Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Regulatory Note REG2004-09

Chondrostereum purpureum Strain PFC2139

Cp-PFC2139 (Technical Grade of Active Ingredient) Chontrol Paste (End-use Product)

The technical grade of active ingredient (TGAI) *Cp*-PFC2139 and associated end-use product (EP) Chontrol Paste, containing the naturally occurring fungus *Chondrostereum purpureum* strain PFC2139, have been granted temporary registration under Section 17 of the Pest Control Products Regulations for use as a herbicide to inhibit stump resprouting in red and Sitka alder.

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

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Foreword

Health Canada's Pest Management Regulatory Agency (PMRA) has issued a temporary registration for the TGAI *Cp*-PFC2139 and its EP Chontrol Paste developed by MycoLogic Inc. Health Canada's PMRA and the United States Environmental Protection Agency (USEPA) have jointly reviewed these products as biopesticides within the North American Free Trade Agreement's Technical Working Group (NAFTA TWG) on Pesticides, Joint Review Program.

Chontrol Paste is a biological herbicide, containing 10^5 to 10^7 CFU/kg (colony forming units per kilogram) *Chondrostereum purpureum* strain PFC2139, and is intended to inhibit stump resprouting on red and Sitka alder. The active microorganism, *C. purpureum*, is a naturally occurring fungus. A different strain of this fungus (HQ-1) has been registered in Canada since 2002 as Myco-Tech Paste (Reg. No. 27020).

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

The TGAI *Cp*-PFC2139 and the EP Chontrol Paste used to inhibit stump resprouting on red and Sitka alder have been granted temporary registration pursuant to Section 17 of the Pest Control Products Regulations.

A summary of the Agency's findings in support of this decision is found in this document.

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1.0 The active substance, its properties and uses

1.1 Identity of the active substance and impurities

Microbial pest control agent (MPCA) identification

Active microorganism	*Chondrostereum purpureum* strain PFC2139
Function	Mycoherbicide
Binomial name	*Chondrostereum purpureum* (Pers. ex. Fr.) Pouzar isolate PFC2139
Taxonomic designation	
Kingdom	Eumycota
Phylum	Dikaryomycota
Subphylum	Basidiomycotina
Class	Holobasidiomycetes
Order	Aphyllophorales
Family	Corticiaceae
Genus	*Chondrostereum*
Species	*purpureum*
Strain	PFC2139
Canadian patent status information	Canadian patent 2171024 American patent 5,587,158 “Biological control for weed trees” (R. Wall et al. 1996)
Nominal purity of active	107
Identity of relevant impurities of toxicological, environmental and/or other significance	The technical product does not contain any impurities or microcontaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The final product must meet microbiological contaminants release standards, and no mammalian toxins are known to be produced by *C. purpureum* or its close relatives in the Corticiaceae family.

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

Technical product: *Cp*-PFC2139

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

EP: Chontrol Paste

Property	Result
Physical state	white paste
Guarantee	nominal 10^6 CFU/kg
Formulants	All formulants in Chontrol Paste are considered relatively non-toxic (i.e., are on either USEPA inerts list 4A or 4B). The product does not contain any USEPA List 1 formulants or formulants known to be TSMP Track 1 substances.
Corrosion character	not reported (non oxidizing or reducing)
Wettability	water dispersable
pH (in solution)	5–8 as a 10% suspension
Moisture content	approximately 40%

1.3 Details of uses and further information *(OECD 2.1.3)*

Chontrol Paste is a formulated product containing living mycelium of the fungus *C. purpureum* isolate PFC2139 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 deliver a dose of 10^5 to 10^7 CFU/kg, with an average of 5 g of Chontrol

Paste applied per stump (approximately 5000 CFU per stump), depending on the stump diameter. Use of the product is proposed across Canada to inhibit resprouting and regrowth from cut stumps of red alder (*Alnus rubra*), Sitka alder (*Alnus sinuata*), speckled alder (*Alnus rugosa*) and trembling aspen (*Populus termuloides*).

The use of *C. purpureum* as a vegetation management tool was first reported in the Netherlands. In Canada, *C. purpureum* has been investigated as a mycoherbicide since 1990; a commercial stump resprouting inhibitor, Myco-Tech Paste, containing *C. purpureum* strain HQ-1 has been registered in Canada since 2002 (Reg. No. 27020).

Chondrostereum purpureum strain PFC2139 was isolated from a canker on a red alder (*Alnus rubra* Bong) on Vancouver Island near Duncan, British Columbia in 1994. The canker developed after the tree was inoculated 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.

C. purpureum is a cosmopolitan species that is globally distributed in temperate zones. It is ubiquitous in Canada and common in the United States as far south as Delaware in the east, and Oregon in the west. *Chondrostereum purpureum* is a white-rot fungus and the causative agent of silver leaf, a disease of fruit trees. It is not host specific, having a wide host range as a wound pathogen of broadleaf trees, but 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 or sporophores) that usually appear between one and three years after the infection is initiated. 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 they are 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 spores on fresh stem wounds or on the surface of cut stumps. Only in fresh wounds are the small basidiospores drawn by capillary action up to 20 mm into the xylem tissues where they are free from competition with other fungi. In this environment, the spores can germinate within 24 hours at optimum temperatures of 25 to 27°C and rapidly colonize xylem tissues.

Chondrostereum purpureum is heterothallic. This type of sexual reproduction promotes outbreeding because successful conjugation occurs only between genetically distinct, but compatible, mycelia. The complete compatibility among Canadian isolates and between

Canadian and European isolates 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. Considerable heterogeneity was indeed observed in studies in which the isolates from across North America were tested using randomly amplified polymorphic DNA (RAPD) analysis and sequence characterized amplified region (SCAR) analysis as described in Section 2.1.1. Genetic diversity appears to be continuously distributed across North America. Although single restriction site polymorphisms (nuclear types) showed a polarized distribution across the continent, 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.

2.0 Methods of analysis

2.1 Methods for analysis of the microorganism as manufactured

2.1.1 Methods for identification of the microorganism

To differentiate PFC2139 from other strains of *C. purpureum*, a polymerase chain reaction based technique is used. An unusual SCAR primer was derived from a RAPD analysis of *C. purpureum* strains. The RAPD technique directly detects DNA polymorphism by amplifying genomic DNA at low stringency using short (10 base pair) randomly generated primers. Multiple DNA fragments are amplified, and DNA polymorphism is observed on electrophoresis as strain-specific banding patterns. In the SCAR technique, RAPD fragments are sequenced, and longer primers, incorporating the original RAPD primer as well as a portion of the amplified sequence are designed. As such, SCAR primers are expected to specifically amplify the RAPD fragment from which they were designed.

During screening of a series of SCAR primers designed from RAPD fragments generated from strain PFC2139 using the RAPD primer OPD13, one SCAR, designated AP-D13, was fortuitously discovered to amplify multiple genomic DNA fragments from *C. purpureum*. On separation by agarose gel electrophoresis, these fragments created a pattern of bands that was unique to each strain. This characteristic lent the chief advantage of the RAPD technique, a strain-specific banding pattern generated from genomic DNA, to a high stringency primer. On amplification with the AP-D13 primers, strain PFC2139 uniquely yields fragments of 1640, 1400, 1200, 1000, 810, 760, 540, and 420 base pairs in length. The AP-D13 SCAR primer can be used to differentiate the Chontrol Paste MPCA from other *C. purpureum* strains, both in manufacturing and in the field.

2.1.2 Methods for establishment of purity of seed stock

The original master stock of the MPCA was a pure culture isolate grown on 1.5% malt extract agar. Agar plugs are stored in liquid nitrogen at MycoLogic and at the Pacific

Forestry Centre as well as in deposits at the Biosystematics Research Institute (Ref No. 84M-89) of Agriculture Canada and at the American Type Culture Collection (ATCC 60854). The master seed stock is replenished from working stock of *Chondrostereum purpureum* strain PFC2139, after re-isolation from cut red alder stems. The culture is tested for viability by plating on malt extract agar; for purity using the 3M Petrifilm Aerobic Count (total mesophiles) and Yeast and Mould plates; as well as for genetic stability using SCAR strain-specific markers. If these quality control points are passed, the culture is used to inoculate malt extract agar plates. Cultures that are 10–15 days old are preserved as agar plugs in liquid nitrogen. Batches of 100 to 200 cryovials are prepared at once, and used within one year, at which time a new production stock is prepared. Only production stocks that are viable, free of contamination and show positive strain identification as PFC2139 are released for production use.

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

The TGAI is the product of a two-stage process: a primary submerged fermentation followed by a solid-state fermentation in bags. Quality control checks throughout manufacturing ensure that culture purity and MPCA titre are within acceptable limits. Genetic identity is confirmed using strain-specific SCAR markers as described in Section 2.1.1. Viable *C. purpureum* titres are calculated by plating serial dilutions of the TGAI or EP. The TGAI must have a *C. purpureum* titre between 10^7 and 5×10^8 CFU/kg. Although the product guarantee for the EP is 10^5–10^7 CFU/kg, a titre of 10^6–10^7 CFU/kg will be required for product release as a condition of registration to compensate for loss of titre with storage.

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

Neither *C. purpureum* nor its close taxonomic relatives in the Corticiaceae family are known to produce mammalian toxins. The mode of action of *C. purpureum* as a plant pathogen is attributed in part to its ability to produce sesquiterpenoid plant toxins or their derivatives. None of the sesquiterpenoids produced in liquid culture are known to be toxic to mammals. *Chondrostereum purpureum* has not been reported to produce genotoxins. Consequently, analytical methods for detection and quantification of these compounds in *Cp*-PFC2139 preparations are not considered necessary.

The product is screened for microbial contamination using 3M Aerobic Count Petrifilm plates (3M Microbiology Products) and Yeast and Mould Count Petrifilm plates according to 3M protocols. The product must have a total microbial contaminant burden of less than 10^6 CFU/kg (less than 10^3 CFU/g).

2.1.5 Methods to show absence of any human and mammalian pathogens

Contaminants testing is done by serial dilution of the EP, followed by plating on selective media to detect pathogens. In general, these media are recommended for the isolation of

specific pathogens from foods. Fecal streptococci/enterococci are enumerated using Difco Kenner Fecal (KF) Streptococcus agar, as recommended by Difco for enumerating enterococci in foods. The following 3M Petrifilm plates are used in contaminants testing: Enterobacteriaceae, coliform bacteria, *Escherichia coli* and other coliforms, staphylococci and *Staphylococcus aureus*. *Salmonella* and *Shigella* spp. are detected using Difco SS agar. For the detection of *Vibrio* spp., samples are diluted in an enrichment medium (alkaline peptone water) and incubated for six hours at 35 to 37°C prior to plating on thiosulphate citrate bile sucrose agar (TCBS Agar, Difco). Release standards for the EP, Chontrol Paste, are less than 10^6 CFU/kg total contaminants, and require the absence of detectable human or animal pathogens. Note that the limit of detection for pathogens is between 10^2 and 10^5 CFU/kg (assuming that a single colony constitutes detection).

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

A storage stability study showed a significant loss in potency over 90 days. Five batches of the EP were assayed before and after 90 days of storage. Titres were calculated from viable plate counts. The mean titre was 7.9×10^6 CFU/kg at day zero, and mean residual titre after 90 days was 2.8×10^6, or 35% of the initial potency. Although both starting and 90-day titres were within the guarantee limits of 10^5 to 10^7 CFU/kg, if they had had a lower starting potency (still within guarantee limits), then the lower limit might have been exceeded after 90 days of storage. The applicant must, therefore, ensure that the product-release standards include a titre of at least 10^6 CFU/kg. Storage statements on the TGAI and EP label are to reflect the limited storage stability of this product.

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

The proposed uses of Chontrol Paste do not include application to food or feed. Calculation of a maximum residue limit (MRL) is, therefore, not required.

3.0 Impact on human and animal health

See Appendix I, Table 1, for summary table.

3.1 Integrated toxicity and infectivity summary

The information and data submitted by Mycologic Inc. in support of registration of *Cp*-PFC2139 and Chontrol Paste, were reviewed from the viewpoint of human health and safety and was determined to be sufficiently complete to permit a decision on registration. The information provided to address the characterization of the active ingredient as well as the manufacturing process and quality control adequately addressed the potential human health and safety concerns associated with *C. purpureum* strain PFC2139 and bacterial/fungal contaminants introduced during production.

The acute toxicity and infectivity studies submitted in support of registration of *Cp*-PFC2139 and Chontrol Paste were reviewed. The data set included acceptable acute oral, acute dermal toxicity/pathology and acute eye irritation studies as well as a supplemental acute pulmonary toxicity/infectivity study. The acute dermal toxicity/pathology also contained sufficient data to make a decision on primary dermal irritation. No overt signs of toxicity were noted when *C. purpureum* strain PFC2139 was administered to rats and rabbits via the oral and dermal routes of exposure. *Chondrostereum purpureum* strain PFC2139 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 mortality noted on Day 2. In rabbits, Chontrol Paste was slightly irritating when applied dermally and was minimally irritating when instilled into the eye. *Chondrostereum purpureum* is not known to produce mammalian toxins.

3.2 Hypersensitivity incidence

There have been no reported incidents of hypersensitivity in manufacturing, formulating or applying Chontrol Paste. However, in common with all microorganisms, *C. purpureum* is considered to be a potential sensitizing agent. Continued surveillance and reporting of hypersensitivity incidents is required.

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

3.3.1 Occupational and bystander exposure assessment

The human health and safety studies reviewed showed that Chontrol Paste and *Cp*-PFC2139 are 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 are no reports of hypersensitivity. Irritation studies in rabbits showed that Chontrol Paste was slightly irritating to the skin and minimally irritating to the eyes.

As described in Section 1.3, the proposed use for *C. purpureum* is as biological herbicide for control of sprouting or regrowth of red alder, Sitka alder, speckled alder and trembling aspen in rights-of-way and forest vegetation management situations. Chontrol Paste is to be applied topically as a paste to freshly cut stumps during summer or autumn at an average rate of 5 g/stump (approximately 5000 CFU/stump) depending on the stump diameter. When handled according to the label instructions, the potential for applicator exposure is limited to the dermal route. The potential for bystander exposure is minimal during application though it increases significantly after fructification has occurred. The potential for bystander exposure following fructification is possible via inhalation of the released basidiospores. However, the intentional deployment of the active ingredient is

unlikely to result in a significant increase in the natural background levels of basidiospores produced by this species, as it is abundant throughout Canada.

On the basis of its biological properties, lack of toxicity and pathogenicity as well as the proposed use pattern for Chontrol Paste, it is recommended that the label include standard personal protective equipment (including gloves) when handling this product. For the TGAI, it is also recommended that the label include standard personal protective equipment, in addition to a dust/mist filtering respirator (MSH/NIOSH approval number prefix TC-21C) or a NIOSH approved respirator with any –95, R-95, P-95 or HE filter for biological products to prevent inhalation exposure. This label statement is recommended because the acute pulmonary toxicity/infectivity study was considered to be supplemental.

4.0 Residues

4.1 Residue summary

As Chontrol Paste is not intended for application to food or feed crops, the establishment of an MRL is not required for *C. purpureum* strain PFC2139 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulations. 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 rats and there are no reports of mammalian toxins being produced by the MPCA. Although heavy rainfall might carry *C. purpureum* into aquatic environments (e.g., runoff from treated stumps), the MPCA is not expected to proliferate in aquatic habitats. Moreover, percolation through soil and municipal treatment of drinking water would reduce the possibility of significant transfer of *C. purpureum* strain PFC2139 to drinking water.

5.0 Fate and behaviour in the environment

5.1 Summary of fate and behaviour in the terrestrial environment

The active ingredient, *C. purpureum*, is a ubiquitous organism with a continuously distributed population across Canada. The extensive genetic diversity and out-crossing nature of *C. purpureum* isolates indicate that deployment of a single isolate across Canada 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 following the deployment of *Chondrostereum purpureum* strain PFC2139 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*.

Although the Netherlands considered imposing a 100–500 m buffer zone around fruit trees and orchards in a withdrawn application to register *C. purpureum*, a critical review of the submitted literature and data indicates that a buffer zone is not required. The Netherlands proposal may have been based on a study in which assumptions were greatly biased towards an overestimation of risk and in which methods relied heavily on environmental modelling. Empirical studies indicate that the additional spore load due to deployment of *C. purpureum* would be of the same order of magnitude, or less than, the natural spore load. Furthermore, it is tree wounding, not spore load, that is the primary determinant of infection. Therefore, no buffer zone around fruit trees or ornamentals that may be pruned or grafted is required because non-target healthy trees are at negligible risk, while wounded trees would likely be equally vulnerable to resident populations of *C. purpureum* as they would to *Chondrostereum purpureum* strain PFC2139.

6.0 Effects on non-target species

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

6.1 Summary of effects on non-target species

Chondrostereum purpureum is a cosmopolitan fungus that is 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 (south to Virginia in the east and to 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, but numerous reports of adverse effects on various terrestrial plants. 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 ‘silver leaf’ 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), as well as 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 six months and three years.

In a recent study, Setliff (2002) noted the potential for widespread outbreak of silver leaf 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 pruned often, such as orchards, should be avoided. These statements are largely based on the ability of *C. purpureum* 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 metre 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 of the same order of magnitude as, or lower than, naturally occurring levels. 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, therefore, 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; environmental fate models of *C. purpureum* sporulation and spore dispersal suggest that the additional spore density, due to deployment of *C. purpureum* strain PFC2139 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.

6.2 Integrated environmental fate and toxicology summary

Several published papers and field trials describing the environmental effects of *C. purpureum* strain PFC2139 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, which predicted sporulation and spore dispersal patterns.

The active ingredient, *C. purpureum*, is a ubiquitous organism with a continuously distributed population across Canada. 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, yet 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 silver leaf disease in terrestrial plants. Acute mammalian toxicity/infectivity studies showed that *C. purpureum* strain PFC2139 is not toxic when administered orally; whereas, on pulmonary exposure, it is slightly toxic but did not appear to be pathogenic or infective. Chontrol Paste (EP) 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 pathogenic to mammals or birds. Adverse effects in birds, wild mammals, fish, arthropods, non-arthropod invertebrates and aquatic plants due to the proposed use of Chontrol Paste are not expected, 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.

The risk to non-target terrestrial plants was addressed in several studies. Chontrol Paste is to be applied as a paste to stumps immediately after cutting. Its formulation is expected to minimize the exposure of non-target plants to *C. purpureum* mycelia. This was demonstrated in an article showing no recovery of *C. purpureum* strain PFC2139 from areas adjacent to a site treated with mycelial paste. Non-target trees are more likely to be infected by spores from fruiting bodies growing on treated 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 because non-target healthy trees are at negligible risk, while wounded trees are as vulnerable to resident populations of *C. purpureum* as they are to introduced biocontrol strains.

7.0 Efficacy data and information

7.1 Effectiveness

7.1.1 Intended use

Chontrol Paste is intended for use on cut stumps of selected deciduous tree species, including red alder, Sitka alder, speckled alder and trembling aspen 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 that is spread over the entire surface of the freshly cut stump as part of the cutting operation conducted during the summer or fall. One bottle of Chontrol Paste will treat approximately 200 cut stumps with a cut surface diameter of 2 to 6 cm (approximately 5 g, or about 5000 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.

7.1.2 Mode of action

Chondrostereum purpureum is a basidiomycete fungus belonging to the Aphyllophorales order of the Corticiaceae family. *Chondrostereum purpureum* strain PFC2139 was isolated from a canker on red alder near Duncan, British Columbia in 1994. 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 that are girdled or cut). The pathogenicity of *C. purpureum* is expressed as silver leaf symptom of some trees and vascular discolouration and necrosis with resulting stem cankers. This species is a pioneer pathogen, rarely surviving more than three years in host tissue and is replaced by other decay organisms.

7.1.3 Nature of pest problem

Much of the vegetation that requires control in both rights-of-way and forest vegetation management consists of deciduous hardwoods trees such as alders, birches (*Betula* spp.), maples (*Acer* spp.) and poplars. These fast-growing species suppress the more economically desirable softwood species that are the foundation of Canada's lumber and pulpwood industries (MacLean and Morgan 1982, Haeuschler and Coates 1986 and Smith 1988).

7.1.4 Effectiveness against pests

7.1.4.1 Isolate selection

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

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

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

A greenhouse study was conducted over a one-year period to evaluate the virulence of several isolates of *C. purpureum* on black cottonwood and red alder seedlings. Ten isolates of *C. purpureum* were inoculated onto black cottonwood seedlings, while twelve isolates of *C. purpureum* were inoculated onto red alder seedlings. One year after inoculation, the study reported a significant difference in virulence among the *C. purpureum* isolates tested. The results also demonstrated that there was a significant difference in the ability of *C. 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 *C. 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.

7.1.4.2 Efficacy on selected species

Red alder (*Alnus rubra*)
Red alder is exclusively found along the coastal region of British Columbia and along the coastal region of the northwestern United States (Canadian Biodiversity website 2003, Hosie 1979 and Little 1971). One shade house trial and three operational trials reported the performance of Chontrol Paste on red alder.

The greenhouse trial was conducted over one year (1995–1996) near Victoria, British Columbia, in which 12 one-year-old red alder stumps were inoculated with 1 of 12 isolates of *C. purpureum*. Measurements were recorded on a monthly basis and included the number of living shoots, stem dieback and percent mortality. One year after inoculation, all *C. purpureum* isolates provided a positive infection; however, significant

differences in virulence were noted among isolates. Of the 12 isolates tested, 3 isolates (2128u, 2139 and 3 × -8u) provided a more consistent level of red alder growth suppression. Overall, the results support the infectivity of the fungal pathogen and the difference between isolate virulences, with isolate 2139 performing well.

Three operational trials conducted over two years (one trial in 1994 and two trials in 1995) reported the growth response parameters such as percent mortality as well as the number of stems per stump in the year following treatment (three trials), two years following treatment (two trials) and three years after treatment (one 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 support 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 British Columbia and extends its range into the western portion of Alberta and the northwestern United States (Canadian Biodiversity website 2003, Hosie 1979 and Little 1976).

One operational trial was conducted over two years (1995–1997) near Ripperto Creek, British Columbia, in which Sitka alder clumps received one of eight treatments, including Chontrol Paste, Paste blank, cut only, and a stump application of triclopyr. The results collected one year after treatment are found in Table 7.1.4.2.1, whereas the results collected two years after treatment are found in Table 7.1.4.2.2.

Table 7.1.4.2.1 Treatments to Sitka alder clumps – one year post treatment

	Chontrol Paste	Paste blank treatment	Cut only treatment	Stump application of triclopyr
Mean mortality	80%	4%	16%	100%
Mean sprouts per clump	1.8	14.1	10.2	0

Table 7.1.4.2.2 Treatments to Sitka alder clumps – two year post treatment

	Chontrol Paste	Paste blank treatment	Cut only treatment	Stump application of triclopyr
Mean mortality	88%	7.4%	11.2%	98.2%
Mean sprouts per clump	0.7	16.4	12.1	0.1

With the overlapping and exclusive range of red alder and Sitka alder in 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 support 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 regional of British Columbia. Its range also extends into the northern states that surround the Great Lakes and throughout the New England states (Canadian Biodiversity website 2003, Hosie 1979).

One operational field trial was conducted over two years (1995–1997) near Thessalon, Ontario, in which speckled alder clumps received one of eight treatments including Chontrol Paste, Paste blank, cut only, and a stump application of triclopyr. The results collected one year after treatment are found in Table 7.1.4.2.3, whereas the results collected two years after treatment are found in Table 7.1.4.2.4.

Table 7.1.4.2.3 Treatments to speckled alder clumps – one year post treatment

	Chontrol Paste	Paste blank treatment	Cut only treatment	Stump application of triclopyr
Mean mortality	12%	0%	0%	94%
Mean sprouts per clump	8.9	21.6	21.4	1.2

Table 7.1.4.2.4 Treatments to speckled alder clumps – two years post treatment

	Chontrol Paste	Paste blank treatment	Cut only treatment	Stump application of triclopyr
Mean mortality	26%	0%	0%	92%
Mean sprouts per clump	5.5	16	15.4	1.1

Considering the distribution of speckled alder throughout Canada, insufficient data were made available on which to base a scientific conclusion as to the ability of Chontrol Paste to inhibit resprouting and regrowth of speckled alder. Therefore, speckled alder must be removed from the product label.

Trembling aspen (*Populus tremuloides*)
Trembling aspen is widely distributed across Canada and into the northern United States (Hosie 1979, Little 1971).

Three operational trials were conducted at two sites in British Columbia and one site in Ontario, which reported trembling aspen growth responses following an application of Chontrol Paste, one and two years after treatment.

A study conducted near Chetwynd, British Columbia, over one year (1996–1997) showed 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, British Columbia, showed that Chontrol Paste provided a significant treatment effect on stump mortality when compared to the blank formulation treatment and the cut only treatment. The results, after two years of treatments, are shown in Table 7.1.4.2.5. Based on the data provided, Chontrol Paste performed significantly better than the formulation blank and the cut only treatments as well as the triclopyr treatment.

Table 7.1.4.2.5 Treatments to trembling aspen clumps – two years post treatment

	Chontrol Paste	Paste blank treatment	Cut only treatment	Stump application of triclopyr
Mean mortality	84%	14%	31%	97%
Mean sprouts per m^2	2.2	3.4	4.2	0.4

A two-year study (1995–1997) conducted north of Iron Bridge, Ontario, showed that Chontrol Paste caused a reduction in the number of root suckers per metre (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 metre, the stem sprout height per square metre or the root sucker height per square metre, 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 those reported in the two British Columbia trials. There is concern that the inconsistency may be associated with the virulence of the fungal isolate PFC2139, which was isolated from a canker on red alder near Duncan, British Columbia. As *C. purpureum* is a living organism, it is possible that the virulence of PFC2139 was diminished due to the unfavourable environmental conditions found outside of its natural British Columbia 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 provide the basis for a scientific conclusion as to the ability of Chontrol Paste to inhibit resprouting and regrowth of trembling aspen and claims to this effect must therefore be removed from the product label.

7.2 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).

The summary provided by the applicant concerning the environmental fate of *C. purpureum* 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 *C. purpureum* can be expected through airborne basidiospores. Because 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 *C. 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 North America and west and central North America, but higher variation between the 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 specifically from *C. purpureum*. Diagnostic molecular genetic markers were used to estimate infection frequency following a treatment with *C. 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 that 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 *C. purpureum* is highly target specific.

Should damage to the desired conifers occur during a forest vegetation management release operation, the only active source of inoculum would be the Chontrol Paste, which will not have activity on the conifer species unless applied directly to the wound. Any increased source of inoculum, i.e., via spore release from infected deciduous stumps, would occur subsequent to the treatment period. Conifer wounds would be healed by then, thereby minimizing the likelihood of infection.

7.3 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. Furthermore, 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.

7.4 Sustainability

It is expected that the use of Chontrol Paste will result in a reduction in herbicide use in the proposed use pattern of forestry rights-of-way and forest vegetation 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.

Chontrol Paste represents a non-chemical control alternative for situations in which chemical treatment is no longer acceptable.

7.4.1 Survey of alternatives

7.4.1.1 Non-chemical control practices

Mechanical clearing techniques are commonly used for control of weedy deciduous species in utility rights-of-way and forest vegetation 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.

7.4.1.2 Chemical control practices

Table 7.4.1.2.1 Alternative herbicides for brush control in rights-of-way and conifer release

TGAI	EP	Herbicide classification		Application rate
		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

7.4.2 Compatibility with current management practices including integrated pest management

The common management practices for vegetation control in rights-of-way and conifer release sites rely largely on herbicide use. In certain settings, however, the use of herbicides is no longer acceptable, with brush saw cutting offering the only viable option

for weedy brush control. As such, Chontrol Paste is compatible with the current management systems in its role of enhancing the activity of a brush control operation.

7.4.3 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 forest vegetation management settings.

7.4.4 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 *C. purpureum*.

7.5 Conclusions

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

7.5.1 Summary

Table 7.5.1.1 Summary of label proposals and recommendations

Directions for use	Proposed	Recommendation (based on value assessment)	Comments
Application timing	"Chontrol Paste is best suited for use on fresh cut during summer or autumn."	Same	With clarification on the label as follows: "Apply Chontrol Paste to freshly cut stumps during the growing season, from summer to early fall, and when conditions are conducive for fungal growth and infection."

Directions for use	Proposed	Recommendation (based on value assessment)	Comments
Number of applications	1 per year	Same	
Application method	"Apply the Paste on the entire surface of cut stump."	Same	With clarification on the label as follows: "Apply a thin layer of Paste on the entire surface of cut stump within 30 minutes of cutting."
Crops	N/A		
Weeds	Inhibition of resprouting and regrowth from cut stumps of red alder, Sitka alder, speckled alder and trembling aspen.	Yes	Adequate efficacy demonstrated to accept claim of inhibition of resprouting and regrowth of cut stumps of red alder and Sitka alder.
		No	Insufficient data submitted to demonstrate acceptability of the proposed label claim for speckled alder and trembling aspen.
Spray volume	N/A		

8.0 Toxic Substances Management Policy considerations

During the review of Chontrol Paste, the PMRA has taken into account the federal TSMP[1] and has followed its Regulatory Directive DIR99-03[2]. It has been determined that this product does not meet TSMP Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. Furthermore, the MPCA does not contain any byproducts or microcontaminants that meet the TSMP Track 1 criteria. Impurities of toxicological concern are not expected to be present in the raw materials nor are they expected to be generated in sufficient quantities during the

1 The federal TSMP is available through Environment Canada's website at www.ec.gc.ca/toxics

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

manufacturing process to present a risk to human health and safety. Also, there are no formulants of toxicological concern present in *Cp*-PFC2139 or Chontrol Paste.

9.0 Regulatory decision

The TGAI *Cp*-PFC2139 and associated EP Chontrol Paste containing *C. purpureum* strain PFC2139 to inhibit stump resprouting on red and Sitka alder have been granted temporary registration pursuant to Section 17 of the Pest Control Products Regulations, subject to the following:

- assurance from the company that a minimum product release titre of 10^6 CFU/kg will be maintained; and

- generation of a replacement acute pulmonary toxicity/pathogenicity study or an acute intraperitoneal injection infectivity study.

List of abbreviations

ATCC	American Type Culture Collection
a.i.	active ingredient
bw	body weight
CFU	colony forming unit
DNA	deoxyribonucleic acid
EP	end-use product
KF	Kenner Fecal
kg	kilogram
KTS	killed test substance
LOAEL	lowest observed adverse effect level
LD_{50}	lethal dose 50%
MAS	maximum average score
MIS	maximum irritation score
MPCA	microbial pest control agent
MRL	maximum residue limit
NAFTA TWG	North American Free Trade Agreement's Technical Working Group
NC	naive controls
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NZW	New Zealand white
PCR	polymerase chain reaction
PMRA	Pest Management Regulatory Agency
RAPD	randomly amplified polymorphic DNA
SC	shelf controls
SCAR	sequence characterized amplified region
TCBS	thiosulphate citrate bile sucrose agar
TGAI	technical grade of the active ingredient
TSMP	Toxic Substances Management Policy
TS	test substance
USEPA	United States Environmental Protection Agency

Appendix I Toxicology

Table 1 Summary of toxicity and infectivity studies with *C. purpureum* strain PFC2139 and Chontrol Paste

STUDY	SPECIES/STRAIN AND DOSES	LD_{50} NOEL/NOAEL AND LOAEL	TARGET ORGAN/ SIGNIFICANT EFFECTS/COMMENTS
ACUTE STUDIES			
Oral toxicity/ pathogenicity	Rat – CD® (SD) – 5/sex treated with undiluted MPCA in sterile water, 5 g/kg bw or 1.2×10^6 CFU/kg bw.	LD_{50} greater than 5 g/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
Pulmonary toxicity/ pathogenicity	Rat – CD® (SD) – 20/sex treated with live MPCA in sterile water (TS), 0.1 mL or ~ 1.6×10^4 CFU/animal – 20/sex treated with heat-killed MPCA (KTS), 0.1 mL – 20/sex naive controls (NC) – 5/sex shelf controls (SC)	LD_{50} greater than 1.6×10^4 CFU/animal	– One male TS rat died on Day 2. – Clinical signs of toxicity included rough hair coat (5/20 ♂ TS and 1/20 ♀ TS) on days 2–4, laboured respirations (6/20 ♂ TS, 3/20 ♀ TS and 2/20 ♀ KTS) on days 0–2, nasal discharge (1/20 ♂ TS) on Day 0, and hunched posture (1/20 ♀ TS) on Day 0. – Between days 0 and 7, body weight losses were reported in 8/15 ♂ TS, 1/15 ♂ KTS, 1/5 ♂ SC, 3/15 ♀ TS, 4/15 ♀ KTS and 2/5 ♀ SC. Body weight losses were also reported in 1/10 ♀ TS, 3/10 ♀ KTS between days 7 and 14. – At necropsy, mottled/pale lung parenchyma, mottled lung intermediate lobe, and/or mottled left lungs were noted on most TS and KTS rats, including the male TS rat found dead on Day 2. No gross lesions were noted in animals from NC or SC groups. Significantly increased lung and associated lymph node and decreased kidney weights were reported in TS and KTS groups through Day 14. On Day 14, increased lung and lymph node and decreased liver weights were observed for male SC rats.

STUDY	SPECIES/STRAIN AND DOSES	LD_{50} NOEL/NOAEL AND LOAEL	TARGET ORGAN/ SIGNIFICANT EFFECTS/COMMENTS
ACUTE STUDIES			
			– *C. purpureum* was detected in lungs and associated lymph nodes of TS rats after dosing. *C. purpureum* was not detected in any of the tissues collected from the male TS rat found dead on Day 2 or in any of the tissues collected on days 7 and 14. – In the female NC group, 1/20 appeared lethargic and thin starting on Day 7, with hunched posture appearing on Day 9 and rough hair coat on Day 13. Body weight losses were observed in 5/15 between days 0 and 7, and in 4/10 between days 7 and 14–16. One female NC rat steadily lost weight while 4/10 recorded overall body weight losses. SLIGHTLY TOXIC, NOT PATHOGENIC SUPPLEMENTAL
Dermal toxicity	Rabbit – NZW – 5/sex treated with Chontrol Paste at 2000 mg/kg bw (3.4×10^4 CFU/kg bw) on ~150 cm^2 area of the back, occluded for 24 hours, then washed off.	LD_{50} greater than 3.4×10^4 CFU/kg bw	No mortalities and no abnormalities on necropsy. Following unwrapping , very slight erythema in 3/5 ♂ and 3/5 ♀ and well-defined erythema in 1/5 ♂ and 2/5 ♀ was observed. Irritation cleared by 72 hours. LOW TOXICITY
Dermal irritation	Rabbit – NZW – See dermal toxicity study above.	MIS 1.2/8 (1 h) MAS 0.6/8 (24, 48, 72 h)	See comments above. SLIGHTLY IRRITATING
Eye irritation	Rabbit – NZW – 3 females treated with undiluted Chontrol Paste, 0.1 mL, right eye	MIS 1.3/110 (1 h) MAS 0.7/110 (24, 48, 72 h)	Mild conjunctival redness was noted in one animal at the 24 and 48 hour timepoints, and in a second animal at the 24 hour timepoint. MINIMALLY IRRITATING

Appendix II Environmental assessment

Table 1 Risks of *Chondrostereum purpureum* strain PFC2139 to non-target terrestrial organisms

Organism	Exposure	Test substance	Conclusions
Arthropods	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED**, based on the limited potential for risk.
Birds	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED,** based on the limited potential for risk.
Wild mammals	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED,** based on the limited potential for risk.
Plants	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED,** based on information and data showing that the risks should be no greater than those caused by resident populations of *C. purpureum.*
Soil microorganisms	Acute	Not required	This data requirement was not triggered.
Non-arthropod invertebrates	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED,** based on the limited potential for risk.

Table 2 Risks of *Chondrostereum purpureum* strain PFC2139 to non-target aquatic organisms

Organism	Exposure	Test substance	Conclusions
Freshwater arthropods	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED,** based on the limited potential for risk.
Freshwater fish	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED,** based on the limited potential for risk.
Freshwater plants	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED,** based on the limited potential for risk.
Estuarine and marine arthropods	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED,** based on the limited potential for risk.
Estuarine and marine fish	Acute	Waiver rationale submitted in lieu of data	The waiver rationale submitted by the applicant was **ACCEPTED,** based on the limited potential for risk.

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