Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Regulatory Note

REG2004-05

Ophiostoma piliferum Strain D97

Sylvanex Technical (TGAI)
Sylvanex (EP)

The microbial active ingredient *Ophiostoma piliferum* strain D97 (SYLVANEX TECHNICAL) and the associated end-use product SYLVANEX have been granted temporary registration under Section 17 of the Pest Control Products (PCP) Regulations for use as a biological antisapstain product to control blue-staining fungi on freshly felled lodgepole pine and red pine logs at the felling site.

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

(publié aussi en français)

30 March 2004

This document is published by the Alternative Strategies and Regulatory Affairs Division, Pest Management Regulatory Agency. For further information, please contact:

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

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

ISBN: 0-662-36737-5 (0-662-36738-3)
Catalogue number: H113-7/2004-5E (H113-7/2004-5E-PDF)

Foreword

The submissions for the registration of the technical grade active ingredient *Ophiostoma piliferum* strain D97 SYLVANEX TECHNICAL and its end-use product SYLVANEX for control of blue-staining fungi on freshly felled lodgepole pine and red pine logs at the felling site, produced by AgraSol Inc., have been reviewed by Health Canada's Pest Management Regulatory Agency (PMRA).

SYLVANEX is a microbial antisapstain product, containing 76% (w/w) *Ophiostoma piliferum* strain D97, intended for the control of blue-staining fungi on freshly felled lodgepole pine and red pine logs at the felling site. The active microorganism, *Ophiostoma piliferum* strain D97, is a naturally occurring fungus and is not currently registered in the United States for use as an antisapstain product. There is no antisapstain product currently registered for Sylvanex's proposed use pattern.

AgraSol Inc. will be carrying out additional studies as a condition of this temporary registration. Following the review of this information, the PMRA will publish a proposed regulatory decision document and request comments from interested parties before proceeding with a final regulatory decision.

Table of Contents

1.0 The active substance, its properties and uses

1.1 Identity of the active substance and impurities

TGAI Identitification

Active microorganism	*Ophiostoma piliferum* strain D97
Function	Biological antisapstain
Binomial name	*Ophiostoma piliferum*
Taxonomic designation	
Kingdom:	Eumycota
Phylum:	Dikaryomycota
Subphylum:	Ascomycotina
Class:	Ascomycetes
Order:	Ophiostomatales
Family:	Ophiostomataceae
Genus:	*Ophiostoma*
Species:	*piliferum*
Strain:	WZ5803D97 (abbreviated as D97)
Canadian patent status information	A patent (Canadian Patent Application Number 2009622) was issued on 30 November 1999 under the former Cartapip 97 trade name for reducing the pitch content in wood pulp. Cartapip 97 has also been applied for patent status as a biological control for wood products and debarking (Canadian Patent Application Number 2149808) and as a fungus for pitch reduction (Canadian Patent Application Number 2047900).
Nominal purity of active	Sylvanex Technical (TGAI) consists of 57% active ingredient and 1.5% spent fermentation medium corresponding to 20–50 activity units (AU)/kg [where 1 AU is defined as 1×10^{12} colony forming units (CFU) of *Ophiostoma piliferum* strain D97]. 76% w/w (equivalent to 30–50 AU/kg) in Sylvanex (end-use product [EP]).

Identity of relevant impurities of toxicological, environmental and/or other significance	The technical product does not contain any impurities or microcontaminants known to be TSMP Track 1 substances. Production cultures are tested for albinism and microbial growth prior to being used as inoculum. The final product is analyzed for microbial contamination (*Salmonella* spp., *Staphylococcus aureus*, total coliforms, fecal coliforms, yeast and mould count, fecal streptococci). Batches that surpass the established limits with respect to microbial contaminants must be destroyed. No mammalian toxins are known to be produced by strain D97.

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

Technical product: Sylvanex technical

Property	**Result**
Physical state	suspension
pH in distilled water	6–7
Density	1000 kg/m^3
Viscosity	400 cps

End-use product: Sylvanex

Property	**Result**
Colour	tan brown
Physical state	flake
Guarantee	76% w/w (equivalent to 30–50 AU/kg)
Formulants	All formulants in Sylvanex are considered relatively non-toxic (i.e., are on either USEPA inerts list 4A or 4B). The product does not contain any USEPA List 1 formulants or formulants known to be TSMP Track 1 substances.
Density	180 kg/m^3
pH in distilled water	6–7

Property	Result
Corrosion character	not irritating to skin; mildly irritating to eyes
Wettability	readily suspended if vortexed
Moisture content	1-3%

1.3 Details of uses and further information

Sylvanex is a wettable powder EP containing the active ingredient *Ophiostoma piliferum* strain D97 proposed for use as a biological antisapstain product on freshly felled lodgepole pine and red pine logs at the felling site only. Sylvanex is to be diluted in water and applied during harvesting of the wood using a hydraulic spray system that is attached to the harvesting equipment. There is no antisapstain product currently registered for this proposed use pattern.

Ophiostoma piliferum has been isolated in Europe, Australia, the United States and Canada. In the United States, *Ophiostoma piliferum* is widespread on coniferous lumber (Hunt, 1956) and it is reported as one of the most important of the staining fungi in the southern states (Verrall, 1939). In Canada, *Ophiostoma piliferum* is indigenous to a number of ecozones. *Ophiostoma piliferum* has been found on logs and/or lumber from lodgepole pine, white spruce, jack pine and black spruce in British Columbia, Alberta, Saskatchewan, Ontario and Quebec (Uzunovic et al., 1999).

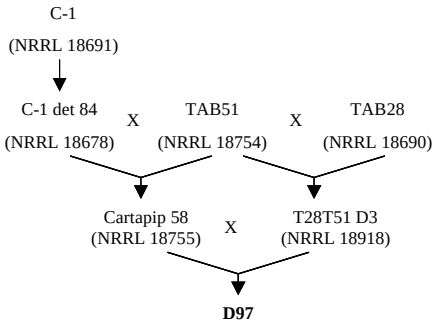
Ophiostoma piliferum is a saprophytic primary colonizer of fresh sapwood in cut trees and lumber. The fungus colonizes only dead woody materials and has a preference for softwoods over hardwoods. Wild-type pigmented strains of the fungus cause discolouration of sapwood.

Ophiostoma piliferum strain D97 was derived from matings of wild-type strains of *Ophiostoma piliferum* (Figure 1.3.1), including the faded grey wild-type C-1 strain of *Ophiostoma piliferum*. Homokaryotic strains were obtained from strain C-1 by single-spore ascospore isolation. The ascospores were allowed to germinate resulting in individual hyphal colonies, which were visually screened. A white or colourless isolate, C-1 det 84, was selected for further matings.

Darkly pigmented (black) strains such as TAB51 and TAB28 are associated with rapid colonization and are used in crosses with colourless strains in order to produce offspring that are both colourless and fast-growing. C-1 det 84 was subsequently crossed with the pigmented wild-type strain, TAB51. From the resulting colonies, Cartapip 58 was selected for further mating based on its colourless and growth properties.

In a similar manner, a light brown variant, T28T51 D3, was a single ascospore isolate resulting from a cross of the two wild-type strains, TAB28 and TAB51. A final cross between T28T51 D3 and Cartapip 58 yielded the single ascospore colourless D97 strain used in Sylvanex. The D97 strain was selected as the active ingredient for Sylvanex based on its growth on woods such as pine, its white or colourless characteristics and its ability to degrade pitch.

Figure 1.3.1 Family tree for *Ophiostoma piliferum* strain D97



Ophiostoma piliferum strain D97 differs from most other strains of this species in that it lacks melanin within its fungal cell walls, thus inferring a colourless quality. Sylvanex is to be used to control blue-staining fungi and prevent sapstain. The white or colourless strain in Sylvanex competes with pigmented fungi for limited food and nutrient sources in freshly felled wood. The natural level of blue-staining fungi cannot colonize and outgrow the high concentration of the albino strain that is applied.

C-1 det 5, another colourless single ascospore isolate of *Ophiostoma piliferum* strain C-1, was used in all human health and safety studies, while Cartapip 58 was the test substance in most environmental toxicology studies. Since there is no indication that there are subspecies or varieties within this species, C-1 det 5, Cartapip 58 and Cartapip 97 can be considered biologically equivalent.

This control agent has previously been assessed in 1998 by Environment Canada and Health Canada and found acceptable for non-pesticidal use as a woodchip pitch control agent for the pulp and paper industry (New Substance Notification, NSN #6858).

2.0 Methods of analysis

2.1 Methods for analysis of the active substance as manufactured

2.1.1 Methods for identification of the microorganism

Ophiostoma species can be differentiated from closely related organisms in the genus *Ceratocystis* based on morphology (e.g., ascocarps, hyphae, conidiophores). Sensitivity to the antibiotic cycloheximide can also distinguish between *Ceratocystis* and *Ophiostoma*. While linear growth of *Ophiostoma piliferum* was observed on malt extract agar amended with cycloheximide at concentrations of up to 1000 ppm, none of the *Ceratocystis* species grew on malt extract agar with >50 ppm cycloheximide (Harrington, 1981).

Restriction fragment length polymorphism (RFLP) patterns generated by *Hae*III DNA-restriction digestion of the 26S rRNA gene can be used to distinguish *Ophiostoma piliferum* from other sapstain *Ophiostoma* species.

Amplification of the β-*tubulin* gene with a specially designed primer pair (Cat1–Cat2), differentiates *Ophiostoma piliferum* strain D97 from most other *Ophiostoma piliferum* strains. Strain D97, in addition to strains from Quebec, Saskatchewan and the United States, yield a characteristic 180 base pair DNA fragment while strains from Europe, New Zealand, British Columbia and Alberta fail to yield this fragment. The use of an original archival seed culture for all production batches of Sylvanex along with other identifying properties of the D97 strain (e.g., colourlessness) alleviates concerns associated with differentiation of the D97 strain from natural or wild-type strains of *Ophiostoma piliferum*.

2.1.2 Methods for establishment of purity of seed stock

The master seed stock of *Ophiostoma piliferum* strain D97 used in production of Sylvanex is stored in vials at -70°C, in AgraSol's laboratory in Raleigh, North Carolina and is also deposited in the United States Department of Agriculture's (USDA) Northern Regional Research Laboratory (NRRL) culture collection (NRRL No. 18917).

Production vials are prepared by aseptically inoculating a 250 mL shake flask containing 100 mL yeast malt extract (20 g/L malt extract, 2 g/L yeast extract) with 1 mL of frozen *Ophiostoma piliferum* strain D97 stock. The flask is then incubated on a shaker set at 120 rpm and 28°C for 24 hours. The contents of the shake flask are then transferred to a 2 L flask containing 900 mL of yeast malt extract and incubated on the shaker once again for 24–48 hours. The incubation is stopped when sporulation occurs, as determined by light microscopy. When grown in this manner, the fermentation product consists of 60–70% blastospores.

Sterilized glycerol is added to the fermentation product to a final concentration of 20% v/v and the mixture is aseptically transferred to sterile 1 mL cryopreservation vials that are stored at -70°C. Each batch of production vials is assigned a code for the purposes of tracking during inoculum quality assurance (QA) and production.

Production cultures are tested for albinism and microbial growth prior to being used as inoculum for production of Sylvanex. To test for albinism, samples from at least two vials are diluted and plated on potato dextrose agar (PDA) and incubated at 28°C for 96 hours. The batch of production vials pass the albinism test only if no pigmented colonies are observed among 1000 plated colonies. No other test for the integrity and consistency of the strain is employed.

To test for unintended microbes, a sample of at least 10^8 colony forming units (CFU) of *Ophiostoma piliferum* strain D97 are plated onto PDA or Luria agar. The plates are incubated at 37°C, which is non-permissive for growth of *Ophiostoma piliferum*. After 24, 48, 72 and 96 hours, the plates are examined for the presence of bacterial, fungal or yeast colonies. The batch of production vials pass the unintended microbes test if no colonies are observed on any of the PDA or Luria agar plates.

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

The viability of the intermediate spore slurry is assayed using one of two methods. The first method uses a specialized spiral plate dispenser that distributes 50 µL of diluted sample (four dilutions) on the surface of a rotating 10 cm PDA plate from the centre to the edge in decreasing amount, such that the volume of sample on any portion of the plate is known. The plates are incubated at 32°C for 60–65 hours and then the total counts are determined. Each plate yields three total plate count readings. The resulting 12 total plate count readings are averaged to determine the final count. Alternatively, a standard spread plate method may be employed. Dilutions of the slurry are plated onto PDA, in replicates of five plates per dilution, and incubated upside down at 25°C for three to four days. Only plates with 25–200 colonies are included in the count determination. The intermediate slurry is acceptable if the cell count by plate assay is at least 5×10^9 CFU/g. The intermediate slurry is generally near $2x10^{10}$ CFU/g.

After drying, the viability of the product is once again checked using the standard spread plate method. The sample is prepared by hydrating 5 g Sylvanex in 100 mL of water at 25°C and pH 5–6 with 30 minutes of shaking or stirring. Five replicates of the hydrated Sylvanex are prepared. The hydrated Sylvanex is serially diluted and 100 µL of each dilution (typically the 10^5, 10^6, 10^7 and 10^8 dilutions) is plated onto a PDA plate. The plates are incubated upside down at 25°C for three to four days. Plates with 25–200 colonies are enumerated and the results are expressed as CFU/mL and activity units (AU)/kg. One activity unit is defined as being equivalent to 10^{12} CFU. The potency data suggest that the range can be further limited to 30–50 AU/kg. The nature of the manufacturing process may result in a wide potency range among various batches. The

applicant proposes to provide the consumer with the total number of AUs per shipping carton along with the net weight of each carton. The user can then calculate the AU/kg for the contents of each shipping carton and determine the amount of product to be applied. This proposal is acceptable. A new product specification form indicating the limits imposed on the guarantee are required for the EP.

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

Plates from Sylvanex potency assays are incubated for an additional 6–7 days to ensure that none of the colonies are pigmented.

Assays for salmonellae, *Staphylococcus aureus*, total coliforms, fecal coliforms, yeasts and moulds as well as fecal streptococci were performed according to recognized guidelines (AOAC, USFDA, American Public Health Association). Quality control data from five batches of Sylvanex were provided. All batches tested negative for salmonellae (/10 g) and *Staphylococcus aureus* (/1g). The highest count of any class of unintended microorganisms tested was the fecal streptococci count in one batch of 1.15×10^4 CFU/g, but this was below the established limit of 10^5 CFU/g established for other microbial pesticide products. The total coliform counts for two batches ranged from 2.2 to 6.6×10^3 CFU/g exceeded the limit of 10^3 CFU/g set for other microbial pesticide products. Representative fecal coliform colonies isolated from these two batches were identified as *Enterobacter* sp., *Proteus mirabilis* and *Klebsiella pneumoniae*. Screening for microbial contaminants must continue for all production batches of Sylvanex and any batches that surpass the established limits with respect to microbial contaminants must be destroyed.

Sylvanex is manufactured in a facility that also produces a number of other *Bacillus* products, primarily *Bacillus thuringiensis* subsp. *kurstaki* (Btk) products. Btk is a registered microbial pest control agent (MPCA) that is highly specific for lepidopteran pests. Btk poses minimal risk to humans or other animals. A potential exists, in the manufacturing plant, for contamination of the Sylvanex product with Btk spores. The applicant claims that the expected residual range for Btk spores in Sylvanex will not exceed 10^3 CFU/g.

Ophiostoma piliferum is not known to produce mammalian toxins. Lack of mammalian toxicity is supported by the lack of reports of adverse effects in humans or animals despite the ubiquitous nature of *Ophiostoma piliferum* in a number of ecozones in Canada.

2.1.5 Methods to show absence of any human and mammalian pathogens

As discussed in Section 2.1.4, the quality assurance program for the production of Sylvanex must include the destruction of batches that exceed the established limits for certain microbial contaminants.

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

The stability of the product was determined at various timepoints after storage at -20°C using the standard spread plate method for viability testing described in Section 2.1.3. The storage stability data submitted for Sylvanex were of limited value since the manufacturing and testing were conducted by a number of different facilities and because only two lots were tested over time. It was unclear whether the variability in retained activity was due to the nature of the assay, the location the lot was manufactured, the testing facility (in some cases, the initial activity was assayed by one facility and the re-assay was conducted by a second facility), or differences in stability among different lots. Based on the limited data, a storage period of up to 18 months at -20°C may be supported. Reference to storage at room temperature for a few weeks must be removed from the label. Additional storage stability data will be required to amend the label to include higher storage temperatures. Such data must include lots that are analyzed intermittently over a suitable period of time on batches stored under expected operational conditions (e.g., 25°C). Other storage temperatures should also be considered. In order to minimize variability, a homogenization step should be included in the protocol for determining potency.

2.2 Method for formulation analysis

Sylvanex is not intended for food or feed use and, therefore, the establishment of a Maximum Residue Limit (MRL) is not required for *Ophiostoma piliferum* strain D97. Consequently, no method(s) to quantify *Ophiostoma piliferum* strain D97 residues in food and feed are required.

3.0 Impact on human and animal health

See Appendix I, Table 1, for summary table.

3.1 Integrated toxicity and infectivity summary

The information and data submitted by AgraSol Inc. in support of registration of *Ophiostoma piliferum* strain D97 and Sylvanex, were reviewed from the viewpoint of human health and safety and were 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 *Ophiostoma piliferum* strain D97 and bacterial/fungal contaminants introduced during production.

The acute toxicity and infectivity studies submitted in support of registration of *Ophiostoma piliferum* strain D97 (Sylvanex Technical) and Sylvanex were reviewed. The data set comprised studies conducted on a closely related strain C-1 det 5 (see Section 1.3), and included acceptable acute oral and pulmonary toxicity/pathogenicity studies, a supplemental dermal toxicity/pathology study, which contained sufficient data to make a decision on primary dermal irritation, as well as a supplemental eye irritation study.

No signs of toxicity or pathogenicity were noted when the *Ophiostoma piliferum* strain C-1 det 5 was administered to rats via the oral or intratracheal routes. *Ophiostoma piliferum* strain C-1 det 5 was not irritating when applied dermally to rabbits, but toxicity results were equivocal because the guideline dose requirements for dermal toxicity testing were not met. Because of this uncertainty, standard personal protective equipment (PPE) to guard against dermal exposure will be required, and a new dermal toxicity study using the EP at the required dose will be required to complete the health and safety assessment. Slight conjunctival redness was observed after administration of *Ophiostoma piliferum* strain C-1 det 5 to the eyes of rabbits. The irritation potential of Sylvanex is expected to be greater than that of the test substance used due to the physical properties of its powder formulation. Standard label statements will be required to mitigate the risk to eyes. *Ophiostoma piliferum* has not been reported to produce any mammalian toxins. A database search yielded no evidence of mammalian toxin production. This finding was confirmed in a letter of expert opinion from a researcher in the field of ophiostomatoid fungi who, after extensively reviewing the literature on *Ophiostoma piliferum* and its metabolites, stated that he knew of no toxins or antibiotics produced by *Ophiostoma piliferum*.

3.2 Hypersensitivity incidence

The Canadian pulp and paper industry has used Sylvanex since 1995 as a pitch reducing agent. There have been no reported incidents of hypersensitivity associated with manufacturing, formulating, or applying Sylvanex for this purpose. However, in common with all microorganisms, *Ophiostoma piliferum* is considered to be a potential sensitizing agent.

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

3.3.1 Occupational and bystander exposure assessment

The review of human health and safety studies showed that Sylvanex (*Ophiostoma piliferum* strain D97) is of low acute toxicity, and is not pathogenic by oral gavage and intratracheal instillation. Like all microbial pesticides, *Ophiostoma piliferum* is considered to be a potential sensitizer, though there were no reports of hypersensitivity. An acute eye irritation study showed that *Ophiostoma piliferum* was minimally irritating to the eyes as a suspension, though its potential for irritation as a powder is unknown.

Furthermore, this product has been used as a pitch control agent in the pulp and paper industry since 1995. No human health or safety concerns have been raised since that time.

The proposed use for *Ophiostoma piliferum* strain D97 is as an antisapstain product on freshly felled lodgepole pine and red pine logs to be applied as a spray at a rate of 10^{11} CFU (100–300 g) per m^3 using a sprayer attached to the cutting apparatus of the feller–buncher, delimber, or harvester machines. Occupational exposure could occur during mixing of the product and loading into the spray tank and from spray drift from the harvester. However, machine operators are protected from spray within an enclosed cab. On the basis of its biological properties, lack of toxicity and pathogenicity and its proposed use pattern, it is recommended that the product label include standard personal protective equipment.

4.0 Residues

4.1 Residue summary

Sylvanex will not be applied to food or feed crops; therefore, the establishment of a maximum residue limit (MRL) is not required for *Ophiostoma piliferum* strain D97 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulations. *Ophiostoma piliferum* is a naturally occurring microorganism that is widespread in the environment. It is not known as an aquatic fungus and therefore is not expected to proliferate in aquatic habitats. Moreover, the risk from non-occupational exposure is considered minimal as there is no evidence of adverse effects from oral, dermal or inhalation exposure. Drinking water is accordingly not being screened for *Ophiostoma piliferum* as a potential indicator of microbial contamination or as a direct pathogenic contaminant. Both percolation through soil and municipal treatment of drinking water reduce the possibility of exposure to *Ophiostoma piliferum* strain D97 through drinking water. Therefore, the potential of significant transfer to drinking water is minimal to non-existent, and the risk from consuming drinking water containing *Ophiostoma piliferum* strain D97 is minimal as there is no evidence of adverse effects from oral exposure to this agent.

5.0 Fate and behaviour in the environment

Environmental fate data (Tier II) were not triggered as adverse effects to non-target organisms are not expected from the proposed use of *Ophiostoma piliferum* strain D97.

6.0 Effects on non-target species

See Appendix II, tables 1 and 2, for summary tables.

6.1 Integrated environmental toxicology summary

Ophiostoma piliferum is one of the most commonly found sapstain fungi of coniferous lumber in the southern United States. This fungus is reported to be a widespread common sapstain fungus of coniferous wood in Europe. In Canada, *Ophiostoma piliferum* is found in logs and lumber of various pine and spruce species originating from numerous ecozones in Canada. The published literature concerning *Ophiostoma piliferum* indicates that this fungus is a saprophyte of dead wood or wood whose host-resistance mechanisms are severely impaired. It can colonize both hardwoods and softwoods, although the latter may be considered the preferred substrate. *Ophiostoma piliferum* and other sapstaining fungi are intimately linked with bark beetles that act as vectors. Spores of sapstain fungi are carried both externally on the body surface and internally within the intestinal tract. Some bark beetles have a specialized feature called a mycangium, which is used for transporting fungal spores.

Ophiostoma piliferum can metabolize the pitch components, i.e., triglycerides, fatty and diterpenoid resin acids, sterols and waxes. According to the literature, no cellulolytic or ligninolytic enzymes are produced. Usually, the growth of sapstain fungi causes a superficial discolouration of the wood. This discolouration is caused by the darkly pigmented hyphae of sapstain fungi. In *Ophiostoma piliferum* strain D97, no staining occurs as the pigments responsible for the stain are not produced, i.e., it is a pigmentless or albino strain. Pigment synthesis is blocked in the 1,8-dihydroxynaphthalene melanin synthetic pathway. *Ophiostoma piliferum* strain D97 does not destroy other sapstain fungi. It merely competes with the other sapstain fungi for the readily metabolizable sugars associated with fresh sapwood. Once the niche is occupied and the nutrients are captured, susbequent colonization by sapstain fungi does not occur. *Ophiostoma piliferum* is not considered to be a pathogen nor is it capable of parasitizing either plants or animals. No reports of adverse effects on birds, wild mammals, fish, arthropods, non-arthropod invertebrates, microorganisms or plants have been reported in the published literature. Furthermore, no overt signs of toxicity or pathogenicity were noted in any of the environmental toxicology studies submitted for review.

According to the proposed use pattern, Sylvanex is to be applied to lodgepole pine and red pine logs at forest-harvesting sites using a hydraulic spray system attached to various cutting and delimbing equipment. Consequently, the potential for exposure to terrestrial non-target species is high. However, the risk to non-target species is expected to be low based on the results of submitted studies and an absence of adverse effects reported in the published scientific literature. Consequently, *Ophiostoma piliferum* strain D97 is expected to pose little environmental risk when used in accordance with the proposed label directions. Furthermore, the formulants in the EP do not pose an environmental risk when used at the proposed application rates.

7.0 Efficacy

7.1 Effectiveness

7.1.1 Intended use

Sylvanex is a flaked end-use product containing the active ingredient *Ophiostoma piliferum* strain D97 proposed for use as a biological antisapstain product on freshly felled lodgepole pine and red pine logs at the felling site only. Sylvanex is to be diluted in water and applied during harvesting of the wood using a hydraulic spray system, attached to the harvesting equipment. There is no antisapstain product currently registered for this proposed use pattern.

7.1.2 Mode of action

Sylvanex contains blastospores of an albino (non-pigmented) strain of the sapstain fungus *Ophiostoma piliferum*. Colourless isolates occur naturally in forests and on wood products but are not commonly found because the lack of melanin makes them unfit for long-term survival in nature. Melanin would be essential for UV light protection, perithecial development and protection from mite and insect predation. The discolouration of wood typically associated with the growth of *Ophiostoma piliferum* does not occur with Sylvanex because the Sylvanex strain is blocked in the 1,8-dihydroxynaphthalene melanin synthetic pathway.

The principle behind the use of Sylvanex is a competition with the wild type pigmented sapstain fungi for the limited food source found in dead or dying wood. As Sylvanex is applied at a high concentration soon after felling, it is the first fungus to colonize the fresh sapwood. It can easily assimilate nutrients in sapwood before the blue-staining fungi present at natural background levels have a chance to establish themselves. Once nutrient resources are captured and the niche occupied, subsequent colonization by wild type pigmented fungi does not occur.

7.1.3 Nature of the pest problem

Sapstain is a grey, black or bluish discolouration of sapwood caused by the presence of black yeast, dark mould and blue-staining fungi. The discolouration greatly reduces the aesthetic quality and commercial value of timber but has little effect on wood strength. The superficial discolouration of wood by these fungi is caused by their dark pigmented hyphae. No actual staining of the wood cell walls occurs.

The blue-staining fungi commonly found in Canada are: *Aureobasidium pullulans, Ceratocystis adiposa, Ceratocystis coerulescens, Ophiostoma flexuosum, Ophiostoma floccosum, Ophiostoma ips, Ophiostoma minus, Ophiostoma piceae, Ophiostoma piceaperdum, Ophiostoma piliferum* and *Phacidium coniferarum*.

Spores of sapstain fungi are distributed mainly by insects, but distribution by wind and rain can also occur as well as during harvest and processing operations. Bark beetles may introduce blue stain through the bark of the logs. Dry air is ineffective in causing conidium dispersal, but both mist-laden air and splash droplets would dislodge and disseminate conidia relatively easily.

7.1.4 Effectiveness against pests

Efficacy trials were conducted in 2000 in Skookumchuck River Valley, British Columbia, and in 2000 and 2001 in the Elk River/Brazeau River Valley area of Alberta.

Lodgepole pine trees from these sites were felled and the stems delimbed using mechanical equipment. This process resulted in a moderate amount of bark damage. Test logs three to five metres long and free of bluestain and bark beetle attack were selected.

The entire surface of each log was sprayed to saturation (540 mL of Sylvanex suspension per square metre of log surface) with a Sylvanex suspension using a backpack sprayer fitted with a flat fan spray nozzle. The untreated controls were sprayed with water. After spraying, experimental logs were piled on bearers and covered with a layer of cover logs. These logs are used in order to compensate for the small size of the experimental piles. Temperatures inside piles of lumber are lower than outside air temperature during hot weather because of the cooling effect of evaporative moisture from the drying wood. Since this cooling effect is limited by the small size of the piles, the researcher added cover logs. In 2000, each treatment was applied to three piles of 10 logs and in 2001 each treatment was applied to two piles of 20 logs.

Six weeks after treatment, cover logs were removed and experimental logs from each pile were selected randomly. The remaining logs were covered again with the protective logs and sampled after an additional six weeks of storage. Fifty millimetre thick disks were taken along the length of each log. Additional discs were taken for moisture content determination. The extent of bluestain colonization was assessed by estimating the visible stain area on the disc surface using a transparent grid template with 2.5 × 2.5 mm units. The sapwood and heartwood diameter were measured and the amount of stain was expressed as a percentage of the sapwood area of each disc.

Staining results from the 2000 Alberta trials

Treatment	Mean sapwood surface area affected by sapstain (%)	
	1st sampling (10 Aug. 2000)	2nd sampling (19 Sept. 2000)
Control (water)	Negligible	11.41 ± 15.47
5×10^7 CFU/mL of Sylvanex	Negligible	1.54 ± 3.28*
1.5×10^7 CFU/mL of Sylvanex	Negligible	10.62 ± 12.18

* Statistically different from control ($p<0.05$)

Negligible stain was seen during the first sampling in Alberta and British Columbia (six weeks after treatment). The dry weather would have dried out logs quickly, thus preventing fungal growth. The report stated that the sapwood moisture content of logs was too low to support bluestain fungi growth. Statistical analysis (ANOVA) showed that there was no significant difference between untreated and treated logs.

Stain was found during the second Alberta sampling (13 weeks after treatment). The second British Columbia sampling was not carried on for unknown reasons. Statistical analysis (ANOVA) of the data showed that significantly less stain was found on logs treated with the highest concentration of Sylvanex than on the control logs ($p < 0.05$). The lowest Sylvanex treatment did not show a difference in stain from the untreated control.

The results from the above trials do not clearly demonstrate the efficacy of Sylvanex, possibly due to an absence of pest pressure. Since Sylvanex requires the same conditions as the wild type fungi to grow, it is not expected to perform as well under conditions that are not favourable to the pest.

Staining results from the 2001 Alberta trial

Treatment	Mean sapwood surface area affected by sapstain (%)	
	6 weeks	13 weeks
Control (water)	32.4 ± 17.7	64.4 ± 19.75
Sylvanex (5×10^7 CFU/mL)	0.9 ± 2.1	27.39 ± 23.8

The 2001 trial used larger piles of logs than the first trials, which better represents normal harvesting operations where logs are less likely to dry out quickly. Only the high concentration suspension was tested (5×10^7 CFU/mL). The pest pressure seen in 2001 was greater than in 2000 as discs from untreated logs had a greater percentage of their surface stained. Statistical analysis of the 2001 data indicated that there was significantly less stain on the Sylvanex-treated logs than on the untreated logs. Treated logs were essentially stain free after the first 6 weeks and had less than half the stain coverage of control logs after 13 weeks.

It is not possible to compare the efficacy of Sylvanex against an established performance standard as these have not been developed for antisapstain products used on logs. Sylvanex is not expected to meet the stringent commercial standard for antisapstain products used on lumber (six month protection) as the data showed too much stain on treated logs. A comparison with a registered product is not possible as there is no currently available alternative for logs. Although the Sylvanex treatment did not provide the same level of efficacy in all submitted trials, the results did show an improvement over untreated logs. The efficacy of Sylvanex against sapstain is not expected to be constant as sapstain damage varies from year to year and fungal growth is dependent on environmental conditions. The results showed a statistically significant reduction of staining over a 13-week period. As logs are processed as soon as possible to avoid staining, the 13-week period of protection conferred by Sylvanex should be adequate in most cases. It should be noted that a retreatment after 13 weeks is not expected to make a difference as most of the nutrients will have been consumed by then.

The efficacy data only covered lodgepole pine. The applicant did provide some published articles indicating that Sylvanex reduces colonization of sapstain fungi on aspen (*Populus tremuloides*) and red pine (*Pinus resinosa*) but the reduction in percentages of surface stained were not reported. Although the paper by Uzunovic et al. (1999) indicates that *Ophiostoma piliferum* was isolated on four of the five wood species sampled, it was found on only 50 logs of a total of 878 sampled logs (6%). As it is not encountered at a high frequency, it may not be the most competitive sapstain fungi and it is not possible to predict if it would grow well enough on other wood species to confer protection against sapstain. Consequently, the efficacy data submitted support application of Sylvanex to lodgepole pine only. Sylvanex may also be used on red pine as a published paper showed its potential to grow on red pine but confirmatory data will be required. The report from the study conducted in 2002 on red pine under a research permit needs to be submitted to support full registration of the use of Sylvanex on red pine. The following will have to be added to the label: *"Sylvanex is to be used on freshly-felled lodgepole pine and red pine logs as a preventative treatment against sapstain."*

In order to be efficacious, Sylvanex needs to establish itself on logs as soon as possible to avoid colonization by wild type sapstain fungi and this requires the application of a large number of spores per square metre. The trial rates do not appear to be excessive since the low application rate used in the first trial did not show a statistically significant difference from the untreated control. Therefore, the trial application rate of 540 mL of

a 5×10^7 CFU/mL (0.05 AU/L) suspension per square metre of wood surface tested is acceptable. However, the label proposed rate is expressed in activity units per ton of wood rather than in volume of suspension per square metre of surface. As the amount of Sylvanex used will depend on the surface area to be treated and not the weight of the wood, the label rate will have to be expressed as in the trials. A specific guarantee reflecting the actual number of AU of each batch will be given on the label in order to calculate the dilution required to obtain the 0.05 AU/L concentration of the Sylvanex suspension. Sylvanex is not expected to prevent the growth of sapstain fungi once they have been established.

The submitted documents indicate that a hydraulic spray system, attached to various cutting and delimbing equipment, must be used for application at the logging site. This application method should ensure full coverage of the logs and is acceptable from an efficacy point of view.

It should be noted that there is no reason to believe that Sylvanex could revert to a coloured phenotype as it has been studied and used as a wood pitch control agent for many years. Any instability in the phenotype would have been identified.

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

Not applicable as Sylvanex is only applied once the tree has been cut.

7.3 Observations on undesirable or unintended side effects

Sapstain is caused by pigmented fungi that invade wood cells and consume some of the tree components to support their growth. Although it is generally accepted that sapstain fungi do not affect the functional properties of the wood, some species can produce enzymes that can damage wood. A report on the effects of Sylvanex and other blue-staining fungi on the toughness and bending strength of lodgepole pine was submitted. A published study on the effects of bluestain on wood toughness and weight was also submitted. These studies did not find any significant impact of staining fungi on toughness, weight and bending strength of stained/treated wood. Therefore, the use of Sylvanex on freshly felled logs is not expected to result in adverse effects to the wood being treated.

7.4 Economics

This has not been assessed. However, it is generally recognized that a significant percentage of wood can be stained by sapstaining fungi and that stained wood has significantly less market value.

7.5 Sustainability

7.5.1 Survey of alternatives

7.5.1.1 Non-chemical control practices

The applicant indicated that control of bluestain fungi has previously been accomplished by sprinkling logs with water, which lowered oxygen content resulting in some inhibition of fungal colonization. However, this required the use of large volume of water, which raised environmental concerns. Harvested trees can be sometimes floated down river to lumber mills or stored in ponds, which also result in some inhibition of fungal colonization. Trees may also be harvested in winter, where possible, when fungal inoculum and insect activity are lowest.

7.5.1.2 Chemical control practices

There are no currently registered control products for the control of sapstain on freshly felled logs. Currently registered antisapstain control products can only be applied on freshly sawn lumber.

7.5.2 Compatibility with current management practices including integrated pest management

There are no current pest management practices used for the control of sapstain on freshly felled logs other than those identified in Section 7.5.1.1.

7.5.3 Contribution to risk reduction

There are no currently registered control products for the control of sapstain on freshly felled logs.

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

Sylvanex contains living fungi that are applied at a high concentration to limit the development of sapstain on freshly felled logs. The mode of action consists of consuming the available nutrient from the logs preventing the establishment of wild type sapstain fungi. As Sylvanex is competitive rather than toxic to wild type fungi, resistance is not expected to develop.

7.6 Conclusions

The efficacy data support the claim that Sylvanex will control blue-staining/sapstain fungi on freshly felled lodgepole pine logs. Confirmatory data described in Section 9.0 is required to support full registration.

Value summary

Proposed		Recommendation (based on value assessment)	Comments
Site/pest	**Details**		
Sapstain on freshly felled logs.	Typically used at a rate of 0.05–0.1 AU/ton of wood, or approximately 1×10^{11} CFU per cubic metre of wood. Apply to freshly cut wood at the felling site or soon thereafter at a log storage yard.	First determine the volume in litres of Sylvanex suspension required for daily operation by multiplying the total surface area in square metres of logs to be treated by 0.54 L. Then, determine the quantity of Sylvanex powder to be added to the water by using the following formula: Number of grams of Sylvanex required = [Volume, in litres, of Sylvanex suspension required for daily operation] × 50 ÷ [label guarantee (in AU/kg)]. Weigh the required quantity of dried powder and rehydrate it by mixing it with water that is 4–30°C and pH 5–8. Use a stirrer to get a vortex. Allow sufficient time for the mixture to become homogeneous before using it. The final Sylvanex suspension will have a concentration of 0.05 AU/L (5.0×10^{10} CFU per litre). This suspension must be used within 24 hours of mixing. The 0.05 AU/L suspension of Sylvanex is to be applied to logs immediately after the harvesting of the wood and cutting/delimbing of branches. The entire surface of each log must be sprayed at a rate of 540 mL per square metre of log surface. A hydraulic spray system, attached to the various cutting and delimbing equipment, must be used for application at the logging site.	a) To be used in the control of blue-staining sapstain fungi on freshly felled lodgepole pine and red pine logs at the felling site only. NOT for use on lumber or debarked logs. b) Sylvanex is to be used on freshly felled lodgepole pine and red pine logs as a preventative treatment against sapstain. c) A hydraulic spray system, attached to the various cutting and delimbing equipment, must be used for application at the logging site.

8.0 Toxic Substances Management Policy considerations

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

9.0 Regulatory decision

The active ingredient *Ophiostoma piliferum* strain D97 (Sylvanex Technical) and the associated end-use product Sylvanex have been granted temporary registration pursuant to Section 17 of the Pest Control Products Regulations for use as a biological antisapstain product to control blue-staining fungi on freshly felled logs at the felling site, subject to the following conditions:

- A new product specification form
- Annual Certificates of analysis
- Hypersensitivity incident reporting
- Additional storage stability
- A dermal toxicity study
- Efficacy data.

---

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

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

List of abbreviations

ANOVA	analysis of variance
AU	activity unit
CFU	colony forming unit
cps	centipoise
EP	end-use product
LOAEL	lowest observed adverse effect level
MAS	maximum average score
MIS	maximum irritation score
MPCA	microbial pest control agent
MRL	maximum residue limit
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NRRL	Northern Regional Research Laboratory
NZW	New Zealand white
PCP	pest control product
PDA	potato dextrose agar
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
QA	quality assurance
RFLP	restriction fragment length polymorphism
TGAI	technical grade active ingredient
TSMP	Toxic Substances Management Policy
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drugs Administration
UV	ultraviolet

Appendix I Toxicology

Table 1 Summary of toxicity and infectivity studies with *Ophiostoma piliferum* Strain C-1 det 5

STUDY	SPECIES/STRAIN AND DOSES	LD_{50} NOEL/NOAEL AND LOAEL	TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS
ACUTE STUDIES			
Oral toxicity/ pathogenicity	Rat—Crl: CD® (SD) BR VAF PLUS 15/sex treated with live C-1 det 5, 2 mL/kg bw, or ~ 10^9 CFU/kg bw 5/sex treated with heat-killed C-1 det 5, 2 mL/kg bw	LD_{50} greater than10^9 CFU/kg bw	No clinical signs indicative of toxicity or pathogenicity, no mortalities, no abnormalities on necropsy, no recovery of *Ophiostoma piliferum* from tissues (brain, heart, liver, lungs, spleen, kidneys, mesenteric lymph nodes), from gastrointestinal tract contents (stomach, small intestine or cecum), urine or feces on Sabouraud-Dextrose agar[1]. LOW TOXICITY, NOT PATHOGENIC
Pulmonary toxicity/ pathogenicity	Rat—Crl: CD® (SD) BR VAF PLUS 18/sex treated with live C-1 det 5, 1.2 mL/kg bw, or ~ 10^9 CFU/kg bw 5/sex treated with heat-killed C-1 det 5 1.2 mL/kg bw	LD_{50} greater than10^9 CFU/kg bw	No clinical signs indicative of toxicity or pathogenicity, no mortalities, no abnormalities on necropsy. Transient recovery of *Ophiostoma piliferum* from lungs (cleared by day 4). No recovery of *Ophiostoma piliferum* from other tissues (brain, heart, liver, spleen, kidneys, mesenteric lymph nodes), from gastrointestinal tract contents (stomach, small intestine or cecum), urine or feces on Sabouraud-Dextrose agar[1]. LOW TOXICITY, NOT PATHOGENIC
Dermal toxicity	Rabbit—NZW 5/sex treated with 2 mL live C-1 det 5 (2.3×10^9 CFU) on a 10 cm^2 area of the back, occluded for 24 hours, then washed off.	LD_{50} greater than 8×10^8 CFU/kg bw	No mortalities. No signs of skin irritation (see below). Definitive conclusions could not be drawn because too low a dose was administered. Note that a suspension of *Ophiostoma piliferum* strain C-1 det 5 was used in testing, not the EP. This is acceptable because no additional ingredients are incorporated in formulation of the EP and both strains are biologically equivalent. SUPPLEMENTAL
Dermal irritation	Rabbit—NZW See dermal toxicity study, above.	MIS[2] 0/8 (all timepoints) MAS[3] 0/8 (24, 48, 72 h)	No signs of dermal irritation. NON-IRRITATING

STUDY	SPECIES/STRAIN AND DOSES	LD_{50} NOEL/NOAEL AND LOAEL	TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS
Eye irritation	Rabbit—NZW 3/sex treated with 0.1 mL live C-1 det 5 (1.15×10^8 CFU)	MIS[2] 4.67/110 (1 h) MAS[3] 1.11/110 (24, 48, 72 h)	No corneal or iridial damage was observed. Conjunctival redness resolved by day 4. Note that the TGAI was used in testing, not the EP. Although no additional ingredients are incorporated during formulation of the EP, as a powder, the EP is potentially abrasive and therefore irritating to the eyes. SUPPLEMENTAL

[1] Although the sensitivity of detection of *Ophiostoma piliferum* on Sabouraud-Dextrose agar was not independently evaluated, the same medium was used effectively for titration of the inoculum, and recovery of *Ophiostoma piliferum* was reported from the lungs in the acute pulmonary study.

[2] MIS = maximum irritation score

[3] MAS = maximum average score

Appendix II Environmental assessment

Table 1 Risks of *Ophiostoma piliferum* strain D97 to non-target terrestrial organisms

Organism	Exposure	Test substance	Conclusions
Arthopods	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED** based on a limited potential for risk.
Birds	Oral/ Pulmonary/ Inhalation/ Injection	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED** based on a limited potential for risk.
Wild mammals	Acute	Not required	This guideline requirement was not triggered based on the lack of significant adverse effects in the published literature and in the toxicological studies described in Section 3.
Plants	Acute— *Pinus resinosa* *Pinus banksiana* *Pinus sylvestris* *Pinus taeda* *Pinus elliottii* *Pinus palustris*	*Ophiostoma piliferum* strain D97	Study: None of the wounded trees died during the study period and all the wounds were closed with the exception of two trees. In *P. taeda*, 1/10 wounds treated with *Ophiostoma piliferum* strain D97 and 1/10 wounds treated with the wild strain of *Ophiostoma piliferum* had callus tissue but pitch exudate prevented complete closure. Based on these results, *Ophiostoma piliferum* strain D97 is not pathogenic to six species of pine. This pathogenicity study is classified as acceptable but does not completely satisfy the PMRA's requirement for terrestrial toxicity/pathogenicity study, as no adequate observations were made to assess sublethal toxicity (e.g., growth vigour). Waiver rationale: The waiver rationale submitted by the applicant was **ACCEPTED** based on a limited potential for risk.
	Acute	Waiver rationale submitted in lieu of data	
Soil microorganisms	Acute	Not required	This data requirement was not triggered.
Non-arthropod invertebrates	Acute	Not required	This data requirement was not triggered.

Table 2 Risks of *Ophiostoma piliferum* strain D97 to non-target aquatic organisms

Organism	Exposure	Test substance	Conclusions
Freshwater arthropods	Acute—*Daphnia magna*	Cartapip 58 1000 mg/L (Nominal)	*Daphnia magna* study: No adverse effects were noted for any of the daphnids throughout the study. The LC_{50} was greater than 1000 mg/L. This study is classified as acceptable but does not fully satisfy the PMRA's requirement for an aquatic arthropod toxicity/pathogenicity study, no definitive conclusions could be made regarding the MPCA's potential for infectivity and pathogenicity. *Ceriodaphnia dubia* study: After 24 hours, 5/20 daphnids died in the group exposed to 100% effluent from treated wood chips. After 48 hours, a daphnid (1/20) died in each of the groups exposed to 50% and 100% effluent from untreated wood chips and 6/20 daphnids died in the group exposed to 100% effluent from wood chips treated with Cartapip 58. This study is classified as acceptable but does not fully satisfy the PMRA's requirement for an aquatic arthropod toxicity/pathogenicity study, as the test substance was neither the TGAI nor the EP and no definitive conclusions could be made regarding the MPCA's potential for infectivity and pathogenicity. No replacement study is required based on *Ophiostoma piliferum*'s biological properties and the absence of adverse effects in the published literature.
	Acute—*Ceriodaphnia dubia*	Effluent from Cartapip-treated wood chips 100, 50, 25, 12.5, 6.2 and 0%	

Organism	Exposure	Test substance	Conclusions
Freshwater fish	Acute	Cartapip 58 1000 mg/L (nominal)	No adverse effects were noted in any of the fish throughout the duration of the study. The LC_{50} was greater than 1000 mg/L. The rainbow trout toxicity study is classified as acceptable, but it does not completely satisfy the PMRA's requirement for a freshwater fish toxicity/pathogenicity study, as no definitive conclusions could be made regarding the MPCA's potential for infectivity and pathogenicity. No replacement study is required based on *Ophiostoma piliferum*'s biological properties and the absence of adverse effects in the published literature.
Freshwater plants	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED** based on a limited potential for risk.
Estuarine and marine arthropods	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED** based on a limited potential for risk.
Estuarine and marine fish	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED** based on a limited potential for risk.

References

Harrington, T. C. 1981. Cycloheximide sensitivity as a taxonomic character in *Ceratocystis*. *Mycologia*. 73:1123–1129.

Hunt, J. 1956. Taxonomy of the genus *Ceratocystis*. *Lloydia.* 19(1):1–30.

Uzunovic, A., D.-Q. Yang, P. Gagné, C. Breuil, L. Bernier, A. Byrne, M. Gignac, and S. H. Kim. 1999. Fungi that cause sapstain in Canadian softwoods. *Canadian Journal of Microbiology.* 45:914–922.

Verrall, A. F. 1939. Relative importance and seasonal prevalence of wood-staining fungi in the southern states. *Phytopathology.* 29:1031–1051.