



US Environmental Protection Agency Office of Pesticide Programs

BIOPESTICIDES REGISTRATION ACTION DOCUMENT

Verticillium Isolate WCS850 (PC Code)

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(PC Code 081305)

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division
Verticillium Isolate WCS850
(PC Code 081305)

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I. EXECUTIVE SUMMARY/FACT SHEET

Active Ingredient and Proposed Use

Verticillium Isolate WCS850 is considered to be a hyaline, avirulent mutant of naturally occurring *Verticillium* sp. (either *V. dahliae* or *V. albo-atrum*). The end-product, Dutch Trig®, is used to control Dutch Elm disease in elm trees used in landscapes. Dutch Trig is injected into the vascular tissue (xylem) of the elm tree via a closed injection system, resulting in the induction of inherent resistance mechanisms in the tree. Once applied, *Verticillium* Isolate WCS850 appears to be restricted to the annual ring of the elm and cannot be isolated from elm trees one year after treatment.

Human Exposure and Risks

Evaluations of mammalian toxicology data and waivers are sufficient to support the unconditional registration of this microbial pesticide for the proposed uses. Acute intraperitoneal toxicity/pathogenicity studies demonstrated no toxicity or pathogenicity potential for *Verticillium* Isolate WCS850. The waivers were based on the following rationale. 1) The product exhibited no toxicity or pathogenicity potential based on the acute intraperitoneal study. 2) The applicator and public are not exposed to the fungus because application of the product is made via a closed system, and once injected, is contained in the annual ring of the elm tree. The closed system involves the product being contained in sealed vials, which are used in the gouge pistol. 3) The vials that contain Dutch Trig are made of non-breakable plastic; therefore, there is a very low likelihood of exposure to the applicator or the public. 4) The product can only be applied by Professional Arborists or Certified Applicators who have received ARCARDIS training which further reduces the likelihood of accidental exposure. 5) The fungus has been approved for use in the Netherlands since 1992 and there have been no adverse effects reports. No dietary, drinking water, or non-occupational exposure is anticipated based on the closed system application method. Occupational exposure is mitigated by the closed system application method and the use of non-breakable plastic vials. If used according to the label, it is anticipated that occupational exposure and risk will be minimal. Based on the review of the submitted human health data and waiver rationales, it is expected that there will be no unreasonable adverse effects to human health.

Ecological and Environmental Exposure and Risks

The submitted ecological data and waiver rationales support a conclusion that there will be no unreasonable adverse effects to nontarget organisms or to the environment expected as a result of the intended use of *Verticillium* Isolate WCS850 (Dutch Trig). *Verticillium* Isolate WCS850 is a hyaline, avirulent mutant of naturally occurring *Verticillium* sp. (either *V. dahliae*

or *V. albo-atrum*). The application of Dutch Trig is made via a closed system, and once injected, is contained in the annual ring of the elm tree. No microsclerotia are produced. The *Verticillium Isolate WCS850* does not grow nor survive at avian body temperatures.

Data Gaps and Requirements/Labeling

There are no data deficiencies for *Verticillium Isolate WCS850*. The registrant must provide the Agency a new certification of deposit from the Centraalbureau voor Schimmelcultures, The Netherlands, certifying the new taxonomic identification of the active ingredient as *Verticillium Isolate WCS850*. However, additional information and data may be required on a case-by-case basis for end products formulated with this active ingredient.

II. OVERVIEW

A. Product Overview

Biological Name:	<i>Verticillium Isolate WCS850</i>
Culture Collection Number:	CBS 276.92 <i>Verticillium dahliae</i> WCS 850, Centraalbureau voor Schimmelcultures, Institute of the Royal Netherlands Academy of Arts and Sciences (KNAW) The Netherlands
Trade and Other Names:	Dutch Trig®
OPP Chemical Code:	081305
Basic Manufacturer:	ARCADIS Geraghty & Miller 1131 Benfield Boulevard, Suite A Millersville, MD 21108

B. Use Profile

The following is information on the proposed uses with an overview of use sites and application methods.

Type of Pesticide:	Fungicide
Use Sites:	Elm trees (<i>Ulmus americana</i> , <i>Ulmus</i> spp.)

Target Pests: *Ophiostoma novo-ulmi* (causative organism of Dutch elm disease)

Formulation Types: Liquid suspension

Method and Rates

of Application: Injected into the vascular system of elm trees at a rate of 3 drops every 3 to 4 inches around the tree circumference at a comfortable waist height.

Use Practice Limitations: Used by professional arborists/certified applicators only.

C. Estimated Usage

An estimate of commercial usage cannot be made because this is the first registration containing *Verticillium Isolate WCS850* as the active ingredient.

D. Data Requirements

The data and information submitted in support of this unconditional registration under section 3(c)(5) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) have been reviewed by the Biopesticides and Pollution Prevention Division (BPPD). For *Verticillium Isolate WCS850*, the product identity and analysis data, as well as the information submitted for acute mammalian toxicity and ecological effects are sufficient to support registration. Based on evaluations of the submitted data and information, as discussed in this document, the Agency foresees no unreasonable adverse effects to human health and the environment from the use of *Verticillium Isolate WCS850*, when used as labeled.

E. Regulatory History

Experimental Use

Three Experimental Use Permits (EUPs) [EPA Reg. No. 71927-EUP-1] have been issued for *Verticillium Isolate WCS850*. The original EUP was granted for 8 lbs a.i. (789 lbs formulated product) to treat 44,700 trees per year (500 acres/year) in the following states: Connecticut, Illinois, Colorado, Maryland, Massachusetts, Michigan, Minnesota, North Carolina, New Jersey, Ohio, and Pennsylvania from the period April 15, 1999 to June 1, 2001. The second EUP was granted for 11 lbs ai. (1054 lbs formulated product) to treat 59,700 trees per year (668 acres/year) in the following states: Connecticut, Illinois, Colorado, Maryland, Massachusetts, Michigan, Minnesota, North Carolina, New Jersey, Ohio, Pennsylvania, California, Washington,

and Wisconsin from the period March 1, 2001 to June 30, 2003. The third EUP was granted for 11 lbs ai. (1054 lbs formulated product) to treat 59,700 trees per year (668 acres/year) in the following states: Connecticut, Illinois, Colorado, Maryland, Massachusetts, Michigan, Minnesota, North Carolina, New Jersey, Ohio, Pennsylvania, California, Washington, and Wisconsin from the period July 1, 2003 to June 30, 2005.

Section 3 Registration

EPA received an application from ARCADIS, Geraghty & Miller on March 1, 2002 to register the active ingredient *Verticillium Isolate WCS850* (originally identified as *Verticillium dahliae* Isolate WCS850). A deficiency letter regarding the taxonomy, host range, and microsclerotia production was issued on December 6, 2002. A new registration package was submitted to the Agency on January 28, 2005. When the application package was deemed complete, the receipt of the application for the new active ingredient was published in the Federal Register [FR: August, 10, 2005, Vol. 70, No. 153, pp. 46507-46508]. No public comments to the FR announcement were received by the Agency.

III. SCIENCE ASSESSMENT

A. Physical and Chemical Properties Assessment

The data submitted in support of product identity requirements for *Verticillium Isolate WCS850* are sufficient for the proposed use patterns of the microbial pesticide. A summary of the product identity and the manufacturing process information for *Verticillium Isolate WCS850* is provided in **Table 1**. All studies were found to be ACCEPTABLE. A summary of the physical and chemical properties of *Verticillium Isolate WCS850* is found in **Table 2**.

1. Product Identity and Mode of Action

Verticillium Isolate WCS850 belongs to the: Division - *Deuteromycota*; Class - *Deuteromycetes*; Order - *Moniliales* (Hyphomycetes); Genus - *Verticillium*. The MPCA was originally isolated from a diseased potato plant in the Netherlands and is deposited at the Centraalbureau voor Schimmelcultures, The Netherlands. *Verticillium* sp. cause “wilt” diseases in a range of hosts, including dicotyledonous plants, ornamental plants and trees, weeds, and woody hosts including trees. Transmission can occur as the pathogen persists in the soil in/on debris of infected plant material and for longer periods as free microsclerotia, which can infect roots upon contact. *Verticillium dahliae* cultures grow rapidly on PDA and malt agar (MA) at 23°C and produce hyaline hyphae. Hyaline sectors arise in colonies which are generally white. Conidiophores are abundant and more or less erect, hyaline, and verticillately branched with 3-4

phialides arising from each node. Dark brown resting mycelium is only formed in association with microsclerotia, which are dark brown to black and arise centrally in cultures. Each microsclerotium arises from a single hypha by repeated budding. Microsclerotia vary in shape and size.

The active ingredient was originally identified as *Verticillium dahliae* Isolate WCS850 and tested in Virginia and North Carolina under an USDA-APHIS permit. Under certain environmental conditions *Verticillium dahliae* readily produce microsclerotia which are small black overwintering/resting structures that may be infective and pathogenic to several susceptible plant genera. *V. dahliae* and *V. albo-atrum* are closely related species. Both species are well-known and thoroughly characterized plant pathogens. *V. dahliae* produces microsclerotia and *V. albo-atrum* does not. After further taxonomic characterization (including Amplified Fragment Length polymorphism analysis) of the isolate, it is now considered to be a hyaline, avirulent mutant of naturally occurring *Verticillium* sp. (either *V. dahliae* or *V. albo-atrum*). The active ingredient is now identified as *Verticillium* Isolate WCS850.

ARCADIS provided studies that confirmed that *Verticillium* Isolate WCS850 did not produce microsclerotia. Two isolates of *V. dahliae* from rose and okra produced microsclerotia when grown on two agar media (corn meal and Czapek-Dox which promote microsclerotia development), but no microsclerotia were produced by *Verticillium* Isolate WCS850 (MRIDs 46416302 and 464811-01). In comparison with other Dutch *Verticillium* isolates, *Verticillium* Isolate WCS850 did not appear to be more pathogenic on tomatoes than the other isolates. In addition, the isolate did not cause wilting when it was injected into maple, ash, and oak trees. Under an EUP (US EPA) in 2001-2003, no disease symptoms developed in *Verticillium*-susceptible trees [19 species including 3 *Ulmus* (elm) species] when challenged with inoculum of Dutch Trig due to the low virulence of *Verticillium* Isolate WCS850 (MRIDs 457207-04 and 464613-03). *Verticillium* Isolate WCS850 could not be re-isolated from elm tree cores of the injection site(s), at 2, 4, and 6 weeks after treatment, indicating the ephemeral nature of the fungal active ingredient (MRID 457207-04).

The microbial pesticide has no direct adverse effects on the Dutch elm pathogen (*Ophiostoma novo-ulmi*). Its mode of action is to induce inherent resistance mechanisms in the tree (MRID 46461303). The application method involves a special injector apparatus (“gouge pistol”), a closed system, that directly injects the *Verticillium* Isolate WCS850 conidial suspension into the trunk of an elm tree (3 drops every 3 or 4 inches around the tree trunk’s circumference at approximately waist height). The *Verticillium* spores are contained in a small glass vial, which is never opened. These vials are “loaded” into the pistol (MRID 457207-04). Because of the “under pressure” in a tree’s xylem tissue, the conidia (suspended in water) are sucked up immediately. Once applied, *Verticillium* Isolate WCS850 appears to be restricted to the annual ring of the elm, and cannot be isolated from elm trees one year after treatment (MRIDs 457207-04, 464613-03).

Table 1: Product Identity & Manufacturing Process for *Verticillium Isolate WCS850*

Guideline	Study	Result	MRID #
151-10 *885.1100	Product Identity	<i>Verticillium Isolate WCS850</i> was isolated, identified, and stored in the collection of the Centraalbureau voor Schimmelcultures in The Netherlands. ACCEPTABLE	447771-01 446729-01
151-11 *885.1200	Manufacturing Process	The manufacturing process includes quality control steps to prevent bacterial and fungal contamination in the technical grade active ingredient and the end-use product batches ACCEPTABLE	447771-01 446729-01
151-12 *885.1300	Discussion of Formation of Unintentional Ingredients	Proper aseptic techniques are practiced throughout manufacture. Fungal cultures and the end-use product are monitored for contamination. The registrant indicates that even if a contaminant was introduced in the beginning of the production process, it would be overwhelmed by the amount of <i>Verticillium Isolate WCS850</i> inoculum used. Any potential contamination would not likely compete under these conditions. ACCEPTABLE	447771-01 446729-01
151-13 *885.1400	Analysis of Samples	Analyses were performed on the final product ACCEPTABLE	447771-02 446729-02
151-15 *885.1500	Certification of limits	Certified limits are within OPPTS guidelines. ACCEPTABLE	447771-02 446729-02 CSF

*OPPTS Harmonized Guidelines

2. Physical and Chemical Properties Assessment

Table 2: Physical & Chemical Properties of *Verticillium* Isolate WCS850 (MRID# 446729-02)

Color	Clear
Physical State	Liquid
Odor	None
Density/Specific Gravity	1.0
pH	7-8
Storage Stability	8-10 weeks @ <4° C [33 to 40° F]
Viscosity	N/A
Miscibility	N/A
Corrosion Characteristics	Not Corrosive

B. Human Health Assessment

1. Food Clearances/Tolerances

N/A

2. Toxicology Assessment

a. Acute Intraperitoneal (IP) Toxicity Study in Rats (885.3200)

Three female and three male rats were dosed with 2 ml of the Dutch Trig preparation which contained approximately 1.7×10^7 CFU of the *Verticillium* Isolate WCS850. Two untreated male and two untreated female rats served as controls. One untreated animal (#932) displayed weight loss between the Day 1 and Day 8 observations. None of the animals displayed any effects due to the dosing. None of the other animals lost weight, and no abnormal clinical nor gross necropsy observations were seen.

CLASSIFICATION: ACCEPTABLE

b. Data Waiver Requests: Tier I Acute Mammalian Toxicity Testing

Data waivers were requested for the following Tier I studies:

- Acute Oral Toxicity/Pathogenicity (152A-10)
- Acute Dermal Toxicity (152A-11)
- Acute Eye Irritation (152A-14)
- Acute Pulmonary Toxicity/Pathogenicity (152A-12)

The following waiver justifications provided by the registrant were found to be “ACCEPTABLE” by the Agency.

1. An acute intraperitoneal test was performed (see discussion above) which showed that a single intraperitoneal dose of Dutch Trig® at 1.7×10^7 CFU per animal (Sprague-Dawley rats) did not produce any clinical signs. No abnormalities were observed upon necropsy.
2. The applicator and public are not exposed to the fungus because application of the product is made via a closed system. Once injected, the product is contained in the annual ring of the elm tree. The closed system is comprised of the product in sealed vials which are then loaded into the gouge pistol and then injected into the elm tree.
3. The vials that contain Dutch Trig are made of non-breakable plastic, resulting in a very low likelihood of exposure to the applicator or the public.
4. The product can only be applied by Professional Arborists or Certified Applicators who have received ARCARDIS training. This further reduces the likelihood of accidental exposure.
5. The fungus has been approved for use in the Netherlands since 1992 and there have been no adverse effects reported.

c. Data Waiver Requests for Hypersensitivity and Immune Response Studies

(i.) Hypersensitivity Study (OPPTS 870.2600)

The registrant has reported that there have been no hypersensitivity incidents during production and testing of *Verticillium* Isolate 850. In addition, the submitted pathogenicity/toxicity study (as discussed above) has shown little toxicity or potential effects for *Verticillium* Isolate WCS850. The registrant must also report any adverse incidents to the Agency under FIFRA section 6(a)(2). Therefore, the data waiver request for the hypersensitivity study is granted.

(ii.) Immune response (OPPTS 885.3800)

An acute intraperitoneal toxicity study showed no animals with an immunological response caused by the injected doses. Treated animals displayed no significant clinical signs and there were no unusual presentation at necropsy. Therefore, the data waiver request for immune response testing is granted.

d. Summary of Tier I Data Waivers for Human Health Toxicity Testing

A summary of the Tier I data waivers for the human health toxicity data for *Verticillium* Isolate WCS 850 is provided below in **Table 3**.

Table 3: Tier I - Data Waivers: Human Health Toxicity Studies for *Verticillium* Isolate WCS850

Guideline	Study	Toxicity Category	Comments	MRID No.
152-10	Acute Oral Toxicity/Pathogenicity	N/A	Waived	N/A
152-11	Acute Dermal Toxicity	N/A	Waived	N/A
152-14	Acute Eye Irritation	N/A	Waived	N/A
152A-12	Acute Pulmonary Toxicity/Pathogenicity	N/A	Waived	N/A
152-36 *870.2600	Hypersensitivity Study	N/A	Waived	N/A
152-38 *870.3800	Immune Response	N/A	Waived	N/A

***OPPTS Harmonized Guideline Numbers.**

e. Subchronic Toxicity, Chronic Toxicity and Oncogenicity

Based on the data generated in accordance with the Tier I data requirements (40 CFR §158.740(c)), Tier II tests (Guidelines 152B-40 through 152B-49) for acute oral, acute inhalation, subchronic oral, acute intraperitoneal/intracerebral, primary dermal, primary eye,

immune response, teratogenicity, virulence enhancement, and mammalian mutagenicity were not required. As a result, Tier III tests (Guidelines 152-50 through 53) for chronic testing, oncogenicity testing, mutagenicity, and teratogenicity also were not required.

f. Effects on the Immune and Endocrine Systems

The Agency is not requiring information on the endocrine effects of this active ingredient, *Verticillium* Isolate 850, at this time. The Agency has considered, among other relevant factors, available information concerning whether the microorganism may have an effect in humans similar to an effect produced by a naturally occurring estrogen or other endocrine effects. There is no known metabolite produced by this microorganism that acts as an “endocrine disrupter.” The submitted toxicity/pathogenicity studies in the rodent (required for microbial pesticides) indicate that following injection and pulmonary routes of exposure, the immune system is still intact and able to process and clear the active ingredient. In addition, because there is no expected associated with the proposed use of this product, the Agency concludes that there will be no incremental adverse effects to the endocrine or immune systems.

3. Occupational and Residential Exposure and Risk Characterization

a. Non-occupational Residential, School and Day Care Exposure and Risk Characterization

The proposed product is an end-use product with no food uses. No mortality was observed at the dosing rate of 1.7×10^7 cfu/animal. No non-occupational residential, school or day care exposure is anticipated because of the use of the closed injection system for this product. The use of the pesticide product, Dutch Trig, should result in minimal to non-existent non-occupational risk. No indoor residential, school or daycare uses currently appear on the label.

b. Occupational Exposure and Risk

The potential for exposure to the pesticide, Dutch Trig, exists for the applicators and the end-product manufacturers. Because of the lack of mammalian toxicity, worker exposure data on *Verticillium* Isolate WCS850 are not required. The “caution” statement and hazard and first aid statements on the label are sufficient to protect from any adverse reactions that may occur from exposure to *Verticillium* Isolate WCS850.

4. Drinking Water Exposure and Risk Characterization

No drinking water exposure is anticipated because of the use pattern. There are no

aquatic use sites for the pesticide, so exposure in drinking water is not expected. In addition, there is no evidence of adverse effects from exposure to this microbial agent. Exposure from the proposed use of *Verticillium* Isolate WCS850 is not likely to pose any incremental risk via consumption of drinking water to adult humans, infants and children.

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

The potential aggregate exposure, derived from (a) dietary (oral) exposure from treated food/feed commodities and from drinking water, potentially exposed secondary to treatment of sites with this pesticide, and (b) dermal and inhalation non-occupational and occupational exposure of populations to *Verticillium* Isolate WCS850, is not expected or should be adequately mitigated, as long as the pesticide is used as labeled.

6. Cumulative Effects

The Agency has considered the potential for cumulative effects of *Verticillium* Isolate WCS850 and other substances in relation to a common mechanism of toxicity. These considerations include the possible cumulative effects of such residues on infants and children. As demonstrated in Section III.B.2 above, *Verticillium* Isolate WCS850 is non-toxic and non-pathogenic to mammals. Because no mechanism of pathogenicity or toxicity in mammals has been identified for this organism, no cumulative effects from the residues of this product with other related microbial pesticides are anticipated.

C. Environmental Assessment

1. Ecological Effects Hazard Assessment

Below is a summary of the ecological effects database evaluated in support of this action (**Table 4**). A determination of reasonable certainty was made that no exposure will take place to wild mammals, avian species, insects including beneficial insects, freshwater fish, aquatic invertebrates, estuarine and marine animals or plants from the intended application method and the fate of the MPCA in the elm tree. The database consists of acceptable waiver requests, submitted data, and other information that support the conclusion that there are no incremental hazards to non-target organisms as a result of intended uses of *Verticillium* Isolate WCS850.

Table 4: Eco-Toxicology Summary

Guideline No.	Study	Status, Classification & Comments	MRID Nos.
154-16 *885.4050	Avian oral toxicity/pathogenicity	Waiver rationale is acceptable. Birds will not be exposed to intended applications of the MPCA. The MPCA does not grow nor survive at avian body temperatures.	46461302, 46461303, 45720704
154-17 *885.4100	Avian Inhalation Toxicity/Pathogenicity	Waiver rationale is acceptable. Birds will not be exposed to intended applications of the MPCA. The MPCA does not grow nor survive at avian body temperatures.	46461302, 46461303, 45720704
154-18 *885.4150	Wild Mammal Testing	Waiver rationale is acceptable. Wild mammals will not be exposed to intended applications of the MPCA. The MPCA does not grow nor survive at temperatures > 30°C.	46461302, 46461303, 45720704
154-19 *885.4200	Freshwater fish testing	Waiver rationale is acceptable. Freshwater fish will not be exposed to the MPCA, because intended applications are restricted to injections into elm trees.	45720704; 46461303
154-20 *885.4240	Freshwater aquatic invertebrate testing	Waiver rationale is acceptable. Freshwater fish will not be exposed to the MPCA, because intended applications are restricted to injections into elm trees.	45720704; 46461303
154-21 *885.4280	Estuarine and Marine Animal Testing	Waiver rationale is acceptable. Estuarine or marine animals will not be exposed to intended applications of the MPCA.	45720704; 46461303
154-22 *885.4300	Non-target plant studies	Waiver rationale is acceptable. The directed application method, and a lack of potential for inoculum (of the MPCA) survival and proliferation outside the intended use (injected into annual ring of the elm tree), preclude hazards to non-target plants. The MPCA is a hyaline mutant of <i>Verticillium</i> sp, and does not produce microsclerotia <i>in vitro</i> .	45720704, 46461303, 46481101

Guideline No.	Study	Status, Classification & Comments	MRID Nos.
154-23 *885.4340	Non-target insect testing	Waiver rationale is acceptable. There are no published reports of toxicity or pathogenicity of the MPCA to insects; and the vast majority of these organisms will not be exposed to the MPCA, because intended applications are restricted to injections into elm trees. Once applied, <i>Verticillium</i> Isolate WCS850 appears to be restricted to the annual ring of the elm and cannot be isolated from elm trees one year after treatment.	45720704; 46461303
154-24 *885.4380	Honeybee testing	Waiver rationale is acceptable. Honeybees will not be exposed to applications of the MPCA and there are no published reports of toxicity or pathogenicity to honeybees.	45720704; 46461303

*885 series = OPPTS Microbial Pesticide Test Guideline Numbers.

2. Environmental Fate, Ecological Exposure, and Environmental Expression Risk Characterization

Verticillium Isolate WCS850 is considered a hyaline, avirulent mutant of naturally occurring *Verticillium* sp. (either *V. dahliae* or *V. albo-atrum*), which are well-known and thoroughly characterized plant pathogens. This Isolate was originally isolated from a diseased potato plant in the Netherlands and is deposited at the Centraalbureau voor Schimmelcultures, The Netherlands. In comparison with other Dutch isolates, *Verticillium* Isolate WCS850 did not appear to be more pathogenic on tomatoes than the other isolates. The MPCA does not produce microsclerotia. The microbial pesticide has no direct effect on the targeted diseased tree (*Ulmus spp*; elm trees with Dutch elm disease), but works by inducing resistance mechanisms in the tree (MRID 464613-03). Once applied, *Verticillium* Isolate WCS850 appears to be restricted to the annual ring of the elm and cannot be isolated from elm trees one year after treatment (MRID 457207-04).

The ecological data (as summarized in section III.C.1 above) support a conclusion that no incremental hazards to non-target organisms or to the environment are expected as a result of the intended use of *Verticillium* Isolate WCS850 (Dutch Trig). The end-use product is intended for directed injections into elm trees in urban landscapes annually in the Spring. No further testing

for ecological effects or environmental expression is required for this use.

D. Efficacy Data

Efficacy data were not reviewed by the Agency because the products are intended for control of the fungal pathogen, *Ophiostoma novo-ulmi* (causative organism of Dutch elm disease) in urban landscaped American elms (*Ulmus americana*) and will not be formulated into products for control of public health pests.

E. Endangered Species Assessment

An environmental risk assessment was performed on non-target birds, wild mammals, plants, freshwater fish, aquatic invertebrates, estuarine and marine animals, honeybees and non-target insects using information as submitted by USDA-IR4 on behalf of ARCADIS. The information and data regarding product identity and characterization support a risk characterization and a conclusion of a reasonable certainty that there are no incremental hazards to non-target organisms, including endangered and threatened species, as a result of the intended uses of *Verticillium* Isolate WCS850.

This opinion is based on a general rationale pertaining to the biology and ecology of the MPCA and the Dutch elm disease complex from the open literature, data from host range studies, and the lack of exposures to non-target organisms because of the directed application method. The intended application method of direct injection of *Verticillium* Isolate WCS850 involves an injector apparatus (gouge pistol), a closed system in which the *Verticillium* conidia (spores) are injected into the trunk of an elm tree. Requests to waive wild mammal toxicity/pathogenicity studies were reviewed and determined acceptable based on associative rationale substantiating a reasonable certainty that wild mammals will not be exposed to intended applications of the MPCA. In addition, direct testing of rodent species for human health assessment purposes did not reveal any hazards. A determination of reasonable certainty was made that no exposure will take place to avian species, freshwater fish, aquatic invertebrates, or estuarine and marine animals and plants from the intended application method and the fate of the MPCA in the tree. The majority of insect taxa, including honey bees, will not be exposed to the intended applications of the MPCA and there are no published reports of toxicity or pathogenicity to honey bees or other insects. The elm bark beetle, *Scolytus scolytus*; *S. europa*, is the vector for the pathogen, *O. novo-ulmi*, and may be exposed; however, there is no adverse risk concern for this insect species.

Verticillium spp. are widely present in soil, as a common inhabitant of crop debris. Only two species of the genus are regarded as plant pathogens. The *Verticillium* Isolate WCS850 appears to be an avirulent mutant of either *Verticillium dahliae* or *V. albo-atrum*, incapable of

inciting disease in *Verticillium*-susceptible plant hosts. In 2001-2003, under an EUP (US EPA), no disease symptoms developed in *Verticillium*-susceptible trees [19 species, including 3 *Ulmus* (elm) species] when challenged with inoculum of Dutch Trig (MRIDs 457207-04 and 464613-03). Furthermore, there is a reasonable certainty that non-target plants will not be exposed to intended injections of the MPCA. There is a lack of potential for inoculum (*Verticillium* Isolate WCS850) to survival and to proliferate outside the intended use environment. Once applied, *Verticillium* Isolate WCS850 appears to be restricted to the annual ring of the elm, and cannot be isolated from elm trees one year after treatment (MRID 457207-04). The microbial pesticide has no direct effect on the targeted plant pathogen (*Ophiostoma novo-ulmi*). Its mode of action is via induced resistance mechanisms in the elm tree.

From a regulatory perspective, the MPCA will be labeled for use by Professional Arborists or Certified Applicators only. All production, distribution, application, and disposal of *Verticillium* Isolate WCS850 are under the guidance and direction of ARCADIS. The product, Dutch Trig, has been used in the Netherlands since 1992 and in the U.S. since 1999 under an EUP. No adverse effects were observed in workers or to the environment.

This analysis supports a no “may effect” opinion to Federally listed Threatened and Endangered species from the direct injection of of *Verticillium* Isolate WCS850 (Dutch Trig) into elm trees as a “vaccination” to induce resistance mechanisms to the Dutch elm disease complex in susceptible elm species in U.S. urban landscapes (MRID 46461303).

Detailed rationale and justification are on file with the EPA as specified in the Memorandum:

“Requests for Waivers from Toxicity and Pathogenicity Testing on Non-target Organisms (Avian Oral, Avian Inhalation, Wild Mammals, Freshwater Fish, Aquatic Invertebrates, Terrestrial and Aquatic Plants, Insects and Honeybees, and Estuarine and Marine Invertebrates) for the Microbial Pesticide (TGAI), *Verticillium* Isolate 850.”

IV. RISK MANAGEMENT AND REGISTRATION DECISION

A. Determination of Eligibility

Section 3(c)(5) of FIFRA provides for the registration of a new active ingredient 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, *Verticillium* Isolate WCS850 has well known properties. The Agency has no knowledge that would contradict the claims made on the label of this product and the active ingredient is not expected to cause unreasonable adverse effects when used according to label instructions. 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, and is likely to provide protection as claimed, satisfying Criterion C. Criterion D is satisfied in that *Verticillium* Isolate WCS850 is not expected to cause unreasonable adverse effects when used according to label instructions.

Therefore, *Verticillium* Isolate WCS850 is eligible for registration. The uses are listed in **Table 5, Appendix A**.

B. Regulatory Position

1. Unconditional Registration

The data requirements are fulfilled and BPPD recommends the unconditional registration of products that contain *Verticillium* Isolate WCS850 as a new active ingredient (Dutch Trig).

2. Tolerances for Food Uses and /or exemptions

Not applicable, non-food use.

3. CODEX Harmonization

Not applicable.

4. Non-food Re/Registrations

The proposed product is for a non-food use of a new active ingredient that is not the subject of reregistration at this time.

5. Risk Mitigation

There is minimal or negligible potential risk to non-target organisms (plants and wildlife), and to ground and surface water contamination through the proposed use of products containing *Verticillium* Isolate WCS850 as discussed in this document. No mitigation measures are required at this time. Appropriate personal protective equipment is required for pesticide handlers. The product label will also bear Environmental Hazards text to mitigate any potential risk as determined by reviewed data and use sites.

6. Endangered Species Statement

The proposed product, Dutch Trig, is an end-use product that is injected into elm trees via the ARCADIS gouge pistol (a closed system). There are no direct applications to the environment. Thus, no labeling is required for endangered species at this time.

C. Labeling Rationale

It is the Agency's position that the labeling for Dutch Trig, containing 1% *Verticillium* Isolate WCS850, complies with the current pesticide labeling requirements.

1. Human Health Hazard

a. Worker Protection Standard

This product does not come under the provisions of the Worker Protection Standards (WPS) because it is a non-food use pesticide intended for aesthetic landscape uses only.

b. Non-Worker Protection Standard

There are no non-WPS human health hazard issues.

c. Precautionary Labeling

The Agency has examined the toxicological data base for *Verticillium* Isolate WCS850 and concluded that the proposed precautionary labeling adequately mitigates the risks associated

with the proposed uses of the end-use product, Dutch Trig (i.e. Signal Word, First Aid Statements and other label statements).

End-Use Product Precautionary Labeling: For Dutch Trig: “Caution”. “KEEP OUT OF REACH OF CHILDREN.”

d. Spray Drift Advisory

No spray drift advisory statement is necessary for this use.

2. Environmental Hazards Labeling

End-Use Product Environmental Hazards Labeling: For Dutch Trig: "Do not apply directly to water, or areas where surface water is present or to intertidal areas below the mean water mark. Do not contaminate water when cleaning equipment or disposing of equipment wash waters.”

3. Application Rate

Dutch Trig is injected in the trunk of the elm in the early spring after bud break with the ARCADIS gouge pistol. The spores of *Verticillium* Isolate WCS850 (1×10^7 conidiospores per ml) are transferred as inoculum from the sealed vial into the trunk of an elm tree using the ARCADIS gouge pistol (a closed system). The product contains at least 8×10^6 conidia per ml of formulated product.

D. Labeling

1. End Use Product

Product name: Dutch Trig

Ingredient Statement:

<i>Verticillium</i> Isolate WCS850	1.0 %
Inert (Distilled water) Ingredients	<u>99.0 %</u>
Total	100.0 %

Based on the evaluation of potential toxicity and pathogenicity for this active ingredient for the unconditional registration of the product, containing *Verticillium* Isolate WCS850, the signal word is "Caution". In addition, the product label shall contain the following information:

- Product Name

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

V. ACTIONS REQUIRED BY REGISTRANTS

This is an unconditional registration under FIFRA 3(c)(5). Reports of incidents of adverse effects to humans or domestic animals are required under FIFRA, Section 6(a)(2) and incidents of hypersensitivity under 40 CFR Part 158.690(c), guideline reference number 152-16. There are no data requirements, label changes, and other responses necessary for the reregistration of the end-use product because the product is being registered after November 1984 and is, therefore, not subject to reregistration. For the same reason, there are also no existing stocks provisions at this time. Before releasing these products for shipment, the registrant is required to provide appropriate labels and other Agency requirements as discussed in this Biopesticide Registration Action Document. The registrant must provide the Agency a new certification of deposit from the Centraalbureau voor Schimmelcultures, The Netherlands, certifying the new taxonomic identification of the active ingredient as *Verticillium* Isolate WCS850.

VI. APPENDICES

APPENDIX A - Use sites

Table 5 lists the use sites for the product. The registrant must comply with the appropriate labeling requirements before releasing products containing *Verticillium* Isolate WCS850 as the active ingredient for shipment.

Table 5: Use Site Unconditional Registration

DutchTrig® <u>Use Sites</u> The end-use product is intended for injection into American elm trees to protect against Dutch elm disease.	Official date registered: October 19, 2005
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APPENDIX B - Citations Considered to be part of the database supporting the unconditional registration of *Verticillium* Isolate WCS850.

CITATIONS/BIBLIOGRAPHY

Studies submitted in support of this registration action

MRID: 45720700

Citation: Arcadis G&M, Inc. (2002) Submission of Product Chemistry, Toxicity, and Efficacy Data in Support of the Application for Registration of *Verticillium dahliae* Isolate WCS 850. Transmittal of 5 Studies.

MRID: 45720701

Citation: Voeten, J. (2002) *Verticillium dahliae* Isolate WCS 850--Product Identity and Disclosure of Ingredients, Manufacturing Process and Discussion on the Formation of Unintentional Ingredients, Analysis of Samples, Certification of Ingredient Limits, Analytical Methods for Certified Limits: Lab Project Number: 86B: PR 86B. Unpublished study prepared by ARCADIS Geraghty and Miller, Inc. 74 p.

MRID: 45720702

Citation: Voeten, J.; Braverman, M. (2002) *Verticillium dahliae* Isolate WCS 850: Acute Intrapertoneal Toxicity: Safety Information: Hypersensitivity: Lab Project Number: 86B: PR 86B: M98AR75.6P31. Unpublished study prepared by Arcadis Tree Services and IR-4 Project. 84 p. {OPPTS 885.3200}

MRID: 45720703

Citation: Voeten, J.; Braverman, M.; Baron, J. (2002) *Verticillium dahliae* Isolate WCS 850: Non-target Organism and Environmental Safety Information: Lab Project Number: 86B: PR 86B: 517/1238/PDB/HR. Unpublished study prepared by Arcadis Tree Services, Arcadis Geraghty, and Miller, Inc., and IR-4 Project. 40 p.

MRID: 45720704

Citation: Voeten, J.; Barron, J. (2002) *Verticillium dahliae* Isolate WCS 850: Non-target Plant and Host Range Studies: Lab Project Number: 86B: PR 86B: 517/1238/PDB/HR. Unpublished study prepared by Arcadis Tree Services, Arcadis Geraghty, and Miller, Inc., and IR-4 Project. 92 p.

MRID: 45720705

Citation: Voeten, J.; Baron, J. (2002) *Verticillium dahliae* Isolate WCS 850--Product

Performance Data: Lab Project Number: 86B: PR 86B: 514AD00/1463/GB. Unpublished study prepared by Arcadis Tree Services, Arcadis Geraghty, and Miller, Inc., and IR-4 Project. 60 p.

MRID: 46461300

Citation: Arcadis Geraghty & Miller, Inc. (2005) Submission of Product Chemistry and Toxicity Data in Support of the Application for Registration of *Verticillium* Isolate WCS 850 Dutch Trig. Transmittal of 2 Studies.

MRID: 46461302

Citation: Voten, J.; Baron, J. (2005) *Verticillium* Isolate WCS 850 Product Characterization/Identity. Project Number: 86B. Unpublished study prepared by Arcadis Geraghty & Miller. 43 p.

MRID: 46461303

Citation: Voten, J.; Baron, J.; Braverman, M. (2005) *Verticillium* Isolate WCS 850 Non-Target Plant and Host Range Studies. Project Number: 86B. Unpublished study prepared by Arcadis Geraghty & Miller, Inc. and IR-4 Northeast Analytical Laboratory. 92 p.

MRID: 46481100

Citation: IR-4 Project (2005) Submission of Toxicity Data in Support of the Application for Registration of Dutch Trig *Verticillium* isolate WCS 850. Transmittal of 1 Study.

MRID: 46481101

Citation: Voten, J.; Baron, J.; Braverman, M. (2005) *Verticillium* Isolate WCS 850: Non-Target Organism and Environmental Safety Information. Project Number: 86B. Unpublished study prepared by Interregional Research Project No. 4. 16 p.

Federal Register Publications

1. Federal Register: August 10, 2005 (Volume 70, Number 153) (Notices) (Page 46507-46508). *Verticillium dahliae* isolate WCS 850 ; Pesticide Products; Registration Applications.
2. Registration of a New Active Ingredient (to be published in 2005).

BPPD Data Evaluation Records/Reviews

Michael Watson and John Kough, U.S. EPA, OPP/BPPD. February 8, 1999.
Physical and Chemical Products, Acute Intraperitoneal Toxicity, and Hypersensitivity

Incident Data, submitted by ARCADIS Geraghty & Miller to Support an EUP for Dutch Trig; MRID#s 446689-01 & -02, 446729-01 & -02.

Michael Watson and John Kough, U.S. EPA, OPP/BPPD. April 5, 1999.

BPPD review of Supplemental Information submitted by ARCADIS Geraghty & Miller to Address Deficiencies Described in Review (Watson to Matten, 2/8/99) of their Previous EUP Submission for Dutch Trig; MRID#s 447771-01 & -02.

Doug Gurian-Sherman and Gail Tomimatsu, U.S. EPA, OPP/BPPD. February 16, 1999.

Review of Data Submitted by IR-4/ARCADIS in Support of an Experimental Use Permit for *Verticillium dahliae* isolate WCS 850 and Request for Waiver of all Non-Target Organism Testin ; MRID# 446998-01, other studies had no MRIDs.

Gail Tomimatsu., U.S. EPA, OPP/BPPD. August 24, 2005.

Requests for Waivers from Toxicity and Pathogenicity Testing on Non-target Organisms (Avian Oral, Avian Inhalation, Wild Mammals, Freshwater Fish, Aquatic Invertebrates, Terrestrial and Aquatic Plants, Insects and Honeybees, and Estuarine and Marine Invertebrates) for the Microbial Pesticide (TGAI), *Verticillium* Isolate 850; MRID# 464811-01.