus licheniformis* strain FMCH001and *Bacillus subtilis* strain FMCH002 and the end-use product F4018-4 (based on the Science Evaluation section of this consultation document). In addition, the 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 licheniformis strain FMCH001 and *Bacillus subtilis* strain FMCH002

1.0 The Active Substance, its Properties and Uses

1.1 Identity of the Active Ingredients

Active mircoorganisms	*Bacillus licheniformis* strain FMCH001	*Bacillus subtilis* strain FMCH002
Function	Biological fungicide–For the partial suppression of seed rot and seedling blight caused by *Rhizoctonia solani* when applied as a seed treatment on corn, soybean and sunflower seed. Biological nematicide–For the partial suppression of root knot nematode when applied as a seed treatment on corn and soybean seed and for the partial suppression of soybean cyst nematode when applied as a seed treatment on soybean seed.	
Binomial name	*Bacillus licheniformis* strain FMCH001	*Bacillus subtilis* strain FMCH002
Taxonomic designation[5]		
Domain	Bacteria	
Phylum	Firmicutes	
Class	Bacilli	
Order	Bacillalaes	
Family	Bacillaceae	
Genus	*Bacillus*	
Species	*licheniformis*	*subtilis*
Strain	FMCH001	FMCH002
Patent Status information	World Intellectual Property Organization applications WO/2018-045041 and WO/2017/045063	
Nominal purity of active	Technical grade active ingredient: minimum of 7.5×10^{11} viable spores/g	Technical grade active ingredient: minimum of 3.0×10^{11} viable spores/g
	End-use Product : minimum of 2.3×10^{10} viable spores/mL *Bacillus licheniformis* strain FMCH001 and minimum of 2.3×10^{10} viable spores/mL *Bacillus subtilis* strain FMCH002	
Identity of relevant	The technical grade active ingredient does not contain any impurities or micro contaminants known to be Toxic Substances	

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

impurities of toxicological, environmental and/or significance.	Management Policy Track 1 substances. The product must meet microbiological contaminant release standards. Most strains of *Bacillus licheniformis* produce a potentially toxic lipopeptide called lichenysin A. Its presence in F4018-4 and FMCH001 Technical, however, was not determined.

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

Property	FMCH001 Technical	FMCH002 Technical	F4018-4
Colour	Light brown or beige		Brown
Physical state	Solid powder		Liquid suspension
Stability to normal/elevated temperature, metals and metal ions	Expected to be stable at temperatures less than or equal to 4 °C.		-
pH	6.74 (1% aqueous solution)	6.90 (1% aqueous solution)	4.80 (1% weight/volume preparation)
Density/Specific gravity	1.7100 g/mL	1.6536 g/mL	1.1695
Viscosity	-	-	56.77 centistokes at 20 °C 44.46 centistokes at 40 °C

1.3 Directions for Use

F4018-4 is applied to seed of corn, soybean and sunflower for partial suppression of early season diseases caused by *Rhizoctonia solani* and for partial suppression of both root knot nematodes (corn and soybean) and soybean cyst nematodes (soybean). The product is applied at 5.0×10^6 colony forming units (CFU)/seed, equating to 8.7 mL/80,000 seeds of corn, 15.2 mL/140,000 seeds of soybean and 10.9 mL/100,000 seeds of sunflower in sufficient liquid to achieve uniform coverage. F4018-4 may also be applied with other conventional and non-conventional seed treatment products provided that compatibility is first verified.

1.4 Mode of Action

Bacillus licheniformis strain FMCH001 and *Bacillus subtilis* strain FMCH002 are soil bacteria that colonize the rhizosphere surrounding the seed and growing seedling. The bacteria produce secondary metabolites that are believed to be responsible for the antagonistic properties against *Rhizoctonia solani*. These bacteria also reduce nematode infection via the direct effect of secondary metabolites on nematode eggs and juveniles, and through reductions in root penetration of the nematode.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

Acceptable methodologies for detection, isolation and enumeration of the active ingredients, *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002, were submitted by the applicant. The microbial pest control agents (MPCAs) have been fully characterized with respect to their strain origins, natural occurrence and biological properties. *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 can be distinguished from other *Bacillus* species and strains based on 16S rDNA sequencing and by comparison of specific gene sequences.

2.2 Methods for Establishment of Purity of Seed Stock

Bacillus licheniformis strain FMCH001 and *Bacillus subtilis* strain FMCH002 are deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH of the Leibniz Institute under the deposit numbers DSM 32154 and DSM 32155, respectively. A master cell bank of each isolate is maintained at -80 °C. Working cell banks are propagated from the master cell banks and are used in the manufacturing process.

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

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

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

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

As noted above, acceptable methods are available to enumerate the microorganisms and to distinguish these microbial pest control agents (MPCAs) from other *Bacillus* species.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures used to limit contaminating microorganisms during the manufacture of FMCH001 Technical and FMCH002 Technical and the end-use product, F4018-4, 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.

Complete microbial contaminant analysis data were submitted for five batches each of FMCH001 Technical and FMCH002 Technical and five pilot-scale batches of F4018-4 using standard methods for detecting and enumerating microbial contaminants of concern. The data demonstrated the absence of human pathogens and below-threshold levels of contaminating microorganisms in the technical grade active ingredients and the end-use product. All batches of technical grade active ingredient must be screened for the microbial contaminants and conform to the limits set out in the Organisation for Economic Co-operation and Development issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43].

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

Storage stability data were not provided for FMCH001 Technical, FMCH002 Technical or F4018-4. In lieu of data, default storage statements will be applied to the label to specify that the technical grade active ingredients and end-use product must be stored in sealed containers at ≤ 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 the two technical grade active ingredients, FMCH001 Technical and FMCH002 Technical, and the associated end-use product, F4018-4.

3.1.1.1 FMCH001 Technical (containing *Bacillus licheniformis* strain FMCH001)

To address the health hazard requirements for FMCH001 Technical acute pulmonary infectivity/toxicity, acute intravenous infectivity, acute oral toxicity, acute dermal toxicity, bacterial reverse mutation, dermal irritation and eye irritation studies were submitted by the applicant.

In the acute pulmonary infectivity/toxicity study, a group of young adult Sprague Dawley CD rats (26/sex) was exposed by the intratracheal route to *Bacillus licheniformis* strain FMCH001 (technical grade active ingredient; 2.2×10^{12} CFU/g) in sterile phosphate buffered saline at a measured dose of 3.63×10^{8} CFU per animal. Animals were observed for a period up to 86 days. There were no mortalities noted during the study. Slight to moderate moist rales were noted in 22/26 male and female rats shortly after dose administration on Day 1 which were no longer present in most animals on Day 2 and absent from all animals by Day 3. There were no treatment-related effects on body weight or body weight gain for either male or female rats and no macroscopic findings were noted at necropsy. At scheduled sacrifice, *Bacillus licheniformis* strain FMCH001 colonies were detected predominantly in lung and lymph node samples. Low sporadic counts were also observed in other samples recovered from spleen and liver. Counts generally fell throughout the study period and a pattern of clearance was established by study termination on Day 86.

In the acute intravenous infectivity study, young adult Wistar Han rats (15/sex) were given a single intravenous injection of F4005 (equivalent to FMCH001 Technical; 1.05×10^{12} CFU/g) in sterile phosphate buffered saline at a dose of 1.04×10^{8} CFU/animal (1 mL). Animals were observed for a period of 45 days. There were no mortalities, clinical signs or effects on body weight noted during the study. At necropsy, macroscopic findings included enlarged spleens for animals sacrificed on Day 8 (1/3 males and 1/3 females), Day 25 (2/3 males and 2/3 females) and Day 45 (3/3males and 1/3 females). One male sacrificed on Day 25 showed enlarged lymph nodes and an enlarged liver. The findings for the spleen, lymph nodes and liver were considered to be a normal immune response to a foreign material. Incidental findings included a flaccid brain, observed in one male sacrificed on Day 25, which is incidentally seen in rats of this age and strain. Since there were no other clinical signs were noted for this animal, no toxicological relevance was attached to this finding. Following treatment, *Bacillus licheniformis* strain FMCH001 was detected in the majority of tissues from the animals sacrificed on Day 1. By Day 25, the MPCA was cleared from the majority of the tissues except for the liver and spleen. On Day 45, further clearance was observed in these tissues and a pattern of clearance was established.

In the acute oral toxicity study, three fasted young adult Sprague Dawley-derived rats were given a single dose of *Bacillus licheniformis* strain FMCH001 (technical grade active ingredient; 2.2×10^{12} CFU/g) in distilled water at a limit of 5000 mg/kg body weight. The treated animals were observed for a period of 14 days. There were no mortalities and all animals gained body weight throughout the study period. At necropsy, there were no observable abnormalities.

In the acute dermal toxicity study, a group of young adult Sprague Dawley-derived rats (5/sex) were exposed to *Bacillus licheniformis* strain FMCH001 (technical grade active ingredient; 2.2×10^{12} CFU/g) at the limit dose of 5000 mg/kg body weight for 24 hours. Following exposure, the animals were observed for a period of 14 days. There were no mortalities or effects on body weight gain. All treated animals exhibited erythema following patch removal on Day 1. All irritation cleared by Day 10. At necropsy, no gross abnormalities were noted for any of the animals at study termination on Day 14.

In the bacterial reverse mutation study, F4005 (equivalent to FMCH001 Technical; 1.05×10^{12} CFU/g) was assayed in five histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102), both in the absence and in the presence of metabolic activation (Aroclor 1254-induced rat liver post-mitochondrial fraction [S-9]). All treatments in this study were performed using aqueous extracts of the test material, F4005. The extract was prepared by suspending the test material in purified water, shaking the resulting suspensions at 37°C for 24 hours, centrifugation at 13000 rpm for 30 minutes and then filtration of the resulting supernatant. The assays were performed at 55.5, 167, 555, 1670, 5550, 16700 and 55555 µg/plate. Following treatment, evidence of toxicity was observed at 16700 µg equivalent/plate or above in strain TA102 in the absence or presence of S-9, at 55555 µg equivalent/plate in strain TA98 in the absence of S-9 and in strains TA100 and TA1537 in the absence and presence of S-9. The test article was completely soluble in the aqueous assay system at all concentrations tested. No increases in revertant numbers were observed in any of the tested strains.

In the dermal irritation study, three young adult female New Zealand white rabbits were exposed to 0.5 g of *Bacillus licheniformis* strain FMCH001 (technical grade active ingredient; 2.2×10^{12} CFU/g) in distilled water for 4 hours. Animals were observed for 10 days. Irritation was scored by the method of Draize. There were no mortalities noted throughout the study period and all animals appeared normal and healthy. Within 30–60 minutes of patch removal, two treated sites exhibited very slight erythema and one treated site exhibited very slight edema within 24 hours of patch removal. The two affected animals were free of dermal irritation by Day 10 (study termination).

The MAS (Maximum Average Score) was 0.78/8 and the MIS (Maximum Irritation Score) was 1/8 (at 24 h). In this study, *Bacillus licheniformis* strain FMCH001 was slightly irritating to the skin following acute exposure.

In the eye irritation study, *Bacillus licheniformis* strain FMCH001 (technical grade active ingredient; 2.2×10^{12} CFU/g) was instilled (0.049 g) into the conjunctival sac of the right eye of three young adult female New Zealand white rabbits. Animals were observed for 72 hours.Irritation was scored by the method of Draize. There were no mortalities noted throughout the study period and all animals appeared normal and healthy. One hour after test material instillation, positive conjunctivitis was noted for one treated eye, which cleared by 24 hours. The MAS was 0/110 and the MIS was 8.7/110 (at 1 h). In this study, *Bacillus licheniformis* strain FMCH001 was minimally irritating to the eye following acute exposure.

3.1.1.2 FMCH002 Technical (containing *Bacillus subtilis* strain FMCH002)

To address the health hazard requirements for FMCH002 Technical, acute pulmonary infectivity/toxicity, acute oral toxicity, acute dermal toxicity, dermal irritation and eye irritation studies were submitted by applicant.

In the acute pulmonary infectivity and toxicity study, young adult Sprague Dawley CD rats (26/sex) were exposed by the intratracheal route to *Bacillus subtilis* strain FMCH002 (technical grade active ingredient; 4.9×10^{11} CFU/g) in sterile phosphate buffered saline at a measured dose of 4.61×10^{8} CFU per animal. Animals were observed for up to 86 days. There were no test substance-related effects or other mortalities during this study. Slight to moderate moist or dry rales were noted in 4/26 male and female rats shortly after dose administration on Day 1 which were no longer present in most animals on Day 2 and absent from all animals by Day 5. There were no test material-related effects on body weight or body weight gain for either male or female rats during the course of the study and no macroscopic findings were noted at necropsy. At scheduled sacrifice, *Bacillus subtilis* strain FMCH002 colonies were detected predominantly in lung and lymph node samples. Low sporadic counts were also observed in other samples recovered from spleen, brain and liver. Counts generally fell throughout the study period and a pattern of clearance was established by study termination on Day 86.

In the acute oral toxicity study, three fasted young adult Sprague Dawley-derived rats were given a single dose of *Bacillus subtilis* strain FMCH002 (technical grade active ingredient; 4.9×10^{11} CFU/g) in distilled water at a limit of 5000 mg/kg body weight. The animals were observed for a period of 14 days. There were no mortalities and all animals gained body weight throughout the study period. At necropsy, there were no observable abnormalities.

In the acute dermal toxicity study, a group of young adult Sprague Dawley-derived rats (5/sex) was exposed to *Bacillus subtilis* strain FMCH002 (technical grade active ingredient; 4.9×10^{11} CFU/g) at the limit dose of 5000 mg/kg body weight for 24 hours. Following exposure, the animals were observed for a period of 14 days. There were no mortalities or effects on body weight gain. All animals exhibited erythema following patch removal on Day 1. One female rat exhibited desquamation on Day 4. All irritation cleared by Day 5. At necropsy, no gross abnormalities were noted for any of the animals at study termination on Day 14.

In the dermal irritation study, three young adult female New Zealand white rabbits were exposed to 0.5 g of *Bacillus subtilis* strain FMCH002 (technical grade active ingredient; 4.9×10^{11} CFU/g) in distilled water for 4 hours. Animals were observed for 72 hours. Irritation was scored by the method of Draize. There were no mortalities noted throughout the study period and all animals appeared normal and healthy. No erythema or edema were observed at any treated site during the course of the study, however, desquamation was noted at the dose site of one animal after 24 and 48 hours. The affected animal was free of desquamation after 72 hours. The MAS and MIS were 0/8. In this study, *Bacillus subtilis* strain FMCH002. In this study, *Bacillus subtilis* strain FMCH002 was minimally irritating to the skin.

In the eye irritation study, *Bacillus subtilis* strain FMCH002 (technical grade active ingredient; 4.9×10^{11} CFU/g) was instilled (0.045 g) into the conjunctival sac of the right eye of three young adult female New Zealand white rabbits. Animals were observed for 72 hours. Irritation was scored by the method of Draize. There were no mortalities noted throughout the study period and all animals appeared normal and healthy. One hour after test material instillation, positive conjunctivitis was noted for one treated eye which cleared by 24 hours. The MAS was 0/110 and the MIS was 6/110 (at 1 h). In this study, *Bacillus subtilis* strain FMCH002 was minimally irritating to the eye following acute exposure.

3.1.1.3 F4018-4 (containing *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002)

To address the health hazard requirements for the end-use product, F4018-4, the applicant submitted dermal irritation and eye irritation studies.

In the dermal irritation study, three young adult female New Zealand white rabbits were exposed to 0.5 g of F4018-4 (containing 2.1×10^{10} CFU/g *Bacillus licheniformis* strain FMCH001 and 2.1×10^{10} CFU/g *Bacillus subtilis* strain FMCH002) in distilled water for 4 hours. Animals then were observed for 72 hours. Irritation was scored by the method of Draize. There were no mortalities noted throughout the study period and all animals appeared normal and healthy. Very slight to well-defined erythema was observed in all three animals and very slight edema was observed in two animals within 30-60 minutes of patch removal. Desquamation was noted in all

three animals at 72 hours. All animals were free of irritation by Day 7. The calculated MIS was 2.33/8 at 1 hour and the MAS was 1.11/8 at 24, 48 and 72 hours. In this study, F4018-4 was mildly irritating to skin.

In the primary eye irritation study, F4018-4 (containing 2.1×10^{10} CFU/g *Bacillus licheniformis* strain FMCH001 and 2.1×10^{10} CFU/g *Bacillus subtilis* strain FMCH002) was instilled (0.1 mL) into the conjunctival sac of the right eye of three young adult female New Zealand white rabbits. Animals were observed for 72 hours. Irritation was scored by the method of Draize. There were no mortalities noted throughout the study period and all animals appeared normal and healthy. One hour after test material instillation, minimal conjunctivitis was noted in all three treated eyes which cleared by 48 hours. There was no corneal opacity or iritis observed in any treated eye. The MAS was 0.44/110 and the MIS was 6/110 (at 1 h). In this study, F4018-4 was minimally irritating to the eye following acute exposure.

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

3.1.2 Additional Information

Scientific rationales were provided to waive the technical grade active ingredient requirements for acute oral infectivity and acute injection on FMCH002 Technical as well as to waive the end-use product requirement for acute dermal toxicity. Additional scientific rationales were also provided to waive studies that are not on the PMRA's list of requirements for MPCAs, including acute inhalation studies for the technical grade active ingredient and end-use product as well as an acute oral toxicity study for end-use product.

The scientific rationales were largely based on the biological properties of the two MPCAs, *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 (see Section 4.1 for details), a lack of toxic and infectious effects in existing studies for these two MPCAs (see Section 3.1.1 for additional details on testing), and the identities of the formulation ingredients in the end-use product.

Surveys of the published scientific literature uncovered no reports of adverse effects for either MPCA under review. However, there were reports of infections (for example, endocarditis, bacteremia) for other strains of *Bacillus licheniformis* and *Bacillus subtilis* reported in published literature. In many of these reports, infections occurred in immunocompromised patients or in patients that suffered trauma through injury or medical procedures (for example, intravenous catheters or lumbar puncture surgery). Few cases of infection were reported in immuno-competent individuals. Of the few reports of *Bacillus licheniformis* infection in immuno-competent individuals, one concerned a young girl, the cause of which was determined to be a plant thorn that had remained deeply embedded in the skin for several days. The infection was treated successfully with a 10-day course of cotrimoxazole. A similar historical case involved a cutaneous infection, identified as *Bacillus licheniformis*, from a wicker splinter. In other cases, a recurring sepsis and sinusitis in apparently immuno-competent patients were reported in which *Bacillus licheniformis* was proposed as the causal agent.

There has only been one reported human fatality associated with *Bacillus licheniformis*, relating to an instance of contaminated baby-milk powder. Lichenysin A, a cyclic lipopeptide, was the only toxic substance isolated from cell extracts of the contaminant strains, and exhibited cytotoxicity towards boar spermatozoa. Other cases were related to drug abuse, as narcotics are often contaminated with bacilli.

In veterinary medicine, bovine mastitis, as well as reproductive disorders in goats and canine endocarditis have been related to *Bacillus subtilis* and *Bacillus licheniformis.* In a retrospective study of bovine abortions associated with *Bacillus licheniformis*, researchers re-examined 81 cases that had originally been ascribed to *Bacillus licheniformis* infection out of a total of 2445 submitted for diagnosis from Danish dairy herds between 1986 and 1993. Using immunohistochemical techniques, 47 (1.9% of all reported cases) tested positive for *Bacillus licheniformis* as tissue lesions with immunostained bacteria being present. Also, abortions from *Bacillus licheniformis* were concluded to be haematogenous in origin with subsequent transplacental spread to the fetus. While *Bacillus licheniformis* is one of several organisms associated with abortion in cattle, pigs and sheep, it is by no means the most prevalent, and the extent to which it constitutes a causal agent is also unclear from the available data, much of which are contradictory. *Bacillus licheniformis* and *Bacillus subtilis* are approved for use as probiotics in the United States and Europe. No adverse effects have been reported in any of the published trials of probiotics, and evaluations undertaken by the European Food Safety Authority revealed no animal safety concerns.

Rope spoilage in bread is also associated with *Bacillus subtilis* and *Bacillus licheniformis* and foodborne illness has occasionally been reported for these two microorganisms. Other food poisoning incidents related to *Bacillus licheniformis* and *Bacillus subtilis* are rare. Neither species is included in the Public Health Agency of Canada's list of pathogens or in the list of foodborne illness index by the United States Centers for Disease Control and Prevention. Also, no signs of toxicity or disease were observed when *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 were administered to rats via the intratracheal route and no signs of disease were observed after *Bacillus licheniformis* strain FMCH001 was intravenously injected into rats. In addition, no signs of toxicity were observed in rats fed acute doses of *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 (see Section 3.1.1 for additional details).

3.1.3 Incident Reports Related to Human and Animal Health

A search of incident reports was conducted for registered strains of *Bacillus amyloliquefaciens*, *Bacillus subtilis*, and *Bacillus licheniformis*. As of 12 June 2018, the PMRA received one human incident involving the active *Bacillus subtilis* that occurred in Canada. In this incident, a person reported minor symptoms of rash and cough following application of aUnited States product containing *Bacillus subtilis*. Given that it was a minor incident involving a United States product that occurred in Canada, no additional risk mitigation measures are recommended. The incident information was incorporated into the evaluation of *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002.

3.1.4 Hazard Analysis

The data package submitted in support of FMCH001 Technical, FMCH002 Technical and F4018-4 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 ingredients, FMCH001 Technical and FMCH002 are of low toxicity and not infective by the pulmonary route, and are of low toxicity by the oral and dermal routes. FMCH001 Technical was also not infective by the intravenous route and no evidence of bacterial reverse mutation was observed. In irritation studies, FMCH001 Technical was slightly irritating to the skin and minimally irritating to the eyes, and FMCH002 Technical was minimally irritating to the skin and eyes. Both MPCAs are considered to be potential sensitizers. Consequently, the hazard statement “POTENTIAL SENSITIZER” will appear on the principal display panel of each technical grade active ingredient. The statement, "May cause sensitization." is also required on the secondary panel of each label under the "PRECAUTIONS" section.

The end-use product, F4018-4, was minimally irritating to the skin and eyes. As the formulation contains a microorganism, the hazard statement "POTENTIAL SENSITIZER" will appear on the principal display panel of the end-use product label. The following statement must also be included under the "PRECAUTIONS" section on the end-use product label’s secondary display panel: "May cause sensitization. Avoid contact with eyes, skin and clothing. Avoid inhaling/breathing mist."

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 infectivity or pathogenicity in any animals tested with the MPCA in Tier I studies.

Within the available scientific literature, there are no reports that suggest *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 have 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 these MPCAs.

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 licheniformis* and *Bacillus subtilis* have not frequently been identified as dermal wound pathogens and there is no indication that it could penetrate intact skin of healthy individuals. Furthermore, toxicity testing with the technical grade active ingredients,

FMCH001 Technical and FMCH002 Technical, showed no toxicity via the oral, pulmonary and dermal routes and no infectivity via the pulmonary route. FMCH001 Technical was slightly irritating to skin and minimally irritating to the eye. FMCH002 Technical was minimally irritating to the skin and eye. The end-use product, F4018-4, was also minimally irritating to the skin and eye. 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 particulate filtering facepiece respirator, 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 F4018-4 and no unacceptable 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 F4018-4, the low infectivity/pathogenicity profiles for FMCH001 Technical and FMCH002 Technical, and the expectation that the label will be followed by applicators in the use of the end-use product. As well, *Bacillus licheniformis* and *Bacillus subtilis* are common species in the environment and the use of F4018-4, as a seed treatment, is not expected to cause sustained increases in exposure to bystanders beyond natural levels. Consequently, the health risk to infants and children is acceptable.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

The use pattern (seed treatment) is not expected to result in direct dietary exposure, and thus, there is no health risk of concern for the general population, including infants and children, or animals. The product will not be applied to the edible portions of crops and the seed treatment applications of *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 are not expected to yield any growth on the edible portions of the crops. Also, the two MPCAs demonstrated no pathogenicity or infectivity in Tier I acute pulmonary (intratracheal) studies and no oral toxicity in the acute toxicity studies.

3.3.2 Drinking Water

Health risks are not expected from exposure to *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 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 label for F4018-4 instructs 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 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 licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 are 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 licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 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 licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 to the general Canadian population, including infants and children, when the end-use product is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. 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 *Bacillus licheniformis* and *Bacillus subtilis* encountered in the environment have been reported in the open scientific literature. Even if there is an increase in exposure to *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 from the use of F4018-4, 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 licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 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 licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002.

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, the PMRA considers both the taxonomy of the MPCA and the production of any potentially toxic metabolites. For the current evaluation, the PMRA has determined that *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 share 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 licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 with these other registered MPCAs are acceptable when used as labelled given their low toxicity and pathogenicity.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

No studies were submitted to address the environmental fate and behaviour of *Bacillus licheniformis* strain FMCH001 or *Bacillus subtilis* strain FMCH002; 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. Both *Bacillus licheniformis* and *Bacillus subtilis* have also been isolated from the plumage of wild birds. Certain strains of *Bacillus licheniformis* are capable of degrading the beta-keratin in feathers and may play a role in the evolution of molt and plumage pigmentation.

The seed treatment application of F4018-4 is expected to result in slight increases of *Bacillus* species in the rhizosphere of treated plants. While levels of *Bacillus licheniformis* and *Bacillus subtilis* may vary from soil to soil and are not well characterized, the localized sowing of treated seed is not expected to increase the overall levels of these species in the environment beyond naturally occurring levels. Also, the localized elevated populations of *Bacillus licheniformis* and *Bacillus subtilis* in the rhizosphere of plants are expected to return to naturally sustainable levels over time.

F4018-4 is not intended to be applied directly to water. As a result, exposure to aquatic environments should be low and limited to run-off after the seeds are sowed in fields. While *Bacillus licheniformis* and *Bacillus subtilis* are not considered to be aquatic species nor expected to grow in this environment, the endospores of these microorganisms are likely to persist in sediment. The seed treatment application of F4018-4 is not expected to significantly increase the overall environmental levels of these species in sediment above naturally occurring levels. As noted previously, any localized increases of *Bacillus licheniformis* and *Bacillus subtilis* in aquatic environments are expected to return to naturally sustainable levels over time.

4.2 Effects on Non-Target Species

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

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

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

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

The PMRA conducted a detailed review of the environmental toxicology studies and scientific rationales submitted in support of the two technical grade active ingredients, FMCH001 Technical and FMCH002 Technical.

4.2.1.1 FMCH001 Technical (containing Bacillus licheniformis strain FMCH001)

Two studies were submitted to address the hazards of *Bacillus licheniformis* strain FMCH001 to birds and honey bees.

The acute oral toxicity/pathogenicity of FMCH001 Technical to 3-week-old Bobwhite quail (*Colinus virginianus*) was assessed over 30 days. FMCH001 Technical was administered to the birds (30 mixed sex) by oral gavage at 5 mL/kg bw (each equivalent to 6.6×10^{10} CFU per day or 1×10^{12} CFU/kg bw) once per day for five consecutive days. There were no treatment related signs of toxicity or pathogenicity. The 30-day acute oral LD_{50} was greater than 1×10^{12} CFU/kg bw. The 30-day NOEL for *Bacillus licheniformis* FMCH001 Technical, based on mortality, general health, body weight, and feed consumption was greater than 1×10^{12} CFU/kg bw.

In a 12-day dietary toxicity/pathogenicity study, newly emerged adult honey bees (*Apis mellifera*) were fed FMCH001 Technical ad libitum at a concentration of 1.8 mg/L (6.3×10^{7} CFU/mL) in a 50% weight/volume sugar solution for the duration of the study. There was no effect on mortality and on Day 12 when mortality in the untreated control group reached 20% and the study was terminated. The 12-day dietary LC_{50} was greater than 1.8 mg/L.

4.2.1.2 FMCH002 Technical (containing *Bacillus subtilis* strain FMCH002)

Two studies were submitted to address the hazards of *Bacillus subtilis* strain FMCH002 to birds and honey bees.

The acute oral toxicity/pathogenicity of FMCH002 Technical to Bobwhite quail (*Colinus virginianus*) from 2-weeks to 3-weeks old was assessed over 30 days. FMCH002 Technical was administered to the birds (30 mixed sex) by oral gavage at 5 mL/kg bw (each equivalent to 1.63×10^{10} CFUs per day or 2.4×10^{11} CFU/kg bw) once per day for five consecutive days.

There were no treatment related signs of toxicity or pathogenicity. The 30-day acute oral LD_{50} was greater than 2.4×10^{11} CFU/kg bw. The 30-day NOEL for FMCH002 Technical, based on mortality, general health, body weight, and feed consumption was greater than 2.4×10^{11} CFU/kg bw.

In a 17-day dietary toxicity/pathogenicity study, newly emerged adult honey bees (*Apis mellifera*) were fed FMCH002 Technical ad libitum at a concentration of 8 mg/L (2.6×10^6 CFU/mL) in a 50% weight/volume sugar solution for the duration of the study. There was no effect on mortality on Day 17 when mortality in the untreated control group reached 20% and the study was terminated. The 17-day dietary LC_{50} was greater than 8 mg/L.

In studies conducted to satisfy the human health and safety requirements, it was determined that technical grade active ingredients for *Bacillus licheniformis* strain FMCH002 and *Bacillus subtilis* strain FMCH002 are of low toxicity and not infective by the pulmonary route, and are of low toxicity by the oral and dermal routes. FMCH001 Technical is also not infective by the intravenous route and no evidence of reverse mutation is observed in bacteria.

Scientific rationales were also submitted in support of requests to waive testing on remaining terrestrial Tier I requirements. These scientific rationales 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 strains of *Bacillus subtilis* and *Bacillus licheniformis* in the environment and the low potential for exposure from the use of *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 as a seed treatment. *Bacillus licheniformis* and *Bacillus subtilis* occur naturally in soils and in association with plants, and organic/inorganic materials. These microorganisms are ubiquitous in the environment. While some *Bacillus* species are opportunistic or obligate pathogens of animals, including mammals (for example, *Bacillus anthracis*), and insects (for example, *Bacillus thuringiensis*), neither *Bacillus subtilis* nor *Bacillus licheniformis* are considered to be pathogens. Furthermore, the use of strains FMCH001 and FMCH002 is only expected to result in minimal increases of these 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, these localized elevated populations of *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 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" and "bacillus licheniformis 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 licheniformis* and *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 licheniformis* and *Bacillus subtilis* as probiotics in animal feed.

Based on all the available information on the effects of *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 to terrestrial non-target organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, terrestrial arthropods, non-arthropod invertebrates, plants or to other non-target microorganisms from the use of F4018-4 as a seed treatment. 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 *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 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 licheniformis* and *Bacillus subtilis* are ubiquitous microorganisms that occur naturally in soils and in association with plants, and organic/inorganic materials. Consequently, these microorganisms naturally migrate into aquatic habitats through run-off and, despite this natural exposure, *Bacillus licheniformis* and *Bacillus subtilis* are not considered to be pathogens of aquatic species. Rather, these species are often studied for use as probiotics in feed for fish and shrimp. In studies from the open scientific literature submitted by the applicant, no adverse effects were reported for *Bacillus licheniformis* and *Bacillus subtilis* in koi carp (*Cyprinus rubrofuscus*), common carp (*Cyprinus carpio*), tilapia nilotica (*Oreochromis-niloticus*), gilthead sea bream (*Sparus aurata*), triangular bream (*Megalobrama terminalis*), tiger shrimp (*Penaeus monodon*) and giant freshwater prawn (*Macrobrachium rosenbergii*). Also, the use of F4018-4 as a seed treatment is not expected to significantly increase the overall environmental levels of *Bacillus licheniformis* and *Bacillus subtilis* above naturally occurring levels in either terrestrial or aquatic environments (see Section 4.1).

A search in PubMed using the keywords "bacillus licheniformis pathogen" and "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 these bacilli 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* and *Bacillus licheniformis* as a probiotic in animal feed, including fish feed.

Based on all the available information on the effects of *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 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 use of F4018-4 as a seed treatment. 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 amyloliquefaciens*, *Bacillus subtilis*, and *Bacillus licheniformis*. As of 12 June 2018, the PMRA did not receive any incident reports related to the environment. No additional risk mitigation measures are recommended for *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002.

5.0 Value

Greenhouse and field studies were submitted in support of the proposed claims. Multiple greenhouse studies conducted on corn and sunflower inoculated with *Rhizoctonia solani* demonstrated that F4018-4 applied at the labelled rate of 5.0×10^6 CFU/seed can be expected to reduce seed rot and seedling blight caused by this pathogen. This was consistent with the results of several inoculated field studies in which F4018-4 was observed to increase seedling survival and crop yield in corn and soybean.

Greenhouse studies conducted on corn and soybean and which were inoculated with either root knot nematodes or soybean cyst nematodes, demonstrated that the labelled rate of F4018-4 reduced the number of nematode eggs, galls or cysts and penetration sites on roots.

Several studies included one or more treatments of F4018-4 mixed with conventional seed treatment fungicides that are registered for the control of seed rot and seedling blight caused by one or more pathogens, including *Rhizoctonia solani*. A comparison of these treatments to a separate treatment of the conventional fungicides without F4018-4 demonstrated that F4018-4 will modestly augment the level of control provided by conventional fungicides. The observed yield increases achieved with the addition of F4018-4 suggests that tank-mixing with a conventional seed treatment product does not alter the biological functions of *Bacillus licheniformis* strain FMCH001 or *Bacillus subtilis* strain FMCH002.

F4018-4 applied at or above the labelled rate did not reduce germination of corn and soybean seed. No phytotoxicity to the crop was observed in any of the field or greenhouse studies, including those conducted on sunflower.

F4018-4 may be used as a component of an integrated pest management program aimed at reducing the loss of seeds and seedlings of corn, soybean and sunflower as a result of infection by *Rhizoctonia solani* as well as by root knot nematodes in corn and soybean and soybean cyst nematodes in soybean. It is anticipated that F4018-4 would typically be mixed with conventional fungicides with differing modes-of-action to combat seed rot and seedling blight caused not only by one or more *Rhizoctonia* species, but also by other pathogens. Similarly, F4018-4 may also be applied in combination with other nematicides that are registered for use in corn or soybean.

The risk of resistance development by *Rhizoctonia solani* and by either root knot nematodes or soybean cyst nematodes to F4018-4 is not a significant concern given that it is a microbial product containing two *Bacillus* species that are already present in soil.

F4018-4 is effective in partially suppressing seed rot and seedling blight caused by *Rhizoctonia solani* in corn, soybean and sunflower as well as major nematode species on corn and soybean for which there are few registered nematicides. F4018-4 is one of the few seed treatment products for use on sunflower to reduce seed rot and seedling blight by *Rhizoctonia solani*. Therefore, F4018-4 represents a useful tool to reduce loss of seeds and seedlings thereby increasing plant stand and crop yield potential.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The Toxic Substances Management Policy is a federal government policy developed to provide direction on the management of substances of concern that are released into the environment. The Toxic Substances Management Policy calls for the virtual elimination of Track 1 substances [those that meet all four criteria outlined in the policy, in other words, 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*.

FMCH001 Technical, FMCH002 Technical and F4018-4 were assessed in accordance with the PMRA Regulatory Directive DIR99-03.[6]

- FMCH001 Technical and FMCH002 Technical do not meet the Track 1 criteria because the active ingredients are biological organisms and hence are not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.

- There are also no formulants, contaminants or impurities present in the end-use product that would meet the Toxic Substances Management Policy 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.*[7] The list is used as described in the PMRA Notice of Intent NOI2005-01[8] and is based on existing policies

---

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

7 *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.*

8 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.*

and regulations including DIR99-03 and DIR2006-02,[9] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

- The technical grade active ingredients, FMCH001 Technical and FMCH002 Technical, 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 and Contaminants of Health or Environmental Concern*.

- The end-use product, F4018-4, 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 of Health or Environmental Concern*.

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

7.0 Summary

7.1 Methods for Analysis of the Microorganism as Manufactured

The product characterization data for FMCH001 Technical, FMCH002 Technical and F4018-4 were judged to be adequate to assess their potential human health and environmental risks. The technical grade active ingredients were characterized and the specifications of the technical grade active ingredients and end-use product were supported by the analyses of a sufficient number of commercial-scale and pilot-scale batches, respectively. All batches of FMCH001 Technical and FMCH002 Technical must be screened for the microbial contaminants and conform to the limits set out in the Organisation for Economic Co-operation and Development issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43].

As no storage stability studies were submitted in support of registration, default storage statements will be applied to the FMCH001 Technical, FMCH002 Technical and F4018-4 labels. 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.

7.2 Human Health and Safety

The acute toxicity and infectivity studies and other relevant information submitted in support of FMCH001 Technical, FMCH002 Technical and F4018-4 were determined to be acceptable. Based on all the available information, the technical grade active ingredients, FMCH001 Technical and FMCH002 Technical are of low toxicity and not infective by the pulmonary route, and are of low toxicity by the oral and dermal routes. FMCH001 Technical was also not infective

[9] Regulatory Directive DIR2006-02, *PMRA Formulants Policy and Implementation Guidance Document.*

by the intravenous route and no evidence of bacterial reverse mutation was observed. In irritation studies, FMCH001 Technical was slightly irritating to the skin and minimally irritating to the eyes and FMCH002 Technical was minimally irritating to the skin and eyes. Also, the MPCAs are considered to be potential sensitizers. The end-use product, F4018-4, was minimally irritating to the skin and eyes. The signal words, "POTENTIAL SENSITIZER" are required on the principal display panel of each technical grade active ingredient and the end-use product; and the following precautionary statements are required on the secondary panel of each technical grade active ingredient and the end-use product: "May cause sensitization.", "Avoid contact with skin and clothing.", and "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 these MPCAs, contain substances that are potential sensitizers. Therefore, users handling or applying F4018-4 must wear waterproof gloves, long-sleeved shirt, long pants, a NIOSH-approved particulate filtering facepiece respirator, and shoes with socks.

The health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is acceptable since minimal dietary and residential exposures are expected from the use of F4018-4 as a commercial seed treatment. The specification of an MRL under the *Pest Control Products Act* is not required for *Bacillus licheniformis* strain FMCH001 or *Bacillus subtilis* strain FMCH002.

7.3 Environmental Risk

The non-target organism tests, scientific rationales and supporting published scientific literature submitted in support of *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 and the associated end-use product F4018-4, were determined to be sufficiently complete to permit a decision on registration. The use of F4018-4 as a seed treatment is 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.

7.4 Value

The data submitted to register F4018-4 are adequate to support the following claims:

- partial suppression of seed rot and seedling blight caused by *Rhizoctonia solani* in corn, soybean and sunflower;
- partial suppression of root-knot nematodes on corn and soybean; and
- partial suppression of soybean cyst nematode on soybean

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing registration for the sale and use of the technical products *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002 and the end-use product F4018-4 (containing *Bacillus licheniformis* strain FMCH001 and *Bacillus subtilis* strain FMCH002) to suppress certain fungal diseases and nematodes in specific crops.

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 product(s) are acceptable.

Additional Information Being Requested

Since the end-use product formulated only at pilot-scale prior to registration, guarantee data representing five commercial-scale production batches from each formulating site will be required as post-market information after registration.

List of Abbreviations

µg	micrograms
bw	body weight
DNA	deoxyribonucleic acid
g	gram
kg	kilogram
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
mg	milligram
mL	millilitre
MAS	maximum average score
MIS	maximum irritation score
MRL	maximum residue limit
NOEL	no observed effect level
PMRA	Pest Management Regulatory Agency
TRR	total radioactive residue
USEPA	United States Environmental Protection Agency

Appendix I Tables and Figures

Table 1 Toxicity Profile of FMCH001 Technical

Study Type/Animal/PMRA #	Study Results
86-day acute pulmonary infectivity and toxicity Sprague Dawley rat PMRA #2710446	There were no mortalities. Slight to moderate moist rales were observed in 22/26 male and female rats shortly after dose administration on Day 1 which were no longer present in most animals on Day 2 and absent from all animals by Day 3. There were no effects on body weight or body weight gain for either male or female rats and no macroscopic findings noted at necropsy. At scheduled sacrifice, colonies were detected predominantly in lung and lymph node samples. Low sporadic counts were also observed in other samples recovered from spleen and liver. Counts in treated animals generally fell throughout the study period (86 days). While complete clearance was not achieved by Day 86, a definitive pattern of clearance was established. The technical grade active ingredient was not toxic or infective via pulmonary exposure at 3.63×10^8 CFU/rat.
45-day acute intravenous injection infectivity Wistar Han rat PMRA #2876711	There were no mortalities. No clinical signs of toxicity and no effects on body weight gain were noted in any of the animals during the study. At necropsy, enlarged spleens were noted in treated animals sacrificed on Day 8 (1/3 males and 1/3 females), Day 25 (2/3 males and 2/3 females) and Day 45 (3/3 males and 1/3 females). One male sacrificed on Day 25 showed enlarged lymph nodes and an enlarged liver. The noted observations for the spleen, lymph nodes and liver were considered to be a normal immune response. A flaccid brain was noted in one male sacrificed on Day 25. This observation is incidentally observed in rats of this age and strain. No other clinical signs were noted for this animal. Therefore, no toxicological relevance was attached to this finding. At scheduled sacrifice, colonies were recovered from the majority of tissues sacrificed on Day. By Day 25, the MPCA cleared from the majority of the tissues except for the liver and spleen. By Day

Study Type/Animal/PMRA #	Study Results
	45, fewer colonies were recovered from the liver and spleen. A definitive pattern of clearance was established by Day 45. The technical grade active ingredient was not infective via intravenous injection at 1.04×10^8 CFU/rat.
14-day acute oral toxicity Sprague Dawley rat PMRA #2710444	There were no mortalities and all animals appeared normal during the study. There were no observable abnormalities upon gross necropsy. The acute oral LD_{50} was greater than limit dose of 5000 mg/kg bw (equivalent to nominal dose of 1.1×10^{13} CFU/kg bw).
14-day acute dermal toxicity Sprague Dawley rat PMRA #2710448	There were no mortalities or effects on body weight gain. All treated animals exhibited erythema following patch removal on Day 1. All irritation cleared by Day 10. At necropsy, there were no observable abnormalities noted. The acute dermal LD_{50} was greater than 5000 mg/kg bw (equivalent to nominal dose of 1.1×10^{13} CFU/kg bw).
10-day dermal irritation New Zealand white rabbit, female PMRA #2710451	Within 30–60 minutes of patch removal, two treated sites exhibited very slight erythema and one treated site exhibited very slight edema within 24 hours of patch removal. All irritation cleared by Day 10. The calculated MIS was 1/8 at 24 hours. TheMAS was 0.78/8 over 24, 48 and 72 hours. The technical grade active ingredient was slightly irritating to skin.
7-day eye irritation New Zealand white PMRA #2748268	There was no corneal opacity or iritis observed in the treated eyes. Redness and discharge was visible in the eyes of all three animals at the 1-hour time point. All irritation cleared by 24 hours. The calculated MIS was 6.0/110 at 1 hour. The MAS was 0/110 over 24, 48 and 72 hours. The technical grade active ingredient was minimally irritating to the eyes.
Bacterial reverse mutation *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and	Aqueous extracts were assayed at equivalent concentrations of 55.5, 167, 555, 1670, 5550, 16 700 and 55 555 µg/plate. Evidence of toxicity was observed at 16 700 µg/plate or above in strain TA102 in the absence or presence of S-9, at 55 555 µg/plate in strain TA98 in the absence of S-9 and in strains

Study Type/Animal/PMRA #	Study Results
TA102 PMRA #2876757	TA100 and TA1537 in the absence and presence of S-9. The extract was completely soluble in the aqueous assay system at all test concentrations. No increases in revertant numbers were observed.

Table 2 Toxicity Profile of FMCH002 Technical

Study Type/Animal/PMRA #	Study Results
86-day acute pulmonary infectivity and toxicity Sprague Dawley rat PMRA #2710447	There were no mortalities. Slight to moderate moist or dry rales were observed in 4/26 male and female rats shortly after dose administration on Day 1 which were no longer present in most animals on Day 2 and absent from all animals by Day 5. There were no effects on body weight or body weight gain for either male or female rats and no macroscopic findings noted at necropsy. At scheduled sacrifice, colonies were detected predominantly in lung and lymph node samples. Low sporadic counts were also observed in other samples recovered from spleen, brain and liver. Counts in treated animals generally fell throughout the study period (86 days). While complete clearance was not achieved from the lung by Day 86, a definitive pattern of clearance was established. The technical grade active ingredient was not toxic or infective via pulmonary exposure at 4.61×10^8 CFU/rat.
14-day acute oral toxicity Sprague Dawley rat, female PMRA #2710445	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 (equivalent to nominal dose of 2.45×10^{12} CFU/kg bw).
14-day acute dermal toxicity Sprague Dawley rat	There were no mortalities or effects on body weight gain. All treated animals exhibited erythema following patch removal on Day 1. One female rat exhibited desquamation on Day 4. All irritation cleared by Day 5.

Study Type/Animal/PMRA #	Study Results
PMRA #2710449	At necropsy, there were no observable abnormalities noted. The acute dermal LD_{50} was greater than 5000 mg/kg bw (equivalent to nominal dose of 2.45×10^{12} CFU/kg bw).
72-hour dermal irritation New Zealand white rabbit, female PMRA #2710452	There was no erythema or edema observed at any treated site during the course of the study. Desquamation was noted at the dose site of one animal at 24 and 48 hours. The effected animal was free of irritation by 72 hours. The calculated MIS and MAS was 0/8. The technical grade active ingredient was minimally irritating to skin.
7-day eye irritation New Zealand white PMRA #2710455	There was no corneal opacity or iritis observed in the treated eyes. Redness, chemosis and/or discharge was noted 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 over 24, 48 and 72 hours. The technical grade active ingredient was minimally irritating to the eyes.

Table 3 Toxicity Profile of F4018-4

Study Type/Animal/PMRA #	Study Results
7-day dermal irritation New Zealand white rabbit PMRA #2710450	Very slight to well-defined erythema was observed in all three animals and very slight edema was observed in two animals within 30–60 minutes of patch removal. The overall incidence and severity of irritation decreased gradually with time. Desquamation was noted in all three animals at the 72-hour time point. All animals were free of irritation by Day 7. The calculated MIS was 2.33/8 at 1 hour. The MAS was 1.11/8 over 24, 48 and 72 hours. F4018-4 was mildly irritating to skin.
7-day eye irritation New Zealand white rabbit PMRA #2710455	There was no corneal opacity or iritis observed in the treated eyes. Redness, chemosis and/or discharge was noted in the eyes of all three animals at the 1-hour time point. All irritation cleared by 48 hours.

Study Type/Animal/PMRA #	Study Results
	The calculated MIS was 6.0/110 at 1 hour. The MAS was 0.44/110 over 24, 48 and 72 hours. The end-use product was minimally irritating to the eyes.

Table 4 Toxicity/Pathogenicity of FMCH001 Technical to Non-Target Species

Organism	Exposure	Significant Effect, Comments	Reference
Terrestrial Organisms			
Vertebrates			
Birds			
Bobwhite quail (*Colinus virginianus*), 25-day-old	5-day – Dietary exposure	There were no treatment related toxicity effects. 30-day acute oral LC_{50} was greater than 1×10^{12} CFU/kg bw. **LOW TOXICITY** **NOT PATHOGENIC**	PMRA #2710456
Invertebrates			
Arthropods			
Honeybees (*Apis mellifera*), young adult worker	12-day dietary toxicity/pathogenicity study	There was no effect on mortality. The 12-day dietary LC_{50} was greater than 1.8 mg/L. **LOW TOXICITY** **NOT PATHOGENIC**	PMRA #2710458

Table 5 Toxicity/Pathogenicity of FMCH002 Technical to Non-Target Species

Organism	Exposure	Significant Effect, Comments	Reference
Terrestrial Organisms			
Vertebrates			
Birds			
Bobwhite quail (*Colinus virginianus*), 25-day-old	5-day – Dietary exposre	There were no treatment related toxicity effects. 30-day acute oral LC_{50} was greater than 2.4×10^{11} CFU/kg bw.	PMRA #2710457

Organism	Exposure	Significant Effect, Comments	Reference
		LOW TOXICITY **NOT PATHOGENIC**	
Invertebrates			
Arthropods			
Honeybees (*Apis mellifera*), young adult worker	17-day dietary toxicity/pathogenicity study	There was no effect on mortality. The 9-day dietary LC_{50} was greater than 8 mg/L. **LOW TOXICITY** **NOT PATHOGENIC**	PMRA #2710459

Table 6 Registered Seed Treatment Alternatives based on mode of action as of July 2018)

Crop	Pest	Non-Conventional Alternatives (FRAC Mode of Action Code)	Conventional Alternatives (FRAC Mode of Action Code)
Corn	seed rot and seedling blight caused by *Rhizoctonia solani* or *Rhizoctonia* spp.	• *Bacillus amyloquefaciens* strain MBI 600 (44)	• M, 3, 7, 11, 12
Soybean		• *Bacillus subtilis* GB03 (44) • *Bacillus amyloquefaciens* strain MBI 600 (44) • *Trichoderma harzianum* Rifai strain KRL-AG2 (BM02) • Saponins of *Chenopodium quinoa*	• M, 3, 7, 11, 12
Sunflower			• 7, 12
Corn	root knot nematode	• *Bacillus firmus* strain I-1582 (44)	• tioxazafen
Soybean			
Soybean	soybean cyst nematode	• *Bacillus firmus* strain I-1582 (44) • *Pasteuria nishizawae Pn1*	• 7 • tioxazafen

Table 7 List of Supported Uses

Items	Supported label claims
Host crops	Corn (field, sweet, pop and seed), soybean and sunflower
Application method and timing	Application to seed using standard seed treating equipment (either on-farm or commercial seed treatment facility) and applied prior to planting
Number of applications	one/year
Application rates	5.0×10^6 CFU/seed (8.7 mL/80 000 seeds of corn; 15.2 mL/140 000 seeds of soybean; 10.9 mL/100 000 seeds of sunflower)
Efficacy claims	Partial suppression of seed rot and seedling blight caused by *Rhizoctonia solani*
	Partial suppression of root knot nematode (corn and soybean only)
	Partial suppression of soybean cyst nematode (soybean only)
Rotational cropping restrictions	None

References

A. List of Studies/Information Submitted by Registrant

1.0 Product Characterization and Analysis

2710440 2016, ORIGIN, DERIVATION, AND IDENTIFICATION OF MPCA(S), DACO: M2.7.1, M2.7.2 CBI

2748483 2016, ACTIVE INGREDIENT OR MPCA, DACO: M2.10.1 CBI

2748484 2016, ANALYSIS FOR MICROBIAL CONTAMINANTS, DACO: M2.10.2, M2.9.2, M2.9.3 CBI

2748486 2016, SUMMARY OF PHYSICAL AND CHEMICAL PROPERTIES, DACO: M2.12 CBI

2748487 2017, ORIGIN, DERIVATION, AND IDENTIFICATION OF MPCA(S), DACO: M2.7.1 CBI

2748547 2016, ACTIVE INGREDIENT OR MPCA, DACO: M2.10.1 CBI

2748548 2016, ANALYSIS FOR MICROBIAL CONTAMINANTS, DACO: M2.10.2, M2.9.2, M2.9.3 CBI

2748550 2016, SUMMARY OF PHYSICAL AND CHEMICAL PROPERTIES, DACO: M2.12 CBI

2748551 2017, ORIGIN, DERIVATION, AND IDENTIFICATION OF MPCA(S), DACO: M2.7.1 CBI

2748819 2016, NAME AND ADDRESS OF FORMULATING PLANT, DACO: M2.12, M2.2, M2.3, M2.4, M2.5, M2.7.2, M2.8, M2.9.1, M2.9.2, M2.9.3 CBI

2748823 2016, NAME AND ADDRESS OF FORMULATING PLANT, DACO: M2.12, M2.2, M2.3, M2.4, M2.5, M2.7.2, M2.8, M2.9.1, M2.9.2 ,M2.9.3 CBI

2749886 2016, ACTIVE INGREDIENT OR MPCA, DACO: M2.10.1 CBI

2749887 2016, ANALYSIS FOR MICROBIAL CONTAMINANTS, DACO: M2.10.2 CBI

2749888 2016, SUMMARY OF PHYSICAL AND CHEMICAL PROPERTIES, DACO: M2.12 CBI

2749889 2017, POTENCY ESTIMATION AND PRODUCT GUARANTEE, DACO: M2.11, M2.12 , M2.8, M2.9, M2.9.1, M2.9.2, M2.9.3 CBI

2749890 2016, POTENCY ESTIMATION AND PRODUCT GUARANTEE, DACO: M2.11, M2.12, M2.8, M2.9, M2.9.1, M2.9.2, M2.9.3 CBI

2875637 2015, UK Standards for Microbiology Investigations Identification of, DACO: M2.7.2

2875638 2018, Response to the PMRA regarding question: Provide the cited reference and summarize the methods for distinguishing *B. subtilis* strain FMCH002 can be distinguished from *B. cereus* and *B. anthracis*, DACO: M2.7.2

2875639 2009, Phylogeny and molecular taxonomy of the *Bacillus subtilis* species complex and description of *Bacillus subtilis* subsp. *inaquosorum* subsp. nov., DACO: M2.7.2

2876692 2018, White Paper, DACO: M2.7.2

2876693 2018, Growth Temperature optimum - FMCH001 and FMCH002, DACO: M2.7.2

2876694 de Clerck, E. and P. de Vos, 2004, Genotypic diversity among *Bacillus licheniformis* strains from various sources, DACO: M2.7.2

2876695 2001, *Bacillus sonorensis* sp. nov., a close relative of *Bacillus licheniformis*, isolated from soil in the Sonoran Desert, Arizona, DACO: M2.7.2

2876696 2016, Identification Certificate, DACO: M2.7.2

2876697 2018, Response to PMRA request for additional information on pathogenicity and metabolite toxicity in *Bacillus licheniformis*, DACO: M2.7.2

2876699 Salkinoja-Salonen, M.S., Vuorio, R., Andersson, M.A., Kampfer, P., Andersson, M.C., Honkanen-Buzalski, T., and Scoging, A.C. , 1999, Toxigenic strains of *Bacillus licheniformis* related to food poisoning, DACO: M2.7.2

2876700 Mikkola, R., Kolari, M., Andersson, M.A., Helin, J., and Salkinoja-Salonen, M.S., 2000, Toxic lactonic lipopeptide from food poisoning isolates of *Bacillus licheniformis*, DACO: M2.7.2

2876701 Price, Neil P. J.; Rooney, Alejandro P.; Swezey, James L.; Perry, Elizabeth; Cohan, Frederick M. , 2007, Mass spectrometric analysis of lipopeptides from *Bacillus* strains isolated from diverse geographical locations , DACO: M2.7.2

2876702 Dhakal, Rajat; Seale, R. Brent; Deeth, Hilton C.; Craven, Heather; Turner, Mark S. , 2014, Draft genome comparison of representatives of the three dominant genotype groups of dairy *Bacillus licheniformis* strains. , DACO: M2.7.2

2876704 Adimpong, David B.; Thorsen, Line; Nielsen, Dennis S.; Jespersen, Lene; Sorensen, Kim I.; Stuer-Lauridsen, Birgitte; Derkx, Patrick M. F.; Abdelgadir, Warda S. , 2012, Antimicrobial susceptibility of *bacillus* strains isolated from primary starters for african traditional bread production and characterization of the bacitracin operon and bacitracin biosynthesis. , DACO: M2.7.2

2876712 Agerholm, J.S., Jensen, N.E., Dantzer, V., Jensen, H. E. & Aarestrup, F.M., 1999, Experimental Infection of Pregnant Cows with *Bacillus licheniformis* , DACO: M2.7.2

2876713 Agerholm, J.S., Willadsen, C.M., Nielsen, T.K., Giese, S.B., Holm, E., Jensen, L. & Agger, J.F., 1997, Diagnostic Studies of Abortion in Danish Dairy Herds, DACO: M2.7.2

2876714 Ameur, M.A., Dubrous, P. & Koeck, J.L., 2005, *Bacillus licheniformis*: an unusual cause of erysipelosis. , DACO: M2.7.2

2876715 Aranda, F.J., Teruel, J.A. & Ortiz, A., 2005, Further aspects on the haemolytic activity of the antibiotic lipopeptide iturin A, DACO: M2.7.2

2876716 Arguelles-Arias, A., Ongena, M., Halimi, B., Lara, Y., Brans, A., Joris, B. and Fickers, P., 2009, *Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens., DACO: M2.7.2

2876717 Arima, K., Kakinuma, A. and Tamura, G., 1968, Surfactin, a crystalline peptidelipid surfactant produced by *Bacillus subtilis*: isolation, characterization and its inhibition of fibrin clot formation, DACO: M2.7.2

2876719 Carrillo, C., Teruel, J.A., Aranda, F.J. and Ortiz, A., 2003, Molecular mechanism of membrane permeabilization by the peptide antibiotic surfactin, DACO: M2.7.2

2876722 Cutting, S.M., 2010, *Bacillus* probiotics, DACO: M2.7.2

2876723 Deng, W., Dong, X.F., Tong, J.M. and Zhang, Q., 2011, The probiotic *Bacillus licheniformis* ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens., DACO: M2.7.2

2876724 EFSA, 2015, Scientific Opinion on the safety and efficacy of Cibenza EP150 (a preparation of *Bacillus licheniformis* (ATCC 53757) and its protease (EC 3.4.21.19)) as a feed additive for chickens for fattening, chickens reared for laying and minor avian species for fattening and to point of lay and ornamental birds, DACO: M2.7.2

2876725 EFSA, 2016, Safety and efficacy of BioPlus 2B (*Bacillus subtilis* DSM 5750 and *Bacillus licheniformis* DSM 5749) as a feed additive for sows, piglets, pigs for fattening, turkeys for fattening and calves, DACO: M2.7.2

2876728 EFSA, 2016, Safety and efficacy of B-Act (*Bacillus licheniformis* DSM 28710) for chickens for fattening and chickens reared for laying, DACO: M2.7.2

2876729 EFSA, 2017, Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 6: suitability of taxonomic units notified to EFSA until March 2017, DACO: M2.7.2

2876730 EFSA, 2011, Scientific Opinion on the safety and efficacy of BioPlus 2B (*Bacillus licheniformis* DSM 5749 and *Bacillus subtilis* DSM 5750) as a feed additive for sows, DACO: M2.7.2

2876735 Galiero, G., 2016, Causes of infectious abortion in the Mediterranean buffalo., DACO: M2.7.2

2876736 Hannah, W. N., Jr and Ender, P. T., 1999, Persistent *Bacillus licheniformis* bacteremia associated with an intentional injection of organic drain cleaner., DACO: M2.7.2

2876737 Haydushka, I.A., Markova, N., Kirina, V. and Atanassova, M., 2012, Recurrent Sepsis Due To *Bacillus Licheniformis*, DACO: M2.7.2

2876738 Idelevich, E.A., Pogoda, C.A., Ballhausen, B., Wullenweber, J., Eckardt, L., Baumgartner, H., Waltenberger, J., Peters, G. and Becker, K., 2013, Pacemaker lead infection and related bacteraemia caused by normal and small colony variant phenotypes of *Bacillus licheniformis*, DACO: M2.7.2

2876741 Jeon Y.L., Yang J.J., Kim M.J., Lim G., Cho S.Y., Park T.S., Suh J.-T., Park Y.H., Lee M.S., Kim S.C., Lee H.J., 2012, Combined *Bacillus licheniformis* and *Bacillus subtilis* infection in a patient with oesophageal perforation, DACO: M2.7.2

2876744 Kim, H.S., Lee, E.J., Bae, E.J., Kim, M.K., Hur, J., Cho, O. H., Kang, D.H., Kim, S., Jun, J.B. and Bae, I.G., 2012, A Case of *Bacillus licheniformis* Spondylitis and Bacteremia in a Patient with Lung Cancer, DACO: M2.7.2

2876745 Kowalski, Z.M., Górka, P., Schlagheck, A., Jagusiak, W., Micek, P. & Strzetelski, J., 2009, Performance of Holstein calves fed milk-replacer and starter mixture supplemented with probiotic feed additive, DACO: M2.7.2

2876746 Kritas, S.K., Govaris, A., Christodoulopoulos, G. & Burriel, A.R., 2005, Effect of *Bacillus licheniformis* and *Bacillus subtilis* Supplementation of Ewe's Feed on Sheep Milk Production and Young Lamb Mortality., DACO: M2.7.2

2876748 Lépine, A., Michel, F., Nicaise, C., Imbert, G., Vialet, R., Thomachot, L., Di Marco, J-N., Lagier, P. and Martin, C., 2008, *Bacillus licheniformis* Septicemia in a Very-Low-Birth-Weight Neonate: A Case Report, DACO: M2.7.2

2876749 Logan, N.A., 2011, *Bacillus* and relatives in foodborne illness, DACO: M2.7.2

2876751 Lucking, G., Stoeckel, M., Atamer, Z., Hinrichs, J. and Ehling-Schulz, M., 2013, Characterization of aerobic spore-forming bacteria associated with industrial dairy processing environments and product spoilage, DACO: M2.7.2

2876752 Madslien, E.H., Rönning, H.T., Lindback, T., Hassel, B., Andersson, M.A. and Granum, P.E., 2013, Lichenysin is produced by most *Bacillus licheniformis* strains. , DACO: M2.7.2

2876753 Maget-Dana, R., Thimon, L., Peypoux, F. and Ptak, M, 1992, Surfactin/iturin A interactions may explain the synergistic effect of surfactin on the biological properties of iturin A, DACO: M2.7.2

2876754 Maget-Dana, R. and Peypoux, F., 1993, Iturins, a special class of pore-forming lipopeptides: biological and physicochemical properties, DACO: M2.7.2

2876755 Nicholson, W.L., Munakata, N., Horneck, G., Melosh, H.J. and Setlow, P. , 2000, Resistance of *Bacillus* Endospores to Extreme Terrestrial and Extraterrestrial Environments, DACO: M2.7.2

2876756 Nieminen, T., Rintaluoma, N., Andersson, M., Taimisto, A.-M., Ali-Vehmas, T., Seppala, A., Priha, O. and Salkinoja-Salonen, M., 2007, Toxinogenic *Bacillus pumilus* and *Bacillus licheniformis* from mastitic milk, DACO: M2.7.2

2876763 2011, Secondary metabolites of soil *Bacillus* spp., DACO: M2.7.2

2876764 1995, *Bacillus licheniformis* prosthetic aortic valve endocarditis, DACO: M2.7.2

2876765 2004, Developments in the use of *Bacillus* species for industrial production, DACO: M2.7.2

2876767 2007, The Safety of Two *Bacillus* Probiotic Strains for Human Use. , DACO: M2.7.2

2876769 2005, *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. , DACO: M2.7.2

2876770 1977, *Bacillus licheniformis* sepsis., DACO: M2.7.2

2876771 2007, Causes of bovine abortion, stillbirth and neonatal death in Finland 1999-2006. , DACO: M2.7.2

2876772 1970, A novel protoplast-bursting factor (surfactin) obtained from *Bacillus subtilis* IAM 1213. I. The effects of surfactin on *Bacilllus megaterium* KM., DACO: M2.7.2

2876776 2014, *Bacillus licheniformis* as a cause of a deep skin abscess in a 5-year old girl: An exceptional case following a plant thorn injury. , DACO: M2.7.2

2876777 2016, Oral Administration of a Select Mixture of *Bacillus* Probiotics Affects the Gut Microbiota and Goblet Cell Function following *Escherichia coli* Challenge in Newly Weaned Pigs of Genotype MUC4 That Are Supposed To Be Enterotoxigenic *E. coli* F4ab/ac Receptor Negative, DACO: M2.7.2

2876778 2016, Effects of *Bacillus licheniformis* on the growth performance and expression of lipid metabolism-related genes in broiler chickens challenged with *Clostridium perfringens*-induced necrotic enteritis. , DACO: M2.7.2

2876780 1995, A retrospective study of bovine abortions associated with *Bacillus licheniformis*. , DACO: M2.7.2

2876781 1995, *Bacillus licheniformis* bacteremia: five cases associated with indwelling central venous catheters, DACO: M2.7.2

2876782 2015, Toxin producing ability among *Bacillus* spp. outside the *Bacillus cereus* group. , DACO: M2.7.2

2883600 2015, Maxillary sinus infection by *Bacillus licheniformis*: A case report from Djibouti. , DACO: M2.7.2

2883602 1989, Identification and characterization of *Bacillus cereus* and other *Bacillus* species associated with food poisoning. , DACO: M2.7.2

2883603 1970, Human Infections Caused by Organisms of the *Bacillus* Species, DACO: M2.7.2
2883604 1999, Bacillary endophthalmitis. Four case reports, DACO: M2.7.2

2.0 Human And Animal Health

2710441 2016, Response to Tier 1 Microbial Pesticide Data Requirements for F4018-4, DACO: M4.1
2710442 2016, Response to Tier 1 Microbial Pesticide Data Requirements for *Bacillus licheniformis* strain FMCH001 Technical, DACO: M4.1
2710443 2016, Response to Tier 1 Microbial Pesticide Data Requirements for *Bacillus subtilis* strain FMCH002 Technical, DACO: M4.1
2710444 2016, *Bacillus licheniformis* FMCH001: Acute Oral Toxicity - Up-And-Down Procedure in Rats, DACO: M4.2.2
2710445 2016, *Bacillus subtilis* FMCH002: Acute Oral Toxicity - Up-And-Down Procedure in Rats, DACO: M4.2.2
2710446 2016, *Bacillus licheniformis* FMCH001: Acute Pulmonary Toxicity/Pathogenicity in Rats, DACO: M4.2.3
2710447 2016, *Bacillus subtilis* FMCH002: Acute Pulmonary Toxicity/Pathogenicity in Rats, DACO: M4.2.3
2710448 2016, *Bacillus licheniformis* FMCH001: Acute Dermal Toxicity in Rats, DACO: M4.4
2710449 2016, *Bacillus subtilis* FMCH002: Acute Dermal Toxicity in Rats, DACO: M4.4
2710450 2016, F4018-4: Primary Skin Irritation in Rabbits, DACO: M4.5.2
2710451 2016, *Bacillus licheniformis* FMCH001: Primary Skin Irritation in Rabbits, DACO: M4.5.2
2710452 2016, *Bacillus subtilis* FMCH002: Primary Skin Irritation in Rabbits, DACO: M4.5.2
2710453 2016, F4018-4: Primary Eye Irritation in Rabbits, DACO: M4.9
2710454 2016, *Bacillus licheniformis* FMCH001: Primary Eye Irritation in Rabbits, DACO: M4.9
2710455 2016, *Bacillus subtilis* FMCH002: Primary Eye Irritation in Rabbits, DACO: M4.9
2748488 2017, Summary - Infectivity, pathogenicity and toxicity, DACO: M4.2.1, M4.2.2, M4.2.3
2748489 2017, Summary - Acute Infectivity, DACO: M4.3.1, M4.3.2, M4.3.3
2748554 2017, Summary - Infectivity, pathogenicity and toxicity, DACO: M4.2.1, M4.2.2, M4.2.3
2748557 2017, Summary - Acute Infectivity, DACO: M4.3.1, M4.3.2, M4.3.3
2788208 1975, An evaluation of the inhalation toxicity of one commercial proteolytic enzyme preparation, DACO: M4.2.3
2749892 2017, Summary - Irritation, DACO: M4.5.1, M4.5.2
2876711 2017, F4005 (*Bacillus licheniformis* strain FMCH001): An Acute Injection Toxicity/Pathogenicity Study with a Single Intravenous Injection in the Rat, DACO: M2.7.2

2876757 2018, F4005 (*Bacillus licheniformis* strain FMCH001): Bacterial Reverse Mutation Assay, DACO: M2.7.2

3.0 Environment

2710456 2016, *Bacillus licheniformis* FMCH001: Acute Oral Toxicity Study in Bobwhite Quail, DACO: M9.2.1
2710457 2016, *Bacillus subtilis* FMCH002: Acute Oral Toxicity Study in Bobwhite Quail, DACO: M9.2.1
2710458 2016, *Bacillus licheniformis* FMCH001: Honey Bee, *Apis mellifera*, Non-target Insect Microbial Testing, DACO: M9.5.1
2710459 2016, *Bacillus subtilis* FMCH002: Honey Bee, *Apis mellifera*, Non-target Insect Microbial Testing, DACO: M9.5.1
2748490 2017, Summary - Environmental Toxicology/Nontarget Organism Testing, DACO: M9.1, M9.2.1, M9.2.2, M9.3, M9.4.1, M9.4.2, M9.5.1, M9.5.2, M9.6, M9.7, M9.8.1, M9.8.2
2748493 2017, Response to DACO 9.6, *Bacillus licheniformis* strain FMCH001 Technical, DACO: M9.6
2748561 2017, Summary - Environmental Toxicology/Nontarget Organism Testing, DACO: M9.1, M9.2.1, M9.2.2, M9.3, M9.4.1, M9.4.2, M9.5.1, M9.5.2, M9.6, M9.7, M9.8.1, M9.8.2
2748566 2017, Response to DACO 9.6, *Bacillus licheniformis* strain FMCH001 Technical, DACO: M9.6
2788209 1967, Application of the Fluorescent-Antibody Technique to an Ecological Study of Bacteria in Soil, DACO: M9.2.2, M9.3, M9.4.1, M9.5.1, M9.5.2, M9.8.1
2788210 2004, Field evaluation of the efficacy of a probiotic containing *Bacillus licheniformis* and *Bacillus subtilis* spores, on the health status and performance of sows and their litters, DACO: M9.3
2788210 2004, Field evaluation of the efficacy of a probiotic containing *Bacillus licheniformis* and *Bacillus subtilis* spores, on the health status and performance of sows and their litters, DACO: M9.3
2788211 2005, Effect of *Bacillus licheniformis* and *Bacillus subtilis* Supplementation of Ewe's Feed on Sheep Milk Production and Young Lamb Mortality, DACO: M9.3
2788211 2005, Effect of *Bacillus licheniformis* and *Bacillus subtilis* Supplementation of Ewe's Feed on Sheep Milk Production and Young Lamb Mortality, DACO: M9.3
2788212 2008, Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of *Tilapia nilotica* (*Oreochromis niloticus*) to challenge infections, DACO: M9.4.1
2788213 2011, Evaluation of probiotic strain *Bacillus subtilis* C-3102 as a feed supplement for koi carp (*Cyprinus carpio*), DACO: M9.4.1
2788214 2002, Thermostable alpha-1,4 and alpha-1,6-glucosidase enzymes from *Bacillus* sp. isolated from a marine environment, DACO: M9.4.2
2788215 1999, Characterization of *Bacillus* strains of marine origin, DACO: M9.4.2
2788216 2004, Screening of bacterial associates of marine sponges for single cell oil and PUFA, DACO: M9.4.2

2788217 2004, Dietary administration of *Lactobacillus delbrueckii* and *Bacillus subtilis*, single or combined, on gilthead seabream cellular innate immune responses, DACO: M9.4.2

2788218 2002, Control of pathogenic *Vibrio* spp. by *Bacillus subtilis* BT23, a possible probiotic treatment for black tiger shrimp *Penaeus monodon*, DACO: M9.4.2

2788219 2011, Probiotic Activity of *Bacillus subtilis* in juvenile freshwater prawn, *Macrobrachium rosenbergii* (de Man) at different methods of administration to the feed, DACO: M9.4.2, M9.5.2

2788220 2015, Exposure to ZnO-NPs enhanced gut-associated microbial activity in *Eisenia fetida*, DACO: M9.6

2788221 2013, Enumeration and detection of phosphate solubilizing bacteria from the gut of earthworm varieties, DACO: M9.6

2788222 2005, Diversity of microflora in the gut and casts of tropical composting earthworms reared on different substrates., DACO: M9.6

2788223 2000, Biological Control of *Fusarium moniliforme* in Maize, DACO: M9.8.1

2788224 2000, Integrated Biological and Chemical Control of Damping-Off Caused by *Rhizoctonia solani* Using *Bacillus subtilis* RB14-C and Flutolanil, DACO: M9.8.1

2788225 1997, Improvement of Arbuscular Mycorrhiza Development by Inoculation of Soil with Phosphate-Solubilizing Rhizobacteria To Improve Rock Phosphate Bioavailability (32P) and Nutrient Cycling, DACO: M9.8.1

2788231 Gunderson, A.R., 2008, Feather-degrading Bacteria: A New Frontier in Avian and Host-Parasite Research?, DACO: M9.2.2

2788232 Ruiz-Rodriguez, M., Valdiva, E., Soler, J.J., Martin-Vivaldi, M., Martin-Platero, A.M., Martinez-Bueno, M., 2009, Symbiotic bacteria living in the hoopoes uropygial gland prevent feather degradation, DACO: M9.2.2

2788233 Whitaker, J.M., Cristol, D.A., Forsyth, M.H., 2004, Prevalence and genetic diversity of *Bacillus licheniformis* in avian plumage, DACO: M9.2.2

2788234 Zhang, C.N., Li, X.F., Xu, W.N., Jiang, G.Z., Lu, K.L., Wang, L.N., 2013, Combined effects of dietary fructooligosaccharide and *Bacillus licheniformis* on innate immunity, antioxidant capability and disease resistance of triangular bream (*Megalobrama terminalis*), DACO: M9.2.2

2788235 Aanniz, T., Ouadghiri, M., Melloul, M., Swings, J., Elfahime, E., Ibijbijen, J., Ismaili, M., Amar, A., 2014, Thermophilic bacteria in Moroccan hot springs, salt marshes and desert soils, DACO: M9.3

2788237 Buehner, K.P., Anand, S., Djira, G.D., Garcia, A., 2014, Corrigendum to Prevalence of thermoduric bacteria and spores on 10 Midwest dairy farms, DACO: M9.3

2788239 Gupta, A., Gupta, P., Dhawan, A., 2014, Dietary supplementation of probiotics affects growth, immune response and disease resistance of *Cyprinus carpio* fry, DACO: M9.4.1

2788240 Li, K., Zheng, T., Tian, Y., Xi, F., Yuan, J., Zhang, G., Hong, H., 2006, Beneficial effects of *Bacillus licheniformis* on the intestinal microflora and immunity of the white shrimp, *Litopenaeus vannamei*, DACO: M9.4.1

2788241 Kumar, N.R., Raman, R.P., Jadhao, S.B,., Brahmchari, R.K., Kumar, K., Dash, G., 2012, Effect of *Bacillus licheniformis* and *Bacillus subtilis* Supplementation of Ewe's Feed on Sheep Milk Production and Young Lamb Mortality, DACO: M9.5.2

2788242 Dedeke, G.A., Omemu, O., Aladesida, A.A. and Museliu, F., 2010, Comparative Microbial Analysis of Earthworm Casts Collected From Ikenne, Ogun State, Nigeria, DACO: M9.6

2788243 Sung Wook Hong, Ju Sam Lee, Kun Sub Chung, 2011, Effect of enzyme producing microorganisms on the biomass of epigeic earthworms (*Eisenia fetida*) in vermicompost, DACO: M9.6

2788244 Ibrahim, D., Zhu, H.L., Yusof, N., Hong, I., Hong, L.S., 2013, *Bacillus licheniformis* BT5.9 Isolated from Changar Hot Spring, Malang, Indonesia, as a Potential Producer of Thermostable alpha-amylase, DACO: M9.6

2788245 Voigt, B., Antelmann, H., Albrecht, D., Ehrenreich, A., Maurer, K.H., Evers, S., Gottschalk, G., van Dijl, J.M., Schweder, T., Hecker, M., 2008, Cell physiology and protein secretion of *Bacillus licheniformis* compared to *Bacillus subtilis*, DACO: M9.8

5.0 Value

2749854	2017, Eficcia do nematicida Nemix High Load (BFB 079) associado a diferentes inseticidas no controle do nematoide das galhas (Meloidogyne javanica) na cultura da soja., DACO: M10.2.1
2749855	2015, Effectiveness of different biofungicides applied to soybeans (natural soil), DACO: M10.2.2
2749856	2015, Effectiveness of different biofungicides applied to soybeans (Rhizoctonia inoculated), DACO: M10.2.2
2749857	2015, Impact of seed treatment fungicides and biologicals applied to soybeans inoculated with Rhizoctonia WI, DACO: M10.2.2
2749858	2014, Impact of seed treatment fungicides and biologicals applied to soybeans in WI, DACO: M10.2.2
2749859	2017, Impact of F4018 and F4007/F4009 seed treatments on protection against Rhizoctonia of soybean WI 2014 and 2015, DACO: M10.2.2
2749860	2017, Impact of seed treatment fungicides and biologicals applied to soybeans in WI (Average of 2 trials 2014), DACO: M10.2.2
2749861	2014, Impact of biological seed treatments on Rhizoctonia of corn, DACO: M10.2.2
2749862	2014, Impact of biological seed treatments on corn, DACO: M10.2.2
2749863	2014, Biological seed treatment impact on corn growth and yield inoculated with Rhizoctonia, DACO: M10.2.2
2749864	2015, Biological seed treatment impact on corn growth and yield, DACO: M10.2.2
2749865	2015, Biological seed treatment impact on corn growth and yield (Rhizoctonia inoculated), DACO: M10.2.2
2749866	2017, Impact of F4121 seed treatment on Rhizoctonia of corn WI: Average of 5 trials 2014, DACO: M10.2.2
2749867	2017, Average performance of corn WI- Rhizoctonia, DACO: M10.2.2

2749868	2014, Impact of biological seed treatments on Fusarium of corn, DACO: M10.2.2
2749869	2015, Biological seed treatment impact on corn growth and yield (Fusarium), DACO: M10.2.2
2749870	2015, Biological seed treatment impact on corn growth and yield (Fusarium inoculated), DACO: M10.2.2
2749871	2016, Impact of biological seed treatments on growth and yield of corn Fusarium graminearum, DACO: M10.2.2
2749872	2017, F4018/F4121 protection against Fusarium (Average of 3 trials WI 2014 to 2016), DACO: M10.2.2
2749873	2017, F4018/F4121 impact on Fusarium of corn (N=4 trials 2014 to 2016), DACO: M10.2.2
2749874	2014, Average Performance of Seed Treatments of Corn at 3 locations in WI 2014, DACO: M10.2.2
2749875	2015, Impact of formulations of F4018 and F4121 on germination of corn seed, DACO: M10.3.1
2749876	2015, Impact of F4018 and F4121 on germination of soybean seed, DACO: M10.3.1
2749877	2015, of formulations of F4018 and F4121 on germination of soybean seed, DACO: M10.3.1
2749878	2016, Germination of soybean on paper towel: Biovision Seed Laboratories, DACO: M10.3.1
2876669	2016, Impact of biological seed treatments on growth and yield of soybean (Fusarium virguliforme), DACO: 10.2.3.3,M10.2.1
2876670	2016, Efficacy of different strains/combination of strains applied as seed treatment in on Corn against Rhizoctonia solani, soil born disease, greenhouse screening pp1/125(4), 2016, DACO: 10.2.3.3,M10.2.1
2876671	2016, Efficacy of different strains/combination of strains applied as seed treatment in on Corn against Rhizoctonia solani, soil born disease, greenhouse screening pp1/125(4), 2016, DACO: 10.2.3.3,M10.2.1
2876672	2017, Efficacy of different strains/combination of strains applied as seed treatment in on Corn against Rhizoctonia solani, soil born disease, greenhouse screening pp1/125(4), 2017, DACO: 10.2.3.3,M10.2.1
2876673	2018, Pool results from 3 greenhouse trials on corn conducted in Europe treated with the label rate of F4018, DACO: 10.2.3.3,M10.2.1
2876674	2018, Pool results from 2 trials on corn conducted in Europe evaluating rates of F4018 (Rhizoctonia, DACO: 10.2.3.3,M10.2.1
2876675	2018, Pool results from 4 trials on sunflowers conducted in Europe with the label rate of F4018, DACO: 10.2.3.3,M10.2.1
2876676	2018, Pool results from 2 trials on sunflowers conducted in Europe evaluating rates of F4018 (Rhizoctonia), DACO: 10.2.3.3,M10.2.1
2876677	2018, Application of 3 rates of F4018 on corn and sunflowers in Rhizoctonia inoculated greenhouse trials N=4 trials, DACO: 10.2.3.3,M10.2.1

2876678	2018, Average performance of the label rate of F4018 providing Rhizoctonia protection on corn, soybeans and sunflowers (N=8 trials), DACO: 10.2.3.3,M10.2.1
2876679	2014, Effectiveness of different rates and combinations of Nemix seed treatment on soybean cyst nematode (Greenhouse study University of Arkansas), DACO: 10.2.3.3,M10.2.1
2876680	2018, Evaluation of the effect of the seed treatment on soybean cyst nematode (Heterodera glycine) under greenhouse conditions, DACO: 10.2.3.3,M10.2.1
2876681	2016, Efficacy of different strains/combination of strains applied as seed treatment in on Sunflower against Rhizoctonia, soil born disease, greenhouse screening pp1/125(4), 2016, DACO: 10.2.3.3,M10.2.1
2876682	2016, Efficacy of different strains/combination of strains applied as seed treatment in on Sunflower against Rhizoctonia, soil born disease, greenhouse screening pp1/125(4), 2016, DACO: 10.2.3.3,M10.2.1
2876683	2017, Efficacy of soil treatment against Rhizoctonia solani on sunflower seedlings. Greenhouse experiment (nursery). France 2016-2017., DACO: 10.2.3.3,M10.2.1
2876684	2017, Efficacy of different strains/combination of strains applied as seed treatment in on Sunflower against Rhizoctonia, soil born disease, greenhouse screening pp1/125(4), 2017, DACO: 10.2.3.3,M10.2.1

B. Additional Information Considered

i) Published Information

1.0 Product Characterization and Analysis

2835732 Apertroaie-Constantin, C., Mikkola, R., Andersson, M.A., Teplova, V., Suominin, I., Johansson, T. and Salkinoja-Salonen, M., 2008, *Bacillus subtilis* and *B. mojavensis* strains connected to food poisoning produce the heat stable toxin amylosin, 2009. J Appl Microbiol 106, 1976-1985., DACO: M2.14, M4.9

2835736 Biagini, R.E. R. J. Driscoll, D. I. Bernstein, T. G. Wilcox, G. M. Henningsen, B. A. MacKenzie, G. A. Burr, J. D. Scinto, and E. S. Baumgardner, 1995, Hypersensitivity reactions and specific antibodies in workers exposed to industrial enzymes at a biotechnology plant, 1996. J. Appl. Toxicol. 16(2): 139-145., DACO: M2.14, M4.9

2835737 De Boer, A. S. and B. Diderichsen, 1991, On the safety of *Bacillus subtilis* and *B. amyloliquefaciens*: a review, Appl. Microbiol. Biotechnol. 36: 1-4., DACO: M2.14, M4.9

2835738 Duc, L.H., Logan, N.A., Sutherland, A.D., Taylor, J. and Cutting, S.M., 2004, Cases of emesis associated with bacterial contamination of an infant breakfast cereal product, Int J Food Microbiol 102, 245-251, DACO: M2.14,M4.9

2835739 Dutkiewicz, J, C. Skorska, J. Milanowski, B. Mackiewicz, E. Krysinska-Traczyk, E. Dutkiwicz, A. Matuszyk, J. Sitkowska, and M. Golec, 2001, Response of herb processing workers to work-related airborne allergens, Ann. Agric. Environ. Med. 8: 275-283, DACO: M2.14, M4.9

2835745 Fossum, K. H. Kerikstad, M. Binde, and K-E. Pettersen, 1986, Isolation of *Bacillus subtilis* in connection with bovine mastitis, Nord Bet Med. 38: 233-236., DACO: M2.14, M4.9

2835748 Aoki, T, H. Sunahara, K. Sugimoto, T. Ito, E. Kanai, and Y. Fuji, 2014, Infective endocarditis of the aortic valve in a Border collie dog with patent ductus arteriosuscured sausages, J. Vet Med. Sci. 73(3): 331-33., DACO: M2.14,M4.9

2835749 Oggioni, M. R., G. Pozzi, P. E. Valensin, P. E. Galieni and C. Bigazzi, Recurrent septicemia in an immunocompromised patient due to probiotic strains of *Bacillus subtilis*, H. Clin. Microbiol. 36(1): 325-326., DACO: M2.14,M4.9

2836295 Thomas. M. and H. Whittet. 1991., Atypical meningitis complicating a penetrating head injury. , J. Neuro. Neurosurg. Psychia. 54(1): 91-92., DACO: M2.14, M4.9

2836296 Raza, T. A, R. A. Chaudry, N. U. Khan, T. N. Pasha, M. Ahmad. 1993, Comparison of vaginal bacterial flora in teddy goats with and without reproductive disorders, Indian J. Dairy Sci. 46: 1-5, DACO: 2.14, M4.9

2836297 Stickel, F., Droz, S., Patsenker, E., Bo¨gli-Stuber, K., Aebi, B. and Leib, S.L. 2009., 2008, Severe hepatotoxicity following ingestion of Herbalife[a] nutritional supplements contaminated with *Bacillus subtilis*, J Hepatol 50, 111-117., DACO: 2.14, M4.9

2838730 Rosenkvist, H, and A. Hansen, 1994, Contamination profiles and characterisation of *Bacillus* species in wheat bread and raw materials for bread production, Int. J. Food Micro. 26: 353-363., DACO: M2.14, M4.9

2838731 Johnson, C. L., I. L. Berstein, J. S. Gallagher, P. F. Boventre, and S. M. Brooks, 1980, Familial hypersensitivity pneumonitis induced by *Bacillus subtilis*, Am. Rev. Resp. Dis. 122: 339-348., DACO: M2.14, M4.9