Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Proposed Regulatory Decision Document PRDD2003-07

Mycostop Biofungicide
Streptomyces griseoviridis strain K61

The microorganism, *Streptomyces griseoviridis* strain K61 and the associated end-use product Mycostop Biofungicide are proposed for full registration under Section 13 of the Pest Control Products (PCP) Regulations for control of diseases on greenhouse cucumbers, tomatoes, peppers and greenhouse ornamentals. This product was submitted to Health Canada's PMRA under the User Requested Minor Use Registration (URMUR) program.

This Proposed Regulatory Decision Document provides a summary of data received and the rationale for the proposed full registration of these products. The Pest Management Regulatory Agency (PMRA) will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to the Publications Coordinator at the address below.

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Foreword

The submission for full registration of the microbial *Streptomyces griseoviridis* strain K61 and the end-use product Mycostop Biofungicide, a microbial biopesticide developed by Kemira Agro Oy for control of damping-off, root and stem rot, and wilt caused by *Fusarium* on greenhouse cucumbers, tomatoes, peppers and greenhouse ornamentals has been reviewed by the Pest Management Regulatory Agency (PMRA) of Health Canada.

The PMRA had previously issued a temporary registration (Regulatory Note REG2002-04) for these products with the requirement that Kemira Agro Oy carry out additional studies. These studies have now been completed. This product was submitted to the PMRA under the User Requested Minor Use Registration (URMUR) program.

Microbial pest control agents are increasingly being investigated for use as alternatives to conventional pesticides because they are thought to pose a lower potential risk to human health and the environment, compared with conventional pesticides. Mycostop Biofungicide represents a reduced risk option to chemical fungicide management tools.

The PMRA has carried out an assessment of available information in accordance with Section 9 of the Pest Control Products (PCP) Regulations and has found it sufficient pursuant to Section 18(*b*), to allow a determination of the safety, merit and value of the technical active ingredient Mycostop Biofungicide Technical and the end-use product Mycostop Biofungicide. The Agency has concluded that the use of the microorganism, *Streptomyces griseoviridis* strain K61, in the technical active ingredient Mycostop Biofungicide Technical and the end-use product Mycostop Biofungicide in accordance with the label has merit and value consistent with Section 18(*c*) of the PCP Regulations and does not entail an unacceptable risk of harm pursuant to Section 18(*d*). Therefore, based on the considerations outlined above, the use of the microbial *Streptomyces griseoviridis* strain K61 and Mycostop Biofungicide for control of damping-off, root and stem rot, and wilt caused by *Fusarium* on greenhouse cucumbers, tomatoes, peppers and greenhouse ornamentals are proposed for full registration, pursuant to Section 13 of the PCP Regulations.

The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document to allow interested parties an opportunity to provide input into the proposed registration decision for these products.

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1.0 Product characterization and analysis

1.1 Physical/chemical properties of the end-use product Mycostop

Property	Mycostop Biofungicide
Physical state at 25°C	Finely divided powder; solid at room temperature
Colour	Brown to tan; standard Munsell 2.5Y 9/2
Odour	Fishy, putrid, woody, yeasty, sewery
pH in distilled water	1% slurry = 5.63; 10% slurry = 5.17
Density	459.4 kg/m^3
Viscosity	Not applicable
Corrosion character	Not applicable
Suspendability	No evaluation performed
Moisture content	No evaluation performed
Storage stability	1 year at 8°C

The guarantee for Mycostop Biofungicide is described as 30% *Streptomyces griseoviridis* strain K61 by weight or minimum 10^8 colony forming units (CFU)/g. A replacement Statement of Product Specifications Form (SPSF), indicating the minimum and maximum guarantee, was completed for Mycostop Biofungicide. Acceptable storage stability data were submitted for 13 batches of Mycostop Biofungicide stored at 8°C. Mycostop Biofungicide retained its guarantee of a minimum of 1×10^8 CFU/g for up to one year.

1.2 Details of uses

Mycostop Biofungicide is a biological fungicide containing dried mycelium and spores of the active ingredient *Streptomyces griseoviridis* strain K61, which was isolated in Finland from *Sphagnum* peat moss. Mycostop is a wettable powder formulation that has a guarantee of 1×10^8 colony forming units (CFU) per gram of product and will be marketed in foil-lined packets. The active ingredient, strain K61, has not been genetically modified or engineered through recombinant nucleic procedures.

Mycostop was first registered in Finland in 1990 and has since been registered in Bulgaria, Chile, Denmark, Estonia, Guatemala, Hungary, Iceland, Latvia, Norway, Sweden, Switzerland, Italy, Lithuania, Spain and The Netherlands. Mycostop was first registered in the U.S. (United States of America) in 1993 and registration in Canada is being sought under the URMUR program for control of damping-off, root and stem rot

and wilt caused by *Fusarium* in greenhouse ornamentals and herbs, and in greenhouse vegetables (cucumbers, tomatoes, and peppers).

The proposed use includes seed treatment of vegetables, ornamentals and herbs, and the treatment of growth medium of vegetables and ornamentals (cut flowers and potted plants), bulbs and corms. Mycostop is to be applied directly to the root zone of the plant either by spraying, soil drenching, drip irrigation, dip treatment or as a seed treatment at rates that are to vary according to the crop and (or) growth stage of the crop to be treated (e.g., 10–50 mg/plant, 2–10 mg/plant pot, 2–20 g/100 m^2, 0.01% suspension, 2–8 g/kg seed), but generally in the range of 1.1–1.9 kg product/hectare (ha). Treatments are to be repeated at intervals of three to six weeks depending on disease pressure and the growth substrate. The number of applications will depend on the length of the growing period and disease pressure.

1.3 Biological properties of *Streptomyces griseoviridis*

Streptomyces griseoviridis strain K61 is a naturally occurring soil bacterium belonging to the family Streptomycetacea in the order Actinomycetales. The streptomycetes are widely distributed in terrestrial and aquatic habitats. They occur in almost all soil types. The number of streptomycetes living in soil varies greatly, both absolutely and proportionately, depending on soil type, moisture level, aeration, pH, depth in soil, amount of organic matter, cultivation measure, plant stand and time of the year. Soil, fodder and composts appear to be the primary reservoirs for streptomycetes. Most are strict saprophytes, but some form parasitic associations with plants or animals. Streptomycetes are typical soil microorganisms — their population is higher near the root surface as compared to non-rhizosphere soil at lower depths. Surprisingly little is known about the role of streptomycetes in natural environments, although evidence of their occurrence and numbers in habitats is extensive. Of the actinomycetes isolated from various soil types, the Streptomyces dominate, and a significant proportion of these are able to secrete antibiotics, restricting growth of other microbes. No specific data/information was provided on the natural occurrence of strain K61 other than that *S. griseoviridis*, in general, is a soil bacterium that favours soils high in humus content but can also thrive in other media.

Streptomycetes are also widely distributed in aquatic habitats, but the possibility of their wash-in from surrounding terrestrial habitats must always be considered. The ecology of streptomycetes in aquatic environments has not been well studied, with most studies focussing only on determining the numbers of streptomycetes in aquatic habitats by applying procedures designed for the selection of strains from terrestrial sources. Streptomycete spores may be washed into freshwater and marine habitats, but there is little evidence of their growth in river, lake or marine sediments.

The mode of action of *S. griseoviridis* strain K61 is based on a combination of mechanisms including colonization of rhizosphere in advance of the pathogen; competition for living space and nutrients; production of antifungal metabolites; lysis of

the pathogen cell wall followed by hyperparasitism; and general growth stimulation of the plant.

Streptomyces griseoviridis strain K61 in Mycostop is a preventative, not a curative, biofungicide. If the first application is made early enough (e.g., immediately after transplanting) the streptomycetes colonize the rhizosphere in advance of the pathogens and, thus, effectively compete with them for living space and nutrients. Strain K61 produces small quantities of an antifungal polyene compound that has been classified as a heptaene. However, it is not known whether this compound is involved in the disease control in rhizosphere. The mode of action of heptaenes is known to be binding to sterols of the cell membranes, thus, influencing the ion permeability across the membrane. The extracellular enzymes produced by streptomycetes, such as chitinase, degrade cell walls of fungal pathogens completely or partially, and may finally result in hyperparasitism.

2.0 Methods of analysis

2.1 Analysis for the active ingredient/microbial pest control agent

Appropriate methodologies for detection, isolation and enumeration of the active ingredient, strain K61, were detailed by the applicant. The active ingredient is differentiated from other bacteria and species of *Streptomyces* using non-molecular based methodologies, as these have yet to be adequately developed for the streptomycetes. Instead, the strain is differentiated from other species based on its carbon substrate utilization patterns, pigment formation, colony morphology on standard growth media, morphology of spore chains and spore surface structure.

2.2 Analysis for microbial contaminants

No human or animal pathogens are likely to occur during the manufacture or formulation of Mycostop. The microbiological purity of each production batch is monitored during fermentation by culture on solid growth media and by microscopic analysis and gram-stain preparations. Fermentations are routinely checked by visual inspection to ensure no abnormalities appear in colour, odour and foaming. The spread plate tests on agar growth media to determine viability of the active ingredient in each batch of dry end-use product are also capable of detecting microbiological contaminants. Using standard microbiological methods and selective growth media, analysis data from five batches of Mycostop revealed no contamination by bacteria or fungi at detection limits of <10 CFUs/g of dried product.

2.3 Analysis for other unintentional ingredients

No known toxic metabolites or hazardous substances are present in the technical or end-use products. All the components in the fermentation medium and all the formulation ingredients used are of food grade quality. Impurities in raw materials of fermentation and formulation, therefore, can be considered insignificant. Purity of the unformulated

microbial pest control agent (MPCA) is ensured by rinsing the equipment with 70% ethanol before mixing the formulation ingredients with the MPCA.

Although no toxins were detected in the technical or end-use products containing *S. griseoviridis* strain K61, other species of *Streptomyces* have been shown to be pathogenic to humans. Results of an extensive literature search for the keywords *Streptomyces griseoviridis* and toxin were submitted. The literature search did not yield any reports of toxins being produced by *Streptomyces griseoviridis* strain K61.

3.0 Human health and safety testing

3.1 Summary of toxicity and pathogenicity studies with Mycostop

See Appendix I.

3.2 Exposure assessment

The product has been used in Finland and other countries for over 16 years with no reports of adverse effects in users and other workers who would come into contact with the product under normal operational conditions. Biological dust emitted during handling of the dry end-product, however, may cause hypersensitivity in certain individuals. Two reports of hypersensitivity in workers, possibly initiated from pulmonary exposure to biological dust of the product, have been reported. Recommended protective measures were not followed in either incident and the adverse reactions were short-lived. The wearing of appropriate safety equipment should lower this potential risk to handlers and users of the product.

The information submitted on lack of significant toxicity during the use and application of Mycostop indicate that the acute pulmonary toxicity observed in the rat has not translated into similar observed human toxicity, with the possible exception being the two incidents involving human pulmonary reaction to biological dust. These incidents and the observed pulmonary effects in the rat indicate that appropriate respiratory tract coverings are to be worn during times of potential exposure.

3.3 Food and feed residue studies

An exemption from the requirement of a residue limit under the *Food and Drugs Act* (FDA) and Regulations is proposed for the residues of *S. griseoviridis* strain K61 in or on all raw agricultural commodities when used as a biofungicide for the treatment of seeds and cuttings of greenhouse crops (cucumber, tomato, pepper) and ornamentals. Based on the low level of toxicity of this organism in the acute oral toxicity study and the lack of production of known mammalian toxins, an exemption from the establishment of a residue limit is warranted. *Streptomyces griseoviridis* is a naturally occurring soil microbe and does not colonize plant tissues above the soil. Furthermore, Mycostop is used for inoculating growing media and seeds and no edible portion of the crop is treated directly

with the product. Tests conducted on cucumber found no detectable residues of *S. griseoviridis* strain K61.

Although a heptaenic antibiotic resembling candicidin and amphotericin have been identified as antifungal exudates of *S. griseoviridis* strain K61, very low concentrations are found in the end product (0.005% weight per weight), and production of this compound in soil following the application of Mycostop is expected to remain at concentrations comparable to background levels naturally present in soil. The heptaenes are also degraded by both visible and UV light. Furthermore, because heptaenic antibiotics are known to be poorly absorbed in the gastrointestinal tract and do not pose a particular human health concern, the establishment of a residue limit under the FDA and Regulations is not warranted for these compounds. The absence of adverse effects in test animals receiving topical and oral applications of Mycostop further supports this conclusion.

3.4 Overall human health summary

The registration package submitted by Kemira Agro Oy in support of registering Mycostop Biofungicide, containing the bacterium *S. griseoviridis* strain K61 as the active ingredient, under the URMUR program was reviewed and determined to be acceptable, though product characterization deficiencies were identified. The information provided to address the characterization of the active ingredient as well as the manufacturing process and quality control adequately addressed the potential human health and safety concerns associated with *S. griseoviridis* strain K61 and bacterial/fungal contaminants introduced during production. The PMRA concurs with the submitted U.S. Environmental Protection Agency (EPA) Data Evaluation Reports (DERs) that *S. griseoviridis* strain K61 was neither pathogenic nor infective to rats when orally dosed with 3.0×10^9 CFU per animal (rat). No signs of toxicity or disease were present and clearance through the caecum was established. In the acute dermal toxicity test, the test organism was not toxic to rabbits when a single 2 g/kg dose was administered dermally. Intratracheal instillation of the test organism showed toxicity in rats dosed at about 3.5×10^7 CFU per animal. The test organism caused death in 54% of treated male and 48% of treated female rats. The organism was not pathogenic or infective. The LD_{50} value for the organism following intraperitoneal injection was 1306 mg/kg and 870 mg/kg in male and female mice, respectively. Since all clinical signs were resolved by day 3 of the test, the test organism was not considered infective. Toxicity was possibly due to the excessive quantity of the test material and the large size of the organism. A mild conjunctival irritation was elicited in the rabbit eye following administration of the test organism. No infectivity was noted. An overall moderate skin sensitization reaction was noted in treated guinea pigs 24 and 48 hours after treatment. In a reverse gene mutation assay, *S. griseoviridis* strain K61 was not genotoxic and a literature search found no reports of other strains of this species producing genotoxins. Two reports of hypersensitivity, possibly initiated from exposure to the product, have been reported. Recommended protective measures were not followed. The product has been used in Finland and other countries for over 16 years with

no reports of adverse effects in users and other workers who would come into contact with the product under normal operational conditions.

An exemption from the requirement of a maximum residue limit (MRL) is proposed to be established for the residues of *S. griseoviridis* strain K61 in or on all raw agricultural commodities when used as a fungicide for the treatment of seeds, cuttings, transplants and plants of greenhouse food crops. Based upon the low level of toxicity of this organism in the Tier I mammalian toxicity/pathogenicity studies, an exemption from MRL requirements is warranted.

The formulants in Mycostop Biofungicide are of food grade quality and do not pose any toxicological concerns.

4.0 Environmental toxicology studies

4.1 Summary of toxicity and pathogenicity of Mycostop to non-target organisms

See Appendix II.

4.2 Ecological risk assessment

4.2.1 Birds

Oral toxicity and pathogenicity studies on the northern bobwhite quail and mallard duck revealed no treatment-related toxicity or pathogenicity at total dosage levels of 12 500 mg/kg bw (1.22×10^{10} CFU/kg) administered at doses of 2500 mg/kg body weight (bw) per day for five consecutive days. The acute oral LD_{50} was determined to be greater than 2500 mg/kg bw and was classified as being practically non-toxic in both avian species.

The results of these studies indicate that Mycostop is practically non-toxic to terrestrial and aquatic avian species. Because intended uses involve greenhouse applications only, the possibility of direct avian exposure to *S. griseoviridis* strain K61 is extremely low; therefore, no avian risk is expected from the proposed uses of this product.

4.2.2 Wild mammals

The data submitted in Part M4, *Human Health and Safety*, indicated that there is no significant toxicity to rodents from acute oral testing at the maximum hazard dose. In light of these results and the restriction to greenhouse use, risks to mammalian wildlife is expected to be minimal to non-existent.

4.2.3 Fish

In a 30-day static renewal toxicity and pathogenicity study on the rainbow trout, the LC_{50} was found to be greater than 12.4 mg/L (7.1×10^5 CFU/mL) and the NOEL was 8.7 mg/L (5.0×10^4 CFU/mL). There was no evidence of infectivity. The LC_{50} value indicates that Mycostop is classified as slightly toxic to the rainbow trout.

In Canada, it is difficult to predict how much aquatic habitat will be exposed to products that are applied in greenhouses. The term "greenhouse" is somewhat ambiguous and, thus, problematic. Greenhouse structures may be very well-contained glasshouses, spanning over several hectares or they may be open, "lathe" houses covering only small areas. In view of the wide range of potential exposures of the MPCA to surface water or groundwater runoff from greenhouses, especially following drip irrigation, and because populations of *S. griseoviridis* strain K61 may survive in aquatic systems, some aquatic non-target organisms are likely to be exposed. Exposures are likely to be less dramatic if intended uses of the MPCA are limited to contained soil, plants and seeds, and if runoff can be adequately contained. Larger commercial greenhouse operations are also likely to use automatic watering systems, trough irrigation, spaghetti tubes and drip irrigation, so that watering crops to runoff may not be a widespread occurrence. In situations where there is runoff, it is highly likely that the majority of the MPCA will eventually drain or percolate into soil beneath or around greenhouses. Also, because streptomycetes are largely confined to the rhizosphere, *S. griseoviridis* strain K61 is less likely to be found in lower soil depths where it might percolate to groundwater.

To mitigate the risk of Mycostop to non-target fish, label use directions should be limited to contained soil and plants, or to seed treatments, and an environmental hazards statement should be added to the label indicating the product is toxic to fish.

4.2.4 Arthropods

In a dietary toxicity and pathogenicity study on the honey bee, the LC_{50} was found to be greater than 2400 mg/L (2.4×10^6 CFU/mL), which indicates that Mycostop is practically non-toxic to the honey bee. Even though the study was only supplemental due to nonspecific mortality in the controls, the results are reliable enough to indicate a low probability of adverse effects. Testing the toxicity/pathogenicity of the MPCA to other beneficial arthropods (i.e., predators and parasites) was not included in the submission.

Because intended uses involve directed applications as a soil drench, in drip irrigation systems, or as a seed treatment in greenhouse environments, the possibility of exposing the applied MPCA to a natural arthropod population is extremely low. Although it is difficult to characterize or assess the risks of *S. griseoviridis* strain K61 without additional toxicity/pathogenicity data, it appears unlikely that there would be irreversible adverse effects to beneficial arthropod releases resulting from unintended exposures, based upon the limited information regarding the ecology of streptomycetes in the soil environment.

In a static/renewal toxicity and pathogenicity study on the aquatic invertebrate *D. magna*, the EC_{50} was found to be 190 mg/L (1.9×10^5 CFU/mL), which indicates that Mycostop is practically non-toxic to aquatic invertebrate species. Any aquatic exposures resulting from greenhouse runoff are not likely to present a risk to aquatic invertebrate species.

4.2.5 Non-arthropod invertebrates

In a 14-day toxicity study on the earthworm, there were no treatment-related mortalities or other adverse effects. The NOEC was found to be greater than 1000 mg/kg of soil, the highest concentration tested. A critical review of the study was not conducted because it was not triggered under the current registration requirements for microbial pest control products. Considering that the intended uses of the product involve directed applications in greenhouses, coupled with the fact that species of *Streptomyces* comprise a significant proportion of the native microbial population in soils, the risks to non-arthropod invertebrate species is likely to be low to nonexistent. This conclusion is supported further if the results of the toxicity study are accepted as an indication of the toxicity of *S. griseoviridis* strain K61 to the earthworm. For example, the expected environmental concentration (EEC) in soil following direct application at the highest proposed label rate of 20 g/100 m^2, assuming a soil depth of 15 cm and density of 1.5 g/cm^3, would be 0.89 mg/kg. This EEC in soil is over three orders of magnitude lower than the NOEC value for the earthworm indicating that Mycostop would present a very low risk to terrestrial non-arthropod invertebrates should runoff from greenhouses occur.

4.2.6 Microorganisms

A waiver for microorganism testing was submitted based on the rationale that *S. griseoviridis* strain K61 is a naturally occurring soil microbe that lives in balance with other soil microorganisms; no adverse effects have been observed on non-target microorganisms exposed to this MPCA during the use and in field tests; and in the rhizosphere the MPCA has been shown to be fungistatic or suppressive against a limited number of plant pathogenic fungi. Data on the compatibility of Mycostop with two other biofungicide products were also submitted.

The submitted rationale is sufficient to waive non-target soil microorganism testing for the intended greenhouse uses of *S. griseoviridis* strain K61, though the MPCA is expected to produce adverse effects on other beneficial soil microorganisms, particularly fungi. Because the product is intended for greenhouse uses, the potential for significant off-target exposure to terrestrial environments is relatively low and there are unlikely to be irreversible effects on beneficial soil microorganisms resulting from unexpected exposures.

4.2.7 Non-target plants

No testing was conducted on non-target terrestrial or aquatic plant species. Waivers from plant testing were based on the rationale that *S. griseoviridis* strain K61 is a naturally

occurring soil microorganism and that no adverse effects have been observed in plants exposed to this organism during field trials in the U.S. and Finland. Of over 150 plants assessed for phytotoxicity after treatment with Mycostop, the only adverse effect was a slight reduction in germination in selected varieties of sweet corn, the ornamental *Centaurea cineraria* (Dusty Miller) and cantaloupe when the product was applied as a seed treatment alone or as a seed treatment followed by a drench or soil spray. Given the lack of toxicity/pathogenicity of Mycostop to a wide variety of plant types and the low potential for seeds of non-target plants to be exposed as a result of greenhouse applications, additional testing is considered unnecessary to conclude that Mycostop presents a low risk to non-target plants.

5.0 Value assessment

5.1 Efficacy data and information

Mycostop Biofungicide is a dry inoculant containing 1×10^8 CFU per g of *S. griseoviridis* strain K61, present as mycelium and spores. The product is applied as powder seed treatment, or is suspended in water for seedling spray, bulb dip, soil drip or drench treatment. It is intended for greenhouse use only.

Mycostop Biofungicide is proposed for control or suppression of soilborne diseases in greenhouse cucumber, tomato, pepper, and ornamentals. Label claims are:

- Has been shown to promote the growth and yield of plants even in healthy crops.
- For control of damping-off, root and stem rot and wilt caused by *Fusarium*.

Mycostop is applied dry as a seed treatment or suspended in water for soil spray, drench, drip irrigation or dip. Proposed use rates are:

- 5–50 mg product per plant in rockwool or pots
- 2–20 g product per 100 m^2 of growing medium in beds
- 0.01% suspension for dipping bulbs, corms or cuttings
- 2–8 g product per kg of seed

Soilborne pathogens are currently controlled by steaming (pasteurizing) growth mix, hot water treating of seed and greenhouse sanitation practices such as removing all plant debris, disinfecting equipment, adequate ventilation and drainage, and controlled light and humidity. There are several chemical products available for greenhouse soil treatments and some resistant/tolerant cultivars are available for *Fusarium* diseases. Limitations to the registered products include development of resistance, need for multiple applications in almost continuous production systems, and incompatibility with use of biological insect control.

In support of Mycostop, fourteen trials were submitted where *Fusarium* sp. was known to be present on greenhouse cucumber, pepper, tomato, carnation, cyclamen, primrose or

narcissus. Mycostop applied as a soil spray or drench at proposed rates (5–50 mg/plant; 100–200 mg/m^2 soil) was effective in reducing root rot, wilt, stunting, seedling death and *Fusarium* populations near roots. Yield was typically improved in treated plants (up to 132% of check). Seed treatment was also effective in improving emergence and yield. These effects were comparable to results obtained with chemical treatments. Rate and timing of applications was similar to that proposed, although a two-week interval was included in some trials.

Two methods of application were not accepted: bulb dip (narcissus) and cutting dip (carnation) with Mycostop solutions were not shown to be effective in reducing disease on their own and in some cases were noted as detrimental (see below).

In general, reduced emergence or other symptoms of phytotoxicity or pathogenicity were not observed in the efficacy trials with various crops. Treatment with Mycostop theoretically increases the population of this *Streptomyces* strain in soil by <0.1% and the applicant reported that this returns to base level after several months.

Some adverse response has been reported for specific situations. Dipping carnation cuttings in Mycostop suspensions of greater than 0.01% was noted as potentially phytotoxic, and reduced plant height, weight and flower yields were noted in trials where this method was used, even at 0.01%. The company has also indicated that reduced emergence is observed with dry product treatment rates of 5 g and 8 g/kg lettuce seed or with 8 g/kg gerbera or sweet pepper seed; however, data were not provided for these seed types. As a result, the above are not recommended for the label. It is not possible to test all ornamentals and, as a precaution, the grower should be directed to test the reaction of any new cultivars to Mycostop prior to using the product for commercial scale production.

Aside from cutting dips, yield effects of Mycostop were typically neutral or positive (e.g., 10–12% for seed treatment) even in the absence of known pathogens, and the claim for promotion of growth and yield of healthy plants is acceptable.

The claim of control of damping-off, root and stem rot and wilt caused by *Fusarium* is accepted for greenhouse cucumber, pepper, tomato and ornamentals at proposed rates. Cutting dip and bulb dip are not recommended. Seed treatment use is accepted with the exception of lettuce, gerbera and sweet pepper. The claim for promotion of growth and yield in healthy plants is accepted.

Mycostop is not expected to be prone to resistance, but has a role in reducing pathogen populations that might otherwise become resistant to chemical fungicides. It should not be tankmixed directly with fungicides but is believed to be compatible with products that would commonly be used in greenhouse production. The grower is referred to the manufacturer for a list of products tested for compatibility in lab conditions.

6.0 Toxic substances management policy

During the review of Mycostop Biofungicide, the federal Toxic Substances Management Policy[1] has been taken into account in that Regulatory Directive DIR99-03[2] has been followed. It has been determined that this product does not meet TSMP 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. Furthermore, the active ingredient (technical grade) does not contain any by-products or microcontaminants that meet the TSMP Track-1 criteria. Impurities of toxicological concern are not expected to be present in the raw materials nor are they expected to be generated in sufficient quantities during the manufacturing process to present a risk to human health and safety. Also, there are no formulants of toxicological concern present in the Mycostop Biofungicide end-use formulation.

7.0 Proposed regulatory decision

The PMRA has carried out an assessment of available information in accordance with Section 9 of the Pest Control Products (PCP) Regulations and has found it sufficient pursuant to Section 18(*b*), to allow a determination of the safety, merit, and value of the microbial *Streptomyces griseoviridis* strain K61 and Mycostop Biofungicide. The Agency has concluded that the use of the active microorganism, *Streptomyces griseoviridis* strain K61, in the technical grade active ingredient Mycostop Biofungicide Technical and the end-use product Mycostop Biofungicide in accordance with the label has merit and value consistent with Section 18(*c*) of the PCP Regulations and does not entail an unacceptable risk of harm pursuant to Section 18(*d*). Therefore, based on the considerations outlined above, the use of the microbial *Streptomyces griseoviridis* strain K61 and Mycostop Biofungicide for control of damping-off, root and stem rot, and wilt caused by *Fusarium* on greenhouse cucumbers, tomatoes, peppers and greenhouse ornamentals are proposed for full registration, under Section 13 of the PCP Regulations.

The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document to allow interested parties an opportunity to provide input into the proposed decision for full registration this product.

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1 The federal Toxic Substances Management Policy is available through Environment Canada's Web Site at: http://www.ec.gc.ca/toxics

2 *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy (TSMP)*, DIR99-03, is available through the Pest Management Information Services: Phone 1-800-267-6315 within Canada or 1-613-736-3799 outside Canada (long distance charges apply); Fax (613) 736-3798; E-mail pminfoserv@hc-sc.gc.ca or through our Web Site at http://www.hc-sc.gc.ca/pmra-arla

List of Abbreviations

bw	body weight
CFU	colony forming units
EC_{50}	effective concentration 50%
EEC	expected environmental concentration
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LOEC	lowest observable effect concentration
LOEL	lowest observable effect level
MAS	maximum average score
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
PCP	Pest Control Products
SPSF	Statement of Product Specification Form
U.S.	United States of America
URMUR	User Requested Minor Use Registration

Appendix I Summary of toxicity and pathogenicity studies with Mycostop

Study	Species/strain and doses	LD_{50}, NOEL/NOAEL and LOEL*	Target organ/significant effects/ comments
Acute studies			
Oral	Rat, CD, 11/sex 3.0E+09 CFU/animal	LD_{50} > 3.0E+09 CFU/animal	Agent cleared from the gastrointestinal tract within three days of dosing and was not detected in the urine, blood or organs at any time. No mortalities and no clinical signs of toxicity or pathogenicity/infectivity. **NOT TOXIC OR PATHOGENIC**
Pulmonary	Rat, CD, 60/sex Males: 3.5E+07 CFU/animal Females: 3.02E+07 CFU/animal	$LD_{50} \leq$ 3.5E+07 CFU/animal (males) $LD_{50} \geq$ 3.02E+07 CFU/animal (females)	High mortality was observed in male (54%) and female (48%) rats treated with the live MPCA. Necropsy results revealed signs of lung irritation (red, inflated and autolysed tissue) in the pulmonary tract. Histopathological observations revealed further evidence of a severe pulmonary reaction to the MPCA. Early clearance from the lungs and lack of detection in the body fluids, organs and tissues of any test animal suggested MPCA was unlikely to be a pathogen. **TOXIC BUT NOT PATHOGENIC**
Injection	Intraperitoneal Injection Mouse, CD-1, 5/sex/group, 5000, 1000, 500 and 100 mg/kg bw (equal to 3.1–3.9E+06, 5.5–7.4E+06, 2.9–3.9E+06 and 5.8–7.7E+05 CFU/animal)	LD_{50} = 1306 mg/kg bw (males) LD_{50} = 870 mg/kg bw (females)	**5000 mg/kg bw:** 100% mortality in males and females. **1000 mg/kg bw:** 40% mortality in males. **500 mg/kg bw:** 40% mortality in females. Clinical observations consisted of rough hair coat, lethargy, hunched posture, closed eyes, laboured respiration, ocular discharge, subcutaneous lesions in males and females. All signs resolved by day 4; thus, MPCA was not considered infective. The large size of the MPCA and quantity of test material administered to the animals was a strong influence in the toxicity. **TOXIC BUT NOT PATHOGENIC**
Dermal toxicity and irritation	Rabbit, NZW, 5/sex, 2 g/kg bw (equal to 2.9E+09 CFU/kg bw)	LD_{50} > 2 g/kg bw	No mortalities, signs of systemic toxicity or dermal irritation. **NOT TOXIC OR IRRITATING**

Study	Species/strain and doses	LD_{50}, NOEL/NOAEL and LOEL*	Target organ/significant effects/ comments
Eye irritation	Rabbit, NZW, 6 males, 100 mg dose (equal to 1.46E+08 CFU)	Maximum average score (MAS) = 6/110 (after one hour) MAS = 4/110 (at day 1)	**MILDLY IRRITATING**
Skin sensitization (Magnusson-Kligman Maximization Test)	Guinea pig, Dunkin-Hartley, 20 females, test material 5% intradermal injection (induction) followed by a topical application of 50% (challenge). No positive control tested.	After 48 hours, 55% of the test animals showed positive skin reactions with four having slight, but confluent or moderately patchy redness and seven having moderate redness.	**MODERATE SENSITIZER**
Mutagenicity			
Salmonella/ Ames test	*S. typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538	5, 15, 50, 150 and 500 µg/plate, ±S9	Negative

* LD_{50}: Lethal Dose 50%
NOEL: No observed effect level
NOAEL: No observed adverse effect level
LOEL: Lowest observable effect level

Appendix II Summary of toxicity and pathogenicity of Mycostop to non-target organisms

Study	Species/strain and doses	LD_{50}, NOEL/NOAEL and LOEL	Target organ/significant effects/ comments
Tier I studies			
30-day avian oral	Bobwhite Quail (*Colinus virginianus*), 30 birds, 2500 mg/kg bw (equal to 2.45E+09 CFU/kg bw) per day for five consecutive days	LD_{50} > 2500 mg/kg bw	No treatment-related mortalities, clinical signs of toxicity or pathogenicity/infectivity. **NOT TOXIC OR PATHOGENIC**
30-day avian oral	Mallard Duck (*Anus platyrhynchos*), 30 birds, 2500 mg/kg bw (equal to 2.45E+09 CFU/kg bw) per day for five consecutive days	LD_{50} > 2500 mg/kg bw	No treatment-related mortalities, clinical signs of toxicity or pathogenicity/infectivity. **NOT TOXIC OR PATHOGENIC**
30-day freshwater fish (static renewal)	Rainbow Trout (*Oncorhynchus mykiss*), 10/group, 5 concentrations (2.5E+04 to 4.0E+05 CFU/mL)	LC_{50}* > 7.1E+04 CFU/mL (equal to 12.4 mg/L) NOEC = 5.0E+04 (equal to 8.7 mg/L)	Signs of toxicity included quiescence, surfacing, laboured respiration, discolouration, equilibrium loss. No signs of infectivity. **SLIGHTLY TOXIC**
5-day terrestrial arthropod (dietary)	Honey Bee (*Apis millifera*), 2 groups, 25/group, 240, 760 and 2400 mg/L (equal to 2.4E+05, 7.5E+05 and 2.4E+06 CFU/mL)	LC_{50} > 2400 mg/L NOEC** = 2400 mg/L	Mortalities observed in all dose groups and untreated controls. Mortality not dose responsive or treatment related. **PRACTICALLY NON-TOXIC**
21-day aquatic arthropod (static renewal)	Freshwater Daphnid (*Daphnia magna*), 4 groups, 5/group, 10, 100 and 1000 mg/L (equal to 1.0E+04, 1.0E+05 and 1.0E+06 CFU/mL)	EC_{50}*** = 190 mg/L (1.9E+05 CFU/mL) LOEC**** = 100 mg/L (1.0E+05 CFU/mL) NOEC = 10 mg/L (1.0E+04 CFU/mL)	No treatment-related effects for reproduction and time to first brood. 100%, 25% and 0% mortality at 1000, 100 and 10 mg/L, respectively. 100% mortality in heat-inactivated control. **PRACTICALLY NON-TOXIC**

* LC_{50}: Lethal Concentration 50%
** NOEC: No observed effect concentration
*** EC_{50}: Effect Concentration 50%
**** LOEC: Lowest observable effect concentration