US Environmental Protection Agency
Office of Pesticide Programs

BIOPESTICIDES REGISTRATION ACTION DOCUMENT

Coniothyrium minitans strain CON/M/91-08 (PC Code 028836)

BIOPESTICIDES REGISTRATION ACTION DOCUMENT

Coniothyrium minitans strain CON/M/91-08

(PC Code 028836)

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division
Coniothyrium minitans strain CON/M/91-08
(PC Code 028836)

TABLE OF CONTENTS

BIOPESTICIDES REGISTRATION ACTION DOCUMENT TEAM

Office of Pesticide Programs:

Biopesticides and Pollution Prevention Division

Microbials and Plant Pesticides Branch

Phil Hutton	Entomologist, Branch Chief
Roy Sjoblad	Biologist, Senior Scientist
Carl Etsitty	Microbiologist, Health Effects
Doug Gurian-Sherman	Microbiologist, Ecological Effects
Gail Tomimatsu	Plant Pathologist, Ecological Effects
Susanne Cerrelli	Biologist, Regulatory Action Leader

I. EXECUTIVE SUMMARY

The fungal active ingredient *Coniothyrium minitans* strain CON/M/91-08 is a naturally occurring strain of the *Coniothyrium* fungal species. *Coniothyrium minitans* is a highly specialized antifungal agent that targets *Sclerotinia sclerotiorum* and *Sclerotinia minor*, common plant pathogens. The sole end-use product (EP) Contans®WG which contains a minimum of 1×10^9 spores per gram, is manufactured by PROPHYTA Biologischer Pflanzenschutz GmbH. This pesticide product is registered in Germany, Switzerland, Austria, Hungary, Luxembourg and Poland. Several acute toxicity studies (eye, dermal, oral, and intraperitoneal) using a dose greater than 10^7 colony forming units (CFU) of *Coniothyrium minitans* strain CON/M/91-08 were conducted, with no adverse effects being observed. *Coniothyrium minitans* has not been reported as a pathogen of any organism in public literature other than *Sclerotinia*, and occasionally several other closely related sclerotia producing fungi. Exposure of humans, birds, fish, aquatic invertebrates, and honey bees to Contans®WG is anticipated to be minimal because the product is incorporated into the soil. However, appropriate personal protection equipment is required as prudent to protect applicators, mixers and other pesticide handlers from unnecessary exposure to microorganisms.

II. OVERVIEW

A. Product Overview

- **Microbial Pesticide Name:** *Coniothyrium minitans* strain CON/M/91-08
- **Trade Name(s):** Contans®WG, Intercept®WG
- **OPP Chemical Code**: 028836
- **Basic Manufacturer:** PROPHYTA Biologischer Pflanzenschutz GmbH
- **US Agent:** Technology Sciences Group, Inc.

B. Use Profile

Type of Pesticide: Biological control agent, Microbial Fungicide

Mechanism of action :
Coniothyrium minitans enters through the small pores or a lacerated surface of the target organism, and/or lysing by chitinase and $-1,3 glucanase for entry. Upon entry, *Coniothyrium minitans* will penetrate the inter- and intra-cellular sub-cortex and medulla, producing fruiting bodies, through asexual reproduction. The infection process will cause

the cell to shrink, due to high osmotic potential, and the membrane will be detached and/or degraded. *Coniothyrium minitans* is highly specialized antifungal agent that targets sclerotia of Ascomycotina and Deuteromycotina (*e.g., Sclerotinia sclerotiorum* and *Sclerotinia minor*). *Coniothyrium minitans* requires a host to be in a vegetative stage.

Use Sites: *Coniothyrium minitans* strain CON/M/91-08 is approved for use in agricultural soils.

Target Pests for Active Ingredient: *Sclerotinia sclerotiorum* and *S. minor*

Formulation Types Registered:

Type:End use Product
Form:Wettable granules
Concentration: 5.30% (1 X 10^9 spores per gram)

Method and Rates of Application:

Types of Treatment Application is by spraying onto soil and incorporating into upper soil layer (approximately 1-2 inches).

Equipment: Conventional spray equipment, tractor mounted sprayers, spray booms equipped with fan nozzles; disc; rotary hoe; or similar equipment.

Timing.
Pre-plant: Apply product to the soil 3 to 4 months prior to the onset of disease.
Postharvest: Apply product to harvest residues and incorporate into the soil.

Contans®WG is less effective at temperatures above 30 °C. Plowing before planting may raise untreated pest fungi closer to the soil surface where they may infect the crop, and is therefore not recommended

Rates of Application:
0.053 - 0.212 lbs of active ingredient per acre
(2.4 x 10^{10} to 9.5 x 10^{10} spores per acre)

Method of Application:
Product is applied as a spray to the soil, followed with mechanical incorporation (i.e., rotating) into the first one to two inches of the topsoil layer.

C. Regulatory History

PROPHYTA Biologischer Pflanzenschutz GmbH submitted an application July 1, 1999 for registration of *Coniothyrium minitans* strain CON/M/91-08. On July 19, 1999 a pesticide petition (9F6038) which proposed establishing an exemption from the requirement of a tolerance for residues of the microbial pesticide, *Coniothyrium minitans* strain CON/M/91-08, on growing crops was submitted. A notice of filing for this pesticide petition was published in the Federal Register on January 24, 2000 (FR Vol. 65, No. 15, pp. 3696-3699).

CONTANS®WG has been previously reviewed and approved (as a non-plant pathogen) for importation for testing by the USDA-APHIS-Plant Protection and Quarantine section. CONTANS®WG is currently registered for use in Germany, Switzerland, Austria and Poland. An application for inclusion of the active substance (*Coniothyrium minitans*) in Annex I to Council Directive 91/414/EEC marketing of plant-protection products was sanctioned in a decision by the European Commission on November 17th, 1998, and was published in the European Union Gazette. This decision by the European Commission means that provisional authorization in the Union Member States may be applied for plant-protection products containing the active ingredient *Coniothyrium minitans*.

III. SCIENCE ASSESSMENT

A. Physical and Chemical Properties Assessment

Product Identity: Contans®WG contains living *Coniothyrium minitans* strain CON/M/91-08 as the active ingredient. *Coniothyrium minitans* strain CON/M/91-08, active ingredient of CONTANS®WG, is a naturally-occurring, nonpathogenic soil microorganism. *Coniothyrium minitans* strain CON/M/91-08 has been classified as Coelomycetes, subdivision Deuteromycotina, with the following physical characteristics: Dark pycnidial wall; conidia size – 4.0-6.0 x 3.5-4.0 ：m; 3 wall layers; a vacuolated cytoplasm; and, verrucose surface. The strain has been recognized by Deutsche Sammlumng von Microorganismen cultural collection: DSM ID 9669.

The host range for *Coniothyrium minitans* strain CON/M/91-08 is semi-broad spectrum, where it mainly attacks fungal sclerotia, Ascomycotina and Deuteromycotina (*e.g., Sclerotinia, Botrytis,* and *Sclerotinia cepivorum*). The *Coniothyrium minitans* enters through the small pores or a lacerated surface of the target organism. It then penetrates the inter- and intra-cellular sub-cortex and medulla, producing fruiting bodies, through asexual reproduction. The optimum sporulation will occur at 25 to 30°C, with pH of 4.5 to 5.6. The formulated product, CONTANS®WG, contains 6.7 x 10^7 CFU, at 5.3% by weight of *Coniothyrium minitans* strain

CON/M/91-08. Product chemistry data which support the registration of *Coniothyrium minitans* strain CON/M/91-08 are summarized in Table 1.

Table 1. Physical and Chemical Properties for *Coniothyrium minitans* strain CON/M/91-08

GUIDELINE Number	STUDY	RESULT	MRID#
151-20 *885.1100	Product Identity and Disclosure of Ingredients	Acceptable	44868701, 45088700
151-21 *885.1200	Manufacturing Process	Acceptable	44868701, 45088700
151-22 *885.1300	Formation of Unintentional Ingredients	Acceptable	44868701, 45088700, 45088701
151-23 *885.1400	Analysis of Samples	Acceptable	44868701, 45088700, 45088701
151-25 *885.1500	Certification of Limits	Acceptable	44868701, 45088700, 45088701
151-26	Physical/Chemical Properties:	Acceptable	44868701, 45088700
*830.6302	color	black	
*830.6303	physical state	non-dusty, water dispersible granules	
*830.6304	odor	mushroom like	
*830.6315	flammability	non-flammable	
*830.6317	Storage stability	6 months	
*830.7000	pH	7.0 ± 0.1	
*885.7300	bulk density	0.46 - 0.5 kg/m^3	

* =OPPTS Microbial Pesticide Test Guidelines

B. Human Risk Assessment

There is a reasonable certainty that no harm will result from exposure to *Coniothyrium minitans* strain CON/M/91-08. This includes all anticipated dietary exposures and all other exposures for which there is reliable information.

1. Human Toxicity Assessment

a. Acute Toxicity

Environmental and dietary exposure to *Coniothyrium minitans* is highly probable for most individuals because this fungus is a ubiquitous and naturally-occurring soil microorganism. *Coniothyrium minitans* was first described by Campbell (1947), after being isolated from sclerotia in California. No toxicological or pathogenic effects by *Coniothyrium minitans* in mammals have been reported in available public literature. *Coniothyrium minitans* has never been reported to be a pathogen of any organism other than *Sclerotinia* and occasionally several other closely related sclerotia producing fungi. No reports of pathogenicity or toxicity from *Coniothyrium minitans* are reported on the electronic databases Agricola, Toxline, or Medline. Furthermore, Prophyta Biologischer Pflanzenschutz Gmbh has submitted several acute toxicity studies (eye, dermal, oral, and intraperitoneal) using a dose that is greater than 10^7 CFU of *Coniothyrium minitans* strain CON/M/91-08, with no adverse effects being observed. In addition, certain biological characteristics of *Coniothyrium minitans* strain CON/M/91-08, which include, its temperature requirements for germination and mycelium growth, and host (*Sclerotinia*) dependence are further indications that this microbial pest control agent would not be pathogenic to mammals.

The submitted exposure studies demonstrate that conidia germinate at 24 and 27°C at 24, 36, 48 and 72 hours, with no conidia germination at 30, 33 and 36°C; and 42.3 mm of mycelium growth at 24°C at day 15, 3.8 mm of growth at 27°C at day 15, 0.9 mm of growth at 30°C at day 15 and no mycelium growth at 33 and 36°C. The *Coniothyrium minitans* data submitted demonstrated no conidia germination at 30°C or above, and no mycelium growth at 33°C or above. Therefore, the fungus would not likely be able to infect humans at their normal body temperatures. All mammalian toxicology data requirements have been adequately satisfied to support registration. Results of the acute toxicity studies are summarized in Table 2.

Table 2. Toxicity Data Requirements

GUIDELINE NUMBER	STUDY	RESULT	MRID#
152-30 *885.3050	Acute Oral Toxicity/ Pathogenicity	*Coniothyrium minitans* strain CON/M/91-08 does not appear to be toxic in rats, when dosed at 2,000 and 2,500 mg A.I./kg bw, and representing a dose of $2.0x10^8$ CFU of *Coniothyrium minitans*/animal **ACCEPTABLE,** **Toxicity Category III**	44868702 44956901 45088700
152-31 *885.3100	Acute Dermal Toxicity/ Pathogenicity	No deaths observed. LD_{50} , 2,5000 mg/kg of *Coniothyrium minitans* strain CON/M/91-08 in rats **ACCEPTABLE**, **Toxicity Category III**	44868703 44956901 45088700

GUIDELINE NUMBER	STUDY	RESULT	MRID#
152-32 *870.1300	Acute Inhalation (End-Use Product WP)	*Coniothyrium minitans* strain CON/M/91-08 does not appear to be toxic in rats, when dosed at 6.04 and 12.74 mg CON/M/91-08 per L air in 5 males and 5 females per concentrations, for 4 h in a nose exposure (chamber). Respirable particles (*e.g.,* # 4 ：m) were determined to be at 0.82 and 1.89 mg/L, respectively. No abnormal clinical signs and mortality were observed in the test subjects, and no gross necropsy studies were performed. On the selected test concentration, the particle size and distribution analysis showed a mass median aerodynamic diameter of 24.12 ：m and 23.51 ：m Potential pathogenicity was not assessed in this study. However, the infectivity component of the testing is adequately addressed by dust/ mist filtering respirator requirement and the lack of adverse effects noted in the acute toxicity studies. No additional data are required. **ACCEPTABLE,** **Toxicity Category IV**	44868704, 44956901, 45088700
152-33 *885.3200	Acute Intraperitoneal Toxicity/Pathogenicity	No abnormal clinical signs and mortality were observed in the test subjects, and no abnormal necropsy results. LD_{50} ,2000 mg/kg (2.0 x 10^7 condia/animal) of *Coniothyrium minitans* strain CON/M/91-08. **ACCEPTABLE** **Toxicity Category IV**	44868705, 44956901, 45088700

GUIDELINE NUMBER	STUDY	RESULT	MRID#
152-35 *870.2400	Primary Eye Irritation	*Coniothyrium minitans* strain CON/M/91-08 showed no signs of irritation, when dosed at 0.1 ml/eye. In addition, the presented data showed no mortality results, and no abnormal clinical signs observed in the test subjects. **ACCEPTABLE ,** **Toxicity Category IV**	44868706, 44956901, 45088700
152-34 *870.2500	Primary Dermal Irritation	*Coniothyrium minitans* strain CON/M/91-08 showed no signs of irritation, when dosed (0.5 ml/animal) on the skin. In addition, the presented data showed no mortality results, and no abnormal clinical signs observed in the test subjects. **ACCEPTABLE,** **Toxicity Category IV**	44868707, 44956901, 45088700
152-36 *870.2600	Delayed Contact Hypersensitivity (MANUSSON & KLIGMANN) in Guinea Pigs	*Coniothyrium minitans* strain CON/M/91-08 showed no signs of irritation, when dosed at 0.1 ml/animal. The Agency requires the registrant to report any hypersensitivity incidents to the Agency under Section 6(a)2. **ACCEPTABLE**	44868708, 44956901, 45088700
152-38	Immune Response	The lack of pathogenicity observed in humans allows the conclusion that the immune system is not adversely affected from exposure to *Coniothyrium minitans*. **DATA WAIVED**	

* =OPPTS Microbial Pesticide Test Guidelines

b. Subchronic Toxicity and Chronic Toxicity

Tier II and III Subchronic and chronic toxicity studies are not required because of the low acute toxicity of the microbial pesticide, and no indications of infectivity or pathogenicity in mammals.

2. Effects on the Immune and Endocrine Systems

EPA is required under the FFDCA, as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients)" may have an effect in humans that is similar to an effect produced by a naturally-occurring estrogen, or other such endocrine effects as the Administrator may designate." Following the recommendations of its Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), EPA determined that there was scientific basis for including, as part of the program, the androgen- and thyroid hormone systems, in additions to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use FIFRA and to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA authority to require wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

Within the available scientific literature, there are no reports (MRID 44868713) that suggest *Coniothyrium minitans* has the potential to cause adverse effects on the endocrine and/or immune systems of animals. When the appropriate screening and or testing protocols being considered under the Agency's Endocrine Disruptor Screening Program have been developed, *Coniothyrium minitans* strain CON/M/91-08 may be subjected to additional screening and/or testing to better characterize effects related to endocrine disruption. Based on the weight of the evidence of available data, no endocrine system-related effects have been identified.

3. Dose Response Assessment

No toxicological endpoints are identified.

4. Aggregate Exposures

In examining aggregate exposure, FFDCA section 408 directs EPA to consider available information concerning exposures from the pesticide residue in food and all other non-occupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses). The potential aggregate non-occupational exposure to *Coniothyrium minitans* strain CON/M/91-08, derived from dermal, inhalation and dietary exposure from drinking water and treated produce containing this organism, should be minimal and should fall well below the currently tested microbial safety levels.

a. Dietary Exposure

(i) *Food*. Dietary exposure is expected to be minimal because the microbial product is incorporated in the soil prior to planting or after harvest. Thus no increase in fungal exposure is anticipated. In addition, standard practices of washing, peeling, cooking, or processing fruits and vegetables will reduce residues of *Coniothyrium minitans* strain CON/M/91-08 and further minimize dietary exposure. The risk posed to adults, infants and children is likely to be minimal because of the low acute toxicity of the microbial pesticide and no reported cases in the literature of disease or injury to humans.

(ii) *Drinking water exposure*. A submitted study showed that the likelihood for *Coniothyrium minitans* passage through a soil medium to groundwater is minimal to none. Also, the survival of *Coniothyrium minitans* in a municipal water treatment is unlikely. This microorganism would not tolerate the conditions the water is subject to in a drinking-water treatment facility (including: chlorination, pH adjustments, high temperatures, and/or anaerobic conditions). Furthermore, the results of the acute toxicity studies using a high dose of the fungus suggest there will not be any adverse effects to humans and there have been no reported cases in the literature of disease or injury to humans.

b. Other Non-Occupational Exposure

Coniothyrium minitans is a naturally-occurring fungus. Dermal and inhalation exposure to *Coniothyrium minitans* pesticide product is expected to be limited to those who apply or handle the pesticide in an agricultural environment. Therefore, no other non-occupational exposure is expected.

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

Coniothyrium minitans is a naturally-occurring fungus. The historical safe-use as a biological control in a lab-control setting has been referenced in scientific literature to control *Sclerotinia* spp. *Coniothyrium minitans* have been shown to have a limited host range to fungal species. The product will be applied at usage rates of 1 to 6 lbs/acre to the soil. There is no indication of indoor/residential, school and/or daycare uses on the label. With *Coniothyrium minitans*' limited host range, and its low acute toxicity, the potential of increased non-occupational exposure to *Coniothyrium minitans* will be limited with no potential human health effects. Dermal and inhalation exposure to *Coniothyrium minitans* pesticide product is expected to be limited to those who apply or handle the pesticide on farms. To mitigate dermal, and inhalation

exposure and any risk to workers, the Agency requires personal protective equipment (PPE) as described in **Section V** under the **"Precautionary Labeling"** section.

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

Soil microorganisms, such as *Coniothyrium minitans,* are naturally occurring and ubiquitous in the environment, with a highly probable, prior human exposure. The toxicity testing conducted by Prophyta Biologischer Pflanzenschutz Gmbh indicates an inability of the microbe to grow at 33°C or above and a lack of potential toxic, pathogenic, or allergic effects to humans. In addition, no potential for toxic or pathogenic effects of *Coniothyrium minitans* to mammals were reported in published literature.

Based on the acute toxicity information discussed above, EPA concludes that there is a reasonable certainty that no harm will result from aggregate exposure to the United States population, including infants and children, to residues of *Coniothyrium minitans* strain CON/M/91-08. This includes all anticipated dietary exposures and all other exposures for which there is reliable information.

FFDCA section 408 provides that EPA shall apply an additional ten-fold margin of exposure (safety) for infants and children in the case of threshold effects to account for pre- and post-natal toxicity and the completeness of the database, unless EPA determines that a different margin of exposure (safety) will be safe for infants and children. Margins of exposure (safety) are often referred to as uncertainty (safety) factors. In this instance, the Agency believes there is reliable data to support the conclusion that *Coniothyrium minitans* strain CON/M/91-08 is practically non-toxic to mammals, including infants and children, and, thus, there are no threshold effects. Therefore, the provision requiring an additional margin of exposure (safety) does not apply. As a result, EPA has not used a margin of exposure (safety) approach to assess the safety of *Coniothyrium minitans* strain CON/M/91-08.

7. Cumulative Effects

No mechanism of toxicity in mammals has been identified for *Coniothyrium minitans* strain CON/M/91-08. Therefore no cumulative effect with other related organisms is anticipated. Because the data demonstrate low toxicity/pathogenicity potential of the active ingredient, the likelihood of adverse dietary effects is expected to be minimal.

C. Environmental Assessment

1. Environmental Fate

Contans®WG, containing the active ingredient *Coniothyrium minitans* strain CON/M/91-08, has been examined as a potential biocontrol agent for sclerotia producing fungi of the genus *Sclerotinia* for over 20 years. The organism was first reported from the United States in 1947 [Campbell, W.A. (1947)], and has since been found in at least 29 countries in most parts of the world. Almost all isolations have been from sclerotia of susceptible fungal species, with a low percentage of isolations from soil. There is no evidence that *Coniothyrium minitans* grows in other environments, although it grows well in artificial media. Maximum growth temperature is reported to be around 32 °C.

Studies in which *Coniothyrium minitans* was isolated from soil using sclerotia as a "trap" indicate the presence of *Coniothyrium minitans* in soil. It is unclear from these studies whether the presence of *Coniothyrium minitans* was associated with *Sclerotinia*. The evidence does suggest that *Coniothyrium minitans* is a poor competitor as a free living soil organism, and probably persists only at relatively low levels, if at all, for more than several years in the absence of host fungi. However, the host fungi are widespread, and therefore it is likely that *Coniothyrium minitans* is also widely distributed, if not always abundant. Using sclerotia as a bait, C. Sandys-Winsch et al. (Mycol Res., v. 97: 1175-1178, 1993) found *Coniothyrium minitans* in 10.2 % of soils examined from a number of countries.

2. Ecological Toxicity

It has been argued that because *Coniothyrium minitans* appears to be a specialized pathogen, or hyperparasite, of *Sclerotinia* sclerotia, it is unlikely to be a pathogen of other organisms. However, parasitism or symbiosis of one group of organisms does not preclude pathogenicity on other organisms which may not be closely related. *Coniothyrium minitans* has not been reported to produce toxins or antibiotics.

Coniothyrium minitans has never been reported to be a pathogen of any organism other than *Sclerotinia* and occasionally several other closely related sclerotia producing fungi. No reports of pathogenicity or toxicity from *Coniothyrium minitans* are reported on the electronic databases Agricola, Toxline, or Medline. Maximum reported growth at around 32 °C makes pathogenicity of homeothermic animals unlikely. The results of the ecological toxicity testing are summarized in Table 3.

Table 3: Eco-Toxicology Summary

Guideline No.	Study	Status, Classification & Comments	MRID #
154-16 *885-4050 and 154-17 *885-4100	Avian oral toxicity/ pathogenicity and Avian injection test	**WAIVED** The maximum growth temperature (32 °C) of *Coniothyrium minitans* makes avian pathogenicity extremely unlikely. The method of application, spraying a water soluble suspension onto soil followed by incorporation (rotation) into the soil, makes exposure of birds to concentrations of *Coniothyrium minitans* strain CON/M/91-08 that may be of toxicological concern unlikely.	44868700
154-18 *885-4150	Wild Mammal Testing	**NOT REQUIRED**. *Coniothyrium minitans* has no history of pathogenicity to any organism except the target fungal species. Maximum reported growth at around 32 °C makes pathogenicity of homeothermic animals unlikely. In addition, there is no evidence that *Coniothyrium minitans* grows in environments other than soil, and *Coniothyrium minitans* cannot survive long term in soil without its specific fungal hosts.	44956902, 44868700
154-19 *885-4200	Fresh water fish toxicity/ pathogenicity	**ACCEPTABLE** (meets toxicity testing requirements, but not those for pathogenicity.) Additional data are not required because aquatic exposure is expected to be minimal since the product is incorporated into the soil, and no aquatic sites are registered.	44868712
154-20 *885-4240	Fresh water aquatic invertebrate toxicity/pathogenicity	**ACCEPTABLE (**meets toxicity testing requirements, but not those for pathogenicity) Additional data are not required because aquatic exposure is expected to be minimal. The product is incorporated. into the soil. No aquatic sites are registered.	44868711
154-22 *885-4300	Nontarget plant toxicity/pathogenicity	**WAIVED.** *Coniothyrium minitans* has no history of pathogenicity to any organism except the target fungi and related species (GJ Turner and HT Tribe, 1976; and DF Farr et al,1989).	44868700; & public literature
154-23 *885-4340	Nontarget insect toxicity/pathogenicity	**WAIVED.** *Coniothyrium minitans* has no history of pathogenicity to any organism except the target fungi and related species.	44868700
154-24 *885-4380	Honeybee toxicological/pathogenicity	**WAIVED**. *Coniothyrium minitans* has no history of pathogenicity to any organism except the target fungi and related species. Exposure to honey bees is expected to be extremely limited because the product is incorporated into the soil.	44868700

*885 series = OPPTS Microbial Pesticide Test Guideline Numbers.

a. Toxicity to Terrestrial Animals

(i) Avian Species: Acute Toxicity/Pathogenicity: The low maximum growth temperature of *Coniothyrium minitans* makes avian pathogenicity extremely unlikely. The method of application, spraying a water soluble suspension onto soil with incorporation, makes exposure of birds to concentrations of *Coniothyrium minitans* strain CON/M/91-08 that may be of toxicological concern unlikely. In combination with the data from other studies cited above, waiver of these studies is adequately supported and waiver of the avian tests is granted.

(ii) Wild Mammals and Other Terrestrial Animals: Acute Toxicity/Pathogenicity: This data requirement is not required because *Coniothyrium minitans* has never been reported to be a pathogen of any organism other than *Sclerotinia* and occasionally several other closely related sclerotia producing fungi. No reports of pathogenicity or toxicity from *Coniothyrium minitans* are reported on the electronic databases Agricola, Toxline, or Medline. Maximum reported growth at around 32 °C (MRID No. 44956902) makes pathogenicity of homeothermic animals unlikely. In addition, there is no evidence that *Coniothyrium minitans* grows in environments other than soil, and *Coniothyrium minitans* cannot survive long term in soil without its specific fungal hosts. (MRID No. 44868700).

b. Toxicity to Aquatic Animals

(i) Freshwater Fish: Seven *Leuciscus idus melanatus* (golden ide), 5-7 cm. in length were tested by suspension. Dosage administered was 9.4 X 10E7 (t=96 hr) to 1.0 X 10E8 (t=0) spores/ml of technical product (active ingredient) for 96 hours. Negative controls without the spores were also run, but it is not clear whether seven fish were also exposed in the negative control. No mortality was observed in either the test or control animals after four days.

The study deviates from EPA guidelines in several respects. The test material was not supplied by an oral route in addition to suspension in the water; fewer than the 30 required test animals were used, and the study was conducted for four rather than the required 30 days. The test species, *Leuciscus idus melanatus* (golden ide) is indigenous to Germany and has a history of use as a test species for toxicity testing there. However, no justification was given for the use of this species rather than the preferred rainbow trout. The test dose was about 100 fold higher than the usual minimum required. Mortality as the only toxic endpoint is not as sensitive as inclusion of aberrant behavior.

The submitted test method is not ideal for establishing toxicity, i.e. whether there is a hazard, as opposed to combining hazard and exposure assessment in one test, as is done in the submitted test. The duration of the test is inappropriately short, and the number of animals too low to determine pathogenicity or all instances of acute toxicity.

Despite the noted deficiencies, no additional data are required. The Agency is waiving the requirement for additional toxicity testing on freshwater fish because exposure to fish is expected to be extremely limited and because *Coniothyrium minitans* has no history of pathogenicity to any organism except the target fungal species. Little aquatic exposure is expected because the microbial pest control agent has no registered aquatic use sites, and the product must be incorporated into the soil after application. In addition, *Coniothyrium minitans* is not reported to be an aquatic organism.

(ii) Freshwater Aquatic Invertebrate

The submitted Daphnia acute toxicity study (MRID NO: 44868711) deviates from EPA guidelines in several respects. OPPTS guidelines require 100 test animals for a single test concentration, and a duration of at least 21 days, rather than the 30 animals and two day test duration of the submitted study. Mortality, the only toxic endpoint considered, does not include OPPTS requirements of assessing behavior change. The test is minimally adequate to assess acute toxicity, but not pathogenicity.

No immobility was observed for test animals in the final test or the initial tests, with the exception of the undiluted sample test, in which case all animals were immobile. This latter result was attributed to oxygen deprivation as a likely cause, based on measurements in another experiment, although no data was presented to support this assertion. The time of appearance of immobility was not disclosed.

For the proposed uses, the amounts of *Coniothyrium minitans* in the aquatic environment is expected to be very low. Aquatic exposure is expected to be minimal in most circumstances because there are no direct aquatic applications for the product and no terrestrial crops that would allow a substantial aquatic exposure (e.g. rice). In addition, because the product must be incorporated into the soil after application, little aquatic exposure is expected. The microbial pest control agent is not reported in the literature to inhabit aquatic environments.

c. Toxicity to Plants

A comprehensive review of the biology of *Coniothyrium minitans* (GJ Turner and HT Tribe, Trans. British Mycol. Soc., v. 66: 97-105, 1976) reported no pathogenicity resulted from tests on 17 host plant species, mostly known hosts of *Sclerotinia* spp. No other reports of plant pathogenicity have been reported in electronic data bases, including Agricola. *Coniothyrium minitans* is also not reported to be a plant pathogen in "Fungi on Plants and Plant Products in the United States" (DF Farr et al, eds, APS Press,1989, p.1252, St. Paul, MN), although many other species of *Coniothyrium* are known plant pathogens, as well as species in the closely related genus, *Microsphaeropsis*.

The suspension of Contans®WG is applied to soil and incorporated. Therefore foliar exposure to non-target plants above levels encountered due to the natural distribution of *Coniothyrium minitans* in the soil is unlikely. Levels of *Coniothyrium minitans* strain CON/M/91-08 that non-target plant species might encounter would not be expected to cause phytotoxicity above that caused by background levels of *Coniothyrium minitans*. In addition, no strains of *Coniothyrium minitans* have displayed any plant pathogenicity despite testing on a number of plant species. The methodology of these latter tests were not disclosed, but consisted mostly of food and feed species as well as several types of ornamental plants. These species are generally susceptible to *Sclerotinia* and are therefore species on which Contans®WG may be applied. Turner and Tribe (Turner G. J. and Tribe H.T. (1976) Trans. Brit. Mycolog. Soc., 66 (1) 97-105) reported pathogenicity tests of *Coniothyrium minitans* on the following 17 plant species with no evidence of disease: *Antirrhinum majus, Apium graveolens, Beta vulgaris, Brassica oleracea, Cheiranthus cheiri, Cichorium intybus, Coleus blumei, Cucumis sativus, Daucus carota, Helianthus annuus, Lactuca sativa, Lupinus regalis, Lycopersicon esculentum, Solanum melongena, Solanum tuberosum, Trifolium repens, Vicia fava.* There is no indication the isolate or isolates of *Coniothyrium minitans* used in these pathogenicity tests were the same as the isolate used in Contans®WG. However, in combination with the lack of pathogenicity reports from other sources, it appears unlikely that adverse incidents to non-target plants due to Coniothyrium minitans strain CON/M/91-08 will occur. Therefore waiver of non-target plant studies is acceptable for the intended uses.

d. Toxicity/Pathogenicity to Honeybees and Nontarget Insects

There are several reports of dissemination of *Coniothyrium minitans* in the soil after contact or ingestion by several invertebrates. This transmissability is not necessarily synonymous with lack of pathogenicity or toxicity to these invertebrates. However, one study (JM Whipps, Mycol. Res., v. 97: 1277-1280, 1993) reported excellent growth and population increase of the collembolan, *Folsomia candida*, over a period of 80 days on pure culture of *Coniothyrium minitans*. *Folsomia candida* is a common and abundant soil invertebrate and is often used as a test indicator of toxicity and pathogenicity of the microbial pest control agent for soil invertebrates. This paper also reports subculture of *F. candida* on *Coniothyrium minitans* over a period of three years without noted adverse effects. While this study did not directly compare growth or mortality of this collembolan on *Coniothyrium minitans* and the standard yeast based growth medium, the reported results are inconsistent with any significant toxicity or pathogenicity of *Coniothyrium minitans* toward this soil organism.

Non-target insect testing is usually required in cases where the mode of action of the microbial pesticide is by insect pathogenicity or toxicity. Contans®WG is intended to control a limited number of fungal pathogen species and has no history of use in controlling

insects. Furthermore, as noted above, there are no reports in the literature indicating toxicity or pathogenicity of *Coniothyrium minitans* to insects, and non-guideline studies with collembola indicate no pathogenicity or toxicity. Therefore, a reasonable certainty of no harm may be established without further testing and the non-target insect tests may be waived.

Although the honeybee testing is typically required, the reasoning and data above are also pertinent for honeybees. In addition, Contans®WG is not applied to the above ground parts of plants, including flowers, but rather to soil before plants have emerged. Therefore, honeybee exposure is expected to be minimal, and honeybee testing requirements are waived.

D. Efficacy Data

In general, *Coniothyrium minitans* has demonstrated good efficacy in the control of white mold diseases caused by *Sclerotinia sclerotiorum* and *S. minor* on the crops tested under a limited number of conditions. Control was often comparable to that of standard fungicides in the few cases where compared. In at least one case, fungicidal treatment with iprodione was applied to the soil, which is comparable to the typical methods of applying *Coniothyrium minitans*, but may not be the most effective means of using the fungicide, which typically is used as a foliar treatment. In addition, most of the testing was performed under greenhouse conditions, which are often not reflective of conditions in the field.

Several of the studies used artificially infested soil. It is not clear in any of these studies whether more than one strain of the pathogen was used. Variation in virulence between strains is common for pathogens, so use of a single strain reduces the likely variation in virulence that may be encountered in the field. Differences in the susceptibility of different isolates of the pathogen to *Coniothyrium minitans* has been noted. In addition, if these strains were grown for prolonged periods on artificial media, their virulence may be reduced compared to other strains, as is commonly seen with a number of fungal pathogens.

The strain of *Coniothyrium minitans* used in Contans®WG was only tested on four crop species (canola, lettuce, gerbera and chrysanthemum), and only in one crop under field conditions (canola). Although lettuce may be grown in the greenhouse, it is typically grown in the field in the U.S., while the flower species are often propagated in greenhouses. The other reported tests were conducted using strains of *Coniothyrium minitans* that may differ from the Contans®WG strain. Differences in efficacy between different strains of a biocontrol species is common.

Several studies indicate that *Coniothyrium minitans* is effective in reducing primary inoculum in the form of sclerotia, but is not effective in preventing secondary spread

between plants after primary infection has occurred. Therefore, in conditions where secondary mycelium inoculum may be important, e.g. longer growing seasons where the disease cycle may have longer to progress and where more senescent tissue or weather conditions may favor spread, or where plants are grown in close proximity, *Coniothyrium minitans* may be relatively less effective than fungicidal treatments. In addition, under conditions where ascospores form the primary inoculum, enough inoculum may be available to significantly limit the efficacy of *Coniothyrium minitans* compared to standard fungicide treatments.

IV. RISK MANAGEMENT AND RE/REGISTRATION DECISION

A. Determination of Eligibility

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

To satisfy criterion "A" above, *Coniothyrium minitans* strain CON/M/91-08 has well known properties. The Agency has no knowledge that would contradict the claims made on the label of this product. Criterion "B" is satisfied by the current label and by the data presented in this document. It is believed that this new pesticidal active ingredient will not cause any unreasonable adverse effects, is a broad spectrum microbial fungicide, and does provide protection as claimed satisfying criterion "C". Criterion "D" is satisfied in that *Coniothyrium minitans* strain CON/M/91-08 is not expected to cause unreasonable adverse effects when used according to label instructions.

Therefore, *Coniothyrium minitans* strain CON/M/91-08 is eligible for registration. The uses are listed in the Section II, B. Use Profile. There are no ineligible uses for *Coniothyrium minitans* strain CON/M/91-08.

B. Regulatory Position

1. Unconditional Registration

The data requirements are fulfilled and the Biopesticides and Pollution Prevention Division recommends unconditional registration of products that contain *Coniothyrium minitans* strain CON/M/91-08 as the sole Active ingredient

(*Coniothyrium minitans* strain CON/M/91-08).

2. Tolerances for Food Uses and /or exemptions

EPA received a pesticide petition (PF 6038) from PROPHYTA Biologischer Pflanzenschutz GmbH, proposing [pursuant to section 408(d) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. section 346a(d)], to amend 40 CFR part 180 by establishing an exemption from the requirement of a tolerance for the microbial pesticide, *Coniothyrium minitans* strain CON/M/91-08.

EPA is issuing a notice establishing an exemption from the requirements of a tolerance for residues of *Coniothyrium minitans* strain CON/M/91-08 in or on all food commodities.

3. CODEX Harmonization

There are no CODEX values for *Coniothyrium minitans* strain CON/M/91-08.

4. Risk Mitigation

Other than standard personal protective equipment (PPE) to protect applicators and other handlers that are listed below in section V, no risk mitigation measures were required.

5. Endangered Species Statement

Given the specificity of this microbial pesticide and based on the intended use pattern, and the results of toxicity and exposure data from public scientific literature and from the data submitted by the applicant, the Agency has determined that this action will have no effect on currently listed endangered and threatened species.

C. Labeling Rational

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

Coniothyrium minitans strain CON/M/91-08 products with commercial use sites are subject to the Worker Protection Standard. Because of the low toxicity of *Coniothyrium minitans* strain CON/M/91-08, the Re-Entry Interval for uses within the scope of WPS is 4 hours. Precautionary statements and personal protective equipment as specified below are required based on the acute toxicity categories of this organism.

2. Environmental Hazard

Precautionary labeling is required as indicated below.

V. ACTIONS REQUIRED BY REGISTRANTS

A. Precautionary Labeling

Coniothyrium minitans strain CON/M/91-08 products must state the following under the heading "Precautionary Statements":

Personal Protective Equipment required for Applicators and other handlers must wear:

Long sleeved shirt and long pants. Waterproof gloves. Shoes plus socks. Dust/mist filtering respirator (MSHA/NIOSH approval number prefix TC-21C), or a NIOSH approved respirator with any N, P, R, or HE filter."

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

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

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

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

B. Environmental Hazards Labeling

Provided the following statement is placed into the environmental hazards statement, the risk of *Coniothyrium minitans* strain CON/M/91-08 is minimal to nonexistent to non-target organisms including endangered species.

1. End-Use Product Environmental Hazards Labeling

"Do not apply directly to water, or to areas where surface water is present or to intertidal

areas below the mean high water mark. Do not contaminate water by cleaning of equipment or disposal of equipment washwaters. "

2. Manufacturing-Use Product Environmental Hazards Labeling

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

3. Application Rate

It is the Agency's position that the labeling for the pesticide products containing *Coniothyrium minitans* strain CON/M/91-08 as the active ingredient complies with the current pesticide labeling requirements. The Agency has not required a maximum number of applications per a season of this active ingredient.

C. Labeling

The attached label for Contans®WG conforms with the labeling requirements for *Coniothyrium minitans* strain CON/M/91-08. Some of the essential label requirements are highlighted below.

Signal word is "Caution," based on (toxicity category III). The product shall contain the following information:

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

BIBLIOGRAPHY: Citations Considered to be part of the Data Base Supporting the

Registration of *Coniothyrium minitans* strain CON/M/91-08.

44868700 Prophyta Biologischer Pflanzenschutz GmbH (1999) Submission of Product Chemistry, Toxicity, Environmental Fate, Risk Assessment and Exposure Data in Support of the Application for Registration of Contans WG. Transmittal of 13 Studies.

44868701 Roberts, A. (1999) Product Chemistry of Contans WG. Unpublished study prepared by Technology Sciences Group, Inc. 48 p. {OPPTS 885.1100, 830.6302, 830.6303, 830.6304, 830.6315, 830.700, 830.7300, 885.1200}

44868702 Leuschner, P. (1994) Acute Toxicity Study of CON/M/91-08 by Oral Administration to Sprague-Dawley Rats: Lab Project Number: 8659/94: CON/M/9108. Unpublished study prepared by LPT Laboratory of Pharmacology and Toxicology. 23 p. {OPPTS 870.1100}

44868703 Leuschner, P. (1994) Acute Toxicity Study of CON/M/91-08 by Dermal Administration to Sprague-Dawley Rats: Lab Project Number: 8660/94. Unpublished study prepared by LPT Laboratory of Pharmacology and Toxicology. 23 p. {OPPTS 870.1200}

44868704 Leuschner, P. (1995) Acute Inhalation Toxicity Study of CON/M/91-08 in Sprague-Dawley Rats: Lab Project Number: 8887/94. Unpublished study prepared by LPT Laboratory of Pharmacology and Toxicology. 39 p. {OPPTS 870.1300}

44868705 Leuschner, P. (1995) Acute Toxicity Study of CON/M/91-08 Intraperitoneal Administration to Sprague-Dawley Rats: Lab Project Number: 9480/95. Unpublished study prepared by LPT Laboratory of Pharmacology and Toxicology. 23 p. {OPPTS 885.3200}

44868706 Leuschner, P. (1994) Acute Eye Irritation Study of CON/M/91-08 by Instillation into the Conjunctival Sac of Rabbits: Lab Project Number: 8662/94. Unpublished study prepared by LPT Laboratory of Pharmacology and Toxicology. 25 p. {OPPTS 870.2400}

44868707 Leuschner, P. (1994) Acute Skin Irritation Test (Patch-Test) of

CON/M/91-08 in Rabbits: Lab Project Number: 8661/94. Unpublished study prepared by LPT Laboratory of Pharmacology and Toxicology. 22 p. {OPPTS 870.2500}

44868708 Leuschner, P. (1995) Examination of CON/M/91-08 in the Skin Sensitization Test in Guinea Pigs According to Magnusson and Klingmann: Lab Project Number: 8888/94. Unpublished study prepared by LPT Laboratory of Pharmacology and Toxicology. 40 p. {OPPTS 870.2600}

44868709 Lebertz, H. (1995) Investigation of the Behavior of the Environment-Leaching Behavior and Side Effects on Soil Microflora of Spore Isolate CON/M/91-08: Lab Project Number: IF-95/02315-00. Unpublished study prepared by Institut Fresenius. 32 p.

44868710 Kamp, N. (1995) Study on the Toxicity Towards Algae of Spore Isolate CON/M/91-08 According to OECD Test Guideline 201: Lab Project Number: IF-94/06075-01. Unpublished study prepared by Institut Fresenius. 18 p.

44868711 Kamp, N. (1995) Study on the Toxicity Towards Daphnia of Spore Isolate CON/M/91-08 According to OECD Test Guideline 202: Lab Project Number: 1F-94/06075-02. Unpublished study prepared by Institut Fresenius. 19 p.

44868712 Kamp, N. (1995) Study on the Toxicity Towards Fish of Spore Isolate CON/M/91-08 According to OECD Test Guideline 203: Lab Project Number: IF-94/06075-03. Unpublished study prepared by Institut Fresenius. 17 p.

44868713 Oellrich, W.; Lueth, P. (1999) Summary of Contans WG: Lab Project Number: PRO 99001-01. Unpublished study prepared by GAB Consulting GmbH. 60 p.

44956900 Prophyta Biologischer Pflanzenschutz GmbH (1999) Submission of Toxicity and Environmental Fate Data in Support of the Application for Registration of Contans WG. Transmittal of 2 Studies.

44956901 Roberts, A. (1999) Supplemental Data on Acute Toxicity Studies

for CON/M/91-08. Unpublished study prepared by Prophyta Biologischer Pflanzenschutz GmbH 273 p

44956902 Dengler, D. (1999) Influence of Temperature on Germination Capacity and Mycelium Growth of Conidia of Coniothyrium minitans Strain CON/M/91-08: Lab Project Number: 99028/01. Unpublished study prepared by Prophyta Biologischer Pflanzenschutz GmbH GAB Biotechnologie GmbH 39 p.

45088700 Prophyta Biologischer Pflanzenschutz GmbH (2000) Submission of Product Chemistry Data in Support of the Application for Registration of Contans WG. Transmittal of 1 Study.

45088701 Dengler, D. (1999) Determination of Purity of Five Batches of Contans WG: Lab Project Number: 99028/02-ALMP. Unpublished study prepared by GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH. 14 p. {OPPTS 885.1400}

45106100 Prophyta Biologischer Pflanzenschutz GmbH (2000) Submission of Efficacy Data in Support of the Application for Registration of Contans WG. Transmittal of 1 Study.

45106101 Roberts, A. (2000) Efficacy Data for Contans WG. Unpublished study prepared by Technology Sciences Group, Inc. 113 p.

Campbell, W.A. (1947). A new species of *Coniothyrium* parasitic on Sclerotia. Mycologia 39:190-195.

"Fungi on Plants and Plant Products in the United States" (DF Farr et al, eds, APS Press,1989, p.1252, St. Paul, MN)

Turner, G. J. and Tribe H.T. (1976) Trans. British. Mycol.. Soc., 66 (1) 97-105

Whipps, JM. Mycol. Res., v. 97: 1277-1280, (1993)