Product Monograph

Pseudozyma flocculosa strain PF-A22 UL
(PC Code 119196)

Pseudozyma flocculosa strain PF-A22 UL (TGAI)
SPORODEX L (EP)

The active ingredient *Pseudozyma flocculosa* strain PF-A22 UL and associated end-use product SPORODEX L, for the control of powdery mildew on roses and cucumbers, are proposed for temporary registration under Section 17 of the Pest Control Products Regulations (Canada) and a conditional registration under Section 3(c)(7)(C) of the Federal Insecticide Fungicide and Rodenticide Act (United States).

This Product Monograph provides a summary of data reviewed and the rationale for the proposed Section 17 (Canada) and Section 3(c)(7)(C) (U.S.) registration of these products.

September 2002

Foreword

The submissions for the registration of the technical grade active ingredient, *Pseudozyma flocculosa* strain PF-A22 UL, and its end-use product, SPORODEX L, manufactured by Plant Products Co., have been jointly reviewed by Health Canada's Pest Management Regulatory Agency (PMRA) and the U.S. Environmental Protection Agency (EPA). Plant Products Co. was granted a temporary registration (Section 17) in Canada on June 3, 2002 for use of *P. flocculosa* strain PF-A22 UL, and its end-use product, SPORODEX L.

SPORODEX L is a biological fungicide, containing 1.3% (w/w) *P. flocculosa* strain PF-A22 UL, intended for the control of powdery mildew on greenhouse roses and cucumbers. The active microorganism, *Pseudozyma flocculosa*, is a naturally occurring fungus and is not currently registered in the U.S. or Canada.

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

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 (Canada) and a conditional registration pursuant to Section 3(c)(7)(C) of the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) on the condition that confirmatory data are submitted.

A summary of PMRA's and EPA's findings in support of this decision is found in this Monograph. A copy of PMRA's Sporodex Regulatory Note can be found on the PMRA internet site at the following address:
http://www.hc-sc.gc.ca/pmra-arla/english/pdf/reg/reg2002-02-e.pdf.

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Chapter 1 The active micro-organism, its properties and uses

1.1 Identity of the active micro-organism and preparation containing it

Table 1.1-1 TGAI Identification

Active Micro-organism	*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
Nominal purity of active	*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 min. 3×10^8 CFU/mL) in SPORODEX L (EP).
Identity of relevant impurities of toxicological, environmental and/or other significance	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 gram), *Staphylococcus aureus* (absence in 1 gram), coliforms (<10 CFU/mL), *Escherichia coli* (absence in 1 gram) and *Salmonella* (absence in 1 gram), 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).

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 and non-food crops, namely cucumber and roses. SPORODEX L is to be applied in an aqueous solution prepared by diluting 500 mL of product per 100 L of water (or 64 U.S. fl oz per 100 U.S. gallons 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 run-off at weekly intervals. Up to 1500 L of spray solution is to be applied per hectare (or 150 U.S. gallons of spray mixture per acre) for cut roses or cucumbers or about 1000 L/ha (or 100 U.S. gallons per acre) for potted roses. 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 USA) 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.

Chapter 2 Methods of analysis

2.1 Methods for analysis of the micro-organism as manufactured

2.1.1 Methods for identification of the micro-organism

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 PCR (RAMS). 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 methods for establishing the purity of the mother colonies were 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 gram), *Staphylococcus aureus* (absence in 1

gram), coliforms (<10 CFU/mL), *Escherichia coli* (absence in 1 gram) and *Salmonella* (absence in 1 gram). If any of the proposed bioburden limits are exceeded, the entire batch is rejected.

2.1.3 Methods to define the content of the micro-organism 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 a 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 Canadian Pest Management Regulatory Agency (PMRA) and the U. S. Environmental Protection Agency (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) are exceeded in the formulated product.

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

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 micro-organism 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 cucumbers, no adverse effects from dietary exposure have been attributed to natural populations of *P. flocculosa*. Given that there are no significant adverse effects reported in acute oral toxicity/pathogenicity study and that there are no reports in literature suggesting *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(s) to quantify *Pseudozyma flocculosa* strain PF-A22 UL residues in food and feed are 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*.

Chapter 3 Impact on human health and safety

P. flocculosa strain PF-A22 UL was considered of low toxicity and no pathogenicity based on the results of the Tier I toxicology studies. Tier II and Tier III studies were not required because the results from the Tier I studies were sufficient to satisfy guideline requirements. On the basis of the studies submitted, it was considered a Toxicity Category III pesticide for acute oral effects due to the amount dosed only, and Toxicity Category IV for dermal and primary dermal irritation health effects. These and additional toxicology studies are summarized below.

3.1 Toxicity and infectivity summaries (Tier I acute studies)

3.1.1 Acute oral toxicity / pathogenicity study (OPPTS 885.3050) (MRID#s 451152-04 453634-01)

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

In an acute oral toxicity study, groups of fasted 6-7 week old Fisher 344 rats (12/sex) were administered a single oral dose of SPORODEX WP in USP sterile water for injection at doses of 5.8×10^8 colony-forming units (CFU) per animal for males and 5.6×10^8 CFU per animal for females. An equal number of animals were dosed with heat-killed test substance and four animals/sex served as untreated controls. The animals were then observed for a period of up to 21 days with interim scheduled sacrifices. No effect on body weight gain and no apparent signs of treatment-related toxicity, infectivity or pathogenicity were observed in any of the treated animals during the study period. Clearance of the test organism occurred by, or prior to, post-treatment day 7. Based on the results of this study, SPORODEX L and its active ingredient, *P. flocculosa*, is not considered toxic or pathogenic to male or female Fisher 344 rats.

The test substance used in this study was a wettable powder formulation of SPORODEX. A change in the intended formulation of the end-use products from a wettable powder to a liquid formulation (SPORODEX L), however, triggered the need for a rationale for the test substance. The applicant requested a waiver from submitting a replacement acute oral study using the TGAI or the liquid formulation based on the fact that the new formulants found in SPORODEX L are of food grade quality and that the levels of other formulants have been significantly reduced. The toxicity of the liquid formulation is, therefore, expected to be less than that of the wettable powder formulation that was tested.

3.1.2 Acute pulmonary toxicity / pathogenicity study (OPPTS 885.3150) (MRID#s 451152-06, 453634-01)

The potential toxicity and pathogenicity of *P. flocculosa* was tested by observing the effects following a single intratracheal instillation of 3.2×10^7 CFU of the test organism (TS) to each of 12 male and 12 female CD rats. An equal number of animals were treated with heat-killed test substance (KTS) and four animals/sex served as untreated controls. Animals were observed for up to 14 days with interim scheduled sacrifices.

A total of 15 rats (3/8 male and 2/8 female TS-dosed rats and 6/8 male and 4/8 female KTS-dosed rats) died on days 2 and 3. Laboured respiration, rough hair coat, ocular discharge and nasal discharge were observed in both TS- and KTS-dosed rats. Hunched posture and lethargy were also observed in one female and one male TS-dosed rat, respectively. The presence or absence of clinical symptoms were not indicative of spontaneous deaths.

Due to the large number of spontaneous deaths and a number of missed data collections, data for evaluating effects on body weights, food consumption and relative organ weight were limited. At the end of the 14-day long study, administration of *P. flocculosa* did not have a statistically significant effect on body weight. Analyses of daily food consumption and relative organ weights were skewed as they were either not determined or did not include animals that died prior to their scheduled sacrifice dates.

At necropsy, liver lesions and lesions and enlargement of the lung and spleen were observed in both TS- and KTS-dosed rats. Confluent dark areas were also seen in the kidneys of a single male TS-dosed rat. These necropsy findings were considered consistent with the method of dosing and the body's normal immunological response to a foreign substance.

Pseudozyma flocculosa was detected in the lungs and lymph nodes and the stomach and small intestine of TS-dosed animals only. Counts in these tissues were below the limit of detection by day 7.

Based on this study, *P. flocculosa* is toxic, but not infective or pathogenic, at the dose administered when introduced by the intratracheal route to male and female CD rats. This acute pulmonary study, however, was originally classified as unacceptable due to major deficiencies in the collected toxicity data and a possible dosing error, as indicated by the presence of the MPCA in the stomach and small intestines on the day of dosing. However, there was relevant pathogenicity information that indicated clearance of the MCPA. Thus, this study is considered to be supplemental because it provides acceptable information regarding infectivity/pathogenicity; however, this study does not differentiate the cause of certain mortalities in the TS and KTS treatments. A confirmatory acute pulmonary toxicity / pathogenicity study using the TGAI and testing of the sterile filtrate from the production culture will therefore be required to provide this additional information as a condition of registration.

3.1.3 Acute pulmonary range-finding study (OPPTS 885.3150) (MRID#s 451152-07, 453634-01)

In order to determine whether the test substance (in both its viable and non-viable forms), *P. flocculosa*, was the cause of the deaths, a subsequent acute pulmonary range-finding toxicity study was conducted. In this range-finding study, groups of young adult CD rats (5/sex/dose level) were exposed by the intratracheal route to *P. flocculosa* (4.2×10^7 CFU/mL) in ASTM Type 1 water at doses of 4.2×10^7, 3.4×10^7, 6.8×10^6 and 3.4×10^6 CFU/animal. Animals were then observed for 14 days. There were no mortalities and all animals gained weight during the study. Rough hair coat occurred in a dose-dependent manner with all 5 animals/sex exhibiting this symptom at the highest dose of 4.2×10^7 CFU/animal. One female dosed with 4.2×10^7 CFU experienced tremors, closed eyes and rough hair coat. *Pseudozyma flocculosa* was classified as being of slight toxicity (EPA Toxicity Category IV) based on adverse effects observed in some test animals.

This acute pulmonary study was considered supplemental. According to USEPA OPPTS

885.3150, the minimum dose is 10^8 units of the MPCA per test animal. The maximum dose level used in this study, however, was only 4.2 x 10^7 CFU/animal. The maximum dose level used in this study, however, was only 4.2 x 10^7 CFU/animal. Furthermore, infectivity was not addressed; however, the acute pulmonary toxicity/pathogenicity study did address infectivity sufficiently. Consequently, this study does not satisfy the guideline requirement for an acute pulmonary study (OPPTS 885.3150) in the rat. EPA, in considering the two studies together, believes that there are sufficient data with which to determine the toxicity and pathogenicity of *Pseudozyma flocculosa*. As any potential inhalation risk that is raised by these studies is primarily a worker risk, EPA is requiring that a respirator be worn by workers to limit any inhalation exposures. In addition, a Restricted-Entry Interval (REI) of 4 hours is required for early entry (post-application) workers or other persons entering treated greenhouses. Finally, a confirmatory acute pulmonary toxicity / pathogenicity study using the TGAI and testing of the sterile filtrate from the production culture will be required as a condition of registration.

3.1.4 Intraperitoneal toxicity / infectivity study (OPPTS 885.3200) (MRID#s 451152-08, 453634-01)

In an acute intraperitoneal toxicity/infectivity study, groups of young adult CD rats (4/sex/scheduled sacrifice date) were exposed by the intraperitoneal route to an undiluted suspension of *P. flocculosa* (TS) at a dose of 3.5 x 10^7 CFU/animal (in 1.0 mL). Animals were then observed for up to 14 days. An equal number of young adult CD rats were similarly injected with heat-killed test substance (KTS). An undosed naive control (NC) group consisting of 4 rats/sex was also included in the study. Cage side observation for clinical symptoms was performed daily and animal body weights and food consumption were monitored.

No unscheduled deaths occurred. Designated animals from the TS and KTS groups were sacrificed on days 0, 7 and 14 and gross necropsies were performed. The NC group of animals was sacrificed and necropsied at the end of the 14 day study. Infectivity and clearance were assessed by quantitatively recovering the MPCA from the blood, lungs and lymph nodes, spleen, kidneys, liver, heart, stomach and small intestine, peritoneal fluid, caecum and brain.

No adverse clinical signs were observed at any point of the study in any of the groups of rats. Body weight gain of TS-dosed male rats was significantly decreased while this group's food consumption was significantly increased compared to NC animals. There was no significant difference between KTS-dosed and NC animals in terms of body weight, body weight gain or food consumption. Upon necropsy of TS- and KTS-dosed animals, white nodules and higher relative spleen weights were observed and attributed to a normal immune response to a foreign substance. The detection of *P. flocculosa* in the peritoneal fluid lavage of TS-dosed male rats was consistent with the method of administration. Clearance of *P. flocculosa* from all other tissues and fluids occurred by day 7. No test substance was detected from any of the organs of the KTS-dosed or NC animals.

At the dose administered, *P. flocculosa* was slightly toxic but not pathogenic to male and female CD rats when introduced by the intraperitoneal route.

3.1.5 Acute dermal toxicity / irritation study (OPPTS 885.3100) (MRID#s 451152-09, 453634-01)

In an acute dermal toxicity study, a single group of New Zealand White rabbits (5/sex) was dermally exposed to 1.2 x 10^7 CFU *P. flocculosa* (equivalent to approximately 0.82-0.90 g/kg bw for males and 0.80-0.91 g/kg bw for females), for 24 hours to an area equivalent to approximately 10% of the dorsal skin surface. Following exposure, the animals were observed for a period of 14 days.

No treatment-related signs of toxicity or skin irritation were observed in any animal during the 14-day observation period. At the dose administered, *P. flocculosa* was not considered toxic or irritating to the skin.

The recommended test substance for acute dermal toxicity and acute dermal irritation studies is the end-use product. Instead, the test substance was produced by the test facility using a method different from the proposed manufacturing method. An acceptable waiver rationale was submitted to address the toxicity and/or irritation potential of the formulation ingredients. The waiver rationale was based on the formulation ingredients being of food-grade quality or considered as relatively non-toxic.

3.1.6 Primary eye irritation study (OPPTS 870.2400) (MRID#s 451152-10, 453634-01)

Administration of 0.1 g of SPORODEX WP to the eyes of rabbits resulted in slight conjunctival redness in 5/6 animals at the 1-hour scoring interval and in 2/6 rabbits at the 24-hour scoring interval. By the 48-hour scoring interval, all signs of ocular irritation had subsided. There were no other adverse clinical symptoms or mortalities during the 7-day observation period. The maximum irritation score (MIS) was 1.7 at the 1-hour scoring interval and the maximum average score (MAS) was 0.22 over the 24-, 48- and 72-hour scoring intervals. Based on the MAS, SPORODEX WP was classified as minimally irritating.

The test substance used in this study was a wettable powder formulation containing a potential ocular irritant. The current formulation, SPORODEX L, contains a much lower level of the potential irritant. Therefore, SPORODEX L is expected to be less irritating to the eye than SPORODEX WP.

3.1.7 Subchronic, chronic toxicity and oncogenicity

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.

3.1.8 Effects on the immune and endocrine systems

EPA does not have any information regarding endocrine effects of this microbial pesticide at this time. There is no evidence to suggest that use of *P. flocculosa* strain PF A-22 UL at the proposed concentrations will adversely affect the endocrine or immune systems. 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 effects to the immune and endocrine systems are known or expected. Based on this rationale, the registrant waiver request for OPPTS 880.3800 (Immune Response) was found to be acceptable.

Table 3.1 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 (MRID#s 451152-04, 453634-01)	Rat - Fisher 344, 12/sex, % 5.8 x 10^8 CFU[1]/animal & 5.6 x 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 other organs at any time. No significant findings observed at necropsy. **LOW TOXICITY AND NO PATHOGENICITY.**

Pulmonary (MRID#s 451152-06 453634-01)	Rat - CD, 12/sex, 3.2 x 10^7 CFU/animal *Pseudozyma flocculosa*	LD_{50} > 3.2 × 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 **SUPPLMENTAL. This study is considered to be supplemental because it provides acceptable information regarding infectivity/pathogenicity; however, this study does not differentiate the cause of certain mortalities in the TS and KTS treatments. Confirmatory acute pulmonary toxicity/pathogenicity study is required using the TGAI and testing of the sterile filtrate from production batches.**
Pulmonary - Range Finding (MRID# 451152-07 453634-01)	Rat - CD, 5/sex/dose level 4.2 x 10^7 CFU/animal 3.4 x 10^7 CFU/animal 6.8 x 10^6 CFU/animal 3.4 x 10^6 CFU/animal *Pseudozyma flocculosa*	LD_{50} > 4.2 x 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 x 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. **Confirmatory acute pulmonary toxicity/pathogenicity study is required using the TGAI and testing of the sterile filtrate from production batches.**

Intraperitoneal Injection (MRID#s 451152-08 453634-01)	Rat - Sprague Dawley, 12/sex, 3.5 x 10^7 CFU/animal *Pseudozyma flocculosa*	LD_{50} > 3.5 × 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 and 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 (MRID#s 451152-09, 453634-01)	Rabbit - New Zealand White, 5/sex 1.2 x 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 × 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 (MRID#s 451152-10, 453634-01)	Rabbit - New Zealand White, 6 females, 0.1 g (equivalent to 5.7 x 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)

3.1.9 Integrated toxicity and infectivity summary

The registration package submitted by Plant Products Co. in support of registering the technical grade active ingredient (TGAI) *Pseudozyma flocculosa* strain PF-A22 UL and the end-use product (EP) 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 signs of toxicity or pathogenicity were 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 TS- and KTS-dosed animals. Presence of the test organism in the stomach and small intestines indicated a potential 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 complete acute pulmonary toxicity / infectivity study, using the TGAI, 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.

P. 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 of food-grade quality or classified as 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 toxins. 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.

3.2 Hypersensitivity (derminal sensitization study OPPTS 870.2600 and reports of incidents OPPTS 885.3400)

The applicant has also submitted an acceptable 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.

Skin sensitizing studies are not considered substitutes for timely reports of hypersensitivity incidents subsequent to registration approval. No adverse effects have been noted among researchers who have worked closely with *P. flocculosa* strain PF-A22 UL for up to 10 years. 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

When handled according to the label instructions, the pulmonary, dermal and ocular routes are potential routes of applicator and bystander exposure. Occupational exposure is of particular concern as the product will be used in an enclosed environment.

U.S. EPA and Canada/PMRA, however, do not expect that occupational exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile. While submitted acute pulmonary toxicity/infectivity studies were found to be lacking, inhalation exposure is not of concern if the required respirator is worn by workers. To mitigate dermal and inhalation exposure and risk to workers, use of appropriate Personal Protective Equipment (PPE) will be required. Furthermore a Restricted-Entry Interval (REI) of 4 hours is required for early entry (post-application) workers or other persons entering treated greenhouses.

Assuming that most microorganisms contain substances that would elicit positive hypersensitivity reactions, *P. flocculosa* strain PF-A22 UL is considered a potential sensitizing agent, and a "POTENTIAL SENSITIZER" statement will be required on the principal display panel of the TGAI and end-use formulation labels.

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.

Chapter 4 Residues

In examining aggregate exposure, FFDCA section 408 directs U.S.EPA to consider available information concerning exposures from the pesticide residue in food and all other non-occupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

Based on the data and analyses outlined above, U.S. EPA has concluded that there is a reasonable certainty that no harm will result from aggregate exposure to the U.S. population, including infants and children, to residues of *P. flocculosa* strain PF-A22 UL arising from use on greenhouse-grown cucumbers and roses. This includes all anticipated dietary exposures and all other exposures for which there is reliable information.

4.1 Residues relevant to consumer safety

4.1.1 Dietary exposure and risk assessment

The proposed food use pattern is likely to result in residues in or on food and feed. 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, however, EPA believes that dietary exposure to the microbial agent will result in negligible to no risk to consumers. 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 effects from dietary exposure have been attributed to natural populations of *Pseudozyma flocculosa*. Furthermore, no adverse effects were observed at maximum hazard dose levels in the acute oral toxicity / pathogenicity study and there are no reports of known mammalian toxins being produced by the MPCA. Subchronic and chronic dietary exposure studies were not required because 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 potential exposure of the MPCA expected for the proposed uses, there is no concern for chronic risks posed by dietary exposure for the general population or sensitive subpopulations, such as infants and children. In addition, an extensive literature search yielded no reports of mammalian toxins being produced by *P. flocculosa* (see section 3.1.9). 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. Therefore, EPA expects negligible to no dietary risk from exposure to naturally-occurring and isolated *P. flocculosa* strain PF-A22 UL residues.

4.1.2 Drinking water exposure and risk assessment

Although heavy rainfall likely carries *P. flocculosa* into neighboring aquatic environments, growth and survival of terrestrial fungi such as *P. flocculosa* is 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 because of minimal to non-existent toxicity. 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 to humans via drinking water is likely to be minimal to non-existent for this MPCA.

4.1.3 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 effects from dietary exposure have been attributed to natural populations of *Pseudozyma flocculosa*. Furthermore, no adverse effects were observed in the acute oral toxicity / pathogenicity study and there are no reports of known mammalian toxins being produced by the MPCA. Therefore, the establishment of a tolerance or maximum allowable residue limit is not required for *P. flocculosa* strain PF-A22 UL under Section 408 of the Federal Food Drug and Cosmetic Act.

4.2 Aggregate exposure from multiple routes including oral, dermal, and inhalation

The current label does not allow applications to turf, residential or recreational areas. Because the use sites are in greenhouses, exposure to the U.S. population including infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk posed by *P. flocculosa* strain PF A-22 UL from non-occupational dermal and inhalation exposures to the general public, including infants and children, is expected to be negligible to non-existent. Any concerns for potential inhalation risk is for occupational exposures, and as mentioned previously, will be mitigated by the requirement of a respirator and restriction of the reentry interval.

4.2.1 Oral

Oral exposure would occur primarily from eating treated foods and from drinking water. Residues of the active microorganism can be easily removed from treated commodities by washing, cooking, peeling and processing. Transfer of the active microogranism to drinking water is not likely as discussed previously. Thus dietary exposure and risk are likely to be minimal to non-existent.

4.2.2 Dermal

Non-occupational dermal exposure and risk to adults, infants and children are not likely if the pesticide is used as labeled. The label does not allow applications to turf, residential or recreational areas. The only use sites are for greenhouse-grown cucumbers and roses.

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. *P. 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 PMRA's and EPA'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 ameliorated if they wear long-sleeved shirts, long pants, shoes and socks.

4.2.3 Inhalation

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 the NIOSH prefix N-95, R-95, P-95 or HE filter for biological products.

4.3 Cumulative effects

The Agency has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. EPA is not aware of any other bacteria or other substances, besides naturally-occurring strains of *Pseudozyma*, that share a common mechanism of toxicity with this active ingredient. Given the low toxicity and pathogenicity profile of *P. flocculosa*, even if there were any other substances with which *P. flocculosa* shared a common mechanism of toxicity, no adverse cumulative effects are expected.

4.4 Determination of safety for U.S. population, infants and children

Based on the toxicology data submitted and other relevant information in the Agency's files, there is reasonable certainty no harm will result from aggregate exposure of residues of *Pseudozyma flocculosa* strain PF-A22 UL to the U.S. population, including infants and children, under reasonably foreseeable circumstances when the microbial pesticide product is

used as labeled. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion based on data submitted demonstrating low toxicity at the maximum doses tested and a lack of information showing adverse effects from exposure to naturally occurring *P. flocculosa* as well as a consideration of the product as currently registered and labeled. As a result, EPA establishes an exemption from tolerance requirements pursuant to FFDCA 408(c) and (d) for residues of *Pseudozyma flocculosa* strain PF-A22 UL in or on all food commodities.

FFDCA section 408 provides that EPA shall apply an additional tenfold margin of exposure (safety) for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of the data base unless EPA determines that a different margin of exposure (safety) will be safe for infants and children. Margins of exposure (safety) are often referred to as uncertainty (safety) factors. In this instance, based on all the available information, the Agency concludes that *P. flocculosa* strain PF-A22 UL is practically non-toxic to mammals, including infants and children. Thus, there are no threshold effects of concern and, as a result the provision requiring an additional margin of safety does not apply. Further, the provisions of consumption patterns, special susceptibility, and cumulative effects do not apply. As a result, EPA has not used a margin of exposure (safety) approach to assess the safety of *P. flocculosa* strain PF-A22 UL.

Chapter 5 Fate and behavior 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-A22UL. Environmental fate data waiver requestsby Plant Products Co. Ltd. were found to be acceptable (MRID# 451152-11).

Chapter 6 Effects on non-target species

6.1 Birds

6.1.1 Avian oral

6.1.2 Avian pulmonary/inhalation/injection (OPPTS 885.4050 and OPPTS 885.4100, MRID# 451152-12)

Plant Products Co. Ltd. submitted an acceptable justification for a data waiver from avian oral toxicity studies and avian pulmonary/inhalation/injection studies. The waiver request was based on the rationale that *P. flocculosa* is a naturally-occurring soil microorganism whose level in the environment will not significantly increase with greenhouse use of SPORODEX L and that an extensive literature search yielded no reports of adverse effects in birds.

Use of SPORODEX L in commercial greenhouses is not expected to result in increased

exposure or adverse effects in birds. Birds will not be directly exposed to the product at the time of application. Greenhouse practices designed to limit exposure to the outside environment will limit post-application exposure to birds. Furthermore, while the body temperature of duck and quail species is approximately 40°C, *Pseudozyma* species do not grow at temperatures beyond 37°C. Consequently, testing is considered unnecessary to assess the risks of SPORODEX L to avian wildlife.

6.2 Wild mammals (OPPTS 885.4150, MRID# 451152-12)

Plant Products Co. Ltd. submitted an acceptable justification for a data waiver from wild mammal studies. The waiver request was based on the rationale that *P. flocculosa* is a naturally-occurring soil microorganism whose level in the environment will not significantly increase with greenhouse use of SPORODEX L and that an extensive literature search and the studies submitted to address human health issues yielded no reports or evidence of significant adverse effects in wild mammals.

Use of SPORODEX L in commercial greenhouses are not expected to result in increased exposure or adverse effects in wild mammals. Wild mammals will not be directly exposed to the product at the time of application. Greenhouse practices designed to limit exposure to the outside environment will limit post-application exposure to wild mammals. Consequently, testing is considered unnecessary to assess the risks of SPORODEX L to wild mammals.

6.3 Fish

6.3.1 Freshwater fish and Estuarine/Marine animals (OPPTS 885.4200 and OPPTS 885.4280, MRID# 451152-12)

Plant Products Co. Ltd. submitted an acceptable justification for a data waiver from freshwater fish and estuarine/marine aniimal studies. The waiver request was based on the rationale that *P. flocculosa* is a naturally-occurring microorganism whose level in the environment will not significantly increase with greenhouse use of SPORODEX L and that an extensive literature search yielded no reports of adverse effects in freshwater fish and estuarine/marine animals.

Use of SPORODEX L in commercial greenhouses is not expected to result in increased exposure or adverse effects in freshwater fish and estuarine/marine animals. Consequently, testing is considered unnecessary to assess the risks of SPORODEX L to freshwater fish and estuarine/marine animals.

6.4 Arthropods

6.4.1 Terrestrial arthropods (OPPTS 885.4340 and 885.4380, MRID# 451152-12)

Plant Products Co. Ltd. submitted an acceptable justification for a data waiver from terrestrial arthropod and honey bee testing. The waiver request was based on the rationale that *P. flocculosa* is a naturally-occurring microorganism whose level in the environment will not significantly increase with greenhouse use of SPORODEX L and that an extensive literature search yielded no reports of adverse effects in terrestrial arthropods.

Terrestrial arthropods, outside of greenhouse facilities using SPORODEX L, will not be directly exposed to the product at the time of application. Greenhouse practices designed to limit exposure to the outside environment will limit post-application exposure to terrestrial arthropods. Use of SPORODEX L in commercial greenhouses is not expected to result in increased exposure or adverse effects in terrestrial arthropods and honeybees. Consequently, testing is considered unnecessary to assess the risks of SPORODEX L to terrestrial arthropods and honey bees.

6.4.2 Aquatic arthropods (OPPTS 885.4240, MRID# 451152.12)

Plant Products Co. Ltd. submitted an acceptable justification for a data waiver from aquatic arthropod studies. The waiver request was based on the rationale that *P. flocculosa* is a naturally-occurring microorganism whose level in the environment will not significantly increase with greenhouse use of SPORODEX L and that an extensive literature search yielded no reports of adverse effects in aquatic arthropods.

Use of SPORODEX L in commercial greenhouses is not expected to result in increased exposure or adverse effects in aquatic arthropods. Consequently, testing is considered unnecessary to assess the risks of SPORODEX L to aquatic arthropods.

6.5 Non-arthropod invertebrates (OPPTS 885.4240 and 885.4340, MRID# 451152-12)

Plant Products Co. Ltd. submitted an acceptable justification for a data waiver from non-arthropod invertebrate studies. The waiver request was based on the rationale that *P. flocculosa* is a naturally-occurring microorganism whose level in the environment will not significantly increase with greenhouse use of SPORODEX L and that an extensive literature search yielded no reports of adverse effects in non-arthropod invertebrates.

Greenhouse practices designed to limit exposure to the outside environment will limit post-application exposure to non-arthropod invertebrates. Use of SPORODEX L in commercial greenhouses is not expected to result in increased exposure or adverse effects in non-arthropod invertebrates. Consequently, testing is considered unnecessary to assess the risks of SPORODEX L to non-arthropod invertebrates.

6.6 Microorganisms (OPPTS 885.1100, MRID# 451152-12)

In place of submitting microorganism studies, Plant Products Co. Ltd. submitted a summary of the host range and mode of action for *P. flocculosa*. This information has been reviewed for Part M2, *Product Characterization and Analysis*. The applicant has also based a waiver rationale on the natural occurrence and limited additional exposure which will be expected due to the proposed uses of SPORODEX L.

A potential exists, particularly in a greenhouse environment where conditions are optimal for growth (e.g., high relative humidity, controlled temperatures), for *P. flocculosa* to adversely affect non-target microorganisms. Based on the natural occurrence, mode of action and limited host range of *P. flocculosa*, however, non-target microorganism testing with SPORODEX L will not be required to assess the magnitude of this impact.

6.7 Plants (OPPTS 885.4300, MRID# 451152-12)

6.7.1 Aquatic plants

Plant Products Co. Ltd. submitted an acceptable justification for a data waiver from aquatic plant testing. The waiver request was based on observations of no adverse effects in greenhouse trials on target crops, the natural occurrence of *P. flocculosa*, the proposed use pattern and an extensive literature search which yielded no reports of adverse effects in aquatic plants.

Use of SPORODEX L in commercial greenhouses is not expected to result in increased exposure or adverse effects in aquatic plants. Adequate containment measures, aimed at minimizing exposure to the outside environment, are in place. Consequently, testing is considered unnecessary to assess the risks of SPORODEX L to aquatic plants.

6.7.2 Terrestrial plants

Plant Products Co. Ltd. submitted an acceptable justification for a data waiver from terrestrial plant studies. The waiver request was based on observations of no adverse effects in greenhouse trials, the natural occurrence of *P. flocculosa*, the proposed use pattern and an extensive literature search which yielded no reports of adverse effects in terrestrial plants.

Despite the common occurrence of *P. flocculosa*, no incidents of adverse effects on terrestrial plants have been noted. The strain of *P. flocculosa* used in SPORODEX L was, in fact, isolated from the leaves of red clover grown in Harrow, Ontario. Furthermore, visual inspections of cucumber, rose and tomato plants, treated with *P. flocculosa* for numerous efficacy trials, yielded no signs of phytotoxicity.

SPORODEX L will be used in commercial greenhouses only. Non-target terrestrial plants will not be directly exposed to the product at the time of application. Greenhouse practices designed to limit exposure to the outside environment will limit post-application exposure to non-target terrestrial plants. Use of SPORODEX L in commercial greenhouses is not expected to result in increased exposure or adverse effects in terrestrial plants. Consequently, testing is considered

unnecessary to assess the risks of SPORODEX L to non-target terrestrial plants.

Table 6.1 Risks 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.

6.8 Integrated environmental toxicology summary

Acceptable waiver rationales were submitted to address environmental toxicology requirements. These waivers were based on minimal increased exposure of non-target organisms resulting from greenhouse use of SPORODEX L. No reports of adverse effects on birds, wild mammals, freshwater fish, aquatic and terrestrial arthropods, non-arthropod invertebrates, and aquatic and terrestrial plants organisms have 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. Rapid death and collapse of susceptible host cells occurs 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 effects 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-use product 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.

Chapter 7 Efficacy data and information

A summary of the efficacy data and information are provided below.

7.1 Effectiveness

7.1.1 Intended use

For control of powdery mildew in greenhouse crops: cut roses or cucumbers or potted roses. Mix 500 mL of SPORODEX L for each 100 L water (or 64 U.S. fl oz per 100 U.S. gallons of water) (equivalent to approximately 10^5 to 10^6 CFU/mL). Add a wetting agent at 0.02%.

Apply up to 1500 L of water per ha (or 150 U.S. gallons of spray mixture per acre) for cut roses or cucumbers and 1000 L per ha for potted roses (or 100 U.S. gallons per acre) . 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 less 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 x 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. Optimum conditions for infection of host fungi are 26^{o} C and > 70% RH. *Pseudozyma* will colonise 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 cucumber 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 (% diseased leaf area on whole plants) was assessed at intervals and an area under disease progress curve (AUDPC) 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 L formulation showed that it is as effective as myclobutanil against powdery mildew on cucumber. In this trial, an application of pine oil (fertiliser) in midseason adversely affected SPORODEX L which resulted in lack of disease control on lower leaves and showed that this product is not compatible. Results are available from two additional trials in the Netherlands and B.C., which 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 were 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 x 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

Mix 500 mL of SPORODEX L for each 100 L water (or 64 U.S. fl oz per 100 U.S. gallons of water) (equivalent to approximately 10^5 to 10^6 CFU/mL). Add a wetting agent at 0.02%.

Apply up to 1500 L of water per ha (or 150 U.S. gallons of spray mixture per acre) for cut roses or cucumbers and 1000 L per ha for potted roses (or 100 U.S. gallons per acre) . 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.

7.2 Phytotoxicity to target plants (including different cultivars), or to target plant products (OECD 7.4)

No adverse effects of SPORODEX formulations were noted in greenhouse trials with cucumber or rose. The additive paraffin oil (1%) used with SPORODEX was noted to cause a slight oedema (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 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 $100-200 per ha annually. There are about 24 ha of greenhouse roses in Canada, mostly in Ontario, with some operations in BC, 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 price of roses as there is zero tolerance for the presence of white mildew spots on the leaves and blooms and 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 $6,000 per ha. Fungicides are currently used to

control mildew but can have adverse effects on yield, fruit and flower quality.

7.5 Sustainability

7.5.1 Survey of alternatives

Powdery mildew is currently managed by environment control, tolerant cultivars, sanitation and 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, ie.shading, overcrowding, overwatering or overfertilizing. The fungal spores will not survive long outside of 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; however, 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 dependant 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. Benomyl, myclobutanil, and sulfur are registered in Canada for cucumber and dodemorph-acetate and copper are registered for roses. The most effective products are systemic, must be applied frequently, may be toxic to beneficials 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. Benomyl is registered but not expected to be marketed beyond 2001. Silicon has been investigated as a disease preventative, 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	
Benomyl	Benlate 50WP (+ Manzate)	tubulin (multisite)	550-850 g 2.25 kg		No longer marketed prone to resistance
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 these 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 crop 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 aleviate or defer the need for chemical fungicide applications thus reducing the associated risks of pesticide resistance, effects to workers and to 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 colonise leaves in the absence of powdery mildew but undergoes rapid reproduction only when the disease is present. For these reasons, it is not anticipated that resistance to *P. flocculosa* will develop; however, as a general principal, it is best not to rely continuously on any one product for disease control.

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 more site-specific fungicides.

7.6 Conclusions

7.6.1 Summary

SPORODEX L is a liquid formulation containing *Pseudozyma flocculosa* at 3 x 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 (or 64 U.S. fl oz per 100 U.S. gallons of water), applied to runoff (1500 L/ha for cut roses and cucumbers (or 150 U.S. gallons of spray mixture per acre) and 1000 L/ha for potted roses (or 100 U.S. gallons per acre)).

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 Columbian greenhouses. 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 were 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	as proposed
	Roses (potted or cut)	as proposed
Rate	500 mL /100L of water (or 64 U.S. fl oz per 100 U.S. gallons of water) with 20 mL wetting agent (3 U.S. fl oz). Use up to 1500 L spray per ha for cut roses, cucumbers (or 150 U.S. gallons of spray mixture per acre) , up to 1000 L for potted roses (or 100 U.S. gallons per acre)	as proposed
Application method	Diluted spray applied to foliage to runoff	as proposed
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

Chapter 8 Overall risk assessment conclusions

8.1 Product characterization and analysis

The product characterization data for both *Pseudozyma flocculosa* strain PF-A22 UL and SPORODEX L are adequate to assess their safety to human health. The technical material was fully characterized and the specifications were supported by the analysis of a sufficient number of batches. Quality control procedures employed during product manufacture and formulation are adequate to ensure an absence of contaminating microorganisms of concern including primary human and animal pathogens. However, due to the microbial contamination noted in the representative quality control data, the submission of certificates of analysis will be required for all production batches of SPORODEX L as a condition of registration by the PMRA and the

EPA.

Additional storage stability data on pilot-scale or production-scale batches are required to ensure product performance and safety. Until these data are available, an expiration date of 3 months when the product is stored at -20°C is required on the product labels.

8.2 Toxicity and infectivity

The acute toxicity and infectivity studies submitted in support of *P. flocculosa* and of SPORODEX L were determined to be sufficiently complete to permit a decision on registration. *Pseudozyma flocculosa* was of low toxicity in the rat when administered via the oral and dermal routes. Slight toxicity was observed when *P. flocculosa* was administered via the pulmonary and intraperitoneal routes. *Pseudozyma flocculosa* was not pathogenic or infective via the oral and intraperitoneal routes. Pathogenicity via the pulmonary route could not be determined. No dermal irritation was observed but mild ocular irritation was noted. Waiver requests were submitted to address the use of alternative test substances (e.g., SPORODEX WP, MPCA produced by the test facility) in the acute toxicity and infectivity studies. These waivers were based on the nature of the formulation ingredients in SPORODEX L and the reduced irritation potential of a liquid formulation compared to that of a wettable powder formulation.

8.3 Exposure

The potential for dermal, eye and inhalation exposure for pesticide handlers exists, with the major source of exposure to workers being generally dermal. To mitigate dermal and inhalation exposure and risk to workers during mixing/loading, application and post-application activities, use of appropriate Personal Protective Equipment (PPE) will be required. PPE will include long-sleeved shirts, long pants, waterproof gloves, dust/filter respirator, shoes and socks. Furthermore a Restricted-Entry Interval (REI) of 4 hours is required for early entry (post-application) workers or other persons entering treated greenhouses.

It is assumed that most microorganisms contain substances that would elicit positive hypersensitivity reactions. *Pseudozyma flocculosa* strain PF-A22 UL is considered a potential sensitizing agent, and a "POTENTIAL SENSITIZER" statement will be required on the principal display panel of the TGAI and end-use formulation labels.

8.4 Food and feed residues

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 effects from dietary exposure have been attributed to natural populations of *Pseudozyma flocculosa*. Residues of the active micoorganism can be easily removed from treated commodities by washing, cooking, peeling and processsing. 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. Furthermore, an extensive literature search yielded no reports of mammalian toxins being

produced by *P. flocculosa*. Based on the low level of toxicity and lack of pathogenic potential for the active microorganism observed in the Tier I acute oral study, there are no acute, subchronic or chronic toxicological concerns for *P. flocculosa*. Therefore, the establishment of a a tolerance for residues of *P. flocculosa* strain PF-A22 UL is not required (rather an exemption from the requirement of a tolerance will be granted) as defined under Section 408 of the Federal Food Drug and Cosmetic Act.

8.5 Environmental assessment

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 reports of adverse effects on these non-target organisms have 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. Rapid death and collapse of susceptible host cells occurs 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 effects 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.

8.6 Efficacy assessment

SPORODEX L applied at proposed label rates can be effective in controlling powdery mildew on greenhouse cucumber and roses provided that high humidity is maintained. Further work is needed on managing the greenhouse environment for full disease control benefits of SPORODEX L to be realized. 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.

Chapter 9 Risk Management Considerations

9.1 Public interest finding

The Agency believes the use of *Pseudozyma flocculosa* strain PF-A22 UL under this conditional registration would be in the public interest. The criteria for Agency evaluation of public interest findings is outlined in 51 CFR No. 43, Wednesday March 5, 1986. Under part IV.A., the proposed product may qualify for an automatic presumptive finding if it is for minor use, is a unique replacement for pesticides of concern, or is for use against a public health pest.

Pseudozyma flocculosa strain PF-A22 UL is intended for formulation into end-use products for control of powdery mildew on greenhouse-grown cucumbers and roses. These uses are for minor use crops and, therefore, the product qualifies for an automatic presumptive finding and its use is presumed to be in the public interest.

9.2 Determination of 3(c)(7)(C) eligibility

Pursuant to FIFRA section 3(c)(7)(C), EPA may conditionally register a new pesticide active ingredient if: 1) insufficient time has elapsed since the imposition of the data requirement for those data to be developed and all other required data have been submitted, 2) the use of the pesticide product during the period of the conditional registration will not cause any unreasonable adverse effect on human health and the environment, and 3) the registration and the use of the pesticide during the conditional registration is in the public interest. The Agency believes that all these criteria have been fulfilled.

For *Pseudozyma flocculosa* strain PF-A22 UL and the end-product, SPORODEX L, the first criterion under FIFRA section 3(c)(7)(C) mentioned above has been met. Insufficient time has elapsed since the imposition of the following outstanding confirmatory data requirement: Acute Pulmonary Toxicity/Pathogenicity. Agency scientists have reviewed the data submitted or cited by Plant Products Co. Ltd. with respect to health effects and ecological effects and have identified no unreasonable adverse effects to human health or to non-target organisms. However, to confirm these expected results, the additional data described below will be required as a condition of registration.

> A complete acute pulmonary toxicity / pathogenicity study (U.S. EPA OPPTS 885.3150) must be conducted using the technical grade active ingredient (TGAI) (*Pseudozyma flocculosa* strain PF-A22 UL) and the sterile filtrate of the production culture.

The two acute pulmonary studies that were submitted did not have fully acceptable toxicity and pathogenicity results contained in a single study. Both of these studies were considered supplemental. However, taken together, parts of each study were acceptable for making a regulatory decision. That is, the acute pulmonary toxicity/pathogenicity study had acceptable pathogenicity data, but not toxicity data and the acute pulmonary toxicity/pathogenicity range

finding study had acceptable toxicity data, but did not address pathogenicity. In addition, the Agency decided that it would be prudent to test the sterile filtrate of the production batch to determine whether there were any toxic components of concern. Testing the sterile filtrate would not have been foreseeable by the registrant and a period reasonable sufficient for generation of the data has not elapsed. Thus, a confirmatory acute pulmonary toxicity / pathogenicity study using the TGAI and testing of the sterile filtrate from the production culture will be required to provide this additional information as a condition of registration. This study must be submitted to the EPA no later than October 3, 2003.

All other required data for registration have been submitted. The Agency is also imposing a continuing monitoring requirement on the registrant as a term of registration; this requirement is not a 3(c)(7)(C) condition and is not connected to the two year term of the conditional registration. In addition, the registrant may submit further storage stability studies to support a change in the labeling to increase the labeled shelf life beyond three months. The submitted storage stability study, however, is sufficient to support the current 3 month label language.

The applicant has submitted or cited data to allow EPA to make the finding necessary to satisfy the second criterion for conditional registration under FIFRA 3(c)(7)(C) as mentioned above. Plant Products Co. Ltd. submitted and/or cited satisfactory data pertaining to the proposed use. The human health effects data and non-target organism effects data are considered sufficient for the period of the conditional registration (2 years). These data demonstrate that no foreseeable human health hazards or ecological effects are likely to arise from the use of the product and, under the terms and conditions of the registration. As any potential inhalation risk that is raised by the supplementary acute pulmonary toxicity/pathogenicity data is primarily a worker risk, EPA is requiring that a respirator be worn by workers to limit any inhalation exposures. In addition, a Restricted-Entry Interval (REI) of 4 hours is required for early entry (post-application) workers or other persons entering treated greenhouses. Accordingly, the Agency has concluded that use of the pesticide product during the period of the conditional registration will not cause any unreasonable adverse effect on human health and the environment

The Agency also believes that the third criterion for a FIFRA 3(c)(7)(C) conditional registration has been fulfilled because the use of *Pseudozyma flocculosa* strain PF-A22 UL under this registration would be in the public interest. The criteria for Agency evaluation of public interest findings is outlined in 51 FR 7628 (Mar. 5, 1986). The proposed product qualifies for an automatic presumptive finding because all intended uses are for minor use crops, i.e., greenhouse-grown cucumbers and roses. In addition, the Agency is not aware of any other information which would alter EPA's presumption that use of this pesticide during the period of conditional registration would be in the public interest.

Therefore, *Pseudozyma flocculosa* strain PF-A22 UL is eligible for a FIFRA 3(c)(7)(C) conditional registration. The proposed registration sites are for greenhouse-grown cucumbers and roses.

9.3 Terms and conditions of registration

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 (Canada/PMRA) and a conditional registration under Section 3(c)(7)(C) of the Federal Insecticide Fungicide and Rodenticide Act (U.S. EPA).

This FIFRA 3(c)(7)(C) conditional registration will automatically expire at midnight, September 30, 2004.

The following list gives the terms and conditions of the registration.

1) A complete acute pulmonary toxicity / pathogenicity study (U.S. EPA OPPTS 885.3150) must be conducted using the technical grade active ingredient (TGAI) (*Pseudozyma flocculosa* strain PF-A22 UL) and the sterile filtrate of the production culture. This study must be submitted to the EPA no later than October 3, 2003.

2) Certificates of analysis must be submitted to the Agency prior to the release of all production batches of SPORODEX L biological fungicide (U.S. EPA OPPTS 885-1100 through 885-1600). Bioassay results, conidial, total aerobic flora, enterobacteria, enterococci, fecal coliform, *E. coli*, *Staphylococci* and *Salmonella* counts must be determined for each production batch and be included in each certificate of analysis. Certificates of analysis must be submitted until sufficient consistency with regards to microbial contaminants has been established.

3) Additional storage stability data (OPPTS 830.6317) derived from at least five production-scale or pilot-scale batches are required to ensure product performance and safety during the shelf-life of the product. SPORODEX L Biological Fungicide should be tested over a period of time and in accordance with the desired storage and use conditions appearing on the product label. An interim expiration date of 3 months from the date of manufacture when SPORODEX L Biological Fungicide is stored at -20°C is required until additional data are submitted and approved by the Agency. For consideration of these data prior to the expiration date of the conditional registration, additional data should be submitted to the Agency no later than October 3, 2003.

4) Finally, although a skin sensitization study on the microbial active ingredient *Pseudozyma flocculosa* strain PF-A22 UL was not required by the Agency, the reporting of any incidents of hypersensitivity subsequent to registration is a standard practice for microbial products. The registrant will be expected to report any subsequent findings of hypersensitivity or other health incidents to workers, applicators, or bystanders exposed to the MPCA (microbial pest control agent) as an ongoing condition of registration. Incident reports under FIFRA section 6(a)2 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.

9.4 Tolerance

EPA has established an exemption from the requirement of a tolerance for residues of the *Pseudozyma flocculosa* strain PF-A22 UL in or on all food commodities. Based on the

toxicology data submitted and other relevant information in the Agency's files, there is reasonable certainty no harm will result from aggregate exposure of residues of *Pseudozyma flocculosa* strain PF-A22 UL to the U.S. population, including infants and children, under reasonably foreseeable circumstances when the microbial pesticide product is used as labeled. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion based on data submitted demonstrating low toxicity at the maximum doses tested and a lack of information showing adverse effects from exposure to naturally occurring *P. flocculosa* as well as a consideration of the product as currently registered and labeled. As a result, EPA establishes an exemption from tolerance requirements pursuant to FFDCA 408(c) and (d) for residues of *Pseudozyma flocculosa* strain PF-A22 UL in or on all food commodities.

FFDCA section 408 provides that EPA shall apply an additional tenfold margin of exposure (safety) for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of the data base unless EPA determines that a different margin of exposure (safety) will be safe for infants and children. Margins of exposure (safety) are often referred to as uncertainty (safety) factors. In this instance, based on all the available information, the Agency concludes that *P. flocculosa* strain PF-A22 UL is practically non-toxic to mammals, including infants and children. Thus, there are no threshold effects of concern and, as a result the provision requiring an additional margin of safety does not apply. Further, the provisions of consumption patterns, special susceptibility, and cumulative effects do not apply. As a result, EPA has not used a margin of exposure (safety) approach to assess the safety of *P. flocculosa* strain PF-A22 UL.

9.5 Codex harmonization

There are no Codex harmonization considerations since there are no Codex Maximum Residue Limits set for food use of this active ingredient.

9.6 Risk mitigation

There is minimal or negligible potential risk to wildlife, or ground and surface water contamination for products containing this active ingredient. Dietary risk will be adequately mitigated by washing, peeling, cooking and processing of treated foods. To mitigate dermal and inhalation exposure and risk to workers during mixing/loading, application and post-application activities, use of appropriate Personal Protective Equipment (PPE) will be required. PPE will include long-sleeved shirts, long pants, waterproof gloves, dust/filter respirator, shoes and socks. Furthermore a Restricted-Entry Interval (REI) of 4 hours is required for early entry (post-application) workers or other persons entering treated greenhouses.

It is assumed that most microorganisms contain substances that would elicit positive hypersensitivity reactions. *Pseudozyma flocculosa* strain PF-A22 UL is considered a potential sensitizing agent, and a "POTENTIAL SENSITIZER" statement will be required on the principal display panel of the TGAI and end-use formulation labels.

No special precautionary or environmental hazard statements are required on the label for SPORODEX L. There are no reports of adverse effects due to the use of *P. flocculosa*. Exposure to *P. flocculosa* due to use of SPORODEX L in commercial greenhouses will be limited. Studies to assess the effect of *P. flocculosa* on these organisms are not 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.

9.7 Endangered species

The Agency has no evidence to believe that any endangered of threatened species will be adversely affected if products containing *Pseudozyma flocculosa* strain PF-A22 UL are used as labeled (i.e., greenhouse-grown cucumbers and roses). Therefore, the Agency has determined that this action will have "no effect"on listed species. No specific labeling is required.

9.8 Labels and labeling

It is Canada/PMRA's and U.S. EPA's position that the labeling for products containing *Pseudozyma flocculosa* strain PL-A22 UL must comply with the current pesticide labeling requirements. SPORODEX® L is manufactured following a continuous manufacturing process that does not involve an intermediate stand-alone technical product.

9.8.1 End-use product (SPORODEX® L)

A joint U.S./Canada NAFTA label containing the necessary regulatory language for SPORODEX® L is provided below. This label was approved by Canada/PMRA, June 3, 2002.

DRAFT U.S./CANADA LABEL

(Front Panel)

SPORODEX L BIOLOGICAL FUNGICIDE
For Control of Powdery Mildew on Greenhouse Roses and Cucumbers.

COMMERCIAL

READ THE LABEL BEFORE USING

POTENTIAL SENSITIZER

ACTIVE INGREDIENT/GUARANTEE: *Pseudozyma flocculosa* strain PF-A22 UL . . 1.3%
OTHER INGREDIENTS: 98.7%
TOTAL: 100.0%
(Contains a minimum of 3×10^8 colony forming units/mL)

KEEP OUT OF REACH OF CHILDREN
CAUTION

U.S. EPA Registration No.: (Pending as File Symbol 69697-3)
U.S. EPA Establishment: 69697-CAN-001

REGISTRATION NUMBER XXXXX PEST CONTROL PRODUCTS ACT

Net Contents: 1 L (32 U.S. fl oz)

Manufactured by: Plant Products Co. Ltd.
314 Orenda Road
Brampton, Ontario L6T 1G1, Canada
905-793-7900

Distributed by: Plant Products Co. Ltd.
6160 Riverside Dr.
Suite 103
Dublin, OH 43017

Lot Number: [XXX]

Date of manufacture: [XXX]
Use within 3 months of the date of manufacture

KEEP FROZEN UNTIL USE

(Back Panel)

PRECAUTIONS / PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS AND DOMESTIC ANIMALS. May cause sensitization. Avoid contact with skin, eyes or clothing. Avoid breathing mist. Wash thoroughly with soap and water after handling.

PERSONAL PROTECTIVE EQUIPMENT (PPE): Applicators and other handlers must wear long-sleeved shirt and long pants, waterproof gloves and shoes plus socks. All mixers/loaders and applicators must wear a dust/mist-filtering respirator (MSHA/NIOSH approval number prefix TC-21C), or a NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products. Remove contaminated clothing and follow manufacturer's instructions for cleaning / maintaining PPE before reuse. If no such instructions are available use clothing detergent and hot water for cleaning all washable PPE. Keep and wash PPE separately from other laundry.

USER SAFETY RECOMMENDATIONS: Users should wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

ENVIRONMENTAL HAZARDS

Do not apply directly to water, or to areas where surface water is present, or to intertidal areas below the mean highwater mark. Do not contaminate water by cleaning of equipment or disposal of equipment washwaters.

FIRST AID	
If on skin or clothing	C Take off contaminated clothing. C Rinse skin immediately with plenty of water for 15 – 20 minutes. C Call a poison control center or doctor for treatment advice.
If inhaled	C Move person to fresh air. C If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible. C Call a poison control center or doctor for treatment advice.
If in eyes	C Hold eye open and rinse slowly and gently with water for 15 - 20 minutes. C Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. C Call a poison control center or doctor for treatment advice.
If swallowed	C Call a poison control center or doctor immediately for treatment advice. C Have person sip a glass of water if able to swallow. C Do not induce vomiting unless told to do so by the poison control center or doctor. C Do not give anything by mouth to an unconscious person.

Seek medical attention IMMEDIATELY if irritation occurs and persists or is severe. Have container, label or product name and product registration number with you when calling a poison control center or doctor, or when seeking medical attention.

TOXICOLOGICAL INFORMATION / NOTE TO PHYSICIAN

No specific antidote is available. Treat the patient symptomatically.

DIRECTIONS FOR USE

In the U.S. - It is a violation of Federal law to use this product in a manner inconsistent with its labeling. For any requirements specific to your State or Tribe, consult the agency responsible for pesticide regulation. Do not apply this product in a way that will contact workers or other persons, either directly or thorough drift. Only protected handlers may be in the area during application.

In Canada - NOTICE TO USER: This control product is to be use only in accordance with the directions on this label. It is an offence under the PEST CONTROL PRODUCTS ACT to use a control product under unsafe conditions.

In the U.S. -

Agricultural Use Requirements

Use this product only in accordance with its labeling and with the Worker Protection Standard, 40 CFR Part 170. This standard contains requirements for the protection of agricultural workers on farms, forests, nurseries and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this label about personal protective equipment (PPE), and restricted entry intervals (REI). The requirements in this box only apply to uses of this product that are covered by the Worker Protection Standard.

Do not enter or allow worker entry into treated areas during the restricted entry interval of 4 hours.

PPE requirement for early entry to treated areas that is permitted under the Worker Protection Standard and that involves contact with anything that has been treated, such as plants, soil or water, is coveralls, waterproof gloves and shoes plus socks.

In Canada -

Do not enter or allow worker entry into treated areas during the restricted entry interval (REI) of 4 hours. Workers can enter treated areas during the REI if appropriate PPE is worn, including a long sleeved shirt, long pants, shoes plus socks and waterproof gloves as well as a NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products.

GENERAL INFORMATION

SPORODEX L is a naturally occurring fungus which is an antagonist to the powdery mildew disease organism. SPORODEX L is an aqueous liquid formulation of *Pseudozyma flocculosa* formulated to control powdery mildew disease on the listed crops.

APPLICATION RATES

CROP	DISEASE	RATE
Greenhouse roses Greenhouse cucumbers	Powdery mildew disease	**In U.S. and Canada:** 500 mL per 100 L of water. Add 20 mL of an appropriate wetting agent per 100 L of spray mixture. or 64 U.S. fl oz per 100 U.S. gallons of water. Add 3 U.S. fl oz of an appropriate wetting agent per 100 U.S. gallons of spray mixture.

Add SPORODEX L to water. Spray foliage to run-off at weekly intervals, beginning when environmental conditions favor development of powdery mildew, or at first sign of disease.

Apply up to 1500 L of spray mixture per hectare (150 U.S. gallons of spray mixture per acre) for cut roses, cucumbers or about 1000 L per hectare (100 U.S. gallons per acre) for potted roses.

Maintain relative humidity above 70% for 12 hours after application, for example, by using shade curtain or by applying SPORODEX L late in the day or during prolonged cloudy conditions.

NOTE: Use of chemicals at the same time as SPORODEX L may inhibit this product's activity against powdery mildew. Do not tank mix SPORODEX L with chemical pesticides.

SPORODEX L has not been tested for compatibility with all chemical and biological products (including biological control insects and arthropods) used in greenhouse production. For details on compatibility contact the distributor or manufacturer, or test effectiveness on a small number of plants prior to commercial scale use.

STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage or disposal.
Pesticide Storage: Store frozen at -20°C (-4°F) or less and keep away from food or feed. Keep product in original container during storage and keep container lid tightly closed when not in use. This product should be used within 3 months of the date of manufacture when stored at -20°C (-4°F). Thaw at room temperature prior to using.

In the U.S.-
Pesticide Disposal: Wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility.
Container Disposal: Completely empty package into application equipment. Then dispose of empty package in a sanitary landfill or by incineration, or, if allowed by State and local authorities, by burning. If burned, stay out of smoke. Do not reuse container.

In Canada-
DISPOSAL

1. Triple- or pressure-rinse the empty container. Add the rinsings to the spray to the spray mixture in the tank.
2. Follow provincial instruction for any required additional cleaning of the container prior to its disposal.
3. Make the container unsuitable for further use.
4. Dispose of the container in accordance with provincial requirements.
5. For information on the disposal of unused, unwanted product, contact the manufacturer or the provincial regulatory agency. Contact the manufacturer and the provincial regulatory agency in case of a spill, and for clean-up of spills.

In the U.S. - NOTICE TO USER: Seller makes no warranty, express or implied, of merchantability, fitness or otherwise concerning the use of this product other than indicated on the label. User assumes all risks of use, storage, or handling not in strict accordance with label instructions.

In Canada - NOTICE TO BUYER: Seller's guarantee shall be limited to the terms set out on the label and, subject thereto, the buyer assumes the risk to persons or property arising from the use or handling of this product and accepts the product on that condition.

SPORODEX L is a trademark of Plant Products Co. Ltd.

9.8.2 Manufacturing-use product

Manufacturing Use Label

NOT TO BE USED DIRECTLY FOR TREATMENT OF PESTS

PSEUDOZYMA FLOCCULOSA Strain PF-A22
Technical Grade Active Ingredient for Manufacturing Use Only.

ACTIVE INGREDIENT: *Pseudozyma flocculosa* Strain PF-A22 UL 9.00%
OTHER INGREDIENTS: 91.00%
TOTAL: 100.0%
(Contains a minimum of 2×10^9 colony forming units/mL)

KEEP OUT OF REACH OF CHILDREN
CAUTION

U.S. EPA Registration No.: (Pending as File Symbol 69697-R)
U.S. EPA Establishment: 69697-CAN-001

Net Contents: (XX)

Manufactured by: Plant Products Co. Ltd.
314 Orenda Road
Brampton, Ontario L6T 1G1, Canada
905-793-7000

Lot Number: [XXX]

Date of manufacture: [XXX]
Use within 3 months of the date of manufacture

PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS AND DOMESTIC ANIMALS. CAUTION. May cause sensitization. Avoid contact with skin, eyes or clothing. Avoid breathing mist. Wash thoroughly with soap and water after handling.

PERSONAL PROTECTIVE EQUIPMENT (PPE): Wear a dust/mist-filtering respirator (MSHA/NIOSH approval number prefix TC-21C), or a NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products. Remove contaminated clothing and follow manufacturer's instructions for cleaning / maintaining PPE before reuse. If no such instructions are available use clothing detergent and hot water for cleaning all washable PPE. Keep and wash PPE separately from other laundry.

ENVIRONMENTAL HAZARDS

Do not discharge effluent containing the product into lakes, streams, ponds, estuaries, oceans, or other waters unless in accordance with the requirements of the National Pollutant Discharge Elimination System (NDPES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance, contact your State Water Board or Regional Office of the EPA.

FIRST AID	
If on skin or clothing	C Take off contaminated clothing. C Rinse skin immediately with plenty of water for 15 – 20 minutes. C Call a poison control center or doctor for treatment advice.
If inhaled	C Move person to fresh air. C If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible. C Call a poison control center or doctor for treatment advice.
If in eyes	C Hold eye open and rinse slowly and gently with water for 15 - 20 minutes. C Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. C Call a poison control center or doctor for treatment advice.
If swallowed	C Call a poison control center or doctor immediately for treatment advice. C Have person sip a glass of water if able to swallow. C Do not induce vomiting unless told to do so by the poison control center or doctor. C Do not give anything by mouth to an unconscious person.

Have container, label or product name and product registration number with you when calling a poison control center or doctor, or when seeking medical attention.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

FOR MANUFACTURING USE ONLY. Only for formulation into end-use products, for use to control plant diseases in/on agricultural commodities. Not for direct treatment of pests. Do not use from damaged, punctured or unsealed containers

STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage or disposal.

Pesticide Storage: Store refrigerated and keep away from food or feed.

Pesticide Disposal: Wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility.

Container Disposal: Triple rinse (or equivalent). Then offer for recycling or reconditioning, or puncture and dispose of in a sanitary landfill, or incineration, or, if allowed by state and local authorities, by burning. If burned, stay out of smoke. Completely empty package into application equipment.

NOTICE TO USER: Seller makes no warranty, express or implied, of merchantability, fitness or otherwise concerning the use of this product other than indicated on the label. User assumes all risks of use, storage, or handling not in strict accordance with label instructions.

Chapter 10 List of abbreviations

AUDPC	area under disease pressure curve
bw	body weight
CFU	colony forming units
DNA	deoxyribonucleic acid
dw	dry weight
EPA	Environmental Protection Agency (U.S.)
FFDCA	Federal Food Drug and Cosmetic Act (U.S.)
FIFRA	Federal Insecticide Fungicide Rodenticide Act (U.S.)
FQPA	Food Quality Protection Act (U.S.)
EP	end-use product
IPM	integrated pest management
KTS	killed test-substance
LD_{50}	lethal dose 50%
MA	Martin's agar
MAS	maximum average score
MIS	maximum irritation score
MPCA	microbial pest control agent
MRL	maximum residue limit (Canada)
NC	naive control
PCA	plate count agar
PCR	polymerase chain reaction
PDA	potato dextrose agar
PMRA	Pest Management Regulatory Agency (Canada)
PPE	personal protective equipment
RAMS	random amplified microsatellites
REI	restricted entry interval
RH	relative humidity
TGAI	technical grade of the active ingredient
TS	test substance
U.S.	United States of America
U.S. EPA	United States Environmental Protection Agency
YM	yeast malt agar