Proposed Registration Decision PRD2011-21

Trichoderma asperellum strain T34

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Overview

Proposed Registration Decision for *Trichoderma asperellum* strain T34

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 T34 Biocontrol Technical and T34 Biocontrol, containing the technical grade active ingredient *Trichoderma asperellum* strain T34, to suppress fusarium wilt caused by *Fusarium oxysporum* on greenhouse ornamentals.

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 Section provides detailed technical information on the human health, environmental and value assessments of T34 Biocontrol Technical and T34 Biocontrol.

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 (e.g. children) as well as organisms in the environment (e.g. 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 PMRA's website at healthcanada.gc.ca/pmra. Before making a final registration decision on *Trichoderma asperellum* strain T34, the PMRA will consider all comments received from the public in response to this consultation document[3]. The PMRA will then publish a

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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."

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

Registration Decision[4] on *Trichoderma asperellum* strain T34, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

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

What Is *Trichoderma asperellum* strain T34?

Trichoderma asperellum strain T34 is a fungus which is used as a microbial pest control agent (MPCA) to control fusarium wilt caused by *Fusarium oxysporum* on greenhouse ornamentals including carnation plants. *Trichoderma asperellum* strain T34 acts by competing directly for space and/or nutrients, by parasitizing the pathogenic fungus, and by inducing plant resistance. The T34 strain of *T. asperellum* was originally isolated from a natural suppressive compost-peat mix in Spain.

Health Considerations

Can Approved Uses of *Trichoderma asperellum* strain T34 Affect Human Health?

Trichoderma asperellum strain T34 is unlikely to affect your health when T34 Biocontrol is used according to the label directions

People can be exposed to *T. asperellum* strain T34 when handling and applying T34 Biocontrol. When assessing health risks, several key factors are considered: the microorganism's biological properties (for example, production of toxic byproducts); 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.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns. When *T. asperellum* strain T34 was tested on laboratory animals, there were no signs that it caused disease, nor any toxicity other than minor effects that were quickly resolved. Furthermore *T. asperellum* strain T34 showed no growth at and above 37°C. No adverse effects from *T. asperellum* strain T34 were reported in the published scientific literature.

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

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 established as a maximum residue limit (MRL) under the *Pest Control Products Act* (PCPA) for the purposes of the adulteration provision of the *Food and Drugs Act* (FDA). Health Canada sets science-based MRLs to ensure that the food Canadians eat is safe.

As there are no direct applications to food, there is no concern for risks posed by dietary exposure of the general population, including infants and children, or animals to *T. asperellum* strain T34.

Occupational Risks From Handling T34 Biocontrol

Occupational risks are not of concern when T34 Biocontrol is used according to label directions, which include protective measures

Workers using T34 Biocontrol can come into direct contact with *T. asperellum* strain T34 on the skin, in the eyes, or by inhalation. For this reason, the label will specify that workers exposed to T34 Biocontrol must wear waterproof gloves, long-sleeved shirt, long pants, shoes plus socks, eye goggles when handling and a dust/mist filtering respirator/mask (NIOSH approval number prefix TC-21) or NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter).

For bystanders, exposure is expected to be much less than that of workers involved in loading and application activities and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When *Trichoderma asperellum* strain T34 Is Introduced Into the Environment?

Environmental risks are not of concern

Following application, *Trichoderma asperellum* strain T34 is likely able to survive in the environment under favourable environmental conditions (i.e., temperature, humidity) but that over time populations of *T. asperellum* strain T34 are expected to return to natural background levels.

The effects of *T. asperellum* strain T34 on soil microorganisms, mushrooms and horticultural crops were examined. These studies showed that *T. asperellum* strain T34 was not toxic or infectious to horticultural crops, and no significant adverse effects were noted on mushrooms and soil microorganisms.

Although avian pulmonary/inhalation/injection, wild mammal, fish, aquatic insect, and earthworms testing were not conducted, adequate information was available to determine that significant adverse effects to these non-target organisms are not expected. There are no published reports of disease associated with *T. asperellum* strain T34 in birds, wild mammals, fish, aquatic insects, and earthworms. Also, minimal exposure to non-target organisms is anticipated from the proposed use of T34 Biocontrol to control *Fusarium oxysporum* in greenhouse ornamentals including carnations.

Value Considerations

What Is the Value of T34 Biocontrol?

T34 Biocontrol is a microbial fungicide that suppresses fusarium wilt caused by *Fusarium oxysporum* on greenhouse ornamentals.

This product represents an additional disease management tool which could reduce the reliance on conventional fungicides in greenhouse ornamental production.

Measures to Minimize Risk

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

The key risk-reduction measures being proposed on the label of T34 Biocontrol to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

As with all microbial pest control products, there are concerns with users developing allergic reactions through repeated high exposures to *T. asperellum* strain T34. Therefore, anyone handling, mixing/loading, or involved in clean-up/repair activities of T34 Biocontrol must wear waterproof gloves, long-sleeved shirt, long pants, shoes plus socks, and a dust/mist filtering respirator/mask (NIOSH approval number prefix TC-21) or NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter).

Environment

As a general precaution, the label prohibits the direct application of the product to aquatic habitats (such as lakes, streams and ponds). The label also directs growers to not allow effluent or run-off from greenhouses containing this product to enter lakes, streams, ponds or other waters and to avoid contaminating surface water by disposal of equipment wash waters.

Next Steps

Before making a final registration decision on *Trichoderma asperellum* strain T34, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Trichoderma asperellum* strain T34 (based on the Science Evaluation Section of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Trichoderma asperellum strain T34

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Trichoderma asperellum* strain T34
Function	control fusarium wilt caused by *Fusarium oxysporum* on greenhouse ornamentals (including carnations)
Binomial name	*Trichoderma asperellum* strain T34
Taxonomic designation	
Kingdom	Fungi
Phylum	Ascomycota
Class	Sordariomycetes
Order	Hypocreales
Genus	*Trichoderma*
Species	*asperellum*
Strain	T34
Patent Status information	No patents are held by the applicant in Canada.
Minimum purity of active	TGAI: 8.0×10^9 colony forming units (CFU)/g EP: 1.0×10^9 colony forming units (CFU)/g
Identity of relevant impurities of toxicological, environmental and/or significance.	The technical grade active ingredient does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards. *Trichoderma asperellum s*train T34 is not known to produce potentially toxic secondary metabolites including T2 toxin and harzianum A (see Section 3.0).

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

Technical Product– T34 Biocontrol Technical and End-Use Product – T34 Biocontrol

Properties	T34 Biocontrol Technical	T34 Biocontrol
Physical state	wettable powder	wettable powder
Colour	Greenish grey	Greenish grey
Odour	No odour	No odour
Density	Not reported	Not reported
pH	5.8–6.1	5.75–6.16
Guarantee	8×10^9 CFU/g, 38.8%	1×10^9 CFU/g, 12%
Corrosion Character	None	None
Suspendibility	Suspendable	Suspendable
Viscosity	Not applicable	Not applicable

1.3 Directions for Use

T34 Biocontrol, containing 12.0% dried conidia of *T. asperellum* strain T34, is to be used for suppression of wilt caused by *F. oxysporum* on greenhouse ornamentals. It is to be applied preventatively as a soil drench to the propagation growing medium, a root dip for cuttings and by chemigation at planting and during the growing season.

1.4 Mode of Action

T. asperellum strain T34 is a fungus whose multi-site mode of action may involve antibiosis, enzyme secretion, mycoparasitism, nutrient competition, site exclusion as well as the induction of systemic resistance in plants.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

Trichoderma asprellum strain T34 can be identified to the species level through microscopic examination of standard morphological features, such as ovoidal conidia along with molecular techniques. The molecular techniques include the polymerase chain reaction (PCR) to amplify internal transcribe spacer 1 (ITS1) adjacent to the 5.8 rDNA gene as well as the ITS sequences of the transcription elongation factor 1 (tef1) gene. A single nucleotide polymorphism in the tef1 gene and the use of DNA fingerprinting with various primers are both methods that enable the distinction of the T34 strain from other strains of *T. asperellum.*

2.2 Methods for Establishment of Purity of Seed Stock

Trichoderma asperellum strain T34 is officially kept in lyophilized form in the Spanish Collection of Type Cultures (CECT No. 20417). To ensure the purity of the starter culture, Biocontrol technologies characterizes the stock culture of T34 received from CECT using the randomly amplified polymorphic DNA (RAPD) technique that shows a T34 strain specific banding pattern.

Practices for ensuring the purity of the 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 potency (CFU/g) of the technical grade active ingredient and the end-use products will be determined by direct counting using microscopy and plate counting on Tryptone Soya Agar (TSA).

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

T34 Biocontrol is not intended for use on food or feed crops. As there are no direct applications to food, no methods to determine and quantify the MPCA and relevant metabolites are required.

In the event it becomes required to analyze for residues of *T. asperellum* strain T34 in plants, DNA isolated from single spore isolations and then the PCR methods developed to identify the MPCA in section 2.1 could be used to analyze for the MPCA.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The manufacturing process described was for commercial scale production and includes steps for quality assurance. The quality assurance procedures that will be used to limit contaminating microorganisms during manufacture of T34 Biocontrol Technical and T34 Biocontrol are acceptable.

During manufacturing, several approaches will be used to limit microbial contamination in the technical product and associated end-use products (EP). These approaches will include frequent purity checks on agar media, sterilization of all equipment and media, and sanitization of recovery equipment.

The absence of human pathogens and below-threshold levels of contaminants were shown in the microbial screening of five production batches using pathogen-specific growth media. Microbe-specific screening methods for enteric bacteria/total coliforms, yeasts/moulds, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., and *Pseudomonas aeruginosa* are adequate 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. No known toxic metabolites or hazardous substances are present in T34 Biocontrol. However, *Trichoderma* spp. are known to produce secondary metabolites which act either as antifungal or antibacterial agents or plant growth regulators. Of special interest is a unique class of linear hydrophobic polypeptides called peptaibols. Peptaibols function as antibotics and contain a high proportion of α,α-dimethylisobutiric acid. Under specific conditions, a different strain, *Trichoderma asperellum* strain T32 has been shown to produce two such peptaibols, specifically trichotoxin 1704E and trichotoxin 1717A which showed antibiotic activity. Although *T. asperellum* and closely related species (*T. viride* and *T. hamatum*) are not known to produce toxic secondary metabolites, the applicant provided analytical data that showed *T. asperellum* strain T34 did not produce the known toxins T2 and harzianum A. Additional analysis for trichodermin was inconclusive, but the data suggested *T. asperellum* strain T34 produced significantly lower concentrations compared to a known fungal producer of the toxin. Analytical methods to detect peptaibols in the end-use products are available, but their analysis will not be required in the manufacturer's quality assurance program since the likelihood of adverse toxicological effects is expected to be acceptably low.

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

Results from storage stability testing of five batches showed that the end-use product is stable when stored at 4–15°C for one year.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

The PMRA conducted a detailed review of the toxicological database for *T. asperellum* strain T34. See Appendix I, Table 1 for a summary table of the test data. The database is complete, consisting of laboratory animal (*in vivo*) toxicity and/or infectivity studies (acute oral toxicity and infectivity, acute oral toxicity, acute pulmonary toxicity/infectivity, acute intraperitoneal infectivity, acute inhalation toxicity, and acute dermal toxicity), as well as irritation studies (dermal irritation and eye irritation) required for health hazard assessment purposes which were carried out in accordance with currently accepted international testing protocols and Good Laboratory Practices. In all of the studies submitted, the test substance was comprised of the EP, T34 Biocontrol. The scientific quality of the data is high and the database is considered sufficient to characterize the toxicity and infectivity of this pest control agent and product.

In an acute oral toxicity/infectivity study, no significant toxicity was observed in Wistar-Hanover rats following oral gavage with the EP at a dose of 1.1×10^8 CFU of *T. asperellum* strain T34 per animal. The MPCA was initially detected in the brain, liver, lymph nodes and lung. The MPCA was cleared from all organs and tissues by Day 21. At necropsy, colour changes were seen in the adrenal glands and the pancreas in the treated animals but were also seen to a lesser extent in some of the untreated control animals. Based on the results of this study *T. asperellum* strain T34 is of low toxicity and is non-infective in the rat when challenged via the oral route.

In an acute oral toxicity study, no mortalities and no signs of toxicity were observed in Wistar-Hanover rats following oral gavage with 2000 mg/kg bw of the end-use product (*T. asperellum* strain T34 at 3.6×10^9 CFU/kg bw).

In an acute inhalation toxicity study, no mortalities and no signs of toxicity were observed in Sprague-Dawley rats following nose-only inhalation exposure for 4 hours with 2.03 mg/L of the end-use product (*T. asperellum* strain T34 at 3.65×10^6 CFU/animal).

In a pulmonary toxicity/infectivity study, no significant toxicity was observed in Wistar-Hanover rats following intratracheal treatment with the EP at a dose of 1.0×10^7 CFU of *T. asperellum* strain T34 per animal. At necropsy, most animals showed changes in the lung and reddish colour and swellings of the pancreas. Day 7 females showed colour changes in the adrenal glands. Five animals from the study showed an enlarged thymus. Two males from Day 14 showed irregular shaped fringe of the spleen and one lobe of the liver. One female rat had significant body loss by Day 3. Necropsy showed empty colon and colour changes in the liver with brightened fringe. Necropsy did not show any hyphal growth. These observations from necropsy were attributed to the dosing method and the high dose level rather than with the actual test substance. On Day 0, the MPCA was only substantially present in the lungs and minimally in other organs or tissues. A pattern of clearance was seen by Day 21 where minimal amounts were only in the lungs. Based on the results of this study *T. asperellum* strain T34 is of low toxicity and is non-infective in the rat when challenged via the pulmonary route.

In an intraperitoneal infectivity study, clinical signs of toxicity, including reduced spontaneous activity, recumbency, wasp waist, kyphosis, piloerection, eye closure and a reduction in body weight, were initially seen in Wistar-Hanover rats following injection with the EP at a dose of 7.3×10^7 CFU/animal of *T. asperellum* strain T34. However, affected animals recovered from the findings within three weeks. One rat died at Day 6 and another at Day 15, where histopathology indicated death was as a result of peritonitis. At Day 3, the MPCA was initially recovered in the lungs, spleen, liver, kidneys and lymph nodes. At Day 21, microbial counts had diminished, establishing a pattern of clearance. The deaths, observed clinical signs of toxicity and pattern of clearance indicate that the high dose of the EP may have caused an immune response rather than resulting from infectivity. Based on the results of this study *T. asperellum* strain T34 is of low toxicity and is non-infective in the rat when challenged via the intraperitoneal route.

In an acute dermal toxicity study, no mortalities and no signs of toxicity were observed in rabbits treated with the EP at a dose of 2000 mg/kg body weight over 10% of total body surface after a 24-hour exposure period.

In a dermal irritation study, no signs of irritation were observed in rabbits treated with the EP at a dose of 0.5 g/animal over 6 cm^2 of body surface after a 4-hour exposure.

In an eye irritation study, minimal irritation of the conjunctivae was observed one hour after 0.1 g of the ground EP was instilled into the conjunctival sac of the right eye of New Zealand white rabbits. Irritation diminished by Day 3 and completely resolved by Day 6 of the treatment period. The EP is minimally irritating to the eye based on the maximum irritation score (MIS) of 11.3/110 (at 1 hour). Consequently neither eye irritation signal words nor label precautions are required on the principal display panel of T34 Biocontrol.

In a dermal sensitization study, sensitization was observed in only 2 of the 10 guinea pigs tested after a first induction with a saline solution containing 2.5% of the EP and after a second induction with a saline solution containing 50% of the EP. Nevertheless, because most microorganisms contain substances that elicit positive hypersensitivity reactions in humans, *T. asperellum* strain T34 is considered to be a potential sensitizing agent. Consequently the signals words "POTENTIAL SENSITIZER" are required on the principal display panels of T34 Biocontrol Technical (TGAI) and T34 Biocontrol.

A statement summarizing the number of people who were potentially exposed to *T. asperellum* strain T34 during production from 2005 to 2010 indicated that no incidence of hypersensitivity occurred. Although a hypersensitivity study is not required, any incidents of hypersensitivity in workers or bystanders must be reported to the PMRA as a condition of registration according to Section 13 of the *Pest Control Products Act*.

A survey of published scientific literature revealed no cases of adverse effects caused by *T. asperellum*. Some cases of infection caused by phylogenetically related *T. viride* were reported in immunocompromised patients in hospital.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute 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 tests.

Within the available scientific literature, there are no reports to suggest that *T. asperellum* strain T34 has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *T. asperellum* strain T34.

3.2 Occupational / Bystander Exposure and Risk Assessment

3.2.1 Occupational

When handled according to the label instructions, the potential for dermal, inhalation and eye exposure for applicators, mixer/loaders, and handlers exist, with the primary source of exposure to workers being exposure to the skin or inhalation of dusts.

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. *Trichoderma asperellum* has not been identified as a wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Also, *T. asperellum* strain T34 demonstrated low toxicity and minimal irritation in the dermal toxicity/irritation studies.

Workers resuspending the wettable powder formulation for application are at risk for inhalation of dusts and/or particulate matter. Inhalation exposure from applications is expected to be minimal. Based on the toxicological profile for *T. asperellum* strain T34, exposure to a large single quantity of the MPCA via the pulmonary route is not of concern.

The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Label statements (i.e., Potential Sensitizer) and risk mitigation measures such as the wearing of personal protective equipment, including waterproof gloves, long-sleeved shirt, long pants, shoes plus socks, and a dust/mist filtering respirator/mask (NIOSH approval number prefix TC-21) or NIOSH approved respirators (with any N-95, P-95, R-95 or HE filter) are required to minimize exposure and protect mixer/loaders, applicators and handlers who are most likely to be exposed repeatedly to the product.

Based on the results from the eye irritation study which showed the EP to be minimally irritating, label statements and risk mitigation measures such as the wearing of eye goggles when handling are not required.

3.2.2 Bystander

Based on the low toxicity/pathogenicity profile for *T. asperellum* strain T34, and the use of T34 Biocontrol for commercial greenhouses only, bystander exposure is not of concern.

3.3 Incident Reports Related to Human and Animal Health

Since April 26, 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Health Canada website. Incidents from Canada and the United States were searched and reviewed for products containing *Trichoderma asperellum*. As of July 22, 2011, there were no health-related incident reports submitted to the PMRA, nor summarized by the U.S. EPA or the California Department of Pesticide Regulation (CalDPR), for end-use products containing *Trichoderma asperellum*.

3.4 Dietary Exposure and Risk Assessment

3.4.1 Food

As there are no direct applications to food, there is no concern for risks posed by dietary exposure of the general population, including infants and children, or animals to *T. asperellum* strain T34.

3.4.2 Drinking Water

The EP is a powder formulation which will be applied as a liquid suspension, to potting growth media or rooting plant cuttings in greenhouse ornamentals by spray, root dip, or chemigation. No risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and there were no harmful effects observed in animals that were exposed orally in Tier I acute oral toxicity and infectivity testing. The T34 Biocontrol label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes. Also, municipal treatment of drinking water will likely remove the transfer of residues to drinking water. Therefore, potential exposure to *T. asperellum* strain T34 in surface and drinking water is negligible.

3.4.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARfDs) and acceptable daily intakes (ADIs) are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e., no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the Agency concludes that the MPCA is of low toxicity is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCA than the general population. Thus there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability. 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 Agency has not used a margin of exposure (safety) approach to assess the risks of this MPCA to human health.

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 established as a maximum residue limit (MRL) under the *Pest Control Products Act* (PCPA) for the purposes of the adulteration provision of the *Food and Drugs Act* (FDA). Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

As there are no direct applications to food crops the establishment of a MRL is not required for *T. asperellum* strain T34.

3.6 Aggregate Exposure

Based on the toxicity and infectivity test data submitted for the technical grade active ingredient and the end-use product and other relevant information in the Agency's files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *T. asperellum* strain T34 to the general Canadian population, including infants and children, when the pest control product is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Even if there is an increase in exposure to *T. asperellum* from the uses of T34 Biocontrol, there should not be any increase in potential human health risk as evidenced in the low toxicity data seen in the dermal and pulmonary studies.

3.7 Cumulative Effects

The PMRA has considered the available information on the cumulative effects of residues and other substances that have a common mechanism of toxicity and action. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Besides naturally occurring strains of *Trichoderma asperellum* in the environment, the PMRA is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity or action with this microbial active ingredient. No cumulative effects are anticipated if the residues of the *T. asperellum* strain T34 found in T34 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Species of *Trichoderma* are cosmopolitan soil inhabiting filamentous fungi present in nearly all soils and other diverse habitats such as decaying wood. In soil, *Trichoderma* spp. frequently are the most prevalent culturable fungi. *Trichoderma asperellum* strain T34 was isolated from a natural suppressive compost-peat mix in Barcelona, Spain. The compost used was commercially available, and was made from vegetable and animal market wastes, sewage sludge and yard wastes.

Environmental fate testing is intended to demonstrate whether a microbial pest control agent (MPCA) is capable of surviving or replicating in the environment to which it is applied, and could provide an indication of which non-target organisms may be exposed to the MPCA as well as provide an indication of the extent of exposure. Environmental fate data are not normally required for Tier I risk assessment purposes and are only triggered if significant toxicological effects in non-target organisms are noted in Tier I testing. Since no significant toxicological effects are expected from the greenhouse use of T34 Biocontrol, no fate data are required to complete the environmental risk assessment of T34 Biocontrol Technical and T34 Biocontrol.

However, four studies were submitted by the applicant to address the environmental fate of *T. asperellum* strain T34 in soil, plant growth media (i.e., coir fibre and peat) and water. Results of these fate studies were used to formulate rationales to waive many of the terrestrial and aquatic non-target organism toxicology study requirements (Tier I). *Trichoderma asperellum* strain T34 was found to persist in soil through a two-year sampling period and in soilless plant growth media through four months of sampling. In simulated river and ground water, *T. asperellum* strain T34 was found to persist throughout a sampling period of 23 days. While not generally recognized as an aquatic fungus, growth of *T. asperellum* strain T34 could occur if sufficient nutrients were present in water (e.g., in presence of decaying plant material).

4.2 Effects on Non-Target Species

4.2.1 Effects on Terrestrial Organisms

Four studies were submitted to address the hazards of *T. asperellum* strain T34 to soil microorganisms, mushrooms and terrestrial horticultural crops.

In one study, the effect of *T. asperellum* strain T34 on the response of soil microbial communities was evaluated when plant growth media wastes (coir fibre and peat, see environmental fate studies on plant growth media under Section 4.1), with or without *T. asperellum* strain T34, were added to soil as an amendment. Soil samples were taken from the top layer (0–20 cm) of soil previously cultivated with horticultural crops. Soil was mixed with plant growth media (5% w/v) and incubated for 7 days at 25ºC. Aliquots of each treatment were used immediately for biological assays (i.e., microbial diversity, microbial biomass and community level physiological profiles). The amendment of a horticultural soil with coir fibre wastes or peat wastes treated with *T. asperellum* strain T34 reduced the populations of fluorescent *Pseudomonas* spp. and cellulolytic actinomycetes, respectively. The growth media wastes and treatment with *T. asperellum* strain T34 affected none of the other microbial econutritive groups in the amended soil. The plant growth media waste did not affect the soil microbial biomass, but it was reduced when the growth media were treated with *T. asperellum* strain T34. However, the microbial richness/diversity and groups of carbon sources used by the microbial community in the amended soil were more affected by type of plant growth media added to the soil than treatment with *T. asperellum* strain T34. Moreover, the effect of the amendment with plant growth media treated with T34 on the community level physiological profiles depended on the type of growth media added to the soil. Overall, the effect of the plant growth media waste used as a soil amendment on the soil microbial communities had a greater

effect than the presence of *T. asperellum* strain T34. Based on the results of this study, *T. asperellum* strain T34 is not likely to greatly impact soil microbial communities.

In another study, the ability of *T. asperellum* strain T34 to induce disease in mushrooms (*Agaricus bisporus*) was studied. It is widely known that several strains of *Trichoderma agressivum* (that belong to the Harzianum clade of *Hypocrea/Trichoderma*) are pathogens of mushrooms causing green mould disease of the commercial mushroom *Agaricus bisporus*. Other recently described *Trichoderma* species, *Trichoderma pleurotum* and *Trichoderma pleuricola* (which also belong to the Harzianum clade of *Hypocrea*/Trichoderma) are also pathogens of the commercial oyster mushroom *Pleurotus ostreatus*. The ability of *T. asperellum* strain T34 to cause disease in *A. bisporus* was evaluated by adding the microbial pest control agent to the mushroom growth media (compost) at final concentrations of 10^3 and 10^4 CFU/mL 10 days before the addition of *A. bisporus*. Populations of *Trichoderma* spp. were assayed at the beginning of the study, on Day 10 (before the addition of *A. bisporus*) and at the end of the study by plating dilutions on selective media. After 10 days, *T. asperellum* strain T34 colonized the mushroom growing medium to a concentration of 10^5 CFU/mL in both treatment groups. However, *T. asperellum* strain T34 seemed to be inhibited by the presence of the host mushroom mycelia (*A. bisporus*) since the populations of *T. asperellum* strain T34 decreased two orders of magnitude (from ~10^5 CFU/mL to ~10^3 CFU/mL) after 2 months. The study author also noted that, after 2 months, *T. asperellum* strain T34 was not pathogenic to mushrooms since no disease was obtained in mushroom growth media inoculated with *T. asperellum* strain T34. However, too few data were provided to support the study author's conclusions that *T. asperellum* strain T34 was not pathogenic to *A. bisporus*. The study report did not describe how disease was assessed in the host crop. The study report, however, did show that *T. asperellum* strain T34 could not outcompete the host crop since a significant reduction in *Trichoderma* populations were noted after the host crop was introduced into the compost medium despite significant increases in populations in the first 10 days of the study. Based on the results of this study, *T. asperellum* strain T34 did not outcompete *A. bisporus* in compost medium and is therefore not likely to cause disease in this host crop.

In two plant studies, the phytotoxicity and pathogenicity of *T. asperellum* strain T34 was evaluated in cucumber, lettuce, tomato and radish grown in either coir fibre or peat-based soilless media. Seeds of these plants were sown in coir fibre or peat that was previously used to grow carnation plants for two months and which received multiple treatments of T34 as follows. Carnation cuttings were submerged overnight in a T34 suspension containing either 1×10^3, 1×10^4 or 1×10^5 CFU/mL. Following the overnight submersion, the carnation cuttings were planted in T34-enriched plant growth medium, coir fibre or peat, at 1×10^3, 1×10^4 and 1×10^5 CFU/mL. After one week, *T. asperellum* strain T34 was applied to the pots by irrigation with a half-dose treatment of *T. asperellum* strain T34 (i.e., 0.5×10^3, 0.5×10^4 and 0.5×10^5 CFU/mL). Eight weeks after planting, the full dose of *T. asperellum* strain T34 (i.e., 1×10^3, 1×10^4 and 1×10^5 CFU/mL) was applied to the pots. Some of these carnation plants were also treated with various combinations of the soil pathogen *Fusarium oxysporum* f.sp. *dianthi*, himexazol (5-methyl 3-isoxazol 36% w/v), and Trianum-P (containing *Trichoderma harzianum* strain T22). After removal of the carnation plants, cucumber, lettuce, tomato and radish seeds were sown in the T34-treated plant growth media and allowed to grow. The crops were not treated further with *T. asperellum* strain T34. At the end of the study, the fresh weight of the aerial part of plants, dry

weight (cucumber only) and chlorophyll content of cucumber plants were measured. *Trichoderma asperellum* strain T34 did not produce any dose-dependent negative effects to cucumber, tomato, lettuce and radish plants compared to untreated control plants.
In addition to the above non-target soil microorganism and crop plant studies, a scientific rationale was submitted to waive testing on birds, mammals, arthropods, non-arthropod invertebrates, microorganisms and non-target plants based on the properties of the MPCA, its similarities and differences to other species of *Trichoderma,* and the limited potential for environmental exposure from the proposed use of T34 Biocontrol in greenhouses.

Trichoderma asperellum was first described following an integrated morphological and molecular analysis of the paraphyletic group *T. viride*. The analysis confirmed that *T. viride* types I and II should be redefined into two separate species. Type I, being the true *T. viride* species, includes the anamorph of *Hypocrea rufa*, and is grouped together with strains of *T. atroviride* and *T. koningii*. Type II represents the new species, *T. asperellum* , which has ovoidal rather than globose conidiation and also darker and faster conidiation. Until the use of molecular techniques, *T. asperellum* was considered as *T. viride*. In terms of molecular characteristics, it was shown that *T. asperellum* is closer to *T. hamatum* rather than to *T. viride*.

Similar to other biological control isolates of *Trichoderma* spp., the mode of action of *T. asperellum* strain T34 is highly complex. Many *Trichoderma* spp. such as T34 have suppressive effects on the soil-born pathogens such as *Fusarium oxysporum*, by directly competing for space and/or nutrients, and by parasitizing the host fungi through the release various fungitoxic metabolites and/or enzymes. *Trichoderma* spp., including strain T34, are now also considered to be opportunistic avirulent plant symbionts. These fungi invade the first layers of the root hair and stimulate resistance to attack by pathogens. The ability of *T. asperellum* strain T34 to stimulate resistance to attack by soil pathogens was demonstrated in two studies. In these studies, two separate phenomena were observed. *T. asperellum* strain T34 acted as a vaccine when it was applied to the roots of plants at standard concentrations. No apparent changes were detected in the plants after the treatment, however, when the plants encountered a pathogen, the plant defense mechanisms were quickly activated. The signalling pathway involved in T34-induced resistance was elucidated using mutant/transgenic plants. T34-induced resistance shares the same signalling pathway than non-pathogenic rhizobacteria-induced resistance also known as induced systemic resistance (ISR), when applied at the standard concentrations. However, if higher concentrations of *T. asperellum* strain T34 were applied to the roots of the plants, a series of alterations could be observed on the systemic tissue. For instance, an increase of the peroxidase activity peaks of defense hormones salicylic and jasmonic acids were detected. In addition, the proteome of the plant was reoriented to switch from an assimilatory metabolism to a non-assimilatory defensive state. This higher treatment concentration was also able to protect the plant from the attack of the pathogen, *Pseudomonas syringae,* and was similar to systemic acquired resistance (SAR). This phenomenon is expected to be found only transiently at certain times of the interaction between plants and *T. asperellum* strain T34 as the concentrations needed to elicit this physiological response in plants do not usually occur naturally.

At the time of application, negligible exposure to *T. asperellum* strain T34 is expected from the proposed use of T34 Biocontrol in greenhouses. The product is to be applied in a water solution by drip irrigation or root bath to inhibit diseases caused by *Fusarium oxysporum*. Microscopical studies have shown that colonization of T34 is limited to the epidermis of the plant roots, as other *Trichoderma* spp., and was never observed to colonize the cortex nor in the vessels of the roots. *T. asperellum* strain T34 cannot be translocated to the upper parts of the plant by the xylem or phloem and, thus, it cannot colonize the flowers (nectar, pollen, etc) and seeds. However, soil insects and other non-arthropod invertebrates may be exposed to *T. asperellum* strain T34 through contact with treated plant growth material during composting. As noted in Section 4.1, *Trichoderma* populations were generally maintained in spent plant growth medium after 4 months. Environmental fate data also showed that *T. asperellum* strain T34 is likely to persist in the upper layers of soil with decreasing populations at increasing depths. Despite this possibility for exposure, few adverse effects are expected. A search in the databases of PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and AGRIS (http://agris.fao.org/) using the keywords "trichoderma asperellum", "trichoderma hamatum" and "trichoderma viride" found numerous reports of adverse effects of these species on soil-borne fungal pathogens. Research attempts to biologically control the root-knot nematode, *Meloidogyne incognita*, with these species were also found in the open literature, but *T. asperellum* is generally considered to be non-pathogenic to non-arthropod invertebrates. Furthermore, the survival of conidia or mycelia ingested via feed or direct colonization of birds is unlikely because no growth was observed in *T. asperellum* strain FE 9901 at temperatures greater than or equal to 37°C. Acute mammalian toxicity and pathogenicity testing with *T. asperellum* strain T34 produced no pathogenic effects (see Section 3.1).

Based on all the available data and information on the effects of *T. asperellum* strain T34 to non-target organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, plants and other microorganisms from the proposed use of T34 Biocontrol.

4.2.2 Effects on Aquatic Organisms

No studies were submitted to address the hazards of *T. asperellum* strain T34 to non-target aquatic organisms. Instead, a scientific rationale was submitted to waive testing on fish, aquatic arthropods, aquatic non-arthropod invertebrates and aquatic plants. This rationale was also based on the properties of the MPCA, and the limited potential for exposure from the use of T34 Biocontrol in greenhouses.

Based on the proposed use of T34 Biocontrol, the primary method of dissemination to aquatic environments is expected to occur via leaching and runoff from treated plant growth material. As described in Section 4.1, *T. asperellum* strain T34 is expected to leach and run-off from treated plant growth media albeit at limited concentrations, i.e., 80 – 130 times less concentrated than in the spent plant growth media. Growth of *T. asperellum* strain T34, however, is not expected in natural aquatic environments unless significant nutrients are present. Furthermore, aquatic exposure to *T. asperellum* strain T34 will be greatly reduced by the addition of standard label statements prohibiting the contamination aquatic habitats during application, clean-up and repair, as well as preventing effluent and run-off of treated greenhouses from entering lakes, streams, ponds or other waters.

No reports of adverse effects on aquatic organisms were found in the published scientific literature following a search in the databases of PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and AGRIS (http://agris.fao.org/) using the keywords "trichoderma asperellum", "trichoderma viride", and "trichoderma hamatum".

Based on the absence of available data on the effects of *T. asperellum* to aquatic organisms, there is reasonable certainty that no harm will be caused to non-target aquatic organisms from the use of T34 Biocontrol. As a precaution, standard label statements will prohibit handlers from contaminating aquatic habitats during application, clean-up and repair, as well as prohibit the effluent and run-off of treated greenhouses from entering lakes, streams, ponds or other waters.

4.3 Incident Reports related to the Environment

Since April 26, 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website http://www.hc-sc.gc.ca/cps-spc/pest/part/protect-proteger/incident/indexeng.php. Only incidents in which the pesticide is determined to be linked to the effects (Canadian causality of highly probable, probable and possible; U.S. causality of highly probable, probable and possible) are considered in the reviews. As of July 25, 2011, there were no environmental incidents reported in the PMRA Incident reporting database nor in the U.S. EPA's Ecological Incident Information System (EIIS) for products containing *Trichoderma* species.

5.0 Value

5.1 Effectiveness Against Pests

5.1.1 Acceptable Efficacy Claims

Four greenhouse trials and six growth chamber trials conducted in Spain from 2005-2008 tested the efficacy of T34 Biocontrol against *Fusarium oxysporum* f. sp. *dianthi* on carnations. Growth chamber trials conducted on tomatoes (*F. oxysporum* f. sp. *lycopersici*) were also considered as supplementary data. Considering that no root assessments were performed in these trials, and that *F. oxysporum* not only infects the roots, but also spreads into stem and petiole xylem vessels, the claim for 'root diseases' was replaced with 'fusarium wilt'.

T34 Biocontrol provided consistent and adequate suppression of *F. oxysporum* f. sp. *dianthi* on carnations under moderate to high disease pressure when applied as a drench to the potting mix, followed with a root dip for cuttings and chemigation applications during the growing season. T34 Biocontrol significantly reduced the incidence and severity of *F. oxysporum* f. sp. *lycopersici* on tomatoes, which shows that this product is efficacious against another *F. oxysporum* formae specialis. Given that fusarial wilts have comparable characteristics of disease development, it is likely that T34 Biocontrol would similarly antagonize the different *F. oxysporum* formae speciales with its broad-spectrum mode of action.

Based on these considerations, the use of T34 Biocontrol is supported for suppression of fusarium wilt caused by *F. oxysporum* on greenhouse ornamentals. Confirmatory data have been requested to confirm the efficacy of T34 Biocontrol in other representative greenhouse ornamentals.

5.2 Economics

No market analysis was performed for this application.

5.3 Sustainability

5.3.1 Survey of Alternatives

Refer to Appendix I, Table 3 for a summary of the active ingredients currently registered for the uses supported with T34 Biocontrol.

5.3.2 Compatibility with Current Management Practices Including Integrated Pest Management

At this time, it is recommended to avoid treatments with chemical fungicides 10 days after or before the application of T34 Biocontrol. Confirmatory data have been requested in order to assess the product compatibility with conventional pesticides.

5.3.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

Based on the multi-site mode of action of *T. asperellum* strain T34, the risk of disease resistance development is not a significant concern.

5.3.4 Contribution to Risk Reduction and Sustainability

T34 Biocontrol represents an additional disease management tool which could reduce the reliance on conventional fungicides such as thiophanate-methyl, which has a high risk for resistance development.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

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

T34 Biocontrol Technical and T34 Biocontrol were assessed in accordance with the PMRA Regulatory Directive DIR99-03[5] and evaluated against the Track 1 criteria. The PMRA has reached the following conclusions:

- T34 Biocontrol 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 product 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:

- T34 Biocontrol Technical and T34 Biocontrol do not contain any formulants or contaminants of health or environmental concern identified in the Canada Gazette.

The use of formulants in registered pest control products is assessed on an ongoing basis through PMRA formulant initiatives and Regulatory Directive DIR2006-02.[9]

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5 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/2005-114 (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 1 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 NOI2005-01, List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern under the New Pest Control Products Act.

8 DIR2006-02, PMRA Formulants Policy.

9 DIR2006-02, PMRA Formulants Policy.

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for T34 Biocontrol Technical and T34 Biocontrol (EP) were deemed adequate to assess their potential human health and environmental risks. T34 Biocontrol Technical was characterized and the specifications of T34 Biocontrol were supported by the analyses of a sufficient number of batches. Storage stability data were sufficient to support a shelf life of one year when stored between 4°C–15°C.

7.2 Human Health and Safety

The acute toxicity and infectivity studies submitted in support of *Trichoderma asperellum* strain T34 were determined to be sufficiently complete to permit a decision on registration for greenhouse use. The EP with conidia of *T. asperellum* strain T34 was of low toxicity in the rat when administered via oral, pulmonary and dermal routes and was not infective via the oral, pulmonary and intraperitoneal routes of exposure with a pattern of clearance established by Day 21. The EP was not irritating to the skin and was minimally irritating to the eyes of rabbits.

When handled according to the label instructions, the potential for dermal, inhalation and eye exposure for applicators, mixer/loaders, and handlers exist, with the primary source of exposure to workers being exposure to the skin or inhalation of dusts. Precautionary statements on the T34 Biocontrol label and the wearing of personal protective equipment (PPE) by workers will adequately mitigate the risks from exposure. Furthermore, precautionary labelling will alert users of the potential sensitization hazard of the product.

7.3 Environmental Risk

The environmental fate studies, non-target studies, scientific rationales and supporting published scientific literature submitted in support of *T. asperellum* strain T34 were determined to be sufficiently complete to permit a decision on registration. The use of T34 Biocontrol containing *T. asperellum* strain T34 is not expected to pose a risk to birds, mammals, arthropods, fish, and plants.

No additional studies were required to address the environmental fate and behaviour of *T. asperellum* strain T34. Environmental fate data are higher tier requirements and are not normally required in the absence of significant toxicological effects in non-target organisms in Tier I testing. Environmental exposure to *T. asperellum* strain T34 is expected to be minimal given that the use of T34 Biocontrol is limited to greenhouses.

As a general precaution, the T34 Biocontrol label prohibits the direct application of T34 Biocontrol to aquatic habitats (such as lakes, streams and ponds) and the release of greenhouse effluent and run-off to natural aquatic systems. The label also directs users to avoid contaminating surface water by disposal of equipment wash waters.

7.4 Value

The data submitted to register T34 Biocontrol are adequate to support its use for suppression of fusarium wilt on greenhouse ornamentals. Additional data are required to confirm the product efficacy in other representative ornamentals as well as its compatibility with conventional pesticides.

7.5 Unsupported Uses

The label claim "suppression of root diseases" was replaced with "suppression of fusarium wilt" given that no root assessments were performed in the trials, and *F. oxysporum* not only infects the roots, but also spreads into stem and petiole xylem vessels.

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 T34 Biocontrol Technical and T34 Biocontrol, containing the technical grade active ingredient *Trichoderma asperellum* strain T34, to suppress fusarium wilt caused by *Fusarium oxysporum* on greenhouse ornamentals.

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

ADI	acceptable daily intake
AGRIS	International System for Agricultural Science and Technology
ALS	acetolactate synthase
ARfD	acute reference dose
bw	body weight
CalDPR	California Department of Pesticide Regulation
CFU	colony forming units
cm	centimetres
DACO	data code
DNA	deoxyribonucleic acid
EIIS	U.S. EPA's Ecological Incident Information System
EP	end-use product
FDA	Food and Drugs Act
g	gram
ISR	Induced Systemic Resistance
ITS	internal transcribe spacer
kg	kilogram
L	litre
LD_{50}	lethal dose 50%
m	metre(s)
mg	milligram
mL	millilitre
MAS	maximum average score
MIS	Maximum Irritation Score
MCPA	microbial pest control agent
MRL	maximum residue limit
NIOSH	National Institute for Occupational Safety and Health
PCR	polymerase chain reaction
PMRA	Pest Management Regulatory Agency
PCPA	Pest Control Product Act
PPE	personal protective equipment
rDNA	ribosomal deoxyribonucleic acid
SAR	systemic acquired resistance
tef	transcription elongation factor
TSA	tryptone soya agar
TGAI	technical grade active ingredient
TSMP	Toxic Substances Management Policy
U.S. EPA	United States Environmental Protection Agency
w/v	weight by volume

Appendix I Tables and Figures

Table 1 Summary of Toxicology Information Submitted for T34 Biocontrol Technical and T34 Biocontrol.

Study Type	Species, Strain, and Doses	Results	Comments	Reference(s)
Acute Toxicity/Infectivity of T34 Biocontrol (End-Use product)				
Acute Oral (acute oral toxicity)	Rat: Wistar-Hanover 3 females, 2000 mg/kg bw (3.6×10^9 CFU/kg bw) single oral dose by gavage (14-day study)	LD_{50} > 2000 mg/kg bw	**LOW TOXICITY ACCEPTABLE**	PMRA 2033838
Acute Oral Infectivity and Toxicity (21-Day study)	Rat: Wistar-Hanover 12/sex, 100 mg/1.5 mL/animal (1.1×10^8 CFU/animal) 4 untreated control (2/sex)	LD_{50}> 1.1×10^8 CFU/animal	**LOW TOXICITY NO PATHOGENICITY NO INFECTIVITY ACCEPTABLE**	PMRA 2033951, 2033957, 2033953
Acute Inhalation study (14-day study)	Rat: Sprague-Dawley rats, 5/sex, 2.03 mg/L/animal (3.65×10^6 CFU/ animal)	LD_{50}> 2.03 mg/L/animal	**LOW TOXICITY ACCEPTABLE**	PMRA 2033842
Pulmonary Toxicity and Infectivity (Intratracheal)	Rat: Wistar-Hanover 14/sex: suspension in sterile distilled water, 1.0×10^7 CFU/0.1mL per animal, interim sacrifices on Days 3, 7, 14 and 21 (21-day study). 5/sex untreated control	LD_{50}> 1.0×10^7 CFU/animal	**LOW TOXICITY NO PATHOGENICITY NO INFECTIVITY ACCEPTABLE**	PMRA 2033959, 2033961, 2033963, 2033941
Intra-peritoneal Infectivity	Rat: Wistar Han 12/sex; suspension in saline (0.9% NaCl) 7.3×10^7 CFU/animal (in 1 mL), interim sacrifices at Days 3, 7, 14, 21; (21-day study) Control: 2/sex; saline only	LD_{50} > 7.3×10^7 CFU/animal	**NO INFECTIVITY ACCEPTABLE**	PMRA 2029870
Acute	Rabbit: New Zealand	LD_{50} > 2000 mg/kg	**LOW TOXICITY**	PMRA 2033840

Study Type	Species, Strain, and Doses	Results	Comments	Reference(s)
Dermal Toxicity	white, 5/sex, undiluted, 1.0×10^9 CFU/g per animal to an area of approximately 10% of total body surface, exposed for 24 hours (14-day study) (2000 mg/kg bw)	bw	**ACCEPTABLE**	
Acute Irritation/Sensitization of End-Use Product				
Dermal Irritation	Rabbit: New Zealand white, 3 females, undiluted, 4.5×10^4 CFU/g to an area of 240 cm^2, exposed for 4 hours, 3-day study	Not a dermal irritant	**ACCEPTABLE**	PMRA 2033844
Eye Irritation	Rabbit: New Zealand white, 3 females, 0.1 g of the ground end-use product (equivalent to 1.8×10^8 CFU/animal), conjunctival sac of right eye, instilled for entire study. Observed for 144 hours (1/3) or 168 hours (2/3) after instillation.	MAS[1]= 6.4/110 (24, 48 and 72 hours) MIS[2] = 11.3/110 (1 hour)	**MINIMALLY IRRITATING** **ACCEPTABLE**	PMRA 2033846
Dermal Sensiti-zation	Guinea pig: Hsd Poc: DH 10 female First induction: 2.5% of EP in isotonic saline Second induction: 50% of EP in isotonic saline 5 females/negative control	Not a dermal sensitizer	As the PMRA assumes most microorganisms contain substances that elicit positive hypersensitivity reactions in humans, *T. asperellum* strain T34 is considered to be a potential sensitizing agent.	PMRA 2033848

[1] MAS = Maximum Average Score
[2] MIS = Maximum Irritation Score

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Organisms				
Vertebrates				
Birds	Oral Pulmonary	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for exposure from the proposed use of T34 Biocontrol in greenhouses. No adverse effects to birds were found in the published scientific literature		PMRA 2034091, 2034100 and 2034912

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
		and *T. asperellum* strain T34 does not grow at temperatures above 37°C. Also, minimal exposure to birds is anticipated from the proposed use of T34 Biocontrol in greenhouses. **WAIVER RATIONALE ACCEPTED**		
Wild Mammals	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for exposure from the proposed use of T34 Biocontrol in greenhouses. No adverse effects to mammals were found in the published scientific literature. In addition, no pathogenicity was observed in acute mammalian toxicity and infectivity testing (see Section 3.1) and minimal exposure to wild mammals is anticipated from the proposed use of T34 Biocontrol in greenhouses. **WAIVER RATIONALE ACCEPTED**			PMRA 2034091, 2034100 and 2034912
Invertebrates				
Arthropods				
Terrestrial Arthropods	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for exposure from the proposed use of T34 Biocontrol in greenhouses. No adverse effects to terrestrial arthropods were found in the published scientific literature. In addition, minimal exposure to terrestrial arthropods is anticipated from the proposed use of T34 Biocontrol in greenhouses. **WAIVER RATIONALE ACCEPTED**			PMRA 2034091, 2034100 and 2034912
Non-arthropods				
Terrestrial Non-Arthropod Invertebrates	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for exposure from the proposed use of T34 Biocontrol in greenhouses. No adverse effects to terrestrial non-arthropod invertebrates were found in the published scientific literature. In addition, minimal exposure to terrestrial non-arthropod invertebrates is anticipated from the proposed use of T34 Biocontrol in greenhouses. **WAIVER RATIONALE ACCEPTED**			PMRA 2034091, 2034100 and 2034912
Microorganisms				
Mushroom	*Agaricus bisporus*	Biocontrol Technologies, S.L. *Trichoderma asperellum*, strain T34 Groups: i. mushroom compost treated with 10^3 CFU/mL (group 1) ii. mushroom compost treated with 10^4 CFU/mL (group 2)	On Day 0: - mushroom compost in groups 1 and 2 contained approximately 5×10^3 and 2.1×10^4 CFU *Trichoderma* spp. per mL, respectively After 10 days: - both treatment groups contained approximately 10^5 CFU *Trichoderma* spp. per mL After 2 months: - mushroom compost in groups 1 and 2 contained approximately 1.4×10^3 and 6.0×10^2 CFU *Trichoderma* spp. per mL	PMRA 2033780

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
		No controls	**ACCEPTABLE**	
Soil Microorganisms	Soil surface (0–20 cm) microbial communities	Biocontrol Technologies, S.L. *Trichoderma asperellum*, strain T34 Groups: i. 5% w/v peat plant growth medium containing ~10^4 CFU/mL, 7 days at 25ºC (treated peat) ii. 5% w/v coir fibre plant growth medium containing ~10^4 CFU/mL, 7 days at 25ºC (treated coir fibre) iii. 5% w/v untreated peat plant growth medium, 7 days at 25ºC iv. 5% w/v untreated coir fibre plant growth medium, 7 days at 25ºC	Microbial diversity: - the addition of strain T34 to both plant growth media induced statistically significant decreases in fluorescent *Pseudomonas* spp. and cellulolytic actinomycetes. Microbial biomass (acridine-orange direct counting): - soil microbial biomass was significantly reduced by the addition of strain T34 to both plant growth media. Functional richness and diversity: - plant growth media had an effect on the functional richness as well as in the diversity of the microbial communities in the amended soil, measured as Shannon (H') and Gini (1-G) indices. - treatments with strain T34 only affect the Gini diversity index. - treatments with strain T34 did not affect the Shanon index or the functional richness of soil microorganisms - a greater effect was attributed to the amendment with growth media waste than treatment with strain T34. **ACCEPTABLE**	PMRA 2033984
Plants				
Crop Plants	Cucumber (*Cucumis sativus* var. Negrito) – 18 seeds Lettuce (*Lactuca sativa* var. Trocadero) – 18 seeds Tomato (*Lycopersicon esculentum* var. Roma) – 27 seeds Radish	Biocontrol Technologies, S.L. *Trichoderma asperellum*, strain T34 Groups: i. untreated coir fibre ii. coir fibre treated with strain T34 at 10^3 CFU/mL iii. coir fibre treated with strain	Cucumber: - fresh weight, dry weight and chlorophyll content of the cucumber plantlets after 18 days did not show any statistically significant differences between treatments. - no symptoms of toxicity or nutritional alteration were observed in cucumber plants for any of the treatments. Lettuce: - fresh weight of the lettuce plantlets after 42 days did not show any statistically significant differences between treatments. - no symptoms of toxicity or	PMRA 2033834, 2033977 and 2033978

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
	(*Raphanus sativus* var. Cumbre) – 27 seeds	T34 at 10^4 CFU/mL iv. coir fibre treated with strain T34 at 10^5 CFU/mL v. coir fibre treated with himexazol (36% w/v, 5-methyl 3-isoxazol) vi. coir fibre treated with Trianum-P (containing *Trichoderma harzianum* strain T22)	nutritional alteration were observed in lettuce plants for any of the treatments. Tomato: - fresh weight of the tomato plantlets after 46 days did not show any statistically significant differences between treatments. - no symptoms of toxicity or nutritional alteration were observed in tomato plants for any of the treatments. Radish: - fresh weight of the radish plantlets after 31 days did not show any statistically significant differences between treatments. - no symptoms of toxicity or nutritional alteration were observed in radish plants for any of the treatments. **ACCEPTABLE**	
Crop Plants	Cucumber (*Cucumis sativus* var. Negrito) – 18 seeds Lettuce (*Lactuca sativa* var. Trocadero) – 18 seeds Tomato (*Lycopersicon esculentum* var. Roma) – 27 seeds Radish (*Raphanus sativus* var. Cumbre) – 27 seeds	Biocontrol Technologies, S.L. *Trichoderma asperellum*, strain T34 Groups: i. untreated peat ii. peat treated with strain T34 at 10^3 CFU/mL iii. peat treated with strain T34 at 10^4 CFU/mL iv. peat treated with strain T34 at 10^5 CFU/mL v. peat treated with himexazol (36% w/v, 5-methyl 3-isoxazol) vi. peat treated with Trianum-P	Cucumber: - fresh weight, dry weight and chlorophyll content of the cucumber plantlets after 18 days did not show any statistically significant differences between treatments. - no symptoms of toxicity or nutritional alteration were observed in cucumber plants for any of the treatments. Lettuce: - fresh weight of the lettuce plantlets after 42 days did not show any statistically significant differences between treatments. - no symptoms of toxicity or nutritional alteration were observed in lettuce plants for any of the treatments. Tomato: - fresh weight of the tomato plantlets after 46 days did not show any statistically significant differences between treatments. - no symptoms of toxicity or nutritional alteration were observed in tomato plants for any	PMRA 2033833, 2033977 and 2033978

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
		(containing *T. harzianum* strain T22)	of the treatments. Radish: - fresh weight of the radish plantlets after 31 days did not show any statistically significant differences compared to untreated controls. - radish plantlets treated with T34 at 10^4 CFU/mL and at 10^5 CFU/mL showed a higher fresh weight than those treated with Himexazol or T34 at 10^3 CFU/mL - no symptoms of toxicity or nutritional alteration were observed in radish plants for any of the treatments. **ACCEPTABLE**	
Non-crop Plants	A request to waive the requirement for non-target plant testing was submitted based on the properties of the MPCA and the limited potential for exposure from the proposed use of T34 Biocontrol in greenhouses. *Trichoderma asperellum* strain T34 is not a plant pathogen as observed in tests conducted on cucumber, lettuce, tomato and radish, and no adverse effects on plants were found in the published scientific literature. In addition, minimal exposure to non-target plants is anticipated from the proposed use of T34 Biocontrol in greenhouses. **WAIVER RATIONALE ACCEPTED**			PMRA 2034091, 2034100 and 2034912
Aquatic Organisms				
		Vertebrates		
Fish	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for exposure from the proposed use of T34 Biocontrol in greenhouses. *Trichoderma asperellum* strain T34 is not a fish pathogen and no adverse effects to fish were found in the published scientific literature. In addition, minimal exposure to fish is anticipated from the proposed use of T34 Biocontrol in greenhouses. **WAIVER RATIONALE ACCEPTED**			PMRA 2034091, 2034100 and 2034912
		Invertebrates		
Aquatic Arthropods and Non-Arthropod Invertebrates	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for exposure from the proposed use of T34 Biocontrol in greenhouses. No adverse effects to aquatic arthropods were found in the published scientific literature. In addition, minimal exposure to aquatic arthropods is anticipated from the proposed use of T34 Biocontrol in greenhouses. **WAIVER RATIONALE ACCEPTED**			PMRA 2034091, 2034100 and 2034912
		Plants		
Aquatic Plants	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for exposure from the proposed use of T34 Biocontrol in greenhouses. *Trichoderma asperellum* strain T34 is not a plant pathogen and no adverse effects to aquatic plants were found in the published scientific literature. In addition, minimal			PMRA 2034091, 2034100 and 2034912

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
	exposure to non-target plants is anticipated from the proposed use of T34 Biocontrol in greenhouses. **WAIVER RATIONALE ACCEPTED**			

Table 3 Summary of Alternatives for the Uses Supported with T34 Biocontrol

Crop	Pests	Active Ingredient and Resistance Management Group
Greenhouse potted ornamentals	Stem, crown and root rots caused by *Fusarium*	thiophanate-methyl (1)
Greenhouse ornamentals	Damping-off, root and crown rot, and wilt caused by *Fusarium*	*Streptomyces* strain K61 (NC)
Greenhouse ornamentals	*Fusarium* root diseases	*Trichoderma harzianum* Rifai strain KRL-AG2 (NC)

Table 4 Use (label) Claims Proposed by Applicant and Whether Acceptable or Unsupported

Proposed claim	Supported claim
For suppression of root diseases caused by *Fusarium oxysporum* on greenhouse ornamentals **Spray of the growing media for propagation**: Apply to the growing media at a dose of 10 grams per m^3 of substrate before potting. **Root bath**: Follow the application to the growing media with a root-dip treatment before planting. Dip the roots of the cuttings several hours or overnight in a solution of water with 0.01 g in 1 L of water. **Chemigation of container-grown crops**: Follow the root-dip treatment with an application of the product by irrigation at 5 grams per 1000 pots of 1L at the planting day. Apply 5 grams per 1000 pots of 1 L every 2-3 months as follow-up treatment. Maximum total concentration: The maximum concentration recommended for the dilution of T34 Biocontrol is 10 g/L water.	Conditionally supported for suppression of fusarium wilt on greenhouse ornamentals.

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B. Additional Information Considered

i) Published Information

1.0 Chemistry

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2.0 Human and Animal Health

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