Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Regulatory Note

REG2002-01

RootShield Biological Fungicide
Trichoderma harzianum Rifai strain KRL-AG2

The active ingredient RootShield Technical Biological Fungicide and associated end-use products RootShield Drench Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide, containing the soil fungus *Trichoderma harzianum* Rifai strain KRL-AG2 for the control of soilborne diseases in greenhouse crops (tomatoes, cucumbers and ornamentals), have been granted temporary registration under Section 17 of the Pest Control Products Regulations.

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

(publié aussi en français)

June 4, 2002

This document is published by the Submission Coordination and Documentation Division, Pest Management Regulatory Agency. For further information, please contact:

Publications Coordinator
Pest Management Regulatory Agency
Health Canada
2720 Riverside Drive
A.L. 6605C
Ottawa, Ontario
K1A 0K9

Internet: pmra_publications@hc-sc.gc.ca
www.hc-sc.gc.ca/pmra-arla/
Information Service:
1-800-267-6315 or (613) 736-3799
Facsimile: (613) 736-3798

ISBN: 0-662-32211-8
Catalogue number: H113-7/2002-1E-IN

Foreword

Health Canada's Pest Management Regulatory Agency (PMRA) has issued temporary registrations for the biopesticide, *Trichoderma harzianum* Rifai strain KRL-AG2 in RootShield Technical Biological Fungicide and its end-use products RootShield Drench Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide, manufactured by BioWorks Inc. for suppression of root pathogens in greenhouse tomatoes, cucumbers and ornamentals.

The submissions for registration of *Trichoderma harzianum* have been reviewed by the PMRA under the User Requested Minor Use Registration Program (URMUR). Reviews from the United States Environmental Protection Agency were provided with the submissions as required for URMURs. User support included the National Greenhouse Vegetable Minor Use Committee.

RootShield Drench and RootShield Granules are microbial products containing 1.15% *Trichoderma harzianum* Rifai strain KRL-AG2, a modified strain of a naturally occurring soil fungus. RootShield end-use products are proposed for suppression of root pathogens in greenhouse crops. RootShield will be especially valuable in the production of greenhouse tomatoes and cucumbers, for which there are presently few disease control products, and for which non-chemical options are preferred.

BioWorks Inc. will be providing confirmatory information as a condition of this temporary registration. Following the review of this new data, the 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 The active microorganism, its properties and uses

1.1 Identity of the active microorganism and preparation containing it

Table 1.1 Technical Grade Active Ingredient Identification

Active microorganism	*Trichoderma harzianum* strain KRL-AG2
Function	Biological fungicide
Binomial name Taxonomic designation Kingdom Phylum Order Genus Species Strain	*Trichoderma harzianum* Rifai strain KRL-AG2 Fungi Deuteromycotina Hyphomycetes (syn. Moniliales) *Trichoderma* *harzianum* KRL-AG2
Canadian Patent status information	None
Nominal purity of active	100% of the "technical" preparation is the active ingredient corresponding to a minimum of 5.0×10^8 colony forming units (CFU)/g dry weight of *T. harzianum* strain KRL-AG2. RootShield end-use products contain 1.15% w/w (equivalent to a minimum 10^7 CFU/g).
Identity of relevant impurities of toxicological, environmental and/or other significance	The technical product does not contain any impurities or microcontaminants known to be Toxic Substances Management Policy (TSMP) Track-1 substances. A bacterial load of $>10^7$ CFU/g or the presence of a human pathogen will result in the rejection of a starter culture, termination of the production process or discarding of the final product. A stock culture is rejected if any fungal or bacterial contamination is found to be present. No mammalian toxins are known to be produced by strain KRL-AG2, but confirmatory data are required to support full registration.

1.2 Physical and chemical properties of technical and end-use product(s)

Table 1.2 Technical Product: RootShield Technical Biological Fungicide

Not applicable. RootShield Drench WP and RootShield Granules Biological Fungicide end-use products are manufactured following a continuous manufacturing process that does not involve an intermediate stand-alone technical product.

Table 1.3 End-Use Product: RootShield Granules Biological Fungicide and RootShield Drench WP Biological Fungicide

Property	RootShield Granular Biological Fungicide	RootShield Drench WP Biological Fungicide
Physical state at 25°C	Coarse granular powder	Fine granular powder
Colour	Grey or green Standard Munsell 7.5Y 5/1.4	Grey or green Standard Munsell 2.5Y 8.3/2
Odour	Odourless to earthy	Odourless to earthy
pH in distilled water	7.05	7.92
Density	Bulk density: 0.61 g/cm^3 Tap density: 0.68 g/cm^3	Bulk density: 0.29 g/cm^3 Tap density: 0.63 g/cm^3
Viscosity	Not applicable	Not applicable
Corrosion character	No evaluation performed	No evaluation performed
Suspendibility	No evaluation performed	No evaluation performed
Moisture content	No evaluation performed	No evaluation performed

1.3 Details of uses

RootShield Drench and RootShield Granules are both proposed for suppression of root diseases caused by *Pythium*, *Rhizoctonia* and *Fusarium* in greenhouse food (Use Site Category (USC) #5) and non-food (USC #6) crops. The products are to be applied to growing medium for tomatoes, cucumbers and ornamental plants and directly to ornamental bulbs. The wettable powder formulation is applied as a drench by combining product with sufficient water to achieve uniform application and spraying (low pressure) onto potting mix. Rate of application is 115–220 g product/m^3 of potting mix. Ornamental bulbs may be dipped in a suspension of 120 g/L prior to planting. The granular formulation is added to potting soil at rate of 600 g product/m^3 of soil by raking or tilling into soil in planting beds or by incorporating during potting mix preparation.

Trichoderma is a genus of filamentous deuteromycetes that is ubiquitous in the environment. Its members are generally found in all soils including forest humus layer as well as in agricultural and orchard soils. *Trichoderma* species are rarely reported to occur on living plants and have not been found as endophytes of living plants. *Trichoderma harzianum* is an antagonist of soil-borne fungal pathogens. Its mode of action is complex involving chemotaxis, antibiosis and parasitism. The initial interaction between the parasite and its host appears to be chemotropic growth. The hypha of the mycoparasite grows directly towards its host in response to secreted lectin(s). Apparently, lectins produced by the host bind to the galactose residues on *T. harzianum*'s cell wall and allow it to locate its prey. The antagonistic process starts in advance of physical contact. *Trichoderma harzianum* secretes a number of cell wall-degrading enzymes and antibiotics. These cell wall-degrading enzymes include β-1,3-glucanases, chitinases and proteinases. Several different volatile and non-volatile antibiotics, such as diterpenes, peptaibols, butenolides, furanones, pyrones and pyridones, have been reported for *T. harzianum*. It is believed that these enzymes and antibiotics provide a synergistic effect on its host. It appears that the weakening of the host cell wall by the enzymes increases the rate of diffusion of the antibiotics through the cell wall. Upon physical contact, the hyphae of *T. harzianum* coil around its host where they proceed with invasive growth. Shortly afterwards, the host hyphae collapse due to a loss of turgor pressure.

Trichoderma harzianum strain KRL-AG2 was derived from the fusion of two auxotrophic strains of *T. harzianum*, strain T12m-2 his^- and strain T95-1 lys^-. Strain T12m-2 his^- is a histidine-deficient auxotroph derived from strain T12m by ultraviolet (UV) light mutation. T12m is a spontaneous derivative of strain T12 (a natural soil isolate from Geneva, New York) obtained by selection without mutation treatment for resistance to cycloheximide as a strain maker. Strain T95-1 lys^- is a lysine-deficient auxotroph derived by UV light mutation and selection from strain T95, a benomyl-resistant mutant derived from a naturally occurring strain isolated from Colombia, South America.

Strain T12 and T95 were selected for protoplast fusion because of their ability to control several plant pathogens. Strain T95 is also capable of colonizing roots of plants grown from inoculated seeds. Strain KRL-AG2 is derived primarily from strain T12. Molecular markers, i.e., four isozyme loci for which the parental strains express different alleles, are of the T12 phenotype. Strain KRL-AG2 is also sensitive to benomyl, as is T12, but not T95. Although both the derivatives of T12 and T95 used for protoplast fusion were auxotrophic, KRL-AG2 is completely prototrophic.

2.0 Methods of analysis

2.1 Methods for analysis of the microorganism as manufactured

2.1.1 Methods for identification of the microorganism

Appropriate methodologies for detection, isolation and enumeration of the active ingredient, strain KRL-AG2 were detailed by the applicant. The microbial pest control agent (MPCA) is identified using two different techniques: isozyme analysis, which is used to evaluate starter cultures prior to use in production, and selective plating on growth media to distinguish between strain KRL-AG2 and other *Trichoderma* strains based on colony morphological traits. Vegetative growth is in the form of hyphae, and asexual reproduction occurs via the production of conidiospores and chlamydospores. Sporulation is influenced by such factors as nutrition, pH and light.

The identification of *T. harzianum* to the species level is achieved using standard mycological techniques for this genus. Additional tests to distinguish strain KRL-AG2 from other *Trichoderma* species and strains of *T. harzianum* included isozyme electrophoresis and colony morphology on differential growth medium developed for strain recognition. The taxonomic position of strain KRL-AG2 was confirmed by microscopic examination of asexual reproductive structures (conidiospores and phialides) according to Rifai's species description.

Using starch gel electrophoresis, strain KRL-AG2 is differentiated from most other strains of *Trichoderma* by comparing 17 isozyme patterns to known allelic profiles. Known exceptions are *T. harzianum* strain T12 (and derivatives) and strain 1892, which express the same allele patterns as KRL-AG2 at the isozyme loci tested. An auxotrophic mutant of strain T12 was used in the production of strain KRL-AG2 by protoplast fusion techniques.

Trichoderma harzianum strain KRL-AG2 is also distinguished from other strains including strain T12 by colony morphology when propagated on a differential medium (CCNS). The CCNS medium is comprised of potato dextrose agar (PDA) amended with cycloheximide, chlortetracycline, nystatin, streptomycin sulfate and Igepal. The CCNS cultures are incubated for 7 days at 25°C with 12-h photoperiods. Colonies of KRL-AG2 are initially off-white in colour, producing little to no diffusible pigment in the surrounding agar medium. After several days of incubation, KRL-AG2 colonies produce green spore masses in diurnal zones of heavy sporulation, whereas other strains produce lighter colour spore masses with little diurnal variation in spore mass density.

The colony morphology of *T. harzianum* strain KRL-AG2 is influenced by such conditions as growth media and light. On PDA, the perimeters of the colonies are white to cottony in appearance with green spores giving a pale to dark green colour to the centre of the colony. Under dense conditions the white aerial hyphae are less extensive giving rise to a denser spore mass.

No known toxic metabolites or hazardous substances are present in the RootShield Drench WP and RootShield Granules end-use products. However, *T. harzianum* is a prolific producer of secondary metabolites that include numerous types of alkyl pyrones, isonitriles, polyketides, oxygen heterocyclic compounds, diketopiperazines, and terpenoids (sesquiterpenes, diterpenes), which act either as antifungal/antibacterial agents or plant growth regulators. Of the many antibacterial and antifungal metabolites produced by *T. harzianum*, only a few present a potential risk to applicators and consumers of treated food crops. Of special interest is a unique class of linear hydrophobic polypeptides called peptaibols, which are produced by most species and strains of *Trichoderma*, including *T. harzianum*. Peptaibols function as antibiotics and contain a high proportion of α,α-dimethylisobutyric acid. Many peptaibols, such as the trichorzianines, trichokindins, trichorzins, trichorozins and harzianins, exhibit a broad range of bioactivities related to cell membrane perturbation. These activities include such *in vitro* effects as hemolysis, uncoupling of oxidative phosphorylation in rat liver mitochondria, inhibition of multiplication of different types of cells, acting as channel agonists in bullfrog cardiac myocytes and enhancing secretion of catecholamine from adrenal chromaffin cells.

There are no known mycotoxins produced by *T. harzianum*, although one report in the literature attributed the production of certain trichothecene mycotoxins (i.e., trichodermin and trichodermol) to a fungus identified as *T. harzianum* but later believed to be a misidentified isolate of *T. atroviride*. *Trichoderma atroviride* is not closely related to *T. harzianum* but is more closely related to *T. viride*, which is a known producer of these mycotoxins.

No analysis has ever been performed on strain KRL-AG2 to determine whether peptaibols, or any other secondary metabolites associated with *T. harzianum,* are produced by this isolate. The applicant claims there have been no reports of any toxic plant or animal effects of KRL-AG2 since its registration in the U.S. and other countries worldwide or from over 18 years of study at Cornell University, where the agent was developed for commercial use. However, the absence of toxic effects in acute animal studies suggests that the manufacturing process either does not favour the production of these potentially toxic secondary metabolites or that the levels produced are too low to elicit an effect in animals administered a high dose of this fungus. In the absence of strain-specific data, it is assumed that *T. harzianum* strain KRL-AG2 has the potential to produce all of the secondary metabolites reported in other strains of this fungus.

2.1.2 Methods for establishment of purity of seed stock

Both the original mother culture and the starter cultures of *T. harzianum* strain KRL-AG2 are stored on grains of silica gel at −20°C at Cornell University, Ithaca, New York. Starter cultures are evaluated for genetic stability and contamination prior to use in production. Potential bacterial contaminants are monitored by plating dilutions of samples onto tryptic soy agar (TSA), whereas fungal contaminants are monitored using PDA. All bacterial and fungal colonies are purified and identified using traditional typing

methods as well as molecular "genetic fingerprint" analysis when necessary. Potential relationship to known bacterial pathogens is determined. A bacterial load of $>10^7$ CFU/g or the presence of a human pathogen will result in the rejection of a starter culture, termination of the production process or discarding of the final product. A stock culture is rejected if any fungal or bacterial contamination is found to be present.

2.1.3 Methods to define the content of the microorganism in the manufactured material used for the production of formulated products

Depending on the end-product formulation being manufactured, between three and five samples are taken from each lot during production and tested (CFU test) using a standardized method to determine the viability of the active ingredient. The CFU test is performed to estimate the number of viable propagules of strain KRL-AG2 per unit mass of sample (CFU/g dry weight). The product guarantee is determined after the milling/de-agglomeration and blending process and is expressed as CFU/g dry weight of product. No bioassays are performed to determine the potency of the final product against targeted seed pathogens.

2.1.4 Methods for the determination of relevant impurities in the manufactured material

The applicant provided a brief discussion on the formation of extraneous material during the manufacturing process. Aside from the normal metabolic products (i.e., carbon dioxide, water and excreted protein), no known or suspected toxic material is produced by KRL-AG2 during the fermentation process. However, since the end products are produced by growing the fungus on matrix media (powder or granular) under non-sterile conditions and the milling and blending processes also occur under non-sterile conditions, contamination is expected to occur. Manufacturing quality-control procedures are designed to both minimize formation of unintentional ingredients and to monitor the level of unintentional ingredients in the final product. These procedures begin with the subculturing of the starter cultures and continue through the blending of the end products.

According to the applicant, the only significant unintentional ingredients in these end products are other soil-dwelling microorganisms, including other fungi (*Trichoderma*, *Penicillium*, *Aspergillus*, *Rhizopus*, yeast) and bacteria (streptomycetes), which are associated with an ingredient in the growth matrix. Contamination specifications, however, are in place to prevent end products from containing levels of microbial contaminants that might affect the product efficacy or storage stability. A human pathogen screening process is also in place. The end-use products do not contain or produce any known human or animal pathogen.

Procedures are in place to monitor the presence of microbial contaminants in the end-use products. Analysis data from five batches each of RootShield Granules and RootShield Drench WP were assessed for the presence and level of contamination by bacteria and fungi. The total bacterial count for RootShield Granules ranged from 1.0×10^5 to 1.1×10^6 CFU/g and the total count for RootShield Drench WP was an order of

magnitude higher at 3.5×10^6 to 1.5×10^7 CFU/g. Fungal contamination in all batches was below 1.0×10^5 CFU/g. Contamination less than this concentration was not considered significant and not recorded. Isolated colonies of bacteria and fungi were not taxonomically identified.

Additional microbial analysis of three production batches each of T-22G Biological Plant Protectant Granules (a granular formulation similar to RootShield Granules) and T-22 Planter Box (a wettable powder formulation similar to RootShield Drench WP) revealed the presence of bacterial and fungal contaminants. In both formulations, total mean populations of bacteria and fungi were within the same order of magnitude of 3.1×10^7 – 5.0×10^7 CFU/g. These contamination levels were equivalent to the microbial background levels found in the formulants. There was no significant difference found in mean population levels between final product and formulants. As an added precaution, cultures of the microbes detected were sent to Cornell University for identification. All were identified as common soil-inhabiting species, including *Trichoderma*, *Penicillium*, *Aspergillus*, yeast and *Streptomyces*. The common airborne fungus *Rhizopus* also was detected. In previous studies, production samples were tested by Rochester General Hospital (Rochester, New York) and found to be free of animal and human pathogens, including *Escherichia coli*, *Staphylococcus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Yersinia* sp., *Salmonella*, *Shigella*, *Clostridium perfringens*, *Bacillus* and *Campylobacter*. No pathogens were detected in any of the five consecutive production batches tested.

2.1.5 Methods to show absence of any human and mammalian pathogens

As discussed above, the quality-assurance program implemented by the applicant for the production of the two RootShield end-use products requires the destruction of the batch if any human or animal pathogens are detected during the manufacturing process.

2.1.6 Methods to determine storage stability, shelf-life of the microorganism

Storage stability testing was conducted on representative batches of RootShield Granules and RootShield Drench WP formulations. Final product samples were stored in the dark at 0–6°C, except for brief periods when samples were being stored or removed for testing. No other environmental factors were controlled or monitored. Fungal viability (CFU) agar plate counts were conducted every 3 months, but only the last test results were reported for each sample. The required minimum specifications for CFU counts were maintained in a majority of the 14–19 batches for up to 12 months of storage. Current directions for use and storage recommend users store the product at temperatures less than 5°C and use the product within 12 months. These recommendations are consistent with the product storage information.

2.2 Methods to determine and quantify residues (viable or non-viable) of the active microorganism and relevant metabolites

As no significant adverse effects were reported in Tier I acute toxicity/pathogenicity studies and no data are available that would suggest the MPCA produces secondary metabolites of potential health concern above levels produced by naturally occurring isolates of *T. harzianum* in the environment, the establishment of a maximum residue limit (MRL) is not required for *Trichoderma harzianum* Rifai strain KRL-AG2. Consequently, no method(s) to quantify *Trichoderma harzianum* Rifai strain KRL-AG2 residues in food and feed are required. However, confirmatory analytical data are required for certain peptaibol antibiotics that may be produced by strain KRL-AG2. Peptaibols required for assay from strain KRL-AG2 shall include the trichorzianines A and B, trichokindins I–VII, trichorzins HA and MA, trichorozins I–IV and harzianins HC. The applicant must culture the fungus in growth media that can support the production of peptaibol antibiotics and employ appropriate analytical techniques for extraction, identification and quantification of residues of these metabolites. Such methods are described in the published scientific literature.

There are no known international tolerances established for this MPCA and its metabolites; the U.S. Environmental Protection Agency (EPA) granted an exemption from the requirement of a tolerance of strain KRL-AG2 in/on all food/feed commodities.

Analytical methods for detecting viable strain KRL-AG2 residues in animal and human body tissues involve blending of tissues and recovery on antibiotic- and fungicide-supplemented Trichoderma Selective Medium which is specially formulated to support only strain KRL-AG2 colony growth.

3.0 Impact on human and animal health

See Appendix I for summary table.

3.1 Integrated toxicity and infectivity summary

The registration package submitted by BioWorks, Inc. in support of RootShield Technical, RootShield Drench WP and RootShield Granules, containing the fungus *Trichoderma harzianum* strain KRL-AG2 as the active ingredient, was reviewed from the viewpoint of human health and safety and was found to be sufficiently complete to permit a decision on registration. 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 *T. harzianum* strain KRL-AG2 and bacterial/fungal contaminants introduced during production. However, additional data (see Section 2.2) are required to determine whether the active MPCA is capable of producing certain polypeptide secondary metabolites, known as peptaibol antibiotics, at levels that could present a risk from commercial applications.

Trichoderma harzianum strain KRL-AG2 did not show any signs of toxicity or pathogenicity when administered to rats via the oral or intravenous routes. The observation of enlarged spleens in treated animals following intravenous injection was considered to be a normal reaction to a high dose of foreign agent. Intratracheal instillation of the test organism showed no apparent signs of treatment-related pathogenicity. Lesions consisting of mottled or enlarged and mottled lungs were observed in the pulmonary tract of treated male and female animals. Although lesions have been known to be associated with the instillation of a large number of microorganisms, particularly fungi, directly into the lungs, the gross lesions in this study appeared to be typical of a normal immune response in healthy animals. A request to waive the actual dermal toxicity and the primary dermal irritation study requirements for RootShield Drench WP and RootShield Granules end-use products was accepted based on a reported absence of adverse effects in workers involved in the manufacture of these products, the non-toxicity and widespread commercial use of the inert formulation ingredients (formulants), and the low toxicity and no pathogenicity ranking of the active microorganism in acute oral, pulmonary and intravenous tests. A purified preparation of the test organism was minimally irritating to the rabbit eye; however, the RootShield end-use products contain formulants that are known to be mild ocular irritants.

Based on the absence of significant adverse effects in the Tier I maximum hazard studies, higher tier toxicity/pathogenicity studies involving subchronic and chronic testing, oncogenicity testing, mutagenicity and teratogenicity are not required for *T. harzianum* strain KRL-AG2. Furthermore, there are no known toxicological concerns associated with any of the formulants contained in RootShield Drench WP and RootShield Granules.

The microbial active ingredient, *T. harzianum* strain KRL-AG2, is not known to be a human pathogen nor an endocrine disrupter. The submitted toxicity/pathogenicity studies in the rodent indicate that, following several routes of exposure, the immune system is still intact and able to process and clear the active ingredient. Therefore, no adverse effects to the endocrine or immune systems are known or expected.

3.2 Hypersensitivity incidents

A skin sensitization study was not submitted on the microbial active ingredient, *Trichoderma harzianum* strain KRL-AG2 as hypersensitivity studies are not required by the PMRA to support the registration of microbial pest control agents. Skin-sensitizing studies are not considered substitutes for timely reports of hypersensitivity incidents subsequent to registration approval.

During product research and development activities as well as operational applications in the U.S. where the active ingredient has been registered since 1990, individuals would have been exposed to both mycelia and spores of the MPCA. Exposure is likely to have occurred via dermal and inhalation routes. No report, or suggestion, of hypersensitivity to this fungus has been noted during this time. Nevertheless, because most microorganisms contain substances that elicit positive hypersensitivity reactions in humans, *T. harzianum*

strain KRL-AG2 is considered to be a potential sensitizing agent. Consequently, the signal words “POTENTIAL SENSITIZER” are required on the principal display panels of the RootShield technical and end-use formulation labels. The use of personal protective equipment will also be required to mitigate against potential dermal sensitization in occupationally exposed workers/handlers. The applicant will be expected to report any subsequent findings of hypersensitivity or other health incidents to workers, applicators or bystanders exposed to the MPCA as a condition of registration. Incident reports are to include details such as a description of the MPCA and formulation, frequency, duration and routes of exposure to the material, clinical observations and any other relevant information.

3.3 Impact on human and animal health arising from exposure to the active substance or to impurities contained in it

3.3.1 Occupational and bystander exposure assessment

The PMRA does not expect that occupational exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for *T. harzianum* strain KRL-AG2 and the assumption that precautionary label statements will be followed in the use of RootShield Drench WP and RootShield Granules.

The potential for dermal, eye and inhalation exposure for pesticide handlers exists, with the major source of exposure to workers being generally 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. *Trichoderma harzianum* strain KRL-AG2 is not known to be a human pathogen nor is it known to produce metabolites that are dermally absorbed. Based on the demonstrated lack of adverse effects in the intravenous study, it is the PMRA’s opinion that even cut skin should not pose a risk to health via entry of absorbed *T. harzianum* strain KRL-AG2 into the body. Furthermore, the pesticide has generated no incidents of skin problems (including hypersensitivity) in workers who have had frequent dermal exposure to strain KRL-AG2. However, as dermal exposure studies conducted on another U.S. EPA-registered Rifai strain of *T. harzianum*, strain T-39, indicated the potential for dermal irritation and delayed contact hypersensitivity, the PMRA has imposed label restrictions and risk mitigation measures to protect populations who are likely to be primarily exposed to strain KRL-AG2 from greenhouse applications of RootShield Drench WP and RootShield Granules. Such exposure to pesticide handlers can be ameliorated if they wear long-sleeved shirts, long pants, waterproof gloves, shoes and socks.

A pure powder preparation of strain KRL-AG2 was shown to be minimally irritating to the rabbit eye, but because the RootShield end-use products both contain ingredients (formulants) that are eye irritants, this concern can be adequately addressed if protective eye goggles are worn by workers.

While submitted studies on strain KRL-AG2 indicated a potential for a mild pulmonary risk, inhalation exposure is not of concern if the required dust/filter respirator is also worn by applicators and early-entry (post-application) workers. Consequently, pesticide handlers must wear a dust/mist filtering respirator with the NIOSH prefix N-95, P-95 or R-95 to mitigate exposure. A restricted entry interval (REI) of 4 h will also be required on the end-product labels for early entry workers or other persons entering treated greenhouses.

The label does not allow applications to turf, residential or recreational areas. Because the use sites are agricultural (greenhouse), 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 to non-existent.

4.0 Residues

4.1 Residue summary

Even though *T. harzianum* is a ubiquitous organism found in most terrestrial environments, *Trichoderma* species are rarely reported to occur on living plants. The proposed food use pattern, therefore, is likely to result in only low levels of dietary exposure or residues on treated food commodities at the time of harvest. Furthermore, any residues of the active microorganism are likely to be removed from treated food by washing, peeling, cooking and processing. Even if residues are not removed, dietary exposure to the microbial agent is unlikely to result in any undue hazard to consumers because no adverse effects were observed at maximum hazard dose levels in the submitted Tier I acute oral study.

The PMRA did not require subchronic and chronic dietary exposure studies, since the Tier I acute oral study demonstrated a low toxicity and no pathogenicity potential for the active microorganism. Because of the low toxicity profile and low exposure potential of the MPCA, there is no concern for chronic risks posed by dietary exposure of sensitive subpopulations, such as infants and children.

Dietary exposure to secondary metabolites produced by *T. harzianum* strain KRL-AG2 is possible, even though no aerial parts of tomato and cucumber crops will be directly treated with the MPCA. Uptake by plant roots and translocation to cucumbers and tomato fruit is possible for metabolites produced by the actively growing fungus in greenhouse media, but no crop residue data were submitted for any of the secondary metabolites that may present a human health concern, specifically peptaibol antibiotics. However, given the long history of safe use of products containing this active ingredient in the U.S. as indicated by the absence of adverse effects reports, residue levels of these metabolites are likely to be sufficiently low in the crop at the time of harvest so as not to provoke a concern for dietary exposure. Analytical data on peptaibol production levels from strain KRL-AG2 relative to at least one other naturally occurring strain of *T. harzianum* are required as confirmation that MPCA-produced peptaibol concentrations are unlikely to

exceed those produced by naturally occurring isolates in the environment. The test strains must be grown in media known to support the production of peptaibol metabolites as reported in the published literature.

Trichoderma harzianum is not generally recognized as an aquatic microorganism. Thus, it is not expected to proliferate in aquatic habitats following incidents of direct or indirect exposure (e.g., runoff from treated greenhouses). Moreover, *T. harzianum* is not considered to be a risk to drinking water. Accordingly, drinking water is not specifically screened for *T. harzianum* as a potential indicator of microbial contamination or as a direct pathogenic contaminant. Both percolation through soil and municipal treatment of drinking water would reduce the possibility of significant transfer of residues to drinking water. Therefore, the potential of exposure and risk via drinking water is likely to be minimal to non-existent for this MPCA.

4.2 Maximum residue limits

As no significant adverse effects were reported in Tier I acute toxicity/pathogenicity studies and no data are available that would suggest the MPCA produces secondary metabolites of potential health concern above levels produced by naturally occurring isolates of *T. harzianum* in the environment, the establishment of a maximum residue limit (MRL) is not required for *Trichoderma harzianum* Rifai strain KRL-AG2 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.

The applicant will be required, however, to supply the PMRA with confirmatory data regarding the identification and quantification of peptaibol antibiotics produced by strain KRL-AG2. Peptaibols required for assay from strain KRL-AG2 include the trichorzianines A and B, trichokindins I–VII, trichorzins HA and MA, trichorozins I–IV and harzianins HC. The fungus must be cultured in media that can support the production of these metabolites, and appropriate analytical techniques for extraction, identification and quantification of such residues must be employed. If residues of these peptaibols are detected in pure culture media, and exceed levels produced by other naturally occurring strains of *T. harzianum*, then additional data may be requested on their occurrence in inoculated greenhouse media and in portions of treated crop plants intended for food or feed use. Depending on the results of such analyses, there may be a need to establish MRLs for the metabolite residues of concern.

The U.S. EPA granted tolerance exemptions for strain KRL-AG2 and another Rifai biological control strain of *T. harzianum*, strain T-39. As well, there is no Codex MRL for any strain of *T. harzianum*.

5.0 Fate and behaviour in the environment

5.1 Summary of fate and behaviour in the terrestrial environment

5.1.1 Field studies

A study was submitted where seeds of corn and soybean treated with conidia of *Trichoderma harzianum* KRL-AG2 were sown in a field along with untreated seeds. The crops were grown following normal agronomic practices with the exception that no fungicides were applied. In the fall, soil and root debris collected from the root zones of treated and untreated plants were placed in microplots located in a field. Those microplots remained in place throughout the winter of 1988–1989. In the spring of 1989, soil samples collected from those overwintered microplots were tested for populations of *T. harzianum* KRL-AG2 and other *Trichoderma* species. Following those assays, untreated seeds of corn and soybean were sown in the microplots. The plants were harvested at maturity then they were tested for root colonization by *T. harzianum* KRL-AG2 and other *Trichoderma* species.

Trichoderma harzianum KRL-AG2 was detected in soil collected from the microplots in the spring of 1989 and, thus, survived over the winter. Its isolation from soils collected from untreated crops in the spring of 1989 suggested that the active ingredient was rapidly disseminated in soil; however, no comparisons could be made between treated and untreated groups. Surviving populations of *T. harzianum* KRL-AG2 were shown to colonize roots of subsequent crops to a limited extent but had no observed effects, either beneficial or deleterious, on subsequent crops.

5.1.2 Conclusions

Trichoderma species are ubiquitous soil-dwellers, inhabiting soil, rotting wood and vegetable matter in virtually all terrestrial environments. They produce copious conidia held together in mucoid spore balls, which can be disseminated by water and by soil fauna such as insects and earthworms. With respect to its abundance relative to other species of *Trichoderma*, *T. harzianum* has been characterized as more characteristic of warm climates; however, it is evident from the field study and published literature that cold-tolerant strains do exist. It is also evident that *T. harzianum* KRL-AG2 will likely disseminate and persist in the Canadian environment following its release. Adverse effects, however, are not expected as *T. harzianum* is a saprophyte, with the exception that it can attack other fungi. Furthermore, *T. harzianum* KRL-AG2 has been used in the U.S. for a number of years with no reports of adverse environmental effects.

6.0 Effects on non-target species

See Appendix I for summary table.

6.1 Integrated environmental toxicology summary

Acceptable waiver rationales were submitted to address the environmental toxicology requirements for birds, wild mammals, freshwater fish, estuarine or marine fish, terrestrial arthropods, aquatic arthropods, non-arthropod invertebrates, microorganisms, aquatic plants and non-target terrestrial plants. These rationales were based on minimal exposure due to the products' proposed use patterns, the ubiquitous nature of *T. harzianum*, lack of reported adverse effects in the literature and the inability of *T. harzianum* to become established in unpolluted aquatic environments. Granted some aggressive biotypes of *T. harzianum* were identified as the causal agent of "green mold disease" in the commercial mushroom industry, but those risks are considered to be low if the products are not used on mushroom farms, and if treated, plant materials are not used as substrate by mushroom growers.

The acute avian oral study was not accepted; however, this data requirement was not considered necessary to assess the risks of *T. harzianum* KRL-AG2 as the potential for exposure via the oral route is expected to be minimal when applied in the greenhouse.

The formulation ingredient identified in Rootshield Drench Biological Fungicide and Rootshield Granules Biological Fungicide do not pose an environmental risk when used at the proposed concentrations and application rates.

6.2 Environmental assessment

Trichoderma species are ubiquitous soil-dwellers, inhabiting soil, rotting wood and vegetable matter in virtually all terrestrial environments. The field study submitted by the applicant demonstrated that *T. harzianum* KRL-AG2 disseminated rapidly in the environment and that it persisted in the environment following its release. Those findings were consistent with published literature; thus, the active ingredient, a fusion product of two strains, behaved as expected of *Trichoderma* species. From the viewpoint of environmental protection, some of the information and data published in the open literature raised some concerns. Its mode of action presented potential non-target effects against beneficial soil microorganisms such as mycorrhizal fungi. Furthermore, the plant-regulating metabolites and cellulolytic enzymes produced by this species could negatively affect non-target plants. Many of those environmental concerns, however, were alleviated by the proposed use patterns for both Rootshield Drench Biological Fungicide and Rootshield Granules Biological Fungicide and by the absence of documented adverse effects in the open literature. *Trichoderma harzianum* KRL-AG2 will only be applied on greenhouse food and non-food crops; hence, the potential for exposure is greatly reduced. In addition, relatively few cases of adverse effects have been reported. Those references reporting adverse effects dealt mostly with the identification of *T. harzianum* as the

causal agent of “green mold disease”. Although the active ingredient’s potential to cause “green mold disease” is unknown, Rootshield Drench Biological Fungicide and Rootshield Granules Biological Fungicide will not be used in mushroom farms. Furthermore, a mitigative statement will prevent greenhouse operators from distributing the treated plant matter to mushroom growers for use as substrate. The remaining few references dealt with the issue of plant growth regulating metabolites. Phytotoxicity was reported; however, those studies used concentrations that were much greater than those expected in natural settings. Phytotoxicity occurrences are unlikely in non-target plants as *T. harzianum* will be applied in greenhouses where exposure to non-target plants is expected to be low.

Based largely on published literature, waivers were submitted for the environmental toxicology requirements. Non-target organisms will face minimal increased exposure to *T. harzianum* as a result of the use of Rootshield Drench Biological Fungicide and Rootshield Granules Biological Fungicide. The formulants in the end products do not pose an environmental risk when used at the proposed concentrations and application rates. Consequently, Rootshield Drench Biological Fungicide and Rootshield Granules Biological Fungicide are expected to pose little environmental risk when used in accordance with the label directions. The following label statement, however, will be required on the secondary panel of the label under the section “ENVIRONMENTAL PRECAUTIONS”:

“The treated plant material must not be used as substrate for mushroom farms.”

7.0 Efficacy

7.1 Effectiveness on selected diseases

RootShield products are proposed for use in greenhouses on tomato, cucumber and ornamental crops, for control of root diseases caused by *Pythium*, *Rhizoctonia* and *Fusarium*. RootShield can be used alone or in conjunction with certain chemical fungicides. RootShield is claimed to provide prolonged protection for the root system extending from stand establishment through to harvest.

The proposed labels indicate that RootShield Drench should be suspended in sufficient water to achieve uniform application and applied at a rate of 115–220 g product/m^3 (loose) of greenhouse potting mix, soil or planting beds. RootShield is to be applied through low-pressure watering nozzles such as fan nozzles or other watering systems. Cuttings or bare root transplants are to be dipped in RootShield Drench dry powder or in a suspension of 480 g product/20 L water. Ornamental bulbs are to be dusted with 0.5–1.5 kg product/50 kg bulbs. RootShield Granules are incorporated into greenhouse potting mix or soil at rate of 600–850 g product/m^3 (loose) mix.

In support of RootShield products, 26 efficacy trials on greenhouse ornamentals, tomatoes, onions, eggplant and pepper were reviewed. In most cases, potting mix was treated with RootShield Drench or RootShield Granules as proposed and then infested with the target pathogens *Pythium* or *Rhizoctonia*. The interval between treatment and inoculation typically varied from 0 to 10 days. For *Fusarium* tests, plants were started in the greenhouse then grown at infested field sites. Plants were assessed for signs of disease such as dead plants or root rot and for growth factors such as plant height, fresh weight, dry weight of tops or roots, and marketable quality of flowering plants. For vegetables, quality and quantity of fruit were assessed. Trials were conducted using 60–90 g RootShield Drench in 100 L water (applied to provide 115–220 g/m^3 potting mix) or 560–600 g RootShield Granules per m^3 of potting mix.

Researchers typically considered RootShield to have an overall positive effect on plant health, although this was not always evident in measured variables. The level of demonstrated benefit in these trials is more appropriately termed *suppression* than control. In *Rhizoctonia* trials, RootShield Drench resulted in greater plant survival (8–44%) compared with the inoculated check, as well as increased plant weight and slight increase in root/shoot grade among ornamentals. In *Pythium* trials, RootShield increased root dry weights and plant fresh weights of certain ornamental varieties and also resulted in improved yield in tomatoes. *Fusarium* trials showed a 25–57% reduction in crown and root rot and increased yield in field tomatoes treated with RootShield prior to transplant. A trial with various treatment methods showed improved yield of onion transplants dipped in RootShield Drench suspension.

The submitted data on RootShield Drench and RootShield Granules suggest that these products have potential to suppress disease due to *Pythium*, *Rhizoctonia* and *Fusarium* and may have a positive effect on plant growth, especially roots, even in the absence of target pathogens (uninoculated checks). The efficacy results of these trials were variable; in some cases there was little measurable effect of the pathogen, in others, both microbial and chemical treatments were ineffective or resulted in reduced growth. This variability is typical of microbial products due to their interaction with other organisms and the soil environment. Nonetheless, there were sufficient positive data to support claims of suppression of these three pathogens on greenhouse tomatoes and ornamentals.

Several factors appear to favour RootShield and improve efficacy. One is timing of application; researchers noted that the product needs to be introduced well before the pathogen. In practice this may mean treating potting mix with RootShield 7–14 days prior to using it for seeding or transplanting, or drenching mix with seedlings *in situ* as soon as is safe after chemical control to give it a head start on colonizing roots compared with pathogens.

Based on submitted data, RootShield Drench and RootShield Granules are supported for suppression of *Rhizoctonia*, *Pythium* and *Fusarium* on tomatoes and for *Rhizoctonia* and *Pythium* on ornamentals. Use for *Fusarium* on ornamentals is limited to drench and bulb dip (120 g/L). There were no efficacy data on cucumber; however, based on tomato and

pepper data, and the broad host range of the three pathogens, RootShield products are also accepted for these claims on cucumber on a temporary basis. The applicant will be expected to conduct confirmatory efficacy trials on this crop (see below).

Use of RootShield Drench as dry product (undiluted powder) for ornamental cuttings or bare-root transplants was not assessed and is not accepted. There were insufficient data to support the higher rate of RootShield Granules (800 g/m^3) on any crop.

In Canadian greenhouses, the highest need for a product such as RootShield is in hydroponic (rockwool and other liquid nutrient culture methods) production of tomatoes and cucumbers. The proposed label does not provide specific directions or rates for this use. Based on results of two preliminary trials with rockwool medium and the fact that application rate is not expected to be as critical for a microbial as for a chemical disease control product, it is possible to accept the use of RootShield Drench on hydroponic greenhouse tomatoes and cucumbers on a temporary basis. The grower will need to estimate application rates from potting mix directions, but the applicant will be expected to conduct confirmatory efficacy trials on tomato or cucumber and to develop appropriate detailed directions for hydroponic use as a condition of further registration.

Based on the efficacy review, the proposed label directions require revision.

7.2 Phytotoxicity/pathogenicity to target plants or to target plant products (OECD 7.4)

Phytotoxicity caused by RootShield products in the absence of soil pathogens was not observed. However, the results of a few trials suggest an interaction with root pathogens may occasionally cause adverse effects to the plant. All ornamental varieties cannot be assessed in research trials; therefore, it is recommended that RootShield be tested on a small sample of each new variety prior to commercial scale use.

7.3 Compatibility with current management practices including integrated pest management

RootShield is believed to be compatible with most chemical products used in greenhouse production. The grower is referred to the manufacturer for a list of products tested for compatibility in laboratory conditions. RootShield application methods require further development for use in integrated pest management (IPM) systems, but it is expected that RootShield will contribute to root disease management without any adverse effect on other IPM tools such as biocontrol insects and sanitation practices.

7.4 Contribution to risk reduction

RootShield contributes to suppression and management of plant diseases that might otherwise require frequent application of fungicides for adequate control. This will reduce fungicide use in greenhouses, with consequent reduction in worker, food and environmental exposure.

7.5 Information on the occurrence or possible occurrence of the development of resistance

The microbial active in RootShield products is not expected to be prone to resistance in target pathogens due to its broad mode of action. It will have a role in competing with pathogens that might otherwise reach high populations around crop roots and require frequent doses of chemical fungicides for control, increasing pressure on these pathogens to develop resistance.

7.6 Conclusions

RootShield Drench and RootShield Granules are accepted for suppression of *Pythium*, *Rhizoctonia* and *Fusarium* on greenhouse tomatoes, cucumbers and ornamentals. Acceptable label rates are 60–90 g RootShield Drench in 100 L water (applied to provide 115–220 g/m^3 potting mix), 120 g/L as an ornamental bulb dip or 600 g RootShield Granules per m^3 of potting mix. The product may be applied in suspension as a drench to potting mix, soil, planting beds or other media, used as a dip for ornamental bulbs or incorporated as a granular into potting media.

As a condition of temporary registration, confirmatory efficacy data are required for claims of disease suppression on cucumber and to develop specific application directions for use in hydroponic production. RootShield products are expected to contribute significantly to root disease management in greenhouse crop production.

8.0 Toxic Substances Management Policy

During the review of RootShield Drench WP Biological Fungicide and RootShield Granules Biological Fungicide, the PMRA has taken into account the federal Toxic Substances Management Policy (TSMP) and has followed its Regulatory Directive DIR99-03.[1] 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

[1] The PMRA's Strategy for Implementing the Toxic Substances Management Policy, DIR99-03, is available through the Pest Management Information Service: 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 www.hc-sc.gc.ca/pmra-arla.

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 RootShield Drench WP and RootShield Granules end-use formulations.

9.0 Regulatory decision

The active ingredient RootShield Technical Biological Fungicide and associated end-use products RootShield Drench Biological Fungicide Wettable Powder and RootShield Granules Biological Fungicide containing *Trichoderma harzianum* Rifai strain KRL-AG2 for suppression of root diseases on greenhouse tomatoes, cucumbers and ornamentals have been granted temporary registration pursuant to Section 17 of the Pest Control Products Regulations, subject to the generation of the following studies:

- confirmatory analysis data on the peptaibol secondary metabolites; and
- confirmatory efficacy trials.

List of abbreviations

bw	body weight
CFU	colony forming units
CNNS	cycloheximide chlortetracycline nystatin streptomycin sulfate (agar medium)
DNA	deoxyribonucleic acid
dw	dry weight
EC_{50}	effect concentration for 50% of the population
EEC	expected environmental concentration
EPA	Environmental Protection Agency (U.S.)
EP	end-use product
ICMSF	International Commission on Microbiological Specifications for Foods
IMEP	Import for Manufacturing and Export Program
IPM	integrated pest management
LD_{50}	lethal dose for 50% of the population
LOEL	lowest observable effect level
MIS	maximum irritation score
MPCA	microbial pest control agent
MRL	maximum residue limit
NOAEL	no observed adverse effect level
NOEL	no observed effect level
PDA	potato dextrose agar
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
REI	restricted entry interval
TGAI	technical grade of the active ingredient
TSA	tryptic soy agar
TSMP	Toxic Substances Management Policy
U.S.	United States of America
USC	Use Site Category

Appendix I Summary Tables

Table 1: Summary of toxicity and pathogenicity studies with RootShield Biological Fungicide (*Trichoderma harzianum* strain KRL-AG2)

STUDY	SPECIES/STRAIN AND DOSES	LD_{50}, NOEL/NOAEL and LOEL	TARGET ORGAN / SIGNIFICANT EFFECTS / COMMENTS
ACUTE STUDIES			
Oral	Rat: Sprague Dawley, 13/sex, approx. 10^8 CFU[1]/animal	$LD_{50} > 1.0 \times 10^8$ CFU/animal	No effect on body weight gain and no clinical signs of treatment-related toxicity, infectivity or pathogenicity. No mortalities. Agent cleared from the gastrointestinal tract within 2 days of dosing and was not detected in the urine, blood or organs at any time. No significant findings observed at necropsy. **LOW TOXICITY AND NO PATHOGENICITY.**
Pulmonary	Rat: Sprague Dawley, 15/sex, approx. 10^8 CFU/animal	$LD_{50} > 1.0 \times 10^8$ CFU/animal	No effect on body weight gain and no clinical signs of toxicity. No mortalities. Necropsy revealed mottled lungs in 11 treated ♂ and ♀ rats, and enlargement of the lungs in 6 treated rats of both sexes. Lesions were typical of a normal immune response in healthy animals to the instillation of a high dose of foreign (antigenic) material. Early clearance of agent observed from body fluids and other organs and clearance by study termination at Day 21 from the lungs. **LOW TOXICITY AND NO PATHOGENICITY.**
Injection	Rat: Sprague Dawley, 15/sex, approx. 10^7 CFU/animal	$LD_{50} > 1.0 \times 10^7$ CFU/animal	No effect on body weight gain and no apparent signs of treatment-related toxicity or pathogenicity. No mortalities. Treatment-related effect was limited to an enlargement of the spleen in male and female animals, which was considered to be a normal reaction to a microbial infection. Following injection, the test microbe was predominantly found in the liver, lungs, spleen, kidney and blood. Enlarged spleens were observed in 9 rats. A drastic reduction in number of test organisms and a distinct pattern of clearance of the agent were demonstrated by post-treatment Day 21. **LOW TOXICITY AND NO PATHOGENICITY.**

STUDY	SPECIES/STRAIN AND DOSES	LD_{50}, NOEL/NOAEL and LOEL	TARGET ORGAN / SIGNIFICANT EFFECTS / COMMENTS
ACUTE STUDIES			
Dermal Toxicity and Irritation	Waiver rationale submitted in lieu of data	Not applicable	Based on an absence of adverse effects reports for workers involved in the manufacture and use of the end-use products in the United States; the non-toxic nature and widespread commercial use of the inert formulation ingredients (formulants); and the low toxicity and no pathogenicity ranking of the active microorganism in acute oral, pulmonary and intravenous tests, a waiver in lieu of actual testing was accepted for RootShield Drench WP and RootShield Granules end-use products. As a precautionary measure, all greenhouse workers who will be exposed to these products during mixing/loading, application and post-application activities will be required to wear appropriate personal protective equipment (long-sleeved shirts, long pants, shoes plus socks and waterproof gloves) to minimize dermal contact.
Eye Irritation	Rabbit: NZW, 6 males, 0.1 g of technical powder (equivalent to 10^8 CFU/animal)	MIS[2]= 2.3/110 (after 1 h)	Slight redness in the conjunctivae was observed in all treated eyes at the 1-h observation interval. All eyes appeared clinically normal by 72 h and no other signs of irritation were observed during the 7-day observation period. Technical powder is minimally irritating. However, certain inert formulation ingredients (formulants) in RootShield end-use products are known to be mild ocular irritants. Protective eyewear (goggles) is recommended for applicators to reduce risk of contact. **TGAI - MINIMALLY IRRITATING** **EP - MILDLY IRRITATING**

[1] CFU = Colony Forming Units
[2] MIS = Maximum Irritation Score

Table 2: Effects of RootShield Biological Fungicide (*Trichoderma harzianum* strain KRL-AG2) on Non-Target Organisms

Organism	Exposure	Test substance	Endpoint value / Comments
Birds: Bobwhite Quail	Acute Oral	Conidia of *T. harzianum* KRL-AG2	30-day $LD_{50} > 4 \times 10^9$ CFU/kg bw (or 11 110 mg a.i./kg bw) No signs of toxicity or pathogenicity. No mortalities. When compared to controls, there were no apparent effects on body weight or feed consumption. At necropsy, one bird in the treated group was noted with enlarged adrenal glands, but this observation was not considered treatment related as the adrenal glands in the other 41 treated birds appeared normal. **SUPPLEMENTAL** Insufficient information and data were provided to determine if the dose received by the birds was viable. A replacement study is not required based on the absence of adverse effects in the open literature and the low potential for avian exposure from greenhouse use.
Birds	Pulmonary / Inhalation / Injection	Waiver rationale submitted in lieu of data	The request for a waiver was supported with the results of the acute bobwhite quail oral (see above) and rat pulmonary studies (see Table 1). Although no treatment-related adverse effects were noted, those results were not sufficient to support the request due to difficulties in extrapolating the results of oral studies to pulmonary studies and of mammals to birds. Rather, the request was supported with literature and the proposed use-patterns for both formulations (i.e., greenhouse only). Based on the absence of adverse effects in the open literature and the low potential for avian exposure, this request for a waiver was **ACCEPTED**.

Organism	Exposure	Test substance	Endpoint value / Comments
Wild Mammals	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported by references to toxicological studies that were previously submitted to the PMRA for the registration of RootShield Granules under IMEP. As indicated in Chapter 3, *T. harzianum* KRL-AG2 did not show any signs of toxicity or pathogenicity when administered to rats via the oral or intravenous routes. Enlarged spleens were noted in treated animals following intravenous injection; however, this observation was considered to be a normal immunological reaction to a foreign particulate. Intratracheal instillation of the test organism showed no apparent signs of treatment-related pathogenicity. Furthermore, *T. harzianum* KRL-AG2 was found to be minimally irritating to the eyes of the rabbit. Based on the absence of significant adverse effects, this request for a waiver was **ACCEPTED**.
Fish	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with a brief literature review. The review described the natural occurrences of species of *Trichoderma*. Although ubiquitous in most terrestrial environments, few references have cited instances of recovery of any species of *Trichoderma* from aqueous environments. All but one of those occurrences involved polluted waters. One reference reported the isolation of *T. harzianum* from a marine sponge. Based on *T. harzianum*'s apparent inability to establish itself in unpolluted waters and on the absence of adverse effects to fish in the open literature, this request for a waiver was **ACCEPTED**.
Arthropods	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with published literature. There are no references in which *T. harzianum* caused infection or any other impact on insects or other invertebrates. In one published article, no adverse effects were reported for bees treated with *T. harzianum* T-39. In another, honey bees and bumble bees were used to disseminate *T. harzianum* KRL-AG2 without ill effects. Other literature reported that insects, especially mites, consumed the hyphae of *Trichoderma* species. Based on the absence of adverse effects in the open literature, this request for a waiver was **ACCEPTED**.

Organism	Exposure	Test substance	Endpoint value / Comments
Non-Arthropod Invertebrates	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with literature. There are no references in which *T. harzianum* caused infection or any other impact on insects or other invertebrates, including non-arthropods. In one published article, earthworms were fed a diet that was infested with *T. harzianum* T3a without ill effects. Based on the absence of adverse effects in the open literature, this request for a waiver was **ACCEPTED**.
Microorganisms	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with published literature. Aggressive strains of *T. harzianum* were identified in the published literature as the cause of "green mold disease". Those strains were grouped into two biotypes, namely TH2 and TH4, according to their ribosomal gene sequences. Studies done on the sequences of several biological control strains of *T. harzianum* showed that they belonged to a third biotype, namely TH1. The biotype of *T. harzianum* KRL-AG2 was not specified in the application; however, this information was not considered essential for its review as it will not be used in the commercial mushroom industry. As there are no other reports of adverse effects in the open literature, this request for a waiver was **ACCEPTED** provided that a mitigative label statement is placed on the label preventing greenhouse operators from distributing the treated plant material to mushroom growers as substrate.
Plants	Acute	Waiver rationale submitted in lieu of data	The waiver request was supported with literature. Even though *T. harzianum* is a ubiquitous organism found in most terrestrial environments, *Trichoderma* species are rarely reported to occur on living plants. Furthermore, its ability to attack living wood or plants is considered weak despite its ability to produce potent enzymes and secondary metabolites with plant growth regulating activities. Although it is possible that those concentrations of enzymes and metabolites may increase above phytotoxic levels following direct inoculation to non-target plants, such occurrences are not expected to occur from the proposed use of the end-use formulations in greenhouses. This request for a waiver was **ACCEPTED**.