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Evaluation Report

Metarhizium anisopliae strain F52

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Overview

Registration Decision for *Metarhizium anisopliae* Strain F52

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, has granted conditional registration for the sale and use of *Metarhizium anisopliae* strain F52 and the end-use product Met52 Granular Bioinsecticide, containing the technical grade active ingredient *Metarhizium anisopliae* strain F52, to control root weevils, specifically black vine weevil and strawberry root weevil, on container-grown 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.

Although the risks and value have been found acceptable when all risk-reduction measures are followed, the applicant must submit additional scientific information as a condition of registration.

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 *Metarhizium anisopliae* strain F52 and Met52 Granular Bioinsecticide.

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 acceptable¹ if there is reasonable certainty that no harm to human health, future generations or the environment will result from use of or exposure to the product under its proposed conditions of registration. The Act also requires that products have value² 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

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."

observed and the uncertainties present when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, as well as on the assessment process and risk-reduction programs, please visit the PMRA's website at www.hc-sc.gc.ca/cps-spc/pest/index-eng.php.

What Is *Metarhizium anisopliae* strain F52?

Metarhizium anisopliae strain F52 is a soil-dwelling fungus that causes a fatal disease in certain insects. Formulated as Met52 Granular Bioinsecticide and incorporated into the growing medium, it can provide control of root weevils, specifically black vine weevil and strawberry root weevil, on container-grown ornamentals.

Health Considerations

Can Approved Uses of *M. anisopliae* strain F52 Affect Human Health?

***Metarhizium anisopliae* strain F52 is unlikely to affect your health when Met52 Granular Bioinsecticide is used according to the label directions.**

People could be exposed to *M. anisopliae* strain F52 when handling and applying the product. When assessing health risks, several key factors are considered: the microorganism's biological properties (e.g., 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.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns. When spores of *M. anisopliae* strain F52 were tested on laboratory animals, no signs of significant toxicity or disease were observed.

Residues in Water and Food

Dietary risks from food and water are not of concern.

The *Food and Drugs Act* prohibits the sale of food containing a pesticide residue that exceeds the established maximum residue limit (MRL). Pesticide MRLs are established for *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act*. Each MRL value determines the maximum concentration in parts per million (ppm) of a pesticide allowed in or on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

As there are no direct applications to food and as no adverse effects were reported in laboratory studies, the establishment of an MRL is not required for *M. anisopliae* strain F52 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulations. In addition, the likelihood of residues of *M. anisopliae* strain F52 contaminating drinking water supplies is negligible. Consequently, dietary exposure and risk are minimal to non-existent.

Occupational Risks From Handling Met52 Granular Bioinsecticide

Occupational risks are not of concern when Met52 Granular Bioinsecticide is used according to label directions, which include protective measures.

Workers using Met52 Granular Bioinsecticide can come into direct contact with *M. anisopliae* strain F52 on the skin, in the eyes, or by inhalation. For this reason, the label will specify that users exposed to Met52 Granular Bioinsecticide must wear waterproof gloves, eye goggles, a long-sleeved shirt, long pants, shoes plus socks and a dust/mist filtering respirator/mask (MSH/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator/mask with any N-95, R-95, P-95 or HE filter.

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

Environmental Considerations

What Happens When Met52 Granular Bioinsecticide Is Introduced Into the Environment?

Environmental risks are not of concern.

Metarhizium anisopliae strain F52 is a non-indigenous soil microorganism that is pathogenic to specific host insects. Since the reproduction of conidiospores is reliant upon infection of a suitable host under conditions of high humidity, the proliferation of *M. anisopliae* strain F52 in the environment would be limited. It is likely that levels of *M. anisopliae* strain F52 would return to levels comparable to native populations of *M. anisopliae*.

Toxicity testing on non-target organisms shows that *M. anisopliae* strain F52 is capable of causing some adverse effects to certain aquatic organisms when exposed to high concentrations. However, the incorporation of Met52 Granular Bioinsecticide into the growing medium of potted plants is unlikely to result in significant contamination of aquatic environments. Therefore, the risk to aquatic organisms from the use of Met52 Granular Bioinsecticide is very low. Toxicity testing also shows that terrestrial non-target organisms, other than target insect species, were not adversely affected by *M. anisopliae* strain F52 when exposed to high concentrations.

Value Considerations

What Is the Value of Met52 Granular Bioinsecticide?

Incorporated into the growing medium, Met52 Granular Bioinsecticide can provide control of black vine weevil and strawberry root weevil on container-grown ornamentals.

The value of Met52 Granular Bioinsecticide is that it provides a viable alternative for the control of certain serious pests on a variety of crops. Root weevils, particularly black vine weevil and strawberry root weevil, are major pests of many ornamentals and are considered very difficult to control. Few other pest control products are registered in Canada for use against these pests and most are older, conventional chemical insecticides. Met52 Granular Bioinsecticide must be incorporated into the growing medium to achieve acceptable efficacy, but also may remain viable for nine months after application.

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 on the label of Met52 Granular Bioinsecticide to address the potential risks are as follows:

Key Risk-Reduction Measures

Human Health

Due to concerns about users developing allergic reactions through repeated high exposure to *M. anisopliae* strain F52, anyone handling or applying Met52 Granular Bioinsecticide must wear waterproof gloves, eye goggles, a long-sleeved shirt, long pants, shoes plus socks and a dust/mist filtering respirator/mask (MSH/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator/mask with any N-95, R-95, P-95 or HE filter.

Environment

As a general precaution, statements will be added to the label to prohibit handlers from contaminating aquatic habitats or allowing effluent from greenhouses containing this product to enter lakes, streams, ponds or other water bodies.

What Additional Scientific Information is Being Requested?

Although the risks and value have been found acceptable when all risk-reduction measures are followed, the applicant must submit additional scientific information as a condition of registration. More details are presented in the Science Evaluation section of this Evaluation Report or in the Section 12 Notice associated with these conditional registrations. The applicant must submit the following information by 30 September 2010.

Human Health

- An acute oral toxicity study conducted with the end-use product, Met52 Granular Bioinsecticide
- Five microbial contamination tests of full-scale production batches of the end-use product
- A storage stability study conducted with the end-use product

Other Information

As these conditional registrations relate to a decision on which the public must be consulted,³ the PMRA will publish a consultation document when there is a proposed decision on applications to convert the conditional registrations to full registrations or on applications to renew the conditional registrations, whichever occurs first.

The test data cited in this Evaluation Report (i.e., the test data relevant in supporting the registration decision) will be made available for public inspection when the decision is made to convert the conditional registrations to full registrations or to renew the conditional registrations (following public consultation). If more information is required, please contact the PMRA's Pest Management Information Service by phone (1-800-267-6315) or by e-mail (pmra_infoserv@hc-sc.gc.ca).

³ As per subsection 28(1) of the Pest Control Products Act.

Science Evaluation

Metarhizium anisopliae strain F52

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	<i>Metarhizium anisopliae</i> strain F52
Function	control of black vine weevil and strawberry root weevil on container-grown ornamentals, including flowering and foliage plants, shrubs, shade and forest tree seedlings
Binomial name	<i>Metarhizium anisopliae</i> var. <i>anisopliae</i> strain F52
Taxonomic designation¹	
Kingdom	Fungi
Phylum	Dikarya
Subphylum	Ascomycota
Class	Pezizomycotina
Order	Hypocreales
Family	Clavicipitaceae
Genus	<i>Metarhizium</i>
Species	<i>anisopliae</i>
Strain	F52
Patent Status information	No patents are held by the applicant in Canada.
Minimum purity of active	1.0×10^{10} colony forming units (CFU)/g
Identity of relevant impurities of toxicological, environmental and/or other 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. <i>Metarhizium anisopliae</i> strain F52 is known to produce the toxins destruxins and cytochalasins.

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

Technical Grade Active Ingredient–*Metarhizium anisopliae* strain F52

Physical state	Fine homogeneous powder
Guarantee	1.0×10^{10} CFU/g
Colour	Grayish-olive
Odour	Earthy
pH	5.66 in 1% (w/v) reagent water
Density	0.273 g/mL (bulk)

End-Use Product–Met52 Granular Bioinsecticide

Physical state	Granular
Guarantee	9.0×10^8 CFU/g

1.3 Directions for Use

Apply Met52 Granular Bioinsecticide prior to or during planting by thoroughly mixing the product into the growing medium, ensuring even distribution. The growing medium should be moist at the time of application and maintained in a moist condition after application for best performance.

Applications to Growing Media for Container-grown Ornamentals:

Apply 500 g to 1.5 kg/m³ of moist growing medium, using a higher application rate when pest pressure is expected to be high. Uniformly incorporate the Met52 Granular Bioinsecticide throughout the growing medium.

Do not mix fungicides in growing media containing Met52 Granular Bioinsecticide. Keep the plants above 16°C and maintain good drainage. Met52 Granular Bioinsecticide is grown on cereal grains that will decompose in the growing media. Pests likely to be attracted by grains, such as rodents, must be controlled prior to use of this product.

1.4 Mode of Action

Met52 Granular Bioinsecticide is composed of spores of the entomopathogenic fungus *Metarhizium anisopliae* strain F52 on a grain matrix. Once the product is incorporated into the growing medium, insects that come into contact with the fungus become infected. Spores of *Metarhizium anisopliae* adhere to insect cuticle, germinate, and begin to grow. Fungal hyphae penetrate the exoskeleton of the insect, followed by rapid mycelial growth within the insect. Under ideal conditions, insect death may occur within 3-5 days after exposure to the fungus. Other insects that come into contact with infected insects may also become infected.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

Metarhizium anisopliae strain F52 is not substantially different from the classical description of the species. The dominant taxonomic characteristics are the morphological features of the sporulating structures. The genus is defined on the basis of the arrangement of the phialides-bearing chains and columns of dry and generally green, cylindrical or slightly ovoid conidia. The columns are formed by aggregation of the conidial chains. Two forms of *M. anisopliae* are distinguished based on the conidial size: (1) the short-spored form *M. anisopliae* var. *anisopliae*, with conidia of about 5-8 µm long and (2) the long-spored form *M. anisopliae* var. *majus*, with conidia usually between 10 and 14 µm long. A method has been developed to differentiate between strains of *M. anisopliae* var. *anisopliae* by the identification of group-I introns at three different positions within the 28S rDNA gene of the *M. anisopliae* var. *anisopliae*, although this does not appear to be a routine component of the quality assurance program.

2.2 Methods for Establishment of Purity of Seed Stock

Subsamples of the mother culture are frozen in a large number of aliquots at -80°C in 15% glycerol. These aliquots are in sufficient numbers to provide starter cultures for several years of production. Once these starter cultures have been depleted, bioassays are used to determine whether passage through an insect target host such as black vine weevil is necessary to retain pathogenicity. *Metarhizium anisopliae* strain F52 is also deposited in three culture collections: one held by the Centre for Agriculture and Biosciences International (IMI 385045), a second held by the Agricultural Research Service Entomopathogenic Fungi (ARSEF 7711) and a third by the American Type Culture Collection (90448).

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 end-use product is based on the number of viable spores per mass of product. The total spore count, determined by means of a hemacytometer, is multiplied by the germination rate, determined by microscopic examination of culture plates for the development of germ tubes, to give a guarantee measured in CFU/gram of end-use product.

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

Methods to determine and quantify residues of the MPCA and its secondary metabolites (i.e. destruxins and cytochalasins) are not required since this product will not be applied to food crops.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality control procedures used to limit contaminating microorganisms during manufacture of *M. anisopliae* strain F52 and Met52 Granular Bioinsecticide are acceptable. As well, the final product is plated onto a number of selective media for detection of contaminating microorganisms. Batches showing an unacceptable level of microbial growth on the selective media are discarded.

2.6 Methods to Show Absence of Any Human and Mammalian Pathogens

As noted in section 2.5, quality control procedures are used to limit microbial contamination in *M. anisopliae* strain F52 and Met52 Granular Bioinsecticide. These procedures include contamination checks to detect total contaminating fungi and contaminating bacteria including: *Salmonella* spp., *Shigella* spp., *Vibrio* spp., and *Escherichia coli*.

Acceptable microbial contaminant analysis data was submitted for five batches of *M. anisopliae* strain F52 and Met52 Granular Bioinsecticide from each manufacturing site. At one of the manufacturing sites, although contamination levels were found to be acceptable on pilot-scale production batches, confirmatory microbial contamination analysis data on five full-scale production batches of Met52 Granular Bioinsecticide are required as a condition of registration.

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

The viability of *M. anisopliae* strain 52 in an end-use product formulation similar to Met52 Granular Bioinsecticide was assessed by determining the guarantee over a period of time and over a range of storage temperatures.

The submitted storage stability data supports a storage period for Met52 Granular Bioinsecticide of 3 months at 4°C. A confirmatory storage stability study performed using Met52 Granular Bioinsecticide is required as a condition of registration.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

A survey of published literature has revealed that many strains of *M. anisopliae* produce the toxic metabolites called destruxins and cytochalasins. Specific analyses were conducted with *M. anisopliae* strain F52 that confirmed production of these toxic metabolites. Also, the toxic metabolites swainsonine, aurovertins F– H, serinocyclins A and B, and the antibiotic K-582 have been shown to be produced by various strains of *M. anisopliae*, however, none of these have been reported to be produced by *M. anisopliae* strain F52.

A published study showed the production of two mutagenic secondary metabolites (NG-391 and NG-393) by a wildtype *M. anisopliae* strain (ARSEF 2575) and its mutant strain (KOB1-3). The PMRA acquired a study that showed that a crude extract of *M. anisopliae* strain F52 did not produce a mutagenic result when tested using a bacterial reverse mutation assay comparable to the published assay for determining the mutagenicity of NG-391 and NG-393.

No human hypersensitivity reactions to *M. anisopliae* strain F52 have been reported by the applicant. However, studies found in the public literature indicated that various strains of *M. anisopliae* have the potential to cause asthmatic responses; also reported was one incidence of a severe dermal hyperallergenic response to *M. anisopliae* var. *acridum*. The PMRA considers all microbial pest control agents (MPCAs) to be potential sensitizers by default, thereby requiring appropriate label statements and mitigating measures to minimize human exposure.

The PMRA conducted a detailed review of the toxicological database for the spores of *M. anisopliae* strain F52. The database is complete, consisting of laboratory animal (*in vivo*) toxicity studies (acute oral toxicity/pathogenicity, acute pulmonary toxicity/pathogenicity, acute intraperitoneal infectivity, acute dermal toxicity/irritation, dermal sensitization, and eye irritation) currently required for health hazard assessment purposes that were carried out in accordance with currently accepted international testing protocols and GLP. The scientific quality of the data is high and the database is considered sufficient to characterize the infectivity of this pest control agent and product. However, a toxicity study testing the end-use product is required to ensure that the end-use product is toxicologically equivalent to the technical grade active ingredient.

In an oral toxicity/infectivity study, groups of 8-week-old CD[®] rats (15 per sex) were exposed by the oral route to spores of *M. anisopliae* strain F52 (5.61×10^{10} CFU/g). The test material was suspended in ASTM type 1 water and Tween 80. Test group (TG) rats were dosed orally with approximately 1.04×10^8 CFU of *M. anisopliae* strain F52 spores per animal in a 1 mL volume and observed for up to 7 days. Heat killed test substance, shelf control and naive control groups were also used. The oral LD₅₀ for males and females is $> 1.04 \times 10^8$ CFU/animal. There were no deaths observed in any of the dosed or control groups prior to scheduled sacrifice. Mean body weight and mean body-weight gains were not statistically different in any test groups during the study. Two TG female rats lost weight on Day 3. Three male and two female rats in the TG group, three male and three female rats in the heat killed test substance group, and three male and three female rats in the naive control group lost weight on Day 7. No adverse clinical observations were made in any group. No gross lesions were noted on any rat at necropsy. No statistically significant effects on relative organ weights (lungs, spleen, liver, kidneys, and brain) were observed. The test organism was not detected in any sample (blood, brain, lungs, spleen, liver, kidneys, mesenteric lymph nodes, stomach and small intestines, cecum, or feces) from the KTG, NC, or SC rats. *Metarhizium anisopliae* strain F52 was only detected in the stomach and small intestines, cecum, and feces on Day 0 from the TG rats. The test organisms were not detected in any other tissue from TG group rats on Days 0, 4, or 7. This study is classified as acceptable and satisfies the guideline requirement for an acute oral toxicity/infectivity study in the rat for the technical grade active ingredient.

In an acute pulmonary infectivity and toxicity study, groups of 10-week-old CD[®] rats (20 per sex) were exposed by the intratracheal route to spores of *M. anisopliae* strain F52 (5.61×10^{10} CFU/g) in 0.1 mL of water containing 0.1% Tween 80[®] at a dose of 1.17×10^8 CFU per animal. Animals were then observed for up to 35 days. Heat killed test substance, shelf control and naïve control groups were also used. The pulmonary LD₅₀ for males and females is $> 1.17 \times 10^8$ CFU/animal. Based on these results, *M. anisopliae* strain F52 is of low toxicity and is not pathogenic in the rat. Test substance was detected in the lungs and associated lymph nodes of test group (TG) dosed rats on Day 0, with clearance from all organs by Day 35, consistent with intratracheal administration. No test substance was detected in naïve control, heat killed test substance and shelf control group animals. There were some statistically significant differences in lung and associated lymph nodes, spleen, kidney, brain, and liver weights. Increase in lung and associated lymph node weight may be due to the immune response (clearance mechanism) to the test substance. Other statistically significant differences were likely transient and of no biological significance. As well, three female rats had brown and/or mottled lungs which is consistent with intratracheal administration. A statistically significant decrease in body-weight gain was observed for female TG rats between Days 14 and 21. This difference is not believed to be biologically significant. This acute pulmonary infectivity and toxicity study is classified as acceptable and satisfies the guideline requirement for an acute pulmonary infectivity and toxicity study in the rat for the technical grade active ingredient.

In an acute intraperitoneal infectivity study, groups of 8-week-old CD[®] rats (12 per sex) were injected with spores of *M. anisopliae* strain F52 (5.61×10^{10} CFU/g) in 1 mL of water containing 0.1% Tween 80[®] at a dose of 1.0×10^7 CFU per animal. Animals were then observed for up to 14 days. Heat killed test substance, shelf control and naïve control groups were also used. *M. anisopliae* strain F52 is not pathogenic based on these results. Test substance was detected in the blood, spleen, liver, kidneys, mesenteric lymph nodes, caecum and peritoneal lavage fluid of TG dosed rats on Day 0 with clearance from all organs and sites by Day 14, except for the lavage fluid where a small number of colony forming units remained at Day 14 (< 100 CFU/mL for males; < 10 CFU/mL for females). It is reasonable to conclude that clearance would be complete from the lavage fluid in the following days. No test substance was detected in naïve control, shelf control or heat killed test substance group animals. There were some statistically significant differences in spleen, brain, and liver weight. Increases in spleen and liver weight may be due to the immune response (clearance mechanism) to the test substance. Other statistically significant differences were likely transient and of no biological significance. No adverse effects were observed in any of the animals. This intraperitoneal infectivity study is classified as acceptable and satisfies the guideline requirement for an intraperitoneal infectivity study in the rat.

In an acute dermal toxicity study, approximately 10% of the body surface area of groups of 3-month-old New Zealand White rabbits (5/sex) were dermally exposed to *M. anisopliae* strain F52 (7.9×10^9 CFU/g) for 24 hours. Following exposure, the animals were observed for a period of 14 days. The dermal LD₅₀ was found to be > 2 g/kg bw. *Metarhizium anisopliae* strain F52 is of low toxicity based on the absence of overt signs of toxicity and mortality in the tested rabbits. Signs of dermal irritation included erythema, edema and eschar formation. All animals had completely recovered from signs of dermal irritation by Day 9. This acute dermal toxicity study is classified as acceptable and satisfies the guideline requirement for an acute dermal toxicity

study in the rabbit for the technical grade active ingredient. In addition to the utilization of this study as a measure of dermal toxicity, the results will be used to assess dermal irritation in the absence of a specific study. It is noted that the dose used in this toxicity study is 2 g applied for 24 hours versus a dose of 0.5 g for 4 hours that is typical of an irritation study. The highest maximum irritation score (MIS) was 3.7 at 48 hours which indicates that *M. anisopliae* strain F52 is moderately irritating to the skin. As well, this classification should apply to the end-use product, Met52 Granular Bioinsecticide, in the absence of an irritation study testing the end-use product.

In a skin sensitization study with *M. anisopliae* strain F52 (7.9×10^9 CFU/g), young adult Hartley guinea pigs (20 males) were tested using the Buehler method. Hexylcinnamaldehyde was used as the positive control material. There were no mortalities. The study used the spores of *M. anisopliae* strain F52 rather than the end-use product. No skin reactions were observed in the test group during the induction phase although one test group guinea pig exhibited mild erythema at the 48-hour point of the challenge phase. In this study, *M. anisopliae* strain F52 is not a dermal sensitizer. However, as a matter of policy, all MPCAs are considered potential sensitizers by PMRA, as they contain substances that can elicit allergic responses and therefore require the statement 'POTENTIAL SENSITIZER' to appear on the principal display panel of the label. This study is classified as acceptable and satisfies the guideline requirement for a dermal sensitization study in the guinea pig, although the study should have been conducted using the end-use product.

In a primary eye irritation study, 0.1 g of *M. anisopliae* strain F52 spores (6.3×10^9 CFU/g) was instilled into the conjunctival sac of the right eye of young adult New Zealand White rabbits (3/sex) for 24 hours. The treated eyes were rinsed with lukewarm water 24 hours after instillation. Animals then were observed for 21 days. Irritation was scored by the method of Draize. A bacterial infection occurred in the eye of one male rabbit. The most likely causative agent of the infection came from bacteria contaminating the test material. The level of contamination in this production lot of test material was not reported. The source of MPCA proposed for registration in Canada involves a different manufacturer in a different location from the source used in this study. Microbial contamination testing performed on the source of MPCA proposed for Canadian registration showed an acceptable level of microbial contamination. It is acceptable to exclude data from this rabbit for the irritation calculations conducted in this study since the symptoms observed were not a result of irritation caused by the MPCA. The highest MIS based on five rabbits observed during the study was 23.2 (maximum possible score = 110) at the 24-hour scoring interval at which time signs of corneal opacity and iridial irritation were seen in four rabbits and conjunctival erythema and chemosis were seen in all rabbits. Recovery from signs of ocular irritation occurred between 72 and 96 hours following test substance administration. The rabbits completely recovered from ocular irritation by Day 4. In this study, *M. anisopliae* strain F52 is moderately irritating to the eye based on an MIS of 23.2 and the possible contribution of the MPCA to the onset of a bacterial infection in one rabbit. Although the MPCA did not itself cause the infection, it may have had a role in the onset, i.e. through physical damage to the eye. As well, this classification should apply to the end-use product, Met52 Granular Bioinsecticide, in the absence of an irritation study testing the end-use product.

This study is classified as acceptable and satisfies the guideline requirement for a primary eye irritation study in the rabbit.

Higher-tier subchronic and chronic toxicity studies were not required due to the low acute toxicity of the test substance, 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 that suggest *M. anisopliae* strain F52 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 spores of *M. anisopliae* strain F52. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *M. anisopliae* strain F52.

3.2 Occupational/Bystander Exposure and Risk Assessment

3.2.1 Occupational

When handled according to the label instructions, the potential routes of handler exposure to *M. anisopliae* strain F52 are pulmonary, dermal and to some extent ocular.

The potential for dermal, eye and inhalation exposure for applicators, handlers and workers exists, with the primary source of exposure to workers being dermal. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. This MPCA has not been identified as a wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Although metabolites may be present in the end-use product in small amounts, exposure to applicators, handlers and workers expected from the use of this product is relatively low.

Respiratory hypersensitivity could possibly develop upon repeated exposure to the product. Specific label wording to minimize exposure to dusts generated while handling or applying the product are required. Exposure by applicators will be mitigated by a label requirement for personal protective equipment, including a dust/mist filtering respirator/mask. Although no dermal toxicity and moderate dermal irritation are expected based on toxicological studies of the spores of *M. anisopliae* strain F52, all MPCAs are considered potential sensitizers. The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions. Label restrictions and risk mitigation measures are required to protect populations that are likely to be primarily exposed to the products. Such exposure to applicators and handlers can be minimized if they wear waterproof gloves, long-sleeved shirts, long pants, shoes and socks.

In an eye irritation study performed using the technical grade active ingredient, *M. anisopliae* strain F52 was found to be moderately irritating to the eye. Consequently, label restrictions are

required to protect workers that are likely to be exposed to the products. Such exposure can be minimized if applicators and handlers wear eye goggles.

3.2.2 Bystander

Overall the PMRA does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for the spores of *M. anisopliae* strain F52 and the low exposure to bystanders from the use of Met52 Granular Bioinsecticide.

The label does not allow applications to turf, residential or recreational areas; therefore, non-occupational exposure and risks to adults, infants and children are low. Because the use sites are commercial, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

Met52 Granular Bioinsecticide is to be applied to commercial growing media for non-food crops only. Negligible to no risk is expected for the general population, including infants and children, or animals because there are no direct applications of Met52 Granular Bioinsecticide to food or feed crops. Therefore, there is no concern for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children. Furthermore, the use of Met52 Granular Bioinsecticide on food crops is not supported due to the lack of mammalian toxicity test data on the end-use product.

3.3.2 Drinking Water

No risks are expected from exposure to this microorganism via drinking water because exposure will be minimal based on the use pattern and no harmful effects were observed in animals that were exposed orally in Tier I acute oral toxicity and infectivity testing. The label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats by cleaning equipment or disposing wastes. Users are also required to not allow effluent or runoff from greenhouses containing this product to enter lakes, streams, ponds or other water bodies. Runoff from treated potting soil is not expected; therefore, it is unlikely that *M. anisopliae* strain F52 will enter aquatic environments. Moreover, the MPCA is not expected to proliferate in aquatic habitats and percolation through soil and municipal treatment of drinking water would reduce the possibility of significant transfer of *M. anisopliae* strain F52 or its residues to drinking water. Therefore, potential exposure to *M. anisopliae* strain F52 in surface and drinking water is negligible.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculation of acute reference doses (ARfDs) and acceptable daily intakes (ADIs) is not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e., no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that the spores of *M. anisopliae* strain F52 are of low toxicity, are not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCA than the general population. Thus, there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCA and other registered micro-organisms that have a common mechanism of toxicity, do not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *M. anisopliae* strain F52 to human health.

3.4 Maximum Residue Limits

The *Food and Drugs Act* prohibits the sale of adulterated food, that is, food containing a pesticide residue that exceeds the established maximum residue limit (MRL). Pesticide MRLs are established for FDA purposes through the evaluation of scientific data under the Pest Control Products Act (PCPA). Each MRL value defines the maximum concentration in parts per million (ppm) of a pesticide allowed in/on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

As there are no applications to food, the establishment of an maximum residue limit (MRL) is not required for *M. anisopliae* strain F52 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulations.

3.5 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA database, there is reasonable certainty no harm will result from aggregate exposure of residues of *M. anisopliae* strain F52 to the general Canadian population, including infants and children, when the microbial pest control product is used according to label directions. 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. As the product is to be used in commercial growing media and is not allowed for use on turf, residential or recreational areas, dermal and inhalation exposure to the general public will be very low. Furthermore, few adverse effects from exposure to natural populations of *M. anisopliae* in the

environment have been reported. Even if there is an increase in exposure to this microorganism from the use of Met52 Granular Bioinsecticide, there should be no increase in potential human health risk.

3.6 Cumulative Effects

The PMRA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Besides naturally occurring strains of *M. anisopliae* in the environment, the PMRA is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity with this active ingredient. No cumulative effects are anticipated if the residues of *M. anisopliae* strain F52 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Environmental fate testing is intended to demonstrate whether an 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 the extent of exposure.

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.

4.2 Effects on Non-Target Species

4.2.1 Effects on Terrestrial Organisms

A complete ecotoxicology package was submitted to address the risks of *M. anisopliae* strain F52 to terrestrial organisms.

The acute oral toxicity of *M. anisopliae* strain F52 to 21-day-old Northern Bobwhites (*Colinus virginianus*) was assessed over 30 days. *Metarhizium anisopliae* strain F52 was administered to the birds (5 birds/treatment; 6 treatment groups) by oral gavage at 3.5×10^8 CFU/g bw for a 5-day period for a total dosage of 1.75×10^9 CFU/g of bw. The 30-day acute oral LD₅₀ of *M. anisopliae* strain F52 was $>3.5 \times 10^8$ CFU/g bw per day for 5 days. The 30-day NOEL of *M. anisopliae* strain F52 to the Northern Bobwhites, based on symptomatology and the absence of mortalities, was $>3.5 \times 10^8$ CFU/g bw per day for 5 days. There were no signs of illness, abnormal behaviour or pathogenicity noted in the animals. No evidence of pathogenicity or replication of the test substance was observed during gross necropsy at test termination. There were no mortalities. Based on the results of this study, *M. anisopliae* strain F52 is of low toxicity to the Northern Bobwhite via the oral route. This acute oral toxicity study is classified as acceptable. This study satisfies the guideline requirement for an acute oral toxicity study in the Northern Bobwhite.

A rationale to waive the data required to assess the hazard of *M. anisopliae* strain F52 to wild mammals was proposed based upon the results of the data generated to assess human health and safety that show the low mammalian toxicity and pathogenicity of *M. anisopliae* strain F52 (See Section 3.1 – Impact on Human and Animal Health: Toxicity and Infectivity above). The rationale was acceptable.

In a 12-day dietary toxicity study, green lacewing larvae (*Chrysoperla carnea*) were exposed to *M. anisopliae* strain F52 at concentrations of 0, 6.00, 60.0, and 600 ppm (equivalent to 4.2×10^5 , 4.2×10^6 and 4.2×10^7 CFU/g of diet). A negative control group (test material not administered) and an attenuated control (killed test material equivalent to 4.2×10^7 CFU/g) group were maintained concurrently. Larvae of green lacewing did not experience increased mortality or overt signs of toxicity when presented with feed containing *M. anisopliae* strain F52. The dietary LC₅₀ value for green lacewing larvae exposed to *M. anisopliae* strain F52 for 12 days was determined to be greater than 600 ppm (4.2×10^7 CFU/g), the highest concentration tested. The NOEC was 600 ppm (4.2×10^7 CFU/g). This study is classified as acceptable, and satisfies the guideline requirement for a dietary toxicity study for terrestrial arthropods.

In a 22-day dietary toxicity study, adult ladybird beetles (*Hippodamia convergens*) were exposed to *M. anisopliae* strain F52 at concentrations of 0, 6.00, 60.0, and 600 ppm (equivalent to 4.2×10^5 , 4.2×10^6 and 4.2×10^7 CFU/g of diet). A negative control group (test material not administered) and an attenuated control (killed test material equivalent to 4.2×10^7 CFU/g) group were maintained concurrently. This study shows that adult *H. convergens* did not experience a statistically significant increase in mortality or overt signs of toxicity when presented with feed containing *M. anisopliae* strain F52. The dietary LC₅₀ value for ladybird beetles exposed to *M. anisopliae* strain F52 for 22 days was determined to be greater than 600 ppm (4.2×10^7 CFU/g), the highest concentration tested. There was a slight, though not statistically significant, increase in mortalities at 600 ppm, suggesting the possibility of treatment-related effect. However, the statistically determined NOEC was 600 ppm (4.2×10^7 CFU/g). This study is classified as acceptable and satisfies the guideline requirement for a terrestrial arthropod study.

In a 26-day dietary toxicity study, adult parasitic Hymenoptera (*Nasonia vitripennis*) were exposed to *M. anisopliae* strain F52 at concentrations of 0, 6.00, 60.0, and 600 ppm (equivalent to 4.2×10^5 , 4.2×10^6 and 4.2×10^7 CFU/g of diet). A negative control group (test material not administered) and an attenuated control (killed test material equivalent to 4.2×10^7 CFU/g) group were maintained concurrently. This study shows that adult *N. vitripennis* did not experience a statistically significant increase in mortality or overt signs of toxicity when presented with feed containing *M. anisopliae* strain F52. The dietary LC₅₀ value for wasps exposed to *M. anisopliae* strain F52 for 26 days was determined to be greater than 600 ppm (4.2×10^7 CFU/mL), the highest concentration tested. The statistically determined NOEC was 600 ppm (4.2×10^7 CFU/mL). It was noted that there was a significant increase in mortality in the attenuated control group; however, it is unlikely that this increase was due to toxicity because a similar effect was not seen in the test groups and the mortality rate began increasing at approximately Day 20. If the higher mortality rate in the attenuated control group was due a toxic effect, it is more likely that the test groups would have exhibited higher mortality rates and

it is expected that the mortalities would have occurred much earlier in the study. This study is classified as acceptable, and satisfies the guideline requirement for a dietary toxicity study for terrestrial arthropods.

In a 16-day dietary toxicity study, honeybee (*Apis mellifera*) larvae were exposed to *M. anisopliae* strain F52 in a 5 µL dietary dose at a concentration of 1.2×10^6 CFU/mL. The test group was run concurrently with an untreated negative control group and a positive control group treated with a dietary exposure to potassium arsenate. Treated honeybee larvae did not experience a significant increase in mortality when compared to the negative control group after exposure to a single 5 µL dietary dose of *M. anisopliae* strain F52 at a concentration of 1.2×10^6 CFU/mL. The 16-day LC₅₀ is $> 1.2 \times 10^6$ CFU/mL. This study is classified as acceptable and satisfies the guideline requirement for a dietary toxicity study for honeybees.

In a 26-day contact toxicity study, adult honeybees (*A. mellifera*) were exposed to *M. anisopliae* strain F52 by spraying a concentration of 1.0×10^7 CFU/mL at a rate of 2.8×10^8 CFU/m². The test group was run concurrently with an untreated negative control group. Treated adult honeybees did not experience a significant increase in mortality when compared to the negative control group. No behavioural or morphological abnormalities were observed. The 26-day contact LC₅₀ of *M. anisopliae* strain F52 to *A. mellifera* adults is $> 1.0 \times 10^7$ CFU/mL. This study is classified as acceptable and satisfies the guideline requirement for a contact toxicity study for honeybees.

In a 14-day contact toxicity study, earthworms (*Eisenia fetida*) were exposed to *M. anisopliae* strain F52 in artificial soil at rates of 0 (negative control), 130, 216, 360, 600, and 1000 mg/kg dry soil (equivalent to 9.1×10^9 , 1.5×10^{10} , 2.5×10^{10} , 4.2×10^{10} and 7.0×10^{10} CFU/kg dry soil). A negative control group (test material not administered) and an attenuated control (killed test material equivalent to 7.0×10^{10} CFU/kg) group were maintained concurrently. There were no mortalities in any control or treatment group during the 14-day test. All worms were normal in appearance and behaviour throughout the test. The 14-day LC₅₀ value for earthworms exposed to *M. anisopliae* strain F52 in an artificial soil substrate was determined to be > 1000 mg/kg (7.0×10^{10} CFU/kg) dry soil, the highest concentration tested. The NOEC was ≥ 1000 mg/kg (7.0×10^{10} CFU/kg) dry soil. This study is classified as acceptable and satisfies the guideline requirement for a contact toxicity study for terrestrial non-arthropod invertebrates.

A data waiver rationale was submitted for the terrestrial plant toxicity data requirement citing testing of the MCPA on many different crops and plants without phytotoxicity. Also, a search in the United States Department of Agriculture National Agriculture Library using the keywords 'Metarhizium' and 'phytotoxicity' yields no 'hits'. A phytotoxicity study on terrestrial plants is not required.

Based on all the available data and information on the effects of *M. anisopliae* strain F52 on terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, arthropods, non-arthropod invertebrates, plants or to other non-target microorganisms from the use of Met52 Granular Bioinsecticide. Although pathogenicity/infectivity were not adequately assessed in terrestrial organisms (mammals excluded), no further data are required

since the use of Met52 Granular Bioinsecticide in potting media is unlikely to result in significant exposure to terrestrial environments and furthermore the proliferation of *M. anisopliae* strain F52 in terrestrial environments is unlikely due to its reliance on a susceptible insect host for growth.

4.2.2 Effects on Aquatic Organisms

Three toxicity studies were submitted to address the hazards of *M. anisopliae* strain F52 to aquatic non-target organisms. These studies included non-target freshwater fish, aquatic arthropods and freshwater algae.

In a 30-day toxicity study, 60 rainbow trout (*Oncorhynchus mykiss*) were exposed to *M. anisopliae* strain F52 under static conditions at nominal concentrations of 0 (negative control), 3.3, 6.6, 13, 27 and 53 mg/L water (equivalent to 2.32×10^8 , 4.64×10^8 , 9.28×10^8 , 1.86×10^9 and 3.71×10^9 CFU/L). The treated fish were also exposed to *M. anisopliae* strain F52 via feed at a rate of 5.3 mg/kg feed (equivalent to 3.71×10^8 CFU/kg). Mortality in the attenuated control group was 10% (1 death) on Day 14. The cause of death was not determined. There were no other mortalities in either the negative control or treatment groups. Fish in all groups appeared normal and healthy throughout the study. No abnormalities or signs of infection were observed during the gross necropsy. Body weights of the test group and attenuated control group fish were not measured. The 30-day LC₅₀ was > 53 mg/L (3.71×10^9 CFU/L), the highest concentration tested. This study satisfies the guideline requirement for a toxicity study for freshwater fish.

In a 21-day toxicity study, 120 daphnids (*Daphnia magna*) were exposed to *M. anisopliae* strain F52 under static conditions at nominal concentrations of 0 (negative control), 2.5, 5.0, 10, 20 and 40 mg/L water (equivalent to 1.75×10^8 , 3.50×10^8 , 7.00×10^8 , 1.40×10^9 and 2.80×10^9 CFU/L). A negative control group (test material not administered) and an attenuated control (killed test material at a concentration equivalent to 2.80×10^9 CFU/L) group were maintained concurrently. *Daphnia magna* exposed to *M. anisopliae* strain F52 at concentrations up to 5.0 mg/L (3.50×10^8 CFU/L) for 21 days showed no significant reductions in survival, reproduction or growth as compared to the negative control. *Daphnia magna* exposed to concentrations of 20 and 40 mg/L showed significant survival effects. The 21-day EC₅₀ value was calculated to be 17 mg/L (1.19×10^9 CFU/L). *Daphnia magna* exposed to 10 mg/L had reduced reproduction and growth (length and dry weight). Consequently the LOEC, based on reproduction and growth was 10 mg/L (7.00×10^8 CFU/L). The NOEC was 5.0 mg/L (3.50×10^8 CFU/L). *Daphnia magna* exposed to an attenuated control (heat killed test substance) at 40 mg/L showed no effects on survival, but did show significant reductions in reproduction and growth. This study is classified as acceptable and satisfies the guideline requirement for a toxicity study for aquatic arthropods.

The effect of *M. anisopliae* strain F52 on the freshwater alga, *Selenastrum capricornutum*, was studied at nominal concentrations of 78.1, 156, 313, 615, and 1250 mg/L of test substance (equivalent to 5.47×10^9 , 1.09×10^{10} , 2.19×10^{10} , 4.38×10^{10} and 8.75×10^{10} CFU/L) under static conditions. A negative control group (test material not administered) and an attenuated

control (killed test material) group were maintained concurrently. The 96-hour EC₅₀ value, based on cell density, for *S. capricornutum* exposed to *M. anisopliae* strain F52 was 573 mg/L (4.01×10^{10} CFU/L), with 95% confidence limits of 402 and 659 mg/L (2.89×10^{10} CFU/L and 4.61×10^{10} CFU/L). The 96-hour E_bC₅₀ value, based on biomass, was 499 mg/L (3.49×10^{10} CFU/L), with 95% confidence limits of 306 and 603 mg/L (2.14×10^{10} CFU/L and 4.22×10^{10} CFU/L). The 96-hour E_rC₅₀ value, based on growth rate, was >1250 mg/L ($>8.75 \times 10^{10}$ CFU/L), the highest concentration tested. The 96-hour NOEC relative to cell density and growth rate was 313 mg/L (2.19×10^{10} CFU/L). Biomass was the most sensitive parameter measured. Based on the significant difference from the control observed at 96 hours in the 156 mg/L treatment group, the 96-hour NOEC relative to biomass was 78.1 mg/L (5.4×10^9 CFU/L). Based on the growth observed in the recovery phase, the effects on algal growth were found to be algistatic, and not algicidal at the concentrations tested. This study is classified as acceptable and satisfies the guideline requirement for an acute toxicity study for algae.

In a study by Genther et. al. (PMRA # 1600093) it was reported that adverse effects were observed in embryos and newly-hatched fry of the silverside fish, *Menidia beryllina*, when exposed to the conidiospores of *M. anisopliae* strain 1080. Also shown was that *M. anisopliae* strain 1080 was an invasive pathogen of embryos of the grass shrimp, *Palaemonetes pugio*. Toxicity of culture extracts from yet another strain, *M. anisopliae* ARSEF 2575, were also examined on several aquatic species. Toxicity was observed to mysids (*Mysidopsis bahia*), developing grass shrimp (*P. pugio*), and juvenile mosquito fish (*Gambusia affinis*). Although toxicity and pathogenicity have been observed in these particular strains of *M. anisopliae*, the fungal spore dose required to produce an LD₅₀ in susceptible species is high ($10^6 - 10^7$ CFU/mL) and the quantities of toxic metabolites produced *in vivo* are usually much less than those secreted in nutrient rich artificial media. The exposure to marine/estuarine environments from the use of Met52 Granular Bioinsecticide is expected to be negligible and, therefore, would not result in toxicity to marine/estuarine wildlife. As a result, no further data are required to address the risk to estuarine/marine organisms since little or no aquatic exposure is expected from the use of Met52 Granular Bioinsecticide.

Based on all the available data and information on the effects of *M. anisopliae* strain F52 to aquatic organisms, there is reasonable certainty that no harm will be caused to non-target aquatic organisms from the use of Met52 Granular Bioinsecticide. Although there is evidence that *M. anisopliae* strain F52 is capable of adversely affecting certain aquatic non-target organisms, the likelihood of such an event occurring is low. Since Met52 Granular Bioinsecticide is only to be incorporated into potting media of container crops, the risk of accidental exposure to aquatic environments is very low. As a precaution, standard label statements will prohibit handlers from contaminating aquatic habitats or allowing effluent from greenhouses containing this product to enter lakes, streams, ponds or other water bodies.

5.0 Value

5.1 Effectiveness Against Pests

Submitted efficacy data demonstrated that Met52 Granular Bioinsecticide can infect eggs, larvae, and adults of the black vine weevil and provide control of this pest when the product is incorporated into the soil or potting medium of container-grown plants. The product was most effective when used under greenhouse conditions and when incubated for several days prior to planting, but in direct comparisons efficacy was less than 10% lower, on average, when the product was used outdoors in containers or without any incubation period. Efficacy was reduced substantially, however, when the product was applied as a top dressing (0-57% control) rather than a soil mix (76-95% control). No other trial results were submitted to support application of the product to the soil surface in established field crops, and such applications may not deliver the active ingredient into an environment suitable for its survival.

Most of the trials tested only a single application rate of 1 g/L, which is the midpoint of the range of application rates, but rates as low as 0.2 g/L and as high as 10 g/L were tested in different trials. In one trial, acceptable levels of control (86%) were achieved only at application rates of 5 g/L or higher, and control failed (33%) at 2 g/L; however, in other trials, control was acceptable even at application rates of less than 1 g/L. Two trials specifically addressed persistence of the active ingredient and demonstrated that activity persisted for more than one year in the potting media of plants maintained outdoors in Oregon. Although the target pest in all trials was the black vine weevil, efficacy is expected to be comparable for the strawberry root weevil due to their close relationship and biological similarity; however, efficacy data for a single species is insufficient to support a label claim for root weevils in general.

5.1.1 Acceptable Efficacy Claims

The submitted efficacy data support the use of Met52 Granular Bioinsecticide for control of all growth stages of black vine weevil and strawberry root weevil in ornamentals by incorporation into the growing medium at application rates of 500-1500 g/m³. Both greenhouse and outdoor use can be supported, but only for container-grown crops, and a claim for residual control up to nine months also can be supported.

5.2 Phytotoxicity to Host Plants

Efficacy trials were conducted on a wide variety of ornamental plants, with no reports of adverse effects on the host plants.

5.3 Economics

No economic analysis was conducted for this product evaluation. However, there is a demand for alternatives to the few pest control products registered in Canada for use against root weevils.

5.4 Sustainability

5.4.1 Survey of Alternatives

Alternative active ingredients registered in Canada for control of root weevils include bendiocarb, carbaryl, and endosulfan for black vine weevil (Table 3) and malathion, permethrin, and methyl bromide for strawberry root weevil (Table 4). In addition, the entomopathogenic nematode *Steinernema kraussei* is commercially available for control of root weevils.

5.4.2 Compatibility with Current Management Practices Including Integrated Pest Management

Met52 Granular Bioinsecticide is generally compatible with current management practices for container-grown ornamentals. Fungicides should not be applied to growing media containing Met52 Granular Bioinsecticide. Compatibility of Met52 Granular Bioinsecticide with soil-dwelling arthropods used as biological control agents (e.g., the predatory mite *Hypoaspis miles* used for the control of fungus gnats) has not yet been established.

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

The development of resistance to entomopathogenic fungi has not been documented and, due to the relatively complex nature of the mode of action, is not considered likely.

5.4.4 Contribution to Risk-reduction and Sustainability

Met52 Granular Bioinsecticide provides a viable alternative to the broad-spectrum chemical insecticides currently registered in Canada for control of black vine weevil and strawberry root weevil.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The management of toxic substances is guided by the federal government's Toxic Substances Management Policy (TSMP), which puts forward a preventive and precautionary approach to deal with substances that enter the environment and could harm the environment or human health. The policy provides decision makers with direction and sets out a science-based management framework to ensure that federal programs are consistent with its objectives. One of the key management objectives is virtual elimination from the environment of toxic substances that result predominantly from human activity and that are persistent and bioaccumulative. These substances are referred to in the policy as Track 1 substances.

In its review, the PMRA took into account the federal Toxic Substances Management Policy and followed its Regulatory Directive DIR99-03, The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy. Substances associated with its use were also considered, including microcontaminants in the technical product, *Metarhizium anisopliae* strain F52, and formulants in the end-use product, Met52 Granular Bioinsecticide. The PMRA has reached the following conclusions:

Metarhizium anisopliae strain F52 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. Therefore, the use of *Metarhizium anisopliae* strain F52 and Met52 Granular Bioinsecticide is not expected to result in the entry of Track 1 substances into the environment.

6.2 Formulants and Contaminants of Health or Environmental Concern

The technical grade active ingredient (technical grade active ingredient), *Metarhizium anisopliae* strain F52, does not contain any contaminants of health or environmental concern identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641–2643: List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.

The end-use product, Met52 Granular Bioinsecticide, does not contain any contaminants of health or environmental concern identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641–2643: List of Pest Control Product Formulants of Health or Environmental Concern.

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for *M. anisopliae* strain F52 and Met52 Granular Bioinsecticide were judged to be adequate to assess their potential human health and environmental risks. The technical grade active ingredient was fully characterized and the specifications were supported by the analyses of a sufficient number of batches. Storage stability data were sufficient to support a shelf life of three months at 4°C. Although data were sufficiently adequate to permit registration, confirmatory microbial contamination data for one of the manufacturing sites and a confirmatory storage stability study are required as conditions of registration.

7.2 Human Health and Safety

The acute toxicity and infectivity studies submitted in support of *M. anisopliae* strain F52 were determined to be sufficiently complete to permit a decision on registration. Spores of *M. anisopliae* strain F52 were of low toxicity in the rat when administered via oral, pulmonary, and dermal routes and were not pathogenic or infective via the oral, pulmonary and intraperitoneal injection exposure route. A toxicity study testing the end-use product is required to ensure that the end-use product is toxicologically equivalent to the technical grade active ingredient. In the intraperitoneal injection infectivity studies, clearance was established by Day 14. *Metarhizium anisopliae* strain F52 was moderately irritating to the skin and moderately irritating to the eye.

Although *M. anisopliae* strain F52 showed no signs of dermal sensitization in the guinea pig dermal sensitization test, the PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, and exposure to allergens including *M. anisopliae* strain F52 may cause allergies following repeated exposures.

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, handlers and workers exists with the primary source of exposure to workers being dermal. Precautionary label statements and personal protective equipment (PPE) specified on the Met52 Granular Bioinsecticide label will adequately mitigate the risks from exposure.

While *M. anisopliae* strain F52 has the potential to be a sensitizing agent, inhalation and dermal exposure are not a concern if the required dust/mist filtering respirator/mask and appropriate PPE to be stipulated on the end-use product label is worn by applicators and handlers. Furthermore, precautionary labelling will alert users of the potential dermal hazard of the end-use product.

The label does not allow applications to turf, residential or recreational areas; therefore, non-occupational exposure and risks to adults, infants and children are low. Because the use sites are commercial, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

Met52 Granular Bioinsecticide is to be applied to commercial potting media for non-food crops only. Negligible to no risk is expected for the general population, including infants and children, and animals because there are no direct applications of Met52 Granular Bioinsecticide to food or feed crops. Therefore, there is no concern for chronic risks posed by dietary exposure of the general population and sensitive subpopulations such as infants and children.

7.3 Environmental Risk

The non-target studies, scientific rationales and published scientific literature submitted in support of *M. anisopliae* strain F52 were determined to be sufficiently complete to permit a decision on registration.

Environmental effects studies and waiver rationales were submitted to address the hazards of *M. anisopliae* strain F52 to non-target organisms. These studies and other published information showed that the use of Met52 Granular Bioinsecticide containing *M. anisopliae* strain F52 does not pose a significant risk to birds, mammals, arthropods (including honeybees), fish, non-arthropod invertebrates, plants, or algae. Although the submitted aquatic toxicity studies did show some adverse effects to aquatic organisms when exposed to high concentrations of *M. anisopliae* strain F52, there is no risk to these organisms since the exposure to non-target aquatic organisms is expected to be negligible based on the use pattern.

No additional studies were required to address the environmental fate and behaviour of *M. anisopliae* strain F52. Environmental fate data (Tier II/III) are not normally required in the absence of significant toxicological effects in non-target organisms in Tier I testing. Furthermore, *M. anisopliae* strain F52 is not expected to be prolific in the environment due to its dependence upon infection of a suitable host under conditions of high humidity in order to reproduce.

As a precaution, standard label statements will prohibit handlers from contaminating aquatic habitats or allowing effluent from greenhouses containing this product to enter lakes, streams, ponds or other water bodies.

7.4 Value

Met52 Granular Bioinsecticide has value for control of all growth stages of black vine weevil and strawberry root weevil when incorporated into the growing medium of container-grown ornamentals.

7.5 Unsupported Uses

Acceptable efficacy has not been demonstrated when Met52 Granular Bioinsecticide is applied as a top dressing or to the soil in established field crops.

8.0 Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, has granted conditional registration for the sale and use of the technical grade active ingredient *Metarhizium anisopliae* strain F52 and the end-use product MET52 to control root weevils, specifically black vine weevil and strawberry root weevil, on container-grown ornamentals.

An evaluation of available scientific information found that, under the approved conditions of use, the end-use products have value and do not present an unacceptable risk to human health or the environment.

Although the risks and value have been determined to be acceptable when all risk-reduction measures are followed, as a condition of these registrations, additional scientific information is being requested from the applicant as a result of this evaluation to ensure that *Metarhizium*

anisopliae strain F52 will control root weevils, specifically black vine weevil and strawberry root weevil, on container-grown ornamentals. For more details, refer to the Section 12 Notice associated with these conditional registrations.

Human Health

- An acute oral toxicity study conducted with the end-use product, Met52 Granular Bioinsecticide
- Five microbial contamination tests of full-scale production batches of the end-use product
- A storage stability study conducted with the end-use product

NOTE: The PMRA will publish a Consultation Document at the time when there is a proposed decision on applications to convert these conditional registrations to full registrations or on applications to renew the conditional registrations, whichever occurs first.

List of Abbreviations

ADI	acceptable daily intake
ARD	acute reference dose
ARSEF	Agricultural Research Service Entomopathogenic Fungi
BRAD	Biopesticides Registration Action Document
bw	body weight
CFU	colony forming unit
EC ₅₀	effective concentration for 50% of the population
EEC	expected environmental concentration
g	gram
GLP	Good Laboratory Practices
IUPAC	International Union of Pure and Applied Chemistry
kg	kilogram
L	litre
LC ₅₀	lethal concentration for 50% of the population
LD ₅₀	lethal dose for 50% of the population
LOEC	lowest observed effect concentration
m ³	cubic metre
mg	milligram
MHC	maximum hazard concentration
MIS	maximum irritation score
mL	millilitre
MPCA	microbial pest control agent
MRL	maximum residue limit
NIOSH	National Institute of occupational Safety and Health
NOEC	no observed effect concentration
NOEL	no observed effect limit
°C	degree(s) Celcius
PCPA	<i>Pest Control Products Act</i>
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
ppm	part per million
TSMP	Toxic Substances Management Policy
USEPA	United States Environmental Protection Agency

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *Metarhizium anisopliae* strain F52

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of <i>M. anisopliae</i> strain F52				
Acute Oral Toxicity and Infectivity	<p>Rat – CD</p> <p>15/sex/dosed with <i>M. anisopliae</i> strain F52 at 1.04×10^8 CFU/animal</p> <p>Heat-killed test substance group (15/sex), naïve control group (15/sex), shelf control group (3/sex)</p> <p>Sacrifices on Days 0, 3, 7</p>	<p>7-day oral LD₅₀ $> 1.04 \times 10^8$ CFU/animal (males, females)</p>	<p>-no mortalities, no treatment related clinical signs, no necropsy findings, no changes in body-weight gain</p> <p>-clearance of test substance by Day 3</p> <p>LOW TOXICITY, NOT PATHOGENIC</p>	<p>PMRA# 1271526 1271555</p>
Acute Pulmonary Toxicity and Infectivity	<p>Rat – CD</p> <p>20/sex dosed with <i>M. anisopliae</i> strain F52 at 1.17×10^8 CFU/animal</p> <p>Heat-killed test substance group (20/sex), naïve control (20/sex) group, shelf control group (5/sex)</p> <p>Sacrifices on Days 0, 7, 21, 35</p>	<p>35-day pulmonary LD₅₀ $> 1.17 \times 10^8$ CFU/animal (males, females)</p>	<p>-no mortalities, no biologically significant signs of toxicity or changes in body-weight gain</p> <p>-some increases in lung and associated lymph nodes, spleen, kidney, brain and liver weight that were transient in nature or consistent with intratracheal administration of a powder were noted</p> <p>-3 females had brown/mottled lungs consistent with intratracheal administration</p> <p>-clearance of test substance cleared by Day 35</p> <p>LOW TOXICITY, NOT PATHOGENIC</p>	<p>PMRA# 1271531</p>

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Intraperitoneal Injection Infectivity	Rat – CD 12/sex dosed with <i>M. anisopliae</i> strain F52 at 1.0×10^7 CFU/animal Heat-killed test substance group (12/sex), naïve control (12/sex) group, shelf control group (3/sex) Sacrifices on Days 0, 3, 7, 14	Clearance from all organs was determined by Day 14	-no mortalities, no significant toxicity, no changes in body-weight gain -some differences in spleen, brain and liver weight that were transient in nature or consistent with an immune response to the test substance NOT PATHOGENIC	PMRA# 1271532
Acute Dermal Toxicity and Irritation	Rabbit – New Zealand White 5/sex administered 2 g of <i>M. anisopliae</i> strain F52 (7.9×10^9 CFU/g) per kg bw to an area 10% of body surface for 24 hours	14-day LD ₅₀ > 2 g/kg bw (males, females) MIS of 3.7 at 48 hours according to Draize method	-no mortalities, no changes in body-weight gain, no necropsy findings -signs of irritation including erythema, edema, and eschar formation at application site MODERATELY IRRITATING	PMRA# 1271530 1271556
Eye Irritation	Rabbit – New Zealand White 3/sex administered 0.1 g of <i>M. anisopliae</i> strain F52 (6.3×10^9 CFU/g) into the conjunctival sac of the right eye The untreated eye served as the negative control	MIS of 23.2 at 24 hours according to Draize method	-corneal opacity and iridial irritation were seen in four rabbits; conjunctival erythema and chemosis were seen in all rabbits MODERATELY IRRITATING	PMRA# 1271533

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Dermal Sensitization	<p>Guinea Pig - Hartley</p> <p><u>Induction phase:</u></p> <p>i. 20 males administered topical application of 0.3 g <i>M. anisopliae</i> strain F52 (7.9×10^9 CFU/g) for 6 hours once per week for three weeks.</p> <p>ii. Positive control: 10 males animals dosed with α-Hexylcinnamaldehyde</p> <p>iii. Negative control group: not dosed during induction phase</p> <p><u>Challenge phase:</u></p> <p>Three weeks after topical induction doses</p> <p>i. 20 males dosed topically in the flank with 0.3 g <i>M. anisopliae</i> strain F52 (7.9×10^9 CFU/g) for 6 hours</p> <p>ii. Positive control 10 males animals dosed with α-Hexylcinnamaldehyde</p> <p>iii. Negative control group: 5 males dosed topically in the flank with 0.3 g <i>M. anisopliae</i> strain F52 (7.9×10^9 CFU/g) for 6 hours</p>	<p><i>M. anisopliae</i> strain F52 showed no sensitizing properties under the test model of Beuhler</p>	<p>-one male in the test group exhibited mild erythema at the 48 hour point of the challenge phase</p> <p>-no signs of irritation were observed in the negative control group</p> <p>-no mortalities</p> <p>NOT A SENSITIZER</p>	<p>PMRA# 1271535</p>

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Organisms				
Vertebrates				
Birds	Oral	<p>5 birds per replicate; 6 replicates</p> <p>Nominal oral dose: 3.5×10^8 CFU/day for 5 day total dose of 1.75×10^9 CFU</p> <p>Viability of test substance was not measured</p> <p>Carrier: dose prepared in corn oil for a final volume of 10 mL/kg bw</p> <p>Birds were observed for 30 days</p>	<p>No mortalities, no abnormal behaviour, no body weight abnormalities</p> <p>Clearance pattern of MPCA not established</p> <p>30-day oral $LD_{50} > 3.5 \times 10^8$ CFU/g bw per day for 5 days</p> <p>30-day NOEL = 3.5×10^8 CFU/g bw per day for 5 days</p> <p>LOW TOXICITY; PATHOGENICITY NOT ASSESSED</p>	PMRA# 1271539
	Pulmonary	<p>A waiver was not submitted for an avian pulmonary study; however, this data requirement can be waived due to the absence of adverse effects in the avian oral toxicity study.</p> <p>NO FURTHER DATA REQUIRED</p>		
Wild Mammals	<p>A waiver was submitted based on the absence of adverse effects observed in the Part 4, <i>Human Health and Safety Testing</i> data submitted. Acute oral and toxicity, intraperitoneal infectivity and toxicity, and acute pulmonary infectivity and toxicity studies performed on rats were submitted where no treatment related adverse effects were observed and no infectivity was observed.</p> <p>Also cited was the BRAD from the USEPA which determined via their environmental risk assessment that the uses of <i>M. anisopliae</i> strain F52 will have no adverse effects on wild mammals from residential outdoor and institutional premise uses of the product.</p> <p>WAIVER ACCEPTED</p>			PMRA# 1277561 1271560

Invertebrates				
Arthropods				
Green Lacewings (<i>Chrysoperla carnea</i>)	Dietary	<p>30 larvae per dosage group</p> <p>Nominal dietary doses of 4.2×10^5, 4.2×10^6, 4.2×10^7 CFU/g of diet for 12 days</p> <p>Viability of test substance was not measured</p> <p>Carrier: doses were prepared in deionized water and Tween 80 before addition to moth egg meal</p> <p>Lacewings were observed for 12 days</p>	<p>Treatment group mortalities were not significantly different from negative control mortalities</p> <p>No overt signs of toxicity were observed</p> <p>12-day oral LC50 > 4.2×10^7 CFU/g of diet</p> <p>NOEC= 600 ppm (4.2×10^7 CFU/g)</p> <p>LOW TOXICITY; PATHOGENICITY NOT ASSESSED</p>	PMRA# 1271543
Ladybird Beetles (<i>Hippodamia convergens</i>)	Dietary	<p>25 adult beetles per replicate; three replicates per dosage group</p> <p>Nominal dietary doses of 4.2×10^5, 4.2×10^6, 4.2×10^7 CFU/g of diet for 22 days</p> <p>Viability of test substance was not measured</p> <p>Carrier: doses were prepared in deionized water and Tween 80 before addition to honey</p> <p>Beetles were observed for 22 days</p>	<p>Treatment group mortalities were not significantly different from negative control mortalities</p> <p>No overt signs of toxicity were observed</p> <p>22-day oral LC50 > 4.2×10^7 CFU/g of diet</p> <p>NOEC = 600 ppm (4.2×10^7 CFU/g)</p> <p>LOW TOXICITY; PATHOGENICITY NOT ASSESSED</p>	PMRA# 1271544

Parasitic Hymenopterans (<i>Nasonia vitripennis</i>)	Dietary	25 adult wasps per replicate; three replicates per dosage group Nominal dietary doses of 4.2×10^5 , 4.2×10^6 , 4.2×10^7 CFU/g of diet for 26 days Viability of test substance was not measured Carrier: doses were prepared in deionized water and Tween 80 before addition to honey Wasps were observed for 26 days	Treatment group mortalities were not significantly different from negative control mortalities Occasional observations of immobility were noted in the last week of the test in the 4.2×10^7 CFU/g treatment group and negative control group 26-day oral LC50 > 4.2×10^7 CFU/g of diet NOEC = 600 ppm (4.2×10^7 CFU/g) LOW TOXICITY; PATHOGENICITY NOT ASSESSED	PMRA# 1271545
Honeybees (larvae) (<i>Apis mellifera</i>)	Dietary	20 larvae per replicate; four replicates One 5 μ L dose containing a nominal concentration of 1.2×10^6 CFU/mL (6000 CFU/larva) Viability of test substance was not measured Carrier: 30% sucrose solution Larvae were observed for 16 days	Treatment group mortalities were not significantly different from negative control mortalities 16-day oral LC50 > 1.2×10^6 CFU/mL of diet LOW TOXICITY; PATHOGENICITY NOT ASSESSED	PMRA# 1271546
Honeybees (adult) (<i>A. mellifera</i>)	Contact (spray application)	Four replicates containing a total of 111 bees Nominal dose: 1.0×10^7 CFU/mL at rate of 2.8×10^8 CFU/m ² Viability of test substance was not measured Carrier: deionized water and Tween 80 Bees were observed for 26 days	Treatment group mortalities were not significantly different from negative control mortalities No behavioural or morphological abnormalities were observed 26-day contact LC50 > 1.0×10^7 CFU/mL LOW TOXICITY; PATHOGENICITY NOT ASSESSED	PMRA# 1271547
Non-arthropods				
Earthworms	Contact (soil)	10 earthworms per replicate;	No mortalities	PMRA#

<i>(Eisenia fetida)</i>	incorporated)	4 replicates Nominal dose: 7.0×10^{10} CFU/kg dry soil Viability of test substance was not measured Worms were observed for 14 days	Worms were normal in appearance and behaviour 14-day contact LC50 > 7.0×10^{10} CFU/kg dry soil LOW TOXICITY; PATHOGENICITY NOT ASSESSED	1271548
Plants				
Plants	A data waiver rationale was submitted for this data requirement citing the testing of <i>M. anisopliae</i> strain F52 on many different crops without incidence of phytotoxicity. Also, a search in the US Department of Agriculture National Agriculture Library using the keywords 'Metarhizium' and 'phytotoxicity' yields no 'hits'. No further phytotoxicity data on terrestrial plants are required. WAIVER ACCEPTED			PMRA# 1271554

Aquatic Organisms				
Vertebrates				
Rainbow trout (<i>Onchorhynchus mykiss</i>)	Aqueous (static)/Dietary	10 fish per dosage group Nominal aqueous concentrations: 2.32×10^8 , 4.64×10^8 , 9.28×10^8 , 1.86×10^9 , and 3.71×10^9 CFU/L Nominal dietary concentration: 3.71×10^8 CFU/kg food (all test groups) Fish were observed for 30 days	No mortalities All fish appeared normal and healthy No abnormalities or signs of infection 30-day aqueous LC50 > 3.71×10^9 CFU/L LOW TOXICITY; PATHOGENICITY NOT ASSESSED	PMRA# 1271541
Invertebrates				
Arthropods				
Daphnids (<i>Daphnia magna</i>)	Aqueous (static)	5 daphnids per replicate; 4 replicates per dosage group Nominal aqueous concentrations: 1.75×10^8 , 3.50×10^8 , 7.00×10^8 , 1.40×10^9 and 2.80×10^9 CFU/L Viability of test substance was not measured Daphnids were observed for 21 days	Mortalities in 1.40×10^9 and 2.80×10^9 CFU/L treatment groups were significantly higher than control; consequently, reproduction, body length and body weight were not measured in these groups 21-day EC50 = 1.19×10^9 CFU/L NOEC for survival = 7.00×10^8 CFU/L Reproduction, body length and body weight were significantly reduced in the 7.00×10^8 CFU/L treatment group NOEC for reproduction and growth = 3.50×10^8 CFU/L LOW TOXICITY; PATHOGENICITY NOT ASSESSED	PMRA# 1271540

Table 3 Alternative Insecticides Registered for Use Against Black Vine Weevil

Active Ingredient	Insecticide Group	Comments
Bendiocarb	1A	One restricted class product registered for use as a soil drench for larvae in interior landscapes
Carbaryl	1A	Three domestic class products registered for foliar application on ornamentals
Endosulfan	2A	One domestic and five commercial class products registered for application to the trunk, lower branches, and surrounding soil of various greenhouse and outdoor ornamentals

Table 4 Alternative Insecticides Registered for Use Against Strawberry Root Weevil

Active Ingredient	Insecticide Group	Comments
Malathion	1B	One domestic and four commercial class products registered for control of adults on strawberries (four of the five product labels specify B.C. only) and blueberries (only one commercial class product, also B.C. only)
Permethrin	3	Three commercial class products registered for control of adults on conifer seedlings
Methyl Bromide	8A	Four restricted class products registered for fumigation of various sites and commodities

Table 5 Use Claims Proposed by Applicant and Whether Acceptable or Unsupported

Claims proposed by applicant	Acceptable claims	Unsupported claims
INSECTS CONTROLLED Root Weevils, such as: Black Vine Weevil (all growth stages) Strawberry Root Weevil (all growth stages)	INSECTS CONTROLLED Black Vine Weevil (all growth stages) Strawberry Root Weevil (all growth stages)	“Root Weevils, such as:”
SITES FOR USE [with an extensive list under each category] ORNAMENTALS SHRUBS SHADE AND FOREST TREE SEEDLINGS BERRY CROPS	SITES FOR USE Container-grown ornamentals, including flowering and foliage plants, shrubs, and shade and forest tree seedlings. This product may be used in container-grown crops in greenhouses and in outdoor nurseries.	Extensive lists of different ornamentals, shrubs, shade and forest tree seedlings.

Claims proposed by applicant	Acceptable claims	Unsupported claims
<p><u>APPLICATION INSTRUCTIONS</u> Apply Met52 Granular Bioinsecticide prior to or during planting by thoroughly mixing the product into the growing medium, ensuring even distribution. Soil should be moist at the time of application and maintained in a moist condition after application for best performance.</p> <p>Applications to Growing Media for Container-grown Crops: Apply 500 g to 1.5 kg/m³ of moist soil medium. Begin using 600g/m³ and adjust up or down depending upon pest pressure. Uniformly incorporate the Met52 Granular Bioinsecticide throughout the growing medium.</p> <p>Applications to Soils: Apply 0.5 kg to 2.5 kg/100 square meters (1.5 to 5 lb/1000 sq ft) of garden, bed, row or field. Incorporate lightly into the surface 2 to 3 cm of loose soil to create a spore infested area in which the pest will contact spores and become infected as it moves in the treated soil.</p>	<p><u>APPLICATION INSTRUCTIONS</u> Apply Met52 Granular Bioinsecticide prior to or during planting by thoroughly mixing the product into the growing medium, ensuring even distribution. Soil should be moist at the time of application and maintained in a moist condition after application for best performance.</p> <p>Applications to Growing Media for Container-grown Crops: Apply 500 g to 1.5 kg/m³ of moist soil medium, using the higher application rate when pest pressure is expected to be high. Uniformly incorporate the Met52 Granular Bioinsecticide throughout the growing medium.</p>	<p>Begin using 600g/m³ and adjust up or down depending upon pest pressure.</p> <p>Applications to Soils: Apply 0.5 kg to 2.5 kg/100 square meters (1.5 to 5 lb/1000 sq ft) of garden, bed, row or field. Incorporate lightly into the surface 2 to 3 cm of loose soil to create a spore infested area in which the pest will contact spores and become infected as it moves in the treated soil.</p>
<p>Depending on cultural practices, it is not uncommon for control to last up to nine months.</p>	<p>Depending on cultural practices and environmental conditions, control may last up to nine months.</p>	

References

A. LIST OF STUDIES/INFORMATION SUBMITTED BY REGISTRANT

1.0 The Active Ingredient, Its Properties and Uses

1271551 M1.2-2.6, DACO: M1.2,M1.3,M2.1,M2.2,M2.3,M2.4,M2.5,M2.6,M2.7

2.0 Methods of Analysis

1271517 1999, Description of Beginning Materials and Manufacturing Process-1, MRID: 456963-02, 456963-03, 448547-02, 448547-03, DACO: M2.10.3,M2.8,M2.9,M2.9.3 CBI

1271518 1999, Product Identification and Disclosure of Ingredients *Metarhizium anisopliae* Straing F52, MRID: 456963-01, 448547-01, DACO: M2.4, M2.5,M2.7,M2.7.1, M2.7.2,M2.9.1,M2.9.2 CBI

1271519 1999, Description of Beginning Materials and Manufacturing Process-2, *Metarhizium anisopliae* Strain 52, TAE-001 Technical Bioinsecticide, MRID: 456963-02, 456963-03, 448547-02, 448547-03, DACO: M2.10.3,M2.8,M2.9.3 CBI

1271520 1999, Discussion of the Formation of Impurities During the Production of *Metarhizium anisopliae* strain F52, MRID: 448447-04, 456963-03, DACO: M2.10.1, M2.10.2, M2.10.3, M2.8,M2.9.1,M2.9.3 CBI

1271521 1999, Analysis of Samples- Manufacturing Process 2 *Metarhizium anisopliae* Strain F52 TAE-001 Technical Bioinsecticide, MRID: 456963-04, 448447-06, 456963-03, 448447-05, DACO: M2.10.2,M2.8,M2.9.1 CBI

1271523 1999, Certification of Ingredient Limits, MRID: 456963-05, 448447-03, 448447-02, 456963-03, DACO: M2.10.2,M2.10.3,M2.8,M2.9,M2.9.1,M2.9.2 CBI

1271525 1998, Determination of the Color, Physical State, Odor, pH and Density of *Metarhizium anisopliae* technical grade active ingredient, 495C-109, DACO: M2.12 CBI

1271529 1999, Lot Analysis of *Metarhizium anisoplae*, strain F52, L08743 SN24, MRID: 456963-04, 448447-06, 456963-03, 448447-05, DACO: M2.10.2,M2.8,M2.9.1 CBI

1271536 1999, Measurement of Fungal Toxins Destruxin and Cytochalasin in *Metarhizium anisopliae* Growth Media, 8743 SN32, DACO: M4.9,M8.5

1271537 1999, Growth Parameters of *Metarhizium anisopliae*, Strain F52, at Various Temperatures, L08743 SN26, DACO: M2.7.2,M8.5

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- 1271552 1999, Measurement of the Fungal Toxins Destruxin and Cytochalasin in *Metarhizium anisopliae* Test Substance, 8743 SN33, DACO: M2.7.2 CBI
- 1271553 1999, Measurement of the fungal toxins destruxin and cytochalasin in *Metarhizium anisopliae* on Cereal Grain, 8743 SN34, DACO: M2.7.2 CBI
- 1271584 2000, Storage Stability Assessment of New BIO 1020 *Metarhizium anisopliae* (TAE-001 GR) Isolate F52 on Cereal Grain-Matrix, MRID: NA, DACO: M2.11 CBI
- 1600056 Request for Information 2006-4033: DACO 2.7.2, DACO: M2.14,M2.7.2
- 1600057 Measurement of the Fungal Toxins Destruxin and Cytochalasin in *Metarhizium anisopliae* Growth Media, 8743 SN32, DACO: M2.7.2 CBI
- 1600058 Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*, DACO: 2.7.1,M2.7.2
- 1600059 Request for Information 2006-4033: DACO 2.7.1, DACO: M2.7.1
- 1600060 Clonal Success of *Metarhizium anisopliae* var. *anisopliae* in Southwestern British Columbia is Independent of Habitat Association, DACO: M2.7.1
- 1600061 Initial Handling and Diagnosis of Diseased Insects, DACO: M2.7.1
- 1600062 Viridoxins A and B: novel toxins from the fungus *Metarhizium flavoviride*, DACO: 2.14
- 1600063 Production of an indolizidine alkaloid, swainsonine by the filamentous fungus, *Metarhizium anisopliae*, DACO: M2.14
- 1600064 Expressed sequence tag (EST) analysis of two subspecies of *Metarhizium anisopliae* reveals plethora of secreted proteins with potential activity in insect hosts, DACO: M2.14
- 1600065 Effect of pH on physiology of *Metarhizium anisopliae* for production of swainsonine, DACO: M2.14
- 1600066 Analysis of swainsonine and its early metabolic precursors in cultures of *Metarhizium anisopliae*, DACO: M2.14
- 1600067 Production of mutagenic metabolites by *Metarhizium anisopliae*, DACO: M2.14
- 1600068 Allergenic extracts from *Metarhizium anisopliae*: obtainment and characterization, DACO: M2.14
- 1600070 Dose dependent allergic responses to an extract of *Penicillium chrysogenum* in BALB/c mice, DACO: M2.14
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- 1600072 Allergen triggered airway hyperresponsiveness and lung pathology in mice sensitized with the biopesticide *Metarhizium anisopliae*, DACO: M2.14
- 1600073 Comparison of respiratory responses to *Metarhizium anisopliae* extract using two different sensitization protocols, DACO: M2.14
- 1600074 Allergic responses to the biopesticide *Metarhizium anisopliae* in Balb/c mice, DACO: M2.14
- 1600076 IGS Sequence variation, group -I introns and the complete nuclear ribosomal DNA of the entomopathogenic fungus *Metarhizium*: excellent tools for isolate detection and phylogenetic analysis, DACO: M2.14
- 1600077 Influence of nutrition on the production and physiology of sectors produced by the insect pathogenic fungus *Metarhizium anisopliae*, DACO: M2.14
- 1600078 Nutrition influences growth and virulence of the insect pathogenic fungus *Metarhizium anisopliae*, DACO: M2.14
- 1600079 Collembolans as vectors of entomopathogenic fungi, DACO: M2.14
- 1600080 Entomopathogenic fungi for mosquito control, DACO: M2.14
- 1600081 Combination of a new simple preparation strategy and an accelerated high-performance liquid chromatography assay with photodiode array and mass spectrometric detection for the determination of destruxins from *Metarhizium anisopliae* culture broth, DACO: M2
- 1600082 Effect of the cyclopeptolide 90-215 on the production of destruxins and helvolic acid by *Metarhizium anisopliae*, DACO: M2.14
- 1600083 Aurovertins F-H from the entomopathogenic fungus *Metarhizium anisopliae*, DACO: 2.14
- 1600084 Serinocyclins A and B, cyclic heptapeptides from *Metarhizium anisopliae*, DACO: M2.14
- 1600085 A taxonomic revision of *Metarhizium* based on a phylogenetic analysis of ribosomal DNA sequence data, DACO: M2.14
- 1600086 Intraspecific polymorphism in *Metarhizium anisopliae* var. *anisopliae* revealed by analysis of r RNA gene complex and mtDNA RFLPs, DACO: M2.14
- 1600087 Pathogenicity of the hyphomycete fungi *verticillium lecanii* and *Metarhizium anisopliae* to the western flower thrips, *frankliniella occidentalis*, DACO: M2.14
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- 1600088 Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of metarhizium, tolypocladium and beauveria species? DACO: M2.14
- 1600089 Light and electron microscopy of the infection of *Metarhizium anisopliae* on the western flower thrips, DACO: M2.14
- 1600090 Comparison of three commonly used drying technologies with respect to activity and longevity of aerial conidia of *Beauveria brongniartii* and *Metarhizium anisopliae*, DACO: M2.14
- 1600091 Cuticle induced endo/exoacting chitinase CHIT30 from *Metarhizium anisopliae* is encoded by an ortholog of the *chi3* gene, DACO: M2.14
- 1600093 Toxicity and pathogenicity testing of the insect pest control fungus *M. anisopliae*, DACO: M2.14
- 1600094 Acute toxicity of locust insecticides to two indigenous invertebrates from Sahelian temporary ponds, DACO: M2.14
- 1600095 Isolation and Characterization of *Metarhizium anisopliae* and *Beauveria bassiana* from subantarctic macquarie island, DACO: M2.14
- 1600097 Effect of temperature on conidia germination and vegetative growth of *Metarhizium anisopliae*, DACO: M2.14
- 1600101 Studies on the structure of a novel peptide antibiotic, K-582., DACO: M2.14
- 1600102 The genus *Metarhizium*, DACO: M2.14
- 1600103 The role of destruxins in the pathogenicity of *Metarhizium anisopliae* for three species of insects, DACO: M2.14
- 1600104 Distribution and occurrence of four entomopathogenic fungi in Finland: effect of geographical location, habitat type and soil type, DACO: M2.14
- 1600107 DACO: M2.10.2 CBI
- 1609958 A new species of the genus *Cordyceps* and its *Metarhizium* anamorph, DACO: M2.14
- 1609959 Persistence of released *Metarhizium anisopliae* in soil and prevalence in ground rove beetles, DACO: M2.14
- 1609962 A new method for assessment of germination of *Metarhizium* conidia, DACO: M2.14
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1609964 Manufacturing Process for *Metarhizium anisopliae* strain F52 (PMRA 2006-4033) and Met52 (PMRA 2006-4035), DACO: M2.8 CBI

3.0 Impact on Human and Animal Health

- 1271526 1999, Toxicity/Pathogenicity Testing of *Metarhizium anisopliae*, Strain F52, Following Acute Oral Challenge in Rats, L08743 SN13, MRID: 448447-09, 457788-03, DACO: M4.2.2
- 1271530 1998, Acute Dermal Toxicity/Pathology Study of *Metarhizium anisopliae*, Strain F52, in Rabbits, L08743 SN16, MRID: 448447-10, DACO: M4.4
- 1271531 1999, US EPA, Toxicity/Pathogenicity Testing of *Metarhizium anisopliae*, Strain F52, Following Acute Intratracheal Challenge in Rats, L08743 SN14, MRID: 448447-11, DACO: M4.2.3
- 1271532 1999, US EPA, Toxicity/Pathogenicity Testing of *Metarhizium anisopliae*, Strain F52, Following Acute Intraperitoneal Challenge in Rats, L08743 SN15, MRID: 448447-12, DACO: M4.3.3
- 1271533 1998, Primary Eye Irritation Study of *Metarhizium anisopliae*, Strain F52, in Rabbits, L08743 SN17, MRID: 448447-13, DACO: M4.9
- 1271534 1999, Hypersensitivity Incidents Report *Metarhizium Anisopliae* Strain F52, DACO: M4.6
- 1271535 1999, US EPA, Dermal Sensitization Study of *Metarhizium anisopliae*, Strain F52, in Guinea Pigs using the Buehler Method, L08743 SN18, MRID: 448447-15, DACO: M4.9
- 1271538 1999, US EPA, Sensitivity of Detection of *Metarhizium anisopliae*, Strain F52, For Toxicity/Pathogenicity Testing in Rats, L08743 SN12, DACO: M4.2.2, M4.2.3, M4.3.3
- 1271555 2001, Data Evaluation Record: *Metarhizium anisopliae* Strain F52; Acute Oral Toxicity/Pathogenicity- Raat (885.3050), L08743 SN13, MRID: 44844709, DACO: M4.9
- 1271556 1998, Data Evaluation Record; *Metarhizium anisopliae* Strain F52; Acute Dermal Toxicity/Pathology - Rabbit (885.3100), MRID: 448447-10, DACO: M4.9

4.0 Impact on the Environment

- 1271539 1999, US EPA, *Metarhizium anisopliae* Strain F52: An Avian Oral Pathogenicity and Toxicity Study in the Northern Bobwhite, 495-103, MRID: 448447-19, DACO: M9.2.1

-
- 1271540 1999, US EPA, *Metarhizium anisopliae* technical grade active ingredient: A 21-day Life Cycle Toxicity and Pathogenicity Test with the Cladoceran (*Daphnia magna*), 495A-104, MRID: 448447-20, DACO: M9.5.2
- 1271541 1999, US EPA, *Metarhizium anisopliae* technical grade active ingredient: A five-concentration toxicity and pathogenicity test with the rainbow trout (*Oncorhynchus mykiss*), 495A-105, MRID: 448447-21, DACO: M9.4.1
- 1271542 2004, Letter with revised Freshwater Fish Calculations, MRID: NA, DACO: M9.4.1
- 1271543 1999, US EPA, *Metarhizium anisopliae* strain F-52: A dietary pathogenicity and toxicity study with Green Lacewing Larvae (*Chrysoperla arnea*), 495-107, MRID: 448447-22, DACO: M9.5.1
- 1271544 1999, *Metarhizium anisopliae* strain F-52: A dietary pathogenicity and toxicity study with the Ladybird Beetle (*Hippodamia convergens*), 495-105, MRID: 448447-23, DACO: M9.5.1
- 1271545 1999, US EPA, *Metarhizium anisopliae* Strain F52: A dietary pathogenicity and toxicity study with parasitic hymenoptera (*Nasonia vietripennis*), 495-106A, MRID: 448447-24, DACO: M9.5.1
- 1271546 1999, US EPA, Evaluation of the Dietary Effects of *Metarhizium anisopliae* Strain F52 on Honeybee Larvae (*Apis mellifera* L.), CAR 166-98, MRID: 448447-25, DACO: M9.5.1
- 1271547 1999, Evaluation of the Acute Contact Toxicity of *Metarhizium anisopliae* Strain F52 on Adult Honeybees (*Apis mellifera* L.), CAR 168-98, MRID: 448447-26, DACO: M9.5.1
- 1271548 1999, *Metarhizium anisopliae* Strain F52: An Acute Toxicity Study with the Earthworm in an Artificial Soil Substrate, 495-104, MRID: 448447-27, DACO: M9.6
- 1271549 1999, US EPA, *Metarhizium anisopliae* Strain F52: A 96-Hour Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*), 495A-106, MRID: 448447-28, DACO: M9.8.2
- 1271554 Waiver Request for Non-target Plants and Estuarine or Marine Fish, DACO: M9.4.2, M9.8
- 1271561 Waiver Request, DACO: M9.3
- 1600093 Toxicity and pathogenicity testing of the insect pest control fungus *M. anisopliae*, DACO: M2.14
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- 5.0 Value**
- 1271567 2006. Efficacy Studies for MET 52. DACO: M10.2.2
- 1607907 Stenzel K, Holters J, Andersch W, and Smit TAM. 1992. BIO 1020: Granular Metarhizium, a new product for biocontrol of soil pests. Brighton Crop Protection Conference – Pests and Diseases. DACO: M10.2.2
- 1607908 PRR06-430 Year 2 Progress Report. DACO M10.2.2
- 1607910 Bruck DJ and Donahue KM. 2007. Persistence of *Metarhizium anisopliae* incorporated into soilless potting media for control of the black vine weevil, *Otiiorhynchus sulcatus* in container-grown ornamentals. Journal of Invertebrate Pathology, in press. DACO: M10.2.2
- 1607911 Easterbrook MA, Cantwell MP, and Chandler D. 1992. Control of the black vine weevil, *Otiiorhynchus sulcatus*, with the fungus *Metarhizium anisopliae*. Phytoparasitica 20 (supplement): 17S-19S. DACO: M10.2.2
- 1607912 Reinecke P, Andersch W, Stenzel K, and Hartwig J. 1990. BIO 1020, a new microbial insecticide for use in horticultural crops. Brighton Crop Protection Conference – Pests and Diseases. DACO: M10.2.2
- 1607913 Shah FA, Ansari MA, Prasad M, and Butt TM. 2007. Evaluation of black vine weevil (*Otiiorhynchus sulcatus*) control strategies using *Metarhizium anisopliae* with sublethal doses of insecticides in disparate horticultural growing media. Biological Control 40: 246-252. DACO: M10.2.2

B. ADDITIONAL INFORMATION

i) Unpublished Information

- 1609884 2004, Identification of carcinogens, N/A, MRID: N/A, DACO: M2.7.1,M2.7.2,M2.9.3,M4.8 CBI
- 1609886 Eilenberg J.,Zimmermann G., Jung K., Butt T., Nielsen C., Typas M., 2007, The fascinating true story about the famous Metarhizium anisopliae isolate Ma43, alias ATCC 90448, alias BIPESCO 5, alias F52 alias..., N/A, MRID: N/A, DACO: M2.7.1,M2.7.2,M2.9.3,M4

ii) Published Information

- 1609887 Skrobek A., Boss D., Defago G., Butt T.A. & Maurhofer M., 2006, Evaluation of different biological test systems to assess the toxicity of metabolites from fungal biocontrol agents, N/A, MRID: N/A, DACO: M2.7.1,M2.7.2,M2.9.3,M4.8

- 1609888 Skrobek A. & Butt T.A., 2005, Toxicity testing of destruxins and crude extracts from the insect-pathogenic fungus *Metarhizium anisopliae*, N/A, MRID: N/A, DACO: M2.7.1,M2.7.2,M2.9.3,M4.8
- 1609889 Wang, C., Skrobek A. & Butt T.M., 2004, Investigations on the destruxin production of the entomopathogenic fungus *Metarhizium anisopliae*, N/A, Journal of Invertebrate Pathology 85 (2004) 168-174, MRID: N/A, DACO: M2.7.1,M2.7.2,M2.9.3,M4.8