Proposed Registration Decision PRD2015-13

Bacillus amyloliquefaciens strain D747

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Overview

Proposed Registration Decision for *Bacillus amyloliquefaciens* strain D747

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 *Bacillus amyloliquefaciens* strain D747 Technical, Double Nickel 55 and Double Nickel LC, containing the technical grade active ingredient *B. amyloliquefaciens* strain D747, to suppress or partially suppress a variety of fungal or bacterial diseases on cucurbits, fruiting vegetables, grapes, lettuce, pome fruit, potato, soybean and strawberry.

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 *B. amyloliquefaciens* strain D747 Technical, Double Nickel 55 and Double Nickel LC fungicides.

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 (for example, those most sensitive to environmental contaminants). 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.

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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 *B. amyloliquefaciens* strain D747, 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 *B. amyloliquefaciens* strain D747, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA 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 amyloliquefaciens strain D747?

The active ingredient, *B. amyloliquefaciens* strain D747, is a naturally occurring bacterium that is found in close association with roots, leaves and other plant parts. It is reported to prevent the establishment of disease-causing fungi and bacteria by rapidly colonizing plant surfaces. *B. amyloliquefaciens* strain D747 along with granular and liquid formulated end-use products have been registered in the United States since 2011 for the management of a wide range of diseases on various ornamental, horticultural and field crops. Two end-use products containing this active ingredient, Double Nickel 55 and Double Nickel LC, are proposed for registration in Canada for the management of fungal and bacterial diseases on cucurbits, fruiting vegetables, grape, lettuce, pome fruit, potato, soybean and strawberry. They are intended for use as foliar and soil applied treatments.

Health Considerations

Can Approved Uses of *Bacillus amyloliquefaciens* strain D747 Affect Human Health?

***Bacillus amyloliquefaciens* strain D747 is unlikely to affect your health when Double Nickel 55 and Double Nickel LC are used according to the label directions.**

People could be exposed to *B. amyloliquefaciens* strain D747 when handling and applying Double Nickel 55 and Double Nickel LC 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 the active ingredient as well as granular and liquid formulations containing *B. amyloliquefaciens* strain D747 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. amyloliquefaciens* strain D747 on the treated crops, at the time of harvest, are anticipated following foliar applications to agricultural crops. *Bacillus amyloliquefaciens* is a bacterium that is found globally in most terrestrial environments. *Bacillus amyloliquefaciens* strain D747 produced no adverse effects (disease or toxicity) when it was administered orally to rats and it is not known to produce metabolites of toxicological concern. Also, no adverse effects have been reported for this microorganism in the United States where it has been registered as a pesticide since 2011. As well, the likelihood of residues of this microorganism contaminating drinking water supplies 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. amyloliquefaciens* strain D747.

Risks in Residential and Other Non-Occupational Environments

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

Double Nickel 55 and Double Nickel LC are 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. amyloliquefaciens* strain D747. 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 Double Nickel 55 and Double Nickel LC

Occupational risks are not of concern when Double Nickel 55 and Double Nickel LC are used according to label directions, which include protective measures.

Workers handling Double Nickel 55 and Double Nickel LC can come into direct contact with *B. amyloliquefaciens* strain D747 on the skin, in the eyes or by inhalation. For this reason, the product label will specify that workers exposed to the end-use products must wear waterproof gloves, long-sleeved shirts, long pants, a National Institute for Occupational Safety and Health-approved (NIOSH-approved) mist filtering mask or respirator with any N-95, P-95, or R-95 filter, and shoes plus socks. Eye goggles are not required as the eye irritation studies indicated minimal eye irritation potential.

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

Environmental Considerations

What Happens When *Bacillus amyloliquefaciens* strain D747 Is Introduced Into the Environment?

Environmental risks are not of concern.

Bacillus amyloliquefaciens is commonly found in soil. Information available in the scientific literature on the environmental fate of *B. amyloliquefaciens* indicates that, like all *Bacillus* species, *B. amyloliquefaciens* strain D747 produces endospores under adverse environmental conditions that allow it to survive under extreme heat and dry conditions. The ability to produce endospores is a major factor in the widespread occurrence of the organism 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. amyloliquefaciens* strain D747 will be above levels naturally found for this species immediately following application as a pesticide, the population will settle back to natural levels over time.

Bacillus amyloliquefaciens may survive to a limited extent in aquatic habitats given its ability to produce endospores and their potential to adsorb to the sediment layer. Endospores, however, are unlikely to be capable of germinating and multiplying in sediment. Double Nickel LC and Double Nickel 55 are not intended for aquatic use and exposure to aquatic environments from spray drift and runoff (following a rain event) from field application is unlikely to be significant.

Studies were conducted to determine the effects of *B. amyloliquefaciens* strain D747 on birds, terrestrial arthropods (including bees), and plants, as well as fish and aquatic invertebrates. These studies showed that *B. amyloliquefaciens* strain D747 was not toxic or pathogenic to these organisms.

Although non-target testing was not conducted on wild mammals, microorganisms and aquatic plants, adequate information was available in the scientific literature to determine that no significant adverse effects to these non-target organisms are expected when Double Nickel 55 and Double Nickel LC are applied according to directions on the label.

Value Considerations

What Is the Value of Double Nickel 55 and Double Nickel LC?

Double Nickel 55 and Double Nickel LC are broad spectrum biological fungicides/bactericides that suppress diseases on a wide range of host plants.

These products can be a valuable addition to sustainable disease management programs and organic production systems in Canada. The bacterial active ingredient in Double Nickel 55 and Double Nickel LC rapidly colonizes plant surfaces and impedes the development of plant-pathogenic organisms through resource competition and production of antagonistic defence metabolites. Most effective when applied preventatively, these products have been shown to reduce the incidence and severity of different economically important diseases under various levels of pest pressure. Because of the complex nature of its mode of action, resistance to the active ingredient is unlikely to develop and would be useful in an integrated disease management program.

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 Double Nickel 55 and Double Nickel LC 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 Double Nickel 55 and Double Nickel LC, respiratory and dermal sensitivity may possibly develop. All microorganisms, including *B. amyloliquefaciens* strain D747, contain substances that are potential sensitizers. Therefore, anyone handling or applying these products must wear appropriate waterproof gloves, a long-sleeved shirt, long pants, a NIOSH-approved mist filtering mask or respirator with any N-95, P-95, or R-95 filter, 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 Double Nickel LC and Double Nickel 55.

Next Steps

Before making a final registration decision on *B. amyloliquefaciens* strain D747, 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 any 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 *B. amyloliquefaciens* strain D747 (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 Reading Room (located in Ottawa).

Science Evaluation

Bacillus amyloliquefaciens strain D747

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active Ingredient	Spores of *Bacillus amyloliquefaciens* strain D747
Function	Biopesticide – To suppress various fungal and bacterial diseases on greenhouse and field crops.
Binomial Name	*Bacillus amyloliquefaciens* strain D747
Taxonomic Designation[1]	
Kingdom	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacilliales
Family	Bacilliaceae
Genus	*Bacillus*
Species	*amyloliquefaciens*
Strain	D747
Patent Status Information	None
Nominal Purity of Active Ingredient	Technical Grade Active Ingredient: 100.0% w/w *B. amyloliquefaciens* strain D747, 2×10^{11} spores per gram (minimum) End-use Products: Double Nickel 55 contains 25.0% w/w *B. amyloliquefaciens* strain D747, 5×10^{10} spores per gram (minimum); Double Nickel LC contains 98.85% w/w *B. amyloliquefaciens* strain D747, 1×10^{10} spores per gram (minimum)
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. In addition, there are no known mammalian toxins or other known toxic metabolites present in the technical grade active ingredient or end-use products.

[1] Taxonomic Designation, http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi

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

Technical Grade Active Ingredient – *Bacillus amyloliquefaciens* strain D747 Technical

Property	Result
Colour	Beige
Physical state	Fine powder
Odour	Yeast
Stability	Up to one year at 4–25°C
pH	6.5–6.7 (1%)
Bulk density	0.307–0.375 g/mL

End-Use Product – Double Nickel 55

Property	Result
Colour	Pale brown
Physical state	Granular
Odour	Yeast
Stability	Up to one year at 4–25°C
Corrosion characteristics	Does not perforate, darken, or cause leaking at the seam of foil bags after 9 months of storage.
pH	7.5–8.5 (1%)
Density	0.60–0.78 g/cm^3
Particle size distribution (dry sieve test)	0.125–0.5 mm
Wet sieve test	0.02% (200 mesh)
Percent moisture	8.12
Percent suspendibility	74.6
Dust content	Nearly dust-free (1.6 mg)
Specific gravity	0.60–0.78

End-Use Product – Double Nickel LC

Property	Result
Colour	Brown to light brown
Physical state	Liquid
Odour	Yeast
Stability	Up to one year at 4–25°C
Corrosion characteristics	Does not perforate, darken, or cause leaking at the seam of high-density polyethylene bottles after 2.5 months of storage.
pH	4.2–4.3
Bulk density	1.02–1.03 g/mL

Property	Result
Viscosity	4.6–16.0 mPas (25°C)
Percent suspension	> 60%
Wet sieve test	< 100 mesh
Miscibility with hydrocarbon oil	25°C (30 min.): immiscible

1.3 Directions for Use

Double Nickel 55 and Double Nickel LC are to be applied on labelled crops preventatively or at the first appearance of disease. The application rates range from 0.2 to 5.0 kg/ha for Double Nickel 55 and 1.0 to 25.0 L/ha for Double Nickel LC. When disease conditions require repeated treatments, reapplication intervals range from three to ten days when these products are applied as a foliar spray and two to four weeks when used in soil applications.

1.4 Mode of Action

Bacillus amyloliquefaciens strain D747 rapidly colonizes plant surfaces and impedes the development of plant-pathogenic organisms by competing with these for space and nutrients. It has also been found to produce different protective metabolites that directly interfere with proper cellular function in fungal pathogens. The active ingredient is reportedly most effective when used preventatively before establishment of the disease-causing organisms. The Fungicide Resistance Action Committee (FRAC) classifies *B. amyloliquefaciens* strain D747 as a Group 44 fungicide along with other *Bacillus* based fungicides. Plant pathogen resistance to these fungicides is not known.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganisms

Bacillus amyloliquefaciens strain D747 can be identified to the species level using a combination of colony morphologies on agar media and biochemical tests. *Bacillus amyloliquefaciens* strain D747 can also be identified to the strain level by Random Amplified Polymorphic DNA (RAPD) analysis and by ribotyping followed by PvuII restriction endonuclease digestion.

2.2 Methods for Establishment of Purity of Seed Stock

Starter cultures of the production strain are maintained in liquid nitrogen and working cultures are stored at -80^{o}C, or for short periods of time on agar slants. All stocks are tested for microbial contamination and integrity of the microbial pest control agent (MPCA). Practices for ensuring the purity and the integrity of the master seed stock were adequately described in the method of manufacture and quality assurance program.

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

The guarantees of the technical grade active ingredient and the end-use products are expressed as the number of spores per gram or millilitre. Representative data on five batches of each product (such as *B. amyloliquefaciens* strain D747 Technical, Double Nickel 55 and Double Nickel LC) were submitted. Representative data included spore counts.

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

As noted in Section 2.1, the MPCA can be identified to the strain level using RAPD analysis or ribotyping followed by PvuII restriction endonuclease digestion. However, no methods are required to quantify viable or non-viable residues of *B. amyloliquefaciens* strain D747 since it is a ubiquitous microorganism in nature and has been isolated from a wide variety of environments. Furthermore, no signs of toxicity or disease were observed when this microorganism was administered orally to rats.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures that will be used to limit contaminating microorganisms during manufacture of *B. amyloliquefaciens* strain D747 Technical, Double Nickel 55 and Double Nickel LC are acceptable. These approaches will include plating on selective agar media, and sterilization of all equipment and media. Absence of *Bacillus anthracis* in the technical grade active ingredient is confirmed using a mouse intraperitoneal bioassay.

The absence of human pathogens and below-threshold levels of contaminating microorganisms were shown in the microbial screening of production batches using microbe-specific screening methods for detecting and enumerating microbial contaminants of concern. Release standards for microbial contaminants comply with those permitted by the PMRA and are adequate to ensure that the end-use products do not contain unacceptable levels of human and animal disease-causing microorganisms.

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

Based on the results of a two-month storage stability study, the technical grade active ingredient is stable when stored between 4–25°C for a period of up to one year. Similarly, the end-use products were stable when stored at 4–25°C for a period of one year based on the results of 12-month storage stability studies.

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 the technical grade active ingredient, *B. amyloliquefaciens* strain D747 Technical, and the end-use products, Double Nickel 55 and Double Nickel LC.

The studies submitted to fulfill the requirements for health hazard assessment of the technical grade active ingredient, *B. amyloliquefaciens* strain D747 Technical, included acute oral toxicity/pathogenicity, acute pulmonary toxicity/pathogenicity, and acute intravenous infectivity studies.

In an acute oral toxicity and infectivity study, groups of fasted, young, Sprague-Dawley rats (14/sex) were given a single oral dose of the technical grade active ingredient at 1×10^8 CFU/animal. The animals were then observed for a period of up to 21 days with interim scheduled sacrifices on Days 3, 7, 14 and 21 to evaluate clearance. There were no mortalities, no treatment-related clinical signs, necropsy findings or changes in body weight. The MPCA was detected in the feces of treated rats on Day 1, but was cleared by Day 14. The MPCA was not detected in the kidney, brain, lung, liver, spleen, stomach, small intestine, large intestine, mesenteric lymph nodes or blood throughout the experimental period. Based on results of this study, *B. amyloliquefaciens* strain D747 Technical is of low toxicity and is not infective or pathogenic to the rat via the oral route.

In an acute pulmonary toxicity and pathogenicity study, groups of young, Sprague-Dawley rats (20/sex) were exposed by the intratracheal route to the technical grade active ingredient at 1×10^7 CFU/animal. Animals were then observed for up to 60 days with interim sacrifices on Days 3, 7, 14, 21 and Day 60 to evaluate clearance. There were no mortalities, no treatment-related clinical signs, necropsy findings or changes in body weight. Shortly after administration, the MPCA was detected in the lungs, trachea and nasal cavity, and on Day 3 in the bronchial lymph nodes. The MPCA cleared from the nasal cavity by Day 7 and from the bronchial lymph nodes by Day 60. In the lungs and trachea, the MPCA decreased over the experimental period but counts in the lungs were 9.6×10^4 CFU/g and 70 CFU/g in the trachea on Day 60. The test microbe was not detected in any other organs, tissues or blood throughout the experimental period. As the MPCA is a resilient spore-forming bacterium, it is not unexpected that complete clearance from the lungs and trachea was not achieved. As the MPCA also showed no signs of proliferation in the test animals, *B. amyloliquefaciens* strain D747 Technical is not considered infective or pathogenic via the pulmonary route. In addition, this study showed that *B. amyloliquefaciens* strain D747 Technical is also of low toxicity to the rat via the pulmonary route.

In an acute intravenous infectivity study, groups of young, Sprague-Dawley rats (20/sex) were injected with the technical grade active ingredient at 10^7 CFU/animal. Animals were then observed for up to 60 days with interim sacrifices on Days 0, 3, 7, 14, 21 and 60 to evaluate

clearance. There were no reported mortalities, treatment-related clinical signs, necropsy findings or changes in body weight. Shortly after administration, the MPCA was detected in the small intestine, large intestine, inguinal lymph nodes, kidney, liver, spleen and blood. The MPCA cleared from the small intestine, the large intestine and the inguinal lymph nodes by Day 21, from the blood by Day 14 and from the kidney by Day 60. The MPCA counts decreased in liver and spleen over the study period, but it was still detected in the liver ($\leq 8 \times 10^2$ CFU/g) and spleen ($\leq 4.9 \times 10^3$ CFU/g) on Day 60. As previously noted, the MPCA is a resilient spore-forming bacterium, therefore it is not unusual that complete clearance from the liver and spleen was not achieved. As the MPCA showed no signs of proliferation, *B. amyloliquefaciens* strain D747 Technical is not considered infective or pathogenic to the rat via the intravenous route.

The studies submitted to fulfill the requirements for health hazard assessment of the end-use products, Double Nickel LC and Double Nickel 55, included oral toxicity, inhalation toxicity, dermal toxicity, dermal irritation and eye irritation studies. These studies were conducted with granular and liquid formulations of *B. amyloliquefaciens* strain D747, in other words, CX-9030 and CX-9032, respectively. As these formulations were similar but not identical to the formulations proposed for registration, the results of toxicity/irritation testing were considered in conjunction with the toxicity and irritation potential of untested formulants present in Double Nickel 55 and Double Nickel LC.

In an acute oral toxicity study, three young female albino Sprague-Dawley rats were given a single oral dose of CX-9030 (5×10^{10} spores/g) in deionized water at 5000 mg/kg bw and observed for 14 days. There were no treatment-related clinical signs, necropsy findings or changes in body weight. Based on results of this study, CX-9030 is of low toxicity to the rat via the oral route.

In an acute inhalation toxicity study, groups of young Sprague-Dawley rats (5/sex) were exposed by nose-only to an aerosol generated from undiluted CX-9030 (5×10^{10} spores/g) at 2.18 mg/L for four hours and observed for 14 days. Clinical signs included piloerection and a decrease in activity, both of which were no longer evident by Day 3. There were no mortalities, no necropsy findings and no changes in body weight. Based on results of this study, CX-9030 is of low toxicity to the rat via the inhalation route.

In an acute dermal toxicity study, groups of young Sprague-Dawley rats (5/sex) were dermally exposed to CX-9030 (5×10^{10} spores/g; moistened with deionized water at 1.0 mL/g of test substance) at 5050 mg/kg bw for 24 hours. The dose was applied to an area of approximately 10% of the body surface. Animals were observed for 14 days. No mortalities, treatment-related clinical signs, necropsy findings or dermal irritation were noted during the study period. Based on results of this study, CX-9030 is of low toxicity to the rat via the dermal route.

In a primary dermal irritation study, three young adult New Zealand white rabbits (2♂, 1♀) were dermally exposed to 0.5 mL of undiluted CX-9032 (1×10^{10} spores/mL) and covered with a semi-permeable dressing for 4 hours to approximately an 8 cm^2 surface area. Animals were then scored for dermal irritation by the method of Draize after 1, 24, 48 and 72 hours. No dermal irritation was noted during the study period. Based on results of this study, CX-9032 is not irritating to the skin of rabbits.

In an eye irritation study, 0.1 mL of undiluted CX-9030 (5×10^{10} spores/g) was instilled into the conjunctival sac of the right eye of New Zealand white rabbits for 24 hours (1♂, 2♀). All treated eyes were washed with deionized water for one minute immediately after recording the 24-hour observation. Irritation was scored by the method of Draize after 1, 24, 48 and 72 hours. Corneal opacity (grades + to 1) was observed in all treated eyes up to 24 hours. Iridal involvement (grade 1) was observed in all treated eyes after 1 hour but had resolved by 24 hours. Conjunctival redness (grades 1–3), chemosis (grades 2–3) and discharge (grade 2) were observed in all treated eyes after 1 hour. Redness (grades 1–3), chemosis (grades 1–2), and discharge (grades 1–2) were still apparent after 24 hours. Fluorescein staining was observed in all treated eyes after 24 hours. All signs of ocular irritation were resolved by 72 hours or sooner. Based on an MIS of 20.8/110 (1h), CX-9030 is considered mildly irritating to the eyes of rabbits.

In another primary eye irritation study, 0.1 mL of undiluted CX-9032 (1×10^{10} spores/mL) was instilled into the conjunctival sac of the right eye of New Zealand white rabbits (2♂, 1♀) for 24 hours. All treated eyes were washed with deionized water for one minute immediately after recording the 24-hour observation. Irritation was scored by the method of Draize after 1, 24, 48 and 72 hours. Conjunctival discharge was noted in one animal after one hour. All irritation cleared after 24 hours. In this study, CX-9032 is not considered an eye irritant and is classified as practically non-irritating to the eye based on the MIS of 0.7 (1 h).

These studies are summarized in Appendix I, Table 1.

3.1.2 Additional Information

Scientific rationales were provided to waive dermal sensitization testing of Double Nickel 55 and Double Nickel LC. In the published scientific literature, there are no reported allergic reactions in humans resulting from exposure to *B. amyloliquefaciens*, and only a few reports of sensitivity in individuals exposed to the closely related species, *Bacillus subtilis* (for example, to laundry detergents containing enzymes from and wood dust containing spores of *B. subtilis*). The request to waive dermal sensitization testing was accepted as PMRA considers all MPCAs to have sensitization potential.

Materials Safety Data Sheets were provided for all the formulants present in the end-use products. The untested formulants present in the proposed formulations do not pose any significant concerns with respect to toxicity or irritation.

3.1.3 Incident Reports Related to Human and Animal Health

Since 26 April 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. Incidents were reviewed for the active ingredient, *B. amyloliquefaciens* strain D747. As of 29 April 2014, no incident reports involving *B. amyloliquefaciens* strain D747 have been reported to the PMRA, the United States Environmental Protection Agency (USEPA) and the California Pesticide Illness Query database.

3.1.4 Hazard Analysis

The database submitted in support of registering *B. amyloliquefaciens* strain D747 Technical, Double Nickel 55 and Double Nickel LC was reviewed from the viewpoint of human health and safety and was determined to be sufficiently complete to permit a decision on registration.

Bacillus amyloliquefaciens strain D747 Technical was of low toxicity and not infective or pathogenic to rats via the oral, pulmonary and intravenous routes.

Granular and liquid formulations containing *B. amyloliquefaciens* strain D747 (such as CX-9030 and CX-9032) were not toxic and not irritating to skin of rabbits. The liquid formulation was not irritating to the eyes of rabbits, while the granular was mildly irritating to eyes. Additional testing with the granular formulation showed that CX-9030 is of low toxicity to the rat via the oral and pulmonary routes. The untested formulants present in Double Nickel 55 and Double Nickel LC do not pose any significant concerns with respect to toxicity or irritation.

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 products 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 toxicity of the end-use products, and no indications of infectivity, toxicity or pathogenicity of *B. amyloliquefaciens* strain D747 in the test animals treated in the Tier I acute oral, pulmonary, intravenous, toxicity/infectivity tests.

Within the available scientific literature, there are no reports that suggest *B. amyloliquefaciens* strain D747 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. amyloliquefaciens* strain D747.

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 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. *B. amyloliquefaciens* strain D747 is not frequently 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 *B. amyloliquefaciens* strain D747 Technical and surrogate liquid and granular formulations containing *B. amyloliquefaciens* strain D747 showed no significant signs of toxicity via the oral or pulmonary routes of exposure, and no dermal toxicity are anticipated. The submitted dermal and eye irritation studies with a granular formulation containing

B. amyloliquefaciens strain D747 demonstrated mild eye irritation but no skin irritation. Precautionary label statements will be included on the label to warn users of the potential for eye irritation from the granular product. Based on the submitted eye and dermal irritation studies with the liquid formulation containing *B. amyloliquefaciens* strain D747, minimal eye and skin irritation is expected for the liquid formulation.

Although toxicity from dermal or inhalation exposure is considered minimal from the proposed use of the end-use products, 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 Double Nickel LC and Double Nickel 55 must wear waterproof gloves, long-sleeved shirts, long pants, a National Institute for Occupational Safety and Health-approved (NIOSH-approved) mist filtering mask or respirator with any N-95, P-95, or R-95 filter, and shoes plus socks. In addition, all unprotected workers are restricted from entering enclosed areas (including greenhouses) where Double Nickel 55 and Double Nickel LC have been handled or applied until spray has dried.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of Double Nickel 55 and Double Nickel LC, and no significant occupational risks are anticipated for these products.

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 *B. amyloliquefaciens* strain D747 Technical, Double Nickel 55 and Double Nickel LC. The PMRA assumes that precautionary label statements will be followed by commercial applicators in the use of Double Nickel 55 and Double Nickel LC. As well, *B. amyloliquefaciens* is a species that is ubiquitous in the environment and the use of Double Nickel 55 and Double Nickel LC 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 negligible.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

Although the proposed use pattern may result in some dietary exposure with possible residues in or on agricultural commodities, dietary risk is expected to be negligible and of no concern for the general population, including infants and children, or animals. This is because *B. amyloliquefaciens* strain D747 demonstrated no pathogenicity, infectivity or oral toxicity at the maximum dose tested in the Tier I acute oral toxicity/infectivity study. The MPCA is not known to produce 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 and pulmonary 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. amyloliquefaciens* strain D747 via drinking water because exposure will be low 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 (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 *B. amyloliquefaciens* strain D747 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. 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 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. amyloliquefaciens* strain D747 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 files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *B. amyloliquefaciens* strain D747 to the general Canadian population, including infants and children, when the end-use products are used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Furthermore, few adverse effects from exposure to other isolates of *B. amyloliquefaciens* encountered in the environment have been reported. Even if there is an increase in exposure to this active ingredient from the use of Double Nickel 55 and Double Nickel LC, 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 amyloliquefaciens are ubiquitous organisms found in most terrestrial environments. Residues of *B. amyloliquefaciens* strain D747 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. No adverse effects from dietary exposure have been attributed to natural populations of *B. amyloliquefaciens*, and no adverse effects were observed in the acute oral toxicity and pathogenicity study conducted with *B. amyloliquefaciens* strain D747. Also, strain D747 is not known to produce 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. amyloliquefaciens* strain D747.

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 (such as production of antifungal compounds), certain registered MPCAs, as well as naturally occurring bacterial strains, may share a common mode of toxicity with *B. amyloliquefaciens* strain D747. No cumulative effects are anticipated if the residues of *B. amyloliquefaciens* strain D747 interact with these microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Bacillus amyloliquefaciens is a ubiquitous soil-dweller that colonizes plant roots, leaves and other plant surfaces. It is closely related to *B. subtilis*; these two species have only recently been considered distinct. Under adverse environmental conditions, *Bacillus* species produce endospores that allow them to endure extreme conditions of heat and desiccation, and this quality is a major factor in the ubiquity of the organism in the environment. Over time, however, the population of *B. amyloliquefaciens* strain D747 should decline to naturally occurring levels. As *B. amyloliquefaciens* strain D747 spores are sensitive to ultraviolet exposure (sunlight), spores are not expected to persist in the phyllosphere.

Given the ability to produce endospores, *B. amyloliquefaciens* 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. amyloliquefaciens* 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, spray drift from application to soil and developed foliage is unlikely to be significant. Concentrations of *B. amyloliquefaciens* strain D747, which are deposited in surface water bodies via drift and/or runoff 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 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 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, such as 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.

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

Nine studies were submitted to address the hazards of the *B. amyloliquefaciens* strain D747 to terrestrial non-target organisms. These studies included non-target avian species, and terrestrial arthropods species including two studies with honeybees, and a study on various non-target plants. To further supplement the studies, waiver rationales were submitted to support testing on non-target terrestrial arthropods and bees. A waiver rationale in lieu of testing was submitted for wild mammals.

Three of the terrestrial arthropod studies (*Typhlodromus pyri*, *Aphidius rhopalosiphii* and one study on *Apis mellifera*) were conducted with CX-9030, a different granular formulation containing the MPCA. As the formulation of CX-9030 was not identical to the granular formulation proposed for registration, the untested formulants present in Double Nickel 55 were considered with respect to their potential for toxicity to non-target arthropods alongside the results of the studies.

The acute oral toxicity of the technical grade active ingredient to 24-week-old northern bobwhite quail (*Colinus virginianus*) was assessed over 14 days. In the study, a single dose was administered to the birds (10 birds) by oral intubation at 2250 mg a.i./kg bw. 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 2250 mg a.i./kg bw. An avian inhalation toxicity/pathogenicity study was not submitted. However, a study is not required to support registration of *B. amyloliquefaciens* strain D747 based on results of the oral study as well as an absence of reports of adverse effects to birds in the open scientific literature for other strains of *B. amyloliquefaciens* and its closely related species, *B. subtilis*.

The acute dietary toxicity of *B. amyloliquefaciens* strain D747 to larvae of the predaceous flower bug (*Orius strigicollis*; 50 insects) was assessed over 12 days. Larvae were exposed to the test material in glass vials for 24 hours at 5×10^8 CFU/mL and observed for 7 days. On Day 12, a sub-sample of adults was permitted to mate and egg production was monitored. The Day 7 (adjusted) death rate was 8.9%. There was no considerable difference in the eclusion rate (in other words, rate of emergence of adults on Day 10) for the test group and control group and egg production (Day 12) was higher in the test substance group than in the control group. However, the 24-hour exposure period is considerably shorter than recommended in recognized test guidelines for dietary toxicity testing of microorganisms in non-target insects (in other words, dietary exposure for at least 21 days) and the test concentration was below guideline requirements. Nevertheless, the study is acceptable for risk assessment purposes only as a toxicity study since it did not address infectivity and pathogenicity endpoints. The 24-hour dietary LC_{50} was greater than 5×10^8 CFU/mL.

The acute contact and dietary toxicity/pathogenicity of *B. amyloliquefaciens* strain D747 to protonymphs of the predaceous mite *Phytoseiulus persimilis* (50 insects) was assessed over 6 days. A single spray application to the insects as well as the habitat and the food source was made at 2 mL/cm^2 (nominal; equivalent to 1×10^9 CFU/cm^2). There were no deaths observed and all insects reached adulthood. There were no significant effects on egg production. The duration of the study (6-day exposure and observation period) is shorter than recommended in recognized test guidelines for dietary toxicity/pathogenicity testing of microorganisms in non-target insects (in other words, 21-day exposure and observation period). Nevertheless, the study is acceptable for risk assessment purposes as a toxicity study only since it did not address infectivity and pathogenicity endpoints. The acute dietary and contact toxicity LC_{50} was greater than 5×10^8 CFU/mL.

The acute dietary toxicity of *B. amyloliquefaciens* strain D747 to green lacewing larvae (*Chrysoperla carnea*; 30 insects) was assessed over 50 days. A single dose of the test material at 5×10^8 CFU/mL (nominal) was blended into the diet. There was no significant effect on survival (Day 21) or egg production of adult females (Days 22–32) and no abnormal behaviour was observed throughout the duration of the study (Day 50). Although the test concentration was below guideline requirements, the study is acceptable for risk assessment purposes. The 15-day dietary LC_{50} was greater than 5×10^8 CFU/mL.

The acute contact toxicity of CX-9030 to parasitic wasps (*A. rhopalosiphii*; 40 wasps) was assessed over 12 days. The insects were exposed for 48 hours to a glass plate previously sprayed with a single application at 15 kg/ha (unspecified aqueous dilution). There were no effects on mortality (48-hour) or on reproduction. The 48-hour LR_{50} and EC_{50} (fecundity) values were greater than 15 kg/ha.

The acute contact toxicity of CX-9030 to predatory mites (*T. pyri*; 100 insects) was assessed over 14 days. Insects were exposed for 14 days to the test material in a glass plate previously sprayed with a single application at 15 kg/ha (diluted in water at 75 g/L; Limit Test).There were no effects on mortality (Day 7) or reproduction (based on the cumulative number of eggs/female on Day 14). The 14-day LR_{50} and the EC_{50} (fecundity) were greater than 15 kg/ha.

The acute dietary toxicity of *B. amyloliquefaciens* strain D747 to adult European honeybees (*A. mellifera*; 80 bees) was assessed over 17 days. The test material was diluted in 50% sucrose at 1×10^9 CFU/mL and offered to bees for 48 hours. There were no effects on mortality. The 48-hour dietary LC_{50} was greater than 1×10^9 CFU/mL. The scope of the study was limited since dietary consumption was not measured and therefore the dosing level could not be confirmed. Furthermore, the duration of the observation period was shorter than recommended for infectivity testing, and the study did not monitor infectivity, or any sub-lethal effects. Nevertheless, the study is acceptable for risk assessment purposes.

The acute dietary and contact toxicity of CX-9030 to adult worker honeybees (*A. mellifera*) was assessed over 48 hours. Honeybees (50 bees) were exposed to CX-9030 in a diet consisting of 50% w/v sucrose solution at 446.63 µg/bee for 6 hours (measured; limit test). There were no mortalities reported from dietary exposure. The 48-hour dietary LD_{50} was greater than 446.63 µg/bee. Another group of honeybees (50 bees) was exposed by contact to a single

aqueous application of CX-9030 to the thorax at 20, 40, 80, 160 and 320 µg/bee (nominal; 2 µL/bee). Mortality from contact exposure after 48 hours was not dose dependent. Minor signs of toxicity were noted in a few bees (low- and high-dose test groups only). The 48-hour contact LD_{50} was greater than 320 µg/bee. A conservative RQ was calculated for bees (based on dietary LD_{50} > 446.63 µg/bee). As the RQ was below the level of concern, no adverse effects to bees are anticipated from the proposed use of Double Nickel LC and Double Nickel 55.

While the tested end-use product CX-9030 contains different formulants than those present in Double Nickel 55, the study results were accepted. The untested formulants present in Double Nickel 55 do not pose any significant toxicity concerns for terrestrial arthropods. In addition, the formulants in the liquid end-use product, Double Nickel LC, are also not expected to pose toxicity concerns to non-target terrestrial arthropods.

Scientific rationales were submitted to further supplement the terrestrial arthropod and honeybee toxicity studies. The rationales presented information from the open scientific literature that supported the unlikelihood of insecticidal toxin production by the MPCA. A history of use of other members of the *B. subtilis-amyloliquefaciens*-complex in pesticide products without reports of adverse effects to non-target arthropods has also been established.

Overall, the toxicity data from various terrestrial arthropod species, as well as information in published literature on the potential insecticidal toxicity and on the fate of *B. amyloliquefaciens* in the environment, and on the history of use of other related strains in biopesticide products without reports of adverse effects was considered acceptable to address the potential for adverse effects to terrestrial arthropods. Based on the available information, adverse effects to non-target terrestrial arthropods, including honeybees, are not expected from the proposed use of Double Nickel 55 and Double Nickel LC.

A study was conducted to assess the potential for adverse effects of *B. subtilis* D747 (equivalent to technical grade active ingredient) on a variety of non-target plants (corn, wheat, green onion, onion, cabbage, Napa cabbage, spinach, soybean and pumpkin). Approximately 30 days after planting, a foliar application of a *B. amyloliquefaciens* strain D747 at 5.0×10^8 CFU/mL or 5.0×10^9 CFU/mL was applied to each plant type (three plants/type) with re-application seven days later. There were no effects reported in any of the test plants over the seven-day observation period. However, the scope of the study was limited since the duration (seven days) was too short to properly assess the infectivity potential, and standard plant parameters (for example, percent germination, seedling emergence, root weight/length, shoot weight/length) were not monitored. Furthermore, the test concentrations (10^8–10^9 CFU/mL) were below the label guarantees of the end-use product proposed for registration (1×10^{10} CFU/mL). Nevertheless, the study is acceptable for risk assessment purposes only as a toxicity study.

No toxicity/pathogenicity data were considered to address the potential for harm to wild mammals, non-arthropod invertebrates, and microorganisms. A scientific rationale was submitted to address the potential for harm to wild mammals. *Bacillus amyloliquefaciens* is not on any authoritative list of mammalian pathogens. As a ubiquitous soil bacterium, wild mammals are expected to have been exposed to strains similar to the MPCA. Searches of the open scientific literature (through PubMed) revealed no reports of adverse effects to mammals

attributable to *B. amyloliquefaciens* strains although there have been a few reports of adverse effects in livestock animals (for example, bovine mastitis) associated with *B. subtilis*. From the data submitted under Section 3.1.1, it was determined that *B. amyloliquefaciens* strain D747 was not toxic or pathogenic to laboratory mammals via the oral, pulmonary and intravenous routes of administration.

The potential for adverse effects to non-arthropod invertebrates and soil microorganisms was also not directly addressed. Additional testing on non-target microorganisms, however, is not required even though the end-use products are intended to control pest microorganisms (in other words, plant pathogenic fungi). As *B. amyloliquefaciens* is a natural component of soil microflora, any increase in exposure to the MPCA is not expected to adversely affect environmentally or economically important microbial species or microbiologically mediated biogeochemical processes. *Bacillus amyloliquefaciens* strain D747 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. amyloliquefaciens* strain D747 to non-target terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, arthropods (including honeybees), non-arthropod invertebrates, non-target microorganisms and plants from the proposed use of Double Nickel 55 and Double Nickel LC on greenhouse and field crops.

4.2.2 Effects on Aquatic Organisms

Two studies were submitted to address the hazards of the technical grade active ingredient to aquatic non-target organisms, including freshwater fish and benthic aquatic arthropods. A waiver rationale was also submitted to address aquatic plant testing.

The toxicity studies on rainbow trout and daphnids were conducted with CX-9032. This test material represents a liquid formulation containing *B. amyloliquefaciens* strain D747. As the formulation is not identical to the formulation proposed for registration, the untested formulants present in the proposed formulation were considered with respect to the potential for toxicity to non-target aquatic organisms alongside the results of the studies.

The acute toxicity/pathogenicity of CX-9032 to rainbow trout (*Oncorhynchus mykiss*; 20 fish) was assessed under semi-static conditions over 30 days. CX-9032 was added to the water at five concentrations ranging from 1.44×10^{10} to 2.30×10^{11} CFU/L. Effects on survival and growth (length and weight) were observed. The 30-day LC_{50} was 8.1×10^{10} CFU/L and the no observed effect concentration (NOEC) (growth) was 1.44×10^{10} CFU/L. No pathological changes from exposure were observed. As the RQ for freshwater fish was below the level of concern, no adverse effects to freshwater fish are anticipated from the proposed uses of Double Nickel LC and Double Nickel 55.

The acute toxicity of CX-9032 to daphnids (*Daphnia magna*; 20 daphnids) was assessed under semi-static conditions over 21 days. CX-0932 was added to the culture medium at five concentrations ranging from 2.84×10^{8} to 2.3×10^{10} CFU/L. There were no effects on mortality.

The LC_{50} is greater than 2.30×10^{10} CFU/L. Prolonged exposure affected parental body length and inhibited reproduction in the surviving parental generation as well as the time to first brood. The lowest NOEC (reproduction and time to first brood) is 2.84×10^{8} CFU/L. As the RQ for benthic aquatic arthropods was below the level of concern, no adverse effects to benthic aquatic arthropods are anticipated from the proposed uses of Double Nickel LC and Double Nickel 55.

The untested formulants present in Double Nickel LC do not pose any significant concerns with respect to toxicity to aquatic organisms. In addition, the formulants present in the granular end-use product, Double Nickel 55, are also not expected to present concerns to aquatic non-target organisms. Regardless, as a general precaution applied to all pesticides not intended for use in aquatic systems, the label will prohibit the direct application of Double Nickel 55 and Double Nickel LC to aquatic habitats, estuaries or marine habitats, and instruct handlers to not contaminate surface water by disposal of equipment wash waters.

A scientific rationale was submitted to address the potential for harm to aquatic plants. *Bacillus amyloliquefaciens* strain D747 acts as an antagonist specifically against plant pathogenic fungi and no aquatic uses are proposed. A published in vitro study that assessed the effects of another strain of *B. amyloliquefaciens* (strain FZB24) to duck weed (*Lemma minor* ST) reported a reduction in growth at high concentrations of *B. amyloliquefaciens* strain FZB24, but a stimulatory effect at low concentrations. Based on the proposed terrestrial uses of Double Nickel LC and Double Nickel 55, no adverse effects on aquatic plants are expected. No further information is required.

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

Based on all the available data and information on the effects of *B. amyloliquefaciens* strain D747 to non-target aquatic organisms and the precautionary measures required on the labels of Double Nickel 55 and Double Nickel LC, 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 26 April 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. Only incidents in which the pesticide is determined to be linked to the effects (Canadian causality of highly probable, probable and possible; American causality of highly probable, probable and possible) are considered in the reviews.

As of 29 April 2014, there were no environmental incidents reported in the PMRA Incident reporting database or in the United States Ecological Incident Information System (EIIS) for products containing *B. amyloliquefaciens* strain D747 for use as pesticides.

5.0 Value

5.1 Consideration of Benefits

Double Nickel 55 and Double Nickel LC have the potential to become useful elements in integrated disease management programs for a number of different high-value horticultural crops. By impeding disease development, these biofungicides/biobactericides may help to reduce the total amount of conventional chemical fungicides required in a season. Furthermore, they would also provide a valuable new option to manage economically important diseases in organic production and other low input agricultural systems. A survey of the different modes of action from alternative products presented in Appendix I, Table 3 highlights the dearth of microbial or otherwise non-conventional pesticides registered for the management of diseases on the Double Nickel 55 and Double Nickel LC labels. For many of the disease claims in question, active ingredients from only one microbial mode of action group are currently registered.

5.2 Acceptable Claims and Effectiveness Against Pests

Efficacy data from 49 small-scale efficacy trials were reviewed to support the value of 16 disease claims to appear on the labels of both Double Nickel 55 and Double Nickel LC. The trials were conducted at different field and greenhouse locations in various Canadian provinces and in other countries including the United States. Based on the available value information, which focussed primarily on efficacy data, the claims were supported at the suppression or partial suppression level. Evidence from trial data and extrapolations of evidence across claims were accepted in support of the various methods of application. A complete list of supported uses is provided in Appendix I, Table 4.

5.3 Non-Safety Adverse Effects

Phytotoxicity resulting from applications of various *B. amyloliquefaciens* strain D747 products, including Double Nickel 55 and Double Nickel LC, was not reported in any of the trials provided. There is no indication that non-safety adverse effects would result from Double Nickel 55 or Double Nickel LC when applied to crops in accordance with label directions and restrictions.

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, in other words, persistent [in air, soil, water and/or sediment], bio-accumulative, primarily a result of human activity and toxic as defined by the *Canadian Environmental Protection Act*).

Bacillus amyloliquefaciens strain D747 Technical, Double Nickel LC and Double Nickel 55 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:

- *Bacillus amyloliquefaciens* strain D747 Technical does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.
- There are also no formulants, contaminants or impurities present in the end-use products that would meet the TSMP Track-1 criteria

6.2 Formulants and Contaminants of Health or Environmental 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:

- The technical grade active ingredient, *B. amyloliquefaciens* strain D747 Technical, does not contain formulants of health or environmental concern as identified in the *Canada Gazette,* Part II, Volume 139, Number 24, pages 2641-2643: *List of Pest Control Product Formulants of Health or Environmental Concern*.
- Double Nickel 55 and Double Nickel LC do not contain formulants of health or environmental concern as identified in the *Canada Gazette,* Part II, Volume 139, Number 24, pages 2641-2643: *List of Pest Control Product Formulants of Health or Environmental Concern*.

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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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, *Formulants Policy and Implementation Guidance Document.*

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for *B. amyloliquefaciens* strain D747 Technical, Double Nickel 55 and Double Nickel LC were judged to be adequate to assess their potential human health and environmental risks. The technical grade active ingredient was characterized and the specifications of the EP were supported by the analyses of a sufficient number of batches. Storage stability data were sufficient to support a shelf life of up to 12 months for the technical grade active ingredient and end-use products when stored at temperatures 4-25°C.

7.2 Human Health and Safety

The acute toxicity and infectivity studies and other relevant information submitted in support of *B. amyloliquefaciens* strain D747 were determined to be sufficiently complete to permit a decision on registration. Submitted information suggests *B. amyloliquefaciens* strain D747 Technical to be of low toxicity and not infective or pathogenic by the oral, pulmonary and intravenous routes. Granular and liquid formulations containing *B. amyloliquefaciens* strain D747 (in other words, CX-9030 and CX-9032) were not toxic and not irritating to the skin. The liquid formulation was not irritating to the eyes, but the granular formulation was mildly irritating to the eyes. Additional testing with the granular formulation showed that it is of low toxicity to the rat via the oral and pulmonary routes. Since *B. amyloliquefaciens* strain D747 Technical, Double Nickel 55 and Double Nickel LC are considered potential sensitizers, the signal words, “POTENTIAL SENSITIZER”, are required on the principal display panel of all three 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 Double Nickel 55 and Double Nickel LC, respiratory and dermal sensitivity could possibly develop upon repeated exposure to the product since all microorganisms, including *B. amyloliquefaciens* strain D747, contain substances that are potential sensitizers. Therefore, anyone handling or applying Double Nickel 55 and Double Nickel LC must wear waterproof gloves, long-sleeved shirts, long pants, a NIOSH-approved mist filtering mask or respirator with any N-95, P-95, or R-95 filter, and shoes plus socks. In addition, all unprotected workers are restricted from entering enclosed areas (including greenhouses) where Double Nickel LC and Double Nickel 55 have been handled or applied until mist 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 expected to be negligible and of no concern due to the low toxicity/pathogenicity profile for *B. amyloliquefaciens* strain D747 Technical, Double Nickel 55 and Double Nickel LC. The specification of an MRL is not required for *B. amyloliquefaciens* strain D747.

7.3 Environmental Risk

The non-target organism tests, scientific rationales and supporting published scientific literature submitted in support of *B. amyloliquefaciens* strain D747 Technical, Double Nickel 55 and Double Nickel LC were determined to be sufficiently complete to permit a decision on the environmental fate and effects of these products. The use of Double Nickel 55 and Double Nickel LC containing *B. amyloliquefaciens* strain D747 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 Double Nickel 55 and Double Nickel LC to aquatic habitats, estuaries or marine habitats, and direct handlers to not contaminate surface water by disposal of equipment wash waters.

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

7.4 Value

The value information available, which was primarily in the form of efficacy data and considerations of other benefits, was determined to be sufficient to support the value of registering a total of 16 fungal and bacterial disease claims for two new end-use products containing *B. amyloliquefaciens* strain D747. The disease claims include uses on a range of horticultural crops including cucurbits, fruiting vegetables, grapes, lettuce, pome fruit, potato, and strawberry. Registration of these new biofungicide products will provide a biological option to users with demonstrated effectiveness in reducing the impact of a broad range of plant diseases on high value crops. Double Nickel 55 and Double Nickel LC can be integrated into sustainable disease management programs. These products will be of particular value for fruit and vegetable producers and managers aiming to avoid the use of conventional synthetic fungicides.

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 *B. amyloliquefaciens* strain D747 Technical and Double Nickel 55 and Double Nickel LC, containing the technical grade active ingredient *B. amyloliquefaciens* strain D747, to suppress or partially suppress a variety of fungal or bacterial diseases on cucurbits, fruiting vegetables, grapes, lettuce, pome fruit, potato, soybean and strawberry.

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

♀	female
♂	male
ºC	degree(s) Celsius
µg	micrograms
µL	microlitres
a.i.	active ingredient
ADI	acceptable daily intake
ARfD	acute reference dose
bw	body weight
cm	centimetres
DNA	deoxyribonucleic acid
EC_{50}	median effective concentration
EIIS	United States Ecological Incident Information System
FRAC	Fungicide Resistance Action Committee
g	gram
h	hour(s)
ha	hectare(s)
kg	kilogram
L	litre
LC	liquid concentrate
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LR_{50}	median lethal rate
LOEC	low observed effect concentration
m	metre(s)
MAS	maximum average score
mg	milligram
min	minute
MIS	mean irritation score
mL	millilitre
mm	millimetre
mPas	millipascal second
MPCA	microbial pest control agent
MRL	maximum residue limit
NC	not classified
NIOSH	National Institute for Occupational Safety and Health
NOEC	no observed effect concentration
OECD	Organization of Economic Cooperation and Development
PMRA	Pest Management Regulatory Agency
RAPD	random amplified polymorphic DNA
RQ	risk quotient
SD	Sprague-Dawley

TSMP	Toxic Substances Management Policy
USEPA	United States Environmental Protection Agency
w/v	weight per volume
w/w	weight per weight

Appendix I Tables

Table 1 Toxicity and Infectivity of *Bacillus amyloliquefaciens* strain D747 Technical, and Double Nickel 55 and Double Nickel LC

Study Type	Species, Strain and Doses	Results	Comments	Reference(s)
Acute Toxicity/Infectivity of *Bacillus amyloliquefaciens* strain D747 (Technical)				
Acute Oral Infectivity and Toxicity (21-Day Study)	Rat – SD 7/sex, single oral dose in sterile physiological saline, ~10^8 CFU/animal, interim sacrifices on Days 1, 3,7,14 and 21 2/sex, vehicle control group, sacrificed on Day 21 Body weight measured on Days 0 (prior to dosing), 7, 14 and 21.	$LD_{50} > 10^8$ CFU/animal	There were no mortalities, treatment-related clinical signs, necropsy findings or changes in body weight. Total clearance achieved by Day 14. **Low Toxicity, Not Infective**	PMRA 2336335
Acute Pulmonary Infectivity and Toxicity (60-Day Study)	Rat – SD 20/sex, ~10^7 CFU/animal in sterile physiological saline, interim sacrifices on Days 3,7, 14, 21 and 60 2/sex, vehicle control group, sacrificed on Day 21 Body weight measured on Days 0 (prior to administration), 7, 14, 21, 38, 42, 49 and 60	$LD_{50} > 10^7$ CFU/animal	There were no mortalities, treatment-related clinical signs, necropsy findings or changes in body weight. MPCA was detected in the nasal cavity, bronchial lymph nodes, lungs and trachea. By Day 60, clearance was achieved in all organs but lungs and trachea. A pattern of clearance was observed in lungs and trachea by Day 60. MPCA was not detected in blood, kidney, spleen and liver during the test period. **Low Toxicity, Not Infective**	**PMRA 2336336**

Study Type	Species, Strain and Doses	Results	Comments	Reference(s)
Acute Intravenous Infectivity **(60-Day Study)**	Rat – SD 20/sex, intravenous injection in sterile physiological saline, ~10^7 CFU/animal, interim sacrifices on Days 0, 3, 7, 14, 21 and 60 2/sex, vehicle control group, sacrificed on Day 21 **Body weights measured on Days 0, 7, 14, 21, 28, 35, 49, 56 and 60**	$LD_{50} > 10^7$ CFU/animal	There were no mortalities, treatment-related clinical signs, necropsy findings or changes in body weight. MPCA was detected in small intestine, large intestine, inguinal lymph nodes, kidney, spleen, liver and blood. Clearance was achieved in small intestine, large intestine and inguinal lymph nodes by Day 21; blood by Day 14 and kidney by Day 60. By Day 60, low counts remained in liver and spleen. A pattern of clearance was achieved by Day 60. MPCA was not detected in the brain during the test period. **Low Toxicity, Not Infective**	PMRA 2336339
Acute Toxicity and Irritation of Double Nickel 55[1]				
Acute Oral Toxicity (14-Day Study)	Rat – SD 3♀, single oral dose of CX-9030 in deionized water, 5000 mg/kg bw Body weight measured on Days 0 (prior to dosing), 7 and 14	$LD_{50} > 5000$ mg/kg bw	There were no mortalities, treatment-related clinical signs, gross necropsy findings or changes in body weight. **Low Toxicity**	PMRA 2336706
Acute Inhalation Toxicity (14-Day Study)	Rat – SD 5/sex, 2.18 mg/L of CX-9030 (containing 5×10^{10} spores/g of *B. amyloliquefaciens* strain D747, nose-only, 4 h) Body weight measured on Days 0 (prior to administration), 7 and 14	$LD_{50} > 2.18$ mg/L	There were no mortalities, necropsy findings and no changes in body weight. Clinical signs included activity decrease and piloerection, which were resolved by Day 3. **Low Toxicity**	PMRA 2336707

Study Type	Species, Strain and Doses	Results	Comments	Reference(s)
Acute Dermal Toxicity	Rat – SD 5/sex, 24-hour dermal exposure 5050 mg CX-9030/kg bw (containing 5×10^{10} spores/g of *B. amyloliquefaciens* strain D747) moistened with water at 1 g/mL deionized water; 10% body area Observed for 14 days.	MIS = 0/8 MAS = 0/8 (24 h, 48 h and 72 h) LD_{50} > 5050 mg/kg bw	No mortalities, no treatment related clinical signs, and no dermal irritation was noted. **Low Toxicity, Non-Irritating**	PMRA 2362265
Eye Irritation	Rabbit – New Zealand White 1 ♂; 2 ♀, 24-hour ocular exposure 0.1 mL CX-9030/animal (containing 5×10^{10} spores/g of *B. amyloliquefaciens* strain D747), right eye Observed for 72 hours.	MIS = 20.8/110 (1 h) MAS = 6.44 /110 (24 h, 48 h, and 72 h)	Corneal opacity (grades + to 1) observed in 3/3 eyes up to 24 h. Iridal involvement (grade 1) observed in 3/3 eyes at 1 h. Conjunctival redness (grades 1–3) observed in 3/3 eyes at 1 h, 24 h and 48 h. Conjunctival chemosis observed in 3/3 eyes at 1 h (grades 2–3) and at 24 h (grades 1–2). Discharge observed in 3/3 eyes at 1 h (grades 2) and 24 h (grades 1–2). Fluorescein staining observed in 3/3 eyes at 24 h. All ocular effects resolved by 72 h, or sooner. **Mildly Irritating**	PMRA 2336709

Study Type	Species, Strain and Doses	Results	Comments	Reference(s)
Acute Toxicity and Irritation of Double Nickel LC[2]				
Dermal Irritation	Rabbit – New Zealand White 2♂ 1♀, 4-hour dermal exposure 0.5 mL CX-9032/animal (containing 10^{10} spores/mL of *B. amyloliquefaciens* strain D747); 8 cm^2 Observed for 72 hours.	MIS = 0/8 MAS = 0/8 (24 h, 48 h, and 72 h)	No dermal irritation was noted. **Non-Irritating**	PMRA 2336808
Eye Irritation	Rabbit – New Zealand White 2♂ 1♀, 24-hour ocular exposure 0.1 mL CX-9032 /animal (containing 10^{10} spores/mL *B. amyloliquefaciens* strain D747), right eye Observed for 72 hours.	MIS = 0.7/110 (1 h) MAS = 0/110 (24 h, 48 h, and 72 h)	Conjunctival discharge (grade 1) was noted after 1 h in 1/3 eyes. All eye irritation cleared by 24 h. **Practically Non-Irritating**	PMRA 2336810

[1] Toxicity testing to support Double Nickel 55 was conducted with *B. subtilis* CX-9030, a granular formulation containing *B. amyloliquefaciens* strain D747 at 5×10^{10} CFU/g.
[2] Toxicity testing to support Double Nickel LC was conducted with *B. subtilis* CX-9032, a liquid formulation containing *B. amyloliquefaciens* strain D747 at 1×10^{10} CFU/mL.

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Protocol	Significant Effect and Comments	Reference
Terrestrial Organisms				
Vertebrates				
Birds	Oral – Northern Bobwhite Quail (*Colinus virginianus*), 24-week old	Birds (10) were administered a single dose of CX-9025[1] by oral intubation at a dose of 2250 mg a.i./kg bw (4.5×10^{11} spores/kg bw). Other groups of birds (10) administered reverse osmosis water to serve as the negative control. Birds were observed for 14 days.	No signs of toxicity. No mortalities. When compared to controls, there were no apparent effects on body weight or feed consumption. 14-day LD_{50} > 2250 mg a.i./kg bw **Low Toxicity**	PMRA 2336341
	Pulmonary/ Injection	No information was submitted to address the potential harm to avian species via pulmonary exposure. *Bacillus amyloliquefaciens* is not generally considered a pathogen of birds and there were no reports of adverse effects to birds in scientific literature. The avian toxicity study with *B. amyloliquefaciens* strain D747 reported no signs of toxicity via the oral route. No further information is required to assess the risk of harm to birds.		
Wild Mammals	A scientific rationale was submitted to waive testing based on the results of human health and safety testing, the infrequency of adverse effects to mammals in published scientific literature given the ubiquitous nature of the organism and that the MPCA is not on any authoritative list of mammalian pathogens. Based on human health and safety testing, it was determined that *B. amyloliquefaciens* strain D747 was not toxic or pathogenic to mammals via the oral, pulmonary and intravenous routes. No further data are required to assess the risk of harm to wild mammals.			PMRA 2362267

Organism	Exposure	Protocol	Significant Effect and Comments	Reference
Invertebrates				
Arthropods				
Terrestrial Arthropods	Dietary – Predaceous flower bug (*Orius strigicollis*), larvae	Larvae (50) were exposed to *B. amyloliquefaciens* strain D747 in glass vials at 5×10^8 CFU/mL for 24 hours. Another group of larvae (50) were administered distilled water to serve as the negative control. Larvae were monitored for development and on Day 12, adults were permitted to mate and egg production was monitored.	No lethal effects on the survival of larvae and no sub-lethal effects on development or reproduction were observed. 24-h LC_{50} $> 5 \times 10^8$ CFU/mL **Low Toxicity**	PMRA 2336347[2]
Terrestrial Arthropods	Dietary and Contact – Predaceous mite (*Phytoseiulus persimilis*), protonymphs	A single spray of *B. amyloliquefaciens* strain D747 (5×10^8 CFU/mL) was made to protonymphs (50) and to the habitat and feed at 2 mL/cm^2 (10^9 CFU/cm^2). Insects were exposed for 6 days. Other groups of protonymphs (50) were administered distilled water to serve as the negative control. Survival, development and behaviour were monitored for 4 days. Egg production was monitored from Day 5–6.	No lethal effects on the survival of larvae and no sub-lethal effects on development or reproduction were observed. $LC_{50} > 5 \times 10^8$ CFU/mL **Low Toxicity**	PMRA 2336347[2]
Terrestrial Arthropods	Dietary – Green lacewing (*Chrysoperla carnea*), larvae	Larvae (30) were exposed to *B. amyloliquefaciens* strain D747 in the diet at 5×10^8 CFU/mL for 15 days. Another group of larvae (30) were fed unmodified diet to serve as the negative control. Larvae were monitored for survival and development for 15 days.	No lethal effects on the survival of larvae and no sub-lethal effects on egg production (Days 22–32) were observed. 15-day $LC_{50} > 5 \times 10^8$ CFU/mL **Low Toxicity**	PMRA 2336347[2]

Organism	Exposure	Protocol	Significant Effect and Comments	Reference
		Egg production was monitored until Day 50.		
Terrestrial Arthropods	Contact – Parasitic wasp (*Aphidius rhopalosiphii*), adults	Parasitic wasps (40) were exposed for 48 hours to a glass plate previously sprayed with CX-9030[3] (5×10^{10} CFU/g; diluted in water) at a rate of 15 kg/ha. Two other groups of wasps were administered dimethoate (40 wasps; 400 g/L) or deionized water (40 wasps; 200 L/ha) to serve as the positive and negative control groups, respectively. Wasps were monitored for survival for 48 hours. After the exposure period, females were offered aphid-infested barley plants for 24 hours to monitor reproduction (aphid parasitisation) on Day 12.	No lethal effects on the survival and no sub-lethal effects on reproduction were observed. 48-h LR_{50} > 15 kg/ha 48-h EC_{50} (fecundity) > 15 kg/ha **Low Toxicity**	PMRA 2434669
Terrestrial Arthropods	Contact – Predatory mite (*Typhlodromus pyri*), protonymphs	Predatory mites (100) were exposed for 14 days to a glass plate previously sprayed with CX-9030[3] at a rate of 15 kg/ha. Other groups of protonymphs (100/control group) were administered dimethoate (400 g/L) or deionized water to serve as the positive and negative control groups, respectively. Mites were monitored for survival and reproduction for 14 days.	No lethal effects on the survival and no sub-lethal effects on reproduction were observed. 14-day LR_{50} > 15 kg/ha 14-day EC_{50} (fecundity) > 15 kg/ha **Low Toxicity**	PMRA 2434670

Organism	Exposure	Protocol	Significant Effect and Comments	Reference
Terrestrial Arthropods	Dietary – Honeybees (*Apis mellifera*), adult worker	Bees (80) were fed *B. amyloliquefaciens* D747 in 50% sucrose at 10^9 CFU/mL for 48 hours. Another group of bees (80) were fed 50% sucrose to serve as the vehicle control. Bees were monitored for survival for 21 days.	No lethal effects on the survival were observed. 48-h $LC_{50} > 10^9$ CFU/mL **Low Toxicity**	PMRA 2336351
Terrestrial Arthropods	Dietary and Contact – Honeybees (*Apis mellifera*), adult worker	Dietary Toxicity – Bees (50) were exposed to CX-9030[3] in 50% sucrose at 446.63 µg/bee for 6 hours. Other groups of bees were fed dimethoate at (0.07, 0.09, 0.12 or 0.17 µg a.i./bee; 40 bees) or a 50% sucrose solution (10 bees) to serve as the positive and negative controls, respectively. Bees were observed for 48 hours. Contact Toxicity – Bees (50) were topically treated with an aqueous solution of CX-9030[3] at 20, 40, 80, 160 or 320 µg/bee (2 µL/bee). Other groups of bees were treated with dimethoate (0.10, 0.15, 0.23, or 0.34 µg a.i./bee; 40 bees) or tap water (10 bees) to serve as the positive and negative controls, respectively. Bees were observed for 48 hours.	Dietary Toxicity – No lethal effects on the survival of worker bees and no signs of toxicity were observed. 48 h dietary $LD_{50} > 446.63$ µg/bee Contact Toxicity – Mortality was not dose dependent. Minor signs of toxicity were noted in some bees (low- and high-dose group only). 48 h contact $LD_{50} > 320$ µg/bee **Low Toxicity**	PMRA 2434666
Terrestrial Arthropods	A scientific rationale was submitted to supplement the toxicity testing based on the lack of evidence of insecticidal activity for *B. amyloliquefaciens* (including no evidence of insecticidal toxin production) as reported in the published scientific literature and the established history of use of other members of the *B. subtilis-amyloliquefaciens*-complex in pesticide products without reports of adverse effects. No further data are required to assess the risk of harm to terrestrial arthropods.			PMRA 2336348 PMRA 2336352

Organism	Exposure	Protocol	Significant Effect and Comments	Reference
Non-arthropods				
Terrestrial Non-Arthropod Invertebrates	No information was submitted to address the potential harm to terrestrial non-arthropod invertebrates. Testing is required only for MPCAs that are intended to control non-arthropod invertebrates.			
Plants				
Plants	Various plants – corn, wheat, green onion, onion, cabbage, Napa cabbage, spinach, soybean and pumpkin, 30 days after planting	A foliar application of *B. amyloliquefaciens* strain D747 was made to each plant (3 replicates/ crop) at 5×10^8 CFU/mL or 5×10^9 CFU/mL with a re-application 7 days later. Plants were observed for 7 days after the last application.	There were no signs of phytotoxicity in any of the plants.	PMRA 2336354
Microorganisms				
Micro-organisms	A request to waive the requirement for test data was not submitted. *Bacillus amyloliquefaciens* is a bacterial and fungal pathogen that is a natural component of the soil. Any increase in exposure to the MPCA is not expected to adversely affect environmentally or economically important microbial species. No further data are required to assess the risk of harm to microorganisms.			
Aquatic Organisms				
Vertebrates				
Fish	Aquatic exposure – Rainbow trout (*Oncorhynchus mykiss*)	Rainbow trout (20) were exposed to CX-9032[4] for 30 days under semi-static conditions at a series of concentrations: 1.44×10^{10} CFU/L 2.88×10^{10} CFU/L 5.75×10^{10} CFU/L 1.15×10^{11} CFU/L 2.30×10^{11} CFU/L Another group of fish (20) were exposed to dilution water alone to serve as negative control. Fish were monitored for 30 days for mortality, growth and signs of infection.	There was a statistically significant effect on survival and growth (length and weight). No pathological changes were observed. 30-day LC_{50} value = 8.1×10^{10} CFU/L NOEC (growth) = 1.44×10^{10} CFU/L LOEC (growth) $\geq 2.88 \times 10^{10}$ CFU/L **Low Toxicity**	PMRA 2336346

Organism	Exposure	Protocol	Significant Effect and Comments	Reference
Invertebrates				
Aquatic Arthropods	Aquatic exposure – Daphnids (*Daphnia magna*)	Daphnids (20/group) were exposed to CX-9032[4] under semi-static conditions at a series of concentrations: 2.84×10^8 CFU/L 8.52×10^8 CFU/L 2.56×10^8 CFU/L 7.67×10^8 CFU/L 2.3×10^{10} CFU/L Another group of daphnids (20) were exposed to culture medium alone to serve as negative control.	There were no effects on survival. Effects on body length, and reproduction in the surviving parental generation as well as time to first brood were observed. 21-day LC_{50} > 2.30×10^{10} CFU/L NOEC (reproduction and time to first brood) = 2.84×10^8 CFU/L **Low Toxicity**	PMRA 2336353
Aquatic Non-Arthropod Invertebrates	No information was submitted to address the potential harm to aquatic non-arthropod invertebrates. Testing is required only for MPCAs that are intended to control aquatic non-arthropod invertebrates.			
Plants				
Aquatic Plants	A scientific rationale was submitted for aquatic plants based on a published study, which reported adverse effects on duck weed (*Lemma minor*) exposed to high concentrations of another strain of *B. amyloliquefaciens* (strain FZB24) and a stimulatory effect at low concentrations. Based on the proposed terrestrial use pattern, aquatic plants are not expected to be adversely affected. No further data are required to assess the risk of harm to aquatic plants.			PMRA 2336355

[1]CX-9025 is a synonym of the technical active ingredient, *B. amyloliquefaciens* strain D747 (2×10^{11} spores/g).
[2] Three non-target insect studies (*O. strigicollis*, *P. persimilis*, and *C. carnea*) were submitted within one document (PMRA 2336347).
[3]CX-9030 is a granular formulation containing *B. amyloliquefaciens* strain D747 (5×10^{10} CFU/g).
[4]CX-9032 is a liquid formulation containing *B. amyloliquefaciens* strain D747 (1×10^{10} CFU/g).

Table 3 Fungicide Resistance Action Committee Modes of Action Groups of Currently Registered Alternative Products for Use in Greenhouse and/or Field Grown Crops (as of December 2014)

Crop	Disease	Fungicide Resistance Action Committee Mode of Action Groups of Registered Alternatives
Cucurbits	Powdery mildew	3; 7; 7+11; 7+M; 9+12; 11; 11+3; 44*; 46*; M2; M4; M5; P5*; NC (citric acid + lactic acid*; garlic powder*; potassium bicarbonate*, *Streptomyces lydicus* strain WYEC 108*)
	Phytophthora blight	33; 40+45; 43; 45; 44*
Fruiting vegetables (Crop Group 8)	Gray mold	7; 44*; NC (*Gliocladium catenulatum* Strain J1446*; *Trichoderma harzianum* Rifai Strain KRL-AG2*)
	Early blight	3; 7; 44*
	Phytophthora blight	40+45; 45
Grapes	Powdery mildew	3; 7+11; 7+9; 11; 13; 44*; 46*; M2; M3+ 29; M4; P5*; U8; NC (garlic powder*; mineral oil*; potassium bicarbonate*; *Streptomyces lydicus* Strain WYEC 108*)
	Gray mold	2; 7; 7+9; 7+11; 9; 9+12; 17; 44*; 46*; NC (*Aureobasidium pullulans* DSM 14940 & DSM 14941)
Lettuce	Downy mildew	4+M3; 21; 28; 33; 40; 40+45; 43; 44*; 45; M3
	Lettuce drop	2; 7; 14; 44*; NC (*Coniothyrium minitans* strain CON/M/91-08*)
Pome fruit	Fire blight	18; 24; 44*; M2; NC (*Aureobasidium pullulans* DSM 14940* and DSM 14941*; *Pantoea agglomerans* strain C9-1 + strain E325*)
Potato	White mold	3; 7; 7+9; 29; 44*
	Early blight	3; 4 + M3; 4+M5; 7; 7+9; 7+M5; 9; 11; 11+3; 11+27; 11+M2; 22; 22+M3; 40+ M3; 44*; M2; M3; M4; M5
	Black scurf	3+ 7; 3+12; 4+M3; 7; 7+4; 11; 12; 12+M3; 44*; NC (saponins of *Chenopodium quinoa**)
Soybean	White mold	3+11; 7; 7+11; 11; 29; 44*; NC (saponins of *Chenopodium quinoa**)
Strawberry	Powdery mildew	M2; 3; 7; 7+11; 11; 13; 46; P5*; NC (citric acid + lactic acid*; *Streptomyces lydicus* Strain WYEC 108*)
	Gray mold (*botrytis*)	1; 2; 7+11; 7; 9; 17; 44*; M3; M4; M5; NC (*Streptomyces lydicus* Strain WYEC 108*; *Trichoderma harzianum* Rifai Strain KRL-AG2*)

*Microbial or otherwise non-conventional pesticides.

Table 4 List of Supported Uses

Note: Double Nickel 55 rates are indicated in kg/ha. Equivalent rates for Double Nickel LC are presented in parentheses in L/ha.

Proposed Claim	Comment
Powdery mildew (*Sphaerotheca fuliginea*, *Erysiphe cichoracearum*) on cucurbits with foliar applications of 0.5-2.5 kg/ha (2.5-12.5 L/ha) every 3 to 10 days as long as conditions favour disease.	Supported for suppression.
Phytophthora blight, wilt, and neck, collar or root rots (*Phytophthora capsici*) on cucurbits with soil applications of 0.2-0.5 kg/ha (1.0-2.5 L/ha) from planting to maturity every 2 to 4 weeks	Supported for partial suppression of "soil level phytophthora blight infection".
Gray mold (*Botrytis cinerea*) on fruiting vegetables with foliar applications of 0.9-3.6 kg/ha (4.5-18 L/ha) from planting to maturity every 3 to 10 days	Supported for suppression.
Early blight (*Alternaria solani*) on fruiting vegetables with foliar applications of 0.5-2.0 kg/ha (2.5-10.0 L/ha) from flowering to fruiting every 3 to 10 days	Supported for suppression.
Phytophthora blight, wilt, and neck, collar or root rots (*Phytophthora capsici*) on fruiting vegetables with soil applications of 0.1-0.5 kg/ha (0.5-2.5 L/ha) from planting to maturity every 2 to 4 weeks	Supported for partial suppression level for soil-level *phytophthor*a blight infection.
Powdery mildew (*Erysiphe necator*) on grapes with foliar applications of 0.5-2.0 kg/ha (2.5-10.0 L/ha) from when shoots are 1-3 cm, 6-10 cm, 18-20 cm and at 3-10 day intervals	Supported for suppression.
Gray mold (*Botrytis cinerea*) on grapes with foliar applications of 0.6-5.0 kg/ha (3.0-25.0 L/ha) at bloom, before bunch closure, at veraison, and before harvest at 3-7 day intervals	Supported for suppression and an overall application interval range of 3-10 days in line with other *B. cinerea* claims.
Downy mildew (*Bremia lactucae*) on lettuce with foliar applications of 1.0-2.5 kg/ha (5.0-12.5 L/ha) preventatively at 7-10 day intervals	Supported for suppression.

Proposed Claim	Comment
Lettuce drop (*Sclerotinia* spp.) on lettuce with foliar applications of 0.2-2.5 kg/ha (1.0-12.5 L/ha) from planting to head formation at 3-7 day intervals or with soil applications of 0.2-0.5 kg/ha (1.0-2.5 L/ha) at or after planting	Supported for suppression with pathogens specified to *S. minor* and *S. sclerotiorum* and an overall application interval range for foliar applications of 3-10 days in line with other *Sclerotinia* claims.
Fire blight (*Erwinia amylovora*) on pome fruit with foliar applications of 1.0-1.5 kg/ha (5.0-7.5 L/ha) from blossom to end of flowering at 3-7 day intervals	Supported for suppression.
White mold (*Sclerotinia sclerotiorum*) on potato with foliar applications of 0.1-2.5 kg/ha (0.5-12.5 L/ha) preventatively at 3-7 day intervals	Supported for suppression with a low rate 0.2 kg/ha (1 L/ha) instead of 0.1 kg/ha (0.5 L/ha) and an overall application interval range of 3-10 days.
Early blight (*Alternaria solani*) on potato with foliar applications of 0.5-2.0 kg/ha (2.5-10.0 L/ha) from onset of crop cover to tuber formation at 3-10 day intervals	Supported for suppression.
Black scurf (*Rhizoctonia solani*) on potato with a soil application of 0.2-1.0 kg/ha (1.0-5.0 L/ha) at planting	Supported for suppression.
White mold (*Sclerotinia sclerotiorum*) on soybean with foliar applications of 0.5-2.0 kg/ha (2.5-10.0 L/ha) from early flowering to pod set at 3-10 day intervals	Supported for suppression.
Powdery mildew (*Sphaerotheca macularis*, *Erysiphe* spp.) on strawberry foliar applications of 0.5-2.5 kg/ha (2.5-12.5 L/ha) from flowering to fruit maturity at 3-7 day intervals	Supported for suppression with intervals of up to 10 days at lower disease pressure; *Erysiphe* spp. removed as a causal pathogen.
Gray mold (*Botrytis cinerea*) on strawberry with foliar applications of 0.6-2.5 kg/ha (3.0-12.5 L/ha) from flowering to fruit maturity at 3-7 day intervals	Supported for suppression level with intervals of up to 10 days at lower disease pressure.

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A. List of Studies/Information Submitted by Registrant

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B. Additional Information Considered

i) Published Information

1.0 The Active Substance, Its Properties and Uses

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