Proposed Registration Decision PRD2020-07

Bacillus amyloliquefaciens strain PTA-4838 and AVEO EZ Nematicide

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Overview

Proposed Registration Decision for *Bacillus amyloliquefaciens* strain PTA-4838

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act,* is proposing registration for the sale and use of AVEO Technical Powder and AVEO EZ Nematicide, containing the technical grade active ingredient *Bacillus amyloliquefaciens* strain PTA-4838, as a seed treatment for early season protection against particular parasitic nematodes on corn and soybeans.

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

This 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 *Bacillus amyloliquefaciens* strain PTA-4838 and AVEO EZ Nematicide.

What Does Health Canada Consider When Making a Registration Decision?

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

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

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

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

Before making a final registration decision on *Bacillus amyloliquefaciens* strain PTA-4838 and AVEO EZ Nematicide, Health Canada's PMRA will consider any comments received from the public in response to this consultation document.[3] Health Canada will then publish a Registration Decision[4] on *Bacillus amyloliquefaciens* strain PTA-4838 and AVEO EZ Nematicide, which will include the decision, the reasons for it, a summary of comments received on the proposed registration decision and Health Canada's response to these comments.

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

What is Bacillus amyloliquefaciens strain PTA-4838?

Bacillus amyloliquefaciens strain PTA-4838 is a naturally present root colonizing bacterium. It produces enzymes that may impact certain parasitic nematodes. The exact mode of action is unknown.

Health Considerations

Can Approved Uses of *Bacillus amyloliquefaciens* strain PTA-4838 Affect Human Health?

***Bacillus amyloliquefaciens* strain PTA-4838 is unlikely to affect your health when AVEO EZ Nematicide is used according to the label directions.**

Potential exposure to *Bacillus amyloliquefaciens* strain PTA-4838 may occur when handling and applying AVEO EZ Nematicide. When assessing health risks, several key factors are considered:

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

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

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

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

Toxicology studies in laboratory animals describe potential health effects from large doses of exposure to a microorganism and identify any pathogenicity, infectivity and toxicity concerns. When a formulation that is toxicologically equivalent to AVEO EZ Nematicide was tested on laboratory animals, there was low toxicity following oral, inhalation and dermal exposures, no dermal irritation and minimal eye irritation. Furthermore, there was no sign that the microbial pest control agent, *Bacillus amyloliquefaciens* strain PTA-4838, caused any disease.

Residues in Water and Food

Dietary risks from food and water are acceptable.

Based on the lack of dietary exposure resulting from the proposed use of AVEO EZ Nematicide, the health risks are acceptable for all segments of the population, including infants, children, adults and seniors. Even in the event of exposure, the health risk is acceptable since there were no signs that it caused any significant toxicity or disease in studies on laboratory animals.

Occupational Risks From Handling AVEO EZ Nematicide

Occupational risks are acceptable when AVEO EZ Nematicide is used according to label directions, which include protective measures.

Workers handling AVEO EZ Nematicide can come into direct contact with *Bacillus amyloliquefaciens* strain PTA-4838 on the skin, by inhalation, or in the eyes. To protect workers from exposure to AVEO EZ Nematicide, the label states that workers must wear personal protective equipment, including waterproof gloves, long-sleeved shirt, long pants, a NIOSH-approved particulate filtering facepiece respirator, socks and shoes.

Risks in Residential and Other Non-Occupational Environments

Estimated risk for non-occupational exposure is acceptable.

AVEO EZ Nematicide is proposed for use as a commercial seed treatment in commercial facilities only. Residential and non-occupational exposure to AVEO EZ Nematicide is expected to be low when label directions are observed. Consequently, the risk to residents and the general public is acceptable.

Environmental Considerations

What Happens When *Bacillus amyloliquefaciens* strain PTA-4838 is Introduced Into the Environment?

Environmental risks are acceptable.

Bacillus amyloliquefaciens is a common microorganism that is widely distributed in the natural environment. Its habitat is predominantly soil, including soils in water columns and bottom deposits in aquatic environments. Under adverse conditions, this microorganism produces a resilient endospore that allows it to readily survive in soils, dusts and aerosols. If protected from sunlight, endospores may survive for very long periods.

No overt adverse effects to terrestrial and aquatic arthropods, birds or fish were observed during testing. AVEO EZ Nematicide is intended for use as seed treatments on corn (all types) and soybean. The end-use product is not intended for aquatic uses and its use as a seed treatment is not expected to significantly increase the levels of this microorganism in soil. Exposure to aquatic environments is also expected to be low and limited to leaching and run-off after the seeds are sown in fields. While published scientific literature on the environmental fate of this species suggests that strain PTA-4838 will survive in soils and sediment under various environmental conditions, the populations of *Bacillus amyloliquefaciens* strain PTA-4838 in soil and sediment are expected to return to naturally sustainable levels.

Based on a critical review of studies, scientific rationales and information from public sources, no significant effects to birds, wild mammals, fish, terrestrial and aquatic arthropods, and plants are expected when AVEO EZ Nematicide is applied according to directions on the label.

Value Considerations

What Is the Value of AVEO EZ Nematicide?

AVEO EZ Nematicide, containing *Bacillus amyloliquefaciens* strain PTA-4838, is a corn and soybean seed treatment product used for the partial suppression of certain parasitic nematodes.

This microbial product provides an additional option for managing nematodes when used as a part of an integrated pest management strategy that includes crop rotation and the use of nematode resistant varieties, such as those with resistance to soybean cyst nematode. AVEO EZ Nematicide is compatible with certain other chemical seed treatments.

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 AVEO Technical Powder and AVEO EZ Nematicide to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

All microorganisms, including *Bacillus amyloliquefaciens* strain PTA-4838, contain substances that are potential sensitizers and thus, sensitivity may possibly develop in individuals exposed to potentially large quantities of *Bacillus amyloliquefaciens* strain PTA-4838. In turn, workers handling or applying AVEO EZ Nematicide must wear waterproof gloves, a long-sleeved shirt, long pants, a NIOSH-approved particulate filtering facepiece respirator, socks and shoes.

Environment

The end-use product label will include environmental precaution statements to reduce contamination of aquatic systems from the use of AVEO EZ Nematicide.

Next Steps

Before making a final registration decision on *Bacillus amyloliquefaciens* strain PTA-4838 and AVEO EZ Nematicide, Health Canada's PMRA will consider any comments received from the public in response to this consultation document. Health Canada will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). Health Canada will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed decision and Health Canada's response to these comments.

Other Information

When the Health Canada makes its registration decision, it will publish a Registration Decision on *Bacillus amyloliquefaciens* strain PTA-4838 and AVEO EZ Nematicide (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Bacillus amyloliquefaciens strain PTA-4838 and AVEO EZ Nematicide

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Bacillus amyloliquefaciens* strain PTA-4838
Function	Nematicide (partial suppression)
Binomial name	*Bacillus amyloliquefaciens* strain PTA-4838
Taxonomic designation[5]	
Kingdom	Eubacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillalaes
Family	Bacillaceae
Genus	*Bacillus*
Species	*amyloliquefaciens*
Strain	PTA-4838
Patent Status information	US Patent No. 6.995.007
Nominal purity of active	Technical grade active ingredient (TGAI): 5×10^{11} CFU/g End-use Product (EP): 4.1×10^{10} spores/mL (minimum)
Identity of relevant impurities of toxicological, and/or environmental significance	The technical grade active ingredient and end-use product do not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminant release standards. In addition, there are no known mammalian toxins or other toxic metabolites present in the technical grade active ingredient or end-use products.

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

Technical Product—AVEO Technical Powder

Property	Result
Colour	Light brown/sand
Physical State	Powder (solid)
Odour	None

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

Property	Result
pH (1%)	4.6 at 25°C
Relative Density	1.91 g/mL at 20°C

End-Use Product — AVEO EZ Nematicide

Property	Result
Colour	Brown
Physical State	Liquid suspension
Viscosity	393.86 cSt at 20°C 143.67 cSt at 40°C
pH (1%)	7.3
Specific gravity	1.2050 at 20°C

1.3 Directions for Use

AVEO EZ Nematicide is a nematicide seed treatment. It is diluted in water and applied directly to seed of soybean and corn to partially suppress soybean cyst and root knot nematodes in soybean and pin, ring, root knot, root lesion, spiral, and stubby root nematodes in corn. AVEO EZ Nematicide is applied at 4.5 mL per standard bag of 140,000 soybean seeds and per standard bag of 80,000 corn seeds.

1.4 Mode of Action

The mode of action of *B. amyloliquefaciens* strain PTA-4838 on plant parasitic nematodes is not definitely known. As a soil bacterium that produces enzymes that can break down the protein, chitin and lipids, such as found in nematode eggs, it may reduce nematode populations resulting in fewer cysts and galls.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganisms

Acceptable methodologies for detection, isolation and enumeration of the active ingredient, *B. amyloliquefaciens* strain PTA-4838, were submitted by the applicant. The microbial pest control agent (MPCA) has been fully characterized with respect to its origin of strain, natural occurrence and biological properties. *Bacillus amyloliquefaciens* strain PTA-4838 can be distinguished from other isolates based on 16S rRNA gene sequencing and gas chromatographic analysis of fatty acid methyl esters.

2.2 Methods for Establishment of Purity of Seed Stock

The strain has been deposited in the American Type Culture Collection (ATCC) under ATCC No. PTA4838. Lyophilized stock cultures are maintained as a permanent cell bank. A master stock is prepared from the cell bank for manufacturing purposes.

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

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

The guarantee of the technical grade active ingredient is expressed in units of colony forming units (CFU) per gram and the guarantee of the end-use product is expressed in units of spores per mL. Representative data on five batches of technical grade active ingredient and end-use product were submitted. The methods for determining CFU counts and spore counts were adequately described.

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

As noted above, acceptable methods are available to enumerate the microorganism and to distinguish this MPCA from other *Bacillus* species.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures used to limit contaminating microorganisms during the manufacture of the technical grade active ingredient, AVEO Technical Powder, and the end-use product, AVEO EZ Nematicide are acceptable. These procedures include sterilization of all equipment and media as well as frequent sampling of the stock culture and production batches for purity and contamination.

The absence of human pathogens and below-threshold levels of contaminating microorganisms were shown in the microbial screening of batches of AVEO Technical Powder and AVEO EZ Nematicide using standard methods for detecting and enumerating microbial contaminants of concern. All batches of AVEO Technical Powder and all batches of AVEO EZ Nematicide conform to the limits set out in the Organisation for Economic Co-operation and Development (OECD) issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43].

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

Storage stability data were provided for AVEO Technical Powder and AVEO EZ Nematicide. Results support a storage period of 12 months at 20°C.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

3.1.1 Testing

The PMRA conducted a detailed review of the toxicological information submitted in support of AVEO Technical Powder and AVEO EZ Nematicide.

The technical grade active ingredient data package consisted of an acute oral toxicity/infectivity study, an acute pulmonary toxicity/infectivity study, and an intravenous (iv) injection infectivity study. In these studies, the test substance was Varnimo Technical, which was toxicologically equivalent to AVEO Technical Powder.

In the acute oral toxicity/infectivity study, groups of fasted, young adult Sprague Dawley rats (12/sex) were given a single oral dose of Varnimo Technical (1.8×10^{10} CFU/g) in phosphate buffered saline (PBS) by gavage at greater than or equal to 3.5×10^{8} CFU/animal. Animals were observed for 21 days with interim scheduled sacrifices on Days 3, 7, and 14. There were no mortalities, no treatment related clinical signs, or necropsy findings. The test organism cleared the urine by Day 14, and showed a pattern of clearance in the feces by Day 14. The brain and kidney were clear of the test substance by Day 7; the blood and cecum contents were clear of the test substance by Day 14 and Day 21, respectively. By Day 21, a pattern of clearance was established in the liver, spleen and mesenteric lymph nodes (MLN). The MPCA was still recovered in the lungs (2/5 animals) by Day 21. Given the oral route of exposure, the CFU counts in the lungs on Day 3-7 were higher than expected and this suggests inadvertent aspiration of the test substance.

In the acute pulmonary toxicity/infectivity study, groups of fasted, young adult Sprague Dawley rats (18/sex) were given a single dose of Varnimo Technical (5.0×10^{11} CFU/g) in PBS by intratracheal instillation at greater than or equal to 3.1×10^{8} CFU/animal. Animals were observed for up to 21 days with interim scheduled sacrifices on Days 3, 7, and 14. Body weight and body weight gain in female MPCA-treated animals was below that of the untreated control animals, but all animals gained weight throughout the study. There were no mortalities, no treatment related clinical signs, or necropsy findings. The test organism cleared from the blood and cecum by Day 7, from the kidneys and MLN by Day 14, and from the brain, lungs, liver and spleen by Day 21.

In the acute intravenous infectivity study, groups of young adult Sprague Dawley rats (15/sex) were injected with Varnimo Technical (measured: 2.7×10^{8} CFU/ml) in PBS at 2.7×10^{7} CFU/animal. Animals were then observed for up to 21 days with interim scheduled sacrifices on Days 3, 7 and 14. There were no mortalities, no treatment related clinical signs, and no abnormal necropsy findings, nor differences in body weight or body weight gain between treatment groups. The test substance was not recovered at any time point in the blood or cecum contents. In the brain and kidney, the test substance cleared by Day 7 and Day 14, respectively. A pattern of clearance was demonstrated in the lungs, liver, spleen, and MLN by Day 21. There were no mortalities, no signs of pathogenicity noted in any of the test animals and no proliferation of the test substance was observed in these organs.

The end-use product data package included dermal toxicity, dermal irritation and eye irritation studies. Although not required, acute toxicity testing was also performed via the oral and inhalation routes. In these studies, the test substance was VBC-90052, which was toxicologically equivalent to AVEO EZ Nematicide.

In the acute dermal toxicity study, groups of young adult Sprague Dawley rats (5/sex) were dermally exposed to VBC-90052 (3.0×10^{10} CFU/mL) at 5050 mg/kg bw for 24 hours. The dose was applied to an area of approximately 10% of body surface area. Following the 24-hour exposure period, the test area was washed and animals were observed for 14 days. There was no mortality observed during the study, no clinical signs of toxicity and all animals exhibited weight gain throughout the study period. There were no signs of dermal irritation noted during the study period. Gross necropsy revealed no observable abnormalities.

In the primary dermal irritation study, three young adult albino New Zealand White rabbits (2 males; 1 female) were dermally exposed to 0.5 mL of undiluted VBC-90052 (3.0×10^{10} CFU/mL) for 4 hours to a 8 × 8 cm body surface area. The test area was covered with a gauze patch and semi-permeable dressing during the exposure period. After 4 hours, the dressings were removed and the test site was washed with room temperature tap water. Animals were observed for 3 days following removal of the test substance and irritation was scored. There were no signs of irritation at any observation point.

In the primary eye irritation study, 0.1 mL of undiluted VBC-90052 (3.0×10^{10} CFU/mL) was instilled into the conjunctival sac of the right eye of young adult New Zealand White albino rabbits (two males; one female) for 24 hours. After recording the 24-hour observation, all treated eyes were washed with room temperature deionized water for one minute. Prior to dosing, a systemic analgesic (Buprenophine) was administered by injection, and both eyes of each animal were anesthetized with Teracaine Ophthalmic Solution. At approximately 8 hours after dosing, a second Buprenophine and Meloxicam injection was administered for continued analgesia. Animals then were observed for 3 days and irritation was scored. The maximum irritation score (MIS) was 4.7/110 (at 1 h). Based on the Maximum Average Score (MAS; at 24, 48, and 72 h) of 0/110, VBC-90052 is minimally irritating to eyes.

In the acute oral toxicity study, three female fasted, young adult Sprague Dawley rats were given a single oral dose of VBC-90052 (3.0×10^{10} CFU/mL; undiluted) at a single dose of 5000 mg/kg bw. The animals were then observed for a period of up to 14 days. There were no mortalities, no treatment related clinical signs, no abnormal necropsy findings and all animals gained weight during the study period.

In the acute inhalation toxicity study, one group of young adult Sprague Dawley rats (5/sex) were exposed by the nose-only inhalation route to an aerosol generated from VBC-90052 (3.0×10^{10} CFU/mL) for 4 hours at concentration 2.13 mg/L (measured). VBC-90052 was diluted at 50% v/v prior to aerosolization. Animals were observed for 14 days. There were no mortalities, no clinical signs of toxicity and all animals exhibited weight gain during the study period. Gross necropsy revealed no observable abnormalities.

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

3.1.2 Additional Information

A survey of published literature uncovered no reports of adverse effects for *B. amyloliquefaciens* strain PTA-4838. There has been one clinical case involving *B. amyloliquefaciens*. The case involved an outbreak of eosinophilia-myalgia syndrome following ingestion of a tryptophan supplement found to contain a chemical impurity. The impurity was produced by the genetically-engineered strain of *B. amyloliquefaciens* during fermentation under specific manufacturing conditions at the Japanese manufacturing plant.

Members of the broader *B. subtilis* complex have the ability to persist in various habitats provided (see Section 4.1 for further details). They may be contaminants in food and aviation fuel and transient members of the bowel microflora. Some members of the *B. subtilis* complex are used in the fermentation of foods. They form endospores that permit survival in sub-optimal environmental conditions. Numerous physiological variants exist in nature, indicating that members of this complex establish successfully in nearly every environment.

Certain members of the *B. subtilis* complex are occasionally reported to cause disease in susceptible humans, including those with debilitating disease or compromised immunity, young infants and the elderly, but do so rarely in the general population. Some produce extracellular enzymes and toxins that could cause food poisoning. Due to its close relationship to *B. amyloliquefaciens*, cases of *B. subtilis* infection were also considered relevant. There have been rare cases of *B. subtilis*-related endocarditis, bacteremia in immunocompromised patients. In some cases, the organism was introduced into sensitive tissues via intravenous catheters or lumbar puncture surgery. Other cases were related to drug abuse, as narcotics are often contaminated with bacilli. The routine use of *B. subtilis* cultures as a non-specific support for a stable gastrointestinal flora has also been suspected as a source. Single cases each of meningitis, hepatotoxicity, eye infection and a shin-bone infection have also been reported for *B. subtilis*.

Rope spoilage in bread is also associated with *B. subtilis* and foodborne illness has occasionally been reported. Other food poisoning incidents related to *B. subtilis* are rare, and the implicated strains produce a highly heat stable toxin (possibly similar to the *B.* cereus-enterotoxin). *Bacillus amyloliquefaciens* strain PTA-4838 is not reported to produce this toxin, and no such illnesses have been reported for this microorganism. Furthermore, when *B. amyloliquefaciens* strain PTA-4838 was administered orally to rats, no signs of toxicity or disease were observed. On rare occasions, *B. subtilis* was attributed to foodborne illness where no toxin production was detected.

In veterinary medicine, bovine mastitis, as well as reproductive disorders in goats and canine endocarditis have been related to *B. subtilis*. Certain strains of *B. licheniformis* can cause bovine, porcine and ovine abortion as well as mastitis in cattle, but the overall impact of *B. licheniformis* disease in livestock is low.

Hypersensitivity pneumonitis was reported from exposure to *B. subtilis* and *B. lichenformis* spores and vegetative cells released from wood dust in domestic and industrial settings. Production of AVEO Technical Powder is not aimed at enzyme enrichment and there have been no adverse health effects reported in workers at the production site where *B. amyloliquefaciens* strain PTA-4838 is fermented or formulated.

3.1.3 Incident Reports Related to Human and Animal Health

Bacillus amyloliquefaciens strain PTA-4838 is a new active ingredient pending registration for use in Canada, and as of 3 October 2019, no incident reports had been submitted to the PMRA.

There was one human incident involving a related strain, *B. subtilis* QST 713. In this incident, a person reported minor symptoms of rash and cough when applying a product containing *B. subtilis* strain QST 713. The label of the proposed product, AVEO EZ Nematicide, contains appropriate precaution statements and personal protective equipment aimed at reducing pesticide exposure when mixing, loading or applying the product. Hence, no additional mitigation measures are proposed based on the incident report review.

3.1.4 Hazard Analysis

The data package submitted in support of registering AVEO Technical Powder and AVEO EZ Nematicide was reviewed from the viewpoint of human health and safety, and was determined to be acceptable.

AVEO Technical Powder is of low toxicity and not pathogenic or infective by the oral, pulmonary, and intravenous routes. The MPCA is considered to be a potential sensitizer. Consequently, the hazard statements “POTENTIAL SENSITIZER” will appear on the principal display panel of the technical grade active ingredient. The statement, “May cause sensitization. Avoid contact with skin, eyes, and clothing. Avoid inhaling/breathing dust.” is also required on the secondary panel of the label under the “PRECAUTIONS” section.

The end-use product, AVEO EZ Nematicide is of low toxicity by the oral, inhalation, and dermal routes and is minimally irritating to eyes. As the formulation contains a MPCA, the hazard statements “POTENTIAL SENSITIZER” will appear on the principal display panel of the end-use product label. The statement, “May cause sensitization. Avoid contact with skin, eyes, and clothing. Avoid inhaling/breathing spray mist.” is also required on the secondary panel of the label under the “PRECAUTIONS” section.

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

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

3.2 Occupational, Residential and Bystander Risk Assessment

3.2.1 Occupational and Post-Application Exposure and Risk

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with primary exposure routes being dermal. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe was a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Bacillus amyloliquefaciens* has not frequently been identified as a dermal wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Furthermore, technical grade active ingredient testing showed no toxicity and no infectivity via the oral, pulmonary, and intravenous routes. Toxicity testing with the end-use product also showed no toxicity via the oral, dermal or inhalation routes, and is not a skin irritant and is minimally irritating to eyes.

Although AVEO EZ Nematicide was of low toxicity via the oral, inhalation and dermal routes, the PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Consequently, risk mitigation measures, such as personal protective equipment (PPE), including waterproof gloves, a long-sleeved shirt, long pants, a NIOSH-approved particulate filtering facepiece respirator, socks and shoes are required to minimize exposure and protect applicators, mixer/loaders, and handlers that are likely to be exposed.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of AVEO EZ Nematicide. Overall, occupational risks to workers are acceptable when the precautionary statements on the label are followed, which include PPE.

3.2.2 Residential and Bystander Exposure and Risk

The use of AVEO EZ Nematicide as a seed treatment in commercial facilities as described on the label is not anticipated to result in any significant residential and bystander exposure. Also, AVEO EZ Nematicide is of low toxicity and there were no signs that the MPCA, *B. amyloliquefaciens* strain PTA-4838, caused any disease in studies on laboratory animals. Consequently, the health risks to bystanders and individuals in residential areas are acceptable.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

The proposed use pattern (seed treatment) is not expected to result in dietary exposure since the product will not be applied to the edible portions of crops, and the seed treatment applications of *B. amyloliquefaciens* strain PTA-4838 are not expected to yield any growth on the edible portions of the crops. Furthermore, *B. amyloliquefaciens* strain PTA-4838 demonstrated no pathogenicity or infectivity in Tier I studies. In addition, no metabolites of toxicological significance have been shown to be produced by this strain. Consequently, there is no health risk for the general population, including infants and children, or animals.

3.3.2 Drinking Water

Dietary exposure from drinking water is expected to be low as the label has the necessary mitigative measures to limit contamination of drinking water from the proposed uses of AVEO EZ Nematicide. The use of this end-use product is limited to seed treatments in commercial facilities, and the label will instruct users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Municipal treatment of drinking water is also expected to further reduce the transfer of residues to drinking water. Furthermore, *B. amyloliquefaciens* strain PTA-4838 demonstrated no pathogenicity or infectivity in Tier I studies. Health risks from residues of *B. amyloliquefaciens* strain PTA-4838 in drinking water are acceptable due to the low toxicity/pathogenicity profile of AVEO EZ Nematicide and limited exposure following application of the end-use product.

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 PTA-4838 is of low oral toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCAs than the general population. Thus, there are no threshold effects of concern and, as a result, there is no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCA and other registered microorganisms that have a common mechanism of toxicity, does not apply to this MPCA. As a result, the Agency has not used a margin of exposure (safety) approach to assess the risks of *B. amyloliquefaciens* strain PTA-4838 to human health.

3.3.4 Aggregate Exposure and Risk

Based on the toxicity and infectivity test data and other relevant information in the PMRA's files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *B. amyloliquefaciens* strain PTA-4838 to the general Canadian population, including infants and children, when the end-use product is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Dermal and inhalation exposure to the general public will be low since the product is not allowed for use on turf, residential or recreational areas.

Furthermore, the label will only include seed treatments and few adverse effects from exposure to other strains of *B. subtilis* encountered in the environment have been reported in the public literature. Even if there is an increase in exposure to *B. amyloliquefaciens* strain PTA-4838 from the use of AVEO EZ Nematicide, 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.

Dietary risk to humans from the proposed use of *B. amyloliquefaciens* strain PTA-4838 as a seed treatment is acceptable since residues are not anticipated on food crops grown from treated seeds. Therefore, the PMRA has determined that specification of an MRL under the *Pest Control Products Act* is not required for *B. amyloliquefaciens* strain PTA-4838.

3.4 Cumulative Assessment

The *Pest Control Products Act* requires that the PMRA consider the cumulative exposure to pesticides with a common mechanism of toxicity. In its assessment of common mechanism of toxicity, the PMRA considers both the taxonomy of MPCAs and the production of any potentially toxic metabolites. For the current evaluation, the PMRA has determined that *B. amyloliquefaciens* strain PTA-4838 shares a common mechanism of toxicity with other strains of *B. amyloliquefaciens*, *B. subtilis* and *B. licheniformis* that are used as MPCAs: *B. amyloliquefaciens* strain F727, *B. amyloliquefaciens* strain MBI 600, *B. amyloliquefaciens* strain D747, *B. subtilis* strain QST 713, *B. subtilis* strain GB03, *B. subtilis* strain FMCH 001, *B. subtilis* var. *amyloliquefaciens* strain FZB24 and *B. licheniformis* FMCH 002. The potential health risks from cumulative exposure of *B. amyloliquefaciens* strain PTA-4838 and these other MPCAs are acceptable when used as labelled given their low toxicity and pathogenicity.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

No studies were submitted to address the environmental fate and behaviour of *B. amyloliquefaciens* strain PTA-4838; however, environmental fate data (Tier II/III) are not normally required at Tier I, and are only triggered if significant toxicological effects in non-target organisms are noted in Tier I testing.

According to published information, *Bacillus* species are saprophytes that are widely distributed in the natural environment. The habitats of most species are soils of all kinds (for example, temperate, acidic, neutral, alkaline), including soils in water columns and bottom deposits of

fresh and marine waters. Their endospores are very durable and they readily survive in soils, dusts and aerosols. If protected from solar radiation, endospores may survive for very long periods. The presence of spores in a particular environment, however, does not necessarily indicate that the organism is metabolically active in this environment. Most species of *Bacillus* are heterotrophic organisms that have been isolated on complex organic media. Some species will degrade biopolymers such as leather and feathers, with versatilities varying according to species. It is therefore postulated that these species have important roles in the biological cycling of carbon and nitrogen.

The seed treatment application of AVEO EZ Nematicide is expected to result in slight increases of *Bacillus* species in the rhizosphere of treated plants. These localized increases in soil are not expected to significantly increase the overall environmental levels of this species above naturally occurring levels. Also, the localized elevated populations of *B. amyloliquefaciens* strain PTA-4838 in the rhizosphere of plants are expected to return to naturally sustainable levels over time.

The end-use product is not intended to be applied directly to water. As result, exposure to aquatic environments should be low and limited to run-off after the seeds are sown in fields. While *B. amyloliquefaciens* is not considered an aquatic species and is not expected to grow in this environment, the endospores of this microorganism are likely to persist in sediment. The seed treatment application of AVEO EZ Nematicide is not expected to significantly increase the overall environmental levels of this species in sediment above naturally occurring levels. As noted previously, any localized increases of *B. amyloliquefaciens* strain PTA-4838 in aquatic environments are expected to return to naturally sustainable levels over time.

4.2 Effects on Non-Target Species

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

The type of environmental risk assessment conducted on MPCAs varies depending on the tier level that was triggered during testing. For many MPCAs, Tier I studies are sufficient to conduct environmental risk assessments. Tier I studies are designed to represent “worst-case” scenarios where the exposure conditions greatly exceed the expected environmental concentrations. The absence of adverse effects in Tier I studies are interpreted as minimal risk to the group of non-target organisms. However, higher tiered studies will be triggered if significant adverse effects on non-target organisms are identified in Tier I studies.

These studies provide additional information that allows the PMRA to refine the environmental risk assessments. In the absence of adequate environmental fate and/or field studies, a screening level risk assessment can be performed to determine if the MPCA is likely to pose a risk to a group of non-target organisms.

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

If the screening level 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

Five studies were submitted to address the hazards of *B. amyloliquefaciens* strain PTA-4838 to birds and arthropods. These studies were performed with Varnimo Technical, which was toxicologically equivalent to AVEO Technical Powder. A scientific rationale was also submitted in support of requests to waive further testing on birds. Data submitted under human and animal health toxicity testing were considered to assess the risk of harm to wild mammals.

Two studies were submitted to address the hazards to birds. In one avian study, the acute oral toxicity of Varnimo Technical (73.4% *B. amyloliquefaciens* strain PTA-4838) to 16-day-old Bobwhite quail (*Colinus virginianus*) was assessed over 30 days. A suspension of Varnimo Technical in deionized water (approximately 33.1% w/v) was administered to the 30 birds by gavage at a dose of 5 mL/kg body weight per day for five (5) consecutive days. Based on enumerations results, this dose was found to be equivalent to approximately 3.5×10^{11} CFU/kg bw/day. During the study, three animals treated with Varnimo Technical were found dead on Day 1 and a fourth animal was found dead on Day 6. One quail exhibited a decrease in activity on Day 8, suffered a leg injury on Day 9 and was euthanized. There were no other clinical signs and all surviving animals gained weight by study termination. At necropsy, the only observable finding was a broken femur in the bird that was euthanized on Day 9. Insufficient data were available to unequivocally determine the cause of death on Days 1 and 6. As a result, this study was of limited utility in the risk assessment.

In the second avian study, the acute oral toxicity of Varnimo Technical (5×10^{11} CFU/g *B. amyloliquefaciens* strain PTA-4838) to 24-day-old Bobwhite quail (*C. virginianus*) was assessed over 30 days. In this study, a suspension of Varnimo Technical in deionized water (approximately 39.6% w/v) was administered to the 30 birds by gavage at a dose of 2.5 mL/kg body weight per day for five (5) consecutive days. Based on enumerations results, this dose was found to be equivalent to approximately 2.2×10^{11}–4.0×10^{11} CFU/kg bw/day. In this study,

there were no mortalities and no clinical signs noted during the study period. On Day 14, the test group mean body weight was significantly less than the mean body weight of the untreated group. By Day 30, there were no significant differences in body weights for any of the groups. This study did not entirely meet the PMRA requirements for a Tier I study since the dose was lower than the maximum challenge concentration (MCC) of 5 mL/kg bw/day for five (5) consecutive days.

Typically, a multi-concentration study is required to establish a definitive endpoint when adverse effects are noted at MCC. To waive further avian testing, a scientific rationale was provided noting that other isolates of *B. amyloliquefaciens* are approved for use as a probiotic in animals to improve productivity. According to published literature, this microorganism excretes enzymes that help the animals to digest feed and produce more efficiently. This use was reviewed by the European Food Safety Authority (EFSA) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). In its review, the FEEDAP Panel stated that *B. amyloliquefaciens* satisfied qualified presumption of safety (QPS) status. As such the Panel did not require specific demonstration of safety, other than confirming the absence of a toxigenic potential and any determinants of resistance to antibiotics of human and veterinary clinical significance.

No toxic metabolites or determinants of resistance were identified for strain PTA-4838. Also, a screening level risk assessment (see Appendix I, Table 4) performed on the MPCA using a highly conservative endpoint (LC_{50} of 2.5 mL/kg bw/day) did not exceed the level of concern (LOC).

To address potential hazards to non-target arthropods, three studies were provided on honey bees (*Apis mellifera*), ladybird beetles (*Hippodamia convergens*) and green lacewing larvae (*Chrysoperla rufilabris*).

In the honey bee study, honey bees (*A. mellifera*; 50 bees/treatment) were exposed to Varnimo Technical (containing 1.8×10^{10} CFU of *B. amyloliquefaciens* strain PTA-4838/g), at a nominal concentration of 4.1×10^{7} CFU/mL via the dietary route of exposure. The study ended on Day 13 when mortality in the negative control group exceeded 20%. On Day 13, mortality in the treated group was 25.3%. There were no significant differences in mortality or food consumption among any of the treatment groups.

In the ladybird beetle study, adult beetles (150) were fed corn earworm treated with Varnimo Technical (containing 1.09×10^{11} CFU of *B. amyloliquefaciens* strain PTA-4838/g) at a nominal rate of 1.1×10^{8} CFU per cm^2. The test suspension was enumerated on Days 0 and 13 and found to contain mean counts of 8.87×10^{9} and 7.20×10^{9} CFU/mL, respectively. The study was ended on Day 24 when mortality in the control group exceeded 20.0%. On Day 24, mortality in the treated groups was 24.7%. There were no significant differences in mortality and in the average daily egg consumption.

In the green lacewing study, larvae (30) were fed corn earworm treated with Varnimo Technical (containing 1.8×10^{10} CFU of *B. amyloliquefaciens* strain PTA-4838/g) at a nominal rate of 1.1×10^{8} CFU per cm^2. The study ended on Day 19 after all the surviving insects emerged as adults. There were no significant differences among groups in mortality, number of days to

pupation, and days to adult emergence. There were also no physical differences observed between treated and untreated adults at emergence. However, the larvae fed Varnimo Technical ate significantly less eggs overall compared to the control group.

In mammalian studies conducted to satisfy the human health and safety requirements, it was determined that AVEO Technical Powder is of low toxicity and was not pathogenic by the oral, pulmonary, or intravenous routes (see Section 3.1 for details).

No information was provided to address potential hazards to plants and non-target non-arthropod invertebrates. *Bacillus amyloliquefaciens* occurs naturally in soils and in association with plants, particularly in the plant rhizosphere. While *B. amyloliquefaciens* is not generally considered to be a pathogen, there are recent publications implicating this species in nematicidal effects. According to these publications, these nematicidal effects are attributed to secondary metabolites and/or enzymes. While the use of AVEO EZ Nematicide as a seed treatment may potentially affect non-arthropod invertebrates, these adverse effects would be limited since this microorganism is only expected to result in minimal increases of *Bacillus* species in the rhizosphere of treated plants (Section 4.1).

These minimal localized increases in soil are not expected to significantly increase the overall environmental levels of this species above naturally occurring levels. Also, the localized elevated populations of *B. amyloliquefaciens* strain PTA-4838 in the rhizosphere of plants are expected to return to naturally sustainable levels over time.

A search in PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) using the keywords “bacillus amyloliquefaciens effect” yielded very few reports of pathogenicity. The reports of pathogenicity consisted mostly of reports on: i) the ability of *B. amyloliquefaciens* to promote growth and/or to induce systemic resistance in host crops; ii) the biological control of various plant pathogenic fungi; iii) the use of *B. amyloliquefaciens* as a probiotic in animal feed (for example, chickens); and iv) few insecticidal and nematicidal effects. A similar literature search in PubMed using the keywords “bacillus subtilis effect” yielded similar results with the exception of the noted infections in mammals (see Section 3.1.2 for further details).

Based on all the available information on the biological properties of *B. amyloliquefaciens*, the minimal documented effects in non-target terrestrial organisms and the anticipated minimal environmental exposure resulting from the use of strain PTA-4838 as a seed treatment, the risks to birds, wild mammals, terrestrial non-target arthropod invertebrates, non-arthropod invertebrates, and terrestrial plants are acceptable. Furthermore, the formulants are not expected to contribute to potential toxicity of the products.

Test results are summarized in Appendix I, Table 3.

4.2.2 Effects on Aquatic Organisms

Four studies were submitted to address the hazards of *B. amyloliquefaciens* strain PTA-4838 to fish and aquatic arthropods. These studies were performed with Varnimo Technical, which was toxicologically equivalent to AVEO Technical Powder.

Two studies were submitted to address the hazards to freshwater fish. In one study, rainbow trout (*Oncorhynchus mykiss*; 10 fish/test group) were aquatically exposed to Varnimo Technical (containing >5 × 10^{11} CFU *B. amyloliquefaciens* strain PTA-4838/g) at nominal concentrations 10^6 and 10^7 CFU/mL under static-renewal conditions and via the dietary route at nominal concentrations of 10^5 and 10^6 CFU/g diet over a period of 30 days. There were no mortalities observed in fish treated at 10^6 CFU/mL, however, there were no surviving fish in the group treated at 10^7 CFU/mL. Fish treated at 10^7 CFU/mL appeared smaller and lethargic, and they remained at the bottom of the test chamber compared to control fish. At test termination, the mean weight and mean growth of fish treated at 10^6 CFU/mL was statistically lower than control animals. The final mean weight and growth for the fish treated at 10^7 CFU/mL was also significantly less than control animals. At necropsy, fish treated at 10^7 CFU/mL had darker feces, smaller bodies, and paler bodies than control fish. These fish also had sediment/feces attached to skin near hyaline region, and slimy and clumpy food and feces in the gastrointestinal tract. The study design did not include adequate inactivated controls to properly distinguish toxic from pathogenic effects. The limitation in study design was further complicated by an apparent decline in water quality which could be responsible for the observed mortalities in fish treated at 10^7 CFU/mL. Consequently, this study was of limited utility in the risk assessment.

In the second fish study, rainbow trout (*O. mykiss*; 10 fish/test group) were aquatically exposed to Varnimo Technical (containing 1.3 × 10^{11} CFU *B. amyloliquefaciens* strain PTA-4838/g) at nominal concentrations 0.063 × 10^6, 0.13 × 10^6, 0.25 × 10^6, 0.50 × 10^6, and 1.0 × 10^6 CFU/mL under static-renewal conditions and via the dietary route at nominal concentrations of 0.063 × 10^5, 0.13 × 10^5, 0.25 × 10^5, 0.50 × 10^5, and 1.0 × 10^5 CFU/g diet over a period of 42 days. The exposure period was extended by 12 days to compensate for inadequate exposure conditions on Days 0 to 10. During this phase of the study, test suspensions were prepared based on the reported viability of the test material. On Days 11 to 42, the test suspensions were prepared using the measured viability of the test material. This extension ensured that the organisms were exposed to the target test concentrations for a period of at least 30 days. There were no mortalities and no clinical signs noted throughout the study period.

Two studies were provided to address the hazards to freshwater and estuarine arthropods. In the freshwater arthropod study, daphnids (*Daphnia* magna, 50) were aquatically exposed to Varnimo Technical (containing 2.6 × 10^{10} CFU *B. amyloliquefaciens* strain PTA-4838/g) at a nominal concentration of 10^5 CFU/mL under static renewal conditions over a period of 21 days. Mean mobility and growth rates were not found to be significantly different from control group; however, mean reproduction rates were significantly less in adult organisms exposed to Varnimo Technical than in control organisms. While reproduction was slightly impacted in this study, the use of AVEO EZ Nematicide as a seed treatment is not expected to cause any chronic aquatic exposure to *B. amyloliquefaciens* strain PTA-4838 (see Section 4.1 for further information on environmental fate).

In the estuarine invertebrate study, grass shrimp (*Palaemonetes vulgaris*, 30/group) were exposed to Varnimo Technical via the dietary route at nominal concentrations of 10^5 CFU/g and 10^6 CFU/g feed over a period of 30 days under static renewal conditions.

There were no mortalities in any shrimp treated with Varnimo Technical, and there were no significant differences in the mean growth and mean length among all groups. Furthermore, there were no observable abnormalities found in the study, except for on Day 9 where uneaten diet was observed at the bottom of all tanks.

No other information was provided to address potential hazards to aquatic non-target organisms. A search in PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) using the keywords "bacillus amyloliquefaciens effect" and "bacillus subtilis effect" yielded no reports of adverse effects to aquatic organisms.

Based on all the available information on the biological properties of *B. amyloliquefaciens*, the lack of documented effects in non-target aquatic organisms and the anticipated minimal environmental exposure resulting from the use of strain PTA-4838 as a seed treatment, the risks to aquatic organisms are acceptable. Furthermore, the formulants are not expected to contribute to potential toxicity of the end-use product.

Test results are summarized in Appendix I, Table 3.

4.3 Incident Reports related to the Environment

As of 1 October 2019, there was one environment incident reported involving *B. subtilis* (strain unspecified). Mortality was reported in spinach plants following aerial application of a product containing *B. subtilis*. The incident was assigned a certainty index of unlikely. Since the causality of the incident does not meet the criteria of highly probable, probable and possible, the incident was not considered in this review. No additional risk mitigation measures are recommended for *B. amyloliquefaciens* strain PTA-4838.

5.0 Value

Nematode injury in corn and soybean has been increasing in recent years, particularly injury caused by the root lesion nematode in corn and the soybean cyst nematode in soybean, which was first detected in southern Ontario in 1988 and has since become more widespread. Nematodes cause root damage, which not only directly interferes with plant growth but also facilitates entry of disease pathogens, such as *Fusarium virguliforme*, which causes sudden death syndrome, a serious disease of soybean.

In greenhouse and field trials conducted on soybean, Aveo EZ Nematicide generally reduced the number of cysts of soybean cyst nematode on a per plant or per gram of root basis. The results of field trials conducted on corn collectively indicate that Aveo EZ Nematicide may reduce the negative impacts, including root damage, caused by certain nematodes, including root knot, ring and spiral nematodes. By means of extrapolation, claims of partial suppression were extended to include other corn-parasitic nematodes as well as root knot nematode in soybean. No injury to corn or soybean as a result of Aveo EZ Nematicide was reported in any of the efficacy trials.

Aveo EZ Nematicide is a microbial product and will constitute an additional option for corn and soybean growers to manage nematodes when used as a part of an integrated pest management strategy that includes crop rotation and where available, the use of nematode resistant varieties,

such as those with resistance to soybean cyst nematode. Aveo EZ Nematicide may be mixed with other seed treatment products that are registered for use on corn and soybean to combat disease and insect pests.

Details of the supported uses can be found in Appendix I, Table 5.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

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

During the review process, AVEO Technical Powder and AVEO EZ Nematicide were assessed in accordance with the PMRA Regulatory Directive DIR99-03[6] and evaluated against the Track 1 criteria. AVEO Technical Powder and AVEO EZ Nematicide do not meet the Track 1 criteria because the active ingredients are biological organisms and hence are not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.

6.2 Formulants and Contaminants of Health or Environmental Concern

During the review process, contaminants in the technical as well as 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.*[7] The list is used as described in the PMRA Notice of Intent NOI2005-01[8] and is based on existing policies and regulations including: DIR99-03; and DIR2006-02[9] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

- Technical grade AVEO Technical Powder and its end-use product, AVEO EZ Nematicide, do not contain any formulants or contaminants identified in the *List of Pest Control Product Formulants of Health or Environmental Concern.*

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

7 SI/2005-114

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

9 DIR2006-02, *Formulants Policy and Implementation Guidance Document.*

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

7.0 Summary

7.1 Methods for Analysis of the Microorganism as Manufactured

The product characterization data for AVEO Technical Powder and AVEO EZ Nematicide were adequate to assess their potential human health and environmental risks. The technical grade active ingredient was characterized and the specifications of the technical grade active ingredient and end-use product were supported by the analyses of a sufficient number of batches. All batches of AVEO Technical Powder must conform to the limits set out in the OECD issue paper on microbial contaminants for microbial pest control products [ENV/JM/MONO(2011)43]. Storage stability data support storage at 20°C for up to 12 months for the technical grade active ingredient and end-use product.

7.2 Human Health and Safety

The acute toxicity/infectivity studies and other relevant information submitted in support of *B. amyloliquefaciens* strain PTA-4838 and AVEO EZ Nematicide were determined to be acceptable. Based on all the available information, the technical grade active ingredient, AVEO Technical Powder, is of low toxicity and was not pathogenic by the oral, pulmonary, or intravenous routes. The end-use product, AVEO EZ Nematicide, was of low toxicity by the oral, inhalation, and dermal route, was not a dermal irritant, and was minimally irritating to the eyes. The MPCA is considered to be a potential sensitizer. The signal words, “POTENTIAL SENSITIZER” are required on the principal display panel of the technical grade active ingredient and the end-use product; and the precautionary statements: “May cause sensitization.”, “Avoid contact with skin, eyes and clothing.”, “Avoid inhaling/breathing dusts/mists.”.

When handled according to label instructions, the potential for dermal, eye and inhalation exposure for mixer/loaders, applicators, and handlers exists, with the primary source of exposure to workers being dermal. Respiratory and dermal sensitivity could possibly develop upon repeated exposure to the product since all microorganisms, including this MPCA, contain substances that are potential sensitizers. Therefore, users handling or applying AVEO EZ Nematicide must wear waterproof gloves, long-sleeved shirts, long pants, a NIOSH-approved particulate filtering facepiece respirator, shoes and socks. Precautionary statements (for example, wearing of personal protective equipment) on the end-use product label aimed at mitigating exposure are considered adequate to protect individuals from risk due to occupational exposure.

The health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is not expected since the product is proposed for use as a seed treatment in commercial facilities. The specification of an MRL under the *Pest Control Products Act* is not required for *B. amyloliquefaciens* strain PTA-4838.

7.3 Environmental Risk

The non-target organism tests, scientific rationale and supporting published scientific literature submitted in support of *B. amyloliquefaciens* strain PTA-4838 were determined to be acceptable. The risks to non-target organisms from the use of AVEO EZ Nematicide as a seed treatment are acceptable, when the directions for use on the label are followed.

As a general precaution, the end-use product label will include environmental precaution statements to reduce contamination of aquatic systems from the use of AVEO EZ Nematicide.

7.4 Value

The submitted value information is adequate to demonstrate the value of Aveo EZ Nematicide for use as a seed treatment for soybean and corn for the partial suppression of certain parasitic nematodes. This microbial product will serve as an additional option for growers to manage nematodes when used as a part of an integrated pest management strategy.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act*, is proposing registration for the sale and use of Aveo Technical Powder and Aveo EZ Nematicide, containing the technical grade active ingredient *Bacillus amyloliquefaciens* strain PTA-4838, as a seed treatment for early season protection against particular parasitic nematodes on corn and soybeans.

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

List of Abbreviations

˚C	degree(s) Celsius
ADI	acceptable daily intake
ai	active ingredient
ARfD	acute reference dose
ATCC	American Type Culture Collection
bw	body weight
cm	centimetre(s)
cSt	centistoke(s)
CFU	colony forming units
DNA	deoxyribonucleic acid
EC_{50}	effective concentration on 50% of the population
EDE	estimated dietary exposure
EEC	estimated environmental concentration
EFSA	European Food Safety Authority
EP	end-use product
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
FIR	food ingestion rate
g	gram(s)
h	hour(s)
iv	intravenous
kg	kilogram
L	litre(s)
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOC	level of concern
mL	millilitre(s)
MAS	maximum average irritation score
mg	milligram(s)
MIS	maximum irritationsScore
MCC	maximum challenge concentration
MLN	mesenteric lymph node
MPCA	microbial pest control agent
MRL	maximum residue limit
NOEC	no observed effect concentration
OECD	Organisation for Economic Co-operation and Development
PBS	phosphate buffered saline
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
QPS	qualified presumption of safety
RQ	risk quotient
rRNA	ribosomal ribonucleic acid
TGAI	technical grade of the active ingredient
TSMP	Toxic Substances Management Policy
UF	uncertainty factor
USEPA	United States Environmental Protection Agency
v/v	volume per volume dilution

Appendix I Tables and Figures

Table 1 Toxicity Profile of AVEO Technical Powder

Study Type/ Animal/ PMRA#	Study Results
21-day Acute Oral Infectivity and Toxicity[1] Sprague Dawley rat PMRA No. 2863617	Oral LD_{50} (males and females) > 3.5×10^8 CFU/rat (Limit Test) **LOW Toxicity** and not infective via oral gavage.
21-day Acute Pulmonary Infectivity and Toxicity[1] Sprague Dawley rat PMRA No. 2863618	Pulmonary LD_{50} was greater than 3.1×10^8 CFU/rat (Limit Test) **LOW Toxicity** and not infective via pulmonary instillation.
21-day Acute Intravenous Injection Infectivity[1] Sprague Dawley rat PMRA No. 2863619	LD_{50} was greater than 2.7×10^7 CFU/rat (Limit Test) **LOW Toxicity** and not infective via intravenous injection.

[1]The test substance was Varnimo Technical containing *B. amyloliquefaciens* strain PTA-4838 which is considered toxicologically equivalent to AVEO Technical Powder.

Table 2 Toxicity Profile of AVEO EZ Nematicide

Study Type/ Animal/ PMRA#	Study Results
14-day acute oral toxicity[1] Sprague Dawley rat, female PMRA No. 2863552	Up/Down method with VBC-90052 Study 19585-15 female (3 ♀) Acute oral LD_{50} was greater than 5000 mg/kg bw.
14-day acute inhalation toxicity[1] Sprague Dawley rat PMRA No. 2863554	Acute inhalation LC_{50} was greater than 2.13 mg/L (♀, ♂).
14-day acute dermal toxicity[1]	Acute dermal LD_{50} was greater than 5050 mg/kg bw (♀, ♂).

Study Type/ Animal/ PMRA#	Study Results
Sprague Dawley rat PMRA No. 2863553	
72-hour dermal irritation[1] New Zealand white PMRA No. 2863556	Non-irritating to the skin.
72-hour eye irritation[1] New Zealand white, female PMRA No. 2863555	Minimally irritating to the eyes (MIS, 1 h = 4.7/110; MAS=0/110).

[1]The test substance was VBC-90052 containing *B. amyloliquefaciens* strain PTA-4838 which was considered equivalent to AVEO EZ Nematicide.

Table 3 Toxicity of Varnimo Technical (equivalent to TGAI) to Non-Target Species

Organism	Exposure	Significant Effect, Comments	Reference
Terrestrial Organisms			
Vertebrates			
Birds			
Bobwhite Quail (*Colinus virginianus*), 16-day-old	5-day – Dietary exposure	Three birds treated with Varnimo Technical were found dead on Day 1 and a fourth animal was found dead on Day 6. One quail exhibited activity decrease on Day 8, suffered an injured leg on Day 9 and was euthanized in the evening. There were no other clinical signs and all surviving animals gained weight by study termination. The cause of death could not be unequivocally determined, i.e., toxicity vs. infectivity. This study was of limited utility in the risk assessment.	PMRA No. 2925822
Bobwhite Quail (*Colinus virginianus*), 24-day-old	5-day – Dietary exposure	There were no treatment related toxicity effects or signs of pathogenicity observed. The 30-day acute oral LD_{50} of Varnimo Technical to the quail was greater than 2.5 mL/kg bw/day (equivalent to 2.2×10^{11}–	PMRA No. 2863622

Organism	Exposure	Significant Effect, Comments	Reference
		4.0×10^{11} CFU/kg bw/day). **LOW TOXICITY** **NOT PATHOGENIC**	
Invertebrates			
Arthropods			
Ladybird beetle (*Hippodamia convergens*), adult	1.1×10^{8} CFU per cm^2 (nominal)* – Dietary exposure *eggs (20 cm diameter sheet) were sprayed with 5 mL of test suspension containing 7.2–8.87×10^{9} CFU/mL, i.e., ~1.15×10^{8} CFU per cm^2	Study was terminated on Day 24 when mortality in control group exceeded 20%. There were no significant differences between the groups for mortality throughout the study compared to the controls. Other than one moribund ladybird beetle on Day 5 in the inactive control group, there were no other observable effects in surviving ladybird beetles. Average daily egg consumption for the control, inactive and active test groups was 59.1, 70.0 and 73.9, respectively. The 24-day dietary LC_{50} was greater than 1.15×10^{8} CFU per cm^2. The NOEC was 1.15×10^{8} CFU per cm^2. **LOW TOXICITY** **NOT PATHOGENIC**	PMRA No. 2863629
Honey Bee (*Apis mellifera*), young adult	4.1×10^{7} CFU/mL in 50% sucrose/water (nominal)* – Dietary exposure *Diets were prepared based on pre-toxicity viability tests of test material.	Study was terminated on Day 13 when mortality in control group exceeded 20%. There were no significant differences in mortality and in food consumption among treatment groups. The 13-day dietary LC_{50} was greater than 4.1×10^{7} CFU/mL. The NOEC value was 4.1×10^{7} CFU/mL. **LOW TOXICITY** **NOT PATHOGENIC**	PMRA No. 2863630

Organism	Exposure	Significant Effect, Comments	Reference
Green Lacewing (*Chrysoperla rufilabris*), larvae	1.1×10^8 CFU per cm^2 (nominal)* – Dietary exposure *Diets were prepared based on pre-toxicity viability tests of test material.	The study ended on Day 19 after all the surviving insects emerged as adults. There were no significant differences among groups in mortality, number of days to pupation, and days to adult emergence. There were also no physical differences observed between treated and untreated adults at emergence. Larvae fed Varnimo Technical ate significantly less eggs overall compared to the control group. The 19-day dietary LC_{50} was greater than 1.1×10^8 CFU per cm^2. **LOW TOXICITY** **NOT PATHOGENIC**	PMRA No. 2863628
Aquatic Organisms			
Vertebrates			
Fish			
Rainbow Trout (*Oncorhynchus mykiss*), juvenile	Aquatic exposure: 10^6 and 10^7 CFU/mL (nominal) Dietary exposure: 10^5, and 10^6 CFU/g (nominal) Static renewal 30 days	There were no mortalities observed in fish treated at 10^6 CFU/mL. There were no surviving fish in the group treated at 10^7 CFU/mL. All fish died by Day 15. The mean weight and mean growth of fish treated at 10^6 CFU/mL was significantly lower than control fish. The final mean weight and growth for the fish treated at 10^7 CFU/mL was significantly less than control fish. Fish treated at 10^7 CFU/mL appeared to remain at the bottom of the test chamber, appeared more lethargic and appeared smaller than the control fish. At necropsy, fish treated at 10^7 CFU/mL had darker feces, smaller bodies, and paler bodies than control fish. These fish also had	PMRA No. 2863624

Organism	Exposure	Significant Effect, Comments	Reference
		sediment/feces attached to skin near hyaline region, and slimy and clumpy food and feces in the gastrointestinal tract. The cause of death could not be unequivocally determined, i.e., toxicity vs. infectivity. This study was of limited utility in the risk assessment.	
Rainbow Trout (*Oncorhynchus mykiss*), juvenile	Aquatic exposure: 0.063×10^6, 0.13×10^6, 0.25×10^6, 0.50×10^6, and 1.0×10^6 CFU/mL (nominal) Dietary exposure: 0.063×10^5, 0.13×10^5, 0.25×10^5, 0.50×10^5, and 1.0×10^5 CFU/g (nominal) Static renewal 42 days	There were no mortalities and no clinical signs noted throughout the study period. The 42-day LC_{50} was greater than 1.0×10^6 CFU/mL. The NOEC was 1.0×10^6 CFU/mL. **LOW TOXICITY** **NOT PATHOGENIC**	PMRA No. 2925826
Invertebrates			
Arthropod			
Daphnids (*Daphnia magna*) , less than 24 hours old	21-day – Aquatic exposure 10^5 CFU/mL (nominal) Static renewal	At study termination, mean mobility and growth rates were not found to be significantly different from control group. Mean reproduction rates were significantly less in adult daphnids exposed to Varnimo Technical than in control daphnids. The 21-day EC_{50} was greater than 10^5 CFU/mL. **LOW TOXICITY** **NOT PATHOGENIC**	PMRA No. 2863626
Grass Shrimp (*Palaemonetes*	30-day – Dietary exposure:	At study termination, no significant differences in the mean growth and mean	PMRA No. 2863627

Organism	Exposure	Significant Effect, Comments	Reference
vulgaris), mixed ages	10^5 and 10^6 CFU/g diet(nominal) Static renewal	length were observed among all groups. There were no observable abnormalities found in the study except for on Day 9 where uneaten diet was observed at the bottom of all tanks. The 30-day EC_{50} was greater than 10^6 CFU/g diet. The NOEC values was 10^6 CFU/g diet. **LOW TOXICITY** **NOT PATHOGENIC**	

Table 4 Screening avian risk assessment using maximum AVEO EZ Nematicide residue values on the highest crop application rate

	Study Endpoint[1] (mg ai/kg bw/day / UF)	EDE[2] (mg ai/kg bw/day)	RQ
Small bird (0.02 kg)			
Acute	72.73	12.009	0.2
Medium bird (0.10 kg)			
Acute	72.73	9.433	0.1
Large bird (1.00 kg)			
Acute	72.73	2.750	0.0

[1] The endpoint of 2.5 mL/kg bw/day was converted into 727.29 mg/kg bw/day as follows:
Endpoint= 2.5 mL/kg bw/day × Estimated percent test material in dose (39.63%) × Percent active ingredient (73.4 % w/w) × 1000 mg/mL
UF = Uncertainty Factor (0.1)
[2] EDE = Estimated dietary exposure; is calculated using the following formula: (FIR/bw) × EEC, where:
FIR= Food Ingestion Rate. For generic birds with body weight less than or equal to 200 g, the "passerine" equation was used; for generic birds with body weight greater than 200 g, the "all birds" equation was used:
Passerine Equation (body weight <or = 200 g): FIR (g dry weight/day) = $0.398(\text{bw in g})^{0.850}$
All birds Equation (body weight >200 g): FIR (g dry weight/day) = $0.648\ (\text{bw in g})^{0.651}$
bw= Generic Body Weight
EEC= Concentration of pesticide on food item. At the screening level, relevant food items representing the most conservative EEC for each feeding guild are used.

Table 5 List of Supported Uses

Supported Uses	Supported Claims
Soybean Application of Aveo EZ Nematicide at 4.5 mL/unit (standard bag) of 140,000 seeds in commercial seed treatment facilities.	Partial suppression of: - soybean cyst nematode (*Heterodera glycines*) - root knot nematode (*Meloidogyne* spp.)
Corn (field, sweet, pop) Application of Aveo EZ Nematicide at 4.5 mL/unit (standard bag) of 80,000 corn seeds in commercial seed treatment facilities.	Partial suppression of: - root knot nematode (*Meloidogyne* spp.) - pin nematode (*Paratylenchus* spp.) - ring nematode (*Criconemella* spp.) - root lesion nematode (*Pratylenchus* spp.) - spiral nematode (*Helicotylenchus* spp.) - stubby root nematode (*Paratrichodorus* spp.)

References

A. List of Studies/Information Submitted by Registrant

1.0 The Active Substance, Its Properties And Uses

PMRA No.	Reference
2863612	2017, VBC-90060 5-Batch Enumeration and Detection of Microbial Contaminants, DACO: M2.10.1, M2.10.2, M2.10.3
2863613	2014, Varnimo Technical Storage Stability, DACO: M2.11
2863614	2013, Varnimo Technical Physical and Chemical Characteristics, DACO: M2.12
2863615	2017, AVEO Technical - Summary of studies to Determine Phy-Chem Properties, DACO: M2.12
2925820	2014, Varnimo Technical Manufacturing Directions, DACO: M2.7.1,M2.7.2 CBI
2925821	2011, BA-1 AStrain D747 EPA Reg decision, DACO: M2.7.1,M2.7.2
2950071	2019, Deficiency Response for AVEO Technical Powder Submission number 2018-1201, DACO: M2.7.1, M2.7.2, M9.2.1
2950072	2019, Deficiency Response for AVEO EZ Nematicide Submission Number 2018-1198, DACO: M2.9.1, M4.9
2988691	2017, VBC-90060: Product Identity, Manufacturing Process, and Formation of Unintentional Imgredients, DACO: M2.2, M2.8, M2.9, M2.9.1, M2.9.2, M2.9.3 CBI
3024905	2019, Clarification Note for AVEO Technical Powder Sub 2018-1201, DACO: M2.11
2863546	2016, VBC-90052: Manufacturing Process and Formation Ingredients, DACO: M2.10.2, M2.10.3, M2.8, M2.9.1, M2.9.3 CBI
2863547	2016, VBC-90052 5-Batch Enumeration and Detection of Microbial Contaminants, DACO: M2.10.2, M2.10.3, M2.8, M2.9.1, M2.9.2, M2.9.3
2863548	2017, VBC-90052 Storage Stability with Corrosion Characteristics, DACO: M2.11
2863549	2016, VBC-90052 Product Chemistry, DACO: M2.12
2863550	2017, AVEO EZ Nematicide - Summary of studies to Determine Phy-Chem Properties, DACO: M12.5
2981979	2019, BA-1 Strain PTA-4838 Further Identification, DACO: M2.7.1,M2.7.2 CBI

2.0 Human and Animal Health

PMRA No.	Reference
2863616	2017, AVEO Technical - Summary of Studies to Determine Acute Toxicology, DACO: M4.1, M4.2.1, M4.3.1, M4.5.1
2863617	2013, Acute Oral Toxicity/Pathogenicity Study in Rats, DACO: M4.2.2
2863618	2013, Acute Pulmonary Toxicity/Pathogenicity Study in Rats, DACO: M4.2.3
2863619	2013, Intravenous Toxicity/Pathogenicity Study in Rats, DACO: M4.3.2
2863620	2014, Acute Tox Waiver Request, DACO: M4.4, M4.5.2
2863551	2017, AVEO EZ Nematicide - Summary of Studies to Determine Acute Toxicology, DACO: M4.1

PMRA No.	Reference
2863552	2016, VBC-90052 Acute Oral Toxicity (UDP) in Rats, DACO: M4.9
2863553	2016, VBC-90052 Acute Dermal Toxicity in Rats, DACO: M4.4
2863554	2016, VBC-90052 Acute Inhalation Toxicity in Rats, DACO: M4.9
2863555	2016, VBC-90052 Acute Eye Irritation in Rabbits, DACO: M4.9
2863556	2016, VBC-90052 Acute Dermal Irritation in Rabbits, DACO: M4.5.2

3.0 Environment

PMRA No.	Reference
2863621	2017, AVEO Technical Powder - Summary of Studies to Determine Environmental Toxicity, DACO: M9.0, M9.1
2863622	2014, Acute MPCA Oral Toxicity Study in Bobwhite Quail, DACO: M9.2, M9.2.1
2863623	2014, Acute MPCA Oral Toxicity Study in Mallard Duck Waiver Request, DACO: M9.0, M9.2, M9.2.1
2863624	2014, MPCA Tier 1 Freshwater Fish Test with Rainbow Trout, DACO: M9.0, M9.4, M9.4.1
2863626	2014, MPCA Tier 1 Freshwater Aquatic Invertebrate Test with Daphnia magna, DACO: M9.0, M9.6
2863627	2014, MCPA Tier 1 Estuarine and Marine Animal testing with Grass Shrimp, DACO: M9.0, M9.5, M9.5.2
2863628	2014, Nontarget Insect Testing with Green Lacewing, DACO: M9.0, M9.9
2863629	2017, Ladybird Beetle Microbial Oral Toxicity Limit Test, DACO: M9.0, M9.9
2863630	2013, Nontarget Insect Testing with Honey Bee, DACO: M9.0, M9.9
2925822	2013, Varnimo Technical Acute Oral Toxicity Study in Bobwhite Quail, DACO: M9.2.1
2925823	2018, Literature - Benefit of BA Chickens, DACO: M9.2.1
2925824	2010, EFSA Scientific Opinion, DACO: M9.2.1
2925825	2008, EFSA Safety and Efficacy of Ecobiol, DACO: M9.2.1
2925826	2018, Tox Path test with Rainbow Trout, DACO: M9.4.1
2950071	2019, Deficiency Response for AVEO Technical Powder Submission number 2018-1201, DACO: M2.7.1, M2.7.2, M9.2.1

4.0 Value

PMRA No.	Reference
2863543	2017, Appendix 1: Trials Reports for Value Summary for AVEO EZ Nematicide, a Seed Protectant Containing *Bacillus amyloliquefaciens* strain PTA-4838, for Control of various nematodes Affecting Corn and Soybeans in Canada, DACO: 10.1, 10.2.1, 10.2.2, 10.2.3.1, 10.2.3.3, 10.3.1, 10.4, 10.5.1, 10.5.2, 10.5.3, 10.5.4
2950483	2018, Deficiency Response for "Value Summary for Aveo EZ nematicide, a Seed Protectant Containing *Bacillus amyloliquefaciens* strain PTA-4838, for Control of various nematodes Affecting Corn and Soybeans in Canada", DACO: 10.1
2950485	2018, Root Knot Nematode - Corn 2018-09-12, DACO: M10.2.2

PMRA No.	Reference
2950486	2018, Evaluation of the Efficacy of Seed Treatments against nematodes in Corn Grown in Florida, DACO: M10.2.2
2950487	2018, Metabolites of Strain BA1 Progress Report, DACO: M10.2.1,M10.4.1
3033260	2019, Clarification Response for "Value Summary for Aveo EZ nematicide, a Seed Protectant Containing *Bacillus amyloliquefaciens* strain PTA-4838, for Control of various nematodes Affecting Corn and Soybeans in Canada", DACO: 10.1
3033261	2019, Appendix 1 - Trial Reports, DACO: 10.1,10.2.3
3038212	2019, AVEO EZ Combined pdf Files , DACO: 10.1,10.2.3
3040006	2019, Clarification DACO 10.2 Sub 2018-1198, DACO: 10.2

B. Additional Information Considered

i) Published Information

1.0 The Active Substance, Its Properties and Uses

PMRA No.	Reference
2835732	Apertroaie-Constantin, C., Mikkola, R., Andersson, M.A., Teplova, V., Suominin, I., Johansson, T. and Salkinoja-Salonen, M., 2008, *Bacillus subtilis* and *B. mojavensis* strains connected to food poisoning produce the heat stable toxin amylosin, 2009. J Appl Microbiol 106, 1976-1985., DACO: M2.14, M4.9
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PMRA No.	Reference
	subtilis in connection with bovine mastitis, Nord Vet Med. 38: 233-236., DACO: M2.14, M4.9
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