Proposed Registration Decision PRD2016-26

Bacillus mycoides isolate J

(publié aussi en français)

28 September 2016

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. 6607D
Ottawa, Ontario K1A 0K9

Internet: pmra.publications@hc-sc.gc.ca
healthcanada.gc.ca/pmra
Facsimile: 613-736-3758
Information Service:
1-800-267-6315 or 613-736-3799
pmra.infoserv@hc-sc.gc.ca

Canada

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

Catalogue number: H113-9/2016-26E (print version)
H113-9/2016-26E-PDF (PDF version)

Table of Contents

Overview

Proposed Registration Decision for *Bacillus mycoides* isolate J

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of BmJ TGAI and BmJ WG, containing the technical grade active ingredient *Bacillus mycoides* isolate J, for the suppression or partial suppression of certain fungal diseases on food crops.

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

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of BmJ TGAI and BmJ WG.

What Does Health Canada Consider When Making a Registration Decision?

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

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

---

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 mycoides* isolate J, the PMRA will consider any comments received from the public in response to this consultation document.[3] The PMRA will then publish a Registration Decision[4] on *Bacillus mycoides* isolate J, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

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

What Is *Bacillus mycoides* isolate J?

Bacillus mycoides isolate J is a Gram-positive, endospore-forming soil bacterium characterized by a unique rhizoid growth pattern on solid media. It is derived from a naturally occurring microorganism in soils. *Bacillus mycoides* isolate J is the microbial fungicide active ingredient, found in BmJ WG. This end-use product is a commercial-class biological for the suppression of various bacterial and fungal diseases on field and greenhouse-grown food crops. BmJ WG is applied as a foliar spray using ground-application equipment or by chemigation. Application of this product to the leaves of susceptible agricultural crops induces resistance in the host crop, thus reducing plant disease by triggering the plant's natural defense mechanisms. The microorganism itself has no direct effect on plant pathogens, but preventative applications may reduce the development of disease in susceptible crops.

Health Considerations

Can Approved Uses of *Bacillus myciodes* isolate J Affect Human Health?

***Bacillus myciodes* isolate J is unlikely to affect your health when BmJ WG is used according to the label directions.**

People could be exposed to *B. mycoides* isolate J when handling and applying BmJ WG and when ingesting treated produce. 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.

[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*.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns. When the technical grade of active ingredient, as well as granular formulation containing *B. mycoides* isolate J, were tested on laboratory animals, there were no signs that it caused any significant toxicity or disease.

Residues in Water and Food

Dietary risks from food and water are not of concern.

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally 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 that the food Canadians eat is safe.

Residues of *B. mycoides* isolate J on the treated crops, at the time of harvest, are anticipated following foliar applications to agricultural crops. *Bacillus mycoides* is a bacterium that is found globally in most terrestrial environments. *Bacillus mycoides* isolate J produced no adverse effects (disease or toxicity) when it was administered orally to rats and it is not known to contain metabolites of toxicological concern. No adverse effects from dietary exposure have been attributed to natural populations of *B. mycoides*. As well, the likelihood of residues of *B. mycoides* isolate J contaminating drinking water supplies resulting from operational applications as a pesticide is considered to be low. Consequently, dietary risks are considered to be low and not of concern. Therefore, the PMRA has determined that the specification of an MRL under the *Pest Control Products Act* is not required for *B. mycoides* isolate J.

Risks in Residential and Other Non-Occupational Environments

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

BmJ WG is proposed for use on agricultural crops only. The application directions on the product labels include statements to minimize spray drift. Consequently, it is unlikely that adults, youths and toddlers will be exposed to *B. mycoides* isolate J. Even in the event of exposure, risk to the general population is not a concern since there were no signs of disease or toxicity noted in toxicological studies with this microorganism.

Occupational Risks From Handling BmJ WG

Occupational risks are not of concern when BmJ WG is used according to label directions, which include protective measures.

Workers handling BmJ WG can come into direct contact with *B. mycoides* isolate J on the skin, in the eyes or by inhalation. For this reason, the product label will specify that workers exposed to the end-use product must wear waterproof gloves, eye goggles, long-sleeved shirts, long pants, a NIOSH approved mist filtering mask or respirator, and shoes plus socks.

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

Environmental Considerations

What Happens When BmJ WG Is Introduced Into the Environment?

BmJ WG is not expected to pose risks of concern to the environment when used according to label directions.

Bacillus mycoides is commonly found in soil. *Bacillus mycoides* isolate J, like all Bacillus species, produces spores in a dormant phase called endospores under adverse environmental conditions, which allow it to survive under extreme heat and dry conditions. The ability to produce endospores is a major factor in the widespread occurrence of these microorganisms in soil environments. However, most endospores are sensitive to sunlight and consequently Bacillus species are not widely found on plant surfaces. While the population of *B. mycoides* isolate J will be above levels naturally found for this species immediately following application as a pesticide, the population will return to natural levels over time.

In aquatic environments, *B. mycoides* may survive to a limited extent given its ability to produce tough endospores and its potential to bind to sediments. Endospores, however, are unlikely to be capable of germinating and multiplying in sediment. The end-use product, BmJ WG, is not intended for aquatic use and exposure to aquatic environments from spray drift and run-off (following a rain event) from field application is unlikely to be significant.

Based on results of laboratory studies with *B. mycoides* isolate J and a critical review of information in the published scientific literature, no significant effects to birds, wild mammals, aquatic and terrestrial arthropods (including bees), plants and fish are expected when BmJ WG is applied according to directions on the label.

Value Considerations

What Is the Value of BmJ WG?

BmJ WG may delay or reduce the need for conventional fungicides, especially for uses where few non-conventional products are available.

There are several conventional products currently registered for use on tomatoes, peppers, potatoes, spinach and sugarbeets to manage agricultural diseases; however there are few non-conventional products available to growers, especially for organic production. With the exception of products for use on tomatoes, this is the first product with the systemic acquired resistance mode of action to be registered for these uses.

When used in an integrated pest management (IPM) program, BmJ may reduce the need for conventional fungicides and may delay the development of resistance to conventional fungicides.

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 BmJ WG to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

In individuals exposed repeatedly to potentially large quantities of BmJ WG, respiratory and dermal sensitivity may possibly develop. All microorganisms, including *B. mycoides* isolate J, contain substances that are potential sensitizers. Therefore, anyone handling or applying these products must wear appropriate waterproof gloves, eye goggles, a long-sleeved shirt, long pants, a NIOSH approved mist filtering mask or respirator, and shoes plus socks.

Environment

The end-use product labels will include environmental precaution statements that prevent the contamination of aquatic systems from the use of BmJ WG as well as statements indicating that BmJ WG may be toxic to bees.

Next Steps

Before making a final registration decision on *Bacillus mycoides* isolate J, the PMRA will consider any comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover

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

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Bacillus mycoides* isolate J (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Bacillus mycoides isolate J

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active ingredient	*Bacillus mycoides* isolate J
Function	Biological fungicide for use on field and greenhouse food crops
Binomial name	*Bacillus mycoides* isolate J
Taxonomic designation	
Kingdom	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Bacillaceae
Genus	*Bacillus*
Species Group	*Bacillus cereus* group
Species	*mycoides*
Isolate	J
Patent Status information	The following United States patents have been issued for *Bacillus mycoides* isolate J for the induction of disease resistance in treated plants: US 8025875 B2; US 8246965 B2; US 8524222 B2; US 9205115 B2. No application for a Canadian patent has been made.
Nominal purity of active	Technical Grade Active Ingredient: minimum of 1×10^{11} viable spores/g End-Use Product: minimum of 3×10^{10} viable spores/g
Identity of relevant impurities of toxicological, environmental and/or significance.	The technical grade active ingredient does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards.

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

Technical Grade Active Ingredient – BmJ TGAI

Property	Result
Colour	Brown
Physical State	Powder
Odour	Fishy
Miscibility	Immiscible
Corrosion Characteristics	Non-corrosive to HDPP containers
pH	5.0–5.1 at 24°C
Relative Density	0.540 g/cm^3 at 24°C

End-Use Product – BmJ WG

Property	Result
Colour	Brown
Physical State	Granular
Odour	Smoky
Miscibility	Immiscible
Corrosion Characteristics	Non-corrosive to HDPP containers
pH	6.0 at 24°C
Relative Density	0.723–0.750 g/cm^3 at 24°C

1.3 Directions for Use

BmJ WG is applied via foliar application to tomatoes, peppers, potatoes, spinach and sugarbeets for the suppression or partial suppression of certain diseases. It is applied at a concentration of 0.33g/L in spray volumes sufficient to adequately cover the crop. It may be applied with commonly-used ground application equipment or by chemigation.

1.4 Mode of Action

Systemic acquired resistance (SAR) is a type of induced defense in plants that is triggered in response to attack by pathogens. These induced defenses are biochemical in nature and are often expressed throughout the plant thereby resulting in broad-spectrum (i.e., non-specific) resistance to pathogen infection in parts of the plant distant from the point of primary infection. Induction of SAR is characterized by an oxidative burst in which active oxygen species such as hydrogen peroxide, superoxide anion, hydroperoxyl and hydroxyl radicals accumulate rapidly. Hydrogen peroxide is involved in several plant defense mechanisms but in SAR acts to stimulate production of salicylic acid (SA) biosynthetic enzymes leading to accumulation of SA. In the plant cytosol, SA acts to reduce the inactive multimer of non-expressor of pathogenesis related genes 1 (NPR1). The active monomer of NPR1 is translocated from the cytoplasm into the

nucleus where it interacts with transcription factors and activates transcription of pathogenesis-related (PR) genes for expression of chitinase, β-glucanase and peroxidase. Functional analogs of SA such as acibenzolar-S-methyl (ASM) can trigger monomerization of NPR1 and subsequent signal transduction downstream of SA.

Applications of BmJ to plants activate SAR in a manner similar to ASM. Induction of SAR is SA-independent and NPR1 dependent.

Bacillus mycoides itself has no direct effect on plant pathogens, but preventative applications (before infection or appearance of disease symptoms) can reduce the incidence and severity of subsequent disease.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganisms

Bacillus mycoides isolate J can be differentiated from other *Bacillus* species based on 16S rRNA analysis. Random amplified polymorphic deoxyribonucleic acid (RAPD) analysis using specific primers can distinguish BmJ from other isolates of *B. mycoides*.

2.2 Methods for Establishment of Purity of Seed Stock

The production strain is maintained as a master seed stock that is stored in liquid nitrogen. To ensure consistency, samples of the master seed stock are sent for RAPD analysis and also microscopically checked for purity by streaking onto a nutrient agar plate. The master seed stock is replenished using a process that includes multiple checks for purity.

The working stock culture is prepared from the master seed stock and stored at -25°C. The colony morphology of the working stock is checked to confirm identity.

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 ingredient and the end-use product are expressed in units of viable spores/g. Representative data on five batches of TGAI and end-use product were submitted. The method for determining viable spore concentration 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, appropriate methods are available to enumerate viable spores and to distinguish this microbial pest control agent (MPCA) from other *Bacillus* species and other strains of *Bacillus mycoides.*

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 BmJ TGAI and BmJ WG are acceptable. These procedures include sterilization of all equipment and media as well as frequent sampling of the stock culture and production batches for purity and contamination.

The absence of human pathogens and below-threshold levels of contaminating microorganisms were shown in the microbial screening of batches of BmJ TGAI using standard methods for detecting and enumerating microbial contaminants of concern as well as by results of mouse toxicity testing. Although limits for microbial contamination were not proposed, all batches of BmJ TGAI must conform to the limits set out in the Organization for Economic Co-operation and Development (OECD) issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43].

Given the relationship between BmJ and members of the *Bacillus cereus* group, additional analyses were conducted for β-exotoxin, *B. cereus*-like enterotoxins and emetic toxin that are associated with other members of the *B. cereus* group. Although not part of the routine quality assurance program, a housefly larvae assay demonstrated the absence of β-exotoxin in batches of BmJ TGAI. A variety of tests [for example, immunoassays, polymerase chain reaction (PCR) analysis and reverse transcriptase PCR (RT-PCR)] found that BmJ possesses the genes for non-hemolytic enterotoxin (NHE) and, under certain conditions, produces NHE albeit at significantly lower levels than by *B. cereus*. NHE, however, is not present at detectable levels when BmJ WG is produced according to the proposed manufacturing methods and diluted to field application rates. *Bacillus mycoides* isolate J also possesses the gene for enterotoxin T (BceT). It is unclear, however, whether the gene is transcribed and, if so, whether BceT contributes to food-borne disease. However, the risk to human health to NHE and BceT is low since the weight of evidence shows that the MPCA does not have the capacity to cause *B. cereus* type health effects (diarrheal symptoms of food poisoning).

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

The storage stabilities of BmJ TGAI and BmJ WG have been assessed at 4°C and 25°C. Based on the storage stability data, the label of BmJ WG must state that the product must be stored at temperatures below 25°C for up to six months from the date of manufacture.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

3.1.1 Test Studies

The PMRA conducted a detailed review of the toxicological studies submitted in support of BmJ TGAI, and BmJ WG.

The studies submitted to fulfil the requirements for health hazard assessment of the technical grade active ingredient, BmJ TGAI, included acute oral toxicity/pathogenicity, acute pulmonary toxicity and toxicity/pathogenicity, and acute intravenous injection infectivity.

In an acute oral toxicity and pathogenicity study, groups of ~7 week old Sprague-Dawley rats (12/sex) were exposed by oral gavage to *B. mycoides* isolate J (BmJ TGAI) at 6.3×10^7 colony forming units (CFU)/rat. Animals were observed frequently on day of dosing and once daily thereafter for 21 days. Feces and urine sample were plated for isolation of the MPCA on Day 0, every 24 hours through 72 hours, then on Day 14. Tissue and blood samples taken at interim sacrifices from the group receiving the active test substance were cultured on Days 3 and 7 while on Day 14 only kidneys and caecal contents and on Day 21, only caecal contents were assayed. The test substance was isolated from fecal samples through Day 14, peaking at 24 hours then declining to 660 CFU/g by Day 14. In urine samples, the peak was Day 0 with 4.3×10^4 CFU/mL with none detected by Day 14. Caecal contents of all test group animals had low levels of detectable MPCA on Day 3, no animals had detectable MPCA on Day 7, one female had <100 CFU/g at Day 14 and no animals had detectable MPCA at Day 21. On Day 3, one female showed <100 CFU/g in brain and kidney samples, and on male had <100 CFU/g in mesenteric lymph nodes. At Day 7 no tissues showed viable MPCA. On Day 14 only kidneys were assayed; one male and one female had <100 CFU/g in kidney tissues. There were no observed abnormalities during necropsy through Day 21. No evidence of toxicity, infectivity or pathogenicity was observed. Clearance was not established through a definite pattern. Dosing was below guideline level and not all tissues were sampled for MPCA through Day 21.

In an acute pulmonary toxicity and pathogenicity study, groups of ~6 week old CD rats (12/sex) were exposed by the intratracheal route to *B. mycoides* isolate J (BmJ TGAI) washed spore suspension. During a preliminary dosing experiment 5×10^8 CFU/rat (unwashed BmJ TGAI) was administered by the intratracheal route and 2/6 animals died within 26 hours. The test substance was subsequently washed and another preliminary study showed no mortality or clinical signs so the main study was conducted using the washed test material. The test facility confirmation of washed test substance viability resulted in animals receiving a dose of 1.1×10^8 CFU/animal. The animals were observed twice daily during weekdays and once daily on weekends for mortality and signs of pharmacologic and/or toxic effects over 35 days. There were no abnormal general health observations. Tissue and blood samples taken at interim sacrifices were cultured on Days 0, 7, 21, and 35. Some treated animals had lung pigmentation (red, pale, or gray) upon necropsy. On Day 7, 3/6 treated rats, 5/6 on Day 21 and 3/6 on Day 35 had lung pigmentation. One male had red foci on the lungs at Day 7. Organ weights relative to body weights, for lungs and lymph nodes, were significantly increased in treated males at Days 7 and 35, and females at Day 21, when compared to control animals. Spleen weights for treated males on Day 21 were significantly decreased, but significantly increased on Day 35. On Day 21 liver weights were significantly increased in treated females. No live MPCA was recovered in blood from any test animal. In treated animal lungs, recovery approached the dose amount through Day 21 and showed a noticeable decline only on Day 35, and then only approaching one logarithmic reduction. Smaller amounts of live test substance were found in spleen, liver, caecal contents and kidney indicating partial clearance of the test substance. One male had 244 CFU in brain tissue samples on Day 7, while 3 females on Days 0 or 7 showed similar counts, most likely as an artifact of the sampling methodology. Heated samples (70°C, 10 minutes) for some tissue types

had slightly elevated counts or showed viable cells just above the detection limit when standard plating showed no detection, but for other tissue types the reverse was true and recovery after heating was typically lower. Such heat treatment therefore was not useful for discerning if vegetative cells were present, as opposed to surviving spores as dosed. Heat treatment may serve to make some spores more easily culturable that otherwise would not readily grow on the chosen culture medium. *Bacillus mycoides* isolate J is not infective or toxic when instilled by the intratracheal route at 1.1×10^8 CFU/rat; a pattern of clearance was not established. Although toxicity resulting in mortalities from the preliminary assay was not explained, it must be noted that a washing procedure is included in the manufacture of BmJ TGAI.

In an acute pulmonary toxicity study, groups of 9 week old Sprague-Dawley rats (3/sex) were exposed by the intratracheal route to BmJ TGAI (1.6×10^{11} CFU/g) in 0.1 mL of phosphate buffered saline at a dose of 10^8 CFU/animal. Animals were then observed for up to 48 hours. There were no treatment related clinical signs or changes in body weight. The 48-hour acute pulmonary LD_{50} for rats is $> 10^8$ CFU/animal. Based on these results, BmJ TGAI is of low toxicity in the rat.

In an acute intravenous toxicity and pathogenicity study, groups of ~7 – 8 week old Sprauge-Dawley rats (21/sex) were exposed by the intratracheal route to BmJ TGAI at a dose of 1.0×10^7 CFU/rat. The animals were observed frequently on day of dosing for mortality and signs toxicity and once daily thereafter for 58 days. Tissue and blood samples taken at interim sacrifices were cultured on Days 0, 3, 7, 21, 34, 42 and 58. There were no abnormal clinical signs in any animal with the exception of a single male that had piloerection, decreased activity and emaciation starting at Day 21 and becoming less severe before recovery by Day 58. The male animal with clinical signs showed significant weight loss coinciding with symptoms, while one female had slight weight loss at Day 21, and six other females had slight weight loss at Day 28 and recovered by Day 34. Female brain weight was significantly heavier. The test substance was isolated from blood of only one animal at Day 0. At Day 3, 3/6 animals had detectable MPCA in lungs, kidney, spleen, and/or mesenteric lymph nodes with highest concentrations in the liver and spleen. At Day 7, detection was in 6/6 animals and one male had detection in brain and blood at low levels. At Day 21, detection in 4/6 animals was from various tissues but not in kidney, brain or blood. By Day 42, detection in 4/6 animals in liver and/or spleen and one male had low level detection in brain tissue. On Day 58, 1/6 animals had low detection in liver, spleen and brain tissue. Regression of data indicated clearance was likely >120 days for liver and spleen tissues. Detection in brain tissue on Day 7 was from 1 CFU on one of the triplicate plates, on Day 42 from 2 CFU on one of the triplicate plates, and on Day 58 from 3 CFU on one of the triplicate plates, all from undiluted samples. There were no observed abnormalities during necropsies through Day 58. *Bacillus mycoides* isolate J is not toxic, infective, or pathogenic when injected intravenously at 1.0×10^7 CFU/rat; a pattern of clearance was established.

The studies submitted to fulfil the requirements for health hazard assessment of the end-use product, BmJ WG, included oral toxicity, inhalation toxicity (waiver request), dermal toxicity, dermal irritation and eye irritation studies.

In an acute oral toxicity study, three fasted female Sprague-Dawley rats were given a single oral gavage dose of BmJ WG diluted 40% in de-ionized water at 12.5 mL/kg to achieve a concentration of 5000 mg/kg body weight (bw). The resulting dose of *B. mycoides* isolate J was approximately 3.0×10^{10} CFU/animal. The animals were observed for 14 days. All animals survived and appeared normal throughout the study. All animals gained weight normally throughout the study. No observable abnormalities were found at necropsy. The acute oral LD_{50} of BmJ WG for female rats is >5000 mg/kg bw.

A waiver rationale was submitted to address inhalation toxicity. *Bacillus mycoides* is widespread and likely ubiquitously found in all soil types, and in surface waters. Exposure on food products is common with no known health concerns, as demonstrated by a literature review. However, fermentation and production of a concentrated spore preparation is not assumed a natural exposure and so safety by inhalation cannot be inferred from such a literature review concerning natural exposures. *Bacillus* species in soil are known to produce copious amounts of degradative enzymes, such as proteases, useful for utilizing various food sources. Some of these have been produced industrially for products such as drain cleaners and laundry detergents. Likely the mortalities and lung lesions observed in the provided pulmonary toxicity and pathogenicity study (preliminary trial using unwashed spores) and severe eye irritation noted in an acute eye irritation study are the result of these enzymes. An acute inhalation toxicity study conducted with the end-use product is not required since the inhalation hazard from *B. mycoides* isolate J was adequately characterized in the acute pulmonary/toxicity and acute pulmonary toxicity study conducted using BmJ TGAI as the test substance.

In an acute dermal toxicity study, groups of young adult Sprague-Dawley rats (5/sex) were dermally exposed to 5050 mg/kg of BmJ WG containing 3.0×10^{10} CFU/g *B. mycoides* isolate J, moistened with 1g/mL in deionized water, for 24 hours on a clipped dorsal surface comprising approximately 10% of the body surface area. Following exposure, the animals were observed for 14 days. All animals survived, gained weight, appeared normal, and had no signs of dermal irritation throughout the study. Animals did exhibit alopecia at the exposure site through Day 1 for males and Day 7 for females. No observable abnormalities were found in any animal at necropsy. The Dermal LD_{50} for male and female rats is >5050 mg/kg bw.

In a primary dermal irritation study, two male and one female New Zealand White rabbits were dermally exposed to 0.5 g of BmJ WG, containing 3.0×10^{10} CFU/g of *B. mycoides* isolate J, in 0.5 mL deionized water, for 4 hours on an approximately 2.5 cm^2 area of the body surface. The animals were observed at 1, 24, 48, and 72 hours after patch removal. Irritation was scored by the method of Draize. No dermal erythema or edema was noted on any animal at 1, 24, 48, or 72 hours. The primary irritation index was zero. BmJ WG is not irritating to the skin.

In a primary eye irritation study, 0.1 g of BmJ WG containing 3.0×10^{10} CFU/g of *B. mycoides* isolate J, as supplied, was instilled into the conjunctival sac of the right eye of two males and one female New Zealand White rabbits. Animals were observed at 1, 24, 48, and 72 hours after test material instillation. Irritation was scored by the method of Draize and classified by the system of Kay and Calandra. No corneal iritis was noted in any rabbit throughout the study. Corneal opacity was noted in all rabbits immediately following exposure with clearance by 24 hours. Positive conjunctival irritation (score $\geq$ 2) was noted on 3/3 rabbits 24 hours after test material

instillation with resolution by 48 hours. The maximum average score was 52.0 (maximum possible score = 110) at 1 hour after test material instillation, with average scores of 6.0 by 24 hours, 4.0 at 48 hours and 0.0 at 7.2 hours. Fluorescein staining was observed in all treated rabbit eyes at 24 hours but not at 72 hours. BmJ WG is severely irritating to the eye.

These studies are summarized in Appendix I, Tables 1 and 2.

3.1.2 Additional Information

A search of the National Center for Biotechnology Information (NCBI) PubMed database using the key words *Bacillus mycoides* with pathogen, infection, toxic or human yielded three citations. *Bacillus mycoides* has been implicated in an acute case of endophthalmitis following ocular trauma as well as in a case of endogenous endophthalmitis resulting in irreparable retinal detachment. *Bacillus mycoides* was also isolated from food samples associated with a food poisoning incident in which two individuals experienced diarrhea, abdominal cramps and fever. No clinical samples from the outbreak were available for testing. *Bacillus mycoides* was not definitively determined as the cause of the food poisoning incident.

Bacillus mycoides is one of ten species that comprise the *B. cereus* group with the others being *B. cereus, B. anthracis, B. thuringiensis, B. pseudomycoides, B. weihenstephanensis, B. cytotoxicus, B. gaemokensis, B. manliponesis, and B. toyonensis*. Members of this group are known to produce toxic metabolites including the enterotoxins hemolysin B (HBL), NHE, cytotoxin cytK (CytK), BceT and enterotoxin FM (EntFM) which are associated with diarrheal symptoms of food poisoning; the emetic toxin cerulide associated with vomiting from food poisoning; and the three-protein exotoxin associated with the disease anthrax (it is accepted that B. mycoides does not produce this toxin). Analyses were conducted to explore the potential for B. mycoides isolate J to produce these entero- and emetic toxins. It was found that B. mycoides isolate J has the genetic potential to produce the NHE and BceT enterotoxins. It was confirmed that the MPCA produces the NHE enterotoxin under certain conditions, however, NHE was not detected in BmJ WG. Also, although an attempt was made to detect BceT enterotoxin mRNA transcripts produced by the MPCA, the results were inconclusive.

3.1.3 Incident Reports Related to Human and Animal Health

Since April 26, 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the PMRA website. *B. mycoides* isolate J is a new active ingredient pending registration for use in Canada. No human or domestic animal incidents involving the active ingredient have been reported to the PMRA.

3.1.4 Hazard Analysis

The database submitted in support of the registration of BmJ TGAI and BmJ WG was reviewed from the viewpoint of human health and safety and was determined to be sufficiently complete.

BmJ TGAI was of low toxicity and not infective or pathogenic to rats via the oral, pulmonary and intravenous routes.

BmJ WG was not toxic via the oral or dermal routes and was not irritating to skin of rabbits. BmJ was severely irritating to eyes of the rabbit.

Although no reports of hypersensitivity incidents among workers were reported, the signal words "POTENTIAL SENSITIZER" will appear on the labels for the technical grade active ingredient and end-use product as all microorganisms are recognized as being able to produce substances that can elicit allergic reactions after repeated exposure to high concentrations.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute oral and dermal toxicity of the end-use product, and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral, pulmonary and intravenous toxicity/infectivity tests.

Within the available scientific literature, there are no reports that suggest *B. mycoides* isolate J has the potential to cause adverse effects on the endocrine system of animals. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *B. mycoides* isolate J.

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 and/or inhalation. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Bacillus mycoides* isolate J has not been identified as a dermal wound pathogen and does not contain any known toxic secondary metabolites. There is no indication that it could penetrate intact skin of healthy individuals. Furthermore, toxicity testing with BmJ TGAI and BmJ WG showed no signs of toxicity via the oral, pulmonary or dermal routes of exposure. The submitted eye and dermal irritation studies with the BmJ WG demonstrated severe eye irritation and no skin irritation. Precautionary label statements will be included on label to warn users of the potential for severe eye irritation and there will be a requirement on the label that users wear eye goggles.

Although the risk of toxicity from dermal exposure is considered low from the proposed end-use product use pattern, the PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Therefore, anyone handling or applying BmJ WG must wear a long-sleeved shirt, long pants, shoes plus socks, waterproof gloves, eye goggles, as well as a NIOSH approved mist filtering mask or respirator. In addition, all unprotected workers are restricted from entering enclosed areas (including greenhouses) where BmJ WG has been handled or applied until spray has dried.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of BmJ WG, and anticipated occupational risk from the use of this product is low.

3.2.2 Residential and Bystander Exposure and Risk

Overall, the PMRA does not expect that residential and bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for BmJ TGAI and BmJ WG and the assumption that precautionary label statements will be followed by commercial applicators in the use of BmJ WG. As well, *B. mycoides* is a species that is ubiquitous in the environment and the use of BmJ WG is not expected to cause sustained increases in exposure to bystanders beyond natural levels. Consequently, the health risk to infants and children is expected to be low.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

Although the proposed use pattern may result in dietary exposure with residues possible in or on agricultural commodities, dietary risk is expected to be low and not of concern for the general population, including infants and children, or animals. As *B. mycoides* isolate J demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity/infectivity study and BmJ WG is not known to contain mammalian toxins. Furthermore, higher tier subchronic and chronic dietary exposure studies were not required because of the low toxicity of the MPCA and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral, pulmonary and intravenous toxicity/infectivity studies. Therefore, there are no concerns for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.3.2 Drinking Water

Health risks are not expected from exposure to *B. mycoides* isolate J via drinking water because exposure will be low from operational applications and because there were no harmful effects observed in Tier I acute oral toxicity testing and infectivity testing. The end-use product labels instruct users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Users are also requested not to allow effluent or runoff from greenhouses containing this product to enter lakes, streams, ponds or other waters. Furthermore, municipal treatment of drinking water is expected to reduce the transfer of residues to drinking water.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

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

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

3.3.4 Aggregate Exposure and Risk

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA's files, no harm will result from aggregate exposure of residues of *B. mycoides* isolate J 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. Furthermore, few adverse effects from exposure to other isolates of *B. mycoides* encountered in the environment have been reported. Even if there is an increase in exposure to this active ingredient from the use of BmJ WG, 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.

Bacillus mycoides are common and ubiquitous soil organisms. Residues of *B. mycoides* isolate J on treated food crops, at the time of harvest, are anticipated as the active ingredient is comprised of resilient resting structures (spores), which are much more persistent in the environment than vegetative cells. Consequently, the PMRA has applied a hazard-based approach for determining whether an MRL is required for this microorganism. Although, it has been shown that *B. mycoides* isolate J has the potential to produce NHE and BceT enterotoxins, the risk to human health from the dietary exposure to BmJ WG is low since the weight of evidence shows that the MPCA does not have the capacity to cause *B. cereus* type food poisoning. Specifically, no adverse effects were observed in the acute oral toxicity and pathogenicity study conducted with *B. mycoides* isolate J or in the acute oral toxicity study conducted with BmJ WG; no adverse effects from dietary exposure have been definitively attributed to natural populations of *B. mycoides*; and BmJ WG is not known to contain mammalian toxins. In addition, the likelihood of

residues contaminating drinking water supplies is low and not of concern. Therefore, the PMRA has determined that the specification of an MRL under the *Pest Control Products Act* is not required for *B. mycoides* isolate J.

3.4 Cumulative Effects

The PMRA has considered all the available information on the cumulative effects of residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Given the broad mode of action of the MPCA (i.e., inducement of SAR in host plants), certain registered MPCAs, as well as naturally occurring bacterial strains, may share a common mode of action with *B. mycoides* isolate J. No cumulative effects are anticipated if the residues of *B. mycoides* isolate J interact with these microbial species.

4.0 Impact on the Environment

Bacillus mycoides is a Gram-positive, endospore-forming soil bacterium. The species is characterized by a unique rhizoid growth habit on solid media, and as colonies develop, they spread and form thin filaments that curl in a similar direction. *Bacillus mycoides* is a common and ubiquitous soil organism. Under adverse environmental conditions, *Bacillus* species produce endospores which allow them to endure extreme conditions of heat and desiccation, and this quality is a major factor in the ubiquity of these microorganisms in the environment. As *B. mycoides* isolate J spores are sensitive to ultra-violet exposure (sunlight), spores are not expected to persist in the phyllosphere.

Given its ability to produce endospores, *B. mycoides* may survive to a limited extent in water. However, its survival in the natural aquatic environment is influenced by the complex interaction of a number of biological, chemical and physical factors. In particular, solar radiation is likely to destroy *B. mycoides* endospores and vegetative cells in the upper layers of an aquatic system. The adsorption of bacterial cells to the sediment layer in the natural aquatic environment is expected to occur but spores are unlikely to be capable of germinating and multiplying in sediment.

Although there may be some potential for surface water exposure resulting from spray drift from field applications from the use of BmJ WG, use instructions will include directions to limit drift. Concentrations of *B. mycoides* isolate J, which are deposited in surface water bodies via drift and/or run off events, are expected at, or below, naturally occurring background levels.

4.2 Effects on Non-Target Species

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

The type of environmental risk assessment conducted on MPCAs varies depending on the tier level that was triggered during testing. For many MPCAs, Tier I studies are sufficient to conduct environmental risk assessments. Tier I studies are designed to represent "worst-case" scenarios where the exposure conditions greatly exceed the expected environmental concentrations. The absence of adverse effects in Tier I studies are interpreted as minimal risk to the group of non-target organisms. However, higher tiered studies will be triggered if significant adverse effects on non-target organisms are identified in Tier I studies. These studies provide additional information that allows PMRA to refine the environmental risk assessments. In the absence of adequate environmental fate and/or field studies, a screening level risk assessment can be performed to determine if the MPCA is likely to pose a risk to a group of non-target organisms. The screening level risk assessment uses simple methods, conservative exposure scenarios (for example, direct application at a maximum application rate) and sensitive toxicity endpoints. A risk quotient (RQ) is calculated by dividing the exposure estimate by an appropriate toxicity value (RQ = exposure/toxicity), and the risk quotient is then compared to the level of concern (LOC).

If the screening level RQ is below the LOC, the risk is considered negligible and no further risk characterization is necessary. If the screening level RQ is equal to or greater than the LOC, 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

Several studies were submitted to address the hazards of the *B. mycoides* isolate J to terrestrial non-target organisms. These studies included non-target avian species and terrestrial arthropods. To further supplement the studies, waiver rationales were submitted to support testing on non-target terrestrial birds, arthropods including bees, as well as plants. Data submitted under human and animal health toxicity testing were considered to assess the risk of harm to wild mammals.

The acute avian oral toxicity of BmJ TGAI to 33 weeks-old northern bobwhite quail (*Colinus virginianus*) was assessed over 14 days. In the study, a single dose was administered to the birds (5/sex) by oral route with a gelatin capsule containing BmJ TGAI. There were no mortalities, signs of toxicity or apparent effects on body weight or feed consumption. The 14-day acute oral LD_{50} was greater than 8.0×10^{10} CFU/kg body weight which was equivalent to 2250 mg BmJ TGAI/kg body weight.

In a scientific rationale submitted to waive further testing of pathogenicity and infectivity in birds, data were provided showing that *B. mycoides* isolate J does not grow vegetatively at temperatures above 36°C which is below the basic avian body temperature of 38–42°C. A thorough search of the public scientific literature also did not find any reports identifying *B. mycoides* as an avian pathogen. No further information was required to address avian pathogenicity and infectivity.

Three terrestrial arthropod studies were submitted for review. The test species included honeybees (*Apis mellifera*), ladybird beetles (*Hippodamia convergens*), and green lacewing larvae (*Chrysoperla rufilabris*).

In an 11-day dietary study, young honeybees (150 bees/group) were exposed to five oral concentrations of BmJ TGAI. On Day 11, the mean cumulative mortality in the treated bees, were 62.7%, 98.7%, 98.7%, 98.7% and 100% in the five test concentrations of 2.84×10^{10}, 6.63×10^{10}, 1.13×10^{11}, 2.25×10^{11} and 4.5×10^{11} CFU/L sucrose solution, respectively. The study was terminated on Day 11 after the cumulative bee mortality exceeded 20 percent in the untreated controls (34.7%). Although mortality rates were observed in an apparent dose related manner, the unreliable feeding data and the lack of significant differences in mortality rates between the different groups receiving the lower doses imparted little confidence in the reported LD_{50}. In the absence of a replacement honeybee toxicity/pathogenicity study, precautionary measures are required on the BmJ WG label alerting users of the hazards to bees from the use of this product as well as instructions to limit exposure to bees.

A scientific rationale to address potential pathogenicity in honey bees was provided to complement the honeybee study. A search in the published scientific literature did not find any reports identifying *B. mycoides* as an infectious agent of bees, instead reports indicated that *B. mycoides* was one among many bacterial symbionts of honeybees.

In an 18-day dietary toxicity study, adult ladybird beetles (150/group) were exposed to a single limit dose of BmJ TGAI at 4.5×10^{8} CFU/mL through feeding with dosed corn earworm eggs. The study was terminated on Day 18 after the cumulative percent mortality in the control group exceeded 20%. Throughout the 18-day study period, the cumulative percent mortality did not differ significantly between the treatment and the control groups. As such, the study did not indicate that the BmJ TGAI was toxic to adult ladybird beetles. No sublethal effects such as lethargy or immobility were reported and no difference in food consumption was observed.

In a 21-day dietary toxicity study, green lacewing larvae (30/group) were exposed to BmJ TGAI at a single limit dose of 4.5×10^{8} CFU/mL from treated corn earworm eggs. There were no significant differences among treatment and control groups in mortality, number pupated, number emerged and number of days to adult emergence. However, the amount of time until pupation was significantly longer in the group treated with active BmJ group by 0.8 of a day compared to the negative and inactive control groups. Egg consumption was also significantly lower in the group treated with active BmJ compared to the group treated with inactive BmJ. Although not pathogenic, BmJ did have slight toxic effects to green lacewings.

Scientific rationales were submitted to further supplement the toxicity studies in the ladybird beetle, and green lacewing larvae. The scientific rationales reported that searches from the published scientific literature indicated that *B. mycoides* is a common saprophyte and not known to be pathogenic or infective to insects. Instead, *B. mycoides* was shown to be among many bacterial symbionts of honeybees, sand flies, long-horned beetles and earthworms. Furthermore, isolates of *B. mycoides* from an olive orchard did not cause significant larval mortality of lepidopteran or coleopteran pests when fed an artificial diet in laboratory bioassays.

A scientific rationale was also submitted to waive testing on terrestrial plants. The rationale was based on the intended non-toxic mode of action to plant pathogens by triggering plant's defenses; lack of observed phytotoxicity or adverse effects to various crops in small plot efficacy studies; reductions in BmJ populations soon after application; and a current lack of documentation of *Bacillus* species as potential frank plant pathogens.

No toxicity/pathogenicity data were submitted to address the potential for harm to wild mammals, non-arthropod invertebrates, and microorganisms. *Bacillus mycoides* is not on any authoritative list of mammalian pathogens, although its closely related species in the *Bacillus cereus* group are, including *B. cereus* and *B. anthracis*. As a ubiquitous soil bacterium, wild mammals are expected to have been exposed to this species. Searches of the open scientific literature revealed no reports of adverse effects to mammals attributable to *B. mycoides* strains. Overall, reports of adverse effects from *B. mycoides* are very rare considering the ubiquitous nature of the microorganism. From the data submitted under human and animal health toxicity testing, it was determined that BmJ TGAI was not toxic or pathogenic to mammals via the oral, dermal or pulmonary routes. No further data are required to assess the risk of harm to wild mammals.

Additional testing on non-target microorganisms is not required even though the end-use product is intended to control pest microorganisms. *Bacillus mycoides* has a non-toxic mode of action. Also, any increase in exposure to the MPCA is not expected to adversely affect environmentally or economically important microbial species or microbiologically mediated biogeochemical processes since *B. mycoides* is a natural component of soil. *Bacillus mycoides* isolate J is also not expected to adversely affect non-arthropod invertebrates (for example, earthworms) given that it is not related to any known non-arthropod invertebrate pathogen.

Based on all the available data and information on the effects of *B. mycoides* isolate J to non-target terrestrial organisms and the precautionary measures required on the label of BmJ WG, there is reasonable certainty that no harm will be caused to birds, wild mammals, arthropods, non-arthropod invertebrates, non-target microorganisms and plants from the proposed use of BmJ WG on greenhouse and field crops.

4.2.2 Effects on Aquatic Organisms

Two studies were submitted to address the hazards of the TGAI to aquatic non-target organisms, including freshwater fish (rainbow trout) and aquatic arthropods (*Daphnia magna*).

The acute toxicity of BmJ TGAI to rainbow trout (*Oncorhynchus mykiss*; 30 fish) was assessed for 96 hours after exposure to a single limit nominal concentration of BmJ TGAI. No mortalities were observed and all test fish appeared normal throughout the study. The 96-hour LC_{50} was determined as greater than 6.22×10^6 CFU BmJ TGAI/mL.

A scientific rationale was submitted to waive further testing on freshwater fish for pathogenicity and infectivity. The rationale was based on the use pattern where aquatic exposure to the end-use product would be limited, and lack of evidence of irreversible adverse effects anticipated to freshwater fish in the published literature. The rationale referred to one incident where *B. mycoides* was isolated from farm-raised channel catfish, as a result of an epizootic infection. Subsequent experiments involving injections of *B. mycoides* in the muscular layer of the fish required at least 10^4 CFU to reproduce the similar lesions as those seen during the epizootic; and fish inoculated via dermal abrasions healed within 5 days and did not develop *B. mycoides* infection. Since populations of *B. mycoides* isolate J were shown to decline over a 2-week period after foliar application, and with the proposed foliar application, the amount of *B. mycoides* isolate J entering aquatic environments is not expected to increase appreciably to cause irreversible adverse effects to freshwater fish.

The acute toxicity of BmJ TGAI to daphnids (*Daphnia magna*; 20 daphnids) was assessed under static conditions over 48 hours. BmJ TGAI was added to the culture medium at six nominal concentrations of 1.94×10^5, 3.89×10^5, 7.78×10^5, 1.56×10^6, 3.11×10^6, and 6.22×10^6 CFU BmJ TGAI/mL. There were no effects on mortality. The LC_{50} was greater than 6.22×10^6 CFU/mL. BmJ TGAI is of low toxicity to the daphnid.

A scientific rationale was submitted to waive further testing for pathogenicity and infectivity of the MPCA on aquatic arthropod invertebrates. The rationale was based on the use pattern where aquatic exposure to the end-use product would be limited, and the lack of evidence in the published scientific literature of irreversible adverse effects caused by *B. mycoides* to aquatic arthropods.

Independent searches of the open scientific literature (through PubMed) using the various keywords yielded no reports of adverse effects to aquatic arthropods, aquatic non-arthropod invertebrates and aquatic plants.

Based on all the available data and information on the effects of *B. mycoides* isolate J to non-target aquatic organisms and the precautionary measures required on the label of BmJ WG, there is reasonable certainty that no harm will be caused to fish, aquatic arthropods, non-arthropod invertebrates and aquatic plants from their proposed uses on greenhouse and field crops.

4.3 Incident Reports related to the Environment

Since April 26, 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website http://www.hc-sc.gc.ca/cps-spc/pest/part/protect-proteger/incident/indexeng.php.

Only incidents in which the pesticide is determined to be linked to the effects (Canadian causality of highly probable, probable and possible; U.S. causality of highly probable, probable and possible) are considered in the reviews.

Since *B. mycoides* is a new active ingredient pending registration for use in Canada, there are no incident reports. Once products containing *B. mycoides* are registered, the PMRA will monitor for incident reports.

5.0 Value

5.1 Consideration of Benefits

Given the mode of action, the risk for resistance development for *Bacillus mycoides* strain J is considered low. BmJ WG would reduce the need for conventional fungicides. There are several conventional products currently registered for use on tomatoes, peppers, potatoes, spinach and sugarbeets to manage the labeled diseases; however there are few non-conventional products available to producers (see Appendix I, Table 4). For control of downy mildew on spinach, BmJ WG is the only non-conventional product currently available. Registration of BmJ WG will allow Canadian growers, especially organic producers, access to a novel mode of action.

5.2 Effectiveness Against Pests

In support of the proposed claims, efficacy data from a total of 16 relevant trials (crop-pest combinations) were provided. Scientific rationales were used to support extrapolation of certain diseases to crops that had been shown to be responsive to the BmJ treatment. Most proposed uses and claims were supported. The level of efficacy observed ranged from partial suppression to suppression. The product was supported for use under field conditions and under greenhouse conditions, when applicable. Both ground application and chemigation were supported.

5.3 Non-Safety Adverse Effects

No signs of phytotoxicity or any other adverse effects were observed from the use of BmJ WG.

5.4 Supported Uses

The reviewed value information was sufficient to support the claims. Details of the supported uses are provided in Appendix I, Table 5.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

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

BmJ TGAI and BmJ WG (end-use product) were assessed in accordance with the PMRA Regulatory Directive DIR99-03[5] and evaluated against the Track I criteria. The PMRA has reached the following conclusions:

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

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*[6]. The list is used as described in the PMRA Notice of Intent NOI2005-01[7] and is based on existing policies and regulations including: DIR99-03; and DIR2006-02[8] 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:

---

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

6 *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.*

7 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*

8 Regulatory Directive DIR2006-02, *PMRA Formulants Policy*

- The technical grade active ingredient, BmJ TGAI, 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 end-use product, BmJ WG, 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 Micro-organism as Manufactured

The product characterization data for BmJ TGAI and BmJ WG were judged to be adequate to assess their potential human health and environmental risks. The TGAI was characterized and the specifications of the end-use product were supported by the analyses of a sufficient number of batches. All batches of BmJ TGAI must conform to the limits set out in the OECD issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43]. Storage stability data support storage at temperatures under 25°C for up to six months.

7.2 Human Health and Safety

The acute toxicity and infectivity studies and other relevant information submitted in support of *B. mycoides* isolate J were determined to be sufficiently complete to permit a decision on registration. Submitted information suggests BmJ TGAI to be of low toxicity and not infective or pathogenicity by the oral, pulmonary and intravenous routes. BmJ WG was not toxic via the oral or dermal routes and not irritating to the skin. BmJ WG was severely irritating to the eyes. Since BmJ TGAI and BmJ WG are considered potential sensitizers, the signal words, “POTENTIAL SENSITIZER”, are required on the principal display panel of both products.

When handled according to prescribed label instructions, the potential for dermal, eye and inhalation exposure for mixer/loaders, applicators, and other handlers exists, with the primary source of exposure to workers being dermal.

In individuals exposed to large quantities of BmJ WG, respiratory and dermal sensitivity could possibly develop upon repeated exposure to the product since all microorganisms, including *B. mycoides* isolate J, contain substances that are potential sensitizers. Therefore, anyone handling or applying BmJ WG must wear long-sleeved shirt, long pants, shoes plus socks, waterproof gloves, eye goggles, as well as a NIOSH approved mist filtering mask or respirator. In addition, all unprotected workers are restricted from entering enclosed areas (including greenhouses) where BmJ WG have been handled or applied until spray has dried.

The health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is low and not of concern due the low toxicity/pathogenicity profile for BmJ TGAI and BmJ WG. The specification of an MRL under the *Pest Control Products Act* is not required for *B. mycoides* isolate J.

7.3 Value

BmJ WG may provide suppression or partial suppression of certain diseases on tomato, pepper, potatoes, spinach and sugarbeets by triggering the plant's natural defense mechanisms. When used in an IPM program, BmJ WG will reduce the need for conventional fungicides and may delay the development of fungicide resistance.

7.4 Environmental Risk

The non-target organism tests, scientific rationales and supporting published scientific literature submitted in support of BmJ TGAI and BmJ WG were determined to be sufficiently complete to permit a decision on the environmental fate and effects of these products. The use of BmJ TGAI and BmJ WG containing *B. mycoides* isolate J 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 label will prohibit the direct application of BmJ WG to aquatic habitats, estuaries or marine habitats, and direct handlers to not contaminate surface water by disposal of equipment wash waters. Also, precautionary measures on the BmJ WG label will alert users of the hazards to bees from the use of this product as well as instructions to limit exposure to bees.

No other environmental fate studies or non-target organism studies are required for the proposed use pattern on greenhouse and field crops.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of BmJ TGAI and BmJ WG, containing the technical grade active ingredient *Bacillus mycoides* isolate J, for the suppression or partial suppression of certain fungal diseases on food crops.

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

List of Abbreviations

~	not classified
ºC	degree(s) Celsius
µg	micrograms
1/n	exponent for the Freundlich isotherm
a.i.	active ingredient
ADI	acceptable daily intake
ALS	acetolactate synthase
ARfD	acute reference dose
ASM	acibenzolar-S-methyl
atm	atmosphere
BceT	enterotoxin T
BmJ	*Bacillus mycoides* isolate J
bw	bodyweight
C	conventional
CAS	Chemical Abstracts Service
CFU	colony forming unit
cm	centimetres
CytK	cytotoxin cytK
DACO	data code
d	day
DF	dry flowable
DNA	deoxyribonucleic acid
DT_{50}	dissipation time 50% (the dose required to observe a 50% decline in concentration)
DT_{90}	dissipation time 90% (the dose required to observe a 90% decline in concentration)
EC_{25}	effective concentration on 25% of the population
EC_{50}	effective concentration on 50% of the population
EP	end-use product
EntFM	enterotoxin FM
ER_{25}	effective rate for 25% of the population
g	gram
h	hour(s)
ha	hectare(s)
HBL	hemolysin B
HDPP	high density polypropylene
HDT	highest dose tested
Hg	mercury
HPLC	high performance liquid chromatography
IPM	integrated pest management
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
K_d	soil-water partition coefficient
K_F	Freundlich adsorption coefficient
km	kilometre

K_{oc}	organic-carbon partition coefficient
K_{ow}	*n*–octanol-water partition coefficient
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LOAEL	lowest observed adverse effect level
LOC	level of concern
LOEC	low observed effect concentration
LOQ	limit of quantitation
LR_{50}	lethal rate 50%
mg	milligram
mL	millilitre
MAS	maximum average score
MCC	Maximum Challenge Concentration
MPCA	microbial pest control agent
MOE	margin of exposure
MRL	maximum residue limit
mRNA	messenger ribonucleic acid
MS	mass spectrometry
N/A	not applicable
NC	non-conventional
NCBI	National Center for Biotechnology Information
NHE	non-hemolytic enterotoxin
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
NOER	no observed effect rate
NPR1	non-expressor of pathogenesis related genes 1
N/R	not required
NZW	New Zealand white
OC	organic carbon content
OECD	Organization for Economic Co-operation and Development
OM	organic matter content
PBI	plantback interval
PCPA	*Pest Control Products Act*
PCR	polymerase chain reaction
PHI	preharvest interval
p*K*a	dissociation constant
PMRA	Pest Management Regulatory Agency
PR	pathogenesis-related
ppm	parts per million
PubMed	global public domain database on life sciences and biomedical topics which is maintained by the United States National Library of Medicine
RAPD	random amplified polymorphic deoxyribonucleic acid
RQ	risk quotient
RSD	relative standard deviation

RT-PCR	reverse transcriptase PCR
rRNA	ribosomal ribonucleic acid
SA	salicylic acid
SAR	systemic acquired resistance
SC	soluble concentrate
$t_{1/2}$	half-life
T3	tri-iodothyronine
T4	thyroxine
TGAI	technical grade of the active ingredient
TRR	total radioactive residue
TSMP	Toxic Substances Management Policy
UAN	urea ammonium nitrate
UF	uncertainty factor
USEPA	United States Environmental Protection Agency
USPN	United States patent number
UV	ultraviolet
v/v	volume per volume dilution
WG	wettable granules

Appendix I Tables and Figures

Table 1 Toxicity Profile of BmJ TGAI

Study Type/Animal/Reference	Study Results
21-day acute oral toxicity and pathogenicity Sprague-Dawley rat PMRA 2412495	No evidence of toxicity, infectivity or pathogenicity was observed. Clearance was not established through a definite pattern. Dosing was below guideline level.
35-day acute pulmonary toxicity and pathogencity CD rat PMRA 2412496	Two of six animals died within 26 hours of administration during preliminary study (unwashed spores). Study was conducted using BmJ TGAI (washed spores). There were no abnormal general health observations. Effects included lung pigmentation, red foci on lungs. Increase lung, spleen, and lymph node weight relative to body weight. Decreased liver weight. Not infective when instilled at 1.1×10^8 CFU/rat – a pattern of clearance was not established and toxicity resulting in mortalities from the preliminary assay was not explained.
48-hour acute pulmonary toxicity Sprague-Dawley rat PMRA 2619368	Study was conducted using BmJ TGAI (washed spores). There were no mortalities, treatment related clinical signs or changes in body weight.
58-day acute intravenous toxicity and pathogenicity Sprauge-Dawley rat PMRA 2507453	Effects included piloerection, decreased activity, emaciation (1 male); and slight weight loss (6 females). There were no observed abnormalities during necropsies. Not toxic, infective, or pathogenic when injected intravenously at 1.0×10^7 CFU/rat – a pattern of clearance was established.

Table 2 Toxicity Profile of BmJ WG

Study Type/Animal/ Reference	Study Results
14-day acute oral toxicity Sprague-Dawley rat PMRA 2415416	All animals survived and appeared normal throughout the study. All animals gained weight normally throughout the study. No observable abnormalities were found at necropsy. The acute oral LD_{50} of BmJ WG for female rats is > 5000 mg/kg bw.
14-day dermal toxicity Sprague-Dawley rat PMRA 2415408	All animals survived, gained weight, appeared normal, and had no signs of dermal irritation throughout the study. Animals exhibited alopecia. No observable abnormalities were found in any animal at necropsy. The Dermal LD_{50} for male and female rats is >5050 mg/kg bw.
72-hour dermal irritation New Zealand White rabbit PMRA 2415411	No dermal erythema or edema was noted on any animal at 1, 24, 48, or 72 hours. BmJ WG is not irritating to the skin.
72-hour eye irritation New Zealand White rabbit PMRA 2415414	Corneal opacity was noted in all rabbits immediately following exposure with clearance by 24 hours. Positive conjunctival irritation (score $\geq$ 2) was noted on 3/3 rabbits 24 hours after test material instillation with resolution by 48 hours. Fluorescein staining was observed in all treated rabbit eyes at 24 hours but not at 72 hours. BmJ WG is severely irritating to the eye.

Table 3 Toxicity of BmJ TGAI to Non-Target Species

Organism	Exposure	Significant Effect, Comments	Reference
Terrestrial Organisms			
Vertebrates			
Birds			
Northern Bobwhite Quail (*Colinus virginianus*)	14d- Acute Oral	No signs of toxicity. No mortalities. No effects on body weight or feed consumption. LD_{50} > 2250 mg a.i./kg bw **LOW TOXICITY**	PMRA 2412501
	A scientific rationale was submitted to waive further testing on pathogenicity and infectivity. *Bacillus mycoides* grows below basic avian temperatures and is not generally considered an avian pathogen. There were no reports of adverse effects to birds in scientific literature.		PMRA 2441785
Invertebrates			
Arthropods			
Ladybird beetles (*Hippodamia convergens*)	18d-Dietary	Cumulative percent mortality did not differ significantly between the groups. No sublethal effects observed. LC_{50} > 4.5×10^8 CFU/mL **LOW TOXICITY**	PMRA 2412507
Green lacewing (*Chrysoperla rufilabris*), larvae	21d-Dietary	No significant lethal effects on the survival of larvae and no sub-lethal effects on adult emergence (Days 22–32) were observed. Pupation and egg consumption was, however, significantly affected. LC_{50} > 4.5×10^8 CFU/mL **LOW TOXICITY**	PMRA 2412508
Honeybees (*Apis mellifera*), adult worker	11d- Dietary	Reported LD_{50}=0.24 g/L but unreliable feeding data to determine LD_{50}. Mortality rates observed in dose related manner.	PMRA 2412510

Organism	Exposure	Significant Effect, Comments	Reference
Terrestrial Arthropods	Scientific rationales were submitted to supplement the toxicity testing based on the lack of evidence of insecticidal activity for *B. mycoides* as reported in the published scientific literature and the ubiquitous nature of *B. mycoides* as a soil saprophyte, as well as symbionts of insects, without reports of adverse effects.		PMRA 2507400
Plants			
Plants	Scientific rationale was submitted to waive testing on terrestrial plants. The rationale based on the following: intended non-toxic mode of action to plant pathogens by triggering plant's defenses; lack of observed phytotoxicity or adverse effects to various crops in small plot efficacy studies; reductions in BmJ populations soon after application; and lack of documentation of *Bacillus* species as potential frank plant pathogens.		PMRA 2543949
Aquatic Organisms			
Vertebrates			
Rainbow trout (*Oncorhynchus mykiss*)	96h-Aquatic exposure (static conditions)	No mortalities observed, all fish appeared normal throughout study. 96-hour $LC_{50} > 6.22\times10^6$ CFU/mL **LOW TOXICITY**	PMRA 2412505
Fish	A scientific rationale was submitted to waive further testing on freshwater fish for pathogenicity and infectivity, based on limited aquatic exposure and lack of evidence of irreversible adverse effects anticipated to freshwater fish in the published literature.		PMRA 2441787
Invertebrates			
Daphnids (*Daphnia magna*)	48h- Aquatic exposure (static conditions)	There were no effects on survival. 48 hours $LC_{50} > 6.22\times10^6$ CFU/mL **LOW TOXICITY**	PMRA 2412511
Aquatic Arthropods	A scientific rationale was submitted to waive further testing on aquatic arthropods, based on limited aquatic exposure, and lack of evidence of irreversible adverse effects anticipated to aquatic arthropods in the published literature.		PMRA 2441787
Plants			
Aquatic Plants	Although no data was submitted, independent searches of the open scientific literature (through PubMed) using the various keywords yielded no additional reports of adverse effects aquatic plants.		

Organism	Exposure	Significant Effect, Comments	Reference

Table 4 Registered Alternatives Grouped by Mode of Action (as of December 2015).

Crop	Pest	Conventional (C) / Non-Conventional (NC)	Active Ingredient (mode of action group #)
tomato	bacterial leaf spot (*Xanthomonas euvesicatoria*)	C	copper (M1), kasugamycin (24)
		NC	acibenzolar-s-methyl (P1), extract of *Reynoutria sachalinensis* (P5), *Bacillus subtilis* strain QST 713 (44)
	bacterial speck (*Pseudomonas syringae* pv. *tomato*)	C	copper (M1)
		NC	acibenzolar-s-methyl (P1), *Bacillus subtilis* strain QST 713 (44)
	early blight (*Alternaria solani*)	C	copper (M1), mancozeb (M3), metiram (M3), ziram (M3), captan (M4), chlorothalonil (M5), difenoconzole (3), boscalid (7), penthiopyrad (7), fluxapyroxad (7), pyrimethanil (9), azoxystrobin (11), pyraclostrobin (11), trifloxystrobin (11), fenamidone (11), cymoxanil + famoxadone (11+27*)
		NC	*Bacillus subtilis* strain QST 713 (44), *Bacillus amyloliquefaciens* strain D747 (44)
	late blight (*Phytophthora infestans*)	C	copper (M1), mancozeb (M3), metiram (M3), ziram (M3), captan (M4), chlorothalonil (M5), pyraclostrobin (11), fenamidone (11), fluoxastrobin (11), cymoxanil + famoxadone (11+27*), cyazofamid (21), propamocarb hydrochloride (28), dimethomorph (40), mandipropamid (40), fluopicolide (43)
		NC	garlic powder (~), mono- and dibasic sodium (~), potassium and ammonium phosphites (~), mono- and di-potassium salt of phosphorus acid (~), tea tree oil (~), *Bacillus subtilis* var. *amyloliquefaciens* strain FZB24 (~), *Streptomyces griseoviridis* strain K61 (~)
pepper	bacterial leaf spot (*Xanthomonas*	C	copper (M1), kasugamycin (24)
		NC	*Bacillus subtilis* strain QST 713 (44)

Crop	Pest	Conventional (C) / Non-Conventional (NC)	Active Ingredient (mode of action group #)
	euvesicatoria)		
	bacterial speck (*Pseudomonas syringae* pv. *tomato*)	C	copper (M1)
		NC	*Bacillus subtilis* strain QST 713 (44)
	early blight (*Alternaria solani*)	C	copper (M1), difenoconzole (3), boscalid (7), penthiopyrad (7), fluxapyroxad (7), benzovindiflupyr (7), pyraclostrobin (11), trifloxystrobin (11),
		NC	*Bacillus subtilis* strain QST 713 (44), *Bacillus amyloliquefaciens* strain D747 (44)
	late blight (*Phytophthora infestans*)	C	copper (M1), fluoxastrobin (11), dimethomorph (40),
		NC	mono- and di-potassium salt of phosphorus acid (33), *Streptomyces griseoviridis* strain K61 (~)
potato	early blight (*Alternaria solani*)	C	copper (M1), mancozeb (M3), metiram (M3), captan (M4), chlorothalonil (M5), difenoconzole (3), metconazole (3), boscalid (7), penthiopyrad (7), fluopyram (7), fluxapyroxad (7), benzovindiflupyr (7), pyrimethanil (9), azoxystrobin (11), fenamidone (11), pyraclostrobin (11), cymoxanil + famoxadone (11+27*), zoxamide (22)
		NC	*Bacillus subtilis* strain QST 713 (44), *Bacillus amyloliquefaciens* strain D747 (44)
	late blight (*Phytophthora infestans*)	C	copper (M1), mancozeb (M3), metiram (M3), captan (M4), chlorothalonil (M5), azoxystrobin (11), pyraclostrobin (11), fenamidone (11), fluoxastrobin (11), cyazofamid (21), zoxamide (22), cymoxanil (27), propamocarb hydrochloride (28), fluazinam (29), dimethomorph (40), mandipropamid (40), fluopicolide (43), ametoctradin (45)
		NC	mono- and di-potassium salt of phosphorus acid (33), mono- and dibasic sodium, potassium and ammonium phosphites (~), *Bacillus subtilis* strain QST 713 (44)
	White mold (*Sclerotinia*	C	metconazole (3), difenoconazole + azoxystrobin (3 + 11*), fluxapyroxad (7),

Crop	Pest	Conventional (C) / Non-Conventional (NC)	Active Ingredient (mode of action group #)
	sclerotiorum)		fluopyram + pyrimethanil (7 + 9*), fluazinam (29),
		NC	*Bacillus subtilis* strain QST 713 (44), *Bacillus amyloliquefaciens* strain D747 (44)
spinach	downy mildew (*Peronospora farinosa spinaciae*)	C	copper (M1), metalaxyl-m and s-isomer (4), boscalid + pyraclostrobin (7 + 11*), azoxystrobin (11), fenamidone (11), fosetyl-al (33)
sugarbeet	cercospora leaf spot (*Cercospora beticola*)	C	copper (M1), mancozeb (M3), metiram (M3), thiophanate-methyl (1), prothioconazole (3), metconazole (3), difenoconazole (3), tetraconazole (3), fluxapyroxad (7), pyraclostrobin (11),
		NC	*Bacillus subtilis* strain QST 713 (44),

*: not clear which active ingredient or if both active ingredients are efficacious against the disease.
~: unknown mode of action

Table 5 BmJ - List of Supported Uses

Supported Use Claims
Suppression of bacterial leaf spot (*Xanthomonas euvesicatoria*) on tomatoes and peppers at a concentration of 0.33 g/L of water. Supported for field and greenhouse use.
Partial suppression of bacterial speck (*Pseudomonas syringae* pv. *tomato*) on tomatoes and peppers at a concentration of 0.33 g/L of water. Supported for field and greenhouse use.
Suppression of early blight (*Alternaria solani*) on tomatoes and peppers at a concentration of 0.33 g/L of water. Supported for field and greenhouse use.
Suppression of late blight (*Phytophthora infestans*) on tomatoes and peppers at a concentration of 0.33 g/L of water. Supported for field and greenhouse use.
Suppression of early blight (*Alternaria solani*) on potatoes at a concentration of 0.33 g/L of water. Supported for field use only.
Suppression of late blight (*Phytophthora infestans*) on potatoes at a concentration of 0.33 g/L of water. Supported for field use only.
Partial suppression of white mold (*Sclerotinia sclerotiorum*) on potatoes at a concentration of 0.33 g/L of water. Supported for field use only.
Partial suppression of downy mildew (*Peronospora farinosa spinaciae*) on spinach at a concentration of 0.33 g/L of water. Supported for field and greenhouse use.
Suppression of cercospora leaf spot (*Cercospora beticola*) on sugarbeet at a concentration of 0.33 g/L of water. Supported for field use only.
Application methods: ground equipment and chemigation

References

A. List of Studies/Information Submitted by Registrant

1.0 Chemistry

PMRA Document Number	Reference
2412490	2014, *Bacillus mycoides* isolate J TGAI, DACO: M2.1, M2.2, M2.3, M2.4, M2.5, M2.6 CBI
2412491	2014, *Bacillus mycoides* isolate J Technical Powder (CX-10244) End-use Product (CX-10250) Microbial Pesticide Data Requirements - Volume II Part A and Part B, DACO: M2.10.1, M2.10.2 ,M2.10.3, M2.11, M2.7.1, M2.7.2, M2.8, M2.9.2, M2.9.3 CBI
2412492	2014, Physical and Chemical Properties of *Bacillus mycoides* isolate J (BmJ) TGAI (CX-10244) and BmJ WG End Use Product (CX-10250) - Volume III, DACO: M2.12 CBI
2415401	2014, BmJ WG, DACO: M2.1,M2.2,M2.3,M2.4,M2.5,M2.6 CBI
2441786	2014, Transmittal Letter and Volume II *Bacillus mycoides* isolate J Response to Preliminary Technical Screen, DACO: M2.7.1,M2.8,M4.2,M9.5.1 CBI
2454247	2005, *Bacillus mycoides* Isolate "J" General Information on the Organism: Description, Natural Occorence and Mode of Action, DACO: M2.7.1 CBI
2454248	2003, Predominant Bacillus spp. in Agricultural Soil Under Different Management Regimes Detected via PCR-DGGE, DACO: M2.7.2 CBI
2454249	2014, *Bacillus mycoides* Taxonomy, DACO: M2.7.2 CBI
2454250	Michael J. Brumlik et al, 2001, Reference document for MRID 46632904 - Use of Long-Range Repetitive Element Polymorphism-PCR to Differentiate *Bacillus anthracis* Strains, DACO: M2.7.2 CBI
2507399	2015, Request for No Further Testing - Enterotoxin Analysis, DACO: M2.7.2 CBI
2507422	2015, Mode of Action, DACO: 10.2.1,M2.7.2
2507423	Bargabus, R.L., N.K. Zidak, J.E. Sherwood, and B.J., 2003, Reference Document: Oxidative burst elicited by *Bacillus mycoides* isolate Bac J, a biological control agent, occurs independently of hypersensitive cell death in sugar beet. Mol. Plant Microbe Interactions 12: 1145 - 1153., DACO: 10.2.1,M2.7.2
2507456	2015, Microbial Contamination Method, DACO: M2.7.1
2508413	2015, Request for No Further Testing, DACO: M2.7.1 CBI
2520861	2015, Response to Deficiency Review Note of March 23, 2015, DACO: M2.7.2 CBI
2520862	2014, Growth Temperature Chart, DACO: M2.7.2 CBI
2520863	2015, Response to Deficiency Review Note of March 23, 2015, DACO: M2.10

2520864	2015, Protocol used for the β-exotoxin bioassay, DACO: M2.10 CBI
2619366	2016, BmJ TGAI and BmJ WG: Detailed Manufacturing Process and Quality Control Procedures for Bacillus mycoides Isolate J Supplement to MRID 49332401, DACO: M2.8 CBI

2.0 Human and Animal Health

PMRA Document Number	Reference
2412494	2014, Summary Document, DACO: M4.2.1
2412495	2013, Acute Oral Injection Toxicity/Pathogenicity Study in Rats with BmJ TGAI - Volume IV, DACO: M4.2.2
2412496	2005, Toxicity/Pathogenicity Testing of *Bacillus mycoides* isolate J Following Acute Intratraceal Challenge in Rats, DACO: M4.2.3
2412499	2013, BmJ TGAI Intravenous Toxicity/Pathogenicity Study in Rats - Volume V, DACO: M4.3.2
2420198	2014, Petition ProPetition Proposing Exemption from the Requirement for a Tolerance for the Use of the Microbial Pest Control Agent *Bacillus mycoides* isolate, DACO: M7.0
2507453	2015, *Bacillus mycoides* isolate J TGAI Intravenous Toxicity I Pathogenicity Study in Rats, DACO: M4.2
2508414	2015, *Bacillus mycoides* isolate J TGAI Pre and Post Toxicity Analysis of Active Ingredient for an MPCA, DACO: M4.2 CBI
2415408	2014, BmJ WG: Acute Dermal Toxicity in Rats - Volume VII, DACO: M4.4
2415409	2014, Summary Document, DACO: M4.5.1
2415411	2014, BmJ WG: Acute Dermal Irritation in Rabbits - Volume X, DACO: M4.5.2
2415412	2014, Sensitization, DACO: M4.6
2415414	2014, BmJ WG: Acute Eye Irritation in Rabbits - Volume IX, DACO: M4.9
2415416	2014, BmJ WG: Acute Oral Toxcity (UDP) in Rats - Volume VI, DACO: M4.9
2415418	2014, BmJ WG: Acute Inhalation Toxicity and Hypersensitivity Incidents – Volume VIII, DACO: M4.9
2619368	2016, *Bacillus mycoides* Isolate J TGAI Acute Inhalation Toxicity/Pathogenicity in Rats, DACO:M4.3.2

3.0 Environment

PMRA Document Number	Reference
2412501	2013, BmJ TGAI: An Acute Oral Toxicity Study with the Northern Bobwhite - Volume XIII, DACO: M9.2.1
2412505	2013, BmJ TGAI: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*) - Volume XII, DACO: M9.4.1
2412507	2013, BmJ TGAI: Ladybird Beetle Non-garge Insect Microbial Testing - Volume XIV, DACO: M9.5.1
2412508	2013, BmJ TGAI: Green Lacewig Non-target Insect Microbial Test- Volume XV, DACO: M9.5.1
2412510	2013, BmJ TGAI: Definative Honey Bee, *Apis mellifera*, Non-target Insect Microbial Test- Volume XVII, DACO: M9.5.1
2412511	2013, BmJ TGAI: A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*) - Volume XI, DACO: M9.5.2
2441785	2014, Volume I - *Bacillus mycoides* isolate J Request for No Further Testin, DACO: M9.2.1
2441787	2014, *Bacillus mycoides* isolate J Request for no Further Testing, DACO: M9.4.1,M9.5.2
2454258	2005, Reference Document: Microbial Diversity of Wild Bird Feathers Revealed through Culture-Based and Culture-Independent Techniques, DACO: M9.2.1
2507400	2015, Rationale for No Further Testing of *Bacillus mycoides* isolate J for effects on nontarget insects, DACO: M9.5.1
2507401	Blibech, I., M. Ksantini, I. Chaieb, B. Jlassi, A. Rhouma, S. Jaoua, and S. Aifa. , 2011, Reference Document: Isolation of entomopathogenic *Bacillus* from a biodynamic olive farm and their pathogenicity to lepidopteran and coleopteran insect pests. , DACO: M9.5.1
2507404	Evans, J.D., and T.N. Armstrong, 2006, Reference Document: Antagonistic interactions between honey bee bacterial symbionts and implications for disease. BMC Ecology 6 (4), 9 pp. (http://www.biomedcentral.com/1472-6785/6/4)., DACO: M9.5.1
2507407	Hillesland, H., A. Read, B. Subhadra, I. Hurwitz, R. McKelvey, K. Ghosh, P. Das, and R. Durvasula, 2008, Reference Document: Identification of aerobic gut bacteria from the Kala Azar vector, *Phlebotomus argentipes*: A platform for potential paratransgenic manipulation of sand flies. Am. J. Trop. Med. Hyg., 79(6): 881–886., DACO: M9.5.1
2507408	Ihssen, J., M.A. Horn, C. Matthies, A. Gößner, A. Schramm, and H.L. Drake, 2002, Reference Document: . N2O-producing microorganisms in the gut of the earthworm *Aporrectodea caliginosa* are indicative of ingested soil bacteria. Appl. Environ. Microbiol. 69: 1655–1661., DACO: M9.5.1

2507414	Podgwaite, J.D., V. D'Amico, R.T. Zerillo, and H. Schoenfeldt, 2012, Reference Document: Bacteria associated with larvae and adults of the Asian longhorned beetle (Coleoptera: Cerambycidae). J. Entomol. Sci. 48 (2): 128–138., DACO: M9.5.1
2507415	Schuch, R., A.J. Pelzek, S. Kan, and V.A. Fischetti. , 2010, Reference Document: 2010 Prevalence of *Bacillus anthracis*-like organisms and bacteriophages in the intestinal tract of the earthworm Eisenia fetida. Appl. Environ. Microbiol. 76: 2286–2294. , DACO: M9.5.1
2543949	2015, Non-Target Plant Study , DACO: M9.8.1,M9.8.2
2454247	2005, *Bacillus mycoides* Isolate "J" General Information on the Organism: Description, Natural Occorence and Mode of Action, DACO: M2.7.1 CBI

4.0 Value

PMRA Document Number	Reference
2415428	2014, CER-2012-032EB - Tomato - Early Blight - Evaluation of compounds for management of early blight in tomato, fall 2012., DACO: M10.2.2
2415432	2014, CER-2012-035 - Potato - Late Blight - Evaluation of fungicides for control of potato late blight, 2012., DACO: M10.2.2
2415435	2014, CER-2012-056 - Tomato - Powdery Mildew - Efficacy of CX-10250 Alone and Alternated with Cueva on the Control of Bacterial Speck of Tomatoes, DACO: M10.2.2
2415437	2014, CER-2012-065 - Potato - White Mold - Evaluate CX-10250 for white mold control in potatoes grown in Eastern Washington., DACO: M10.2.2
2415447	2014, CER-2013-015 - Tomato - Late Blight - Efficacy of CX-10250 for Control of Phytophthora infestans (Late Blight) in Tomato Trial DACO: M10.2.2
2415451	2014, CER-2013-044 - Potatoe - Late Blight - Evaluation of fungicide programs for potato late blight control: 2013., DACO: M10.2.2
2415455	2014, CER-2013-052 - Tomato - Early Blight and Late Blight - Fungicide evaluation for early blight and late blight on tomato, 2013, DACO: M10.2.2
2415457	2014, CER-2013-053 - Pepper - Bacterial Leaf Spot - Evaluation of chemicals for controlling bacterial spot on bell pepper 2013, DACO: M10.2.2
2415479	2014, IND-2007-094 - Potato - Early Blight - Evaluation of fungicides to control early blight of potato - Hancock, 2007, DACO: M10.2.2
2415481	2014, IND-2010-226 - Potato Early Blight - Comparison of OMRI listed products for the control of early blight on organic potatoes, 2010., DACO: M10.2.2

2415486	2014, MMP-2010-227 - Potato - White Mold - Control of White Mold in Russet Burbank Potatoes, DACO: M10.2.2
2507387	2014, CER1408 - CER-2014-017 - Evaluate CX-10250 and CX-10470 for Control of Downy Mildew in Spinach, DACO: M10.2.2
2507388	2014, CER1407 - CER-2014-016 - Evaluation of the Efficacy of CX-10250 Alone and in Alternation with Cueva Against Bacterial Speck in Tomatoes, DACO: M10.2.2
2533197	Barry J. Jacobson, 1997, fungicide and biological control alternatives to tpth for cercospora leaf spot control, DACO: 10.2.3
2533199	Barry J. Jacobson, 1999, management of cercospora leaf spot in western North Dakota and Eastern Montana, DACO: 10.2.3

B. Additional Information Considered

i) Published Information

1.0 Chemistry

PMRA Document Number	Reference
2558712	Bargabus-Larson, R.L. and Jacobsen, B.J., 2007. Biocontrol elicited systemic resistance in sugarbeet is salicylic acid independent and NPR1 dependent. Journal of Sugar Beet Research 44(1and2):17-33. DACO M2.7.2
2558713	DiFranco, C., Santini, T., Pisaneschi, G., and Beccari, E. 2005. Insights into the genetic organization of the *Bacillus mycoides* cryptic plasmids pDx14.2 and pSin9.7 deduced from their complete nucleotide sequence. Plasmid 54(2005):288-293. DACO: M2.7.2
2558715	Goodwin, A.E., Roy, J.S., Grizzle Jr., J.M., and Goldsby Jr., M.T. 1994. 1994. *Bacillus mycoides*: a bacterial pathogen of channel catfish. Diseases of Aquatic Organisms 18:173-179. DACO: M2.7.2
2558716	Nakamura, L.K., and Jackson, M.A. 1995. Clarification of the taxonomy of *Bacillus mycoides*. Int. J. of Systematic Bacteriology 45(1):46-49. DACO: M2.7.2
2558718	Daffonchio, D., Borin, S., Frova, G. Gallo, R., Mori, E., Fani, R., and Sorlini C. 1999. A randomly amplified polymorphic DNA marker specific for the *Bacillus cereus* group is diagnostic for *Bacillus anthracis*. Applied and Environmental Microbiology, 65(3):1298-1303. DACO: M2.7.2
2558719	Agata, N., Ohta, M., Arakawa, Y., and Mori, M., 1995. The bceT gene of *Bacillus cereus* encodes an enterotoxic protein. Microbiology 141:983-988. DACO: M2.7.2, DACO: M2.7.2
2558720	Choma, C. and Granum, P.E., 2002. The enterotoxin T (BceT) from *Bacillus cereus* can probably not contribute to food poisoning. FEMS Microbiology Letters 217:115-119. DACO: M2.7.2, DACO: M2.7.2

2558722	Ansell, S.E., Lawton, E.B., Mcrae, S., Mortimer, G., and Cursons R.T. 1980. Endophthalmitis due to *Bacillus cereus*: case report. New Zealand Medical J. 92(663)12-3. DACO: M2.7.2
2558723	Sahu, C., Kumar, K., Sinha, M.K., Venkata, A., Majii, A.B., and Jalali, S. 2013. Review of endogenous endophthalmitis during pregnancy including case series. Int. Opathalmol 33:611-618. DACO: M2.7.2
2558724	Mcintyre, L., Bernard, K., Beniac, D., Isaac-Renton, J.L., and Naseby, D.C. 2008. Identification of *Bacillus cereus* group species associated with food poisoning outbreaks in British Columbia, Canada. Applied and Environmental Microbiology 74(23):7451-7453. DACO: M2.7.2
2581406	Jimenez, G., Udriain, M., Cifutenes, A., Lopez-Lopez, A., Blanch, A.R., Tamames, J., Kampfer, P., Kolso, A-B., Ramon, D., Martinez, J.F., Codoner, F.M., and Roseelo-Mora, R. 2013. Description of *Bacillus toyonensis* sp. *nov*., a novel species of the *Bacillus cereus* group, and pairwise genome comparisons of the species of the group by means of ANI calculations. Systematic and Applied Microbiology 36:383-391. M2.7.2
2581407	Gruinebretiere, M-H, Auger, S., Galleron, H., Contzen, M., De Sarrau, B., De Buyser, M-L., Lamberet, G., Fagerlund, A., Granum, P.E., Lereclus, D., De Vos, P., Nguyen-The, C., and Sorokin, A. 2013. *Bacillus cytotoxicus* sp. nov. is a novel thermotolerant species of the Bacillus cereus group occasionally associated with food poisoning. Int. J. of Systematic and Evolutionary Microbiology 63:31-40. DACO: M2.7.2
2581408	Jung, M-Y., Paek, W.K., Park I-S., Han, J-R., Sin, Y., Paek, J., Rhee, M-S., Kim, H., Song, H.S., and Chang, Y-H. 2010. *Bacillus gaemokensis* sp. nov., isolated from forshore tidal flat sediment from the Yellow Sea. The J. of Microbiology. 48(6):867-871. DACO: M2.7.2
2581411	Jung, M-Y, Kim, J-S., Paek, W.K., Lim, J., Lee, H., Kim, P. I., Ma, J.Y., Kim, W., and Chang, Y-H. 2011. *Bacillus manliponensis* sp. nov., a new member of the *Bacillus cereus* group isolated from foreshore tidal flat sediment. The J. of Microbiology 49(6):1027-1032. DACO: M2.7.2

2.0 Environment

PMRA Document Number	Reference
2558715	Goodwin, A.E., Roy, J.S., Grizzle Jr., J.M. and Godlsby Jr., M.T., 1994. *Bacillus mycoides*: a bacterial pathogen of channel catfish. Diseases of Aquatic Organisms 18:173-179. DACO: M2.7.2, DACO: M2.7.2
2598389	2006, US EPA report R143131, DACO: M12.5