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OPP 2004-0390

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: *Chondrostereum purpureum* in Chontrol™; EPA Reg. No. 74200-E / R

TO: Susanne Cerrelli, Regulatory Action Leader
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511C)

MAR 11 2004

FROM: Joel V. Gagliardi, Ph.D., Microbial Ecologist
Microbial Pesticides Branch, Biopesticides and
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Joel V. Gagliardi

THROUGH: John L. Kough, Ph.D., Senior Scientist
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511C)

John L. Kough

ACTION REQUESTED: Review submitted studies, background material and waiver requests to support registration of the end-use product Chontrol™ containing *Chondrostereum purpureum* isolate PFC 2139.

CONCLUSION: Studies for Acute Oral Tox, Acute Dermal Tox/Path and Acute Eye Irritation were **ACCEPTABLE**. A supporting study was **SUPPLEMENTAL** and the Acute Pulmonary Tox/Path study was **SUPPLEMENTAL but upgradeable**. Waiver requests for Acute Injection Tox/Path and Acute Oral Tox/Path were **ACCEPTABLE**, though a waiver request for Hypersensitivity Incidents was **SUPPLEMENTAL** since reporting of any incidents is ongoing.

DATA REVIEW RECORD:

Active Ingredient:	*Chondrostereum purpureum* strain PFC 2139
Product Name:	CHONTROL
Company Name:	Mycologic, Inc.
Company ID No.:	074200
Chemical Number:	081308
Decision Number:	218667
DP Number:	291959
MRID Nos.	454933-03, 454933-04, 455071-02, 455071-03, 455071-04, 455071-01, 460183-02.

BACKGROUND:

The basidiomycete *Chondrostereum purpureum* was originally described in 1774 as *Stereum purpureum*, renamed *Telephora purpurea* between 1801 and 1821, re-established as *Stereum purpureum* in 1838 (Chamuris 1988) then classified into a new genus with *Chondrostereum purpureum* as the sole species by 1959 (Pouzar 1959). Synonyms include the previously separated *Stereum rugosiusculum* described in 1873, *Stereum purpureum* and the current *Chondrostereum purpureum*.

Naturally occurring *Chondrostereum purpureum* isolates are reported as having varying degrees of diversity. In one reference, considerable genetic diversity is reported in 93 isolates from four of five ecozones shown on a map of Canada; analysis with 22 RAPD amplicons showed the isolates were all different (Gosselin et al. 1999). Tommerup et al. (1995) found that RAPD patterns could easily be non-reproducible and extraordinary steps must be taken to reproduce results and compare previous to current results. In another reference, three major nuclear types (I, II and III) using RFLP are reported as prevalent among the 107 studied isolates; type I is found mainly in eastern North America (and in Europe and New Zealand), type II mainly in western North America, and type III only in Europe and New Zealand (Ramsfield et al. 1996). Types I and II coexist in the middle of the North American continent and it is thought the populations will eventually converge naturally by way of airborne basidiospores and subsequent sexual recombination between growing mycelia (Ramsfield et al. 1996).

Reports of *C. purpureum* existence on all continents except Antarctica, and in at least 40 countries, show this organism is relatively common (Commonwealth Mycological Institute 1991). *C. purpureum* was described in several references as ubiquitous to northern North America south to Virginia in the East and to northern California in the west. The natural range of *C. purpureum* is thought limited to temperate, moist zones and research shows growth is inhibited above 35 °C and stops above 37 °C (Wall 1986). Sporulation is reported as optimum below 20 °C with wet conditions (> 90 % relative humidity) (Spiers 1985, Dye 1974), and spores optimally germinate within 24 hours at 17.5 - 28 °C and $\geq$ 75 % water content, though a high germination rate was also noted at 5 °C (Spiers and Hopcroft 1988). Spore-producing basidiocarps are reported to retain viability despite 12 months of dehydration (Buller 1958) and can resume basidiospore production within 6 - 8 hours of re-hydration (Spiers and Hopcroft 1988).

The natural host range of *C. purpureum* includes a variety of deciduous hardwood plants in which it is a pathogen gaining entry mostly through newly created wounds, causing the systemic 'silverleaf' disease in many deciduous plants (Ginns 1986; Setliff 2002). *C. purpureum* was also reported to produce mostly localized cankers in artificially-inoculated, healthy Yellow Birch (Wall 1991). Host plants have been reported to successfully fight off infection if invading *C. purpureum* can be physically compartmentalized. Disease progression 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 variously, extracellular enzymes (Miyairi et al. 1977, 1979), sesquiterpene compounds (Ayer et al. 1981) (e.g. torreyol, sterpurene) and antibiotics (Bianco and Scurti 1977). Ergosterol, a conidial wall constituent and a precursor to vitamin D, is reportedly produced by *C. purpureum*

though its role in plant disease has not been defined.

Conifer trees have had reported cases of infection, though other saprophytes apparently quickly crowd out *C. purpureum* in infected tissues, so it does not lead to disease in these plants (Etheridge and Morin 1963). A recent study by E.C. Setliff (2002) surveyed 561 collections of *C. purpureum* in Canada and the United States. They found infection rates highest in: Betulaceae (45 %) especially *Betula* (27 %) and *Alnus* (15 %); Salicaceae (20 %) with 15 on *Populus* and and 5 % on *Salix*; Rosaceae (11 %) though reflecting a bias of agricultural research in orchards; Fagaceae (6 %); Aceraceae (4 %); Ulmaceae (3 %); from 1 to 3 % for Cornaceae, Juglandaceae, Magnoliaceae and Pinaceae, and < 1 % for Aquifoliaceae, Caprifoliaceae, Cupressaceae, Grossulariaceae, Hamamelidaceae, Hippocastanaceae, Myrtaceae, Oleaceae, Rhamnaceae and Tiliaceae. The author pointed out the potential for epidemic in the Betulaceae and Salicaceae (Birch and Alder) and that significant incidences of infection could follow timber harvesting or storm damage. Setliff also pointed out that application of *C. purpureum* to areas often pruned, such as orchards, should be avoided, as has been the practice with use of *C. purpureum* as a biocontrol agent in The Netherlands.

C. purpureum is considered a primary invader of wounds in plants where it causes disease. Progression by other, more invasive fungi occurs late, often after *C. purpureum* sporulation from basidiocarps on dead tissues when it has killed the host. Disease progression is reported to take from months to several years after infection with life cycle progression and replacement by secondary colonizers and degraders typically reported at 6 - 12 months to 3 years. Rayner (1977) reported that *C. purpureum* was dominant on cut trees in one field study the first two years after infection and sporophores formed within 1 year. In a second field study *C. purpureum* disappeared after 18 months on cut stumps and was replaced by other Basidiomycetes. De Jong (1990) reports spore production 'in the fall following biological control' to black cherry stumps. Apple trees infected through the trunk reportedly die within 2-3 years with fructification in the autumn on dead wood and spore production continuing for about a year, and winter and early spring the periods of highest naturally-acquired infection (Fujita 1990). In a study of several different tree types experimentally inoculated with *C. purpureum* in June, some produced fungal fruiting bodies by fall and most (especially Birch) had them by the following spring, but Trembling Aspen was minimally affected (Wall 1990). Lab studies show that *C. purpureum* is easily overgrown by 8 other fungi (*Bjerkandera fumosa*, *Coriolus versicolor*, *Hypholoma fasciculare*, *Oudemansiella radicata*, *Phanerochaete velutina*, *Phlebia merismoides*, *Piptoporus betulinus* and *Scytalidum album*) co-grown on opposing sides of 3 % malt agar plates, but *C. purpureum* produces basidiocarps and releases spores before complete overgrowth occurs (Rayner 1978). Wound treatments for fresh cuts on trees in orchards are differentially-, or not-effective at preventing *C. purpureum* infection (Wicks et al. 1983).

An extensive literature review of *Chondrostereum purpureum* and similar organisms to show potential contribution to human disease found no reported or inferred causative toxicological or teratogenic effects, but general hypersensitivity to fungi (spores, mycelia or various basidiomycete structures) would probably include reaction to *C. purpureum* though there were no specific reports found that document this. Health effects to humans or wild organisms from

fungal metabolic compounds can therefore only be inferred but should be minimal if exposure is limited. From a search through TOXLINE, some sesquiterpene compounds are reportedly strong insecticides and can have highly varying toxic effects to mammals, though none with this activity were reported involving *Chondrostereum* or *Stereum* species. Since growth of *C. purpureum* reportedly is inhibited at 35 °C and does not occur at higher temperatures, the potential risk for skin, lung or systemic infection in mammals is thought very low. Inhalation contact in the TGAI production system may need to be evaluated though if the end-use product is applied as a paste, inhalation exposure should be minimal though sporulation of the organism after inoculation may result in inhalation exposure long after application during cool, wet conditions.

Chontrol™ paste is proposed for use as 'a biological herbicide for the inhibition of resprouting and regrowth from cut stumps of hardwoods in right of ways and forestry'. Directions from the draft label include 'best suited for use on fresh cut stumps during summer or autumn', 'apply as a thin paste on the entire cut area' and 'do not mix Chontrol™ paste with chemical pesticides or herbicides'. The draft label also directs 'do not use Chontrol™ paste within 50 meters of fruit trees or ornamentals that may be pruned or grafted'. There were some discrepancies in the chemistry and nature of the tested products (TGAI, MP, EP or other) in toxicity and pathogenicity assays, compared to the CSF and label descriptions.

<u>*Chondrostereum purpureum* Formulations and Physical / Chemical Characteristics</u>

MRID 454933-02 page 37 / 714:

MP / TGAI; White Powder, 1.5-2.5 g per cc, slight mushroom odor.

EP; White Powder, 1.0-2.0 g per cc, slight corn-oil mushroom odor.

[NOTE: CHONTROL™ MSDS, MRID 454933-02 page 65 / 714, lists the EP as a white paste]

August 2, 2001 materials:

Chondrostereum CP-PFC2139 Manufacturing Use Product CSF;

1.68 % *Chondrostereum purpureum* isolate PFC2139 *

Also contains [redacted] other carriers, and [redacted] nutrients.

* Final concentration 1 x 10^7 to 3 x 10^8 CFU per Kg.

[NOTE: 1/20/2003 CSF and label indicates 1 x 10^7 to 5 x 10^8 CFU per Kg]

Chontrol™ Paste CSF; *

38.8-41.2 % *Chondrostereum* CP-PFC 2139 Manufacturing Use Product 1.68 %.

Also contains [redacted] as a diluent/carrier plus a nutrient, carrier and sticker.

* (EP) contains at least 1 x 10^5 CFU per Kg *Chondrostereum purpureum* PFC 2139 in 100 lbs.

Chontrol™ Paste Label;

0.67 % *Chondrostereum purpureum* PFC 2139 *

99.33 % Inerts

* Contains a minimum of 1 x 10^2 CFU per g.

[NOTE: 1/20/2003 CSF and label indicates 1 x 10^5 to 1 x 10^7 CFU per Kg]

SUMMARY OF DATA SUBMITTED:

STUDY TYPE: Acute Oral Toxicity - Rats.
OPPTS Guideline: 870.1100
TEST MATERIAL: *Chondrostereum purpureum* isolate PFC 2139 TGAI.
MRID NO.: 454933-03
DISCUSSION:
This study follows the OPPTS 870.1100 guideline. All rats survived the 4-day preliminary and 14-day main study at a dose of 5000 mg (1.2×10^6 CFU) per Kg body weight. All animals, male and female, gained weight steadily during the study, and clinically there were "no signs observed" for any animal. At necropsy there were "no gross lesions" in any animal.
CLASSIFICATION:
ACCEPTABLE - combined oral NOAEL > 5000 mg per Kg (1.2×10^6 CFU per Kg) - TOXICITY CATEGORY IV.

STUDY TYPE: Study supporting Acute Pulmonary Toxicity / Pathogenicity.
OPPTS Guideline: Addresses parts of 885.0001, 885.3100 and 885.3150.
TEST MATERIAL: *Chondrostereum purpureum* isolate PFC 2139 TGAI and MP.
MRID NO.: 455071-01
DISCUSSION:
The data presented, as interpreted, shows the test organism survives the grinding process and is successfully recovered from lungs and caecum at comparable levels. Blending had no consistent effect on recovery of the test organism. In general, either MEA or MA media is suitable for growth and enumeration of the test organism from lung and caecal tissues. Recovery of *Chondrostereum purpureum* after inoculation ranged from 15 - 32 % in blended tissue (lung or caecum) in 0.1 % peptone, or in 0.1 % peptone alone.
CLASSIFICATION:
SUPPLEMENTAL - data will be used for the OPPTS Guideline 885.3150 study.

STUDY TYPE: Acute Pulmonary Toxicity / Pathogenicity - Rat.
OPPTS Guideline: 885.3150
TEST MATERIAL: *Chondrostereum purpureum* isolate PFC 2139 TGAI.
MRID NO.: 455071-02
DISCUSSION:
Detection sensitivity data (MRID 455071-01) showed recovery of *Chondrostereum purpureum* inoculated to 0.1 % peptone or tissue in 0.1 % peptone was 15 - 32 % on either MA or MEA media. Clinical signs of rough hair coat and labored respirations were reported from days 2 - 4 in several rats dosed with either Live or Killed TGAI. One male rat died on day 2 and had mottled lung tissue but no viable *C. purpureum* though the author of the study did not give an explanation for the mortality; one female rat died due to anesthesia and was replaced. *C. purpureum* was detected in lungs and associated lymph nodes after dosing with the TGAI, with clearance by day 7. Mottled / pale lung parenchyma, mottled lung intermediate lobe, and / or mottled left lungs were noted on most Live and Killed TGAI dosed rats tested through day 14. No gross lesions were noted in animals from Naive or Shelf Control groups though one Naive Control female rat appeared sick during the study and four female Naive Control rats, including the sick rat, lost

weight over the 14 day study. Significantly increased lung and associated lymph node and decreased kidney weights reported in TGAI and Killed TGAI groups through day 14 were likely a result of test material administration. Male rats in the Shelf Control group had increased lung and lymph node and decreased liver weights on day 14 though the author did not deem this effect biologically significant. From the weight range of rats reported on the day of dosing (approximately 300 g each) and guideline dosing recommendations (0.3 mL per 100 g body weight) a dose approaching 1 mL (approximately 1.76×10^5 CFU per animal) could have been used. Pulmonary exposure to *C. purpureum* during TGAI manufacture is possible, though high doses are unlikely since the TGAI / MP dilutes the a.i., and the end-use product is a paste that further dilutes the a.i.

CLASSIFICATION:

SUPPLEMENTAL but upgradeable - when the mortality, plus the shelf and naive control clinical effects are explained, or the study is repeated acceptably.

STUDY TYPE: Acute Dermal Toxicity / Pathology - Rabbits.
OPPTS Guideline: 885.3100
TEST MATERIAL: *Chondrostereum purpureum* isolate PFC 2139 MP.
MRID NO.: 455071-03

DISCUSSION:

There were no mortalities or overt clinical signs reported following dosing. There was no edema in the treated areas, though 9 of 10 treated rabbits had mild erythema that cleared by day 5. The remaining rabbit exhibited no signs of irritation. All tested rabbits gained weight steadily throughout the study.

CLASSIFICATION:

ACCEPTABLE - combined LD > 2000 mg per Kg - TOXICITY CATEGORY III for irritation.

STUDY TYPE: Acute Eye Irritation - Rabbits.
OPPTS Guideline: 870.2400
TEST MATERIAL: *Chondrostereum purpureum* isolate PFC 2139 EP.
MRID NO.: 455071-04

DISCUSSION:

No corneal opacity, iritis, or positive conjunctival irritation (score ≥ 2) was noted from any rabbit. Redness of conjunctivae was noted in two animals at 24 and 48 hours. The maximum average score was 1.3, 24 hours after test material instillation, with clearance by 72 hours. Chontrol™ was practically non-irritating. The 0.1 mL test dose apparently contained 56 CFU in 0.1 mL by extrapolation from the sponsor's statement.

CLASSIFICATION:

ACCEPTABLE - TOXICITY CATEGORY IV.

STUDY TYPE: Summaries of: Acute Oral Toxicity / Pathogenicity - Rats; Acute Dermal Toxicity / Pathology - Rabbits; Acute Pulmonary Toxicity / Pathogenicity - Rats; Acute Eye Irritation - Rabbits.
OPPTS Guideline: 885.3050, 885.3100, 885.3150, 870.2400
TEST MATERIAL: **Clarified here from the original MRID's for the listed tests.**

MRID NO.: 460183-02

DISCUSSION:

This report presents a summary of toxicology and / or pathogenicity testing using Chrontrol™ and the TGAI *Chondrostereum purpureum* strain PFC2139. This volume also contains a waiver request for intraperitoneal infectivity testing that is addressed elsewhere.

CLASSIFICATION:

SUPPLEMENTAL - previously reported data.

WAIVER REQUEST: 885.3200; Acute Injection Toxicity / Pathogenicity.
PRIMARY REVIEW BY: Susan Chang, M.S.; Oak Ridge National Laboratory
MRID NO.: None (Volume 4 of submission; Project 94B).

BACKGROUND:

The author indicates that acute pulmonary testing yielded toxicity but not pathogenicity with adverse signs mostly consisting of labored breathing and a rough coat on days 2 - 3 with one mortality of a Live TGAI dosed rat on day 2. The test substance was present on day 0 but cleared from the lungs of the rat that died and was not detectable in any other necropsied rat by day 7, nor in any other tissue. Necropsy examinations on days 0, 7 and 14 found discolored lung parenchyma or lobes in Live TGAI dosed rats. In an acute oral toxicity study of 5g TGAI per Kg body weight, there were no adverse clinical signs or evidence of toxicity or pathogenicity.

DISCUSSION:

Results of OPPTS Guideline 885.3150, Acute Pulmonary Toxicity/Pathogenicity - Rat were: "**ACCEPTABLE** - LD_{50} > approximately 1.6 x 10^4 CFU per animal with one attributed mortality at this dose and some reported toxicity." Results of OPPTS Guideline 870.1100, Acute Oral Toxicity - Rats were: "**ACCEPTABLE** - combined oral NOAEL > 5000 mg per Kg (1.2 x 10^6 CFU per Kg) - TOXICITY CATEGORY IV." Results of OPPTS Guideline 885.3100, Acute Dermal Toxicity/Pathology - Rabbits were: "**ACCEPTABLE** - combined LD_{50} > 2000 mg per Kg - TOXICITY CATEGORY III for irritation." Results of OPPTS Guideline 870.2400, Acute Eye Irritation - Rabbits were: "**ACCEPTABLE** - TOXICITY CATEGORY IV." The most likely exposures are oral, dermal or to the eye and *Chondrostereum purpureum* was not infective in these or in the pulmonary exposure test. *Chondrostereum purpureum* isolate PFC 2139 and the end-use product CHONTROL™ pose a low risk for the expected exposures, and intraperitoneal exposure is unlikely due to the proposed use pattern, i.e. application to cut tree stumps as a paste.

CLASSIFICATION:

ACCEPTABLE

WAIVER REQUEST: 885.3050; Acute Oral Toxicity / Pathogenicity.
MRID NO.: none
Study Title: *Chondrostereum purpureum* isolate PFC 2139 Safety Information-Rationale for Waivers (Volume 4 of 13; page 6 of 25).

BACKGROUND:

The author indicates that an oral toxicity test (OPPTS 870.1100) on *Chondrostereum purpureum* (TGAI only) was performed without observed effects (14 day LD_{50} > 5000 mg per Kg), all proposed uses of Chontrol™ are non-food (primarily for use on forest trees), the TGAI is

naturally occurring in wooded ecosystems, and there already is a commercial product in Europe containing *Chondrostereum purpureum* (BIOCHON by Koppert).

DISCUSSION:

In correspondence (W.A. Sexsmith, PMRA to William Hintz, Mycologic, Inc. from March 22, 2000) an oral toxicity test was required and the oral infectivity test was conditionally waived. EPA notes that the dose in the Oral Toxicity study was 2.5 x 10^5 CFU per mL x 5mL per Kg x 0.290 Kg (mean animal weight) = approximately 3.625 x 10^5 CFU per animal. The dose requirement for this guideline is 10^8 CFU per animal or an explanation why a lower dose is tested. It is apparent a higher CFU concentration in this volume is not likely using the current TGAI production system, and the manufacturing-use and end-use products significantly dilute *Chondrostereum purpureum* levels (label rates indicate 10^2 - 10^4 CFU per g) so the dose tested is close to the maximum achievable guideline exposure for the TGAI (10 - 20 mL per Kg corresponding to 7.25 x 10^5 - 1.45 x 10^6 CFU per animal) with this test. From the Acute Pulmonary Toxicity / Pathogenicity - Rat test, the TGAI was not deemed infective or pathogenic (LD_{50} > approximately 1.6 x 10^4 CFU per animal) and the organism cleared from tissues within 7 days, though there was one attributed mortality at this dose and some reported toxicity. The inert ingredients were not assessed with the OPPTS 870.1100 test, though a review of potential toxicity (MSDS sheets corresponding to the CAS numbers provided) and levels of the inerts in the end-use formulation, indicates that at most mild irritation would result from ingestion.

CLASSIFICATION:

ACCEPTABLE

WAIVER REQUEST: 885.3400; Hypersensitivity Incidents.
PRIMARY REVIEW BY: Susan Chang, M.S.; Oak Ridge National Laboratory
MRID NO.: 454933-04

BACKGROUND:

The author indicates no reports of hypersensitivity incidents were noted during extensive field assessments of this product formulation. The inert ingredients are common industrial or food grade materials and the formulation is a paste. The two major inerts are considered minor respiratory or eye irritants when in dry form, though they should not be implicated in a hypersensitive response as a paste. The active ingredient *Chondrostereum purpureum* consists of mycelia only and reportedly does not form conidia known to induce allergies and hypersensitivity.

DISCUSSION:

The ingredients are not common allergens and the formulation as a water-based paste mitigates exposure likely to lead to hypersensitive or allergic responses. Continued surveillance and reporting of hypersensitivity incidents under this guideline is still required.

CLASSIFICATION:

SUPPLEMENTAL - reporting of hypersensitivity incidents is ongoing, if and when any occur. The registrant is required to report to EPA any incidents of adverse effects resulting from use of this product. This requirement includes any hypersensitivity that may develop after use.

DATA EVALUATION RECORD

Review by: Susan Chang, M.S.; Oak Ridge National Laboratory.
EPA Review by: Joel V. Gagliardi, Ph.D.

Study Type	OPPTS Guideline 870.1100; Acute Oral Toxicity - Rats.
MRID No.	454933-03
Test Material	*Chondrostereum purpureum* isolate PFC 2139 TGAI
Study No.	L08725 SN2
Sponsor	MycoLogic, Inc.; P.O. Box 3020; Victoria, British Columbia; Canada V8W 3N5
Testing Facility	IIT Research Institute; Life Sciences Operation; 10 West 35th St.; Chicago, IL 60616-3799
Title of Report	Acute Toxicity / Limit Testing of *Chondrostereum purpureum* Following Acute Oral Challenge in Rats.
Author	Kelly A. Harrington, B.S.
Study Completed	April 1999
Study Summary	This study follows the OPPTS 870.1100 guideline. All rats survived the 4-day preliminary and 14-day main study at a dose of 5000 mg (1.2×10^6 CFU) per Kg body weight. All animals, male and female, gained weight steadily during the study, and clinically there were "no signs observed" for any animal. At necropsy there were "no gross lesions" in any animal.
Classification	**ACCEPTABLE** - combined oral NOAEL > 5000 mg per Kg (1.2×10^6 CFU per Kg) - TOXICITY CATEGORY IV.
Good Laboratory Practice	GLP compliance statement was signed on 4/21/99 with a noted exception: Test substance formulation was the responsibility of the sponsor.

TEST SUBSTANCE: *C. purpureum* Lot No. PFC2139 TGAI received on cold packs August 5, 1998 dosage 2.4×10^5 CFU per mL; a light gray slurry of mycelia in water.

METHODS:

Test Animals: Eleven each male and female CD rats (Charles River Laboratories, Portage, MI) were received at 54 days old weighing 169-184 g (females) and 206-230 g (males). Rats were housed up to two per plastic cage [10.5 x 18 x 8" with hardwood chip bedding], fed Certified Purina Rodent Chow No. 5002 *ad libitum* except during overnight fasting prior to dosing, and tap water *ad libitum*, re-supplied 2x weekly. Environmental conditions of the animal room were: temperature 18-22 °C; relative humidity 58-79 %; photoperiod 12 hour light / dark cycle using fluorescent bulbs. Air changes per hour were not reported. Animals were quarantined and observed daily for 19 days prior to testing.

Preliminary Dosing:
A preliminary 4 day challenge of 5000 mg per Kg was performed in 3 male and 3 female rats.

Experimental Design:
Remaining test animals were "randomly assigned constrained by body weight" for treatment: 5 male (ear tag nos. 565-569; average predose weight 351.70 g) and 5 female (ear tag nos. 548 and 571-574; average predose weight 226.76 g) rats were selected.

Dosing:
Prior to dosing, concentration of the test material was determined by serial dilution in sterile ASTM 1 water, plating on Malt Extract Agar (MEA) and incubating at approximately 25 °C for 95 hours. Test material in water [5000 mg in 5 mL] was dosed by oral gavage at 5 mL per Kg body weight.

Evaluation:
Body weights were recorded prior to dosing and on days 7 and 14. Test animals were observed for clinical signs of toxicity at least daily for 14 days. All animals were necropsied at day 14.

RESULTS SUMMARY:
Mortality: All rats survived the 4-day preliminary and 14-day main study.
Measured Dose: 2.4×10^5 CFU per mL so 1.2×10^6 CFU per Kg body weight.
Body Weights: All animals, male and female, gained weight steadily during the study.
Clinical Observations: During the study there were "no signs observed" for any animal.
Gross Necropsy: At necropsy there were "no gross lesions" in any animal.

STUDY AUTHOR'S CONCLUSIONS:
Based on the results of this study, *Chordrostereum purpureum* [sic] did not cause mortality or acute toxicity in study animals. Therefore, the acute oral lethal dose of *Chordrostereum purpureum* in male and female rats is greater than 5g/kg body weight (1.2×10^6 cfu/kg).

DISCUSSION:
This study follows the OPPTS 870.1100 guideline. All rats survived the 4-day preliminary and 14-day main study at a dose of 5000 mg (1.2×10^6 CFU) per Kg body weight. All animals, male and female, gained weight steadily during the study, and clinically there were "no signs observed" for any animal. At necropsy there were "no gross lesions" in any animal.

CLASSIFICATION:
ACCEPTABLE - combined oral NOAEL > 5000 mg per Kg (1.2×10^6 CFU per Kg) - TOXICITY CATEGORY IV.

DATA EVALUATION RECORD

Review by: Susan Chang, M.S.; Oak Ridge National Laboratory.
EPA Review by: Joel V. Gagliardi, Ph.D.

Study Type	Addresses parts of OPPTS Guidelines 885.0001, 885.3100 and 885.3150.
MRID No.	455071-01
Test Material	*Chondrostereum purpureum* isolate PFC 2139 TGAI and MP.
Study No.	L08725 SN1
Sponsor	MycoLogic, Inc.; P.O. Box 3020; Victoria, British Columbia; Canada V8W 3N5
Testing Facility	IIT Research Institute; Life Sciences Operation; 10 West 35th St.; Chicago, IL 60616-3799
Title of Report	Sensitivity of Detection of *Chondrostereum purpureum* for Toxicity / Pathogenicity Testing in Rats
Authors	Bruce A. Gingras, Ph.D.
Study Completed	October 1998
Study Summary	The data presented, as interpreted, shows the test organism survives the grinding process and is successfully recovered from lungs and caecum at comparable levels. Blending had no consistent effect on recovery of the test organism. In general, either MEA or MA media is suitable for growth and enumeration of the test organism from lung and caecal tissues. Recovery of *Chondrostereum purpureum* after inoculation ranged from 15 - 32 % in blended tissue (lung or caecum) in 0.1 % peptone, or in 0.1 % peptone alone.
Classification	**SUPPLEMENTAL** - data will be used for the OPPTS Guideline 885.3150 study.
Good Laboratory Practice	GLP compliance statement was signed on 10/26/98 with noted exceptions: the sponsor was responsible for characterization and documentation of the test substance formulation.

TEST SUBSTANCE:

C. purpureum Lot No. PFC2139 TGAI received on cold ice packs July 16, 1998 was determined to contain 1.76 x 10^5 CFU per mL; a light gray slurry of mycelia in sterile water.

METHODS:

Test Animals: Seventy-eight male and seventy-eight female CD® rats were received from Charles River Laboratories, Portage, MI. The rats (approximately 41 - 43 days old) weighed 143

- 156 g (males) and 125 - 154 g (females) on receipt. Animals were housed up to two per cage in plastic cages. The rats had free access to tap water and certified Rodent Chow 5002. The environmental conditions of the animal room were: temperature 19 - 21 °C; relative humidity 45 - 74 %; photoperiod 12 hour light / dark cycle. Air changes per hour were not reported.

Titer: Aliquots were taken from three locations in the tube (top, center, and bottom just above any sediment) and each was plated in triplicate to TSA (Trypticase Soy Agar). This suspension was also examined for fungal (MEA - Malt Extract agar) and bacterial (BHI - Brain Heart Infusion agar) contamination. Growth was compared on MA (Martin's agar) and MEA media after 91 hours.

Assay: Four males and four females were quarantined 47 days and fasted overnight prior to sacrifice. The lungs and caecum were removed and blended with 0.1 % peptone using a Stomacher blender and blending bag. An aliquot was removed from a stirred test organism suspension, ground with a glass grinder, then held on ice. An aliquot of ground test organism suspension was diluted with sterile ASTM Type 1 Purified Water to deliver 2 x 10^4 CFU per ml (actual count 1.7 x 10^4 CFU per ml). This suspension was diluted with water to 2 x 10^2 CFU per ml (actual count 1.7 x 10^2 CFU per ml). The test organism suspensions were placed into blending bags containing either blended lungs or caecum in 0.1 % peptone in volumes standardized to the tissue weight, or to 0.1 % peptone only, then mixed by vortexing. Prior to blending, the suspensions and / or dilutions were enumerated by plating a 0.1 mL aliquot to MEA and MA media (n = 3 each). The suspensions were then re-blended and again enumerated. Colonies were counted after incubation at room temperature (25 - 26 °C) for approximately 141 hours.

RESULTS SUMMARY:

The test organism was approximately 1.76 x 10^5 CFU per ml and the suspension was homogeneous. No bacterial or fungal contamination of the test organism was found. Comparable and sufficient growth occurred on both MEA and MA media after incubation at 25 °C for 91 hours (though slightly better on MA) and the suspension showed no decrease in viability or stability over 2.5 hours.

Results of sensitivity of detection of the test organism using MEA and MA media from lung and caecal tissues in 0.1 % peptone or 0.1 % peptone alone, pre- and post- blending, are shown in Tables 1 and 2. Using MEA, recovery of *Chondrostereum purpureum* was 82 - 122 % from lung tissue and 75 - 99 % from caecal tissue at 10^2 - 10^4 CFU per ml inoculum, respectively, compared to inoculum recovered from peptone only. Using MA, recovery of *Chondrostereum purpureum* was 75 - 100 % from lung tissue and 78 - 86 % from caecal tissue at 10^2 - 10^4 CFU per ml inoculum, respectively, compared to inoculum recovered from peptone only. Table 2 shows sensitivity of detection values from Table 1 compared to actual inoculum levels, on MEA and MA, pre- and post- blending. Blending did not have uniform effects and often decreased recovery slightly compared to the same, non-blended sample. However, blending inoculated caecum increased recovery slightly in three of four assays. Recovery after inoculation ranged from 15 - 32 % of actual inoculum levels, across all treatments.

TABLE 1. Sensitivity of detection of *Chondrostereum purpureum*							
Media [a]	Tissue	Target dose CFU per ml	Pre-blending CFU per ml recovered	Pre-blending [c] recovery % vs. pre-blending no tissue	Post-blending CFU per ml recovered	Post-blending recovery % [d] vs. pre-blending no tissue	Post-blending recovery % [d] vs. pre-blending
MEA	None	2×10^2	3.50×10^1 [b]	**100**	4.75×10^1	136	
	Lung		2.88×10^1	82	2.75×10^1	79	95 [bb]
	Caecum		2.63×10^1	75	3.13×10^1	89	119 [bb]
	None	2×10^4	3.98×10^3	**100**	4.08×10^3	103	
	Lung		4.86×10^3	122	4.21×10^3	106	87
	Caecum		3.93×10^3	99	5.38×10^3	135	137
MA	None	2×10^2	5.00×10^1	**100**	4.25×10^1	85	
	Lung		3.75×10^1	75	3.63×10^1	73	97
	Caecum		3.88×10^1	78	4.38×10^1	88	113
	None	2×10^4	5.08×10^3	**100**	4.83×10^3	95	
	Lung		5.09×10^3	100	3.70×10^3	73	73
	Caecum		4.39×10^3	86	3.66×10^3	72	83

[a] MEA = Malt Extract Agar; MA = Martin's Agar.

[b] N = 2 (no tissue; peptone only), N = 4 tissue (lung or caecum); [bb] N = 3

[c] Pre-blended = sample mixed by vortexing and plated.

[d] Post-blended = sample re-blended and plated.

TABLE 2. Sensitivity of Detection of *Chondrostereum purpureum*							
Medium	Actual Dose [b] CFU per ml	Detected vs. Actual Dose (%) [a]					
		Pre-Blending			Post-Blending		
		No Tissue	Lung	Caecum	No Tissue	Lung	Caecum
MEA	1.7×10^2	21	17	**15**	28	16	18
	1.7×10^4	23	29	23	24	25	**32**
MA	1.7×10^2	29	22	23	25	21	26
	1.7×10^4	30	30	26	28	22	22

[a] Ratio of CFU recovered (Table 1 columns 4 & 6) to Inoculum CFU (Table 2 'Actual Dose').

[b] from MRID 455071-01, pg. 9 of 32.

STUDY AUTHOR'S CONCLUSIONS:

The test substance was found to be homogeneous and stable in aqueous suspension under conditions of this study, which were similar to those to be employed in pathogenicity/toxicity studies. MEA (non-selective) and MA (selective) provide acceptable levels of recovery of the test substance from the lungs of male and female CD rats, while MA provides better selective recovery from the caecum. Recovery from tissue after blending was similar to recovery from tissue before blending. Based on the results of this study, MEA will be employed as the nonselective recovery medium for rat tissues, except the stomach, caecum, and intestinal tract. MA will be employed as the selective recovery medium for the stomach, caecum, and intestinal tract in subsequent pathogenicity/toxicity studies.

DISCUSSION:

The data presented, as interpreted, shows the test organism survives the grinding process and is successfully recovered from lungs and caecum at comparable levels. Blending had no consistent effect on recovery of the test organism. In general, either MEA or MA media is suitable for growth and enumeration of the test organism from lung and caecal tissues. Recovery of *Chondrostereum purpureum* after inoculation ranged from 15 - 32 % in blended tissue (lung or caecum) in 0.1 % peptone, or in 0.1 % peptone alone.

CLASSIFICATION:

SUPPLEMENTAL - data will be used for the OPPTS Guideline 885.3150 study.

DATA EVALUATION RECORD

Review by: Susan Chang, M.S.; Oak Ridge National Laboratory.
EPA Review by: Joel V. Gagliardi, Ph.D.

Study Type	OPPTS Guideline 885.3150; Acute Pulmonary Toxicity / Pathogenicity - Rat
MRID No.	455071-02
Test Material	*Chondrostereum purpureum* isolate PFC 2139 TGAI
Study No.	L08725 SN3
Sponsor	MycoLogic, Inc.; P.O. Box 3020; Victoria, British Columbia; Canada V8W 3N5
Testing Facility	IIT Research Institute; Life Sciences Operation; 10 W. 35th St.; Chicago, IL 60616-3799
Title of Report	Toxicity / Pathogenicity Testing of *Chondrostereum purpureum* Following Acute Intratracheal Challenge in Rats.
Authors	Bruce A. Gingras, Ph.D.
Study Completed	April 1999
Study Summary	Detection sensitivity data (MRID 455071-01) showed recovery of *Chondrostereum purpureum* inoculated to 0.1 % peptone or tissue in 0.1 % peptone was 15 - 32 % on either MA or MEA media. Clinical signs of rough hair coat and labored respirations were reported from days 2 - 4 in several rats dosed with either Live or Killed TGAI. One male rat died on day 2 and had mottled lung tissue but no viable *C. purpureum* though the author of the study did not give an explanation for the mortality; one female rat died due to anesthesia and was replaced. *C. purpureum* was detected in lungs and associated lymph nodes after dosing with the TGAI, with clearance by day 7. Mottled / pale lung parenchyma, mottled lung intermediate lobe, and / or mottled left lungs were noted on most Live and Killed TGAI dosed rats tested through day 14. No gross lesions were noted in animals from Naive or Shelf Control groups though one Naive Control female rat appeared sick during the study and four female Naive Control rats, including the sick rat, lost weight over the 14 day study. Significantly increased lung and associated lymph node and decreased kidney weights reported in TGAI and Killed TGAI groups through day 14 were likely a result of test material administration. Male rats in the Shelf Control group had increased lung and lymph node and decreased liver weights on day 14 though the author did not deem this effect biologically significant. From the weight range of rats reported on the day of dosing (approximately 300 g each) and guideline dosing recommendations (0.3 mL per 100 g body weight) a dose approaching 1 mL (approximately 1.76×10^5 CFU per animal) could have been used. Pulmonary exposure to *C. purpureum* during TGAI manufacture is possible, though high doses are unlikely since the TGAI / MP dilutes the a.i., and the end-use product is a paste that further dilutes the a.i.

Classification	**SUPPLEMENTAL but upgradeable** - when the mortality, plus the shelf and naive control clinical effects are explained, or the study is repeated acceptably.
Good Laboratory Practice	GLP compliance statement was signed on 4/21/99 with a noted exception: sponsor was responsible for characterization of the test substance.

TEST SUBSTANCE: *C. purpureum* Lot No. PFC2139 TGAI received on cold ice packs July 16, 1998 was determined to contain 1.76 x 10^5 CFU per mL; a light gray slurry of mycelia in sterile water.

METHODS

Test Animals: Seventy-eight male and seventy-eight female CD® rats were received from Charles River Laboratories, Raleigh, NC. The male and female rats (approximately 85 days old) were assigned and weighed (446.5 - 573.2 g and 248.5 - 346.8 g respectively) on the day of dosing. Test animals were housed up to two per cage in plastic cages. The rats had free access to tap water and certified Rodent Chow 5002. The environmental conditions of the animal room were: temperature 18 - 21 °C; relative humidity 45 - 74 %; and photoperiod 12 hour light / dark cycle. Air changes per hour was not reported.

Preliminary Assay: Four each male and female rats received an intratracheal dose (see below) of 1.6 x 10^4 CFU per animal in 0.1 mL.

Dosing: Test material was taken from the middle of the container while stirred, then ground in a glass grinder and held on ice. A portion was inactivated by autoclaving (121 °C, 15 minutes). Viability was tested on non-selective Malt Extract Agar (MEA) or selective Martin's Agar (MA) and dilutions were prepared in sterile ASTM Type 1 Purified Water. Test material was administered by a single intratracheal dosage of 0.1 mL (1.6 x 10^4 CFU).

Main Assay: Rats were ear-tagged, assigned to treatment groups (Table 1) and quarantined 44 days prior to dosing. Body weights for surviving rats were recorded on days 0, 7 and 14 (day 16 for two females in the NC group). Test animals were observed for clinical signs of toxicity daily for the duration of the study.

Table 1 - Treatment groups and assigned animal numbers.

Sex	Day	TGAI Dose Group	Killed TGAI [b] Dose Group	Naive Control Group	Shelf Control Group	Total
M	0 [d]	101-105	121-125	141-145	-	15
	7 [d]	106-110	126-130	146-150	-	15
	14 [d]	111-115	131-135	151-155	161-165	20
	28	116-119, 120 [a]	136-140	156-160	-	15
	Total	**20**	**20**	**20**	**5**	**65**
F	0 [d]	166-170	186-190	206-210	-	15
	7 [d]	171-175	191-193, 195, 247 [c]	211-215	-	15
	14 [d]	176-180	196-200	216-220	226-230	20
	28	181-185	201-205	221-222	-	12
	Total	**20**	**20**	**17**	**5**	**62**

M = Male; **F** = Female; [a] Spontaneous death of rat 120 on day 2; [b] Autoclaved 121 °C, 15 minutes; [c] Replacement for rat 194 that died from over-anesthetization; [d] Sacrifice day.

Necropsy: Five rats per sex in the TGAI, Killed TGAI and Naive Control groups were euthanized on each of days 0, 7, and 14; five rats per sex in the Shelf Control group were euthanized on day 14. Brain, lungs, spleen, liver, kidneys, and caecum were removed and examined for gross abnormalities. The lungs and associated lymph nodes, blood, brain, spleen, liver, kidneys and caecum were collected and weighed prior to microbial enumeration. Tissues were blended, aliquots serially diluted in 0.1 % peptone, then 0.1 mL of dilutions were plated. MA medium was used for caecum, and remaining tissues were plated to MEA. Microbial titer (CFU per ml blood or per g tissue) was by counting colonies on duplicate spread plates after a 138 hour incubation at 25 - 26 °C.

RESULTS SUMMARY:

Mortality: One TGAI-dosed male (No. 120) was found dead on day 2. One female in the Killed TGAI group was replaced when it died from over-anesthetization following dosing. All other rats survived to scheduled sacrifice.

Body Weights: Eight surviving male and three female rats in the TGAI group, one male and five female rats in the Killed TGAI group, one male and two female rats in the Shelf Control group and five females in the naive control group lost weight during the first week of the study. From day 7 to days 14 - 16 for remaining rats: one TGAI group female, three Killed TGAI group females, and four Naive Control group females lost weight; one of the TGAI group females and four Naive Control females had an overall weight loss for the study.

Clinical Observations: Rough hair coat and labored respirations were observed in TGAI (Male Nos: 106, 107, 108, 115, 117, 119; Female Nos: 171, 178, 184) and Killed TGAI (Female Nos. 191, 204) dosed rats on days 2 - 4. One Naive Control female (No. 222) had a rough hair coat on day 2, hunched posture on day 6 and appeared lethargic and thin on day 8.

Gross Necropsy: Mottled / pale lung parenchyma, mottled lung intermediate lobe, and / or mottled left lungs were noted in: three males in the TGAI group sacrificed on day 0; the TGAI group male that died on day 2; five males / five females in the TGAI and four males / three females in the Killed TGAI group sacrificed on day 7; five males / five females in the TGAI and three males / four females in the Killed TGAI group sacrificed on day 14. No gross lesions were noted from animals in the Naive Control or Shelf Control groups.

Organ Weights: Significantly increased lung and associated lymph node weights relative to the Naive Control group were noted in males from the TGAI and Killed TGAI groups on days 7 and 14 and in females from the TGAI group on day 14. Significantly decreased kidney weights relative to the Naive Control group were noted in males from the TGAI and Killed TGAI groups on day 7. Male rats in the Shelf Control group had a significant increase in lung and lymph node weight, and decrease in liver weight, relative to the Naive Control group on day 14.

Infectivity Results: No test organisms were detected in any tested tissue samples from the Naive Control, Shelf Control or Killed TGAI groups. The test organism was detected in lung and associated lymph node tissues of male and female rats in the TGAI group on day 0 but not on subsequent sampling days, including from the male rat that died on day 2 (Table 2).

Table 2 - Recovery of *Chondrostereum purpureum* from rat tissues

Tissue	Sex	Mean Viable Recovery (log_{10} CFU per g or mL) [a]		
		Sacrifice Day		
		0	7	14
Blood	Male / Female	BDL [b]	BDL	BDL
Lungs & Associated Lymph Nodes	Male	3.400 *	BDL	BDL
	Female	3.056 *	BDL	BDL
Spleen	Male / Female	BDL	BDL	BDL
Liver	Male / Female	BDL	BDL	BDL
Kidneys	Male / Female	BDL	BDL	BDL
Brain	Male / Female	BDL	BDL	BDL
Caecum	Male / Female	BDL	BDL	BDL

[a] Mean of log_{10} [CFU per g tissue or mL blood + 1], N = 5; [b] BDL = Below Detection Limit [< 30 CFU per g tissue or per mL blood]; * Significantly different from the Naive Control ($p \leq 0.05$).

STUDY AUTHOR'S CONCLUSIONS:

Within the study design parameters measured, a microbial pest control agent, containing *Chondrostereum purpureum*, was found to be slightly toxic, but not pathogenic at the dose administered by the intratracheal route to the male and female CD rats employed in the study. Detection of the test substance from the lungs and associated lymph nodes of TG-dosed rats on Day 0, is consistent with intratracheal administration of a fungi. By Day 7, test substance was cleared from all tissues tested. No test substance was detected in NC, KTG or SC animals.

DISCUSSION:

Detection sensitivity data (MRID 455071-01) showed recovery of *Chondrostereum purpureum* inoculated to 0.1 % peptone or tissue in 0.1 % peptone was 15 - 32 % on either MA or MEA media. Clinical signs of rough hair coat and labored respirations were reported from days 2 - 4 in several rats dosed with either Live or Killed TGAI. One male rat died on day 2 and had mottled lung tissue but no viable *C. purpureum* though the author of the study did not give an explanation for the mortality; one female rat died due to anesthesia and was replaced. *C. purpureum* was detected in lungs and associated lymph nodes after dosing with the TGAI, with clearance by day 7. Mottled / pale lung parenchyma, mottled lung intermediate lobe, and / or mottled left lungs were noted on most Live and Killed TGAI dosed rats tested through day 14. No gross lesions were noted in animals from Naive or Shelf Control groups though one Naive Control female rat appeared sick during the study and four female Naive Control rats, including the sick rat, lost weight over the 14 day study. Significantly increased lung and associated lymph node and decreased kidney weights reported in TGAI and Killed TGAI groups through day 14 were likely a result of test material administration. Male rats in the Shelf Control group had increased lung and lymph node and decreased liver weights on day 14 though the author did not deem this effect biologically significant. From the weight range of rats reported on the day of dosing (approximately 300 g each) and guideline dosing recommendations (0.3 mL per 100 g body weight) a dose approaching 1 mL (approximately 1.76×10^5 CFU per animal) could have been used. Pulmonary exposure to *C. purpureum* during TGAI manufacture is possible, though high doses are unlikely since the TGAI / MP dilutes the a.i., and the end-use product is a paste that further dilutes the a.i.

CLASSIFICATION: SUPPLEMENTAL but upgradeable - when the mortality, plus the shelf and naive control clinical effects are explained, or the study is repeated acceptably.

DATA EVALUATION RECORD

Review by: Susan Chang, M.S.; Oak Ridge National Laboratory.
EPA Review by: Joel V. Gagliardi, Ph.D.

Study Type	OPPTS Guideline 885.3100; Acute Dermal Toxicity / Pathology - Rabbits.
MRID No.	455071-03
Test Material	*Chondrostereum purpureum* isolate PFC 2139 E.P.
Study No.	L08725 SN4
Sponsor	MycoLogic, Inc.; P.O. Box 3020; Victoria, British Columbia; Canada V8W 3N5
Testing Facility	IIT Research Institute; Life Sciences Operation; 10 West 35th St.; Chicago, IL 60616-3799
Title of Report	Acute Dermal Toxicity / Pathology Study of *Chondrostereum purpureum* in Rabbits
Authors	John Findlay, B.S.
Study Completed	August 26, 1998
Study Summary	There were no mortalities or overt clinical signs reported following dosing. There was no edema in the treated areas, though 9 of 10 treated rabbits had mild erythema that cleared by day 5. The remaining rabbit exhibited no signs of irritation. All tested rabbits gained weight steadily throughout the study.
Classification	**ACCEPTABLE** - combined LD > 2000 mg per Kg - TOXICITY CATEGORY III for irritation.
Good Laboratory Practice	GLP compliance statement was signed on 8/26/98 with noted exceptions: the sponsor was responsible for characterization and documentation of the test substance.

TEST SUBSTANCE: End-use product Chontrol™ paste containing *C. purpureum* ID No. PFC2139 received with ice packs July 16, 1998 was determined to contain 1.7 x 10^4 CFU per g; a tan paste.

METHODS:

Test Animals: Five each male and female New Zealand White rabbits were received from Kuiper Rabbit Ranch, Gary, IN. The animals were assigned to groups and weighed (2.13 - 2.26 Kg males, 1.96 - 2.30 Kg females) on the day of treatment. The young adult animals (2.5 months old) were housed individually in suspended stainless steel cages with mesh floors. The animals were fed approximately 150 g of Purina Lab Rabbit Chow HF No. 5325 daily. Tap water was available *ad libitum*. The environmental conditions of the animal room were: temperature 20.5 - 23.0 °C; relative humidity 48 - 73 %; photoperiod 12 hour light / dark cycle. Air changes per hour was not reported.

Assay: Rabbits were ear-tagged (Male Nos. 740 - 744; Female Nos. 745 - 749) and quarantined for one week. MPCA enumeration (1.7×10^4 CFU per g) of the bulk test material prior to treatment was determined by a standard plate counting technique. The test material (2000 mg per Kg body weight; $6.63 - 7.82 \times 10^4$ CFU per animal) was applied to the clipped dorsal trunk in an area approximately 10 % of the body surface. The application site was covered with a 12.8 x 11.5 cm surgical dressing, then plastic film, and secured by a lint-free cloth and elastic adhesive bandage. Coverings were removed after 24 hours and excess test material was removed with water moistened gauze pads. Test animals were observed twice daily for mortality (once during the weekend), and for clinical signs of toxicity frequently after treatment, then at least daily thereafter for 14 days. Rabbits were weighed prior to treatment and on days 7 and 14. Rabbits were euthanized on day 14. No necropsies were performed.

RESULTS SUMMARY:

Clinical Observations: There were no reported clinical signs during the study. Four males and five females had very slight to well defined erythema on days 2 to 4 with clearance by day 5.
Mortality: All rabbits survived the study; no gross necropsies were performed.
Body Weights: All animals had normal body weight gains.

Table 1: Dermal Results (Erythema/Eschar and Edema) at 24 and 72 hours.

Animal No.	Sex	Score: 24 hrs.		Score: 72 hrs.		Total Irritation Score: 24 hrs.	Total Irritation Score: 72 hrs.	Average Irritation Score	Grand Mean by sex	Grand Mean
		eryth/esc	edema	eryth/esc	edema					
740	M	0	0	0	0	0	0	0	0.9	1
741	M	1	0	1	0	1	1	1		
742	M	1	0	1	0	1	1	1		
743	M	1	0	1	0	1	1	1		
744	M	2	0	1	0	2	1	1.5		
745	F	2	0	1	0	2	1	1.5	1.1	
746	F	2	0	1	0	1	1	1		
747	F	1	0	1	0	1	1	1		
748	F	1	0	1	0	1	1	1		
749	F	1	0	1	0	1	1	1		

Table 2: Dermal Irritation Effects Scoring using the Draize Scale.

Reaction	Finding	Score
Erythema and Eschar Formation	None	0
	Very Slight Erythema (barely perceptible)	1
	Well Defined Erythema	2
	Moderate to Severe Erythema	3
	Severe Erythema (beet redness) to Slight Eschar Formation (injuries in depth)	4
Edema Formation	None	0
	Very Slight Edema (barely perceptible)	1
	Slight Edema (edges of area well defined by definite raising)	2
	Moderate Edema (area raised approximately 1 mm)	3
	Severe Edema (raised more than 1 mm and extending beyond the area of exposure)	4

Total Primary Irritation Score at 24 or 72 hours = (Erythema/Eschar Score) + (Edema Score)
Average Irritation Score = ((Score at 24 hours) + (Score at 72 hours)) ÷ 2

Table 3: Interpreting Draize Scoring.

Total Score	Irritation Level	Toxicity Category
0	None	IV
1 or 2	Mild	III
2, 3, 4 or 5	Moderate	II
6, 7 or 8	Severe	I

STUDY AUTHOR'S CONCLUSIONS:
Based upon the results of this study, the acute median lethal dose (LD_{50}) for *Chondrostereum purpureum* in male and female rabbits is greater that 2 g / Kg body weight (6.63×10^{4} to 7.82×10^{4} CFU). No overt signs of systemic toxicity were observed in any rabbit during the study. Very slight to well defined erythema was observed in nine animals following unwrapping on Day 2 and persisted through Day 4. No other clinical signs, including any signs of dermal irritation were observed for the remainder of the study.

DISCUSSION:
There were no mortalities or overt clinical signs reported following dosing. There was no edema in the treated areas, though 9 of 10 treated rabbits had mild erythema that cleared by day 5. The remaining rabbit exhibited no signs of irritation. All tested rabbits gained weight steadily throughout the study.

CLASSIFICATION:
ACCEPTABLE - combined LD > 2000 mg per Kg - TOXICITY CATEGORY III for irritation.

DATA EVALUATION RECORD

Review by: Susan Chang, M.S.; Oak Ridge National Laboratory.
EPA Review by: Joel V. Gagliardi, Ph.D.

Study Type	OPPTS Guideline 870.2400; Acute Eye Irritation - Rabbits.
MRID No.	455071-04
Test Material	*Chondrostereum purpureum* isolate PFC 2139 E.P.
Study No.	1367 SN1
Sponsor	MycoLogic, Inc.; P.O. Box 3020; Victoria, British Columbia; Canada V8W 3N5
Testing Facility	IIT Research Institute; Life Sciences Operation; 10 West 35th St.; Chicago, IL 60616-3799
Title of Report	Acute Eye Irritation Study of the Technical Grade Formulation Containing Viable Mycelia of *Chondrostereum purpureum* in Rabbits.
Authors	J. Brooks Harder, D.V.M.
Study Completed	February 2001
Study Summary	No corneal opacity, iritis, or positive conjunctival irritation (score ≥ 2) was noted from any rabbit. Redness of conjunctivae was noted in two animals at 24 and 48 hours. The maximum average score was 1.3, 24 hours after test material instillation, with clearance by 72 hours. Chontrol™ was practically non-irritating. The 0.1 mL test dose apparently contained 56 CFU in 0.1 mL by extrapolation from the sponsor's statement.
Classification	**ACCEPTABLE** - TOXICITY CATEGORY IV.
Good Laboratory Practice	GLP compliance statement was signed on 8/26/98 with noted exceptions: sponsor was responsible for analysis and documenting characterization and stability of the test substance.

TEST SUBSTANCE: Chontrol™ end-use product with *C. purpureum* isolate PFC-2139 received December 12, 2000 in three 50 mL tubes; claimed by the sponsor to contain 5.6×10^5 CFU per Kg and to be free of contaminants.

METHODS:

Test Animals: Three female young adult New Zealand White rabbits were received from Kuiper Rabbit Ranch, Gary, IN. The animals were housed individually in suspended stainless steel cages with mesh floors. The animals, approximately 27 weeks old, were fed approximately 150 g of Certified lab Diet No. 5325 Hi-Fiber Rabbit food daily. Tap water was available *ad libitum*. The environmental conditions of the animal room were: temperature, 22.0 - 25.0 °C; relative humidity, 34 - 42 %; and photoperiod, 12 hour light / dark cycle. Air changes per hour were not reported.

Assay: Rabbits were ear-tagged (Nos. 452 - 454) and quarantined for approximately 12 weeks. The test material (0.1 mL per eye, per animal) was applied in the everted lower lid of the right eye, and the eye held closed for approximately two seconds. The contralateral eye served as the control. The treated eye was rinsed with lukewarm water 24 hours after test material instillation. The eyes were examined and scored at 1, 24, 48 and 72 hours after test material instillation.

RESULTS SUMMARY:

Mortality:

No animals died during the study.

Ocular Lesions:

Corneal opacity or iris effects were not noted from any animal during the study. Redness of conjunctivae was noted in two animals at 24 and 48 hours (Table 1). The maximum average score was 1.3, 24 hours after test material instillation, with clearance by 72 hours.

TABLE 1: Summary of Reported Eye Irritation Scores.

Tissue		Animal No.	Sex	Score			Draize Score			Average Draize Score		
				24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Conjunctiva	Erythema	452	F	0	0	0	0	0	0	1.3	0.7	0
		453	F	1	1	0	2	2	0			
		454	F	1	0	0	2	0	0			

TABLE 3: Interpreting Ocular Lesions based on the Draize Method.

Toxicity Category	Description	Effects	Average Draize Score	Clears
I	Severely Irritating (or Corrosive)	The undiluted product, when instilled into the eyes of rabbits, produces severe corneal involvement with or without severe iritis.	50.1 - 110.0	> 21 Days
II	Moderately Irritating	The undiluted product, when instilled into the eyes of rabbits, produces moderate corneal involvement with or without severe iritis.	25.1 - 50.0	21 Days
III	Slightly Irritating	The undiluted product, when instilled into the eyes of rabbits, produces slight to moderate conjunctival irritation, slight corneal involvement, and/or slight iritis.	15.1 - 25.0	7 Days
IV	Practically Non-Irritating	The undiluted product, when instilled into the eyes of rabbits, produces no noticeable irritation, or slight transient conjunctival irritation.	0.00 - 15.0	< 7 Days

TABLE 2: Grading Ocular Lesions based on the Draize Method.

CORNEA	**Opacity - degree of density**	None	0
		Scattered or diffuse area; details of iris are clearly visible	* 1
		Easily discernible translucent areas; details of iris slightly obscured	* 2
		Opalescent areas; no details of iris visible, size of pupil barely discernible	* 3
		Opaque; iris invisible	* 4
	Area of cornea involved	None	0
		1-25 %	1
		26-50 %	2
		51-75 %	3
		76-100 %	4
	The opacity reading is taken at the most dense area.		
	Cornea score = (Opacity points) * (Area of cornea involved points) * 5 ; minimum 0 , maximum 80.		
IRIS	No change from normal		0
	Iris still reacts to light (sluggish response is positive). Any or all of the following or any combination: ~ Folds above normal ~ Congestion ~ Swelling ~ Circumcorneal injection		* 1
	Any or all of the following: ~ No reaction to light ~ Hemorrhage ~ Gross destruction		* 2
	Iris score = (Iris points) * 5 ; minimum 0 , maximum 10.		
CONJUNCTIVAE	**Redness**	Normal	0
		Vessels definitely injected above normal	1
		More diffuse deeper crimson red individual vessels not easily discernible	* 2
		Diffuse beefy red	* 3
	Chemosis	Normal	0
		Any swelling above normal including the nictitating membrane	1
		Obvious swelling with partial eversion of lids	* 2
		Swelling with lids about half closed	* 3
		Swelling with lids about half closed to completely closed	* 4
	Discharge	Normal	0
		Any amount different from normal (does not include the small amount observed in inner canthus of normal animals)	1
		Discharge with moistening of the lids and hairs just adjacent to the lids	2
		Discharge with moistening of the lids and considerable area around the eyes	3
	Redness refers to palpebral conjunctivae only (The palpebral conjunctiva covers the posterior surface of the eyelid).		
	Conjunctivae score = ((Redness points) + (Chemosis points) + (Discharge points)) * 2 ; minimum 0 , maximum 20.		
Total score = (Corneal score) + (Iris score) + (Conjunctivae score) ; minimum 0 , maximum 110.			

* Significant or notable irritation per OPPTS Guideline 870.2400.

STUDY AUTHOR'S CONCLUSIONS:
Under the conditions of this study, the test substance was considered to be a nonirritant to the eyes of rabbits, according to the criteria that are based on the EEC guidelines, as described in Appendix 2.

DISCUSSION:
No corneal opacity, iritis, or positive conjunctival irritation (score ≥ 2) was noted from any rabbit. Redness of conjunctivae was noted in two animals at 24 and 48 hours. The maximum average score was 1.3, 24 hours after test material instillation, with clearance by 72 hours. Chontrol™ was practically non-irritating. The 0.1 mL test dose apparently contained 56 CFU in 0.1 mL by extrapolation from the sponsor's statement.

CLASSIFICATION:
ACCEPTABLE - TOXICITY CATEGORY IV.

DATA EVALUATION RECORD

Review by: Susan Chang, M.S.; Oak Ridge National Laboratory.
EPA Review by: Joel V. Gagliardi, Ph.D.

Study Type	Summaries of OPPTS Guidelines: 885-3050, Acute Oral Toxicity/Pathogenicity - Rats; 885.3100, Acute Dermal Toxicity/Pathology - Rabbits; 885.3150, Acute Pulmonary Toxicity/Pathogenicity - Rats; 870.2400, Acute Eye Irritation - Rabbits.
MRID No.	460183-02
Test Material	**Clarified here from the original MRID's for the listed tests.**
Study No.	94 B
Sponsor	MycoLogic, Inc.; P.O. Box 3020; Victoria, British Columbia; Canada V8W 3N5
Testing Facilities	Previously reported.
Title of Report	Toxicology and Pathology Studies. Amendment No. 1 to MRID Nos. 454933-03, 455071-02, 455071-03, 455071-04
Authors	Paul de la Batiste, Ph.D.
Study Completed	March 27, 2003
Study Summary	This report presents a summary of toxicology and / or pathogenicity testing using Chrontrol™ and the TGAI *Chondrostereum purpureum* strain PFC 2139. This volume also contains a waiver request for intraperitoneal infectivity testing that is addressed elsewhere.
Classification	**SUPPLEMENTAL** - previously reported data.
Good Laboratory Practice	Previously reported.

TEST SUBSTANCES: Clarified here from the original MRID's for the listed tests.

METHODS:
Listed in the original MRID for each test.

RESULTS SUMMARY:
Report of results contained in studies for OPPTS Guidelines: 885.3050 Acute Oral Toxicity/Pathogenicity - Rats; 885.3100 Acute Dermal Toxicity/Pathology - Rabbits, 885.3150 Acute Pulmonary Toxicity/Pathogenicity - Rats, and 870.2400 Acute Eye Irritation - Rabbits.

STUDY AUTHOR'S CONCLUSIONS:
None specific to this volume, which contains summary data of previously reviewed studies.

DISCUSSION:
This report presents a summary of toxicology and / or pathogenicity testing using Chrontrol™ and the TGAI *Chondrostereum purpureum* strain PFC 2139. This volume also contains a waiver request for intraperitoneal infectivity testing that is addressed elsewhere.

CLASSIFICATION:
SUPPLEMENTAL - previously reported data.