Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Proposed Regulatory Decision Document PRDD2002-01

Chondrostereum purpureum (HQ1)

The technical active ingredient Chondrostereum purpureum (HQ1) and associated end-use product Myco-Tech™ Paste, containing the naturally-occurring fungus *Chondrostereum purpureum* strain HQ1, for inhibition of sprouting and regrowth in cut stumps of certain deciduous tree species in rights-of-way and conifer release management situations are proposed for registration under Section 13 of the Pest Control Products Regulations.

This Proposed Regulatory Decision Document provides a summary of data reviewed and the rationale for the proposed Section 13 registration of these products. The Pest Management Regulatory Agency (PMRA) will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to the Publications Coordinator at the address listed below.

(publié aussi en français) **January 4, 2002**

This document is published by the Submission Coordination and Documentation Division, Pest Management Regulatory Agency. For further information, please contact:

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

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

ISBN: 0-662-31500-6
Catalogue number: H113-9/2001-7E-IN

Foreword

The submission for the registration of Myco-Tech™ Paste and Chondrostereum purpureum (HQ1), manufactured by Myco-Forestis Corporation, has been reviewed by Health Canada's Pest Management Regulatory Agency (PMRA).

Myco-Tech™ Paste is a vegetation management product, containing 9.1% Condrostereum purpureum (HQ1), intended to inhibit sprouting and regrowth in cut stumps of certain deciduous tree species in rights-of-way and conifer release management situations. The active microorganism, *Chondrostereum purpureum* strain HQ1, is a naturally-occurring fungus and represents a new microbial forest herbicide to Canada.

Microbial pest control agents are increasingly being investigated for use as alternatives to conventional pesticides because they are thought to pose a lower potential risk to human health and the environment, compared with conventional pesticides. Myco-Tech™ Paste represents a reduced risk option to chemical pesticide vegetation management tools.

The PMRA has carried out an assessment of available information in accordance with Section 9 of the Pest Control Products Regulations and has found it sufficient pursuant to Section 18b, to allow a determination of the safety, merit, and value of the technical active ingredient Chondrostereum purpureum (HQ1) and the end-use product Myco-Tech™ Paste. The Agency has concluded that the use of the microorganism, *Chondrostereum purpureum* strain HQ1, in the technical active ingredient Chondrostereum purpureum (HQ1) and the end-use product Myco-Tech™ Paste in accordance with the label has merit and value consistent with Section 18c of the PCP Regulations and does not entail an unacceptable risk of harm pursuant to Section 18d. Based on the considerations outlined above, therefore, the use of the technical active ingredient Chondrostereum purpureum (HQ1) and the end-use product Myco-Tech™ Paste for inhibition of sprouting and regrowth in cut stumps of certain deciduous tree species in rights-of-way and conifer release management situations is proposed for full registration, pursuant to Section 13 of the PCP Regulations.

These products have been granted a one-year limited term registration by the PMRA to allow users access to this low-risk product, while giving concerned Canadians an opportunity to provide input into the decision for full registration through this Proposed Regulatory Decision Document.

Table of Contents

1.0 The active substance, its properties, and uses

1.1 Identity of the active substance and impurities

Table 1.1-1 TGAI Identification

Active Micro-organism	*Chondrostereum purpureum* strain HQ1
Function	Mycoherbicide
Binomial name: Taxonomic designation: Kingdom Phylum Subphylum Class Order Family Genus Species Strain	*Chondrostereum purpureum* Pouzar strain HQ1 Eumycota Dikaryomycota Basidiomycotina Holobasidiomycetes Aphyllophorales Corticiaceae *Chondrostereum* *purpureum* HQ1
Canadian Patent Status Information	Pending
Nominal purity of active	24.8%
Identity of relevant impurities of toxicological, environmental and/or other significance	The technical product does not contain any impurities or microcontaminants known to be TSMP Track-1 substances. Microbiological contaminants are not permitted in the final end-use product 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)

Table 1.2-1 Technical Product: *Chondrostereum purpureum* (HQ1) — Mycelial Suspension

Property	Result
Physical state	liquid suspension
Specific gravity	1.025 g/mL

Property	Result
Viscosity	not yet measured
Corrosion character	non-corrosive
Wettability	not applicable
Suspendability / Water content	fully suspendable / 98%

Table 1.2-2 End-Use Product: Myco-Tech™ Paste

Property	Result
Physical state	solid/gel
Specific gravity	1.09 g/mL
Viscosity	paste or thick gel (similar to mayonnaise)
Corrosion character	non-oxidizing or reducing
Wettability	not applicable
Moisture content	approximately 72%

1.3 Details of uses

Myco-Tech™ Paste is a formulated product containing viably active mycelium of the fungus *Chondrostereum purpureum* strain HQ1 for application to freshly cut stumps of weedy deciduous brush species in rights-of-way (Use Site Category #16), and conifer release management situations (Use Site Category #4). The product is designed to deliver a minimum dose of 10^5 CFU/mL with a range of 0.5 to 2 mL of paste applied per stump, depending on the stump diameter. Myco-Tech™ is applied as a thin layer over the surface area of freshly cut stumps within 30 minutes of cutting. Use of the product is proposed for inhibition of stump sprouting on species including birch, pin-cherry, poplar/aspen, red maple, sugar maple, and speckled alder in the Boreal and Mixed forest regions of Canada, east of the Rocky Mountains. The product will be marketed in plastic containers of 0.5 to 4 L volume. *Chondrostereum purpureum* strain HQ1 was isolated from a naturally infected cut stump of paper birch (*Betula papyrifera*) near Ste. Agathe, Quebec, in 1992. It is naturally occurring and has not been genetically modified.

Chondrostereum purpureum is a lignicolous, naturally-occurring basidiomycete fungus commonly found in temperate deciduous forests. It is a pathogen of various deciduous trees including species of *Acer*, *Aesculus*, *Alnus*, *Betula*, *Crataegus*, *Fagus*, *Larix*, *Malus*, *Ostrya*, *Picea*, *Populus*, *Prunus*, *Salix*, and *Sorbus*. *Chondrostereum purpureum* is the causative agent of a condition known as silverleaf disease (silvering of the leaf blade

sprouts). It is also occasionally observed as a saprophyte on the conifer *Abies balsamea*; however, it has not been reported to cause any diseases in coniferous tree species. The fungus is ubiquitous in Canada and it is commonly found in the United States as far south as Delaware in the east, and Oregon in the west. Fruiting bodies (basidiocarps) have been collected from various types of forest cover as well as urban and agricultural areas representing all but one microbial ecozone (Ecozone 3) in Canada. The fungus also commonly occurs in woodpiles, silvicultural thinnings, and recently deceased trees. It is a primary colonizer of tissues freshly exposed by pruning, wind damage, frost cracks and lightning damage. Stressed trees are also much more susceptible to silverleaf disease than healthy, vigorous trees. Considerable differences in susceptibility to silverleaf disease also exist between species and varieties.

Chondrostereum purpureum is spread via airborne basidiospores. These basidiospores are released in large quantities following a significant rainfall providing an effective strategy for early arrival on exposed sapwood. It invades the xylem tissues of susceptible host species via fresh wounds. Inside its host, the organism avoids the host's defences by extending rapidly throughout the xylem. The extending mycelium often occludes the vessels and induces a water stress reaction. The water stress is responsible for numerous cascading symptoms. A reduction in water supply leads to a decrease in cell turgor. Changes in cell turgor can cause stomatal closure, growth inhibition, and a decrease in photosynthesis. These changes in metabolism can lead to increased respiration, membrane breakdown, protein breakdown, ultrastructural changes, and cell death.

The fungus also releases various phytotoxic compounds that induce silverleaf disease. These compounds include sterpurenes (sesquiterpenes) and a group of isozymes with endo-polygalacturonase activity. The sterpurenes, including sterpuric acid, sterepolide, and dihydrosterepolide, are transported to the leaves where they induce toxicity symptoms such as leaf yellowing. Many other sterpurenes have been isolated from *C. purpureum*; however, they have not been individually tested for toxicity to plants. The endo-polygalacturonase enzymes are responsible for the silvery appearance of affected leaves. These enzymes are also transported to the leaves where they apparently degrade the cell wall components causing the palisadic parenchyma to separate from the epidermis. In silvered leaves, the epidermis is separated from the palisadic parenchyma and the gap between these layers is filled with air. The altered cellular structure causes irregular reflection of light: thus the silvery appearance. If the infection is serious, the foliage dies as a result of water stress in conjunction with toxin action.

Basidiocarps are normally produced one to three years following infection. Each basidiocarp is capable of producing numerous basidiospores that can then be carried significant distances via wind currents. These spores can potentially infect neighbouring deciduous trees and orchards. Rainfall and relative humidity are also important factors governing basidiocarp development and spore release, and not all infections will result in the formation of fruiting bodies. Basidiocarps can also remain desiccated for 12 to 14 months without losing viability and spore-producing capacity after re-hydration.

2.0 Methods of analysis

2.1 Methods for analysis of the micro-organism as manufactured

2.1.1 Methods for identification of the micro-organism

To discriminate the HQ1 strain from other closely related strains of *C. purpureum*, the applicant employs a number of different methods. Random amplified polymorphic DNA (RAPD) analysis is the definitive method used to identify the microbial active ingredient. This technique directly detects DNA polymorphisms by randomly generating DNA fragments with specific 10-base pair (10-mer) primers that are then separated by agarose gel electrophoresis. The following two primers produce amplicons (DNA fragments) that are specific to the HQ1 strain: OPJ15 and OPS13. Amplification with OPJ15 and OPS13 produce two fragments of 540 ($OPJ15_{540}$) and 700 ($OPS13_{700}$) base pairs. A third 580-base pair fragment, from the OPJ15 primer and designated $OPJ15_{580}$, is also unique to HQ1, but is not consistently detected on agarose gels.

In addition, the applicant performs a phenotypic characterization test which checks for typical colony morphological features, including pigmentation, optical characteristics, elevation, growth rate, texture, consistency, and growth habit on potato dextrose agar (PDA) media plates.

2.1.2 Methods for establishment of purity of seed stock

A stock of master seed is maintained in sterile ampoules and stored in liquid nitrogen. Working seed stocks are prepared from master seed stock by plating each of the three mycelial blocks onto separate PDA (potato dextrose agar) growth medium plates (amended with the antibiotics streptomycin and chlortetracycline to inhibit bacteria) and allowing the cultures to grow for 5–7 days, after which the mycelial growth from one plate is cut into blocks and stored in ampoules in liquid nitrogen (working seed). Subsequently, one working seed is used for each production batch. Prior to preservation, a number of quality control tests are performed on working seed cultures, including a check for viability, microbiological purity, typical phenotypic characteristics and, periodically, genotypic (DNA) integrity by RAPD analysis using the two 10-mer primers described above in 2.1.1. RAPD analysis is conducted on one working seed per lot of 50 and the analysis is performed on samples taken during two steps of the manufacturing process. At this testing frequency, DNA analysis is performed on 2% of all production lots.

2.1.3 Methods to define the content of the micro-organism in the manufactured material used for the production of formulated products

A number of methods are employed to ensure purity of the microorganism culture during the manufacturing process and preparation of the end-use product. The first involves visual inspection of colony morphology, following inoculation of working seed stock

onto PDA growth media plates, to ensure that mycelial features (i.e., pigmentation, elevation, texture, optical characteristics) are typical of the HQ1 strain. This test is also performed at several other steps during the manufacturing process including preparation of primary and secondary inocula and at the final stage of growth of the production batch. A second viability test is performed on PDA plates to assess the rate of mycelial growth. This test is conducted at the end of each step of the manufacturing process including the finished end-use product. Bacterial contamination is determined at various stages of production including the finished product to ensure an absence of bacterial microorganisms. The assay is performed on standard plate count agar. Detection of any bacteria results in destruction of the production batch. However, if any changes are made to the protocol such that low levels of contamination may not result in destruction of the batch, then the applicant will be required to implement appropriate methodologies for identification and enumeration of contaminants. Selective (i.e., microbe-specific) media will be required to screen for potential microbial contaminants, including total mesophiles, fecal streptococci/enterococci, total coliforms, fecal coli, staphylococci, salmonellae, and yeast and moulds in Myco-Tech™ production batches. Furthermore, biochemical tests will be used to identify colonies of different morphology on the screening media to ensure the integrity of production cultures. Should ambiguities arise in the biochemical tests, other tests (e.g., fatty acid analysis) are to be performed to provide additional information for confirmation. Acceptable limits will then be established for each identified contaminant in the end-use product; however, each batch must be free of human and animal primary pathogens.

A small-log bioassay is also conducted at the end of the production process to confirm the viability and infectivity of *C. purpureum*. The method involves inoculating the freshly cut ends of small birch stems or branch segments (8–10 cm in diameter and 15 cm long) with mycelial suspension or end-use product and following the progression of disease symptoms. After five days of inoculation, a distinct discolouration and browning of the xylem wood results from positive infections, which proceed to grow further through the birch segments with time. Due to differences in the wood (i.e., percent moisture and growth stage) comparisons between test treatments conducted at different dates are less reliable than test treatments conducted on the same day. The test is therefore used as a subjective measurement of the ability of the test material to infect wood segments. Growth rates for positive infections are typically 0.3 to 1.0 cm/day.

Potency is also expressed in CFUs (colony forming units) per unit volume of technical active ingredient suspension or weight of final end-use product paste after plating these preparations onto malt agar growth medium. This method is used to measure viability of the HQ1, and the end-use product must contain a minimum of 1.0×10^5 CFU per mL to be accepted for release.

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 have been implicated as potential producers of mammalian toxins. The mode of action of *C. purpureum* as a plant pathogen is attributed in part to its ability to produce plant toxins known as sesquiterpenoid compounds or their derivatives. None of the sesquiterpenoids which can be produced in liquid cultures of *C. purpureum* is reported to be toxic to mammals. Consequently, analytical methods for detection and quantification of these compounds in HQ1 preparations are not considered necessary. Also, *C. purpureum* has not been implicated as a producer of genotoxins.

Methods for determining the presence of bacterial contaminants and genetic variants of the HQ1 are described in 2.1.1 and 2.1.3, above.

2.1.5 Methods to show absence of any human and mammalian pathogens

As discussed above, the quality assurance program implemented by the applicant for the production of the TGAI and EP requires the destruction of the batch if any contamination is detected during the manufacturing process. If any changes are planned to allow for low level contamination, then the applicant must comply with the quality control measures required by the PMRA, including the use of selective media to screen for, and enumerate, total mesophiles, total coliforms, fecal coli, fecal streptococci/enterococci, staphylococci, salmonellae, and yeasts and moulds employing methods and criteria consistent with international standards such as those described by the International Commission on Microbiological Specifications for Foods (ICMSF).

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

A number of methods are used to assess the stability of the microorganism during storage. Storage studies include performance of the growth assay on PDA plates, the plate count agar check for bacterial contaminants, the small-log bioassay for viability and infectivity, and the CFU potency test on malt agar. The required minimum specifications for product performance were satisfied for five production batches of Myco-Tech™ Paste held at 2–6EC over an 11-week storage period.

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

No method to quantify HQ1 residues in food and feed is required. Results of human health and safety tests indicated low acute oral toxicity and product characterization information indicated a low potential for mammalian toxin production. Thus, HQ1 did not satisfy the criteria for requiring the establishment of a Maximum Residue Limit (MRL). Also, the exclusive use of the product on deciduous trees negates the need for analytical methodology of HQ1 residues on food and feed.

Analytical methods for detecting viable HQ1 residues in animal and human body tissues involve blending of tissues and recovery on non-selective agar plates. Malt agar is a suitable recovery medium for "sterile" rat tissues (e.g. lung), whereas Martin agar is most suitable for recovering and enumerating HQ1 in the stomach, caecum and intestinal tract.

No analytical method is required for analysis of toxins/metabolites in food, feed, or other sources, although such methods are described in the published literature.

3.0 Impact on human and animal health

3.1 Integrated toxicity and infectivity summary

The acute toxicity and infectivity studies submitted in support of *C. purpureum* HQ1 and Myco-Tech™ Paste were reviewed and determined to be complete and acceptable. *Chondrostereum purpureum* HQ1 was of low acute toxicity in the rat via oral gavage and it was not pathogenic to the rat via intraperitoneal injection. Myco-Tech™ Paste was of low acute toxicity to rabbits following a dermal exposure of 24 hours. It was also non-irritating to the skin of rabbits following a dermal exposure of 4 hours. Acute eye irritation studies are not required for microbial end-use products, as they are all expected to act as mild, reversible, ocular irritants. Concerns about ocular irritation potential are addressed through appropriate precautionary labelling statements and the requirement for protective eyewear for applicators. The requirement for an acute inhalation study was waived due to the low potential for exposure via the inhalation route at the time of application (see 3.3).

With respect to potential effects on the endocrine system, there is no report or indication in the available scientific literature to suggest that *C. purpureum* HQ1 in Myco-Tech™ Paste has caused, or has the potential to cause, adverse effects on the endocrine system of animals. Also, there are no reports in published literature which would implicate *C. purpureum* as a potential producer of genotoxins.

The formulants used in Myco-Tech™ Paste are not of toxicological concern.

3.2 Hypersensitivity

Research to assess *C. purpureum* HQ1 and to develop Myco-Tech™ Paste for use as a vegetation management product was initiated in 1992. Since that time, none of the 45 individuals who have had opportunity for direct dermal and inhalation exposure to *C. purpureum* HQ1 has reported any incidence of hypersensitivity.

There is no reference in the published literature reporting any adverse effect following human exposure to *C. purpureum*. There are, however, many references that report induced allergic reactions following exposure to fungal spores of other species. Most of these references deal with fungal spores of ascomycetes and imperfect fungi. Nevertheless, as many as 25 species of basidiomycetes have been identified as producing

spores causing allergic reactions. However, Myco-Tech™ Paste contains the mycelium of *C. purpureum* HQ1, and according to literature, mycelia usually give weaker skin test reactions than spores. Assuming that most microorganisms contain substances that would elicit hypersensitivity reactions in humans, *C. purpureum* HQ1 is considered to be a potential sensitizing agent.

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

3.3.1 Occupational and bystander exposure assessment

As described in 1.3, the proposed use of *C. purpureum* HQ1 is as a topical application of a paste to freshly cut stumps of deciduous tree species. According to the draft label, the treatment of an area with the average density of 20 000 to 30 000 stems per hectare would require approximately 20 to 30 L of product per hectare, or approximately 1 mL per stem depending on diameter of the stem. In small treatment areas, the paste is to be applied manually using a spreader (e.g., spatula) to ensure thorough coverage, whereas in larger areas, the paste is to be applied with a low-pressure applicator designed to extrude the formulation directly onto the stump surface. The applicant noted that alternative systems were being investigated for the application of the paste (gel type consistency). The applicant also noted that all these systems involved topical application only. When handled according to the label instructions, the potential for applicator exposure is limited to the dermal route. The potential for bystander exposure increases significantly after one to three years following infection when fructification has occurred. The potential for bystander exposure following fructification is possible via inhalation due to the release of basidiospores. However, the intentional introduction 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. Furthermore, most large-scale commercial applications are expected to occur in remote forested areas. Even in small-scale domestic settings (e.g., urban areas) where the abundance of this species may be lower, the concentration of *C. purpureum* basidiospores produced is not expected to be significantly greater than local background levels.

The potential for exposure via the oral route, i.e., ingestion, is unlikely. Although *C. purpureum* is a basidiomycete fungus, it is generally regarded as inedible due mainly to its thin flesh and tough leathery texture rather than to any toxicological concerns. It is, however, closely related to species of the genus *Stereum*, and other members of the Corticiaceae family, used by some cultures for medicinal purposes.

Even though direct exposure to Myco-Tech™ Paste is unlikely to produce any adverse effects in workers or bystanders, it is recommended that applicators follow standard safety procedures by wearing appropriate personal protective equipment, including gloves and protective eyewear, when handling this product.

4.0 Residues

4.1 Residue summary

Myco-Tech™ will be topically applied as a paste to freshly cut stumps of birch species, pin-cherry, poplar/aspen, red maple, sugar maple, and speckled alder in rights-of-way and in coniferous tree plantations. *Chondrostereum purpureum* HQ1 will not be applied to food or feed crops, hence exposure via this route is considered unlikely.

5.0 Fate and behaviour in the environment

5.1 Summary of fate and behaviour in the terrestrial environment

5.1.1 Environmental modelling

Various studies were submitted detailing environmental models used to assess the risk associated with the use of *C. purpureum* as a biological herbicide. These studies relied on a combination of empirical and calculated data. All of the models required certain assumptions, such as the incidence of successful infection on inoculated stubs or the level of acceptable risk, in order to arrive at their various conclusions.

With regard to the level of acceptable risk, papers submitted for Part M2, *Product Characterization and Analysis*, attempted to relate inoculum concentration to host infection. (Critical reviews of these papers were not conducted.) In an initial study by Grosclaude (1969), wounds on plum trees inoculated with 22–44 or 438 spores resulted in approximately an 80% incidence of infection, while inoculum with 4380, 43 800, or 392 000 spores resulted in infection less than 10% of the time. A later study, however, found that 100% infection could be obtained with all inoculum levels (Spiers and Hopcroft, 1988). The infective dose, therefore, could not be established.

In the study conducted by Goulet (1998), spore emission and spore dispersal patterns were both predicted by an Operational Epidemiological Model (OPEM). The author concluded that wounded trees situated as far as 2 km downwind of a treatment site were at potential risk. OPEM, however, was not validated by empirical data and its prediction that the highest daily mean spore concentration would not be in the predominant wind direction renders the model questionable. Furthermore, the maximum threshold for spore density and the values used for daily mean spore concentration were selected in an extremely conservative manner, such that the estimation of risk likely represented an overestimation.

Risk was assessed in a more empirical manner by De Jong *et al*. (1990). In this study, spore emission was measured while spore dispersal patterns were predicted by a Gaussian plume model (GPM). Based on spore dispersal data which were only 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 a radius of 500 m of a treatment site

would be at appreciable risk and those at 5000 m would be at negligible risk. Upon closer inspection of the data, spore densities at distances of both 500 m and 5000 m were predicted to be <1 spore/m^3 for the majority of days. A spore density of <1 spore/m^3 is not considered high enough to cause infection. Once again, the manner in which risk was assessed likely led to an inflated estimate. Additionally, the environmental input data for the GPM were typical of conditions in the Netherlands which, compared to conditions in Canada, are expected to result in higher levels of sporulation.
Canadian conditions were assessed in a second study by De Jong *et al*. (1996) that was conducted in British Columbia. Spore density and spore dispersal patterns were not taken into account, thereby eliminating one component of the risk assessment that was commonly based on environmental models. Instead, only fructification was taken into account, presumably assuming that if there is an increase in fructification, the spore density would also increase. Natural fructification was surveyed in both random (i.e., healthy stands) and non-random plots (i.e., areas with significant tree wounding). The significantly higher incidence of natural fructification found in non-random plots (73–100%), as compared to random plots, indicated that wounding, and not spore density, was the predominant factor determining infection. The level of added fructification, due to use of *C. purpureum*, was estimated and was found to be of the same order of magnitude, or less, than natural fructification.

5.1.2 Genetic analysis

Random amplified polymorphic DNA (RAPD) analysis and restriction fragment length polymorphism (RFLP) analysis were used to detect genetic variation among isolates of *C. purpureum* and to track the fate of deployed strains of *C. purpureum* as they interact with resident populations.

Based on variations within the large non-transcribed spacer (NTS-L) region of the ribosomal DNA repeat, as detected by RFLP analysis, Ramsfield *et al*.(1996) identified two geographically distinct nuclear types in North America. RFLP analysis detects variation within a small region of the genome and is, therefore, less sensitive to variation than RAPD analysis which is capable of detecting variation within the entire genome. Subpopulations of *C. purpureum* (i.e., eastern, western, and central populations) may, in fact, be more heterogeneous than those presented in this paper.

Two studies by Gosselin *et al*. (1995 and 1999a) utilized RAPD analysis to assess the genetic variation among *C. purpureum* isolates from Quebec and from across Canadian ecozones (Ecozones 1, 2, 4 and 5). No RAPD marker specific to host species or regional origin was identified. Greater diversity was noted within ecozones and small geographic areas than among ecozones and larger geographic areas. In contrast to the less sensitive RFLP analysis, these studies indicate that populations of *C. purpureum* are highly heterogeneous.

Gosselin *et al*. (1999b) then examined the fate of deployed strains of *C. purpureum*. In support of the findings of De Jong *et al.* (1996), a significantly higher incidence of disease (approximately 15%) was noted in sampling plots with injured trees, as compared to the incidence for areas with healthy stands of trees (0.3%), indicating that tree wounding is the main determinant of infection. Although RAPD analysis determined that at least 85% of the infections were not attributable to the deployed strains of *C. purpureum*, the impact of the deployed strains may have been underestimated since trees in the managed areas were likely cut prior to spore release.

Becker *et al*. (1999) also studied the fate of a deployed strain of *C. purpureum*. Although the absence of secondary infections by the deployed strain indicated a minimal risk for non-target trees, the findings were once again inconclusive since secondary infections were tested for before fruiting bodies and spore emission resulting from the deployed strain would be expected to occur.

5.1.3 Mating studies

Single-spore isolates of *C. purpureum* readily form compatible mating interactions (Gosselin *et al*., 1995; Wall *et al*., 1996). Given the diverse genetic variation among *C. purpureum* isolates, a single deployed isolate, in the form of Myco-Tech™, will be incorporated into the resident population and the genes introduced by the deployed isolate will be diluted by those of the resident population.

5.1.4 Conclusions

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.

Chondrostereum purpureum has been used in the Netherlands for a number of years with no reports of adverse environmental effects. While the Netherlands has restricted use of *C. purpureum* to areas within 500 m of fruit-growing orchards, a critical review of the submitted literature indicates that a buffer zone is not required. The decision of the Netherlands was likely based on a study (De Jong *et al*., 1990) whose assumptions were greatly biased towards an overestimation of risk and whose methods relied heavily on environmental modelling. Studies which relied more heavily on empirical data (De Jong *et al*., 1996; Gosselin *et al*., 1999b) indicated that, firstly, the additional spore load, due to deployment of *C. purpureum*, will be of the same order of magnitude, or less than, the natural spore load, and that, secondly, tree wounding, and not the spore load, is the primary determinant of infection. Therefore, no buffer zone is required since non-target healthy trees are at negligible risk while wounded trees would likely be vulnerable to resident populations of *C. purpureum*.

6.0 Effects on non-target species

6.1 Birds

6.1.1 Avian oral and
6.1.2 Avian pulmonary/inhalation/injection

The applicant submitted a rationale justifying why avian oral and pulmonary/inhalation/injection testing should not be required. The waiver request was based on the elements of the risk equation: exposure and toxicity.

The natural occurrence, method of application, level of additional spore load, and the spore dispersal pattern of *C. purpureum* all suggest that exposure to *C. purpureum* will not increase significantly with the use of Myco-Tech™. The proposed use pattern for Myco-Tech™ is limited to forests and woodlots to inhibit sprouting and regrowth of mechanically cut trees. The total area on which Myco-Tech™ will be applied is relatively small when compared to the total forested area within Canada. Also, the frequency of application to rights-of-way (ROWs) will only be one to two applications every ten years. Myco-Tech™ is to be applied topically as a gel/paste formulation in order to minimize spread of the product. Spray applications and other non-targeted application methods will not be permitted by the label. Therefore, avian exposure to *C. purpureum* will be minimal at the time of application.

Environmental fate models of *C. purpureum* sporulation and spore dispersal indicate that the additional spore density, due to deployment of *C. purpureum* as a biological control agent, will be equal in magnitude to, or less than, the naturally occurring spore density.

No evidence of infectivity or toxicity to test animals or birds has been noted for *C. purpureum* or its associated active compounds which are known only as plant toxins. Furthermore, *C. purpureum* is not a thermo-tolerant organism and does not grow or survive above temperatures of 37EC.

Given that adverse effects and increased exposure, due to use of *C. purpureum* as a biological control agent, are not expected, testing is considered unnecessary to assess the risks of Myco-Tech™ to birds. The request for a waiver is accepted.

6.2 Fish

6.2.1 Freshwater fish

The applicant submitted a rationale justifying why freshwater fish studies should not be required. The waiver request was based on the elements of the risk equation: exposure and toxicity.

The natural occurrence, method of application, level of additional spore load and the spore dispersal pattern of *C. purpureum* all suggest that exposure to *C. purpureum* will not increase significantly with the use of Myco-Tech™. A literature review also concluded that any spores or propagules of *C. purpureum* which may be deposited in aquatic habitats would not be able to become established in such an environment.

No cases of disease or infection caused by *C. purpureum* in freshwater fish have been reported in the literature.

Given that adverse effects and increased exposure, due to use of *C. purpureum* as a biological control agent, are not expected and that *C. purpureum* does not have the capacity to grow and establish itself in an aquatic habitat, testing is considered unnecessary to assess the risks of Myco-Tech™ to freshwater fish. The request for a waiver is accepted.

6.3 Arthropods

6.3.1 Terrestrial arthropods

The applicant submitted a rationale justifying why terrestrial arthropod testing should not be required. The waiver request was based on the elements of the risk equation: exposure and toxicity.

The natural occurrence, method of application, level of additional spore load, and the spore dispersal pattern of *C. purpureum* all suggest that exposure to *C. purpureum* will not increase significantly with the use of Myco-Tech™.

Chondrostereum purpureum is a ubiquitous organism that is a natural component of the forest ecosystem. No adverse effects on terrestrial arthropods, due to natural populations of *C. purpureum*, has been noted. The incremental increase in spore density is not expected to increase the likelihood of adverse effects on terrestrial arthropods. Therefore, additional testing to assess the risks of Myco-Tech™ to terrestrial arthropods is not required. The request for a waiver is accepted.

6.3.2 Aquatic arthropods

The applicant submitted a rationale justifying why aquatic arthropod studies should not be required. The waiver request was based on the elements of the risk equation: exposure and toxicity.

The natural occurrence, method of application, level of additional spore load, and the spore dispersal pattern of *C. purpureum* all suggest that exposure to *C. purpureum* will not increase significantly with the use of Myco-Tech™. A literature review also concluded that any spores or propagules of *C. purpureum* that may be deposited in aquatic habitats would not be able to become established in such an environment.

No case of disease or infection caused by *C. purpureum* in aquatic arthropods or aquatic plants has been reported in the literature.

Given that adverse effects and increased exposure, due to use of *C. purpureum* as a biological control agent, are not expected and that *C. purpureum* does not have the capacity to grow and establish itself in an aquatic habitat, testing is considered unnecessary to assess the risks of Myco-Tech™ to freshwater fish, aquatic arthropods and aquatic insects. The request for a waiver is accepted.

6.4 Non-arthropod invertebrates

The applicant submitted a rationale justifying why non-arthropod invertebrate testing should not be required. The waiver request was based on the elements of the risk equation: exposure and toxicity.

The natural occurrence, method of application, level of additional spore load, and the spore dispersal pattern of *C. purpureum* all suggest that exposure to *C. purpureum* will not increase significantly with the use of Myco-Tech™.

C. purpureum is a ubiquitous organism. No adverse effect to non-arthropod invertebrates, due to natural populations of *C. purpureum*, has been noted. The incremental increase in spore density is not expected to increase the likelihood of adverse effects on non-arthropod invertebrates. The use of *C. purpureum* is expected to have a beneficial effect on the habitat of non-arthropod invertebrates. *C. purpureum* initiates the wood decay process which contributes to surface litter, moisture retention, and increased levels of organic matter that non-arthropod terrestrial invertebrates prefer. Therefore, testing to assess the risks of Myco-Tech™ to non-arthropod invertebrates is not required. The request for a waiver is accepted.

6.5 Plants

6.5.1 Aquatic plants

The applicant submitted a rationale justifying why aquatic plant studies are not required. The waiver request was based on the elements of the risk equation: exposure and toxicity.

The natural occurrence, method of application, level of additional spore load, and the spore dispersal pattern of *C. purpureum* all suggest that exposure to *C. purpureum* will not increase significantly with the use of Myco-Tech™. A literature review also concluded that any spores or propagules of *C. purpureum* which may be deposited in aquatic habitats would be unable to become established in such an environment.

No case of disease or infection caused by *C. purpureum* in aquatic plants has been reported in the literature.

Given that adverse effects and increased exposure, due to use of *C. purpureum* as a biological control agent, are not expected and that *C. purpureum* does not have the capacity to grow and establish itself in an aquatic habitat, testing is considered unnecessary to assess the risks of Myco-Tech™ to aquatic plants. The request for a waiver is accepted.

6.5.2 Terrestrial plants

The applicant submitted a rationale justifying why terrestrial non-target plant testing should not be required. The waiver request was mainly based on the exposure element of the risk equation.

The natural occurrence, method of application, level of additional spore load, and the spore dispersal pattern of *C. purpureum* all suggest that exposure to *C. purpureum* will not increase significantly with the use of Myco-Tech™. Furthermore, any increase in spore load is not expected to increase the incidence of non-target infection and mortality if there is not a simultaneous increase in the incidence of wounding on non-target plants. A number of studies which determined the susceptibility of non-target tree species (both deciduous and coniferous) to various isolates of *C. purpureum* were reviewed. Under the worst-case scenarios of the studies, the rates of mortality were 1.4–7% and 2.5–76.7% for coniferous and deciduous tree species, respectively. Under natural conditions where the full interaction of such favourable conditions rarely occur, the rates of mortality are expected to be less than those found under experimental conditions.

Chondrostereum purpureum only infects woody-stemmed terrestrial plants (Chamuris, 1988). Therefore, other terrestrial non-woody plants are not at risk and additional testing to assess the risks of Myco-Tech™ to terrestrial non-target plants is not required. The request for a waiver is accepted.

6.6 Integrated environmental toxicology summary

Acceptable waiver rationales were submitted to address the environmental toxicology requirements for birds, freshwater fish, terrestrial arthropods, aquatic arthropods, non-arthropod invertebrates, aquatic plants, and non-target terrestrial plants. These rationales were based on minimal additional exposure, the ubiquitous nature of *C. purpureum*, lack of reported adverse effects in the literature, and, in the case of aquatic organisms, inability of *C. purpureum* to become established in aquatic environments.

The formulants in the end-use product do not pose an environmental risk when used at the proposed concentrations and application rate for control of stump-sprouting.

6.7 Environmental assessment

The environmental fate papers submitted indicated that populations of *C. purpureum* are highly heterogeneous and that compatible mating interactions occur between all combinations of single-spore isolates of *C. purpureum*. Therefore, the effect of releasing a single isolate, in this case HQ1 in the form of Myco-Tech™, is expected to be minimal. The deployed isolate will be incorporated into the resident population and the genes introduced by the deployed isolate will be diluted by those of the resident population.

Chondrostereum purpureum presents a unique situation in which the target species in one situation (e.g., ROWs) are also the non-target plants of concern outside the treatment area. A critical review of the submitted studies, however, has also led to the conclusion that a buffer zone is not required. Neither the spore load, nor the proximity to a spore source, determines whether non-target infection will occur. The main determinant for infection is the presence of a recent wound. *Chondrostereum purpureum*, however, is not considered a threat to hardwood trees in healthy forests but could contribute to the decline of severely stressed trees. The susceptibility of a tree is dependent on its condition, which in turn is dependent on cultural management and season of the year (i.e., weather conditions). The additional risk posed to susceptible injured trees, due to use of Myco-Tech™, is negligible since the additional spore load will not be significantly greater than the natural spore load.

Based largely on the environmental fate data, waivers were submitted for the environmental toxicology requirements. Non-target organisms will face minimally increased exposure to *C. purpureum* as a result of the use of Myco-Tech™. Although *C. purpureum* is ubiquitous, there has been no reported case of adverse effects on birds, freshwater fish, terrestrial arthropods, aquatic arthropods, non-arthropod invertebrates, or aquatic plants.

The formulants in the end-use product, Myco-Tech™, do not pose an environmental risk when used at the proposed concentrations and application rate for control of stump-sprouting. Consequently, Myco-Tech™ is expected to pose little environmental risk when used in accordance with the label directions. Furthermore, no special precautionary or environmental hazard statement is required on the label for Myco-Tech™. Although the applicant has intended that Myco-Tech™ only be used east of the Rockies, there would be no additional risk expected from the use of this product across Canada.

7.0 Efficacy

7.1 Effectiveness on selected species

Birch (*Betula* spp.)
Six trials were conducted over 5 calendar years (1992, 1995, 1996, 1997, and 1998) in the following locations: St. Michel, L'Ascension, Ste. Agathe, and Saguenay, QC. Three of the trials examined control with the CQP1 isolate (on rye grain or Paste 95) of

Chondrostereum purpureum and four of the trials examined control with HQ1 (formulated as Paste 96 or Myco-Tech™). The trials examining the CQP1 isolate, and one of the HQ1 trials, were small-scale trials while the three trials examining Myco-Tech™ were large-scale trials. The trial locations were largely populated by paper birch and yellow birch.

Sprouting frequency (% of cut stumps that sprouted) was reported in 2 small-plot trials with CQP1. In both trials, the sprouting frequency of the *C. purpureum* treated stumps was lower than that of the control cut stumps in the first and second year following treatment (40–100% lower in the first year, 85–100% lower in the second year). One of the trials continued evaluations of the sprouting frequency into the third year following treatment with results indicating an increase in sprouting in the *C. purpureum* treated stumps compared to the control cut (10% sprouting frequency vs. 2.5% for control cut). This trend in decreased sprouting frequency was observed when applications of *C. purpureum* were made early (June) or late in the season (August/September).

The two trials reporting sprouting frequency also recorded sprout production (cm) with the trial corresponding to a complete inhibition of sprouting frequency, in the first two years following treatment, reporting 0 cm for sprout production. The same trial reported sprout production of 1.1 cm for the *C. purpureum* treatment in the third year of evaluation against a corresponding value of 5.0 cm for the control cut treatment. The second trial reported no difference in sprout production between the fungal treatment and control cut one year after treatment (both 56 cm). A decrease of 38% in sprout production of the *C. purpureum* treatment (vs. control cut) occurred the second year following treatment.

The third small-plot trial conducted in L'Ascension in 1995 examined sprouting control by reporting on the number of stems/ha prior to treatment and 1, 2, and 3 years following treatment. The results of this trial indicated that a treatment of isolate CQP1 applied to cut stumps reduced the number of stems/ha by 73%, 72%, and 95% during the first, second, and third year following treatment, respectively. Concurrently, the control cut treatment resulted in an increase of 6.7×, 5.4×, and 3.1× the pre-treatment stem population during the first, second, and third year following treatment, respectively.

All three of the pilot scale trials examined control of birch species with a treatment of HQ1 in the formulation of Myco-Tech™. The first of the three trials examining different timings of application (referred to as the "time trial" with application timings from June to October) suggests that earlier applications of Myco-Tech™ may be more effective for sprout inhibition of birch species, with complete suppression of sprouting occurring (during both the first and second year following treatment) with applications made in June, July, or August, while the late season applications (data available for October timing only) suggest a higher incidence of stump sprouting (for both the treated and control cut treatment).

The remaining pilot-scale trials reported a control ratio value (stem density at evaluation divided by stem density at cutting) ranging from 0 to 0.24 for the Myco-Tech™ treatment as opposed to values ranging from 0.63 to 1.58 for the control cut treatment suggesting that the Myco-Tech™ treatment was efficacious in reducing the incidence of stump sprouting compared to the untreated stumps. Concurrently, one of the trials reported the mean height of the highest sprout per clump with the Myco-Tech™ treatment reporting a sprout height considerably reduced compared to the control cut treatment for both the first and second years following treatment (22 and 0 cm for years 1 and 2 vs. 44 and 109 cm for years 1 and 2, for the Myco-Tech™ and control cut treatments, respectively).

Pin cherry (*Prunus pensylvanica*)
Eight trials were conducted over 7 years (1992–1998, inclusive) at the following locations: Ste. Agathe (4 trials), Hunterstown, Causapscal, and L'Ascension. Five of the trials examined control of pin cherry with isolate CQP1 (on rye grain, Paste 95 or Paste 96), one of the trials examined isolate IB as Paste 95, and three trials examined isolate HQ1(as Paste 95 or Myco-Tech™). Six of the trials were small-scale research trials (all trials with CQP1, IB, or HQ1 as Paste 95) and the remaining two trials were pilot-scale trials (both with isolate HQ1 formulated as Myco-Tech™).

Sprouting frequency was recorded in 5 of the 8 trials. In all 5 trials the sprouting frequency for the *C. purpureum* treated stumps was reduced compared to the control cut stumps one year following treatment, with values ranging from 16% to 84% reduction in sprouting frequency. The trial with the low value of 16% is the trial in which isolate IB was used and it is noted in the trial report that the inoculum concentration was low at 10^3 CFU (as opposed to the proposed concentration of 10^5). Four of the same trials recorded sprouting frequency two years following the fungal application with results ranging from 0 to 100% reduction compared to the control cut. Again, the value of 0% was recorded in the trial using the low concentration of the IB isolate. Two of the trials continued the evaluation of sprouting frequency up to three years following treatment, with similar results obtained with the IB isolate (2% reduction compared to the control cut); however, the second trial reported up to 100% reduction of sprouting frequency compared to the control cut three years following Myco-Tech™ treatment.

Mean sprout height (cm) was recorded in four trials (3 small-plot, and 1 pilot-scale). Values for the fungal treatment ranged from a 6% reduction to 42% reduction compared to the control cut in the first year following treatment. Results from the second year were varied, with a 20% reduction in one trial and an increase in mean sprout height (5%) in the second trial. A single trial examining effects three years following treatment indicated a decrease of 6% compared to the control cut.

Sprout production (cm) was reported in two small plot trials with excellent results obtained from treatments made in June or August. In both the first and second year following treatment, the CQP1 isolate resulted in a reduction in sprout production of over 90% for both June and August treatments. A single trial reporting results three years following treatment indicated continued suppression of sprout production with values of

86% and 100% reduced sprout production, compared to the control cut, for June and August applications respectively.

Three of the small-scale trials reported values for mean number of sprouts per stump. Two trials reported a decrease in the mean number of sprouts per stump, compared to the control cut, one year following treatment. Two years after treatment, two of the three trials reported a decrease in the mean number of sprouts per stump for the *C. purpureum* treatment, compared to the control cut. One of the trials continued evaluation of this variable into the third year following treatment and reported a decrease in the number of sprouts per stump with the proposed treatment compared to the control cut.

A single small-plot trial conducted at L'Ascension in 1995 recorded the number of stems/ha at treatment, and 1, 2, and 3 years following treatment. This trial was conducted with isolate CQP1 formulated as Paste 95. The results from this trial show an increase in the number of stems/ha in all three years following treatment, for both the CQP1 treatment and the control cut. The increases reported are similar between the fungal treatment and the control cut, with values at approximately 65% in year 1, 67% in year 2, and 60–68% in year 3 (for the control cut and fungal treatment, respectively).

The 1997 pilot-scale "time trial" results for pin cherry are varied. The values for control ratio reported in the first and second year following treatment indicate that the treatment of isolate HQ1 as Myco-Tech™ yielded a lower control ratio value compared to the control cut for applications made in June, July, or August. The results for applications made in September and October are a reversal with the Myco-Tech™ treatment resulting in a similar or higher control ratio value than the control cut. Results for control ratio were also reported in a 1998 pilot trial in Ste. Agathe. In this trial the control ratio of the Myco-Tech™ treatment was reported as 2.31 compared to the control cut value of 5.08, in the second year following treatment.

Aspen (*Populus tremuloides*)
Eight trials were conducted over 6 years (1992–1993, 1995–1998) at the following locations: Ste. Agathe, Hervey Jonction, L'Ascension, Abitibi, and Saguenay. Four of the trials examined control of aspen with isolate CQP1 while five of the trials examined control with HQ1. Four of the trials were pilot-scale with the remaining as small-plot trials.

Sprouting frequency was reported in 3 of the small-plot trials. During the first two years following treatment, all three of the trials reported reduced sprouting frequency with the fungal treatment compared to the control cut. Values ranged from 32% to 100% reduced sprouting compared to the control cut in the first two years following treatment. The reduced sprouting was observed with treatments made in June, August, or September. A single trial reported sprouting frequency three years following treatment with a continuation of the trend for reduced sprouting with the *C. purpureum* treatment.

Sprout production was reported in two trials conducted with CQP1 on rye grain inoculum. Both trials examined treatments of cut stumps in June and August. In both trials, and at both treatment timings, the *C. purpureum* treated stumps reduced sprout production compared to the control cut with values ranging from 63% to 100% reduction compared to the control cut treatment. This trend was observed up to three years following treatment.

Mean sprout height (cm) was recorded in two of the trials. One of the trials reported mean sprout height reductions of 32% and 28% during the first and second year following treatment. The second trial recorded an increase in mean sprout height with the *C. purpureum* treatment compared to the control cut (two years after treatment).

The mean number of sprouts per stump, recorded in a single small plot trial, was reduced with a treatment of *C. purpureum* the first year following treatment but equalled or increased compared to the control cut treatment the second year following treatment.

A single trial recorded the number of stems/ha at treatment and up to 3 years following treatment. The results from this trial report an increase in the number of stems/ha with a treatment of *C. purpureum* and also with the control cut. The increase with the fungal treatment is reduced, however, compared to the control cut, with the *C. purpureum* treated stems increasing approximately 10 to 15% compared to the stem population at treatment. The number of stems/ha of the control cut treatment, however, increased 6.5- to 7.8-fold in relation to the population at treatment.

The results of the "time trial" are fairly consistent with the exception of the July treatment in that the control ratio reported for aspen is consistently lower with a treatment of Myco-Tech™ compared to the control cut treatment. The results for July indicate similar control ratios for the fungal treatment and the control cut. These results were observed both the first and second year following treatment.

The remaining three pilot-scale trials exhibited a similar trend to the "time trial" in reporting lower control ratio ratings for the Myco-Tech™ treatment compared to the control cut treatment suggesting increased suppression of resprouting of aspen with the Myco-Tech™ treatment.

Sugar Maple (*Acer saccharum*)

Six trials were conducted over 5 years (1992, 1995–1998) at the following locations: St. Michel, L'Ascension, and Ste. Agathe (4 trials). Two of the trials indicated in the report that the population of maple trees was a combination of sugar and red maple with sugar maple being the predominant species. As a result, these trials were included in the review for sugar maple and only trials identifying red maple as the subject species were included in the review for red maple.

Sprouting frequency was recorded in 3 small-plot trials. In two of the three trials the sprouting frequency recorded for a treatment of *C. purpureum* was reduced compared to the control cut treatment, whether application was made in June or August. The third trial showed little difference between the *C. purpureum* treated stumps and the control cut with similar sprouting frequencies of 98% and 100%, respectively (combination of sugar and red maple in this trial).

Mean sprout height (cm) was recorded in three trials (2 small-plot and 1 pilot-scale). On average, the mean sprout height of the *C. purpureum* treated stumps was 12% lower than that of the control cut stumps (averaged over year 1 and year 2 observations). In two of these trials the mean number of sprouts/stump was recorded. Year 1 data from one of the trials showed a reduction in the mean number of sprouts/stump with the *C. purpureum* treatment (2.9 sprouts/stump) versus the control cut (4.3 sprouts/stump) with no second year results recorded. The second trial which reported year 2 results indicated no difference in the mean number of sprouts/stump for the fungal treatment versus the control cut (each produced 1.5 sprouts/stump on average), however combined with the mean height data (a 12% reduction for the fungal treatment), the results suggest that *C. purpureum* still had an effect in inhibiting sprouting by reducing mean sprout height in this trial.

Sprout production (cm) was recorded in a single trial with the *C. purpureum* treated stumps resulting in much lower values for sprout production than the control cut treatment. This was true for applications made in June or August, during the first, second, and third year following treatment.

A single trial reported stem density/ha at treatment and up to 3 years after treatment. Substantial increases in stem density were recorded for both the *C. purpureum* and control cut treatments. Specifically, the fungal treatment resulted in increases of 7×, 10×, and 11× the density at treatment in the first, second, and third year following treatment, respectively. This increase was not as pronounced as the control cut densities that recorded increases 7×, 12×, and 15× the density at treatment in the first, second, and third year following treatment, respectively.

The “time trial” conducted in 1997 reported control ratio values for Myco-Tech™ that were consistently lower than the control cut treatment (both 1 and 2 years following treatment) for applications made in June, July, August, and September. The reduction in control ratio of the fungal treatment compared to the control cut ranged from 40% to 86% reduction in the first year following treatment and 70% to 89% in the second year. The control ratio values for the October application are virtually identical for Myco-Tech™ and the control cut suggesting that a late season treatment with Myco-Tech™ may not be as efficacious for this species. Control ratio results from a second pilot-scale trial confirmed the findings of the “time trial” in that the control ratio for the Myco-Tech™ treatment was approximately 50% lower than the value for the control cut.

Red Maple (*Acer rubrum*)
A total of four trials examined sprout inhibition of red maple with a treatment of *C. purpureum*. The trials were conducted over a period of three years (1995, 1997, and 1998) and in the following locations: L'Ascension, Ste. Agathe, and Saguenay, QC. One of the trials was a small-plot trial examining isolate CQP1 formulated as Paste 95 while the remaining trials were pilot-scale trials using Myco-Tech™.

The small-scale trial recorded results as the number of stems/ha at treatment and 1, 2, and 3 years following treatment with the results indicating an increase in stem density in all years, with both the fungal and control cut treatments. The magnitude of increase in stem density was not as pronounced for the *C. purpureum* treatment (3-fold increase three years following treatment) as it was for the control cut (7-fold increase three years following treatment).

Mean sprout height (cm) was measured in one of the pilot scale trials with the other trial recording the mean height of the highest sprout/clump. In both cases the height of sprouts, be it the mean or highest sprout, was reduced with a Myco-Tech™ treatment, compared to the control cut. The degree to which the sprout height in the Myco-Tech™ treatments was reduced was 38% and 42% the first year following treatment, and 31% in the second year after treatment.

The results of the "time trial" report lower values for the control ratio for the Myco-Tech™ treatment compared to the control cut, for the first year following treatment when applications were made in June, July, August, and September. Similar results were obtained for the second year following treatment with the exception of the July treatment in which the Myco-Tech™ treatment resulted in a control ratio higher than the control cut (0.59 and 0.26 for Myco-Tech™ and the control cut, respectively). Results for the October treatment timing both 1 and 2 years following treatment suggest a lack of efficacy with Myco-Tech™ resulting in higher control ratio ratings for the fungal treatment compared to the control cut. Additional results for the control ratio reported in two other trials concur with the results of the "time trial" in reporting lower control ratio values for Myco-Tech™ compared to the control cut, both 1 and 2 years following treatment.

Speckled Alder (*Alnus rugosa*)
A single pilot scale research trial conducted at Abitibi in July of 1997 reported the effects of *C. purpureum* in inhibiting sprouting of cut stumps of alder species. During the first year following treatment the control ratio for the fungal treatment was 0.04 which represents a 90% reduction compared to the control cut value of 0.41. Similarly, the second year following treatment yielded control ratio values of 0.06 and 0.29 for the Myco-Tech™ and control cut treatments respectively, representing a reduction of 80% with the fungal treatment.

In addition to the results of the above field trial, the applicant provided published reports documenting control of alder with *C. purpureum*. The first paper (Pitt *et al.*, 1999) examined sprout suppression of speckled alder with a treatment of two isolates of *C. purpureum*, isolate 2139 (from British Columbia) and isolate JAM 6 (from Ontario) at locations in eastern Ontario. Details concerning formulation were not provided apart from the indication that two formulations were used. In addition, details regarding application concentration (i.e., CFU/mL) were also omitted from the published report. The results from this trial indicate that the fungal treatment provided a slight reduction in overall stem density of alder (relative to pre-treatment density) and reduced overall plant vigour as reflected in decreased stem (72% reduction in stem volume index) and crown size (57% reduction in crown volume index) compared to the untreated check.

A similar study to Pitt *et al.* (1999) was conducted in western Canada in 1995. The same isolates and formulations of *C. purpureum* were used to examine the effect of the fungal treatment on sprouting and regrowth of Sitka alder at two locations in the Boundary Forest District of southern British Columbia. Results from this trial indicate that a treatment of Sitka alder stumps with *C. purpureum* is more effective in controlling growth than a cut treatment alone (regardless of isolate or formulation used) providing statistically greater mortality than the control cut treatment alone (11.2%).

The final publication submitted by the applicant in support of the request to include speckled alder on the Myco-Tech™ label is a complement to the work reported by Pitt *et al.* (1999). Becker *et al.* (1999) examined the rate of infection of speckled alder by the JAM6 and 2139 isolates of *C. purpureum* and reported that 80% to 87% of the treated stumps became infected (confirmed via recovery of fungal samples from the treated stumps). These results reflect only disease incidence and not the degree of damage, if any, caused by infection within the treated stump.

The published studies submitted for review in support of speckled alder do not contain sufficient detail concerning the concentration of inoculum, formulation, etc., and consequently are not acceptable.

The applicant has also stated that Alnus and Betula are taxonomically related and as such, this forms part of a rationale for including alder on the Myco-Tech™ label: this rationale is not acceptable.

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

A total of three studies examined the potentially adverse effects of *C. purpureum*. Two of the trials specifically examined effects on conifer species while the third trial examined the effects of an increased source of *C. purpureum* inoculum and the effects on disease incidence. Two of the trials were conducted in a greenhouse setting with a variety of coniferous species, including Balsam fir, Black spruce, Norway spruce, White spruce, and Jack pine. Seedlings were deliberately wounded and inoculated with an actively growing

culture of *C. purpureum*. In both trials control treatments were included for the purpose of comparison. The greenhouse trials were established as either completely randomized or split-plot design. The third trial was established in a hydro right of way in Quebec for the purpose of examining the effects of artificially increasing the *C. purpureum* inoculum source. Effects were recorded for paper birch since this species is particularly susceptible to the fungus.

In the two greenhouse trials conditions were created to examine the impact of the fungus in a "worst case scenario" by deliberately wounding the conifer trees and placing a source of inoculum in direct contact with the wound. The results indicate that infection of various conifer species can occur; however, mortality related to this infection is generally low (i.e., 23% infection rate on Black spruce resulted in 7% mortality while Norway spruce exhibited 19% infection with only 1.4% mortality). In addition, in instances where the infection rate may be quite high, there were no visual differences between the treated and untreated trees with respect to shoot elongation, needle discolouration, necrosis, or chlorosis.

For the third trial in which the effects of augmenting the inoculum source for *C. purpureum* were examined, the results on paper birch indicated that there was little effect on increasing the incidence of infection on cut stumps of this species (in a radius of up to 600 m from the treated area). There was an increase in infection with respect to birch logs placed in the experimental area; this, however, was not unexpected.

While the results of these trials suggest that infection of coniferous species with *C. purpureum* may occur, the effect of the disease on conifers does not appear to coincide with its effect on certain deciduous tree species. In addition, with regard to crop safety in a conifer release setting, it is important to note that, should damage to the desired conifers occur during a release operation, the only active source of inoculum would be the Myco-Tech™ 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 following the treatment period, with conifer wounds having healed, thereby minimizing the likelihood of infection.

7.3 Compatibility with current management practices including IPM

The common management practices for vegetation control in rights-of-way and conifer release management rely largely on herbicide use (chemical pesticide). In certain settings, however, the use of herbicides (chemical pesticides) is no longer acceptable with brush saw cutting offering the only viable option for weedy brush control. As such, Myco-Tech™ is compatible with the current management systems in its role of enhancing the activity of a brush control operation.

7.4 Contribution to risk reduction

The use of Myco-Tech™ 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 conifer release management settings.

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

Based on the mode of action of Myco-Tech™, the development of resistance is unlikely.

7.6 Conclusions

Adequate efficacy data has been provided to support the use of Myco-Tech™ in rights-of-way and conifer release management, as proposed on the product label, for inhibition of sprouting on species of birch, pin-cherry, poplar/aspen, red maple, and sugar maple. The data submitted in support of the use of Myco-Tech™ in conifer release management demonstrates adequate safety to coniferous species.

8.0 Toxic Substances Management Policy considerations

During the review of Myco-Tech™ Paste, the PMRA has taken into account the federal Toxic Substances Management Policy[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 active ingredient (technical grade) does not contain any by-products or microcontaminants that meet the TSMP Track-1 criteria. Impurities of toxicological concern are not expected to be present in the raw materials nor are they expected to be generated during the manufacturing process. Also, there are no formulants of toxicological concern present in the Myco-Tech™ Paste formulation.

---

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

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

9.0 Proposed regulatory decision

The Pest Management Regulatory Agency has carried out an assessment of available information in accordance with Section 9 of the Pest Control Products Regulations and has found it sufficient pursuant to Section 18b, to allow a determination of the safety, merit, and value of the technical active ingredient Chondrostereum purpureum (HQ1) and the end-use product Myco-Tech™ Paste. The Agency has concluded that the use of the active microorganism, *Chondrostereum purpureum* strain HQ1, in the technical active ingredient Chondrostereum purpureum (HQ1) and the end-use product Myco-Tech™ Paste in accordance with the label has merit and value consistent with Section 18c of the PCP Regulations and does not entail an unacceptable risk of harm pursuant to Section 18d. Based on the considerations outlined above, therefore, the use of the technical active ingredient Chondrostereum purpureum (HQ1) and the end-use product Myco-Tech™ Paste for inhibition of sprouting and regrowth in cut stumps of certain deciduous tree species in rights-of-way and conifer release management situations is proposed for full registration, pursuant to Section 13 of the PCP Regulations.

These products have been granted a limited term registration by the PMRA to allow users access to this low-risk product. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document to allow interested parties an opportunity to provide input into the proposed decision for full registration this product.

List of abbreviations

bw	body weight
CFU	colony forming units
DNA	deoxyribonucleic acid
dw	dry weight
EC_{50}	effect concentration 50%
EEC	expected environmental concentration
EP	end-use product
GPM	Gaussian plume model
ICMSF	International Commission on Microbiological Specifications for Foods
KTG	killed test group
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LOEC	lowest observable effect concentration
LOEL	lowest observable effect level
MA	Martin agar
MAS	maximum average score
MEA	malt extract agar
MPCA	microbial pest control agent
MRL	maximum residue limit
NA	nutrient agar
NC	naive control
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
NTS-L	large non-transcribed spacer region
OPEM	Operational Epidemiological Model
PDA	potato dextrose agar
PMRA	Pest Management Regulatory Agency
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
ROWs	rights-of-way
SC	shelf control
TG	test group
TGAI	technical grade of the active ingredient
TSMP	Toxic Substances Management Policy

References

Becker, E.M., L. A. Ball, M.T. Dumas, D.G. Pitt, R.E. Wall, and W.E. Hintz. 1999. *Chondrostereum purpureum* as a biological control agent in forest vegetation management. III. Infection survey of a national field trial. Can. J. For. Res. **29**:859-865.

Becker, E.M., L.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. Biol. Cont. **15**:71-80.

De Jong, M.D., P.C. Scheepends, and J.C. Zadoks. 1990. Risk analysis applied to biological control of a forest weed, using the Gaussian plume model. Grana **29**:139-145.

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

Gosselin, L., R. Jobidon and L. Bernier. 1995. Assessment of genetic variation within *Chondrostereum purpureum* from Quebec by random amplified polymorphic DNA analysis. Mycol. Res. **100(2)**: 151-158.

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

Gosselin, L., R. Jobidon, and L. Bernier. 1999b. Biological control of stump sprouting of broadleaf species in rights-of-way with *Chondrostereum purpureum*: incidence of the disease in non-target hosts. Biol. Cont. **16**:60-67.

Goulet, A. 1988. Epidemiology of the fungus *Chondrostereum purpureum*, a biological control agent of the asexual reproduction of deciduous tree species in forest environment. Master's thesis. Faculté de forestrie et de géomatique. Université Laval. Chapitre 3 - Development of OPEM, a model simulating spore release and dispersal of *Chondrostereum purpureum*.

Grosclaude, C. 1969. La plomb des arbres fruitiers VII. Observations sur les carpophores et les spores du *Stereum purpureum*. Ann. Phytopath **1**:75-85.

Pitt, D.G., M.T. Dumas, R.E. Wall, D.G. Thompson, L. Lanteigne, W. Hintz, G. Sampson, and R.G. Wagner. 1999. *Chondrostereum purpureum* as a biological control agent in forest vegetation management. I. Efficacy on speckled alder, red maple, and aspen in eastern Canada. Can. J. For. Res. **29**:841-851.

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

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

Wall, R.E., D.E.Macey, and E. Sela. 1996. Virulence and infertility of *Chondrostereum purpureum* isolates. Biol. Cont. **7**:205-211.

Appendix I Summary Tables

Table 1 Summary of Toxicity and Infectivity Studies with Myco-Tech™ (*C. purpureum* HQ1)

STUDY	SPECIES/STRAIN AND DOSES	LD_{50}, NOEL/NOAEL and LOEL	TARGET ORGAN/ SIGNIFICANT EFFECTS/ COMMENTS
ACUTE STUDIES			
Oral Toxicity	Rat — outbred Sprague Dawley CD, 3/sex, 5000 mg/kg bw or 5×10^6 CFU/kg bw	$LD_{50} > 5 \times 10^6$ CFU/kg bw	No clinical symptoms and no mortalities. All animals gained weight except for one **&** which lost 2 grams between days 7 and 14. No significant findings observed at necropsy. **LOW TOXICITY**
Intraperitoneal Injection Infectivity	Rat — Outbred Sprague Dawley CD: — 12/sex treated with live active ingredient, 2 mL/100g bw or 0.04 g (dw)/100 g bw; — 12/sex treated with heat-killed active ingredient, 2 mL/100 g bw or 0.04 g (dw) /100 g bw	$LD_{50} > 2$ mL/100 g bw	No mortalities. Statistically significant decrease in body weights and body weight gains compared to negative control noted on day 3 in animals treated with live and heat-killed test substance. No other clinical symptoms noted. At necropsy, multiple masses/adhesions noted in the peritoneal cavity of most animals treated with live and heat-killed test substance. A liver mass was noted on day 7 in one **&** treated with heat-killed test substance, and an enlarged mesenteric lymph node was noted in another **&** in that same group on day 7. On day 7, mean relative spleen weights of**%** rats treated with heat-killed test substance were statistically higher than the negative controls on day 7. Masses/adhesions, enlarged lymph nodes and increased mean relative spleen weights are normal immunological responses to a foreign particulate and are not considered as adverse effects. No significant findings noted at necropsy of negative and shelf control animals. MPCA not recovered from any of the tissues or masses examined other than from caecum collected on day 0 from a single **%** treated with live test substance. On day 0, live test substance was recovered from the peritoneal lavage fluid of all animals treated with live test substance. **NOT PATHOGENIC**
Dermal Toxicity	Rabbit — NZW, 5/sex, 2000 mg/kg bw single dose (approximately 2.44×10^5 CFU/kg bw)	$LD_{50} > 2.44 \times 10^5$ CFU/kg bw ($LD_{50} > 2000$ mg/kg bw)	No mortalities. All animals exhibited grade[1] 1 or grade 2 erythema following unwrapping on day 2. Irritation cleared by day 3 in all animals except for one male which cleared on day 4. No overt signs of toxicity noted in any animal. **LOW TOXICITY**
Dermal Irritation	Rabbit — NZW, 1 male and 2 females, 0.5 g single dose (approximately 6.1×10^4 CFU)	MAS[2] = 0/8 (24h, 48h, and 72h)	No mortalities and no signs of dermal irritation. **NON-IRRITATING**

[1] Erythema: 0 = none, 1 = very slight, 2 = well-defined, 3 = moderate to severe, 4 = severe erythema to slight eschar formation
[2] MAS = Maximum Average Score