Proposed Registration Decision PRD2018-19

Bacillus thuringiensis subsp. *galleriae* Strain SDS-502, grubGONE! G, grubHALT!, beetleGONE!, beetleJUS!

(publié aussi en français)

19 November 2018

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

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

Catalogue number: H113-9/2018-19E (print version)
H113-9/2018-19E-PDF (PDF version)

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Overview

Proposed Registration Decision for *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing registration for the sale and use of Phyllom SDS-502 Technical (containing *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502), the manufacturing product Phyllom SDS-502-MP and the end-use products grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS! containing the technical grade active ingredient *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502, to control specific beetle pests of turf and ornamental plants.

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

This summary describes the key points of the evaluation, while the Science Evaluation section provides detailed technical information on the human health, environmental and value assessments of Phyllom SDS-502 Technical, Phyllom SDS-502-MP and the end-use products grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!.

What Does Health Canada Consider When Making a Registration Decision?

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

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

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

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

Before making a final registration decision on *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502, Phyllom SDS-502-MP and the end-use products grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!, Health Canada's PMRA will consider any comments received from the public in response to this consultation document.[3] Health Canada will then publish a Registration Decision[4] on *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502, Phyllom SDS-502-MP and the end-use products grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!, which will include the decision, the reasons for it, a summary of comments received on the proposed registration decision and Health Canada's response to these comments.

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

What Is *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502?

Bacillus thuringiensis subsp. *galleriae* strain SDS-502 is a bacterium that infects and kills certain types of beetles. It can be used as an insecticide to control these beetle pests of turf and ornamental plants.

Health Considerations

Can Approved Uses of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 Affect Human Health?

***Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 is unlikely to affect your health when grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS! are used according to the label directions.**

Potential exposure to *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 may occur when handling and applying grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!. When assessing health risks, several key factors are considered:

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

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

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

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

Studies in laboratory animals describe potential health effects from large doses of exposure to a microorganism and identify any pathogenicity, infectivity and toxicity concerns. When *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 was tested on laboratory animals, there was no sign that it caused any significant toxicity or disease.

Residues in Water and Food

Dietary risks from food and water are acceptable.

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

Risks in Residential and Other Non-Occupational Environments

Estimated risk for non-occupational exposure is acceptable.

Risk to the general population is not a concern since there were no signs that this active ingredient caused any significant toxicity or disease in studies on laboratory animals. Moreover, the product labels for grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS! include mitigation measures to prevent bystander exposure.

Occupational Risks From Handling grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!

Occupational risks are not of concern when grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS! are used according to label directions, which include protective measures.

Commercial workers handling grubGONE! G and beetleGONE! can come into direct contact with *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 on the skin, in the eyes or by inhalation. For this reason, the product label will specify that commercial workers must wear personal protective equipment, including waterproof gloves, a long-sleeved shirt, long pants, a particulate filtering respirator, and socks with shoes. In addition, all unprotected commercial workers are restricted from entering areas during application and for 4 hours following application or until all sprays have dried or dusts have settled.

Residential users of grubHALT! and beetleJUS! are instructed to prohibit re-entry into treated areas where these end-use products have been applied for 4 hours or until the sprays have dried or dusts have settled.

Environmental Considerations

What Happens When *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 Is Introduced Into the Environment?

Environmental risks are not of concern.

Bacillus thuringiensis subsp. *galleriae* is natural occurring soil microorganism that produces a resilient endospore under adverse conditions. These endospores allow these microorganisms to readily survive in soils, dusts and aerosols. Information on the environmental fate of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 suggests that it is likely to readily survive in soils and sediment after applications of grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!, but the population of this microorganism should return to naturally sustainable levels over time.

There are no published reports of disease associated with natural populations of *Bacillus thuringiensis* subsp. *galleriae* in birds, wild mammals, fish, terrestrial and aquatic arthropods, terrestrial and aquatic non-arthropod invertebrates, or terrestrial and aquatic plants. Also, no adverse effects to birds, bees, and terrestrial arthropods were observed during testing. Toxic effects were noted in daphnids at the highest concentrations tested, however, these effects occurred at levels that exceed the expected exposure levels when grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS! are used according to the label.

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

Value Considerations

What Is the Value of grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!?

These products provide a new mode of action for control of certain beetle pests of turf and ornamental plants.

Both grubGONE! G and grubHALT! control beetle grubs that feed on turf and the roots of ornamental plants. Both beetleGONE! and beetleJUS! control beetle grubs that feed on turf and the roots of ornamental plants and also control adult beetles that feed on the leaves of ornamental plants. These products provide a new mode of action for control of these beetle pests and can help with management of resistance to registered alternatives.

Measures to Minimize Risk

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

The key risk-reduction measures being proposed on the labels of Phyllom SDS-502 Technical, Phyllom SDS-502 MP, grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS! to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

All microorganisms, including *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502, contain substances that are potential sensitizers and thus, respiratory and dermal sensitivity may possibly develop in individuals exposed to potentially large quantities of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502. In turn, commercial workers handling or applying grubGONE! G and beetleGONE! must wear waterproof gloves, a long-sleeved shirt, long pants, a particulate filtering respirator, and socks with shoes. Furthermore, all unprotected workers, users and bystanders are restricted from entering areas treated with grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS! for 4 hours following application or until sprays have dried or dusts have settled.

A standard drift statement is also required on the commercial class end-use products, grubGONE! G and beetleGONE! to minimize the potential for drift to areas of human habitation or areas of human activity such as houses, cottages, schools and recreational areas.

Environment

The end-use product labels will include standard environmental precaution statements to reduce contamination of aquatic systems from the use of grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!. The commercial end-use product labels for grubGONE! G and beetleGONE! will also include a standard environmental precaution statement to prohibit aerial application.

Next Steps

Before making a final registration decision on *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502, Phyllom SDS-502-MP and the end-use products grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!, Health Canada's PMRA will consider any comments received from the public in response to this consultation document. Health Canada will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to PMRA Publications (contact information on the cover page of this document). Health Canada will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed decision and Health Canada's response to these comments.

Other Information

When the Health Canada makes its registration decision, it will publish a Registration Decision on *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502, Phyllom SDS-502-MP and the end-use products grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS! (based on the Science Evaluation section of this consultation document). In addition, the confidential test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Bacillus thuringiensis subsp. *galleriae* strain SDS-502, grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active mircoorganisms	*Bacillus thuringiensis* subsp. *galleriae* strain SDS-502
Function	Control of certain beetle larvae and adults in turf and on ornamentals.
Binomial name	*Bacillus thuringiensis* subsp. *galleriae* strain SDS-502
Taxonomic designation[5]	
Domain	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillalaes
Family	Bacillaceae
Genus	*Bacillus*
Species	*thuringiensis*
Subspecies	*galleriae*
Strain	SDS-502
Patent Status information	A United States patent (US 6 962 977 B2) was issued on 8 November 2005. No application has been made for a Canadian patent.
Nominal purity of active	Technical grade active ingredient: 1.2×10^{10} CFU/g
	Manufacturing Concentrate (MA): 1×10^{10} CFU/g
	End-Use Products: grubGONE! G – 1×10^{9} CFU/g grubHALT! – 1×10^{9} CFU/g beetleGONE! – 8.5×10^{9} CFU/g beetleJUS! – 8.5×10^{9} CFU/g
Identity of relevant impurities of toxicological, environmental	The technical grade active ingredient does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminant release standards. Many subspecies and strains of *Bacillus*

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

and/or significance.	*thuringiensis* produce *Bacillus cereus*-like enterotoxins which have been implicated in cases of food poisoning. The presence of these enterotoxins has not been determined but food uses are not currently proposed.

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

Property	Phyllom SDS-502 MP	grubGONE! G	grubHALT!	beetleGONE!	beetleJUS!
Colour	Light brown	Light brown, brown	Light brown, brown	Light brown, tan	Light brown, tan
Odour	Earthy, yeast-like	Earthy, yeast-like	Earthy, yeast-like	Mild earthy	Mild earthy
Oxidation/ Reduction	None	None	None	N/A	N/A
Flammability	None	None	None	N/A	N/A
Explodability	None	Non-combustible granule	Non-combustible granule	N/A	N/A
Miscibility	Forms suspension in water	Forms suspension in water	Forms suspension in water	Forms a fine-particulate suspension of fine insoluble particles in water	Forms a fine-particulate suspension of fine insoluble particles in water
Corrosion Characteristics	None	None	None	None	None
pH	5.02 (1% solution)	N/A	N/A	N/A	N/A
Viscosity	N/A	N/A	N/A	N/A	N/A
Density/Relative Density/Bulk Density	0.05–0.06 g/mL	0.72 g/mL	0.72 g/mL	0.54 g/mL	0.54 g/mL

1.3 Directions for Use

The granular products grubGONE! G and grubHALT! are applied at 1.12–1.68 kg per 100 square metres with a minimum re-application interval of 7 days and a maximum annual application rate of 3.36 kg per 100 square metres for control of the larvae of several species of beetles that feed in turf or on the roots of ornamental plants. These products should be applied when rainfall is expected or irrigated after application to move the active ingredient into the root zone.

The domestic class product grubHALT! is for outdoor use only but the commercial class product grubGONE! G includes use in nurseries, greenhouses and indoor plantscapes as well as an alternative application rate of 13–17 g per square metre for ornamental plants growing in containers. See labels for details.

The water-dispersible products beetleGONE! and beetleJUS! are applied at 125–195 g per 100 square metres (12.5–19.5 kg/ha) for control of the larvae of several species of beetles that feed in turf or on the roots of ornamental plants or at 15–30 g/L (1.5–3.0 kg per 100 L) for control of adults of four species of beetles that feed on the foliage of ornamental plants. For application of beetleGONE! to turf or soil, there is a minimum re-application interval of 7 days and a maximum annual application rate of 39.5 kg/ha; otherwise, there is no minimum re-application interval or maximum number of applications for these products. The domestic class product beetleJUS! is for use only on turf and landscape ornamentals but the commercial class product beetleGONE! includes use on ornamental plants in nurseries, greenhouses and indoor plantscapes as well as an alternative application method of dipping (submersion of trays or pots to saturate soil) in a solution of 0.75–1.5 kg per 100 L of water. See labels for details.

1.4 Mode of Action

Active ingredients derived from *Bacillus thuringiensis* bacteria are classified as microbial disruptors of insect midgut (IRAC mode-of-action group 11). These bacteria produce dormant spores and crystals of protein. When ingested by an insect, the protein crystals dissolve in the alkaline environment of the insect gut, releasing protein toxins that bind to specific receptors on the insect's midgut cells. Different subspecies of *Bacillus thuringiensis* produce different protein toxins that are specific to different groups of insects (for example, Lepidoptera vs. Diptera vs. Coleoptera); those produced by subspecies *galleriae* are specific to Coleoptera. Binding of the protein toxins disrupts the midgut cell membranes, inducing the insect to stop feeding and allowing germinating spores to invade the insect's hemocoel and proliferate, leading to a lethal septicemia.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

Acceptable methodologies for detection, isolation and enumeration of the active ingredient, *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502, were submitted by the applicant. The microbial pest control agent (MPCA) has been fully characterized with respect to its strain origins, natural occurrence and biological properties. Acceptable methods were provided for distinguishing the active ingredient from other subspecies of *Bacillus thuringiensis* but not for characterizing the active ingredient to the strain level.

2.2 Methods for Establishment of Purity of Seed Stock

Bacillus thuringiensis subsp. *galleriae* strain SDS-502 is deposited at the Laboratory of Microbial Industry and Technology, Institute of Industrial Science Technology, Ministry of International Trade and Industry (now Institute of Advanced Industrial Science and Technology,

Independent Administrative Institution) at AIST Tsukuba Central 6, 1-1, Higashi 1-Chome, Tsukuba-shi, Ibaraki-ken 305-8566 Japan under Accession No. FERM P-17979 and transferred to International Deposition under International Receipt No. FERM BP-7667.

The original deposit was subsequently converted to a deposit under the Budapest Treaty under accession number FERM P-17979.

The active ingredient provided to Phyllom LLC by SDS Biotech K.K. is the same genus, species, subspecies and strain as that described in Asano et al., 2003 and in US Patent No. US 6962977 B2. Working stocks have been propagated from the provided sample and stored at -80 °C.

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

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

The guarantees of the technical grade active ingredient, MA and end-use products are expressed as colony forming units (CFU)/g. The method for determining CFU counts was adequately described. Representative data on batches of MA and each of the end-use products were submitted for each manufacturing and formulating site.

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

Acceptable methods were provided for quantifying the active ingredient and for distinguishing the active ingredient from other subspecies of *Bacillus thuringiensis*.

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 Phyllom SDS-502 Technical, Phyllom SDS-502 MP and the end-use products are acceptable. These procedures include sterilization of all equipment and media as well as frequent sampling of the stock culture and production batches for purity and contamination.

Complete microbial contaminant analysis data were submitted for batches of Phyllom SDS-502 MP produced at each of the manufacturing sites using standard methods for detecting and enumerating microbial contaminants of concern. Additional microbial contaminant data were also submitted for batches of the end-use products produced at each of the formulating sites. The data demonstrated the absence of human pathogens and below-threshold levels of contaminating microorganisms in the MA and the end-use products. All batches of either the technical grade active ingredient or the MA must be screened for the microbial contaminants and conform to the limits set out in the Organisation for Economic Co-operation and Development issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43].

The absence of β-exotoxin and heat-labile exotoxins in either Phyllom SDS-502 MP or Phyllom SDS-502 Technical was confirmed using standard screening methods.

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

Storage stability data were provided for Phyllom SDS-502 MP, grubGONE! G and beetleGONE!. The storage stability of Phyllom SDS-502 MP and grubGONE! G was assessed at ambient temperature for a period of 17 months. The storage stability of beetleGONE! was assessed at ambient temperature for a period of 16 months.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

3.1.1 Testing

The PMRA conducted a detailed review of the toxicity and infectivity studies submitted in support of Phyllom SDS-502 Technical, Phyllom SDS-502 MP, grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!.

To address the health hazard requirements for the technical grade active ingredient, Phyllom SDS-502 Technical, an acute oral toxicity study, an acute pulmonary infectivity and toxicity study, an acute inhalation study, an acute intraperitoneal infectivity study, a dermal toxicity/irritation study, and an eye irritation study were submitted by the applicant.

In the acute oral toxicity study, groups of fasted, 9-week old, Sprague Dawley rats (12/sex) were given a single dose of Phyllom SDS-502 Technical (containing *Bacillus thuringiensis* subsp. *galleriae*, strain SDS-502 at 1.13×10^{10} CFU/g) in phosphate buffered saline by gavage at 2.2×10^{8} CFU/animal. The animals were then observed for a period of up to 21 days with interim scheduled sacrifices on Days 3, 7, and 14. There were no treatment related clinical signs, necropsy findings, changes in body weight, or mortalities.

In the acute pulmonary infectivity and toxicity study, groups of 5-week old, Sprague Dawley rats (17/sex) were given an intratracheal instillation of Phyllom SDS-502 Technical (containing *Bacillus thuringiensis* subsp. *galleriae*, strain SDS-502 at 1.13×10^{10} CFU/g) in phosphate buffered saline at doses of 1.0×10^{8} CFU/animal. Animals were then observed for up to 21 days. Treatment related observations of toxicity included transient body weight loss in 2 male and 3 female rats from the test group, observations of tan and/or red discoloured/mottled lung tissue loss in 3 male and 4 female rats from the test group at necropsy, and one test group female rat was found dead on Day 1. Although the test organism had not cleared completely from the lungs, kidney, brain, liver, spleen, and lymph node tissues and blood and caecum contents, a pattern of clearance was established by Day 21.

In the acute inhalation toxicity study, 2 groups of 5-week old, Sprague Dawley rats (5/sex) were exposed by the inhalation route to Phyllom SDS-502 Technical (containing *Bacillus thuringiensis* subsp. *galleriae*, strain SDS-502 at 6.17×10^{10} CFU/g) for 4 hours to nose only at a concentration of 4.30 mg/L. Animals were then observed for 14 days. There were no treatment related clinical signs, necropsy findings, changes in body weight, or mortalities.

In the acute intraperitoneal infectivity study, groups of 11-week old, Sprague Dawley rats (15/sex) were given a single intraperitoneal injection of Phyllom SDS-502 Technical (containing *Bacillus thuringiensis* subsp. *galleriae*, strain SDS-502 at 1.13×10^{10} CFU/g) in phosphate buffered saline at doses of 2.0×10^{7} CFU/animal. Animals were then observed for up to 21 days. There were no treatment related clinical signs, necropsy findings, changes in body weight, or mortalities. The blood, kidney, brain tissues, and caecum contents were completely cleared of the test organism. Although the test organism had not cleared completely from the lung, liver, spleen, and lymph node tissues, a pattern of clearance was established by Day 21.

In the acute dermal toxicity study, groups of 13-week old New Zealand White albino rabbits (5/sex) were dermally exposed to Phyllom SDS-502 Technical for 24 hours to an area of approximately 10% of body surface area at a dose of 2020 mg/kg body weight (bw). Following exposure, the animals were observed for a period of 14 days. There was very slight erythema on 5 rabbits on Day 1 and very slight erythema on 2 rabbits on Day 3. There were no other treatment related clinical signs, necropsy findings, changes in body weight, or mortalities. The MIS (Maximum Irritation Score) was 0.5/8 (at Day 1). In this study, Phyllom SDS-502 Technical was minimally irritating to the skin following acute exposure.

In the primary eye irritation study, 0.1 mL (31.0 mg) of Phyllom SDS-502 Technical was instilled into the conjunctival sac of the right eye of young adult New Zealand White albino rabbits (2♂; 1♀) Animals were then observed for 72 hours. Irritation was scored by the method of Draize. Iridic irritation was observed in two animals and conjunctival irritation was observed in all animals. All observed irritation had resolved by 72 hours. The MIS was 14.7/110 (at 1 hour). In this study, Phyllom SDS-502 Technical was minimally irritating to the eye following acute exposure.

To address the health hazard requirements for the end-use products, grubGONE! G and grubHALT!, the applicant submitted an acute dermal toxicity/irritation study and an acute eye irritation study. The substance used for testing, grubGONE! G, is considered toxicologically equivalent to grubHALT!.

In the acute dermal toxicity study, groups of 14-week old New Zealand White albino rabbits (5/sex) were dermally exposed to grubGONE! G for 24 hours to an area of approximately 10% of body surface area at a dose of 2020 mg/kg bw. Following exposure, the animals were observed for a period of 14 days. There was very slight to moderate erythema on 9 rabbits on Day 1 and very slight erythema on 4 rabbits on Day 4. All signs of irritation had resolved by Day 7. There were no treatment related clinical signs, necropsy findings, changes in body weight, or mortalities. The MIS was 1.9/8 (at Day 1). In this study, grubGONE! G was mildly irritating to the skin following acute exposure.

In the primary eye irritation study, 100 mg of grubGONE! G was instilled into the conjunctival sac of the right eye of young adult New Zealand White albino rabbits (2♂; 1♀) for 24 hours. Eyes were washed after 24 hours. Animals then were observed for 72 hours. Irritation was scored by the method of Draize.

Slight opacity of the cornea was observed in one animal, and some conjunctival irritation was observed in all animals. All observed irritation had resolved by 24 hours. The MIS was 4.7/110 (at 1 hour). In this study, grubGONE! G was minimally irritating to the eye following acute exposure.

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

3.1.2 Additional Information

Scientific rationales were provided to waive the requirements for acute dermal toxicity and dermal irritation for beetleGONE! and beetleJUS!. These scientific rationales were based on the lack of toxicity of Phyllom SDS-502 Technical by the dermal route and an assessment of the formulation ingredients in beetleGONE! and beetleJUS!. The requests to waive these requirements were accepted.

Although skin and eye irritation, and dermal toxicity were not addressed for Phyllom SDS-502 MP, this MA is not expected to be irritating to the skin or eyes, or toxic via the dermal route since Phyllom SDS-502 Technical, which is contained in the MA, is not a skin or eye irritant and it is not formulated with ingredients that are classified as being irritating to the skin or eyes or toxic via the dermal route.

Although data are not required to address eye irritation, beetleGONE! and beetleJUS! are not expected to be irritating to the eyes since Phyllom SDS-502 Technical, which is contained in the end-use products, is not an eye irritant and they are not formulated with substances that are classified as being irritating to the eye.

A survey of the published scientific literature conducted by the World Health Organization in 1999 has revealed that although *Bacillus thuringiensis* products have been used for a long period of time, *Bacillus thuringiensis* has been isolated in only a few cases of human bacterial infection:

a) A farm worker was splashed with a commercial *Bacillus thuringiensis* subsp. *kurstaki* product and developed a corneal ulcer in one eye. *Bacillus thuringiensis* was subsequently isolated from the affected eye. The eye condition was resolved after treatment with a topical antibiotic and corticosteroid. The possibility that *Bacillus thuringiensis* may have been a non-pathogenic contaminant of the ulcer was not considered. Another worker presented with conjunctivitis after being splashed with a *Bacillus thuringiensis* product.

b) During the investigation of a gastroenteritis outbreak in a chronic care institution, bacteria were isolated from four individuals and were identified as *Bacillus thuringiensis*. The *Bacillus thuringiensis* isolates showed cytotoxic effects characteristic of *Bacillus cereus*.

c) *Bacillus thuringiensis* was isolated from burn wounds in two patients. None of the isolates showed any toxicity to Vero cells.
d) *Bacillus thuringiensis* was isolated from a war wound. The strain (*Bacillus thuringiensis* subsp. *konkukian*) could infect immunosuppressed mice after cutaneous application.
e) A research worker developed a marked local reaction and lymphadenitis following a needle stick injury when handling *Bacillus thuringiensis* subsp. *israelensis*. *Acinetobacter calcoaceticus* and *Bacillus thuringiensis* were cultured from the exudate.
f) *Bacillus thuringiensis* was isolated from the body fluids of 55 patients with different infectious diseases. In 52 of the patients, *Bacillus thuringiensis* was considered a contaminant, while in 3 patients with pre-existing medical conditions, no firm conclusion was established concerning a causal relationship between the infection and *Bacillus thuringiensis*.

Although *Bacillus anthracis* and *Bacillus cereus* are known to be pathogenic to humans and other animals and are closely related to *Bacillus thuringiensis*, *Bacillus thuringiensis* can be distinguished from *Bacillus anthracis* and *Bacillus cereus* by its production of insecticidal crystal proteins.

Also, a search in the PubMed database using "galleriae" as a keyword found no reports of adverse effects in human or animals. Although another search in the PubMed database using "thuringiensis" and "infection" as a keyword revealed some reports including periorbital cellulitis, nosocomial bacteremia in patients with underlying diseases, gastroenteritis and corneal ulcer, the number of reports are quite rare despite the extensive use of products containing *Bacillus thuringiensis*.

Bacillus thuringiensis is closely related to *Bacillus cereus*, the only known difference between the species being the production of the insecticidal crystal proteins. Some strains of *Bacillus cereus* can cause food poisoning and their pathogenic effects are caused by metabolites and are manifested as one of two types of foodborne poisoning:

i. Vomiting which is caused by the ingestion of a heat-stable toxin which consists of a cyclic peptide; and

ii. Diarrhea which is caused by heat-labile enterotoxins.

Given the close relationship between *Bacillus thuringiensis* and *Bacillus cereus*, new subspecies and strains of *Bacillus thuringiensis* must normally be screened for the presence of *Bacillus cereus*-like enterotoxins. *Bacillus cereus*-like enterotoxins have been implicated in cases of food poisoning. The end-use products, however, are not intended for use on food crops. Therefore, screening for *Bacillus cereus*-like enterotoxins is not required for the proposed uses of the end-use products.

Hypersensitivity testing with the technical grade active ingredient was not performed. To date there have been no reports of hypersensitivity during manufacturing of Phyllom SDS-502 Technical. All hypersensitivity incidents will be reported.

3.1.3 Incident Reports Related to Human and Animal Health

As of 9 July 2018, no incident reports for *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 had been submitted to the PMRA, and there were 59 human and 3 domestic animal incident reports for other subspecies of *Bacillus thuringiensis*. Almost all of the reported incidents involved *Bacillus thuringiensis* Berliner (subsp. *kurstaki*, strain HD-1), with one human and one domestic animal incident each involving a product containing Bacillus thuringiensis (serotype H-14).

In 37 of the human incidents, there was some degree of association between the reported symptoms and the suspected pesticide exposure. In the four human cases, individuals exposed in an occupational setting reported minor skin effects. In 34 of the human incidents, 56 individuals reported symptoms following aerial application of products containing *Bacillus thuringiensis* Berliner. Details surrounding their exposures were not provided; rather, the reports for the most part reported that the spraying had occurred and the individuals were experiencing adverse effects. Individuals reported mostly minor respiratory symptoms such as cough, nasal congestion, sore throat, and shortness of breath, with a few individuals reporting asthma. In a few cases, lethargy, headache, and skin and eye irritation were also reported.

Two minor domestic animal incidents were considered to be associated with exposure to *Bacillus thuringiensis*. In one incident, a dog was suspected to have eaten some product, and was lethargic and lost its appetite. In the second incident, a dog was reported to have had an asthma attack following an aerial application.

Based on the mostly minor effects reported, and the uncertainty around the degree of exposure that occurred in human incidents, no additional mitigation measures are recommended based on the incident report review.

3.1.4 Hazard Analysis

The data package submitted in support of registering Phyllom SDS-502 Technical, Phyllom SDS-502 MP, grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS! was reviewed from the viewpoint of human health and safety and was determined to be acceptable.

Based on all the available information, the technical grade active ingredient, Phyllom SDS-502 Technical, is of low toxicity by the oral, inhalation, and dermal routes, and was not pathogenic or infective by the pulmonary or intraperitoneal routes. Some toxicity, however, was observed by the pulmonary route. In irritation studies, the technical grade active ingredient was not irritating to the skin and minimally irritating to the eyes. Also, the MPCA is considered to be a potential sensitizer. Consequently, the hazard statement “POTENTIAL SENSITIZER” will appear on the principal display panel of the technical grade active ingredient. The statement, “May cause sensitization. Avoid inhaling/breathing spray mist (or dust).” is also required on the secondary display panel of the label under the "PRECAUTIONS" section.

Phyllom SDS-502 MP, beetleGONE!, and beetleJUS! are not toxic via the dermal route, are minimally irritating to the skin and minimally irritating to the eyes. GrubGONE! G, and grubHALT! are mildly irritating to the skin and minimally irritating to the eyes and not toxic via the dermal route.

As these formulations contain an MPCA, the hazard statement "POTENTIAL SENSITIZER" will appear on the principal display panels of each MA and end-use product label. The statement, "May cause sensitization. Avoid contact with skin and clothing. Avoid inhaling/breathing spray mist (or dust)." is also required on the secondary display panels of each end-use product label under the "PRECAUTIONS" section.

Additionally, since grubGONE! G and grubHALT! are mildly irritating to the skin, the hazard statement “CAUTION – SKIN IRRITANT” will appear on the principal display panels of these end-use products and the statement “May irritate the skin” is also required on the secondary display panels of each end-use product label under the "PRECAUTIONS" section.

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

Within the available scientific literature, there are no reports that suggest *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 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 this MPCA.

3.2 Occupational, Residential and Bystander Risk Assessment

3.2.1 Occupational Exposure and Risk

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and other handlers exists, with the primary exposure route being dermal. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Bacillus thuringiensis* has not frequently been identified as a dermal wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Furthermore, toxicity testing with the technical grade active ingredient, Phyllom SDS-502 Technical, showed no toxicity via the oral, inhalation and dermal routes, and no signs of infectivity or pathogenicity via the pulmonary or intraperitoneal injection routes. GrubGONE! G, grubHALT!, beetleGONE!, and beetleJUS! are not eye irritants. BeetleGONE!, and beetleJUS! are not dermal irritants. However, grubHALT!, grubGONE! G are mildly irritating to the skin and PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing.

Risk mitigation measures for the commercial class end-use products, grubGONE! G and beetleGONE!, such as personal protective equipment, including waterproof gloves, a long-sleeved shirt, long pants, a NIOSH-approved particulate filtering facepiece respirator with any N, R or P filter, and shoes with socks are required to minimize exposure and protect applicators, mixer/loaders, and handlers that are likely to be exposed. In addition, all unprotected workers that are likely to be exposed are prohibited from entering treated areas where grubGONE! G and beetleGONE!, has been applied for 4 hours or until the sprays have dried or dusts have settled.

Risk mitigation measures for the domestic class end-use products, grubHALT! and beetleJUS!, include a label statement prohibiting re-entry into treated areas where grubGONE! G and BeetleGONE!, has been applied for 4 hours or until the sprays have dried or dusts have settled.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of beetleGONE!, beetleJUS!, grubGONE! G and grubHALT! and no significant occupational risks are anticipated for this product.

3.2.2 Residential and Bystander Exposure and Risk

Overall, the PMRA does not expect that residential and bystander exposures will pose a health risk of concern on the basis of the low toxicity profile for beetleGONE!, beetleJUS!, grubGONE! G and grubHALT!, the low infectivity/pathogenicity profile for Phyllom SDS-502 Technical and the expectation that precautionary label statements will be followed by Commercial and Domestic users of beetleGONE!, beetleJUS!, grubGONE! G and grubHALT!. Although the labels for the end-use products allow for application to turf, dermal and inhalation exposure to the general population is low since entry into the treatment sites (turf, residential and recreational areas) is to be prohibited for 4 hours or until the sprays have dried or dusts have settled. As well, *Bacillus thuringiensis* is a species that is common in the environment and the use of beetleGONE!, beetleJUS!, grubGONE! G and grubHALT! is not expected to cause sustained increases in exposure to bystanders beyond natural levels. Consequently, a health risk to infants and children is not expected.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

The proposed use pattern excludes application to food or feed crops and therefore is not expected to result in dietary exposure. Consequently, there is no health risk of concern for the general population, including infants and children, or animals. Also, *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 demonstrated no pathogenicity or infectivity in Tier I acute pulmonary (intratracheal) and intraperitoneal injection studies; and no oral toxicity in the acute toxicity study.

3.3.2 Drinking Water

Health risks are not expected from exposure to *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 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. The

beetleGONE!, beetleJUS!, grubGONE! G and grubHALT! label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Furthermore, municipal treatment of drinking water is expected to reduce the transfer of residues to drinking water.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses and acceptable daily intakes are not usually possible for predicting acute and long-term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (in other words, no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 is of low oral toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCAs than the general population. Thus, there are no threshold effects of concern and, as a result, there is no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCA and other registered microorganisms that have a common mechanism of toxicity, does not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 to human health.

3.3.4 Aggregate Exposure and Risk

Based on the toxicity and infectivity test data and other relevant information in the PMRA's files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 to the general Canadian population, including infants and children, when the end-use product is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Dermal and inhalation exposure to the general public will be low since the product labels include statements limiting bystander exposure following use on turf, residential or recreational areas. Furthermore, the label will include mitigation measures to reduce spray drift and few adverse effects from exposure to other strains of *Bacillus thuringiensis* encountered in the environment have been reported in the public literature. Even if there is an increase in exposure to *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 from the use of beetleGONE!, beetleJUS!, grubGONE! G and grubHALT!, 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 under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada specifies science-based maximum residue limits to ensure the food Canadians eat is safe.

Residues of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 on food crops are not anticipated following application since the use pattern excludes application to food or feed crops. Therefore, the PMRA has determined that specification of a maximum residue limit under the *Pest Control Products Act* is not required for *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502.

3.4 Cumulative Effects

The *Pest Control Products Act* requires that the PMRA consider the cumulative exposure to pesticides with a common mechanism of toxicity. In its assessment of common mechanism of toxicity, the PMRA considers both the taxonomy of MPCAs and the production of any potentially toxic metabolites. For the current evaluation, the PMRA has determined that *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 shares a common mechanism of toxicity with other strains of *Bacillus thuringiensis* and *Bacillus mycoides* that are registered for use in Canada; *Bacillus thuringiensis* subsp. *tenebrionis*, *Bacillus thuringiensis* subsp. *israelensis*, *Bacillus thuringiensis* subsp. *kurstaki*, *Bacillus thuringiensis* subsp. aizawai, Strain ABTS-1857, *Bacillus mycoides* isolate J, and *Bacillus sphaericus* strain 2362. The potential health risks from cumulative exposure of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 and these other registered MPCAs are not of concern when used as labelled given their low toxicity and pathogenicity.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

No studies were submitted to address the environmental fate and behaviour of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502; however, environmental fate data (Tier II/III) are not normally required at Tier I, and are only triggered if significant toxicological effects in non-target organisms are noted in Tier I testing.

Bacillus species are commonly found in soil and in plant litter where they play an important role in the biological cycling of carbon and nitrogen. Under unfavourable growth conditions, this species can undergo sporulation and create a resilient endospore that can endure many adverse environmental conditions.

While the application of the end-use products, grubHALT!, beetleJUS!, beetleGONE!, and grubGONE! G, is expected to temporarily increase natural populations of *Bacillus thuringiensis* in outdoor terrestrial or aquatic environments, the levels of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 are expected to return to naturally sustainable levels.

The end-use products are not intended to be applied directly to water. As a result, exposure to aquatic environments should be low and limited to run-off after application. While *Bacillus thuringiensis* is not considered an aquatic species and is not expected to grow in this environment, the endospores of this microorganism are likely to persist in sediment. The application of grubHALT!, beetleJUS!, beetleGONE!, and grubGONE! G is not expected to significantly increase the overall environmental levels of this species in sediment above naturally occurring levels. As noted previously, any localized increases of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 in aquatic environments are expected to return to naturally sustainable levels over time.

4.2 Effects on Non-Target Species

PMRA has a four-tiered approach to environmental testing of microbial pesticides. Tier I studies consist of acute studies on up to seven broad taxonomic groups of non-target organisms exposed to a maximum hazard or Maximum Challenge Concentration of the MPCA. The Maximum Challenge Concentration is generally derived from the amount of the MPCA, or its toxin, expected to be available following application at the maximum recommended label rate multiplied by a safety factor. Tier II studies consist of environmental fate (persistence and dispersal) studies as well as additional acute toxicity testing of MPCAs. Tier III studies consist of chronic toxicity studies (in other words, 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 estimated environmental concentrations. The absence of adverse effects in Tier I studies are interpreted as minimal risk to the group of non-target organisms. However, higher tiered studies will be triggered if significant adverse effects on non-target organisms are identified in Tier I studies. These studies provide additional information that allows the PMRA to refine the environmental risk assessments. In the absence of adequate environmental fate and/or field studies, a screening level risk assessment can be performed to determine if the MPCA is likely to pose a risk to a group of non-target organisms.

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

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

4.2.1 Effects on Terrestrial Organisms

Several in vivo studies and other assays were submitted to address the hazards of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 to birds, honey bees and other non-target terrestrial arthropods. Scientific rationales were also submitted in support of a waiver for avian inhalation and terrestrial plants. Data submitted under human and animal health toxicity testing were considered to assess the risk of harm to wild mammals.

The acute oral toxicity/pathogenicity of Phyllom SDS-502 Technical (containing at least 1.0×10^{10} CFU of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502/g) to 21-day-old Mallard ducks (*Anas platyrhynchos*) was assessed over 34 days. Phyllom SDS-502 Technical was administered to the birds in two treatment groups (10 per treatment) by gavage at an average daily dose of 3.6×10^{10} CFU (3600 mg)/kg/day. There were no treatment related toxicity effects or signs of pathogenicity observed.

In a 20-day dietary toxicity study, honey bee larvae (80/test concentration) were exposed to SDS-502 Cry8Da protein toxin (extracted from a culture of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502) via the diet at a dose of 500 ng and 250 ng in 10µL of 30% sucrose solution. Doses were administered to larvae by placing the 10 µL dose in the brood cell, adjacent to the larvae. Bees were observed for emergence (in other words, mortality). There were no significant effects on emergence rates in test bees.

In a 21-day dietary toxicity study, honey bee larvae (*A. mellifera*) were exposed to Phyllom SDS-502 MP (containing 2.00×10^{10} CFU/g of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502) via 10 µL dietary doses at concentrations of 0.00146 g/mL, 0.01825 g/mL and 0.07300 g/mL (14.6 µg, 182.5 µg, and 730 µg or 2.92×10^{5}, 3.65×10^{6}, and 1.46×10^{7} CFU/bee larva). Doses were administered to larvae by placing the 10 µL dose in the brood cell, adjacent to the larvae. Honey bee larvae were observed for emergence (in other words, mortality). There were no treatment related effects on mortality in the test groups.

In a 9-day dietary toxicity study, ladybird beetles (*Hippodamia convergens*) were exposed to Phyllom SDS-502 Technical (containing 2.09×10^{11} CFU/g of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502) via the diet in a 30% honey solution at concentrations of 170, 850 and 1700 ppm. There were no treatment related behavioural abnormalities. There were no significant differences in mortality between the test groups and control groups.

A series of assays was submitted where four species of parasitic hymenopteran wasps (*Spathius agrili*, *Tetrastichus planipennis*, *Oobius agrili*, and *Atanycolus hicoriae*); the Colorado potato beetle (*Leptinotarsa decemlineata*); the spotted cucumber beetle (*Diabrotica undecimpunctata howardi*) were fed a diet spiked with purified SDS-502 Cry8Da protein toxin. Wasps, Colorado

potato beetles, and spotted cucumber beetles were fed SDS-502 Cry8Da protein toxin at concentrations of 4000 ppm, 5000 ppm, and 5000 ppm, respectively. No signs of toxicity or other adverse effects were observed in any of the assays.

Although not required, a scientific rationale to waive inhalation testing on birds was submitted. The rationale was based on the fact that Phyllom SDS-502 Technical was not toxic or pathogenicity to ducks exposed by the oral route.

A scientific rationale to waive testing on terrestrial plants was also submitted. This rationale was based on the known biological properties of *Bacillus thuringiensis*. This species is neither a plant pathogen nor is it related to any plant pathogens and a review of the published scientific literature on *Bacillus thuringiensis* and its byproducts indicate no known detrimental effects on plant life.

A search in the PubMed database (using the keywords “galleriae” and “toxicity”, and “galleriae” and “pathogenicity” found no reports of adverse effects to terrestrial non-target organisms from natural populations of *Bacillus thuringiensis* subsp. *galleriae*.

Based on all the available information on the effects of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 to terrestrial non-target organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, terrestrial arthropods, non-arthropod invertebrates, terrestrial plants or to other non-target microorganisms from the proposed use of grubHALT!, beetleJUS!, beetleGONE!, and grubGONE! G.

Test results are summarized in Appendix I, Table 3, 4 and 5.

4.2.2 Effects on Aquatic Organisms

An *in vivo* laboratory study and a scientific rationale were submitted to address the hazards of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 to daphnids and freshwater fish, respectively.

In a 21-day toxicity/pathogenicity study, groups (20/group) of daphnia (*Daphnia magna*) were exposed to Phyllom SDS-502 Technical at concentrations of 1, 10, 50, and 100 mg/L (equivalent to 2.1×10^7, 2.1×10^8, 1.0×10^9, and 2.1×10^9 CFU of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502/L) under static renewal conditions. Daphnia were observed for survival, reproduction, and behavioural abnormalities. There were no observed effects on survival. However, the mean neonate production per adult value in the highest test group (100 mg/L) was significantly lower than the control group. The 21-day EC_{50} was greater than 100 mg/L (2.1×10^9 CFU/L). The no observed effect concentration value, based on neonate production, was 50 mg/L (1.0×10^9 CFU/L). Since the no observed effect concentration is greater than the exposure estimate, the screening level risk quotient is below the level of concern.

The scientific rationale and supporting published scientific literature submitted by the applicant to address the freshwater fish data requirement for *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 was sufficient to waive the requirement for further testing. The rationale was based on

a history of use and testing of closely related *Bacillus thuringiensis* species, strains, and Cry proteins showing no significant adverse effects in freshwater fish.

A search in the PubMed database using the keywords “galleriae” and “toxicity”, and “galleriae” and “pathogenicity” found no reports of adverse effects to aquatic non-target organisms from natural populations of *Bacillus thuringiensis* subsp. *galleriae*.

Based on all the available information on the effects of *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502 to aquatic non-target organisms, there is reasonable certainty that no harm will be caused to fish, aquatic arthropods, aquatic non-arthropod invertebrates or aquatic plants from the use of grubHALT!, beetleJUS!, beetleGONE!, and grubGONE! G.

Test results are summarized in Appendix I, Table 3.

4.3 Incident Reports related to the Environment

There are no environmental incident reports in the PMRA database for the proposed active ingredient *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502. As of 9 July 2018 there were two incident reports for the related active ingredient *Bacillus thuringiensis* Berliner (subsp. *kurstaki*, strain HD-1).

In one major incident a reported fish kill following a fire at a warehouse where *Bacillus thuringiensis* was stored was considered to be unrelated to the active ingredient. In one minor incident drooping leaves were observed on one cottonwood, one alder, two dogwoods, and some annual plants following drift from the application site.

5.0 Value

Bacillus thuringiensis subsp. *galleriae* strain SDS-502 provides a new mode of action for control of certain beetle pests of turf and ornamental plants. Registered alternatives for the uses of grubGONE! and beetleGONE! products are limited to conventional chemical insecticides from mode of action groups 1, 3, 4 or 28. There are only three or fewer different mode of action groups for any given use, so any new products will have particular importance for resistance management, and for some uses there are no alternatives registered.

There are no reports of resistance to *Bacillus thuringiensis* subsp. *galleriae* in the Arthropod Pesticide Resistance Database. However, there are numerous reports of resistance to other Group 11 insecticides, mostly in Lepidoptera but including single reports of resistance to *Bacillus popilliae* in Japanese beetle and oriental beetle. Therefore, resistance management may be a concern for grubGONE! and beetleGONE! products and resistance management recommendations are included on the labels of both commercial class products.

Support for registration of grubGONE! and beetleGONE! products was provided by results from 7 laboratory trials and 20 field trials testing the products against larvae of annual bluegrass weevil, Asiatic garden beetle, green June beetle, European chafer, Japanese beetle, May or June beetle, northern masked chafer, oriental beetle, and southern masked chafer, and adults of Japanese beetle. The weight of evidence provided by these trials was sufficient to support label

claims for all of the listed species and, along with consideration of biology and feeding habits, extend those claims to include larvae of black turfgrass ataenius and adults of Asiatic garden beetle, oriental beetle, and rose chafer.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The TSMP is a federal government policy developed to provide direction on the management of substances of concern that are released into the environment. The TSMP calls for the virtual elimination of Track 1 substances, in other words, those that meet all four criteria outlined in the policy: persistent (in air, soil, water and/or sediment), bio-accumulative, primarily a result of human activity and toxic as defined by the *Canadian Environmental Protection Act*. The *Pest Control Products Act* requires that the TSMP be given effect in evaluating the risks of a product.

During the review process, Phyllom SDS-502 Technical was assessed in accordance with the PMRA Regulatory Directive DIR99-03[6] and evaluated against the Track 1 criteria. Phyllom

SDS-502 Technical, its manufacturing concentrate, Phyllom SDS-502 MP, and end-use products, grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS! did not meet TSMP criteria.

6.2 Formulants and Contaminants of Health Concern

During the review process, contaminants in the technical as well as formulants and contaminants in the manufacturing concentrate and end-use products are compared against the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.*[7] The list is used as described in the PMRA Notice of Intent NOI2005-013[8] and is based on existing policies and regulations including DIR99-03 and DIR2006-024,[9] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act*, 1999 (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

- Technical grade Phyllom SDS-502 Technical and its manufacturing concentrate and end-use products do not contain formulants or contaminants identified in the *List of Pest Control Product Formulants and Contaminants 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.

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

7 SI/2005-114

8 NOI2005-01, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.*

9 DIR-2006-02, *Formulants Policy and Implementation Guidance Document.*

7.0 Summary

7.1 Methods for Analysis of the Microorganism as Manufactured

The product characterization data for Phyllom SDS-502 Technical, Phyllom SDS-502 MP, grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS! were judged to be adequate to assess their potential human health and environmental risks. The technical grade active ingredient was adequately characterized and the specifications of the technical grade active ingredient, MA and end-use products were supported by the analyses of a sufficient number of batches. All batches of either the technical grade active ingredient or the MA must be screened for the microbial contaminants and conform to the limits set out in the Organisation for Economic Co-operation and Development issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43] before being released for sale or formulation.

Storage stability data for Phyllom SDS-502 MP, grubGONE! G and grubHALT! support storage at ambient temperatures (4–25 °C) for up to 17 months. For beetleGONE! and beetleJUS! (4–25°C), storage stability data support storage at ambient temperatures for up to 16 months.

7.2 Human Health and Safety

The acute toxicity and infectivity studies and other relevant information submitted in support of Phyllom SDS-502 Technical, Phyllom SDS-502 MP, grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS! were determined to be acceptable. Based on all the available information, the technical grade active ingredient, Phyllom SDS-502 Technical, is of low toxicity by the oral, inhalation, and dermal routes, and was not pathogenic or infective by the pulmonary and intraperitoneal routes. The technical grade active ingredient is not irritating to the skin or eyes, and is a potential sensitizer. The signal words, “POTENTIAL SENSITIZER” are required on the principal display panel of the technical grade active ingredient as well as the precautionary statements: “May cause sensitization. Avoid inhaling/breathing spray mist or dust”.

Phyllom SDS-502 MP, beetleGONE!, and beetleJUS! are not toxic via the dermal route, are minimally irritating to the skin and minimally irritating to the eyes. GrubGONE! G, and grubHALT! are mildly irritating to the skin and minimally irritating to the eyes and not toxic via the dermal route. The signal words ‘POTENTIAL SENSITIZER’ are required on the principal display panel of the MA and end-use products, as well as the precautionary statements: “May cause sensitization. Avoid inhaling/breathing spray mist or dust.” Additionally, the signal words “CAUTION – SKIN IRRITANT” are required on the principal display panels of the grubGONE! G, and grubHALT! labels as well as the precautionary statement “May irritate the skin”.

When handled according to prescribed label instructions, the potential for dermal, eye and inhalation exposure for mixer/loaders, applicators, and handlers exists, with the primary source of exposure to workers being dermal. Respiratory and dermal sensitivity could possibly develop upon repeated exposure to the product since all microorganisms, including this MPCA, contain substances that are potential sensitizers. Therefore, commercial workers handling or applying grubGONE! G and beetleGONE! must wear waterproof gloves, a long-sleeved shirt, long pants, a NIOSH-approved particulate filtering facepiece respirator, and shoes with socks to minimize

exposure and protect applicators, mixer/loaders, and handlers that are likely to be exposed. In addition, all unprotected commercial workers, residential users, and bystanders are prohibited from entering treated areas where grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS! has been applied for 4 hours or until the sprays have dried or dusts have settled.

A health risk to the general population, including infants and children, as a result of bystander exposure is not expected due to the low toxicity/pathogenicity profile for *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502, Phyllom SDS-502 Technical, Phyllom SDS-502 MP, grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!. The specification of a maximum residue limit under the *Pest Control Products Act* is not required for *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502.

7.3 Environmental Risk

The non-target organism tests, scientific rationales and supporting published scientific literature submitted in support of Phyllom SDS-502 Technical and its associated MA and end-use products, Phyllom SDS-502 MP, grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!, were determined to be acceptable. The application of grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!, containing *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502, 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 end-use product labels will include environmental precaution statements to reduce contamination of aquatic systems from the use of grubGONE! G, grubHALT!, beetleGONE!, and beetleJUS!. The commercial end-use product labels for grubGONE! G and beetleGONE! will also include a standard environmental precaution statement to prohibit aerial application.

7.4 Value

The grubGONE! and beetleGONE! products provide a new mode of action for control of certain beetle pests of turf and ornamental plants, which may aid in management of resistance to the few conventional chemicals registered for these uses.

8.0 Proposed Regulatory Decision

Health Canada's Pest Management Regulatory Agency, under the authority of the *Pest Control Products Act* and Regulations, is proposing registration for the sale and use of Phyllom SDS-502 Technical (containing *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502) and the end-use products grubGONE! G, grubHALT!, beetleGONE!, beetleJUS! containing the technical grade active ingredient *Bacillus thuringiensis* subsp. *galleriae* strain SDS-502, to control specific beetle pests of turf and ornamental plants.

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

List of Abbreviations

µg	micrograms
bw	body weight
cm	centimetres
EC_{50}	effective concentration on 50% of the population
g	gram
ha	hectare(s)
kg	kilogram
km	kilometre
L	litre
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
MA	Manufacturing Concentrate
mg	milligram
MIS	Maximum Irritation Score
mL	millilitre
Ng	nanogram
PMRA	Pest Management Regulatory Agency
ppm	parts per million
TSMP	Toxic Substances Management Policy

Appendix I Tables and Figures

Table 1 Toxicity Profile of Phyllom SDS-502 Technical (technical grade active ingredient)

Study Type/Animal/PMRA #	Study Results
21-day Acute Oral Toxicity Sprague Dawley rat PMRA No. 2645706	There was no mortality in any group during the study. There were no treatment related clinical signs, necropsy findings, or changes in body weight. The 21-day acute oral LD_{50} is greater than 2.2×10^8 CFU/animal. The technical grade active ingredient was not toxic when instilled at 2.2×10^8 CFU/animal.
21-day Acute Pulmonary Infectivity and Toxicity Sprague Dawley rat PMRA No. 2645707	One test group female rat was found dead on Day 1. There was transient body weight loss in 2 male and 3 female rats from the test group. There were observations of tan and/or red discoloured/mottled lung tissue loss in 3 male and 4 female rats from the test group. A pattern of clearance was achieved by Day 21. The 21-day acute pulmonary LD_{50} is greater than 1.0×10^8 CFU/animal. The technical grade active ingredient was not infective or pathogenic via intratracheal instillation at 1.0×10^8 CFU/animal. There was some toxicity observed.
14-day Acute Inhalation Toxicity Sprague Dawley rat PMRA No. 2645707	There were no treatment related clinical signs, necropsy findings, changes in body weight, or mortalities. The 14-day inhalation LC_{50} is greater than 4.30 mg/L. The technical grade active ingredient was not toxic following inhalation at 4.30 mg/L.
21-day Acute Intraperitoneal Infectivity Sprague Dawley rat PMRA No. 2645708	There were no treatment related clinical signs, necropsy findings, changes in body weight, or mortalities. A pattern of clearance was established by Day 21. The technical grade active ingredient was not infective or pathogenic via intraperitoneal injection at 2.0×10^7 CFU/animal.
14-day Acute Dermal Toxicity/Irritation New Zealand White rabbit PMRA No. 2645709	There was very slight erythema on 5 rabbits on Day 1 and very slight erythema irritation on 2 rabbits on Day 3. There were no treatment related clinical signs, necropsy findings, changes in body weight, or mortalities. The calculated MIS was 0.5/8 at Day 1.

Study Type/Animal/PMRA #	Study Results
	The 14-day dermal LD_{50} is greater than 2020 mg/kg bw. The technical grade active ingredient was not toxic when applied to the skin but was minimally irritating.
76-hour Primary Eye Irritation New Zealand White rabbit PMRA No. 2645710	Iridic irritation was observed in two animals and conjunctival irritation was observed in all animals. All observed irritation resolved by 72h. The calculated MIS was 14.7/110 at 1 hour. The technical grade active ingredient was minimally irritating to the eyes.

Table 2 Toxicity Profile of grubGONE! G; grubHALT! (end-use products)

Study Type/Animal/PMRA#	Study Results
14-day Acute Dermal Toxicity/Irritation New Zealand White rabbit PMRA No. 2645789	There was very slight to moderate erythema on 9 rabbits on Day 1 and very slight erythema irritation on 4 rabbits on Day 4. All signs of irritation resolved by Day 7. The calculated MIS was 1.9/8 at Day 1. The dermal LD_{50} is greater than 2020 mg/kg bw. grubGONE! G and grubHALT! were not toxic when applied to the skin but were slightly irritating.
76-hour Primary Eye Irritation New Zealand White rabbit PMRA No. 2897266	Slight opacity of the cornea was observed in one animal, and some conjunctival irritation was observed in all animals. All observed irritation resolved by 24h. The calculated MIS was 4.7/110 at 1 hour. grubGONE! G and grubHALT! were minimally irritating to the eyes.

Table 3 Toxicity/Pathogenicity of Phyllom SDS-502 Technical (technical grade active ingredient) to Non-Target Species

Organism	Exposure	Significant Effect, Comments	Reference
Terrestrial Organisms			
Vertebrates			
Birds			
Mallard duck	5-day – Dietary	There were no treatment related toxicity effects	PMRA

Organism	Exposure	Significant Effect, Comments	Reference
(*Anas platyrhynchos*), 21-day-old	exposure	or signs of pathogenicity observed. The 34-day acute oral LD_{50} of Phyllom SDS-502 Technical to the duck was greater than 3.6×10^{10} CFU (3600 mg)/kg/day. **LOW TOXICITY** **NOT PATHOGENIC**	2645715
Invertebrates			
Arthropods			
Ladybird beetle (*Hippodamia convergens*), adult	170, 850 and 1700 ppm – Dietary exposure	There were no treatment related behavioural abnormalities. There were no significant differences in mortality between the test groups and control groups. The 9-day dietary LC_{50} and the no observed effect concentration was greater than 1700 ppm. **LOW TOXICITY**	PMRA 2894591
Aquatic Organisms			
Invertebrates			
Arthropod			
Daphnids (*Daphnia magna*)	21-day – Aquatic exposure (static renewal conditions)	There was no effect on survival. Mean neonate production per adult values in the untreated control, 1 mg/L, 10 mg/L, 50 mg/L, and 100 mg/L groups were 273, 236, 265, 244, and 144 respectively. The 21-day EC_{50} was greater than 100 mg/L (2.1×10^9 CFU/L). The no observed effect concentration value, based on neonate production, was 50 mg/L (1.0×10^9 CFU/L). **LOW TOXICITY** **NOT PATHOGENIC**	PMRA 2645721

Table 4 Toxicity/Pathogenicity of Phyllom SDS-502 MP (Manufacturing Concentrate) to Non-Target Species

Organism	Exposure	Significant Effect, Comments	Reference
Terrestrial Organisms			
Invertebrates			
Arthropods			

Organism	Exposure	Significant Effect, Comments	Reference
Honeybees (*Apis mellifera*), young larvae	14.6 µg, 182.5 µg, and 730 µg (2.92×10^5, 3.65×10^6, and 1.46×10^7 CFU) per bee larva – Dietary exposure	There was no effect on emergence (in other words, mortality). The dietary 21-day LD_{50} was greater than 730 µg/bee larva. **LOW TOXICITY** **NOT PATHOGENIC**	PMRA 2886812

Table 5 Toxicity of SDS-502 Cry8Da protein toxin to Non-Target Species

Organism	Exposure	Significant Effect, Comments	Reference
Terrestrial Organisms			
Invertebrates			
Arthropods			
Honeybees (*Apis mellifera*), young larvae	500 and 250 ng per bee larva – Dietary exposure	There was no effect on emergence (in other words, mortality). The dietary 21-day LD_{50} was greater than 500 ng/bee larva. **LOW TOXICITY**	PMRA 2886812

Appendix II Estimated Environmental Concentration

Aquatic

The maximum application rate of grubGONE!-G and grubGONE!-G DOM is 168 kg/ha or 1.68×10^{14} CFU/ha, and the maximum rate of beetleGONE! and beetleJUS! is 19.5 kg/ha or 1.66×10^{14} CFU/ha . Therefore, assuming that the maximum application rate (1.68×10^{14} CFU/ha) were applied to surface water, the aquatic estimated environmental concentration would be 1.11×10^{8} CFU/L.

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4.0 Value