Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Regulatory Note

REG2000-04

Mycostop Biofungicide
Streptomyces griseoviridis

The microorganism *Streptomyces griseoviridis* strain K61 and the formulated product Mycostop Biofungicide have been granted temporary registration under Section 17 of the Pest Control Products Regulations. Mycostop Biofungicide is intended for suppression of diseases on greenhouse cucumbers, tomatoes, peppers and ornamentals. The review of this product was facilitated under the User Requested Minor Use Registration (URMUR) program.

This regulatory note provides a summary of data reviewed and the rationale for the regulatory decision concerning these products.

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Foreword

Health Canada's Pest Management Regulatory Agency (PMRA) has issued a temporary registration for Mycostop Biofungicide, intended for control of damping off, root and stem rot, and wilt caused by *Fusarium* of greenhouse ornamentals and vegatables such as cucumbers, tomatoes and peppers. The review of the active ingredient *Streptomyces griseoviridis* strain K61 and Mycostop Biofungicide, which are Kemira Agro Oy products, was facilitated under the PMRA URMUR program because of the following features/rationale:

- Mycostop will be used for control of damping off, root and stem rot, and wilt caused by *Fusarium* of greenhouse ornamentals and vegetables such as cucumbers, tomatoes and peppers.

- Mycostop has already been registered in the United States (U.S.) and several European countries.

- Mycostop is composed of a naturally occurring soil microorganism, thereby providing a control product that is compatible with an integrated pest management system.

Kemira Agro Oy will be providing confirmatory information as a condition of this temporary registration. Following the review of this new data, PMRA will publish a proposed regulatory decision document and request comments from interested parties before proceeding with a final regulatory decision.

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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 25EC	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	3 months at 28EC

The guarantee for Mycostop Biofungicide is described as 30% *Streptomyces griseoviridis* strain K61 by weight or minimum 10^8 cfu/g. The product specifications should typically include the minimum and maximum guarantee, in terms of cfu/g product, an item which can be corrected as a condition of registration. The proposed directions for the Mycostop label indicated a shelf life of 12 months if stored unopened at below 8 ºC. The study provided, however, did not extend beyond 3 months storage time. Therefore the label is revised to indicate a 3 month maximum, and data supporting a longer storage time may be provided after registration.

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 1.0E+08 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, Russia, Spain and The Netherlands. Mycostop was first registered in the U.S. 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,* and for suppression of root rots of *Pythium*, *Phytophthora* and *Rhizoctonia* in greenhouse ornamentals and herbs, and in greenhouse vegetables (cucumbers, tomatoes, peppers and lettuce).

The proposed use includes seed treatment of lettuce, other 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 thrive also 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; this compound effectively prevents the growth of many fungal pathogens in the 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.

Streptomyces griseoviridis strain K61 is not closely related to any known pathogens of higher plants or animals.

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. Therefore, additional information is required to confirm that *S. griseoviridis* strain K61 does not produce known mammalian toxins. Results of a detailed search of the scientific literature for findings on *S. griseoviridis* and toxins must be submitted to the PMRA for evaluation as a condition of registration.

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 Data Evaluation Reports that *S. griseoviridis* strain K61 was neither pathogenic nor infective to rats when orally dosed with 3.0E+09 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 3.5E+07 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 K61was 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 adverseeffects 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 (1.22E+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 (7.1E+05 CFU/mL) and the NOEL was 8.7 mg/L (5.0E+04 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 also are 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 (2.4E+06 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 (1.9E+05 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 is 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, lettuce 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*.
- Suppression of root rots of *Pythium, Phytophthora* and *Rhizoctonia*.

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 cucumber, pepper, tomato, carnation, cyclamen, primrose or narcissus. Mycostop applied as a soil spray or drench at proposed rates (5–50 g/plant; 10–20 g/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).

Nine trials were reported in which *Pythium, Phytophthora* or *Rhizoctonia* were present on cucumber, pepper, periwinkle or seed of cauliflower, cabbage and rape. While these accounts suggested that Mycostop had potential suppressive effect, the reports were not considered adequate due to insufficient disease pressure, lack of detailed methods or inconsistent results.

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 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 compatible with products that would commonly be used in greenhouse production. The grower is referred to the manufacturer for a list of products tested in for compatibility in lab conditions.

6.0 Overall conclusions

The results of the toxicity/pathogenicity studies submitted indicated that the active ingredient was not pathogenic or infective to treated animals in any of the studies. *Streptomyces griseoviridis* strain K61 was not toxic when administered orally or dermally. Moderate skin sensitization in treated guinea pigs was observed at 24 and 48 hours following treatment. Mild conjunctival irritation was elicited in rabbits in the primary eye irritation study. There have been two reports of hypersensitivity related to biological dust exposure, but in these instances no protective measures had been taken.

Mortality to treated rats was observed in the acute pulmonary toxicity/pathogenicity test, and to treated mice in the acute intraperitoneal toxicity/pathogenicity test. The large size of the microbial active ingredient and the large quantity of test material given to the animals in the intraperitoneal test was a strong influence on the toxicity of the organism. The size of the organism, however, did not appear to be the main factor in animal mortality in the pulmonary test. The necropsy and histopathological observations indicated a severe pulmonary reaction to the presence of the live organism. The high mortality rate observed was sufficient to provoke concern about pulmonary exposure to significant amounts of the organism during handling and use of the product. Consequently, product labelling must advise the user that a dust/mist filter respirator (MSHA/NIOSH TC-21C) must be worn when handling the product. The maximum human exposure to the products would be so far below those levels causing mortality in rats that there should be no adverse human health risk from the use of the organism.

The need for data on the short-term toxicity/pathogenicity and on setting MRLs of food crops was not triggered under current requirements for the proposed product since the organism is naturally occurring and results of initial Tier I toxicology tests did not meet the criteria that would require additional testing or establishment of an MRL. Exemption of an MRL for both the microbial active ingredient and a heptaenic compound involved in the mode of action of the active ingredient is recommended.

The environmental effects studies submitted indicated that *S. griseoviridis* strain K61 is practically nontoxic to terrestrial and aquatic avian species, aquatic invertebrates and to honey bees. The product was slightly toxic to the freshwater fish, *Oncorhynchus mykiss* (rainbow trout). This effect was sufficient to provoke concern about exposure of aquatic

habitats to significant amounts of the organism. Consequently, product labelling must advise the user that the contamination of water bodies is to be avoided.

A short-term toxicity study on the earthworm was submitted, but not reviewed as it was not triggered by current registration requirements. There was sufficient information and data available on the mode of action of streptomycetes against plant pathogenic fungi as well as the absence of any reported adverse effects of other strains of *S. griseoviridis* on non-arthropod invertebrates to conclude that the product is not likely to adversely affect populations of non-arthropod invertebrates in terrestrial or aquatic ecosystems. Non-target microorganism testing requirements were waived based upon the fact that *S. griseoviridis* is a common soil bacterium found throughout the world and because the product is intended for directed applications either by spraying, drenching, drip irrigation, dip treatment, or as a seed treatment in greenhouses where the possibility of exposing the applied bacterium to natural microbial communities is likely to be minimal. Non-target plant testing requirements were also waived based upon the lack of pathogenicity to a wide variety of crop plant types. There 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, but since non-target plants would not be exposed in this manner, additional testing was considered unnecessary. In considering the impact on mammalian wildlife species, toxicological studies on laboratory mammals indicated that there is no significant toxicity to rodents from acute oral testing at the maximum hazard dose, so risk to mammalian wildlife is expected to be minimal.

Sufficient information and data regarding ecological effects were submitted to support registration of *S. griseoviridis* strain K61 and the end-use product, Mycostop Biofungicide, limited to soil drenches or drip irrigation of vegetables and ornamentals and to seed treatment of vegetables in commercial greenhouses. No additional studies or data are required to complete the environmental risk assessment of the product. The need for data on environmental fate or advanced ecotoxicological data (i.e., Tier II) was not triggered under current requirements for the proposed product as results of initial Tier I tests did not meet the criteria that would require additional testing.

Mycostop was shown to be effective in reducing symptoms due to *Fusarium*; however, there were insufficient studies to confirm efficacy against *Pythium, Phytophthora* or *Rhizoctonia*. Rates used in trials were mostly consistent with the proposed label and the application timing of every three to six weeks was appropriate. Soil spray, drench and seed application methods are acceptable. Greenhouse tomatoes, peppers, cucumbers, ornamentals and various seeds (except lettuce, gerbera and pepper) are acceptable host crops. Mycostop is most effective if applied prior to infestation by the pathogen and continued for most of the production period. Mycostop also has limited but positive effect on growth of seedlings in the absence of specific pathogens.

Mycostop was considered acceptable for registration, subject to the provision of confirmatory information noted in sections 1.1 and 2.3 and 7.0.

6.1 Label Revisions

As a result of the reviews detailed above, the following changes have been incorporated on the Mycostop product label. The "Precautions" section of the Mycostop Biofungicide label, has been amended to read:

> *"Wear a long sleeved shirt, long pants, shoes plus socks, chemical resistant gloves, eye goggles and a dust mask (MSHA/NIOSH TC-21C) when handling, mixing/loading or applying the product and during all clean-up/repair activities. Do not enter or allow entry into treated areas for 4 hours after application unless wearing a long sleeved shirt, long pants, shoes plus socks, chemical resistant gloves, eye goggles and a dust mask."*

The proposed "Toxicological Information" statement, "*This product contains the spores and mycelium of a ray fungus which is not known to have any adverse human health effect*.", has been deleted from the product label as it is misleading and contradicts the human health concerns underlying the preceding precautionary statements.

Due to the absence of storage stability data conducted at 8EC, the shelf-life has been set to a maximum period of three months, as this is the only verifiable period of time for which the applicant has submitted storage data.

As *S. griseoviridis* strain K61 is slightly toxic to fish, label use directions are limited to directed applications to contained soil, plants and seeds for production crop use only, and a separate "Environmental Hazards" heading has been added to indicate that the product is toxic to fish:

> "*ENVIRONMENTAL HAZARDS:This product is toxic to fish. Do not apply directly to water or to areas where surface water is present. Do not contaminate any body of water when disposing of equipment wash water.*"

Under the heading "Mixing Instructions", the claim "*MYCOSTOP is harmless to beneficial insects*" has been removed as there is no data available on the toxicity/pathogenicity of *S. griseoviridis* strain K61 to beneficial non-target arthropods (i.e., predators and parasites) other than to the honey bee.

The directions for use on lettuce have been deleted from the label along with directions for use on cuttings at 0.01% suspension dip, and bulbs and corms at 0.01% soaking prior to planting. The list of pathogens affected has been limited to *Fusarium*.

Label directions for growing media treatment have been modified to read:

> *"For seedling production, apply first spray after emergence using lower rate. Mycostop has been shown to be safe on common ornamentals however it is not possible to test all cultivars and some may show delayed germination or*

growth. Mycostop should first be tested on a small lot of plants if the response of the variety is not known, before using in commercial scale production."

7.0 Regulatory Decision

The PMRA has granted temporary registration to December 31, 2000 for Mycostop Biofungicide under PCP Regulations Section17 conditional on submission of the information noted below.

- DACO M2.7.2 Biological Properties of the MPCA
  Detailed literature search to confirm that *S. griseoviridis* strain K61 does not produce known mammalian toxins.
- DACO M 2.9.1 Product Specifications
  Updated product specifications showing minimum and maximum limits of the microbial active expressed as cfu/g dry weight.
- DACO M2.11
  If a storage period of greater than 3 months is to appear on the label, appropriate product stability data at 8 ^{o}C for the proposed storage time are required.

List of Abbreviations

bw	body weight
CFU	colony forming units
EC_{50}	effect 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
U.S.	United States
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} # 3.5E+07 CFU/animal (males) LD_{50} $ 3.02E+07 CFU/animal (females)	High morality 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 further revealed 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