US Environmental Protection Agency
Office of Pesticide Programs

BIOPESTICIDES REGISTRATION ACTION DOCUMENT

Chondrostereum purpureum strain PFC 2139 (PC Code 081308)

September 20, 2004

BIOPESTICIDES REGISTRATION ACTION DOCUMENT

Chondrostereum purpureum strain PFC 2139
(PC Code 081308)

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division
Microbial Pesticides Branch

TABLE OF CONTENTS

BIOPESTICIDE REGISTRATION ACTION DOCUMENT TEAM

Office of Pesticide Programs:

Biopesticides and Pollution Prevention Division

*Microbial Pesticide Branch***

Joel Gagliardi, Ph.D.	*Microbial Ecologist*
John Kough, Ph.D.	*Senior Scientist*
Susanne Cerrelli	Regulatory Action Leader
Jim Downing	Environmental Protection Specialist
Dennis Szuhay	Branch Chief
Brian Steinwand	Joint Review Coordinator, EPA

We wish to acknowledge the staff of **Health Canada, Pest Management Regulatory Agency who reviewed *Chondrostereum purpureum* strain PFC 2139 data jointly with the United States (U.S.) Environmental Protection Agency (EPA) in the Pesticides Joint Review Program.

Pest Management Regulatory Agency (PMRA)

Brian Belliveau, Ph.D.	Head, Biopesticides Evaluation Section Health Evaluation Division
Denis Rochon	Senior Evaluation Officer Biopesticides Evaluation Section Health Evaluation Division
Deborah Ashby	Evaluation Officer Biopesticides Evaluation Section Health Evaluation Division
Andrew Russell	Senior Evaluation Officer, Herbicides Section Efficacy and Sustainability Assessment Division, PMRA
Mark Brohm	Joint Review Coordinator, *PMRA*

I. EXECUTIVE SUMMARY

Cp-PFC 2139, and its end-use product, Chontrol Paste, were developed by MycoLogic, Inc. These biopesticide products were reviewed jointly by Health Canada's Pest Management Regulatory Agency (PMRA) and the United States (U.S.) Environmental Protection Agency (EPA) within the North American Free Trade Agreement's Technical Working Group (NAFTA TWG) on Pesticides Joint Review Program. On September 23, 2004, EPA issued a Conditional Registration for the Manufacturing Use Product (CP-PFC 2139) and its End-Use Product, (Chontrol Paste).

Chontrol Paste is a vegetation management product intended to inhibit sprouting and regrowth in cut stumps of certain deciduous tree species in rights-of-way and forests. *Chondrostereum purpureum* strain PFC 2139, is a naturally-occurring fungus. The only reported ecological niche for *C. purpureum* is in the xylem of living or recently dead broadleaf trees and shrubs. It is not host specific, having a wide host range as a wound pathogen. Despite its broad host range, its impact is limited. *C. purpureum* can invade only through fresh wounds in the xylem, and it is a weak pathogen, affecting only compromised trees. Healthy trees repel fungal infection with antifungal metabolites (phytoalexins) and by compartmentalizing infected tissues. Because the fungus can survive as a saprophyte, there is little selection pressure toward greater virulence or host specialization. *Chondrostereum purpureum* is disseminated through the production of numerous short-lived basidiospores from fertile fruiting bodies (basidiocarps).

A storage stability study showed a significant loss in potency over 90 days. Therefore the applicant must continue to ensure that the product release standards include a titre of at least 10^6 Colony Forming Units (CFU)/kg, and change the product label to reflect the limited storage stability of this product.

Toxicology and Pathogenicity

The acute toxicity and infectivity studies submitted in support of registration of *Cp-PFC 2139* and Chontrol Paste were reviewed. The data set included acceptable acute oral toxicity, acute dermal toxicity and acute eye irritation studies, with a supplemental acute pulmonary toxicity/infectivity study. The acute dermal toxicity data also contained sufficient information to make a decision on primary dermal irritation. No overt signs of toxicity were noted when *C. purpureum* strain PFC 2139 was administered to rats and rabbits via the oral and dermal routes of exposure. *Chondrostereum purpureum* strain PFC 2139 appeared to be slightly toxic in the rat via the intratracheal route, however, additional data and information are required to properly interpret these results as clinical signs (e.g., loss of body weight) were noted in both treated and control rats, and no explanation was provided for the single mortality noted on day 2. In rabbits, *C. purpureum* strain PFC 2139 was slightly irritating when applied dermally, and was minimally irritating when instilled in the eye. Furthermore, *C. purpureum* has not been reported to produce any mammalian toxins and is not known to infect mammalian tissues or grow at or near normal body temperature (37°C).

When handled according to the label instructions, the potential for applicator exposure is limited to the dermal route. On the basis of its biological properties, lack of toxicity and pathogenicity, and the proposed use pattern for Chontrol Paste, it is recommended that the label include standard personal protective equipment including gloves, long-sleeved shirt, long pants, shoes and socks when handling this product. For the TGAI, it is recommended that the label include these standard personal protective equipment (PPE) plus a dust/mist filtering respirator (MSH/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 to prevent inhalation exposure as a general precaution for all microbial products and since the acute pulmonary toxicity/infectivity study was considered to be supplemental.

Environmental assessment

Several published papers and field trials describing the environmental effects of *Chondrostereum purpureum* strain PFC 2139 following its use as a biological herbicide were submitted for review. These included analyses of genetic variation in native populations of *C. purpureum*, environmental fate field trials, environmental toxicology studies and environmental fate models that predicted sporulation and spore dispersal patterns.

The active ingredient, *C. purpureum*, is a ubiquitous organism with a continuously distributed population across North America. Although single restriction site polymorphisms (nuclear types) showed a polarized distribution across North America, both types do occur on either coast, and there is a convergence of types in central populations suggesting that gene flow is continuous across the continent. Where the entire genome was examined, variation was shown to be greater within populations than between geographic locations or host types. A field study measuring genetic similarity between introduced *C. purpureum* biocontrol strains and field-collected *C. purpureum* isolates gathered before and after a field release showed no increase in similarity to biocontrol strains between pre- and post-release field isolates. Taken together, these studies indicate that the application of a single biocontrol strain across North America will have a minimal effect on the genetic diversity of resident *C. purpureum* populations.

Chondrostereum purpureum is ubiquitous in the forest ecosystem, so non-target organisms are naturally exposed to a large number of spores. However, an extensive literature search found no reports of direct adverse effects on birds, wild mammals, fish, arthropods, non-arthropod invertebrates or aquatic plants. As expected, many articles identified *C. purpureum* as the causative agent of silverleaf disease in terrestrial plants. Acute mammalian toxicity testing showed that *C. purpureum* strain PFC 2139 is not toxic when administered orally. It may be slightly toxic but shows no signs of being pathogenic or infective on pulmonary exposure. Chontrol Paste is a slight dermal irritant, but it is practically non-irritating to the eyes. Because *C. purpureum* does not grow at 35°C and is killed by sustained incubation at 37°C, it is unlikely to be a pathogen of mammals or birds. Based on the lack of reported adverse effects, the lack of significant toxicity or infectivity in acute mammalian toxicity/infectivity studies, and the inability of *C. purpureum* to grow at high temperatures, adverse effects due to the use of *C. purpureum* strain PFC 2139 are not expected to birds, wild mammals, fish, arthropods, non-arthropod invertebrates and aquatic plants.

The risk to non-target terrestrial plants was addressed in several studies. Chontrol Paste is to be applied as a mycelial paste to stumps immediately after cutting the tree near the ground. The products paste formulation is expected to minimize the exposure of non-target plants to *C. purpureum* mycelia. This was demonstrated in a research article showing no recovery of *C. purpureum* strain PFC 2139 from areas adjacent to a site treated with mycelial paste. Wounded non-target trees are more likely to be infected by spores from fruiting bodies growing on untreated stumps. Environmental models of sporulation and spore dispersal in *C. purpureum* suggest that the additional spore density contributed by the deployment of biological control strains is equal in magnitude to, or less than, the density of naturally occurring spores from resident *C. purpureum* populations. The incremental increase in spore density due to biocontrol operations is not expected to increase the likelihood of non-target effects. Tree wounding, not spore load, appears to be the primary determinant of infection, and the overall health of a tree appears to determine the extent of disease progression. Although buffer zones have been considered for *C. purpureum* biocontrol products, empirical studies indicate that no buffer zone is required since non-target healthy trees are at negligible risk, while wounded trees are equally vulnerable to resident populations of *C. purpureum* as they are to introduced biocontrol strains.

II. OVERVIEW

A. Product Overview

Microbial Pesticide Name: *Chondrostereum purpureum* strain PFC 2139

Trade Name(s): Chontrol Paste (end-use product)
CP-PFC 2139 (manufacturing use product)

PC Code: 081308

ATCC/Culture
Collection Number: *60854*

Basic Manufacturer: MycoLogic Inc.
Department of Biology
University of Victoria
P.O. Box 3020
Victoria, BC, Canada V8W 3N5

US Agent: Dr. Michael Braverman, IR-4 Project
IR-4 Headquarters
Center for Speciality Crop Pest Management
Rutgers, The State University of New Jersey
681 US #1 South
North Brunswick, NJ 08902-3390

B. Use Profile

Type of Pesticide: Microbial Herbicide

Use Sites: Chontrol Paste is a vegetation management product intended to inhibit sprouting and regrowth from cut stumps of certain deciduous (hardwood) tree species in rights-of-way, and in forest vegetation management.

Target Pests for Active Ingredient: hardwood trees and shrubs including red alder, sitka alder, speckled alder and trembling aspen.

Formulation Types: The Manufacturing Use Product is a wettable powder and the End-Use Product is a paste.

Method and Rates of Application: Topically applied as a mycelial paste formula to entire surface of freshly cut stump during summer or autumn forestry cutting operations. About 5.0 mL of Chontrol Paste is applied with a squeeze bottle to each cut surface of a 2 to 6 cm stump (approximately 5,000 CFU per stump).

C. Estimated Usage

This is the first conditional registration of the active ingredient, so estimated usage data are not available.

D. Data Requirements

The submissions to comply with Agency data requirements for granting this conditional registration under Section 3(c)(7)(C) of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) have been reviewed by the Biopesticides and Pollution Prevention Division (BPPD). For *Chondrostereum purpureum* strain PFC 2139, the product identity and analysis data, as well as the data and information submitted for acute mammalian toxicology and ecological effects, are sufficient to allow the proposed use patterns with only one exception. Based on evaluations of submitted data, as discussed in this document, the Agency foresees no unreasonable adverse effects to human health and the environment from the use of *Chondrostereum purpureum* strain PFC 2139, as long as it is used as labeled.

Conditions of registration for this new active ingredient are replacement of the acute pulmonary infectivity and toxicity study or alternatively submission of an acute intraperitoneal injection infectivity study to satisfactorily address the toxicity and infectivity potential of *Chondrostereum purpureum* strain PFC 2139. If more extensive use patterns are sought including treatment of other sites or crops, additional information and data will be required on a case-by-case basis.

E. Regulatory History

In 1990, the Netherlands had an application for a forestry product based on *Chondrostereum purpureum.* However that product was never registered in the Netherlands because the applicant withdrew the application.

In Canada, *C. purpureum* has been investigated as a mycoherbicide since 1990. Myco-Tech Paste, a stump re-sprouting inhibitor containing *C. purpureum* strain HQ-1 has been registered in Canada since 2002 (Reg. No. 27020).

On April 15, 1999 a formal request was submitted to EPA by Mycologic Incorporated for a Joint Review of the *Chondrostereum purpureum* strain PFC 2139 submission with both the PMRA in Canada and the Environmental Protection Agency in the United States. Subsequently, Mycologic Incorporated submitted an application to EPA on August 2, 2001 for registration of the end-use product Chontrol Paste and CP-PFC 2139 Manufacturing Use Product which contain the active ingredient *Chondrostereum purpureum* strain PFC 2139. On September 23, 2004 , EPA issued a Conditional Registration for the Manufacturing Use Product (CP-PFC 2139) and its End-Use Product (Chontrol Paste).

III. SCIENCE ASSESSMENT

A. Physical and Chemical Properties Assessment

Identity of the active micro-organism

Active Micro-organism	***Chondrostereum purpureum* strain PFC 2139**
Function	Mycoherbicide
Binomial name:	*Chondrostereum purpureum* (Pers. ex. Fr.) Pouzar isolate PFC 2139
Taxonomic designation:	
Kingdom:	Eumycota
Phylum:	Dikaryomycota
Subphylum:	Basidiomycotina
Class:	Holobasidiomycetes
Order:	Aphyllophorales
Family:	Corticiaceae
Genus:	*Chondrostereum*
Species:	*purpureum*
Strain:	PFC 2139
Patent Status Information:	Canadian: None. U.S.: 5,587,158 "Biological control for weed trees" Wall, R. et al. 1996.
Nominal purity of active	10^5 to 10^7 CFU/kg of the End-Use Product, Chontrol Paste

1. Product Identity

The EPA has classified *Chondrostereum purpureum* strain PFC 2139 as a microbial pesticide. Chontrol Paste and Cp-PFC 2139 are products that contain living *Chondrostereum purpureum* strain PFC 2139 as the active ingredient. Chontrol Paste is a formulated product containing living mycelia of the fungus *Chondrostereum purpureum* isolate PFC 2139 intended for application to freshly cut stumps of weedy deciduous brush species in rights-of-way and forest vegetation management situations. The product is designed to contain 10^5 to 10^7 CFU/kg with an average of 5 grams of Chontrol Paste applied per stump (approximately 5,000 CFU per stump), depending on the stump diameter. Use of the product is proposed across Canada and the United States for the inhibition of resprouting and regrowth from cut stumps of deciduous trees and shrubs, including red alder, sitka alder, speckled alder and trembling aspen.
The use of *C. purpureum* as a vegetation management tool was first reported in the Netherlands, where a mycelial inoculum was applied to freshly cut stumps of *Prunus serotina* (black cherry), an introduced tree species in the Netherlands. In Canada, *C. purpureum* has been investigated as a mycoherbicide since 1990. Myco-Tech Paste, a stump resprouting inhibitor containing *C. purpureum* strain HQ-1 has been registered in Canada since 2002.

2. Mode of Action

Chondrostereum purpureum strain PFC 2139 was isolated from a canker on a red alder (*Alnus rubra* Bong) on Vancouver Island near Duncan, British Columbia in 1994. The canker developed as a result of inoculation in July 1993 with strain PFC2090, originally isolated from an apple tree (*Malus* spp.) in Saanicton, British Columbia in 1989. It is naturally occurring and has not been genetically modified.

Chondrostereum purpureum is a cosmopolitan species that is widely distributed in temperate regions of the northern and southern hemispheres. It is ubiquitous in Canada and common in the United States south to Virginia in the east and to northern California in the west. The only reported ecological niche for *C. purpureum* is in the xylem of living or recently dead broadleaf trees and shrubs. It is a white-rot fungus, and the causative agent of silverleaf, an important disease in fruit trees. Silverleaf symptoms arise when the *C. purpureum* mycelia occlude xylem vessels, blocking the transfer of sap to the leaves. The leaf epidermis detaches from the palisadic parenchyma, and the air gap between these layers gives the leaves a “silvery” appearance. Pectinase enzymes released by *C. purpureum* may also contribute to silverleaf symptoms. *Chondrostereum purpureum* also produces sterpurane sesquiterpenes. Several sterpuranes, such as sterpuric acid, sterepolide and dihydrosterepolide, have been reported to induce toxicity symptoms such as leaf yellowing. *Chondrostereum purpureum* is not host specific, having a wide host range as a wound pathogen of deciduous trees and shrubs. In spite of its broad host range, its impact is limited. It can invade only through fresh wounds in the xylem, and it is a weak pathogen causing a mild sapstreak in many infected trees and killing only severely compromised trees. Healthy trees repel fungal infection with antifungal metabolites (phytoalexins) and by compartmentalizing infected tissues. Because the fungus can survive as a saprophyte there is little selection pressure toward greater virulence or host specialization.

Chondrostereum purpureum is disseminated through the production of numerous short-lived basidiospores from fertile fruiting bodies (basidiocarps). Sporophores develop when numerous terminal dikaryotic cells enlarge to form basidia which, after fusion of haploid nuclei, produce four basidiospores. This usually occurs between one and three years after infection is initiated. If the host tree is killed, *C. purpureum* may continue

to fructify on dead tissues until replaced by more competitive saprophytic species. Sporophores only produce spores after immersion in free water, or if grown on a substrate with greater than 75% moisture content. This makes rainfall the most important environmental factor governing spore release. Released spores are sensitive to sunlight and dry conditions, and are considered unlikely to survive for more than five hours. This suggests that long distance translocation of spores is unlikely. Infection of a new host begins with the deposition of basidiospores on fresh stem wounds or the surface of cut stumps. In fresh wounds the small basidiospores are drawn by capillary action up to 20 mm into the xylem tissues where they are free from competition with other fungi. Sporulation is reported as optimum below 20°C with wet conditions (> 90 % relative humidity), and spores optimally germinate within 24 hours at 17.5 - 28°C and >75% water content, though a high germination rate was also noted at 5°C. Spore-producing basidiocarps are reported to retain viability despite 12 months of dehydration and can resume basidiospore production within 6-8 hours of rehydration.

Chondrostereum purpureum is heterothallic. This type of sexual reproduction promotes outbreeding because successful conjugation occurs between two genetically distinct, but compatible, mycelia. Sexual compatibility is controlled by specific genetic determinants known as mating factors, controlled by many alleles, occurring at two loci. Compatibility testing among North American isolates and between North American isolates and those from Sweden and Norway indicates that *C. purpureum* maintains a highly diverse population of mating type alleles. Consequently, a significant level of genetic variation within the species is expected. In studies where isolates from across North America were tested, considerable heterogeneity was indeed observed using randomly amplified polymorphic DNA (RAPD) analysis and sequence characterized amplified region (SCAR) analysis. Genetic diversity appears to be continuously distributed across North America. Although single restriction site polymorphisms (nuclear types) showed a polarized distribution across the continent, both types do occur on either coast and there is a convergence of types in central populations suggesting that gene flow is continuous across the continent. Where the entire genome was examined, variation was shown to be greater within populations than between geographic locations or host types.

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

Technical Product: Cp-PFC 2139

Property	Result
Physical state	white powder
Odor	slight mushroom smell
Specific gravity	1.5-2.5 g/mL
Viscosity	NA
Corrosion character	not reported (non-oxidizing or reducing)
Wettability	water dispersable
pH (in solution)	5-8 as a 10% suspension
Moisture content	~17%

End-Use Product: Chontrol Paste

Property	Result
Physical state	white paste
Odor	slight corn oil and mushroom smell
Specific gravity	1.0 - 2.0 g/mL
Viscosity	paste or thick gel (similar to mayonnaise)
Corrosion character	not reported (non oxidizing or reducing)
Wettability	water dispersable
pH (in solution)	5-8 as a 10% dispersion
Moisture content	~40%

Product chemistry data which support the registration of *Chondrostereum purpureum* strain PFC 2139 as a Microbial Pest Control Agent (MPCA) are summarized below.

OPPTS GUIDELINE Number	STUDY	RESULT	MRID#
885.11	Product Identity and Disclosure of Ingredients	Acceptable	45493302
885.12	Manufacturing Process	Acceptable	45493302
885.13	Formation of Unintentional Ingredients	Acceptable	45493302
885.14	Analysis of Samples	Acceptable	45493302
885.15	Certification of Limits	Acceptable	45493302
830.6302 830.6303 830.6304 830.7000 885.7300	Product Chemistry	Acceptable	45493302

B. Human Health Assessment

There is a reasonable certainty that no harm will result from exposure to *Chondrostereum purpureum* strain PFC 2139. This includes all anticipated dietary exposures and all other exposures for which there is reliable information.

1. Toxicology and Pathogenicity Assessment

a. Acute Toxicity and Pathogenicity

Acute oral toxicity/pathogenicity

The toxicity of *C. purpureum* strain PFC 2139 was assessed by acute oral challenge in rats. In a preliminary test for lethality, fasted male and female rats (3/sex) were challenged by oral gavage with a suspension of *C. purpureum* strain PFC 2139 mixed in sterile water at a dose of 5 g/kg body weight (bw). No signs of toxicity were noted during the 4-day observation period. In the definitive study, male and female rats (5/sex) were fasted overnight and treated by gavage with a single 5g dose per kg bw (containing $1.2x10^6$ CFU) of undiluted suspension of *C. purpureum* strain PFC 2139 (equivalent to $2.5 – 4.4 \times 10^5$ CFU/animal). The animals were observed at least daily for 14 days, and weighed weekly. A gross necropsy was performed on all animals at study termination. All rats survived the14-day period and all animals, male and female, gained weight steadily during the study. No signs of toxicity were noted for any animal and there were no gross lesions noted at necropsy. *Chondrostereum purpureum* strain PFC 2139 was of low toxicity, as the combined LD_{50} was greater than 5g/kg bw (1.2×10^6 CFU/kg bw). The formulant ingredients were not assessed, though a review of potential toxicity (MSDS sheets corresponding to the CAS numbers provided) and levels of the formulants in the EP indicates that, at most, mild irritation would result from ingestion.

The oral exposure studies did not investigate the infective potential of the microbial pest control agent (MPCA). The test dose was lower than that normally required for acute oral toxicity/infectivity testing (10^8 CFU per animal), although the administered volume of 5g/kg bw is in accordance with chemical guideline limit testing for acute oral toxicity. Because the concentration of viable units (CFU) in the TGAI is low, the infectivity guideline dose levels are not achievable in this volume. Note that the TGAI label guarantee is 10^4–10^5 CFU/g, which is lower than the test concentration, and formulation of the EP further dilutes the MPCA. In addition, public literature shows *C. purpureum* is killed at 37°C, and pulmonary infectivity testing suggested that *C. purpureum* strain PFC 2139 is unlikely to be infective or pathogenic, with clearance achieved by 7 days. Based on the results of the acute oral toxicity and acute pulmonary toxicity/infectivity studies, and published literature showing that *C. purpureum* is killed at 37°C, no oral infectivity testing is required to assess the risks of *C. purpureum* strain PFC 2139.

Acute pulmonary toxicity/pathogenicity

The acute pulmonary toxicity and infectivity of *C. purpureum* strain PFC 2139 was assessed by intratracheal instillation in rats. In a preliminary test for lethality, 0.1 mL of an undiluted suspension of *C. purpureum* strain PFC 2139 containing approximately 1.76×10^4 CFU was dosed by intratracheal administration to male and female rats (3/sex). No signs of toxicity were noted during the 4-day observation period. In the definitive study, male and female rats (20/sex) were dosed with approximately 1.6×10^4 CFU of the test substance (TS) and an additional group of male and female rats (20/sex) was treated with autoclaved test substance (KTS). Ten rats (5/sex) served as shelf controls (SC) and 40 rats (20/sex) served as naive controls (NC). Animals were observed daily and weighed weekly for 14 days, with two female NC rats weighed on day 16. Five animals per sex per group from the TS, KTS and NC groups were sacrificed by carbon dioxide asphyxiation on days 0, 7 and 14, and ten rats (5/sex) in the SC group were sacrificed at study termination on day 14. These animals were subject to gross necropsy where macroscopic examinations of cranial, thoracic

and abdominal tissues, and microbiological enumeration of the MPCA in the blood, brain, lungs, liver, spleen, kidneys and cecum were performed.

Clinical signs of rough hair coat and labored respiration were reported from days 2–4 in several rats dosed with either live or autoclaved test substance. One female rat in the NC group appeared lethargic and thin starting on day 7, with hunched posture appearing on day 9 and rough hair coat on day 13. Eight surviving male and three female rats in the TS group, one male and five female rats in the KTS group, one male and two female rats in the SC group and five females in the NC group lost weight during the first week of the study. Between days 7 and 14–16, one female TS rat, three female KTS rats, and four female NC rats lost weight. Overall study weight losses were recorded in one female TS rat and four female NC rats. A male TS rat died on day 2. This rat had clinically affected lung tissue but no viable *C. purpureum*. No explanation was provided on the cause of death. One female KTS rat died due to anesthesia and was replaced.

Chondrostereum purpureum was detected in lungs and associated lymph nodes after dosing with the live substance, with clearance by day 7. On necropsy, findings such as mottled/pale lung parenchyma, mottled lung intermediate lobe, and mottled left lungs were noted in TS- and KTS-treated rats. These findings were consistent with the method of dosing and were attributed to the body's normal immunological response to a foreign substance. No gross lesions were noted in animals from NC or SC groups. Significantly increased lung and associated lymph node and decreased kidney weights reported in TS and KTS groups through day 14 were likely a result of a normal immunological response. On day 14, increased lung and lymph node and decreased liver weights were observed for male rats in the SC group.

The results of this study are difficult to interpret because clinical signs were observed in both treated and control rats. These signs may have been due to an infection in the rat colony, affecting both treated and control animals, which could have obscured the true effects of *C. purpureum* strain PFC 2139. This acute pulmonary toxicity/infectivity study is therefore considered to be supplemental. Additional data are required to properly address the mortality noted on day 2 and the clinical signs (e.g., loss of body weight) noted in female NC rats. Although there was one mortality attributed to treatment at this dose, and signs of toxicity such as weight loss, *C. purpureum* strain PFC 2139 is unlikely to be infective or pathogenic. Clearance was achieved within 7 days in this study, and *C. purpureum* is known to be killed at mammalian body temperatures.

Acute Intraperitoneal Injection Infectivity

Mycologic, Inc. submitted a waiver request in place of an acute intraperitoneal injection infectivity study. This request was based on the rationale that *C. purpureum* strain PFC 2139 is not infectious to mammalian species, and was supported by the results of the acute mammalian testing.

Chondrostereum purpureum strain PFC 2139 was not toxic or overtly irritating in acute mammalian studies, and was not considered to be infectious or overtly toxic in the acute pulmonary toxicity/infectivity study. In addition, *C. purpureum* does not grow at 35°C and is killed at 37°C. Consequently, *C. purpureum* strain PFC 2139 is unlikely to be infective to humans.

Acute dermal toxicity

The acute dermal toxicity of Chontrol Paste was assessed in rabbits. Male and female New Zealand White rabbits (5/sex) were prepared by clipping the dorsal fur of approximately 10% of the body surface. Chontrol Paste was applied to the clipped dorsal trunk at a dose of 2000 mg/kg bw or $6.6 – 7.8 \times 10^4$ CFU/animal. Each treated site was covered with a 12.8 x 11.5 cm surgical dressing and plastic film secured with a lint-free cloth and elastic adhesive bandage. After 24 hours, the dressing was removed and the test substance washed away with a water-moistened gauze pad. The rabbits were observed twice daily for mortality (daily during the weekend), and for clinical signs of toxicity frequently on the day after treatment, then at least daily thereafter for 14 days. At study termination, the animals were sacrificed without necropsy. There were no mortalities or overt clinical signs reported following dosing. There was no edema in the treated areas, though 9 of 10 treated rabbits had mild erythema that cleared by day 5. The remaining rabbit exhibited no signs of irritation. All tested rabbits gained weight steadily throughout the study.

Based on these results, Chontrol Paste is considered to be of low dermal toxicity and the combined LD_{50} was greater than 2000 mg/kg bw.

Primary dermal irritation

As part of the dermal toxicity study, dermal irritation was scored in accordance with standard guidelines (OECD 404; OPPTS 870.2500). The dose and duration of exposure exceeded those specified in the guidelines, and may be considered to represent a worst-case exposure scenario. Very slight to well-defined erythema was noted in 9/10 rabbits treated with Chontrol Paste. Irritation had cleared within 96 hours following patch removal. The remaining rabbit exhibited no signs of irritation. The maximum irritation score (MIS) was 1.2/8 (1 hour)and the maximum average score (MAS) was 0.6/8 (24, 48, 72 hours). Based on the MIS of 1.2/8 (1 hour) and clearance of the symptoms, Chontrol Paste is considered to be slightly irritating. No signal words or precautionary statements are required on the TGAI or EP product labels.

Reporting of hypersensitivity incidence

Mycologic, Inc. reported no incidents of hypersensitivity during extensive field testing. The formulation ingredients are common industrial or food grade materials. Two of these formulants are considered to be minor respiratory or eye irritants when in dry form, though they should not be implicated in a hypersensitive response as a paste. The active ingredient, *C. purpureum* strain PFC 2139, consists of mycelia only and does not form conidia known to induce allergies and hypersensitivity. Continued surveillance and reporting of hypersensitivity incidents is required.

Genotoxic potential

Chondrostereum purpureum has not been reported to produce genotoxins.

Primary eye irritation

The eye irritation potential of Chontrol Paste was assessed by ocular instillation in the rabbit. Three female New Zealand White rabbits were treated with 0.1 mL of undiluted Chontrol Paste (containing 5.6×10^2 CFU/g), in one eye. The untreated eye served as a control. Animals were observed for signs of ocular

irritation one hour, and 1, 2, and 3 days after dosing. No corneal opacity, iritis, or positive conjunctival irritation (score ≥ 2) was noted in any rabbit. Redness of conjunctiva was noted in two animals at 24 and 48 hours. The MIS was 1.3 (24 hours) and the MAS was 0.7 (24, 48 hours), with clearance by 72 hours. Based on the degree and the duration of the irritation noted, Chontrol Paste is minimally irritating to the eyes of rabbits.

Reproductive and developmental toxicity

No reproductive or developmental toxicity studies were submitted. Because survival, replication, infectivity, significant toxicity or persistence of *C. purpureum* strain PFC 2139 were not observed in the Tier I acute pulmonary test, no reproductive or developmental toxic effects are expected upon exposure to *C. purpureum*.

Neurotoxicity (acute, delayed and sub-chronic)

No neurotoxicity studies were submitted. No neurological signs were noted in the course of acute studies, and none are expected to occur as a result of exposure to *C. purpureum*.

Toxicity Data Requirements

OPPTS GUIDELINE NUMBER	STUDY	RESULT	MRID#
885.3050	Acute Oral Toxicity/ Pathogenicity in Rat - strain CD® (SD)	LD_{50} greater than 5g/kg bw or 1.2×10^6 CFU/kg bw No clinical signs indicative of toxicity, no mortalities and no abnormalities on necropsy. LOW TOXICITY Acceptable, Toxicity Category IV Oral Infectivity - WAIVED	45493303
885.3100	Acute Dermal Toxicity/ Pathogenicity in Rabbit - strain NZW	LD_{50} of *Chondrostereum purpureum* strain PFC 2139 in rats is greater than $3.4x10^4$ CFU/kg bw No mortalities or abnormalities prior to study termination. Following unwrapping, very slight erythema in 3/5 females (♀) and 3/5 males (♂) and well-defined erythema in 1/5 ♂ and 2/5 ♀ was observed. Irritation cleared within 96 hours following patch removal. LOW TOXICITY Acceptable, Toxicity Category III	45507103

OPPTS GUIDELINE NUMBER	STUDY	RESULT	MRID#
885.3150	Acute Pulmonary Toxicity/ Pathogenicity in Rat - strain CD® (SD)	LD50 greater than 1.6×10^4 CFU/animal Slightly Toxic, Not Pathogenic **Supplemental**	45507102
870.1300	Acute Inhalation (End-Use Product Chontrol Paste)	Not applicable The end-use product is a non-volatile paste.	NA
885.3200	Acute Intravenous Toxicity/Pathogenicity	There were no signs of pathogenicity in the Acute Pulmonary Toxicity/Pathogenicity or acute oral toxicity studies that were submitted. Intraperitoneal exposure is unlikely from the paste application of *Chondrostereum purpureum* strain PFC 2139 to cut tree stumps. WAIVED	NA
870.2400	Primary Eye Irritation in Rabbit - strain NZW	Mild conjunctival redness was noted in one animal at the 24 and 48 hour time points, and in a second animal at the 24 hour time point. MINIMALLY IRRITATING. Acceptable, Toxicity Category IV	45507104
870.2500	Primary Dermal Irritation in Rabbit - strain NZW	See Acute Dermal Toxicity/Pathogenicity results above MINIMALLY IRRITATING Acceptable, Toxicity Category III	45507103
870.2600	Delayed Contact Hypersensitivity in Guinea Pigs	No incidents reported. Continued surveillance and reporting of hypersensitivity incidents is required. WAIVED	NA

Integrated toxicity and infectivity summary

The acute toxicity and infectivity studies submitted in support of *Cp-PFC 2139* and Chontrol Paste registrations were reviewed. The data set included acceptable acute oral toxicity, acute dermal toxicity and acute eye irritation studies, and a supplemental acute pulmonary toxicity/infectivity study. The acute dermal toxicity study also contained sufficient data to make a decision on primary dermal irritation. No overt signs of toxicity were noted when *C. purpureum* strain PFC 2139 was administered to rats and rabbits via the oral and dermal routes of exposure. *Chondrostereum purpureum* strain PFC 2139 appeared to be slightly toxic in

the rat via the intratracheal route, however, additional data and information are required to properly interpret these results as clinical signs (e.g., loss of body weight) were noted in both treated and control rats, and no explanation was provided for the single mortality on day 2. In rabbits, *C. purpureum* strain PFC 2139 was slightly irritating when applied dermally, and was minimally irritating when instilled into the eye. *Chondrostereum purpureum* is not known to produce mammalian toxins.

b. Subchronic Toxicity and Chronic Toxicity

Subchronic and chronic toxicity testing was not required because survival, replication, infectivity, toxicity, or persistence of the microbial agent were not observed in the test animals treated in the Tier 1 acute pulmonary test.

c. Effects on the Immune and Endocrine Systems

EPA is required under section 408(p) of the FFDCA, as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) "may have an effect in humans that is similar to an effect produced by a naturally-occurring estrogen, or other such endocrine effects as the Administrator may designate." Following the recommendations of its Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), EPA determined that there is no scientific basis for including, as part of the screening program, the androgen and thyroid hormone systems in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use FIFRA and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA authority to require wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP). When the appropriate screening and/or testing protocols being considered under the EPA's EDSP have been developed, *Chondrostereum purpureum* strain PFC 2139 may be subjected to additional screening and/or testing to better characterize effects related to endocrine disruption.

To date, the EPA has no information to suggest that *Chondrostereum purpureum* strain PFC 2139 has an effect on endocrine systems. Moreover, as is expected from a non-pathogenic microorganism that is practically non-toxic to mammals, the submitted toxicity/pathogenicity studies in rodents indicated that following several routes of exposure, the immune system is still intact and able to process and clear the active ingredient.

When the appropriate screening and/or testing protocols being considered under the EPA's Endocrine Disruptor Screening Program have been developed, *Chondrostereum purpureum* strain PFC 2139 may be subjected to additional screening and/or testing to better characterize effects related to endocrine disruption. Based on the weight of the evidence of available data, no endocrine system-related effects have been identified for *Chondrostereum purpureum* strain PFC 2139.

2. Dose Response Assessment

No toxicological endpoints are identified.

3. Dietary Exposure and Risk Characterization (includes drinking water)

a. Dietary Exposure and Risk

In the absence of any toxicological endpoints, risk from the consumption of residues is not expected for the general population, including infants and children. Chontrol Paste will be applied to freshly cut stumps. It will not be applied to food or feed crops, hence exposure from food or feed is unlikely. Although *C. purpureum* is ubiquitous in nature, no adverse effects from dietary exposure have been attributed to this species. Furthermore, no adverse effects were observed in the acute oral toxicity study in mice and there are no reports of known mammalian toxins being produced by this MPCA.

4. Occupational, Residential, School and Day Care Exposure and Risk Characterization

a. Occupational and Residential Exposure and Risk

The human health and safety studies reviewed showed that Chontrol Paste and *C. purpureum* strain PFC 2139 is of low acute toxicity via the oral, pulmonary and dermal routes of exposure, and is not likely to be pathogenic by intratracheal instillation. However, like all microbial pesticides, *C. purpureum* is considered to be a potential sensitizer, though there have been no reports of hypersensitivity incidents. Irritation studies in rabbits showed that Chontrol Paste was slightly irritating to the skin and minimally irritating to the eyes.

The proposed use for *C. purpureum* is as a biological herbicide for control of sprouting or regrowth of deciduous trees and shrubs in rights-of-way and forest vegetation management situations. Chontrol Paste is to be applied topically as a paste on freshly cut stumps during summer or autumn at an average rate of 5 g/stump (approximately 5,000 CFU per stump), depending on the stump diameter. When handled according to label instructions, applicator exposure is limited to the dermal route. The potential for bystander exposure is minimal during application. The potential for human exposure following fructification is possible via inhalation due to the release of basidiospores. However, the intentional use of this active ingredient is unlikely to result in a significant increase in the natural background levels of basidiospores produced by this species, as it is already naturally abundant throughout Canada and the United States.

The required standard personal protective equipment (including gloves) for Chontrol Paste when handling this product will minimize exposure to workers. For the TGAI, it is also recommended that the label include standard personal protective equipment, including a dust/mist filtering respirator (MSH/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 to prevent inhalation exposure as a general precaution for all microbial pesticide products, and since the acute pulmonary toxicity/infectivity study was considered to be supplemental.

b. Residential, School and Day Care Exposure and Risk Characterization

Because no toxic endpoints for mammals have been identified, and because no toxic effects have been reported from limited human exposure, no toxicity or pathogenicity is expected from aggregate exposure of the public via inhalation, dermal, and oral routes of exposure.

5. Drinking Water Exposure and Risk Characterization

Although heavy rainfall might carry *C. purpureum* into aquatic environments (e.g., runoff from treated stumps), this MPCA is not expected to proliferate in aquatic habitats. Moreover, *C. purpureum* is not considered to pose a risk to drinking water. Both percolation through soil and municipal treatment of drinking water would reduce the possibility of significant transfer of residues to drinking water. Accordingly, the use of this MPCA on terrestrial plants is not anticipated to negatively impact the quality of drinking water.

6. Acute and Chronic Dietary Risks for Sensitive Subpopulations, Particularly Infants and Children

There have been no confirmed reports of immediate or delayed allergic reactions to *Chondrostereum purpureum* strain PFC 2139.

Based on the acute toxicity information discussed above, EPA concludes that there is a reasonable certainty that no harm will result from aggregate exposure to the United States population, including infants and children, to residues of *Chondrostereum purpureum* strain PFC 2139. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The EPA has arrived at this conclusion because, as discussed above in Unit B. Human Risk Assessment, *Chondrostereum purpureum* strain PFC 2139 is practically non-toxic to mammals and under reasonably foreseeable circumstances it does not pose a risk.

FFDCA section 408 provides that EPA shall apply an additional ten-fold margin of exposure (safety) for infants and children in the case of threshold effects to account for pre- and post-natal toxicity and the completeness of the database, 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, the EPA believes there is reliable data to support the conclusion that *Chondrostereum purpureum* strain PFC 2139 is practically non-toxic to mammals, including infants and children, and, thus, there are no threshold effects; therefore, EPA has not used a margin of exposure (safety) approach to assess the safety of *Chondrostereum purpureum* strain PFC 2139. As a result, the provision requiring an additional margin of exposure (safety) does not apply.

7. Aggregate Exposure from Multiple Routes Including Dermal, Oral, and Inhalation

Because no toxic endpoints for mammals have been identified, and because no toxic effects have been reported from limited human exposure, no toxicity or pathogenicity is expected from aggregate exposure of the public via inhalation, dermal, and oral routes of exposure. Worker exposure via inhalation and dermal routes will be minimal because the end-use product is a paste and personal protective equipment are required for applicators and other handlers.

Based on the available information, EPA concludes that there is a reasonable certainty that no harm will result from aggregate exposure to the United States population, including infants and children, to residues of *Chondrostereum purpureum* strain PFC 2139. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. EPA has arrived at this conclusion because *Chondrostereum purpureum* strain PFC 2139 shows no evidence of toxicity or infectivity in any animal.

8. Cumulative Effects

Chondrostereum purpureum strain PFC 2139 is practically non-toxic to mammals and has an extremely low use rate. No mechanism of toxicity in mammals has been identified for this organism. Therefore, no cumulative effect with other related organisms is anticipated. Currently, there is only one other *Chondrostereum purpureum* product registered in the U.S.: CP-PFC 2139 (manufacturing use product).

C. Environmental Assessment

1. Environmental Fate

Several published papers and field trials describing the environmental fate of *Chondrostereum purpureum* strain PFC 2139 following its use as a biological herbicide were submitted for review. These included analyses of genetic variation in native populations of *C. purpureum*, environmental fate field trials and environmental fate models which predicted sporulation and spore dispersal patterns.

Chondrostereum purpureum is ubiquitous in northern North America, south to Virginia in the east and to northern California in the west, and in temperate, moist zones around the world. Optimal vegetative growth occurs between 20 and 25°C. No growth occurs at 35°C, but cultures incubated at 35°C can resume normal growth on incubation at 25°C. Incubation at 37°C for 7 days is lethal (Wall 1986; Ekramoddoullah et al. 1993). Rainfall appears to be the primary requirement for sporulation. Spiers (1985) observed sporulation only in basidiocarps that had been immersed in water or in those that had been grown on agar medium and had a water content of ≥75%. Optimum sporulation is reported at 18°C (Spiers 1985; Dye 1974), and spores germinate within 24 hours at temperatures between 17.5 and 28°C on media with ≥75% water content, though germination also occurs at 5°C (Spiers and Hopcroft 1988). Sporophores are reported to retain viability despite 12 months of dehydration (Buller 1958) and can resume basidiospore production within 6–8 hours of re-hydration (Spiers and Hopcroft 1988).

Genetic Characterization of Native Populations of *Chondrostereum purpureum*

The degree of diversity and geographic or host specialization was investigated in naturally occurring *C. purpureum* populations using several molecular techniques.

Ekramoddoullah et al. (1993) found little phenotypic variation in enzyme biochemistry, growth temperature, virulence and protein profiles among isolates of *C. purpureum* collected from New Brunswick, Vancouver Island (British Columbia) and southern mainland British Columbia. Any variation that was observed did not appear to be related to host type or geographical location.

Considerable genetic diversity was reported by Gosselin et al. (1999) in 93 isolates from four of five Canadian microbial pesticide ecozones following analysis of 22 randomly amplified polymorphic DNA (RAPD) amplicons. However, the population appeared to be continuously distributed, and no trends, with respect to ecological or host origins, were identified. Statistical analysis indicated that most of the total gene diversity existed within populations, suggesting that the population is highly heterogeneous, and that molecular variability is continuously distributed across the sample population. Because short, non-specific primers are used at low stringency in RAPD analysis, reaction conditions must be standardized for results to be consistent. Standard reaction conditions were maintained throughout this study.

Restriction fragment length polymorphism (RFLP) in the large non-transcribed spacer (NTS-L) region within the ribosomal DNA (rDNA) repeat of 107 *C. purpureum* isolates collected from Europe, New Zealand and North America, was reported by Ramsfield et al. (1996). Three distinct restriction patterns, or nuclear types (I, II and III), were identified using the restriction endonuclease *Hae*III; type I is found in isolates from North America, Europe and New Zealand, type II in North American isolates only, and type III only in isolates from Europe and New Zealand. Type I predominates in eastern North America, and type II in the west, though both types are found on each coast. Types I and II exist at equal frequency in central North America, suggesting that gene transfer occurs across the continent by way of airborne basidiospores.

Similarly, two mitochondrial haplotypes (types I and II) were identified upon digestion of a mitochondrial DNA-specific sequence characterized amplified region (SCAR) with the restriction endonuclease *Nsi*I (Ramsfield et al. 1999). Both haplotypes were present in all geographic regions surveyed. In New Zealand, the haplotype distribution was roughly equal, whereas in Europe and North America, haplotype I was present at twice the frequency. In North America, type I was predominant in the east, but the haplotype distribution was nearly equal in western isolates.

Although the two studies by Ramsfield et al. (1996 and 1999) suggested geographic polarization of *C. purpureum* populations across North America, the polymorphism identified in each was in a single restriction site in a non-coding portion of the genome where variability might be expected. A polarized distribution with a transcontinental frequency gradient might be expected from a single polymorphism.

Field Studies

RAPD analysis and the SCAR marker APD-13 were used to track the fate of deployed strains of *C. purpureum*.

Becker et al. (1999) developed a species specific marker, APN-1, which amplifies a 500 base pair (bp) fragment from the intergenic region of the rDNA. This marker differentiates *C. purpureum* from other basidiomycetes. As well, a SCAR marker, designated APD-13, was discovered to produce a DNA fingerprint that is unique to each strain of *C. purpureum*. These markers were applied in a field study, in which stump sections of sitka alder (*Alnus sinuata*) and trembling aspen (*P. tremuloides*) were sampled four months after treatment with *C. purpureum* mycelial paste prepared from strains PFC 2139 and JAM6. No *C. purpureum* of any strain was detected using the species-specific APN-1 primer in any of the control stumps. However, the incidence of secondary, spore-borne infection could not be determined since spore-bearing fruiting bodies (sporophores) do not develop for one to three years after primary infection.

The occurrence of silverleaf disease in non-target forest trees surrounding powerline rights-of-way was studied by Gosselin et al. (1999). Non-target infection was assessed after the deployment of two native isolates of *C. purpureum*, strains IB and CQP1, in undisturbed areas and at sites where trees had been cut. The overall infection frequency was 15% in disturbed areas compared to 0.3% in undisturbed areas. It is not clear if this difference was due to wounding, or if it was an artifact of different sampling techniques. At the intact sites, infection was assessed by sporophore counts, whereas at wounded sites wood samples were cultured, and many culture-positive stumps did not display sporophores. There was no correlation between infection frequency and proximity to the release site. RAPD analysis showed that at least 85% of these infections did not originate from the introduced strains, therefore non-target silverleaf disease observed on

sites adjacent to areas where *C. purpureum* has been used as a biological herbicide should not be presumed to be due to the biological treatment.

The potential for spread of *C. purpureum* infection between trees through the root system was studied by the applicant. Stems and roots of trembling aspen (*P. tremuloides*) inoculated with *C. purpureum* PFC 2139 one year earlier were destructively sampled and screened for the presence of the fungus using the species-specific rDNA PCR primer APN-1 described by Becker et al. (1999). Treated stems in PFC 2139-treated plots all demonstrated complete mortality, but no amplification of the PCR product specific to *C. purpureum* was seen in treated or control root samples. A positive control sample extracted from infected alder stemwood showed an amplification product typical of *C. purpureum*, even when diluted 1/6000.

The risk of introducing genetic material into resident *C. purpureum* populations by application of a biocontrol strain as a mycoherbicide was studied by the applicant. Isolates of *C. purpureum* were collected immediately before *C. purpureum* biocontrol strains PFC 2139 and JAM6 were applied as part of a field trial. After 18 months, trees were cut to a height of one meter at distances of 50, 700 and 1500 meters from the treated site to act as "spore traps". These trees were monitored over 12–18 months for the presence of sporophores, and wood samples were periodically taken for culture. The genetic similarity between pre-trial isolates and each of the introduced strains, and between spore trap isolates and the introduced strains, was compared using the APD-13 SCAR primer pair described in Becker et al. (1999). No significant differences between pre- and post-treatment isolates were detected.

Taken together, these studies indicate that there is little risk that the introduction of a single biocontrol isolate will overwhelm the native *C. purpureum* gene pool.

Environmental Modeling

In a study by De Jong et al. (1990), spore emission (release) was measured, and spore dispersal patterns were predicted by a Gaussian Plume Model (GPM). Based on spore dispersal data applicable to a maximum of 9% of the days and a risk limit expected to result in infection only 19% of the time, the study found that non-target trees within 500 m of a treatment site would be at appreciable risk and trees at 5000 m would be at negligible risk of infection with *C. purpureum*. However, spore densities at distances of both 500 and 5000 m were predicted to be < 1 spore/m^3 for the majority of days, a density that is not considered high enough to cause infection. Because the values selected for input into the GPM were extremely conservative, the risk is probably overestimated. As well, the environmental input data for the GPM were typical of conditions in the Netherlands which, compared to conditions in North America, are expected to result in higher levels of sporulation.

North American conditions were assessed in a second study by De Jong et al. (1996) conducted in British Columbia. Instead of modeling spore density and dispersal patterns, sporophores were counted, presumably on the assumption that increased fructification would increase the airborne spore density. Natural fructification was surveyed in both healthy stands and areas with significant tree wounding. The significantly higher incidence of natural fructification found at wounded plots (73–100%), as compared to healthy plots, suggests that wounding, and not spore density, was the predominant factor determining infection. The difference in fructification before and after the introduction of *C. purpureum* was estimated and found to be of the same order of magnitude, or less, than the level of natural fructification.

Integrated Environmental Fate Summary

The active ingredient, *C. purpureum*, is described in several references as ubiquitous to northern North America south to Virginia in the East and to northern California in the west. The extensive genetic diversity and out-crossing nature of *C. purpureum* isolates indicate that deployment of a single isolate across Canada and the United States will have a minimal impact on the resident population.

Chontrol Paste is to be applied topically as a mycelial paste formulation, therefore exposure of terrestrial and aquatic organisms to *C. purpureum* will be minimal at the time of application. Furthermore, environmental fate models of *C. purpureum* sporulation and spore dispersal suggest that the additional spore density due to deployment of *C. purpureum* strain PFC 2139 as a biological control agent will be equal in magnitude to, or less than, the naturally occurring spore density from resident populations of *C. purpureum*.

Empirical studies indicate that the additional spore load due to the use of *C. purpureum* would be of the same order of magnitude, or less than, the natural spore load, and that tree wounding, not spore load is the primary determinant of infection. Therefore, no buffer zone around fruit trees or ornamentals that may be pruned or grafted is required since non-target healthy trees are at negligible risk. Wounded trees would likely be equally vulnerable to resident populations of *C. purpureum* as they would to *C. purpureum* strain PFC 2139.

2. Ecological Toxicity

Freshwater Aquatic Invertebrate Toxicity/Pathogenicity

Mycologic, Inc. submitted a request to waive freshwater aquatic invertebrate toxicity/pathogenicity testing, based on the rationale that the use of *C. purpureum* strain PFC 2139 as a biological control agent is not expected to produce any adverse effects on aquatic invertebrates. This rationale was supported with results of literature searches in various databases. No reports identified *C. purpureum* as a producer of toxins or as a pathogenic/infectious agent that affects aquatic arthropods.

Because Chontrol Paste is to be applied topically as a mycelial paste formulation, and the inoculum will be restricted to the cut surfaces of stumps, no spray drift is expected. Therefore, aquatic exposure to *C. purpureum* will be minimal at the time of application. Heavy rainfall might carry *C. purpureum* into aquatic environments (i.e., runoff from treated stumps), however the MPCA is not expected to proliferate in aquatic habitats. In water, terrestrial fungi are normally present in a mainly dormant state, growing and sporulating only in greatly enriched conditions (Suberkropp and Klug 1976). Fungi imperfecti and ascomycetes are the more frequently encountered fungi in aquatic environments while basidiomycetes play only a minor role (Jones 1982; Rheinheimer 1992). Among the aquatic basidiomycetes are *Leptosporomyces*, *Bulbillomyces*, *Subulicystidium*, *Fibulomyces* and *Sistotrema* ssp.(Jones 1982; Goh and Hyde 1996) which are within the same family (Corticiaceae) as *C. purpureum*. Other aquatic basidiomycetes are more distantly related and belong to the orders Ustilginales, Polyporales, Teremellales or the class Gateromycetes (Jones 1982; Goh and Hyde 1996). Furthermore, environmental fate models of *C. purpureum* sporulation and spore dispersal suggest that the additional spore density due to the use of *C. purpureum* strain PFC 2139 as a biological control agent, will be equal in magnitude to, or less than, the naturally occurring spore density from resident populations of *C. purpureum*. *Chondrostereum purpureum* is a ubiquitous organism in the forest ecosystem and it is likely that a large number of spores are naturally deposited in aquatic environments. Despite this

exposure, no adverse effects on aquatic invertebrate species have been noted in the published literature. The incremental increase in exposure due to run-off subsequent to the application of Chontrol Paste and sporulation in the years following infection is not expected to increase the likelihood of adverse effects on aquatic arthropod species.

Based on predicted minimal increased exposure and the absence of reports in published literature associating *C. purpureum* with disease in aquatic invertebrates, the proposed use of Chontrol Paste is not expected to result in adverse effects on aquatic invertebrates. Consequently, no aquatic invertebrate testing is required to further assess the risks of Chontrol Paste. The request for a waiver of aquatic invertebrate testing is accepted.

Avian Toxicity/Pathogenicity

Mycologic, Inc. submitted a justification for the waiver of avian oral toxicity and avian pulmonary/inhalation/injection studies, based on the rationale that the use of *C. purpureum* strain PFC 2139 as a biological control agent is not expected to produce any adverse effects in birds. This rationale was supported with results of literature searches in various databases. No reports of adverse effects that identified *C. purpureum* as a producer of toxins or as a pathogenic/infectious agent affecting avian fauna, were found. This finding, according to the scientific rationale, is supported by the results of human health and safety testing concerning the infectivity, toxicity and eye irritation potential of *C. purpureum* strain PFC 2139 on laboratory mammals.

Acute mammalian toxicity testing showed that *C. purpureum* strain PFC 2139 is not toxic via the oral route. Via the intratracheal route, *C. purpureum* strain PFC 2139 appeared to be slightly toxic but not pathogenic in the rat, however, additional data and information are required to properly interpret these results as clinical signs (e.g., loss of body weight) were noted in both treated and control rats. Chontrol Paste was minimally toxic (irritating) via the dermal route and was practically non-irritating via the ocular route. Requests to waive mammalian acute oral infectivity, and acute intraperitoneal injection infectivity testing were accepted. Moreover, *C. purpureum* is not thermo-tolerant and does not grow at 35°C. Temperatures over 37°C for sustained periods (7–33 days) are lethal to *C. purpureum*. The normal body temperature of ducks and quails is approximately 40°C.

Based on lack of evidence of adverse effects in the published literature, lack of significant toxicity or infectivity in acute mammalian toxicity/infectivity studies, and the inability of *C. purpureum* to grow at high temperatures, the proposed use pattern of Chontrol Paste is not expected to result in adverse effects on avian species. Consequently, no avian testing is required to further assess the risks of Chontrol Paste on avian wildlife. The request for a waiver of avian oral and avian pulmonary/inhalation/injection testing is accepted.

Freshwater Fish Toxicity/Pathogenicity

Mycologic, Inc. submitted a request to waive fish testing, based on the rationale that the use of *C. purpureum* strain PFC 2139 as a biological control agent is not expected to produce any adverse effects on fish species. This rationale was supported with results of literature searches in various databases. No reports of adverse effects identifying *C. purpureum* as a producer of toxins or as a pathogenic/infectious agent that affects fish were found.

As noted in the discussion in the *Freshwater Aquatic Invertebrate toxicity/pathogenicity section*, exposure to *C. purpureum* strain PFC 2139 will be minimal at the time of application. No adverse effects on fish have been noted in published literature despite its ubiquitous nature. The incremental increase in spore density due to biocontrol operations is not expected to increase the likelihood of adverse effects on fish.

Based on predicted minimal increased exposure and the absence of reports associating *C. purpureum* with disease in fish in published literature, the proposed use of Chontrol Paste is not expected to result in adverse effects on fish species. Consequently, no fish testing is required to further assess the risks of Chontrol Paste. The request for a waiver of fish testing is accepted.

Wild Mammal Toxicity/Pathogenicity

No additional testing is required to further assess the risks of Chontrol Paste to wild mammals, as no significant adverse effects were noted in the published literature or in any of the acute oral toxicity, dermal toxicity, and dermal and eye irritation studies described in *Unit B, Human Risk Assessment*. *Chondrostereum purpureum* strain PFC 2139 appeared to be slightly toxic but not pathogenic in the rat via the intratracheal route, though additional data and information are required to properly interpret these results. *Chondrostereum purpureum* does not grow at 35°C and temperatures above 37°C for sustained periods (7–33 days) are lethal to *C. purpureum*.

Nontarget Plant Toxicity/Pathogenicity

Terrestrial plants: Mycologic, Inc. submitted a justification for a data waiver from terrestrial plant testing. According to the scientific rationale, the impact of *C. purpureum* on non-target terrestrial plants is limited due to: (1) fragile basidiospores (Spiers 1985; Spiers and Hopcroft 1988), (2) the requirement for a wound, preferably a fresh wound, to gain entry into the host (Brooks and Moore 1926), and (3) the ability of healthy hosts to fend off or compartmentalize infections. The rationale also noted that, based on the biological properties of *C. purpureum*, it is unlikely that a highly virulent isolate would survive long as a distinct entity despite the fact that this species varies in virulence (Ekramoddoulah et al. 1993). Its outbreeding tendency and absence of a clonally dispersed propagule such as conidia indicate that highly virulent isolates would recombine with resident populations. Furthermore, selective pressure towards virulence is low because the fungus can survive and proliferate as a saprophyte.

In North America, *C. purpureum* is a ubiquitous organism that is a natural component in the forest ecosystem, but is rarely reported as a disease of fruit trees or forest crops (Browne, 1968). Reported cases of silverleaf disease are often associated with already weakened deciduous trees. Infections on coniferous trees have been reported, but these are limited as other saprophytes apparently crowd out *C. purpureum* from infected tissues. *Chondrostereum purpureum* is not known to be a pathogen of herbaceous plants. In susceptible host species, the presence of a fresh wound, and not the basidiospore load, is the main determinant of a non-target host tree's risk of infection. The susceptibility of a tree is dependent on its health status. Healthy host trees have been reported to successfully fight off infection by physically compartmentalizing invading *C. purpureum* (Wall 1991).

Non-target terrestrial plant exposure is not expected to increase significantly with use of *C. purpureum* as a biological control agent. Chontrol Paste is to be applied topically as a mycelial paste formulation (about 5.0 mL per a cut surface of a 2 to 6 cm stump, approximately 5,000 CFU per stump) and, therefore, exposure to

non-target terrestrial plants will be minimal at the time of application. Furthermore, *C. purpureum* is not likely to spread from treated stumps to neighboring trees via the root system, and environmental fate models of *C. purpureum* sporulation and spore dispersal indicate that the additional spore density due to the use of *C. purpureum* as a biological control agent, will be equal in magnitude to, or less than, the naturally occurring spore density from resident populations of *C. purpureum*.

Based on the above-noted information, *C. purpureum* strain PFC 2139 has the potential to affect non-target terrestrial plant species, particularly deciduous trees, but the risks should be no greater than from background levels of this fungal species. Consequently, no terrestrial plant testing is required to further assess the risks of Chontrol Paste on terrestrial plant species. The request for a waiver of terrestrial plant testing is accepted.

Aquatic plants: In the aquatic plant toxicity/pathogenicity study, the impact of *C. purpureum* spores was assessed by exposing two types of aquatic plant species, *Elodea* sp. and *Lemna minor*, to cut alder logs bearing sporophores of *C. purpureum* strain PFC 2139. This study, however, was classified as unacceptable due to uncertainties regarding the number of *C. purpureum* spores administered during treatment.

The waiver rationale argued that the use of *C. purpureum* strain PFC 2139 as a biological control agent should not be expected to produce any detrimental effects on aquatic plants. This rationale was supported with a review of relevant literature detailing known host range and results of literature searches in pertinent databases. No reports of adverse effects that identified *C. purpureum* as a producer of toxins affecting aquatic plants or as a pathogenic/infectious agent to aquatic plants were found.

Exposure to *C. purpureum* strain PFC 2139 would be minimal to aquatic plants at the time of application. No adverse effects on aquatic plants have been noted from *Chondrostereum purpureum* in the published literature despite its ubiquitous nature. The incremental increase in spore production in the years following application is not expected to increase the likelihood of adverse effects on aquatic plant species.

Based on predicted minimal increased exposure and the absence of reports associating *C. purpureum* with disease in aquatic plants, the proposed use of Chontrol Paste is not expected to result in adverse effects on aquatic plants. Consequently, no additional aquatic plant testing is required. The request for a waiver of aquatic plant testing is accepted.

Nontarget Insect and Honeybee Toxicity/Pathogenicity

Mycologic, Inc. submitted a request to waive terrestrial arthropod and terrestrial insect testing, based on the rationale that the use of *C. purpureum* strain PFC 2139 as a biological control agent is not expected to produce any adverse effects on arthropod and insect species. This rationale was supported with results of literature searches in various databases. No reports of adverse effects which identified *C. purpureum* as a producer of toxins or as a pathogenic/infectious agent that affects arthropods were found.

Because Chontrol Paste is to be applied topically as a paste formulation, the inoculum will be restricted to the cut surfaces of stumps, and no spray drift is expected. Therefore, terrestrial arthropod exposure to *C. purpureum* will be minimal at the time of application. Furthermore, environmental fate models of *C. purpureum* sporulation and spore dispersal suggest that the additional spore density due to the use of *C.*

purpureum as a biological control agent will be equal in magnitude to, or less than, the naturally occurring spore density from resident populations of *C. purpureum*.

Chondrostereum purpureum is a ubiquitous organism that is a natural component of the forest ecosystem and no adverse effects on terrestrial arthropods due to natural populations of *C. purpureum* have been noted in the published literature. The incremental increase in spore density is not expected to increase the likelihood of adverse effects on terrestrial arthropods. Recently felled and treated areas may attract a range of arthropod species including wood-boring insects which might be exposed to the MPCA by feeding in treated stumps. Many insects, however, do not colonize wood until the later stages of decay and it is likely that stumps would be naturally colonized by resident populations of *C. purpureum* in any case, if left untreated.

Based on predicted minimal increased exposure and the absence of reports in published literature associating *C. purpureum* with disease in arthropods, the proposed use of Chontrol Paste is not expected to result in adverse effects in terrestrial arthropods including insects. Consequently, no non-target insect toxicity/pathogenicity testing or honey bee toxicity/pathogenicity testing is required to further assess the risks of Chontrol Paste. The request for a waiver of non-target insect and honey bee toxicity/pathogenicity testing is accepted.

Estuarine and Marine Animals Toxicity/Pathogenicity

No other data or scientific rationales were submitted and no additional data are required to assess the impact of *C. purpureum* strain PFC 2139 on estuarine and marine organisms. Based on existing host-specificity data and the proposed use pattern of Chontrol Paste, the potential for adverse effects of *C. purpureum* strain PFC 2139 on other estuarine and marine non-target organisms is considered minimal to non-existent.

Eco-Toxicology Summary

Guideline	*Study*	*Status*
885.4100	*Avian inhalation toxicity/pathogenicity*	Waived based on a limited potential for risk.
885.4050	*Avian oral toxicity/pathogenicity*	Waived based on a limited potential for risk.
885.4200	*Freshwater fish toxicity/pathogenicity*	Waived based on a limited potential for risk.
885.4240	*Freshwater Aquatic Invertebrate toxicity/pathogenicity*	Waived based on a limited potential for risk.
885.4150	*Wild Mammal toxicity/pathogenicity*	Waived based on a limited potential for risk.
885.4300	*Nontarget Plant toxicity/pathogenicity*	Waived based on a limited potential for risk.
885.4340	*Nontarget Insect toxicity/pathogenicity*	Waived based on a limited potential for risk.
885.4380	*Honey Bee toxicity/pathogenicity*	Waived based on a limited potential for risk.

Guideline	Study	Status
885.4280	Estuarine and Marine Animals toxicity/pathogenicity	Waived based on a limited potential for risk.

Integrated Environmental Toxicology Summary

Chondrostereum purpureum is a cosmopolitan fungus widely distributed in over 40 different countries on all continents except Antarctica. In North America, it can be found in Canada and the northern regions of the United States (north of Virginia in the east and northern California in the west). The natural range of *C. purpureum* is thought to be limited to temperate, moist zones. Extensive literature searches in various databases found no reports of adverse effects on birds, mammals, fish, arthropods, non-arthropod invertebrates and aquatic plants. Numerous reports of adverse effects on various woody terrestrial plants were found.

The natural host range of *C. purpureum* includes a variety of terrestrial plants, particularly deciduous trees in which it is a pathogen, gaining entry mostly through newly created wounds and causing the systemic 'silverleaf' disease. Coniferous trees have had reported cases of infection, though other saprophytes apparently quickly crowd out *C. purpureum* in infected tissues (Etheridge and Morin 1963). Herbaceous plants are reportedly not infected by *C. purpureum*. Disease in infected plants includes occlusion of xylem and subsequent water stress to the plant with a variety of compounds reportedly produced that cause or contribute to disease symptoms (Spiers et al. 1987), including extracellular endo-polygalacturonase enzymes (Miyairi et al. 1977; Miyairi et al. 1979), and sesquiterpene compounds such as torreyol, sterpuric acid, sterepolide, and dihydrosterepolide (Strunz et al. 1997; Ayer et al. 1981). Fructification and replacement by secondary colonizers is typically reported between 6–12 months and 3 years.

In a recent study, Setliff (2002) noted the potential for widespread outbreak of silverleaf disease in the Betulaceae and Salicaceae (birch and alder) following timber harvesting or storm damage. Setliff also noted that application of *C. purpureum* to areas often pruned, such as orchards, should be avoided. These statements are largely based on *C. purpureum*'s ability to colonize fresh wounds and to disseminate in the environment through the production of numerous short-lived basidiospores from fertile sporophores following significant rainfall. Thousands of naturally released basidiospores per cubic meter of air were reported by Spiers (1985) and Dye (1974) and this large reservoir of basidiospores provides an effective strategy for early arrival on potential hosts.

Attempts to estimate the risk of infection to non-target terrestrial plants were made by calculating theoretical spore emissions using environmental data obtained from the Netherlands and southern Vancouver Island, British Columbia by means of the Gaussian plume model. The climate of Vancouver Island, with environmental conditions particularly suitable for fructification and sporulation, represents a worst-case scenario for risk to non-target trees in North America. Using these data, De Jong et al. (1996) estimated that the added number of basidiocarps resulting from introduced *C. purpureum* was in the same order of magnitude as naturally occurring levels, or lower. The presence of a fresh wound, and not the basidiospore load, is the main determinant of a non-target host tree's risk of infection. As well, the susceptibility of a tree is dependent on its health status. Healthy host trees have been reported to successfully fight off infection by physically compartmentalizing invading *C. purpureum* (Wall 1991).

Chontrol Paste is to be applied topically as a paste formulation, so exposure of terrestrial and aquatic organisms to *C. purpureum* will be minimal at the time of application. Furthermore, *C. purpureum* is not likely to spread between trees through the root system and environmental fate models of *C. purpureum* sporulation and spore dispersal suggest that the additional spore density, due to deployment of *C. purpureum* strain PFC 2139 as a biological control agent will be equal in magnitude to, or less than, the naturally occurring spore density from resident populations of *C. purpureum*. The incremental increase in spore density is not expected to increase the likelihood of adverse effects to these non-target organisms.

D. Efficacy Data

The following summary of efficacy data is cited from *Health Canada, Pest Management Regulatory Agency Product Monograph for Chondrostereum purpureum* (PFC 2139) Chontrol Paste. These data were not assessed by EPA.

1. Effectiveness

a. Intended use

Chontrol Paste is intended for use on cut stumps of selected deciduous tree species including red alder (*Alnus rubra*), sitka alder (*Alnus sinuata*), speckled alder (*Alnus rugosa*) and trembling aspen (*Populus tremuloides*) in rights-of-way, and forest vegetation management situations. An application of Chontrol Paste is designed to increase the efficiency of the mechanical cutting operation by inhibiting the resprouting and regrowth potential.

The product is formulated as a paste which is spread over the entire surface of the freshly cut stump during the summer or fall cutting operation. One bottle of Chontrol Paste will treat approximately 200 cut stumps with a cut surface diameter of 2 to 6 cm (approximately 5.0g per stump, or about 5,000 CFU per stump). Successful treatment of cut stumps with Chontrol Paste should result in reduced resprouting and regrowth thereby minimizing the need for subsequent cutting and allowing for the establishment of more desirable shrub species in rights-of-way and forest vegetation management situations.

b. Mode of action

Chondrostereum purpureum is a basidiomycete fungus belonging to the Aphyllophorales order of the Corticiaceae family. *Chondrostereum purpureum* PFC 2139 was isolated from a canker on red alder near Duncan, British Columbia. The fungus is not host specific and has a wide host range with a preference for broad-leaved trees. *Chondrostereum purpureum* invades its tree host through wounds in the xylem and causes mortality of infected trees only if they are severely stressed (e.g. tree stems girdled or cut). The pathogenicity of *Chondrostereum purpureum* is expressed as silver leaf symptoms of some trees and vascular discoloration and necrosis with resulting stem cankers. This species is a pioneer pathogen, rarely surviving more than three years, and is replaced by other decay organisms.

c. Nature of pest problem

Much of the vegetation that requires control in both forestry and rights-of-way management consists of deciduous hardwoods trees such as alders, (*Alnus* spp.), birches (*Betula* spp.), maples (*Acer* spp.) and

poplars (*Populus* spp.). These fast growing species suppress the more economically desirable softwood species that are the foundation of most lumber and pulpwood industries (MacLean and Morgan 1982, Haeuschler and Coates 1986 and Smith 1988).

d. Effectiveness against pest

i. Isolate selection

One of the first steps toward development of the end use product Chontrol Paste was to evaluate several isolates of the fungus *Chondrostereum purpureum* to determine which of the isolates demonstrated optimum virulence to identify an isolate suitable for further testing.

Two research trials (1 laboratory study and 1 greenhouse study) were conducted to determine the virulence of several isolates of *Chondrostereum purpureum* to cause infection and mortality on potential hosts and host tissues.

In the laboratory study, 18 isolates of *Chondrostereum purpureum* were inoculated onto tissue cultures of red alder (*Alnus rubra*), black cottonwood (*Populus balsamifera*) and thimbleberry (*Rubus parviflorus*). The study results indicated that there was a significant difference in the virulence of *Chondrostereum purpureum* among the isolates. The study also reported a significant difference in the virulence of the fungus to woody tissues.

A greenhouse study was conducted over a one year period to evaluate the virulence of several isolates of *Chondrostereum purpureum* to cause infection and mortality on black cottonwood and red alder seedlings. Ten isolates of *Chondrostereum purpureum* were inoculated onto black cottonwood seedlings, while 12 isolates of *Chondrostereum purpureum* were inoculated onto red alder seedlings. One year after inoculation, the study reported a significant difference in the virulence among the *Chondrostereum purpureum* isolates tested. The results also demonstrated that there was a significant difference in the ability of *Chondrostereum purpureum* to infect and cause mortality between the seedlings tested, with black cottonwood being a more challenging species compared to red alder.

The results of the isolate selection trials demonstrate the ability of *Chondrostereum purpureum* to infect various broadleaf tree species and cause a degree of mortality that varies between tree species. As such, these trials are supportive of the proposed use pattern.

ii. Efficacy on selected species

Red alder (*Alnus rubra*)

Red alder is exclusively found along the coastal region of British Columbia (B.C.) and along the coastal region of the northwestern U.S. states (Canadian Biodiversity web site 2003, Hosie 1979, Little 1971). One shade house trial and 3 operational trials reported the performance of Chontrol Paste on red alder.

The greenhouse trial was conducted over 1 year (1995 - 1996) near Victoria, B.C. in which 12 one-year-old red alder stumps were inoculated with one of twelve isolates of *Chondrostereum purpureum*. Measurements were recorded on a monthly basis which included the number of living shoots, stem dieback, and percent

mortality. One year after inoculation, all *Chondrostereum purpureum* isolates provided a positive infection, though significant differences were noted among the virulence of isolates. Of the twelve isolates tested, three isolates (2128u, 2139 and 3x-8u) provided a more consistent level of red alder growth response. Overall, the results do support the infectivity of the fungal pathogen and the differential virulence between isolates, with isolate 2139 performing well.

Three operational trials conducted over 2 years (1 trial in 1994 and 2 trials in 1995) reported the growth response parameters such as percent mortality and the number of stems per stump in the year following treatment (3 trials), 2 years following treatment (2 trials), and 3 years after treatment (1 trial).

One year after treatment, Chontrol Paste provided a mean of 94% mortality (n=3) and a mean of 2.7 sprouts per stump (n=2), while the cut only treatment provided a mean of 49% mortality (n=3) and a mean of 7.9 sprouts per stump (n=2).

Two years after treatment, Chontrol Paste provided a mean of 100% mortality (n=2) and a mean of 0 sprouts per stump (n=1), while the cut only treatment provided a mean of 50.7% mortality (n=2) and a mean of 11.3 sprouts per stump (n=1).

Three years after treatment, Chontrol Paste provided a mean of 100% mortality (n=1), while the cut only treatment provided a mean of 15.4% mortality (n=1).

The data supports a claim that sprouting or regrowth of red alder is inhibited following an application of Chontrol Paste.

Sitka alder (*Alnus sinuata*)

Sitka alder is distributed throughout B.C. and extends its' range into the western portion of Alberta and the northwestern U.S. states (Canadian Biodiversity web site 2003, Hosie 1979, Little 1976).

One operational trial was conducted over 2 years (1995 - 1997) near Ripperto Creek, B.C. in which sitka alder clumps received 1 of 8 treatments including Chontrol Paste, Paste blank, cut only, and a stump application of triclopyr. One year after treatment, Chontrol Paste provided a mean of 80% mortality, and a mean of 1.8 sprouts per clump, the Paste blank treatment provided a mean of 4% mortality, and a mean of 14.1 sprouts per clump, the cut only treatment provided a mean of 16% mortality, and a mean of 10.2 sprouts per clump, the stump application of triclopyr provided a mean of 100% mortality, and a mean of 0 sprouts per clump.

Two years after treatment, Chontrol Paste provided a mean of 88% mortality, and a mean of 0.7 sprouts per clump, the Paste blank treatment provided a mean of 7.4% mortality, and a mean of 16.4 sprouts per clump, the cut only treatment provided a mean of 11.2% mortality, and a mean of 12.1 sprouts per clump, the stump application of triclopyr provided a mean of 98.2% mortality, and a mean of 0.1 sprouts per clump.

With the overlapping and exclusive range of red alder and sitka alder to British Columbia, the environmental conditions, such as temperature, moisture, and light, required for spore germination and mycelium growth would be similar for both alder species. It is therefore reasonable to use the red alder data to support the claim for sitka alder.

The data supports a claim that sprouting or regrowth of sitka alder is inhibited following an application of Chontrol Paste.

Speckled alder (*Alnus rugosa*)

Speckled alder is widely distributed across Canada with the exception of the coastal region of B.C. Its range also extends into the northern U.S. states which surround the Great Lakes, and throughout the New England States (Canadian Biodiversity web site 2003, Hosie 1979).

One operational field trial was conducted over 2 years (1995 - 1997) near Thessalon, Ontario in which speckled alder clumps received 1 of 8 treatments including Chontrol Paste, Paste blank, cut only, and a stump application of triclopyr. One year after treatment, Chontrol Paste provided a mean of 12% mortality, and a mean of 8.9 sprouts per clump, the Paste blank treatment provided a mean of 0% mortality, and a mean of 21.6 sprouts per clump, the cut only treatment provided a mean of 0% mortality, and a mean of 21.4 sprouts per clump, the stump application of triclopyr provided a mean of 94% mortality, and a mean of 1.2 sprouts per clump.

Two years after treatment, Chontrol Paste provided a mean of 26% mortality, and a mean of 5.5 sprouts per clump, the Paste blank treatment provided a mean of 0% mortality, and a mean of 16.0 sprouts per clump, the cut only treatment provided a mean of 0% mortality, and a mean of 15.4 sprouts per clump, the stump application of triclopyr provided a mean of 92% mortality, and a mean of 1.1 sprouts per clump.

Insufficient data were made available, considering the distribution of speckled alder throughout Canada, with which to base a scientific conclusion as to the performance of Chontrol Paste for inhibition of resprouting and regrowth of speckled alder and must therefore be removed from the product label in Canada.

Trembling aspen (*Populus tremuloides*)

Trembling aspen is widely distributed across Canada and into the northern U.S. states (Hosie 1979, Little 1971).

Three operational trials were conducted at 2 sites in B.C. and 1 site in Ontario and reported trembling aspen growth responses following an application of Chontrol Paste 1 or 2 years after treatment.

A study conducted near Chetwynd, B.C. over one year (1996-1997) reported that Chontrol Paste provided a significant treatment effect on stump mortality and stump health index when compared to the blank formulation treatment and the cut only treatments. One year after treatment, Chontrol Paste provided a mean of 37% mortality, the Paste blank treatment provided a mean of 21% mortality, the cut only treatment provided a mean of 15% mortality, and the stump application of triclopyr provided a mean of 100% mortality. Based on the data provided, Chontrol Paste performed significantly better than the formulation blank and the cut only treatments.

A two year study (1995-1997) conducted northwest of Grand Forks, B.C. reported that Chontrol Paste provided a significant treatment effect on stump mortality when compared to the blank formulation treatment and the cut only treatment. Two years after treatment, Chontrol Paste provided a mean of 84% mortality and a mean of 2.2 sprouts per square meter, the Paste blank treatment provided a mean of 14%

mortality and a mean of 3.4 sprouts per square meter, the cut only treatment provided a mean of 31% mortality and a mean of 4.2 sprouts per square meter, and the stump application of triclopyr provided a mean of 97% mortality and a mean of 0.4 sprouts per square meter. Based on the data provided, Chontrol Paste performed significantly better than the formulation blank and the cut only treatments and comparable to the triclopyr treatment.

A two year study (1995-1997) conducted north of Iron Bridge, Ontario reported that Chontrol Paste caused a reduction in the number of root suckers per meter (0.05 root suckers/m^2) when compared to the Paste blank treatment (0.56 root suckers/m^2) and the cut only treatment (0.59 root suckers/m^2). However, the Chontrol Paste treatment did not provide a reduction in the number of stem sprouts per square meter, the stem sprout height per square meter, or the root sucker height per square meter when compared to the Paste blank treatment and the cut only treatment. Mortality was not reported in this study.

The results from the Ontario trial are inconsistent with that reported in the 2 B.C. trials. There is concern that the inconsistency may be associated with the virulence of the fungal isolate PFC 2139, which was isolated from a canker on red alder near Duncan, B.C. As *Chondrostereum purpureum* is a living organism, it is possible that the virulence of PFC 2139 was diminished due to the unfavorable environmental conditions found outside of its' natural B.C. habitat. If environmental conditions found in Ontario contributed to the diminished virulence of Chontrol Paste, different environmental conditions found across Canada may have an influence on the performance of the product to inhibit the sprouting or regrowth of trembling aspen.

Insufficient data were made available to base a scientific conclusion as to the performance of Chontrol Paste to inhibition of resprouting and regrowth of trembling aspen and must therefore be removed from the product label in Canada.

2. Phytotoxicity to target plants (including different cultivars), or to target plant products

Chondrostereum purpureum is pathogenic to a wide variety of species with pathogenicity expressed as sapwood stain, non-girdling cankers, and silver leaf disease, but is seldom lethal unless the host is subjected to severe stress (Bishop 1978, Wall 1996).

In the summary provided concerning the environmental fate of *Chondrostereum purpureum*, the applicant states that the topical application of mycelium to the surface of cut stems would pose little risk to nearby vegetation at the time of application. Local dispersion of *Chondrostereum purpureum* can be expected through airborne basidiospores. Since this fungus requires a fresh wound to enter a host, susceptible non-target vegetation is only at risk following pruning or other activity that introduces wounds during times of active sporulation.

Polymorphisms in the mitochondrial DNA restriction patterns were used to assess genetic variation in the *Chondrostereum purpureum* population. The distribution of DNA types suggests that gene flow has occurred across the entire continent of North America with little variation between east and central or west and central North America, but higher variation between east and west. This implies that central North America acts as a bridge between the coastal populations.

Two field trials were performed to establish that disease symptoms were from *Chondrostereum purpureum* specifically. Diagnostic molecular genetic markers were used to estimate infection frequency following a treatment with *Chondrostereum purpureum* (Becker *et al*. 1999) The two trials were established in British Columbia on sitka alder and trembling aspen (one trial each). Results indicated that the specific isolates released were recovered only from stumps which had been treated with the isolates in question. The applicant indicates that there was no cross-contamination with a Paste formulation suggesting that this method of application of *Chondrostereum purpureum* is highly target specific.

Should damage to the desired conifers occur during a release operation, the only active source of inoculum would be the Chontrol Paste which will not have activity unless applied directly to the wound. Increased source of inoculum, ie. via spore release from infected deciduous stumps, would occur subsequent to the treatment period with conifer wounds having healed, thereby minimizing the likelihood of infection.

3. Economics

The harvest of commercial softwoods in Canada equals or exceeds the annual allowable cut of about 170,000,000 m^3 (Canadian Council of Forest Ministers 1993). According to the applicant, productivity of commercial forest lands needs to be increased through more intensive management in order for industry to maintain its present level. Currently, over 700,000,000 tree seedlings are planted annually in Canada and the total cost for silviculture exceeds $800,000,000 (Canadian Council of Forest Ministers 1993). According to the applicant, this level of activity is likely to increase, requiring environmentally friendly options for vegetation management. Further, the applicant anticipates that the use of Chontrol Paste will increase the efficacy of manual or mechanical control of hardwoods and reduce the reliance on chemical control.

4. Sustainability

It is expected that the use of Chontrol Paste will result in a reduction in conventional herbicide use in the proposed use pattern of rights-of-way and forest management settings. The frequency of mechanical brush control operations should also be reduced with the use of Chontrol Paste due to the increased control of weedy deciduous species, thereby prolonging the time period between mechanical cutting operations. Chontrol Paste represents a non-chemical, biological control alternative for situations in which chemical treatment is no longer acceptable.

a. Survey of alternatives

i. Nonchemical control practices

Mechanical clearing techniques are commonly used for control of weedy deciduous species in utility rights-of-way and conifer release management situations. The frequency of operations depends on the weedy species present at the site and their associated resprouting tendency. Accordingly, sites inhabited by species with a prolific tendency to sprout require more frequent cutting activities.

ii. Chemical control practices

Alternative herbicides for brush control in rights-of-way and conifer release:

Technical Grade Active Ingredient	End-Use Products	Herbicide Classification		Application Rate (g a.i./ha)
		Group	Mode of Action	
Glyphosate	Ezject Herbicide Capsules	9	Inhibitor of 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase	0.15 g a.i. per 5 cm tree stump diameter
Triclopyr	Garlon 4	4	Synthetic auxins	1.9 to 3.8 kg a.i./ha
Picloram + 2,4-D	Tordon 101	4	Synthetic auxins	5.5 to 7.6 kg a.i./ha for broadcast application; 1:1 ratio with water for cut stump treatment
Hexazinone	Velpar	5	Inhibitor of photosynthesis at photosystem II Site A	4 to 8 kg a.i./ha

b. Compatibility with current management practices including integrated pest management

The common management practices for vegetation control in rights-of-way and conifer release management rely largely on conventional herbicide use. In certain settings however, the use of herbicides is no longer acceptable, so brush saw cutting offers the only viable option for weedy brush control. As such, Chontrol Paste is compatible with the current management systems and in fact, could become a vital part of integrated vegetative management in rights-of-way as well as in forestry management.

c. Contribution to risk reduction

The use of Chontrol Paste offers an alternative to traditional chemicals by augmenting the efficacy of a brush cut operation and reducing the number of follow up cutting operations required. As such, this product may contribute to reduced chemical use in rights-of-way and in forest management settings.

d. Information on the occurrence or possible occurrence of the development of resistance

Based on the mode of action of Chontrol Paste, the development of resistance is unlikely. The use of Chontrol Paste in conjunction with conventional herbicides may mitigate, in part, the development of herbicide resistance in hardwoods, as well as minimize the potential for resistance to *Chondrostereum purpureum*.

5. Conclusions

Adequate efficacy data were provided to support the use of Chontrol Paste in rights-of-way and in forest vegetation management, as proposed on the product label, for the inhibition of resprouting and regrowth from cut stumps of red alder and sitka alder. Insufficient data were made available to base a scientific conclusion as to the performance of Chontrol Paste to inhibit resprouting and regrowth of speckled alder and trembling aspen and must therefore be removed from the Canadian product label. Adequate data were provided to address the issue of potential adverse effects to conifer species with the use of Chontrol Paste as proposed in rights-of-way and forest vegetation management situations.

IV. RISK MANAGEMENT AND RE/REGISTRATION DECISION

A. Determination of Eligibility of Registration

Section 3(c)(5) of FIFRA provides for the registration of new active ingredients if it is determined that (A) its composition is such as to warrant the proposed claims for it; (B) its labeling and other materials required to be submitted comply with the requirements of FIFRA; (C) it will perform its intended function without unreasonable adverse effects on the environment; and (D) when used in accordance with widespread and commonly recognized practice, it will not generally cause unreasonable adverse effects on the environment.

To satisfy criterion "A" above, *Chondrostereum purpureum* strain PFC 2139 has well known properties. The EPA has no knowledge that would contradict the claims made on the label of this product. Criterion "B" is satisfied by the current label and by the data presented in this document. It is believed that this new pesticidal active ingredient will not cause any unreasonable adverse effects, is a broad spectrum microbial fungicide, and does provide protection as claimed satisfying criterion "C". Criterion "D" is satisfied in that *Chondrostereum purpureum* strain PFC 2139 is not expected to cause unreasonable adverse effects when used according to label instructions.

Therefore, *Chondrostereum purpureum* strain PFC 2139 is eligible for registration. The eligible uses are listed in the Section II, B. Use Profile. There are no ineligible uses for *Chondrostereum purpureum* strain PFC 2139.

B. Regulatory Position

1. Unconditional/Conditional Registration

With only one exception, the data requirements are fulfilled for registration of products that contain *Chondrostereum purpureum* strain PFC 2139 as the sole active ingredient. On September 23, 2004 , EPA issued a Conditional Registration for the Manufacturing Use Product (CP-PFC 2139 and its End-Use Product, Chontrol Paste). The conditions of registration are spelled out in the notice of registration: replacement of the acute pulmonary infectivity and toxicity study or alternatively, submission of an acute intraperitoneal injection infectivity study to satisfactorily address the infectivity potential of *Chondrostereum purpureum* strain PFC 2139.

2. Tolerances for Food Uses and /Or Exemptions

Chontrol Paste is not to be applied to food or feed. Therefore, the establishment of a tolerance is not required for *C. purpureum* strain PFC 2139.

3. CODEX Harmonization

There are no CODEX values for *Chondrostereum purpureum* strain PFC 2139 .

4. Risk Mitigation

There is minimal or negligible potential hazard to humans and non-target organisms (plants, insects, aquatic freshwater, estuarine and marine animals and wildlife), and to ground and surface water contamination through the use of products containing *Chondrostereum purpureum* strain PFC 2139 as discussed in this document as long as label directions are followed. No further mitigation measures are required at this time beyond the appropriate PPE required for pesticide applicators and other handlers. These include long sleeved shirt, long pants, shoes and socks plus gloves and respirator. The product labels will also include appropriate Environmental Hazards text to mitigate any potential risk.

5. Endangered Species Statement

No adverse effects are expected to any of the deciduous woody-plant species listed by the U.S. Fish and Wildlife Service.

Given the specificity of this microbial pesticide, the application method, the intended use pattern, and *Chondrostereum purpureum's* innate dependency on fresh wounding of trees to be an effective herbicide, the EPA has determined that this action will have no effect on currently listed endangered and threatened species. These findings are supported by the results of toxicity and exposure data from the public scientific literature and from the data submitted by the applicant.

C. Labeling Rationale

1. Human Health Hazard (WPS and non-WPS)

Chondrostereum purpureum strain PFC 2139 products with commercial use sites are subject to the Worker Protection Standard. Because of the low toxicity of *Chondrostereum purpureum strain PFC 2139*, the Re-Entry Interval for uses within the scope of WPS is 4 hours. Precautionary statements and personal protective equipment as specified below are required based on the acute toxicity categories of this organism.

2. Environmental Hazard

Precautionary labeling is required as indicated below in section VI, B.

V. PUBLIC INTEREST FINDING

The Agency believes use of *Chondrostereum purpureum* strain PFC 2139 under this conditional registration would be in the public interest. The criteria for Agency evaluation of public interest findings are outlined in 51 FR No. 43, Wednesday March 5, 1986. Under part IV. A., the proposed product may qualify for an automatic presumptive finding that the proposed conditional registration is in the public interest, if it is for a minor use, is a unique replacement for pesticides of concern, or is for use against a public health pest.

Although forestry use is not a minor use, the uses of *Chondrostereum purpureum* strain PFC 2139 are minor uses in non-minor crops/sites. Currently, there is no biopesticide registered to inhibit sprouting and regrowth in cut stumps of certain deciduous tree species in rights-of-way and forest situations. The registration of *Chondrostereum purpureum* strain PFC 2139 will fulfil a unique and essential user need in forest management situations. The proposed microbial pesticidal active ingredient is indigenous to North America. It is much less toxic to man or the environment and represents reduced exposure to applicators than registered alternatives currently in use within the forest management industry. This new microbial herbicide will actually displace use of other conventional chemical herbicides mentioned above in section III. D. 4. a. This new microbial herbicide presents advantageous environmental fate characteristics and properties as compared to the registered alternatives. Furthermore, no adverse effects have been reported by researchers who have been engaged in research trials with this active ingredient.

Based on these rationales, the Agency has determined that *Chondrostereum purpureum* strain PFC 2139 is likely to provide a cost effective biocontrol herbicide agent for inhibition of sprouting and regrowth in cut stumps of certain deciduous tree species, and availability of this new biopesticide to foresters is in the public interest.

VI. ACTIONS REQUIRED BY REGISTRANTS

Reports of incidents of adverse effects to humans or domestic animals are required under FIFRA, Section 6(a)(2) and incidents of hypersensitivity under 40CFR158.690(c) and guideline reference number 152-16. There are no data requirements, label changes and other responses necessary for the reregistration of the end-use product since the product is being registered after November 1984 and is, therefore, not subject to reregistration. For the same reason, there are also no existing stocks provisions at this time. Before releasing these products for shipment, the registrant is required to provide appropriate labels and other Agency requirements as discussed in this document.

In connection with the Conditional Registration of *Chondrostereum purpureum* strain PFC 2139 products mentioned above, replacement of the acute pulmonary infectivity and toxicity study or alternatively submission of an acute intraperitoneal injection infectivity study to satisfactorily address the infectivity potential of *Chondrostereum purpureum* strain PFC 2139 is required.

A. **Precautionary Labeling**

Chondrostereum purpureum strain PFC 2139 products must state the following under the heading "Precautionary Statements":

Personal Protective Equipment required for Applicators and other handlers:

Long sleeved shirt and long pants. Waterproof gloves. Shoes plus socks. A NIOSH approved respirator with any N, P, R, or HE filter.

WPS labels must state the following under the heading "User Safety Recommendations"

Users should wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet.

Users should remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.

Users should remove PPE immediately after handling this product. If gloves are worn, wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

B. Environmental Hazards Labeling

Provided the following statement is placed into the environmental hazards statement, the risk of *Chondrostereum purpureum* strain PFC 2139 is minimal to nonexistent to non-target organisms including endangered species.

1. End-Use Product Environmental Hazards Labeling

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

2. Manufacturing-Use Product Environmental Hazards Labeling

"Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or public water unless this product is specifically identified and addressed in an NPDES permit. Do not discharge effluent containing this product to sewer systems without previously notifying the sewage treatment plant authority. For guidance, contact your State Water Board or Regional Office of the EPA."

3. Application Rate

It is the EPA's position that the labeling for the pesticide products containing *Chondrostereum purpureum* strain PFC 2139 as the active ingredient complies with the current pesticide labeling requirements. EPA has not required a maximum number of applications per season for this active ingredient.

C. Other Labeling

The attached label conforms with the labeling requirements for *Chondrostereum purpureum* strain PFC 2139 . Some of the essential label requirements are highlighted below.

Signal word is "Caution," based on the acute dermal toxicity/pathogenicity toxicity category III. In addition, the product shall contain the following information:

- Product Name
- Ingredient Statement
- EPA Registration Number
- "Keep Out of Reach of Children" statement
- Signal Word (CAUTION)
- First Aid Statement
- Personal Protective Equipment (PPE)
- Environmental Hazard Statement
- Storage and Disposal Statement
- Agricultural Use Requirements
- Directions for Use

VII. BIBLIOGRAPHY

Citations Considered to be part of the Data Base Supporting the Registration of *Chondrostereum purpureum* strain PFC 2139:

MRID Citation

45493300 Mycologic, Inc. (2001) Submission of Product Chemistry, Efficacy, Toxicity, and Environmental Fate Data in Support of the Application for Registration of Chontrol. Transmittal of 4 Studies.

45493301 Bastide, P. (2001) Chondrostereum purpureum Isolate PFC 2139 (Comprehensive Data Summaries): Lab Project Number: 94B. Unpublished study prepared by MycoLogic, Inc. 33 p.

45493302 Bastide, P. (2001) Chondrostereum purpureum Isolate PFC 2139 Product Identity and Disclosure of Ingredients, Manufacturing Process and Discussion on the Formation of Unintentional Ingredients, Analysis of Samples, Certification of Ingredient Limits, Analytical Methods for Certified Limits and Physical and Chemical Properties: Lab Project Number: 94B. Unpublished study prepared by MycoLogic, Inc. 714 p. {OPPTS 885.1000, 885.1100, 885.1300, 885.1400, 885.1500, 885.1600}

45493303 Harrington, K. (1999) Acute Toxicity/Limit Testing of Chondrostereum purpureum Following Acute Oral Challenge in Rats: Lab Project Number: L08725 SN2. Unpublished study prepared by IIT Research Institute. 30 p. {OPPTS 870.1100}

45493304 Bastide, P. (2001) Chondrostereum purpureum Isoloate PFC 2139 Hypersensitivity Incidents: Lab Project Number: 94B. Unpublished study prepared by Mycologic, Inc. 5 p. {OPPTS 885.3400}

45507100 Mycologic Inc. (2001) Submission of Toxicity and Efficacy Data in Support of the Application for Registration of Chontrol. Transmittal of 5 Studies.

45507101 Gingras, B. (1998) Sensitivity of Detection of Chondrostereum purpureum for Toxicity/Pathogenicity Testing in Rats: Final Report: Lab Project Number: L08725 SN1. Unpublished study prepared by IIT Research Institute. 32 p. {OPPTS 885.001, 885.3100, 885.3150}

45507102 Gingras, B. (1999) Toxicity/Pathogenicity Testing of Chondrostereum purpureum Following Acute Intratracheal Challenge in Rats: Final Report: Lab Project Number: L08725 SN3. Unpublished study prepared by IIT Research Institute. 95 p. {OPPTS 885.3150}

45507103 Findlay, J. (1998) Acute Dermal Toxicity/Pathogenicity Study of Chondrostereum purpureum in Rabbits: Lab Project Number: L08725SN4. Unpublished study prepared by IIT Research Institute. 25 p. {OPPTS 885.3100}

45507104 Harder, J. (2001) Acute Eye Irritation Study of the Technical Grade Formulation Containing Viable Mycelia of Chondrostereum purpeum in Rabbits: Lab Project Number: 1367 SN1. Unpublished study prepared by IIT Research Institute. 25 p. {OPPTS 870.2400}

45507105 Biehn, W. (2001) Chondrostereum purpeum Isolate PFC 2139 Product Performance Data: Lab Project Number: 94B. Unpublished study prepared by The University of Victoria. 477 p.

46018300 Mycologic Inc. (2003) Submission of Product Chemistry and Toxicity Data in Support of the Applications for Registration of CP-PFC 2139 and Chontrol Paste. Transmittal of 3 Studies.

46018301 de la Batiste, P. (2003) Chondostereum purpueum (sic) Isolate PCF 2139 Comprehensive Data Summaries: Amendment #1 to MRID 45493301. Project Number: 94B. Unpublished study prepared by Mycologic, Inc. 35 p.

46018302 de la Batiste, P. (2003) Toxicology and Pathology Studies: Amendment 1: (Chontrol). Project Number: 94B. Unpublished study prepared by Mycologic Inc. 20 p.

46018303 de la Batiste, P. (2003) Environmental Toxicology and Non-Target Studies: (Cp-PFC 2139). Project Number: 94B. Unpublished study prepared by Mycologic Inc. 130 p.

Cited References

Ayer, W.A., Hossein Saeedi-Ghomi, M., Van Engen, D., Tagle, B., and Clardy, J. 1981. The sterpuric acids: A new type of sesquiterpenoid. Tetrahedron Supplement **1**: 379–385.

Becker, E.M., A. Ball, and W.E. Hintz. 1999. PCR-based genetic markers for detection and infection frequency analysis of the biocontrol fungus *Chondrostereum purpureum* on sitka alder and trembling aspen. Biological Control 15: 71-80.

Bishop, G.C. 1978. Studies on silver leaf disease of stone and pome fruit trees. Ph.D. Thesis, University of Adelaide, 128p.

Brooks, F.T., and Moore, W.C. 1926. Silver-leaf disease. V. I. Pomol. and Hort. **5**: 11–97.

Browne, F.G. 1968. Pest and diseases of forest plantation trees. Clarendon Press, Oxford. 976p.

Buller, A. H. R. 1958. Researches on fungi. Volume 1. Hafner Publishing Co., New York, pages 103, 111 and 127.

Canadian Biodiversity. 2003. Canadian biodiversity web site. Available at http:www.canadianbiodiversity.mcgill.ca/ca. Accessed: August 5, 2003.

Canadian Council of Forest Ministers. 1993. Compendium of Forestry Statistics. Forestry Canada, Communications Division, Ottawa, Ontario. 122 p.

De Jong, M.D., Scheepens, P.C., and Zadoks, I.C. 1990. Risk analysis for biological control: a Dutch case study in biocontrol of *Prunus serotina* by the fungus *Chondrostereum purpureum*. Plant Dis. **74**: 189–194.

De Jong, M.D., Sela, E., Shamoun, S.F. and Wall, R.E. 1996. Natural occurrence of *Chondrostereum purpureum* in relation to its uses as a biological control agent in Canadian forests. Biological Control **6**: 347–352.

Dye, M. H. 1974. Basidiocarp development and spore release by *Stereum purpureum* in the field. N. Z. J. Agric. Res. **17**: 93–100.

Ekramoddoullah, A.K.M., Shamoun, S.F., and Wall, R.E. 1993. Comparison of Canadian isolates of *Chondrostereum purpureum* with respect to temperature response, virulence and protein profiles. Can. J. Plant Pathol. **15**: 7–13.

Etheridge, D.E., and Morin, L.A. 1963. Colonization by decay fungi of living and dead stems of balsam fir following artificial injury. Can. J. Bot. **41**: 1532–1534.

Goh, T.K., and Hyde, K.D. 1996. Biodiversity of freshwater fungi. J. Ind. Microbiol. **17**:328–345.

Gosselin, L., Jobidon, R. and Bernier, L. 1999. Genetic variability and structure of Canadian populations of *Chondrostereum purpureum*, a potential biophytocide. Mol. Ecol. **8**: 113–122.

Haeuschler, S. and D. Coates. 1986. Autecological characteristics of selected species that compete with conifers in British Columbia: a literature review. Canada-B.C. Forest Resource Development Agreement (FRDA) Report 001. 180 p.

Hosie, R.C. 1979. Native trees of Canada. 8th edition. Fitzhenry & Whiteside Ltd. Don Mills. Ont. 380 p.

Jones, E.B.G. 1982. Decomposition by basidiomycetes in aquatic environments. *In* Decomposer basidiomycetes: their biology and ecology. eds. Frankland, J.C., Hedger, J.N., and Swift, M.J. Cambridge University Press. pp. 191-212.

Little, E.L., Jr. 1971, Atlas of United States trees, volume 1, conifers and important hardwoods: U.S. Department of Agriculture Miscellaneous Publication 1146, 9 p.

Little, E.L., Jr. 1976, Atlas of United States trees, volume 3, minor Western hardwoods: U.S. Department of Agriculture Miscellaneous Publication 1314, 13 p.

MacLean, D.A. and M.G. Morgan. 1982. Long term growth and yield response of young fir to manual and chemical release from shrub competition. For. Chron. 59: 177-183.

Miyairi, K., Fujita, K., Okuno, T., and Sawai, K. 1977. A toxic protein causative of silver-leaf disease symptoms on apple trees. Agric. Biol. Chem. **41**: 1897–1902.

Miyairi, K., Okuno, T., and Sawai, K. 1979. Studies on isoenzymes of the toxic endopolygalacturonase produced by *Stereum purpureum*. Bull. Fac. Agric. Hirosaki Univ. **31**: 1–10.

Ramsfield, T.D., Becker, E.M., Rathief, S.M., Tang, Y., Vrain, T.C., Shamoun, S.F. and Hintz, W.E. 1996. Geographic variation of *Chondrostereum purpureum* detected by polymorphisms in the ribosomal DNA. Can. J. Bot. **74**: 1919–1929.

Ramsfield, T.D., Becker, E.M., Shamoun S.F., Punja, Z.K. and Hintz, W.E. 1999. Variation in the mitochondrial DNA of the potential biological control agent *Chondrostereum purpureum*. Can. J. Bot. **77:** 1490–1498.

Rheinheimer, G. 1992. Aquatic microbiology. 3rd ed. John Wiley & Sons. Toronto.

Setliff. E.C. 2002. The wound pathogen Chondrostereum purpureum, its history and incidence on trees in North America. Aust. J. Bot. **50**: 645–651.

Smith, S.M. 1988. Regeneration delays and natural yields on untreated backlog forest land in British Columbia. Canada-B.C. Forest Resource Development Agreement (FRDA) Report 043. 130 p.

Spiers, A. G. 1985. Factors affecting basidiospore release by *Chondrostereum purpureum* in New Zealand. Eur. J. For. Pathol. **15**: 111–125.

Spiers, A. G., Edwards, W. R. N. and Hopcroft, D. H. 1987. Effects of silverleaf infection on ultrastructure of foliage of *Prunus, Rosa* and *Populus*. N. Z. J. Bot. **25**: 411–423.

Spiers, A.G., and Hopcroft, D.H. 1988. Ultrastructural studies of basidial and basidiospore development and basidiospore release in *Chondrostereum purpureum*. Eur. J. For. Pathol. **18**: 367–381.

Strunz, G.M., Bethel, R., Dumas, M.T., and Boyonoski, N. 1997. On a new synthesis of sterpurenes and the bioactivity of some related *Chondrostereum purpureum* sesquiterpene metabolites. Can. J. Chem. **75**: 742–753.

Suberkropp, K., and Klug, M.J. 1976. Fungi and bacteria associated with leaves during processing in a woodland stream. Ecology **57**: 707–719.

Wall, R.E. 1986. Pathogenicity of *Chondrostereum purpureum* to yellow birch. Plant Dis. **70**: 158–160.

Wall, R.E. 1991. Pathological effects of *Chondrostereum purpureum* in inoculated yellow birch and beech. Can. J. Plant Pathol. **13**: 81–87.

Wall, R.E. 1996. Pathogenicity of the bioherbicide fungus *Chondrostereum purpureum* to some trees and shrubs of southern Vancouver Island. FDRA Report 246. 18 p.