Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Regulatory Note

REG2002-02

SPORODEX L End-use Product and *Pseudozyma flocculosa* strain PF-A22 UL Technical Grade Active Ingredient

The technical grade active ingredient *Pseudozyma flocculosa* strain PF-A22 UL and associated end-use biopesticide product SPORODEX L, containing the naturally occurring fungus *Pseudozyma flocculosa* strain PF-A22 UL, for the control of powdery mildew on roses and cucumbers, are proposed for 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 5, 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: 0-662-32235-5
Catalogue number: H113-7/2002-2E-IN

Foreword

Health Canada's Pest Management Regulatory Agency (PMRA) has issued temporary registrations for technical grade active ingredient, *Pseudozyma flocculosa* strain PF-A22 UL, and its end-use product, SPORODEX L, developed by Plant Products Co. Canada. These products were reviewed jointly as biopesticide products within the North American Free Trade Agreement's Technical Working Group (NAFTA TWG) on Pesticides Joint Review Program by Health Canada's PMRA and the United States (U.S.) Environmental Protection Agency (EPA). Reviews were shared on an informal basis with the Netherlands Pesticide Regulatory Authority.

SPORODEX L is a biological fungicide, containing 1.3% (w/w) *Pseudozyma flocculosa* strain PF-A22 UL, intended for the control of powdery mildew on greenhouse-grown roses and cucumbers. The active microorganism, *Pseudozyma flocculosa*, is a naturally occurring fungus and represents a new biopesticide registration to the U.S. or Canada.

Microbial pest control agents are being investigated increasingly for use as alternatives to conventional pesticides because they are thought to pose a lower potential risk to human health and the environment, compared with conventional pesticides. SPORODEX L represents a potential biopesticide replacement for chemical fungicides.

Plant Products Co. 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.

Table of Contents

1.0 The active microorganism, its properties and uses

1.1 Identity of the active microorganism and preparation containing it

Table 1.1-1 Technical Grade Active Ingredient Identification

Active microorganism	*Pseudozyma flocculosa* strain PF-A22 UL
Function	Biological fungicide
Binomial name: Taxonomic designation: Kingdom: Phylum: Genus: Species: Strain:	*Pseudozyma flocculosa* (Traquair, J. A., Shaw, L. A., and Jarvis, W. R.) Boekhout, T. and Traquair, J. A., strain PF-A22 UL Fungi Deuteromycotina Dematiaceous Asexual Fungi *Pseudozyma* *flocculosa* PF-A22 UL
Canadian Patent status information	A patent was granted in the name of Her Majesty in right of Canada as represented by the Minister of Agriculture with Plant Products Co. Ltd. as licencee. Canadian Patent Serial No.: 2,011,705 Issued: 1999/06/15 Expires: 2010/03/07 Title: METHOD AND COMPOSITION FOR THE BIOLOGICAL CONTROL OF PLANT DISEASES
Nominal purity of active ingredient	*Pseudozyma flocculosa* strain PF-A22 UL (TGAI) consists of 100% active ingredient in spent fermentation medium corresponding to a minimum of 3×10^8 colony-forming units (CFU)/mL of *Pseudozyma flocculosa* strain PF-A22 UL. 1.3% w/w (equivalent to a minimum 3×10^8 CFU/mL) in SPORODEX L end-use product.

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 stock culture is rejected if biological activity is altered or if mutations are detected. If any contamination is found in the media prior to inoculation, the media is discarded. If contamination exceeds the product release standards for total aerobic flora (<1000 CFU/mL), enterobacteria (<10 CFU/mL), fecal streptococci (absence in 1 g), *Staphylococcus aureus* (absence in 1 g), coliforms (<10 CFU/mL), *Escherichia coli* (absence in 1 g) and *Salmonella* (absence in 1 g), the product is discarded. No mammalian toxins are known to be produced by strain PF-A22 UL.

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

Table 1.2-1 Technical Product: *Pseudozyma flocculosa* strain PF-A22 UL

Not applicable. SPORODEX L is manufactured following a continuous manufacturing process that does not involve an intermediate stand-alone technical product.

Table 1.2-2 End-Use Product: SPORODEX L

Property	SPORODEX L
Physical state at 25°C	Liquid
Colour	Beige
Odour	Faint mushroom smell
pH in distilled water	6.4–6.8
Density	1.05 g/mL
Viscosity	51 centipoise
Corrosion character	None

All formulants in Sporodex L are either of food grade quality or are considered relatively non-toxic (i.e., EPA list 3, 4A or 4B). There is no formulant, contaminant, or impurity present in the end-use product that would meet TSMP Track-1 criteria.

1.3 Details of uses and further information

SPORODEX L is an end-use product containing the active ingredient *Pseudozyma flocculosa* strain PF-A22 UL. SPORODEX L is a liquid proposed for use as a biological fungicide to control powdery mildew fungi (*Sphaerotheca pannosa* var. *rosae* and *Sphaerotheca fuliginea*) on greenhouse food (Use Site Category #5) and non-food crops (Use Site Category #6), namely cucumbers and roses. SPORODEX L is to be applied in an aqueous solution prepared by diluting 500 mL of product per 100 L of water (equivalent to approximately 10^5 to 10^6 CFU/mL). A wetting agent is added to a final concentration of 0.02% to improve its efficacy. Plants are to be treated beginning when environmental conditions favour development of powdery mildew or at the first sign of the disease. Plants are to be sprayed to the point of runoff at weekly intervals. Up to 1500 L of spray solution is to be applied per hectare for cut roses or cucumbers or about 1000 L/ha for potted plants. After application, the relative humidity is to be maintained above 70% for 12 hours.

Pseudozyma flocculosa was isolated in 1986 from the leaves of red clover, *Trifolium pratense*, infected with powdery mildew, *Erysiphe polygoni*, by researchers at Agriculture and Agri-Food Canada, Harrow, Ontario. Initially, this organism was erroneously identified as a new ascomycetous yeast with an anamorphic state in the broad genus *Sporothrix* and a teleomorphic state in the genus *Stephanoascus*. In 1995, its taxon was changed to *Pseudozyma flocculosa* following ribosomal DNA analysis. The genus *Pseudozyma* contains other smut-like anamorphs, including *P. rugulosa* (formerly *Sporothrix rugulosa). Pseudozyma flocculosa* is a phyllosphere epiphyte and hyperparasite of primarily powdery mildew but has been isolated in association with other leaf-surface moulds. It is widely distributed in North America (Canada and the U.S.A.) and in Europe on aerial plant surfaces in field or greenhouse agricultural ecosystems.

Pseudozyma flocculosa antagonizes a number of different powdery mildew fungi (*Sphaerotheca pannosa* var. *rosae*, *Sphaerotheca fulginea, Erysiphe graminis* var. *tritici* and *Erysiphe polygoni*) on many different plants in greenhouse and field environments when the relative humidity is greater or equal to 70%. This fungus is a necrotroph mycoparasite that kills susceptible target host cells upon contact or in close proximity. Rapid death and collapse of host cells without penetration is brought about by the secretion of three fungitoxic unsaturated C-17 fatty acids (9-heptadecenoic acid, 6-methyl-9-heptadecenoic acid and 4-methyl-7,11-heptadecadienoic acid) and an acyclic norterpene (2, 6, 10, 14, 18-pentamethyl-2, 6, 8, 10, 12, 14, 17-nonadecaheptene-1,19-diol). The fungitoxins disrupt susceptible plasma membranes and cytoplasmic organelles within 30 minutes of exposure. The inhibitory response includes a loss of proteins and electrolytes. After 24 hours, the host cells rapidly collapse and die as a result of the activity of the fungitoxins on the host cell's membranes and lipids. Sensitivity to the unsaturated C-17 free fatty acids is related to a high degree of unsaturation of phospholipid fatty acids and a low proportion of sterols.

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 *P. flocculosa* strain PF-A22 UL were detailed by the applicant. The microbial pest control agent (MPCA) is identified using a combination of morphological traits, molecular techniques, and biological activity.

The identification of *Pseudozyma* to the species level is done using a standard mycological approach. *Pseudozyma* species can be differentiated from morphologically similar species such as *Hyalodendron*, *Tilletiopsis*, *Sporobolomyces*, and *Sporothrix*. The branching conidiophores of *Pseudozyma* can be confused with those produced by *Hyalodendron*; however, the whole cell hydrolysates of this filamentous basidiomycete contain xylose, which is not found in *Pseudozyma*. *Tilletiopsis* and *Sporobolomyces*, other saprophytic wild yeasts on aerial plant surfaces, are different from *Pseudozyma* in that they produce spores that are forcibly discharged upon sporulation (ballistospores). Furthermore, *Tilletiopsis* species produce a fungus-degrading β-1,3 glucanase that is not produced by *Pseudozyma* species. The genus *Sporothrix* represents a group of anamorphic ascomycetous yeasts such as *Sporothrix schenckii* (type), an animal pathogen. Physiologically, *Pseudozyma* species differ greatly from *Sporothrix* species. Unlike the ascomycetous *Sporothrix* anamorphs, *P. flocculosa* shows positive reactions in Diazonium Blue B and urease tests typical of all basidiomycetous yeasts. Also, the major ubiquinone is Q-10 rather than Q-8 or Q-9 typical of the ascomycetes, *Saccharomycopsis* and *Stephanoascus*.

Strain PF-A22 UL can be differentiated from other strains of *P. flocculosa* using a DNA-based technique called multiplex polymerase chain reaction (multiplex PCR). The multiplex PCR system is essentially a cocktail of different primers which allows the rapid assessment of numerous DNA fragments in a single PCR amplification. The protocol is based on the amplification of two nuclear regions (ITS and NS), and one mitochondrial region (ML). Those regions were found to be discriminant in the identification of *P. flocculosa* PF-A22 UL.

The integrity and consistency of the MPCA is ensured by two methods. The first method is a DNA-based PCR technique called random amplified microsatellites (RAMS) PCR. Microsatellites are hypervariable non-coding regions of DNA within the genome that evolve more rapidly than coding DNA. The other method is a bioassay that measures biological activity. The biological activity of the MPCA is measured by the inhibition zone created when a susceptible organism is grown next to it. Given that the pest controlled, *Sphaerotheca* species, is an obligate biotroph, it cannot be used directly in this bioassay. Instead, a *Phomopsis* species is used because its sensitivity to *P. flocculosa*'s fungitoxic secretions is similar.

2.1.2 Methods for establishment of purity of seed stock

The mother colony is maintained as slant cultures at 4°C, and as freeze-dried cultures stored at –20°C. The genetic stability of those cultures is verified at least once every six months using RAMS PCR (see Section 2.1.1 for details). The frequency of this analysis is to be increased accordingly if the mother colony begins to show signs of reduced yield.

No method for establishing the purity of the mother colonies was submitted; however, sufficient microbial contaminant screening methods were proposed for the production of *Pseudozyma flocculosa* strain PF-A22 UL and SPORODEX L. There are essentially three types of screening methods involved in the production of *Pseudozyma flocculosa* strain PF-A22 UL and SPORODEX L, namely pre-fermentation sterility tests, MPCA integrity tests, and microbial contaminant screening tests.

Prior to inoculation, all media are screened for the presence of microbial contaminants by plating aliquots of the medium onto plate count agar (PCA) plates. If any microbial contamination is found, the medium is discarded. Similarly, all cultures are monitored for MPCA integrity and microbial contamination by plating various dilutions onto potato dextrose agar (PDA) plates. If significant microbial contamination is detected, the culture is rejected. In case of abnormal colony morphology on PDA, a multiplex PCR analysis (see Section 2.1.1 for details) is performed to properly identify the afflicted colonies. Furthermore, the bioassay method described in Section 2.1.1 is done prior to product formulation to verify its biological control potential. Microbial contaminant screening tests are performed on the formulated end-use product prior to packaging. They are monitored by culturing dilutions of formulated end-use products onto or into various media. The groups of microbial contaminants tested and their proposed product release standards include total aerobic flora (<1000 CFU/mL), enterobacteria (<10 CFU/mL), fecal streptococci (absence in 1 g), *Staphylococcus aureus* (absence in 1 g), coliforms (<10 CFU/mL), *Escherichia coli* (absence in 1 gram) and *Salmonella* (absence in 1 g). If any of the proposed bioburden limits is exceeded, the entire batch is rejected.

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

The concentration of *Pseudozyma flocculosa* strain PF-A22 UL is determined by measuring the number of viable colony-forming units (CFU) per millilitre of formulated product. For this assay, a 25-mL sample is diluted in peptone, then plated onto PDA. Microscopic observations made on the formulated product ensure that the MPCA is under the proper conidial form. According to product specifications, the guarantee is expressed as greater than 3×10^8 CFU/mL. The biological control potential of the MPCA is measured prior to product formulation using the bioassay method described in Section 2.1.1.

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

No known or suspected toxic material is produced by *Pseudozyma flocculosa* strain PF-A22 UL during the fermentation process. Although the majority of the manufacturing process is designed to avoid microbial contamination, some contamination can occur as the end-use product is centrifuged and formulated under non-sterile conditions. As mentioned in Section 2.1.2, there are various methods to monitor the levels of various groups of contaminating microorganisms in the formulated product.

Quality control data from five batches (1 commercial-scale and 4 pilot-scale batches) of SPORODEX L were assessed using the microbial contaminant screening methods described in Section 2.1.1. The total aerobic flora in SPORODEX L ranged from 150 to 2×10^4 CFU/mL. Both the enterobacteria and fecal coliform counts were 0 CFU/mL and no enterococci, *E. coli*, *Staphylococcus aureus*, or *Salmonella* were detected in SPORODEX L. It must be noted that two of the five batches, including the only commercial batch, were destroyed due to microbial contamination. In one of those batches, the total aerobic flora exceeded the product release standard for this group of contaminants, i.e., $< 10^3$ CFU/mL. In the other, significant microbial contamination was detected during an MPCA integrity test on PDA. Both of those batches were rejected.

Given that two batches were destroyed as a result of microbial contamination, the submission of certificates of analysis for all production batches of SPORODEX L will be required as a condition of registration by the PMRA and the U.S. EPA.

2.1.5 Methods to show absence of any human and mammalian pathogens

As discussed in Section 2.1.2, the quality assurance program implemented by the applicant for the production of SPORODEX L requires the destruction of the batch if any of those product release standards (including animal and human pathogens) is exceeded in the formulated product.

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

Storage stability data are required to ensure product performance and safety. The data included in the submission package were derived from a single batch of SPORODEX L over a period of 11 months at -20°C. Additional storage stability data derived from at least five production-scale or pilot-scale batches are required to support label claims and ensure product performance and safety. An expiration date of three months from the date of manufacture is required until additional data are generated.

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

Although *Pseudozyma* species are ubiquitous in nature and have been isolated from a wide variety of plant surfaces including leaf litter, clover, maize, and cucumber, no adverse effect has been attributed to natural populations of *P. flocculosa*. Given that no significant adverse effect is reported in the acute oral toxicity/pathogenicity study and that no report in literature suggests that *Pseudozyma* (*Sporothrix*) *flocculosa* produces mammalian toxins, the establishment of a maximum residue limit (MRL) is not required for *Pseudozyma flocculosa* strain PF-A22 UL. Consequently, no method to quantify *Pseudozyma flocculosa* strain PF-A22 UL residues in food and feed is required.

Analytical methods for detecting viable *Pseudozyma flocculosa* residues in animal and human body tissues involve blending of tissues and recovery on yeast malt agar (YM) or Martin's agar (MA). If needed, a multiplex PCR analysis (see Section 2.1.1 for details) can be performed to discriminate strain PF-A22 UL from other strains of *P. flocculosa*.

3.0 Impact on human and animal health

See Appendix I, Table I, for summary table.

3.1 Integrated toxicology summary

The registration package submitted by Plant Products Co. in support of registering the TGAI *Pseudozyma flocculosa* strain PF-A22 UL and SPORODEX L, was reviewed from the viewpoint of human health and safety and was determined 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 *P. flocculosa* strain PF-A22 UL and bacterial/fungal contaminants introduced during production.

No sign of toxicity or pathogenicity was noted when SPORODEX WP, a wettable powder formulation, was administered to rats via the oral route.

Intratracheal administration of *P. flocculosa* resulted in a significant number of spontaneous deaths among both test substance (TS)- and heat-killed test substance (KTS)-dosed animals. Presence of the test organism in the stomach and small intestines indicated a possible dosing error. In a second pulmonary study, there were no mortalities but rough hair coat occurred in a dose-dependent manner. Based on this study, *P. flocculosa* was classified as slightly toxic. This study was considered supplemental because infectivity was not assessed and because the test substance was not the recommended TGAI. The label must be upgraded with a statement requiring respirators for all users and a full acute pulmonary toxicity/infectivity replacement study, using the TGAI and the sterile filtrate of the production culture, will be required.

Pseudozyma flocculosa was found to be slightly toxic but non-pathogenic when administered to rats via intraperitoneal injection. There were no mortalities or adverse clinical symptoms. White nodules and higher relative spleen weights were noted at necropsy and attributed to a normal immune response to a foreign substance. Male TS-dosed rats, however, exhibited decreased body weight gain despite increased food consumption, indicating that *P. flocculosa* was slightly toxic. Clearance of the test organism occurred within 7 days, indicating lack of pathogenicity.

Pseudozyma flocculosa was not toxic or irritating when applied dermally to rabbits. A waiver rationale was submitted to address the toxicity and/or irritation potential of the formulation ingredients in SPORODEX L. All formulation ingredients are either food-grade or relatively non-toxic. One formulation ingredient may cause irritation of the skin with prolonged contact. Standard personal protective equipment requirements are adequate.

Slight conjunctival redness was observed after administration of SPORODEX WP to the eyes of rabbits. The irritation potential of SPORODEX L is expected to be less than that of SPORODEX WP. Standard label statements, instructing users to avoid contact with eyes, are sufficient.

Pseudozyma flocculosa has not been reported to produce any mammalian toxin. The applicant included computer literature search results to a number of keywords such as pseudozyma*, tilletiopsis, fate, non target, carcin*, mutagen*, toxic*, pathogen*, antibiotic*, polyen*, sporothrix, sporobolomyces, rhodotorula, phyllosphere yeast*, carcinog*, and teratogen*. The literature search covered *AGRICOLA*, Biological Abstracts, CAB Abstracts, CHEMTOX, RTEX, and AGRIS databases from 1980 to 1999. No reports of mammalian toxicity were found in standard biological, chemical, and toxicological abstracts.

Survival, replication, infectivity, significant toxicity, or persistence of the MPCA was not observed in the test animals treated in Tier I acute oral, pulmonary, and intravenous toxicity/infectivity tests. Consequently, higher tier tests involving subchronic and chronic testing, oncogenicity testing, mutagenicity, and teratogenicity were not required, based on the lack of concerns following analysis of Tier I test results.

The active ingredient, *P. flocculosa* strain PF-A22 UL, 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 effect to the endocrine or immune systems is known or expected.

3.2 Hypersensitivity

A skin sensitization study was not submitted on the microbial active ingredient, *P. flocculosa*, 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.

No adverse effect has been noted among researchers who have worked closely with *P. flocculosa* strain PF-A22 UL for up to 10 years. The applicant has submitted a waiver rationale from conducting a dermal sensitization study based on the assumption that most microorganisms contain substances that could elicit a hypersensitivity response. *Pseudozyma flocculosa* is considered a potential sensitizing agent; therefore, the statement "POTENTIAL SENSITIZER" is required on the principal display panels of the 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. As a condition of registration, 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. 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 its impurities

3.3.1 Occupational and bystander exposure assessment

Occupational exposure is of particular concern as the product will be used in an enclosed environment. The PMRA, however, does not expect that occupational exposure will pose an undue risk on the basis of the low toxicity/pathogenicity profile for *P. flocculosa* provided that precautionary label statements are followed. When handled according to the label instructions, the pulmonary, dermal, and ocular routes are potential routes of applicator and bystander exposure.

Dermal exposure via the skin would be the primary route of exposure for applicators. 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. *Pseudozyma flocculosa* is not known to be a human pathogen nor is it known to produce metabolites that are dermally absorbed. Based on the minimal adverse effects in the intraperitoneal study, it is the PMRA's opinion that even cut skin should not pose a significant risk to health via entry of absorbed *P. flocculosa* into the body. Although the MPCA has been found to be non-toxic and non-irritating following dermal exposure, it is a potential sensitizer. Label restrictions and risk mitigation measures are required to protect populations who are likely to be primarily exposed to the pesticide. Such exposure to pesticide handlers can be reduced if they wear long-sleeved shirts, long pants, shoes, and socks.

Inhalation would be another route of exposure for mixer/loader applicators and possibly early-entry workers. Based on the results of the pulmonary study in which lesions were noted on the lungs of some treated animals, pesticide handlers must wear a dust/mist-filtering respirator with National Institute for Occupational Safety and Health (NIOSH) prefix –95, R-95, P-95 or HE filter for biological products. A restricted entry interval of four hours will also be required for early-entry workers or other persons entering treated greenhouses.

SPORODEX WP was found to be minimally irritating to the rabbit eye and, based on the nature of its formulation ingredients, SPORODEX L is expected to be less irritating. Eye protection will not be required.

The label does not allow applications to turf, residential, or recreational areas. Because the use sites are in greenhouses, 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

According to its proposed use pattern, SPORODEX L will be applied by foliar spray to rose and cucumber plants. The proposed food use pattern is likely to result in residues on food and feed. However, residues of the microbial pesticide 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 effect was observed at maximum hazard dose levels in the submitted Tier I acute oral study. Furthermore, an extensive literature search yielded no report of mammalian toxins being produced by *P. flocculosa* (see Section 3.1). The fungitoxic unsaturated C-17 fatty acids and acyclic norterpene produced by the MPCA have not been reported to be toxic to mammals. Neither this organism nor its close relatives are listed among microbial contaminants of food.

Subchronic and chronic dietary exposure studies are not required since the Tier I acute oral study demonstrated a low level of 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.

Although heavy rainfall likely carries *P. flocculosa* into neighbouring aquatic environments, growth and survival of terrestrial fungi such as *P. flocculosa* are limited in such environments. Thus, it is not expected to proliferate in aquatic habitats following incidents of direct or indirect exposure (e.g., runoff from treated greenhouses). Moreover, *P. flocculosa* is not considered to be a risk to drinking water. Accordingly, drinking water is not specifically screened for *P. flocculosa* 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

Although *Pseudozyma* species are ubiquitous in nature and have been isolated from a wide variety of plant surfaces including leaf litter, clover, maize and cucumber, no adverse effect from dietary exposure has been attributed to natural populations of *P. flocculosa*. Furthermore, no adverse effect was observed in the acute oral toxicity/pathogenicity study and there is no report of known mammalian toxins being produced by the MPCA. Therefore, the establishment of an MRL is not required for *P. flocculosa* strain PF-A22 UL 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.

5.0 Fate and behaviour in the environment

Environmental fate data (Tier II) were not triggered as adverse effects on non-target organisms are not expected from the proposed greenhouse use of *P. flocculosa* strain PF-A22 UL.

6.0 Effects on non-target species

See Appendix I, Table II, for summary table.

6.1 Integrated environmental toxicology summary

Acceptable waivers were submitted to address environmental toxicology requirements. Non-target organisms, including birds, wild mammals, freshwater fish, aquatic and terrestrial arthropods, non-arthropod invertebrates, and aquatic and terrestrial plants are not expected to face increased exposure to *P. flocculosa* due to use of SPORODEX L in commercial greenhouses. No adverse effect on these non-target organisms has been reported in the literature. Studies to assess the effect of *P. flocculosa* on these organisms are not required.

Pseudozyma flocculosa is a saprophytic fungal epiphyte and a hyperparasite of powdery mildew. Collapse of susceptible host cells and rapid death occur via the secretion of three fungitoxic unsaturated C-17 fatty acids and an acyclic norterpene. The fungitoxins disrupt susceptible plasma membranes and cytoplasmic organelles while the acyclic norterpene has limited antifungal potential. Despite the mode of action associated with *P. flocculosa*, its host range is limited to mainly powdery mildews (e.g., *Sphaerotheca pannosa* var. *rosae, S. fulginea, Erysiphe graminis* var. *tritici* and *E. polygoni*). Although *in vitro* bioassays have shown that soil-borne fungi such as *Trichoderma, Fusarium, Pythium,*

Phytophthora, and *Rhizoctonia* species and selected Gram-negative (e.g., *Xanthomonas campestris*) and Gram-positive (e.g., *Bacillus subtilis*) bacteria were weakly to moderately susceptible to *P. flocculosa, P. flocculosa* being a phyllosphere epiphyte and a non-rhizosphere competent organism is not expected to have significant effect on soil-borne microorganisms. Based on the limited host range of *P. flocculosa*, no non-target microorganism testing will be required.

The formulants in the end-products, SPORODEX L, do not pose an environmental risk when used at the proposed concentrations and application rate for control of powdery mildew on roses and cucumbers grown in greenhouses. Consequently, SPORODEX L is expected to pose little environmental risk when used in accordance with the label directions. Furthermore, no special precautionary or environmental hazard statement is required on the label for SPORODEX L.

7.0 Efficacy

7.1 Effectiveness

7.1.1 Intended use

For control of powdery mildew in greenhouse crops.
Mix 500 mL of SPORODEX L for each 100 L water. Add a wetting agent at 0.02%.

Apply up to 1500 L of water per ha for cut roses or cucumbers and 1000 L per ha for potted plants. Spray foliage of plants to runoff at weekly intervals, beginning when environmental conditions favour development of powdery mildew or at first sign of the disease.
Maintain RH above 70% for 12 hours after application.

Use of chemical fungicides at the same time as SPORODEX may inhibit this product's activity against powdery mildew.

Keep frozen at -20 ^{o}C or lower in the freezer until use; thaw at room temperature prior to using.

7.1.2 Mode of action

SPORODEX L is a liquid formulation containing *Pseudozyma flocculosa* (synonym *Sporothrix flocculosa*) at 3×10^{8} cfu/mL. The strain of *P. flocculosa* that is the basis of this product was isolated from powdery mildew on weeds near Harrow, Ontario, in 1988. It has been found to be antagonistic to most species of powdery mildew pathogens, and appears to be common in horticultural and agricultural environments where powdery mildews are found. Host range may include antagonism to *Sphaerotheca* and *Erysiphe* but it is less active on *Trichoderma*, *Fusarium*, *Pythium*, *Phytophthora*, and *Rhizoctonia* according to *in vitro* bioassays. *Pseudozyma* destroys the integrity of host cell membranes

through the action of fatty acid metabolism, causing cell leakage, but does not appear to colonize host hyphae. Optimal conditions for infection of host fungi are 26° C and > 70% RH. *Pseudozyma* will colonize leaves in the absence of powdery mildew but undergoes rapid reproduction only when the disease is present.

7.1.3 Crops

SPORODEX L is intended for use on greenhouse roses and cucumbers.

7.1.4 Effectiveness against pest

Eleven trials with *Pseudozyma flocculosa* on cucumbers were conducted in the Netherlands and Canada in research or commercial greenhouses. Plants were grown in rockwool according to normal hydroponic production practices. SPORODEX WP (two early formulations) or SPORODEX L were applied at intervals as per label directions to plants which were usually inoculated with powdery mildew (*Sphaerotheca* spp.) For comparison, commercial fungicide standards were applied as needed. Powdery mildew (percent diseased leaf area on whole plants) was assessed at intervals and an area under disease progress curve for the whole season was generated for comparison of treatments. Cucumbers were harvested and graded, and total yield or first class grade yield were recorded.

In seven cucumber trials, based on total disease over the season, SPORODEX WP provided 18–48% control which was significantly different from the check but less than that provided by the chemical fungicides (44–66%). In two comparative trials under moderate disease pressure, a newer formulation of SPORODEX WP showed significantly better control (>56%) than the check and than the older formulation which was not effective. Yield of SPORODEX-treated cucumbers was generally greater than the check and slightly lower than chemically treated plants.

Rose powdery mildew studies were conducted in Canadian and Columbian greenhouses. SPORODEX WP was comparable in efficacy to various chemical fungicides and often resulted in better quality or yield of roses. The product was less effective where high humidity was not maintained.

One confirmatory trial with the proposed SPORODEX Liquid formulation showed that it is as effective as myclobutanil against powdery mildew on cucumber. In this trial, an application of pine oil (fertilizer) in midseason adversely affected SPORODEX which resulted in lack of disease control on lower leaves and showed that pine oil is not compatible with SPORODEX. Results are available from two additional trials in the Netherlands and British Columbia (B.C.); these showed that reduction of powdery mildew and improved rose yield with SPORODEX L were comparable to results with dodemorph-acetate. Efficacy studies showed a need to maintain high humidity (>70% RH) for continued viability and effectiveness of SPORODEX products.

These studies show that SPORODEX can provide comparable efficacy to chemical fungicide sprays in controlling powdery mildew and improving yield of cucumbers and quality of greenhouse roses. SPORODEX significantly reduced powdery mildew compared with untreated checks. Although the early formulation was not as effective as chemical standards, limited trials with more recent formulations suggest that SPORODEX L will be as effective as chemical standards provided that high humidity is maintained. Further, it does not cause phytotoxic effects which indirectly lowered yields as seen with some chemical treatments.

The proposed rate of SPORODEX L was 500 mL product in 100 L water, applied to runoff (1500 L/ha for cut roses and cucumbers and 1000 L/ha for potted roses). This delivers approximately 1×10^9 cfu/L of *P. flocculosa*, which was the concentration used in most of the efficacy trials with various formulations. A lower rate may also be effective but should not be considered until crop management practices have developed to give more consistent disease control performance at the current rate.

7.1.5 Total spray volume

SPORODEX L is intended to be applied in up to 1500 L water per ha for cut roses and cucumbers and 1000 L for potted plants.

7.2 Phytotoxicity to target plants (including different cultivars) or to target plant products (Organisation for Economic Co-operation and Development [OECD] 7.4)

No adverse effect of SPORODEX formulations was noted in greenhouse trials with cucumber or rose. The additive paraffin oil (1%) used with SPORODEX was noted to cause a slight edema (water blisters) on the underside of rose leaves of one cultivar and the use of oil was discontinued in that trial. The oil was typically used at lower concentrations in other trials and is not recommended on the SPORODEX L label.

7.3 Observations on undesirable or unintended side effects, e.g., on beneficial and other non-target organisms, on succeeding crops, other plants, or parts of treated plants used for propagating purposes (e.g., seed, cutting, runners) (OECD 7.5)

SPORODEX L was tested in commercial greenhouses throughout its development, using typical production practices including integrated pest management (IPM) and biological control organisms. In these efficacy trials, observations suggested that the product has no adverse effect on crop plants, or on beneficial insects or mites with respect to pest control. However, direct assays to confirm no adverse effect of SPORODEX L on specific biocontrol insects and arthropods were not conducted.

7.3.1 Impact on succeeding crops (OECD 7.5.1)

Not applicable to greenhouse use.

7.3.2 Impact on adjacent crops (OECD 7.5.2)

Not applicable to greenhouse use; adjacent crops (if any) are typically grown within separate compartments.

7.3.3 Impact on seed viability (OECD 7.5.3)

Not applicable to proposed crops.

7.4 Economics

According to the applicant, the farm gate value of greenhouse cucumbers in Ontario is $25 million. There are also greenhouse areas in BC, Alberta and Quebec. Crops are worth $625,000–$1,250,000 per ha annually. There are about 24 ha of greenhouse roses in Canada, mostly in Ontario, with some operations in B.C., Alberta, and Quebec. Grade #1 roses are priced at $0.50 per stem.

Although it rarely affects fruit, powdery mildew spreads rapidly on leaves and can cause a loss of photosynthetic area, and water stress, leading to reduced flower production or yield and up to 100% loss. Mildew can also affect marketability and the price of roses as there is zero tolerance for the presence of white mildew spots on the leaves and blooms; they will be downgraded. The price difference to growers for grade #1 to grade #2 roses is $0.15 per stem, and control of mildew could potentially increase revenues by $37,000 per ha. Fungicides are currently used to control mildew but can have adverse effects on yield, and on fruit and flower quality.

7.5 Sustainability

7.5.1 Survey of alternatives

Powdery mildew is currently managed by environmental control, tolerant cultivars, sanitation and chemical fungicides. The trend in greenhouse production is to reduce chemical pesticide use as much as possible. Thus, there is a need for alternative products to use in the disease management program.

7.5.1.1 Non-chemical control practices

Powdery mildew is partly managed by environment control; however, this is difficult to balance because the different stages of disease development are favoured by different conditions. For example, both low humidity and free water on leaves followed by rapid drying have been found to reduce disease, yet daily fluctuations in humidity can increase disease. In general, growers should avoid conditions which lead to succulent foliage, i.e., shading, overcrowding, overwatering, or overfertilizing. The fungal spores will not survive long outside host plant material, so a thorough cleanup and break period of 2–3 weeks between crops can reduce carryover of inoculum. Seedlings which are already

started should be cultivated in isolation from the older producing plants. Teardown and replant of the cucumber crop is a labour intensive operation due to the volume of vine material handled, so the plants are usually maintained as long as is profitable.

Mildew-resistant cucumber and rose cultivars are not available in practice for Canadian conditions; although some tolerance is available, these cultivars may not be commercially desirable. For instance, in B.C. cucumber production, more resistant varieties are grown in fall when mildew pressure is high but they are lower-yielding and more prone to Botrytis and gummy stem than mildew-susceptible varieties grown earlier in the year. Choice of rose varieties is dependent on market acceptability for colour and other characteristics rather than tolerance to powdery mildew.

7.5.1.2 Chemical control practices

Few chemical products are available for powdery mildew control in greenhouses. Myclobutanil and sulfur are registered in Canada for cucumbers, and dodemorph-acetate and copper are registered for roses. The most effective products are systemic, must be applied frequently, may be toxic to beneficial insects, and are prone to development of resistance in the powdery mildew pathogens. Both myclobutanil and dodemorph were noted to cause phytotoxicity to flowers and fruit under some conditions, and reduced leaf size has been reported for both cucumber and rose. Silicon has been investigated as a disease preventive, either applied into hydroponic solution or as a foliage spray, but has not been consistently effective on its own. Milsana is another non-fungicidal product under investigation but not used commercially in Canada. The trend in greenhouse production is to reduce chemical pesticide use as much as possible. Thus, there is a need for alternative products to use in the disease management program.

Table 7.5-1 Alternative disease control products

Active Ingredient	End-Use Products	Fungicide Activity Site	Application Rate (product/1000 L)		Comments
			Cucumber	Rose	
Myclobutanil	Nova 40W	demethylation	340 g/ha		Can affect leaf and fruit growth, prone to resistance
Sulfur	Kumulus, MicroNiasul	multisite	1.2–1.5 kg		Harmful to some beneficial mites, can be phytotoxic
Copper	Phyton 27	multisite		1.25–2.5 L	
Dodemorph-acetate	Meltatox 40EC	isomerase		2.5 L	Can reduce bloom quality

7.5.2 Compatibility with current management practices including IPM

SPORODEX L has potential to reduce or replace chemical fungicide sprays on cucumbers and roses and efficacy trials showed that it can be alternated with some of those products. SPORODEX L also appears to be compatible with IPM practices for control of insects and mites (see Section 7.3). However, SPORODEX L has not been tested for compatibility with all chemical products or with other microbial disease control organisms; therefore, the grower should be referred to the manufacturer for updated information.

IPM practices currently include the monitoring of crops for signs of early disease, which is necessary to ensure that SPORODEX L is applied at the earliest opportunity for maximum effectiveness. At present, the value of SPORODEX L is limited by its susceptibility to changing environmental conditions. Growers and extension staff will need to invest further work in determining the best local production practices for viability and efficacy of SPORODEX L in the greenhouse to obtain optimum powdery mildew control and thereby reduce the need for chemical products.

7.5.3 Contribution to risk reduction

It is expected that SPORODEX L will be used in greenhouses to control powdery mildew in situations of lower disease pressure and at the beginning of the growing season to delay the progress of the disease. In this way it may alleviate or defer the need for chemical fungicide applications thus reducing the associated risks of pesticide resistance, effects to workers and on the environment.

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

Powdery mildew pathogens have been known to develop resistance to chemical fungicides; however, resistance to *Pseudozyma flocculosa* is less likely because of its broad mode of action and lack of persistence on the crop plant. *Pseudozyma flocculosa* destroys the integrity of host cell membranes, causing cell leakage. Optimum conditions for colonization do not occur continuously in the greenhouse environment. It will colonize leaves in the absence of powdery mildew but undergoes rapid reproduction only when the disease is present.

SPORODEX L does have a role in prolonging effectiveness of chemical fungicides. By reducing pathogen populations and the number of fungicide sprays applied for control of powdery mildew, SPORODEX L may reduce pressure on the pathogen to develop resistance to site-specific fungicides.

7.6 Conclusions

7.6.1 Summary

SPORODEX L is a liquid formulation containing *Pseudozyma flocculosa* at 3×10^{8} cfu/mL for control of powdery mildew in greenhouse roses and cucumbers. The proposed rate of SPORODEX L is 500 mL product in 100 L water, applied to runoff (1500 L/ha for cut roses and cucumbers and 1000 L/ha for potted roses). Eleven trials with *Pseudozyma flocculosa* on cucumber were conducted in the Netherlands and Canada in research or commercial greenhouses. Five rose powdery mildew studies were conducted in Canadian and Colombian greenhouses. SPORODEX L significantly reduced powdery mildew compared with untreated checks. Although the early formulation was not as effective as chemical standards, limited trials with more recent formulations suggest that SPORODEX L will be as effective as chemical standards provided that high humidity is maintained. Further, it does not cause phytotoxic effects which indirectly lowered yields, as seen with some chemical treatments. Further work is needed on managing the greenhouse environment for full disease control benefits of SPORODEX L to be realized.

SPORODEX L is a microbial product which may be affected by co-application of fungicides and other products. The label precautions should be expanded to advise the grower of this and include guidance for better performance. SPORODEX L has not been shown to adversely affect other tools such as biocontrol agents, shows no phytotoxic effects on the crop and is generally compatible with IPM practices which are being adopted for greenhouse production.

Table 7.6-1 Summary of label proposals and recommendations

Proposed		Recommendation (based on Value Assessment)
Greenhouse crops	Cucumber Roses (potted or cut)	as proposed
Rate	500 mL /100L of water with 20 mL wetting agent Use up to 1500 L spray per ha for cut roses, cucumbers, up to 1000 L for potted roses	as proposed
Application method	Diluted spray applied to foliage to runoff	as proposed

Proposed		Recommendation (based on Value Assessment)
Timing of applications	Weekly from first disease or when environmental conditions favour development of disease	as proposed
Conditions	Maintain RH >70% for 12 hours Do not apply at same time as chemical fungicides	provide additional details/guidance

8.0 Toxic Substances Management Policy considerations

Pseudozyma flocculosa strain PF-A22 UL in SPORODEX L 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. There is also no formulant, contaminant, or impurity present in the end-use product that would meet the TSMP Track-1 criteria.

9.0 Regulatory decision

The active ingredient *Pseudozyma flocculosa* strain PF-A22 UL, and the formulated product SPORODEX L for control of powdery mildew on greenhouse-grown roses and cucumbers have been granted temporary registration pursuant to Section 17 of the Pest Control Products Regulations, on the condition that the applicant carry out the following:

- submits certificates of analysis for all production batches;
- reports all incidents of hypersensitivity occurring following registration;
- provides storage stability data for at least five pilot-scale or production-scale batches;
- provides a full acute pulmonary toxicity / pathogenicity replacement study.

List of abbreviations

bw	body weight
CFU	colony-forming units
DNA	deoxyribonucleic acid
dw	dry weight
EPA	Environmental Protection Agency (U.S.)
IPM	integrated pest management
KTS	killed test substance
LD_{50}	lethal dose 50%
MAS	maximum average score
MIS	maximum irritation score
MPCA	microbial pest control agent
MRL	maximum residue limit
NC	naive control
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
PDA	potato dextrose agar
PMRA	Pest Management Regulatory Agency (Health Canada)
RAMS	random amplified microsatellites
RH	relative humidity
TGAI	technical grade of the active ingredient
TS	test substance
TSMP	Toxic Substances Management Policy
U.S. or U.S.A.	United States of America

Appendix I Summary tables

Table I Summary of toxicity and pathogenicity studies with *Pseudozyma flocculosa*

STUDY	SPECIES/STRAIN AND DOSES / TEST SUBSTANCE	LD_{50}, MIS/MAS	TARGET ORGAN/ SIGNIFICANT EFFECTS/ COMMENTS
ACUTE STUDIES			
Oral	Rat — Fisher 344, 12/sex, ♂ 5.8×10^8 CFU[1]/animal ♀ 5.6×10^8 CFU/animal SPORODEX WP	♂ $LD_{50} > 5.8 \times 10^8$ CFU/animal ♀ $LD_{50} > 5.6 \times 10^8$ CFU/animal	No effect on body weight gain or feed consumption and no clinical signs of treatment-related toxicity, infectivity, or pathogenicity. No mortalities. Agent cleared from the gastrointestinal tract within seven 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 — CD, 12/sex, 3.2×10^7 CFU/animal *Pseudozyma flocculosa*	$LD_{50} > 3.2 \times 10^7$ CFU/animal	Laboured breathing, rough hair coat, ocular discharge and nasal discharge observed in TS[2]- and KTS[3]-dosed animals. Hunched posture and lethargy observed in one TS-dosed female and one TS-dosed male, respectively. Mortalities included 3 ♂ TS-dosed, 6 ♂ KTS-dosed, 2 ♀ TS-dosed and 4 ♀ KTS-dosed rats. No effect on body weight based on rats sacrificed on day 14. Daily food consumption analysis and relative organ weights either not determined or did not include animals that died prior to their scheduled sacrifice dates. Necropsy findings including lesions and enlargement of the lung, confluent dark areas in the kidneys, lesions and enlargement of the spleen and lung lesions in ♂ and ♀ rats dosed with TS and KTS were attributed to the method of dosing and the body's normal immunological response to a foreign substance. Agent was detected in the lungs and lymph nodes, stomach and small intestines. Clearance from these organs by day 7. Study classified as **UNACCEPTABLE.**
Pulmonary — Range Finding	Rat — CD, 5/sex/dose level 4.2×10^7 CFU/animal 3.4×10^7 CFU/animal 6.8×10^6 CFU/animal 3.4×10^6 CFU/animal *Pseudozyma flocculosa*	$LD_{50} > 4.2 \times 10^7$ CFU/animal	No mortalities. All animals gained weight over the course of the 14-day study. Rough hair coat occurred in a dose-dependent manner. One female rat dosed at 4.2×10^7 CFU presented with tremors, closed eyes, and rough hair coat. **SLIGHTLY TOXIC; PATHOGENICITY NOT DETERMINED.** Study classified as **SUPPLEMENTAL**. Upgraded label statements required.

STUDY	SPECIES/STRAIN AND DOSES / TEST SUBSTANCE	LD_{50}, MIS/MAS	TARGET ORGAN/ SIGNIFICANT EFFECTS/ COMMENTS
ACUTE STUDIES			
Intraperitoneal Injection	Rat — Sprague Dawley, 12/sex, 3.5×10^7 CFU/animal *Pseudozyma flocculosa*	$LD_{50} > 3.5 \times 10^7$ CFU/animal	No effect on body weight but body weight gain significantly lower in TS-dosed ♂ rats despite increased food consumption by TS-dosed ♂ rats. No clinical symptoms or mortalities. White nodules noted on stomach, caecum, liver or small intestine of ♂ and ♀ rats dosed with TS and KTS attributed to normal immunological response to a foreign substance. Increased relative spleen weight in ♀ TS- and KTS-dosed rats also considered to be a normal response. Following injection, the test microbe was recovered from the caecum, kidneys, liver, lungs and associated lymph nodes, spleen, stomach, and small intestines of ♂ and ♀ TS-dosed rats. Clearance of the test organism occurred within 7 days of administration. **SLIGHTLY TOXIC AND NO PATHOGENICITY**
Dermal Toxicity and Irritation	Rabbit — New Zealand White, 5/sex 1.2×10^7 CFU/animal *Pseudozyma flocculosa* (equivalent to approximately 0.82–0.90 g/kg bw for ♂ and 0.80–0.91 g/kg bw for ♀)	$LD_{50} > 1.2 \times 10^7$ CFU/animal (♂ $LD_{50} > 0.82$–0.90 g/kg bw ♀ $LD_{50} > 0.80$–0.91 g/kg bw)	No mortalities. One ♂ rabbit lost weight within the first week but experienced a slight weight gain thereafter. All other animals gained weight. Slight diarrhea observed in one ♂ 7 days after administration. No other adverse clinical symptoms. No signs of dermal irritation. **LOW TOXICITY AND NON-IRRITATING.**
Eye Irritation	Rabbit — New Zealand White, 6 females, 0.1 g (equivalent to 5.7×10^7 CFU/animal) SPORODEX WP	MIS[4] = 1.7 / 110 at the one-hour scoring interval MAS[5] = 0.22	Slight conjunctival redness observed in 5/6 animals at the one-hour scoring interval. By the 24-hour scoring interval, only 2/6 animals continued to exhibit slight conjunctival redness. All signs of ocular irritation were absent at the 48-hour scoring interval. No other signs of ocular irritation or adverse clinical symptoms. No mortalities. SPORODEX WP formulation expected to be more irritating to the eye than SPORODEX L. **MINIMALLY IRRITATING.**

[1] CFU = Colony Forming Units
[2] TS = Test Substance
[3] KTS = Killed Test Substance
[4] MIS = Maximum Irritation Score
[5] MAS = Maximum Average Score (based on scores from 24-, 48- and 72-hour scoring intervals)

Table II Risk of *Pseudozyma flocculosa* strain PF-A22 UL to non-target organisms

Organism	Exposure	Test Substance	Conclusions
Birds	Oral/ Pulmonary / Inhalation / Injection	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the company was **ACCEPTED** based on a limited potential for risk.
Wild mammals	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the company was **ACCEPTED** based on a limited potential for risk.
Freshwater fish	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the company was **ACCEPTED** based on a limited potential for risk.
Arthropods	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the company was **ACCEPTED** based on a limited potential for risk.
Non-arthropod invertebrates	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the company was **ACCEPTED** based on a limited potential for risk.
Microorganisms	Acute	Waiver rationale submitted in lieu of data	Although non-target microorganisms may be at potential risk, the waiver rationale submitted by the company was **ACCEPTED** based on limited host range.
Plants	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the company was **ACCEPTED** based on a limited potential for risk.