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PRD2009-10

Proposed Registration Decision

Verticillium albo-atrum **strain WCS850**

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Overview

Proposed Registration Decision for *Verticillium albo-atrum* strain WCS850

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the [Pest Control Products Act](#) and Regulations, is proposing full registration for the sale and use of Verticillium isolate WCS850 and Dutch Trig, containing the microbial pest control agent *Verticillium albo-atrum* strain WCS850, to prevent Dutch elm disease in uninfected elm trees.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of Verticillium isolate WCS850 and Dutch Trig.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable¹ if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value² when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment (for example, those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides and Pest Management portion of Health Canada's website at healthcanada.gc.ca/pmra.

¹ "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act*.

² "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (a) efficacy; (b) effect on host organisms in connection with which it is intended to be used; and (c) health, safety and environmental benefits and social and economic impact."

Before making a final registration decision on *Verticillium albo-atrum* strain WCS850, the PMRA will consider all comments received from the public in response to this consultation document.³ The PMRA will then publish a Registration Decision⁴ on *Verticillium albo-atrum* strain WCS850, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What Is *Verticillium albo-atrum* isolate WCS850?

Verticillium albo-atrum strain WCS850 is a microbial pest control agent (MPCA) used to prevent Dutch elm disease by inducing the treated tree's natural defense mechanisms, a process called induced systemic resistance. The end-use product, Dutch Trig, is a commercial fungicide product that contains *Verticillium albo-atrum* strain WCS850 as the active ingredient.

Health Considerations

Can Approved Uses of *Verticillium albo-atrum* strain WCS850 Affect Human Health?

***Verticillium albo-atrum* strain WCS850 is unlikely to affect your health when Dutch Trig is used according to the label directions.**

When assessing health risks, several key factors are considered: the microorganism's biological properties (for example, production of toxic byproducts), reports of any adverse incidents, its potential to cause disease or toxicity as determined in toxicological studies and the level to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

Negligible exposure to *Verticillium albo-atrum* strain WCS850 is expected when handling and applying Dutch Trig. Dutch Trig is injected into trees using a completely closed application system and is packaged into thick polyethylene vials that are waterproof and virtually unbreakable.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns. When spores of *Verticillium albo-atrum* strain WCS850 were tested on laboratory animals, there were no signs that it caused any toxicity or disease. Furthermore, *Verticillium albo-atrum* strain WCS850 does not grow at temperatures above 30°C and few adverse effects to *Verticillium* spp. were reported in published scientific literature despite intensive research.

³ "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

⁴ "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

Residues in Water and Food

Dietary risks from food and water are not of concern

The *Food and Drugs Act* prohibits the sale of food containing a pesticide residue that exceeds the established maximum residue limit. Pesticide maximum residue limits are established for the *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act*. Each maximum residue limit value determines the maximum concentration in parts per million (ppm) of a pesticide allowed in or on certain foods. Food containing a pesticide residue that does not exceed the established maximum residue limit does not pose an unacceptable health risk.

As there are no direct applications to food and no significant adverse effects were reported in Tier I acute toxicity/pathogenicity studies, the establishment of a maximum residue limit is not required for *Verticillium albo-atrum* strain WCS850 under section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, section B.15.002 of the Food and Drugs Regulations. In addition, the likelihood of residues of *Verticillium albo-atrum* strain WCS850 contaminating drinking water supplies is negligible. Consequently, dietary exposure and risk are minimal to non-existent

Occupational Risks From Handling Dutch Trig

Occupational risks are not of concern when Dutch Trig is used according to label directions, which include protective measures

Workers using Dutch Trig are not likely to come into direct contact with *Verticillium albo-atrum* strain WCS850 during application. Dutch trig is injected into trees using a specialized injection tool which was designed to prevent spillage. Furthermore, Dutch Trig is packaged in thick polyethylene vials that are waterproof and virtually unbreakable. However, minimal worker exposure to *Verticillium albo-atrum* strain WCS850 may occur via the dermal route during routine maintenance of the injection equipment. To prevent exposure during application and maintenance of the injection equipment, the label will specify that anyone applying or maintaining injection equipment must wear waterproof gloves, long-sleeved shirts, long pants, and shoes plus socks.

For bystanders, exposure is expected to be much less than that of handlers and applicators and is also considered negligible.

Environmental Considerations

What Happens When Dutch Trig is Introduced Into the Environment?

Environmental risks are not of concern

Verticillium albo-atrum strain WCS850 is a natural un-pigmented (white) variant of *Verticillium albo-atrum*. *Verticillium albo-atrum* is a plant pathogen that can infect many different tree species and vegetable crops causing *Verticillium* wilt disease. Strain WCS850 differs from the pigmented wild type in that it has lost its ability to produce resting structures and thus has lost its ability to persist in its natural environment. *Verticillium albo-atrum* strain WCS850 has also lost much of its ability to infect plants. After Dutch Trig is injected into elm trees, *Verticillium albo-atrum* strain WCS850 is contained within a growth ring of the treated elm tree. The WCS850 strain is just pathogenic enough to induce a natural resistance response in the injected elm, but has lost so much of its pathogenicity that it is no longer capable of successfully infecting an elm with *Verticillium* wilt disease. After injection, *Verticillium albo-atrum* strain WCS850 can only be recovered from the injection site, and only up to two weeks after injection.

The risk to terrestrial and aquatic organisms from the use of Dutch Trig is very low based on the negligible potential for environmental exposure.

Value Considerations

What Is the Value of Dutch Trig?

Dutch Trig induces resistance in elm trees to fend off beetle-inflicted Dutch elm disease infections with its own natural defence mechanisms.

Dutch Trig prevents Dutch elm disease in uninfected elms. The injection system is self contained consisting of virtually unbreakable plastic vials that can only be used in the Dutch Trig Tree Injection Tool. No mixing or diluting is required. Treatment with Dutch Trig causes no adverse effects on the tree and does not affect the beetle population or prevent beetles from feeding on elm trees. Dutch Trig can be used in the urban environment (streets, parks, backyards) on trees that provide aesthetic, environmental, economic and psychological benefits.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The PMRA is proposing key risk-reduction measures on the label of Dutch Trig to address the potential risks identified in this assessment.

Key Risk-Reduction Measures

Human Health

To prevent exposure to *Verticillium albo-atrum* strain WCS850 during application and during routine maintenance of the injection equipment, anyone applying Dutch Trig or maintaining injection equipment must wear waterproof gloves, long-sleeved shirts, long pants, and shoes plus socks.

Environment

As a general precaution, statements will be added to the label to prohibit handlers from contaminating aquatic habitats during routine maintenance of the injection system.

Next Steps

Before making a final registration decision on *Verticillium albo-atrum* strain WCS850, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document.

Please forward all comments to PMRA Publications. The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Verticillium albo-atrum* strain WCS850 (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Verticillium albo-atrum strain WCS850

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Microbial Pest Control Agent

Active microorganism	<i>Verticillium albo-atrum</i> strain WCS850
Function	Prevent Dutch elm disease (<i>Ophiostoma novo-ulmi</i>) by inducing the treated tree's systemic resistance.
Binomial name	<i>Verticillium albo-atrum</i> strain WCS850
Taxonomic designation	
Kingdom	Fungi
Sub-kingdom	Dikarya
Phylum	Ascomycota
Sub-phylum	Pezizomycotina
Class	Sordariomycetes
Sub-class	Sordariomycetes incertae sedis
Order	Phyllachorales
Genus	<i>Verticillium</i>
Species	<i>albo-atrum</i>
Strain	WCS850
Patent status information	No patents are held by the applicant in Canada.
Minimum purity of active ingredient	1.0×10^7 colony forming units (CFU)/mL
Identity of relevant impurities of toxicological, environmental and/or significance	The technical grade active ingredient does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards. <i>Verticillium albo-atrum</i> strain WCS850 does not produce any known toxins or any other known toxic metabolites.

1.2 Physical and Chemical Properties of the Active Ingredient and End-Use Product

Technical Grade Active Ingredient–Verticillium isolate WCS850

Property	Result
Physical state	Aqueous suspension
Guarantee	1.0×10^7 CFU/mL (nominal)
Colour	Clear
Odour	Odourless
pH	7.0
Density	1 kg/L (bulk)

End-Use Product–Dutch Trig

Property	Result
Physical state	Aqueous suspension
Guarantee	1.0×10^7 CFU/mL (nominal)
Colour	Clear
Odour	Odourless
pH	7.0
Density	1 kg/L (bulk)

1.3 Directions for Use

Dutch Trig will prevent beetle-vectored infection of healthy elm trees from Dutch elm disease (*Ophiostoma ulmi*, *Ophiostoma novo-ulmi*). Inject one drop from a 40 mL vial every 10 cm of tree circumference using the Dutch Trig tree inoculation tool at a comfortable waist height (refer to illustration on label). Application of Dutch Trig may only be executed with the Dutch Trig tree inoculation tool. Treatments should take place in early spring after bud break and before full leaf (at 50% leaf expansion). One treatment per year is required to protect elm trees from infection throughout the season. Only inject healthy elm trees which show no sign of Dutch elm disease as Dutch Trig has no curative properties. Dutch Trig is to be used on American elm trees only. Dutch Trig must be refrigerated prior to use. Detailed information on storing, transporting and applying Dutch Trig can be found on the product label.

1.4 Mode of Action

Verticillium albo-atrum strain WCS850 is a variant that does not produce resting spores. Infection of elm trees with the organism induces a systemic acquired resistance (SAR) response. SAR is the activation of plant defences in uninfected parts of the plant which makes the entire plant more resistant to secondary infection. The response to this infection is localized at first and subsequently spreads systemically and develops in untreated parts of the tree. The effect is long lasting and often confers broad-based resistance to different pathogens. SAR acts non-specifically throughout the plant and reduces the severity of disease. Host resistance to

Dutch elm disease is associated with the ability of elms to localize infection by limiting the movement of the pathogen in the vascular system.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

The microbial pest control agent (MPCA) in *Verticillium* isolate WCS850 and Dutch Trig is a spontaneous hyaline (white) variant of *Verticillium albo-atrum*. The classical approach of using morphological characteristics in distinguishing this MPCA from a closely-related species, *Verticillium dahliae*, is inadequate because this hyaline strain lacks an important species-determinant, i.e. the presence of microsclerotia or dauermycelium. Instead, DNA-based methods such as whole genome analysis by amplified fragment length polymorphism and single gene analysis of rDNA-ITS (internal transcribed spacers) by species-specific PCR primers are required. In addition, *Verticillium albo-atrum* does not grow on Czapek Dox agar at 30°C whereas *Verticillium dahliae* still grows at 30°C, albeit at a reduced rate. Also, *Verticillium albo-atrum* is incompatible in paired experiments with four reference isolates of *Verticillium dahliae* representing all four vegetative compatibility groups of this species. These methods, however, do not distinguish strain WCS850 from other hyaline strains of this species.

2.2 Methods for Establishment of Purity of Seed Stock

The production stock of *Verticillium albo-atrum* strain WCS850 is maintained at the Central Bureau for Fungi (Centraalbureau voor Schimmelcultures or CBS), an internationally recognized culture collection located in the Netherlands. The methods and tests employed by CBS to ensure the integrity and purity of the MPCA are available through the European Union's Common Access to Biological Resources and Information (CABRI) consortium. These methods were developed for CABRI by the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH or DSMZ), CBS and the Belgian Co-Ordinated Collections of Micro-Organisms. In short, cultures are maintained and preserved by cryopreservation (in or above liquid nitrogen, or at -70°C) or by freeze-drying (shelf freeze-drying, spin freeze-drying, liquid freeze-drying or vacuum freeze-drying). In rare cases, microorganisms that cannot be preserved by one of the metabolically inactive methods above will be preserved as metabolically active cultures by periodic transfer on agar/liquid culture or as agar cultures under mineral oil. Irrespective of the applied preservation method, all preserved stocks are monitored for viability of the strains and the number of available ampoules, capillary tubes or straws. Cultures are also tested for purity and, when appropriate, the identity of the strain. Testing is performed immediately after each culture is preserved.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The guarantee of Dutch Trig is based on the number of viable spores per volume of product. The total spore count is determined by means of a hemacytometer. In addition, the germination rate of each batch must be greater or equal to 80%.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

No known toxins are produced by *Verticillium albo-atrum* despite intensive research on *Verticillium* spp. The mode of action of the MPCA does not involve any direct toxic interactions between the MPCA and the target pest, *Ophiostoma novo-ulmi*. Furthermore, the results of supporting mammalian toxicity and pathogenicity data do not indicate any toxic or pathogenic effects.

Based on the above information and the proposed use of Dutch Trig in elm trees, the establishment of a maximum residue limit (MRL) is not required for *Verticillium albo-atrum* strain WCS850 under section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, section B.15.002 of the Food and Drugs Regulations. As a result, no methods to determine and quantify the MPCA and relevant metabolites are required.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality control procedures used to limit contaminating microorganisms during manufacture of *Verticillium* isolate WCS850 and Dutch Trig are acceptable. Batches showing microbial contamination are discarded.

2.6 Methods to Show Absence of Any Human and Mammalian Pathogens

As noted in Section 2.5, quality control procedures are used to limit microbial contamination in *Verticillium albo-atrum* strain WCS850 and Dutch Trig. These procedures include contamination checks to detect contaminating microbial contamination.

Acceptable microbial contaminant analysis data were submitted for five batches of Dutch Trig.

2.7 Methods to Determine Storage Stability, Shelf-life of the Microorganism

The viability of *Verticillium albo-atrum* strain WCS850 in Dutch Trig was evaluated over a 10-week period at 4°C. The submitted storage stability data support a storage period for Dutch Trig of 10 weeks at 4°C.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

The PMRA conducted a detailed review of the toxicological database for *Verticillium albo-atrum* strain WCS850 in *Verticillium* isolate WCS850 (technical grade active ingredient) and Dutch Trig. The database is considered complete, consisting of published literature, scientific rationales and a laboratory animal (in vivo) infectivity study (intraperitoneal infectivity) that was carried out in accordance with currently accepted international testing protocols and good laboratory practices. Waiver requests were deemed acceptable to address all remaining health data requirements (i.e. acute oral toxicity/infectivity, acute pulmonary toxicity/infectivity, acute dermal toxicity and dermal irritation). The scientific quality of the information and data is high, and the database is considered sufficient to characterize the infectivity of this pest control agent and product.

In an acute intraperitoneal infectivity study, a group of 8-week old Sprague Dawley rats (3 per sex) were injected with Dutch Trig (containing approximately 9.1×10^6 CFU/ mL) at a dose of approximately 1.7×10^7 CFU per animal. Animals were then observed for up to 14 days. An untreated control group (2 per sex) was also included in the study protocol. In this study, *Verticillium albo-atrum* strain WCS850 was not pathogenic to rats. No adverse effects were observed in any of the test animals and no gross findings were reported at necropsy. This intraperitoneal infectivity study is classified as acceptable and satisfies the guideline requirement for an intraperitoneal infectivity study in the rat.

Requests to waive all other health requirements were accepted based on the biological properties of the MPCA, the nature of the end-use formulation and the proposed use pattern of Dutch Trig.

Verticillium spp. are cosmopolitan fungi found in soil and on crop debris left in the soil after harvest. These fungi have a very wide host range on plants, and have been studied in depth because of their important economic impact on agricultural crops. *Verticillium albo-atrum* occurs generally amongst many food crops and plants including potato and tomato. Given these fungi are so common, both people and animals have likely come in contact with them when handling earth, plants, crop residues or dead organic material. Despite the extensive research and the likely exposure, there are only few reports of potential adverse effects in published scientific literature. Only two references to human or animal pathogenicity of *Verticillium* can be found in published scientific literature. In one case, peritonitis was reported in a patient undergoing peritoneal dialysis while in the other case, fungal peritonitis was preceded by at least one episode of bacterial peritonitis. The ability of *Verticillium albo-atrum* strain WCS850 to grow and colonize warm-blooded mammals is doubtful as it does not grow at temperatures above 30°C. In addition, *Verticillium* species are not known to produce toxins or toxic metabolic by-products. A search of published scientific literature has yielded only one obscure citation which reported the production of thiourea by *Verticillium albo-atrum*. This report was published in 1940, and no other such reports were found in more recent scientific publications. Also, the MPCA is not related to any known toxigenic human pathogen.

Verticillium albo-atrum is not known to express any specific sensitizing compounds. However, one report of an allergenic response was reported in combination with the presence of *Verticillium albo-atrum* in greenhouse workers. This publication, however, did not provide any evidence to support *Verticillium albo-atrum* being the causal organism. During spore sampling, *Penicillium*, *Cladosporium* and *Cephalosporium* were the predominant fungi found in the greenhouse. *Verticillium* was also found in lower concentrations as well as *Aspergillus fumigatus*. No other reports of allergic responses to *Verticillium* were found in published scientific literature. The applicant also noted that none of the employees involved in the production of Dutch Trig have ever showed any symptoms of sensitization or allergic responses since production began in 1992. In addition, none of the commercial arborists have ever displayed any dermal or allergic effects from applying Dutch Trig to over 450 000 elms in the Netherlands. The PMRA, however, considers all MPCAs to be potential sensitizers by default.

Verticillium albo-atrum strain WCS850 in Dutch Trig is used to induce systemic resistance in elm trees to protect them against Dutch elm disease. This mode of action does not involve any pathogenicity, toxicity or any other direct interaction between the target pest, *Ophiostoma novo-ulmi*, and the MPCA. Instead, the mode of action of *Verticillium albo-atrum* strain WCS850 relies completely on the exact level of pathogenicity that it has on elm trees. The key to the success of the MPCA is that it is just pathogenic enough to induce a natural resistance in the injected elm, but has lost so much of its pathogenicity that it is no longer capable of successfully infecting an elm with *Verticillium* wilt. To be efficacious, Dutch Trig must be injected in elm trees by trained personnel. The product is shipped in sealed virtually unbreakable vials and it is injected using a closed injection system. This closed injection system prevents spills and data show the injected MPCA is contained in the annual growth ring of treated trees. As a consequence, negligible occupational and bystander exposure are expected from the proposed use of *Verticillium albo-atrum* strain WCS850.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the test substance, and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity tests.

Within the available scientific literature, there are no reports that suggest *Verticillium albo-atrum* strain WCS850 has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the spores of *Verticillium albo-atrum* strain WCS850. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *Verticillium albo-atrum* strain WCS850.

3.2 Occupational/Bystander Exposure and Risk Assessment

3.2.1 Occupational

Negligible worker exposure to *Verticillium albo-atrum* strain WCS850 is expected when Dutch Trig is applied according to the label instructions. The end-use product is shipped in sealed virtually unbreakable vials and it is injected into elm trees by trained personnel using a closed injection system. This closed injection system greatly reduces all potential routes of exposure during application. Worker exposure to *Verticillium albo-atrum* strain WCS850, however, may occur via the dermal route during routine maintenance of the injection equipment. Given unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. This MPCA has not been identified as a wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Exposure to workers during routine maintenance is expected to be low. To prevent exposure during application and during maintenance of the injection equipment, the label will specify that anyone applying or maintaining injection equipment must wear waterproof gloves, long-sleeved shirts, long pants, and shoes plus socks.

The PMRA assumes that all microorganisms contain substances that can elicit allergic reactions upon repeated exposure to any microbial organism. For *Verticillium albo-atrum* strain WCS850, however, no additional label restrictions or risk mitigation measures are necessary to protect workers. Repeated exposures to this MPCA are not likely to occur since the product is injected into elm trees by trained personnel using a closed injection system.

3.2.2 Bystander

Overall the PMRA does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for the spores of *Verticillium albo-atrum* strain WCS850 and the negligible exposure to bystanders from the use of Dutch Trig.

The label does allow applications to trees located in residential and recreational areas; however, *Verticillium albo-atrum* strain WCS850 is contained within a growth ring of the treated elm trees. Data were submitted which showed that, following injection, *Verticillium albo-atrum* strain WCS850 can only be re-isolated from the wood of the treated elm at the site of injection, and only up until two weeks after treatment. After treatment, the treated elm rapidly identifies the infection with *Verticillium albo-atrum* strain WCS850 in its vascular system and uses its natural defense mechanisms to rid its vascular system of this infection. Non-occupational exposure and risks to adults, infants and children are low. Furthermore, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

Dutch Trig is to be injected in elm trees to prevent Dutch elm disease. Negligible to no risk is expected for the general population, including infants and children, or animals because there are no direct applications of Dutch Trig to food or feed crops. Therefore, there is no concern for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.3.2 Drinking Water

No risks are expected from exposure to this microorganism via drinking water because exposure will be negligible. The label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats by cleaning of equipment or disposal of wastes. Furthermore, runoff from treated trees is not expected and therefore it is unlikely that *Verticillium albo-atrum* strain WCS850 will enter aquatic environments. Moreover, the MPCA is not expected to proliferate in aquatic habitats and percolation through soil and municipal treatment of drinking water would reduce the possibility of significant transfer of *Verticillium albo-atrum* strain WCS850 or its residues to drinking water. Therefore, potential exposure to *Verticillium albo-atrum* strain WCS850 in surface and drinking water is negligible.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculation of acute reference doses and acceptable daily intakes are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e. no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that the spores of *Verticillium albo-atrum* strain WCS850 are of low toxicity, are not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCA than the general population. Thus there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intraspecies and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects from prenatal or postnatal exposures, and cumulative effects on infants and children of the MPCA and other registered micro-organisms that have a common mechanism of toxicity, do not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *Verticillium albo-atrum* strain WCS850 to human health.

3.4 Maximum Residue Limits

As there are no applications to food, the establishment of an MRL is not required for *Verticillium albo-atrum* strain WCS850 under section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, section B.15.002 of the Food and Drugs Regulations. The *Food and Drugs Act* prohibits the sale of adulterated food, that is, food containing a pesticide residue that exceeds the established MRL. Pesticide MRLs are established for *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act*. Each MRL value defines the maximum concentration in parts per million (ppm) of a pesticide allowed in/on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

3.5 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information, there is reasonable certainty no harm will result from aggregate exposure of residues of *Verticillium albo-atrum* strain WCS850 to the general Canadian population, including infants and children, when the microbial pest control product is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Given Dutch Trig is to be injected in elm trees, dermal and inhalation exposure to the general public will be very low. Furthermore, few adverse effects from exposure to natural populations of *Verticillium* spp. in the environment have been reported. Even if there is an increase in exposure to this microorganism from the use of Dutch Trig there should not be any increase in potential human health risk.

3.6 Cumulative Effects

The PMRA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Besides naturally occurring strains of *Verticillium albo-atrum* in the environment, the PMRA is not aware of any other microorganisms or other substances that share a common mechanism of toxicity with this active ingredient. No cumulative effects are anticipated if the residues of *Verticillium albo-atrum* strain WCS850 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Environmental fate testing is intended to demonstrate whether a MPCA is capable of surviving or replicating in the environment to which it is applied, and could provide an indication of which non-target organisms may be exposed to the MPCA as well as provide an indication of the extent

of exposure. Environmental fate data (Tier II/III) are not normally required at Tier I and are only triggered if significant toxicological effects in non-target organisms are noted in Tier I testing.

For *Verticillium albo-atrum* strain WCS850, data and information were submitted on the biological and ecological characteristics of parent (pigmented) *Verticillium albo-atrum* as well as results of re-isolation attempts from treated elm trees.

Verticillium albo-atrum is a plant pathogen that can infect many different plant species causing *Verticillium* wilt. This disease is considered a cool-weather disease because growth of *Verticillium albo-atrum* is optimal at approximately 21°C and is halted at temperatures below 10°C and at 30°C and higher. *Verticillium albo-atrum* strain WCS850 is a natural hyaline mutant of *Verticillium albo-atrum*. It differs significantly from the wild type strains because it has lost the ability to produce resting structures. With the loss of its capacity to produce resting structures, *Verticillium albo-atrum* strain WCS850 has lost its ability to persist in soil and crop residues. Hyaline strains of *Verticillium albo-atrum* are also generally regarded as less pathogenic than wild type strains and the mutation is irreversible.

Studies were conducted in the Netherlands and in the United States to assess the location and life-span of *Verticillium albo-atrum* strain WCS850 in elm trees following treatment with Dutch Trig. These studies concluded that *Verticillium albo-atrum* strain WCS850 could be re-isolated from wood samples obtained approximately 5–10 cm (2–4 inches) above the site of injection, but only up to two weeks following injection. After removing the bark, a light grayish-brown discolouration of the vessels indicated the presence of *Verticillium*. At the end of the growing season this discolouration could no longer be found. The studies also concluded that spores were not transported throughout the entire crown by the inoculated xylem vessels because upward transport is halted when the xylem vessel is pierced upon injection at least until the damaged vessels are restored. After treatment, the elm tree apparently identifies the infection in its vascular system and uses its natural defence mechanisms to rid its vascular system of this infection. The injected *Verticillium albo-atrum* strain WCS850 is contained (compartmentalized) within one growth-ring (one growing season) in the elm.

If the MPCA would ever be accidentally released into a suitable environment, strain WCS850 would not likely survive because it has lost the ability to produce resting mycelia necessary for survival. Also, *Verticillium albo-atrum* strain WCS850 would not likely be able to compete with wild type strains of *Verticillium albo-atrum*.

The ability of *Verticillium albo-atrum* to grow and establish itself in water is not known; however, this species is not generally considered a water-borne fungus.

4.2 Effects on Non-Target Species

4.2.1 Effects on Terrestrial Organisms

A study was submitted to address the effects of *Verticillium albo-atrum* strain WCS850 to terrestrial plants. In this study, the phytopathogenic effects of *Verticillium albo-atrum* strain WCS850 were evaluated over a period of 16 weeks after Dutch Trig was injected into various tree species, including *Acer macrophyllum*, *Acer saccharum*, *Aesculus hippocastanum*, *Betula papyrifera*, *Castanea* spp., *Cornus nuttallii*, *Crataegus monogyna*, *Fagus* spp., *Magnolia* spp., *Malus* spp., *Prunus lusitanica*, *Prunus serrata*, *Quercus rubra*, *Rhododendron* spp., *Sorbus aucuparia*, *Ulmus carpinifolia*, *Ulmus laevis*, *Ulmus pumila*, and *Viburnum* spp. Trees were injected in mid-May 2001 in accordance with a USEPA-issued experimental use permit; however, few details were reported in the study. Untreated trees, when available, were selected and served as negative controls. No phytopathogenic (wilting) or phytotoxic effects were noted throughout the study period even though *Verticillium*-susceptible species (*A. macrophyllum*) were included in the study. The study was judged supplemental due to missing information. A replacement study, however, will not be required because non-target plant exposure is expected to be negligible based on the proposed use of Dutch Trig (discussed in greater detail below).

In addition to the above non-target terrestrial plant study, several scientific rationales were submitted to waive testing on birds, mammals, arthropods, non-arthropod invertebrates and microorganisms based on the biological and ecological properties of the MPCA, the limited potential for exposure from the use of Dutch Trig and the nature of the end-use formulation.

Verticillium albo-atrum is a ubiquitous soil-borne fungus with a wide geographical distribution, predominantly in temperate regions. *Verticillium albo-atrum* is known to have a northern distribution in North America and Europe, preferring temperate climates. It is known as a strictly root inhibiting fungus, able to cause wilting symptoms in a wide array of mostly vegetable crops and some susceptible tree species in specific circumstances. As a result, this species has been extensively studied. Despite this extensive research and the organism's ubiquitous nature, no adverse effects to birds, wild mammals, arthropods, non-arthropod invertebrates and microorganisms are attributed to *Verticillium albo-atrum* in published scientific literature.

The original parent culture, from which *Verticillium albo-atrum* strain WCS850 was obtained, was isolated from a diseased potato field in the Netherlands. This strain of *Verticillium albo-atrum* is therefore regarded as indigenous to Northern Europe. As previously noted, *Verticillium albo-atrum* strain WCS850 is a natural hyaline mutant of the wild type *Verticillium albo-atrum*. This strain has lost its ability to produce resting structures and is generally regarded as less pathogenic than the wild type strain. As a result, *Verticillium albo-atrum* strain WCS850 has likely lost its ability to persist in its natural environment and to compete with wild type strains of *Verticillium albo-atrum*. In addition, strain WCS850 does not grow at temperatures of 30°C or higher.

The proposed use of *Verticillium albo-atrum* strain WCS850 is expected to yield negligible environmental exposure. Dutch Trig is directly injected by trained personnel into the xylem tissues of the healthy elm trees in spring, just after bud break. These injections are made with a closed injection system that limits environmental exposure by preventing spills of the formulated product. Following treatment with Dutch Trig, *Verticillium albo-atrum* strain WCS850 is quickly identified and eradicated by the treated elm tree through the use of its natural defence mechanism. The injected MPCA is thus contained (compartmentalized) within one growth-ring in the elm and is not released into the surrounding environment. The re-isolation of *Verticillium albo-atrum* strain WCS850 from the treated elms was demonstrated in a study, but this re-isolation was only possible from wood obtained at the site of injection, and only up until two weeks after treatment. The extent of potential environmental exposure is also further reduced by the packaging of the product, i.e. Dutch Trig is transported and shipped in virtually unbreakable vials with a maximum content of 40 mL. Even if the MPCA would ever be accidentally released into the environment, the impact, if any, would be temporary because *Verticillium albo-atrum* strain WCS850 has virtually no chances of survival because it can't produce the black resting structures that are necessary for long-term survival. *Verticillium albo-atrum* strain WCS850 would also have to cope with competition with wild type strains of *Verticillium albo-atrum*, which are more pathogenic to plants.

The purpose of the Dutch Trig injections is to induce a natural phenomenon in trees known as systemic acquired resistance, a mechanism used by plants to defend themselves from various diseases. This treatment is analogous to human vaccinations. This type of resistance can be induced by inoculating or injecting plants with specific microorganisms which are just sufficiently pathogenic to initiate the tree's defense mechanisms, but are no longer capable of establishing disease. For Dutch Trig, the intent is to reinforce the elm's own natural defence mechanism against a possible Dutch elm disease infection later in the growing season. The mode of action of *Verticillium albo-atrum* strain WCS850 does not rely on any toxin or toxic metabolite interaction between MPCA and targeted Dutch elm disease organism, *Ophiostoma novo-ulmi*. Moreover, no report of *Verticillium albo-atrum* producing any type of toxin or toxic metabolite was found in published scientific.

Based on all the available data and information on the effects of *Verticillium albo-atrum* strain WCS850 to non-target terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, arthropods, non-arthropod invertebrates, microorganisms and plants from the use of Dutch Trig.

4.2.2 Effects on Aquatic Organisms

No studies were submitted to address the hazards of *Verticillium albo-atrum* strain WCS850 to non-target aquatic organisms. Instead, scientific rationales were submitted to waive testing on fish, aquatic arthropods, aquatic arthropod invertebrates and aquatic plants. These rationales were also based on the biological and ecological properties of the MPCA, the limited potential for exposure from the use of Dutch Trig, and the nature of the end-use formulation.

No adverse effect to aquatic organisms or report of any kind of toxic metabolite was found in the published scientific literature. The ability of *Verticillium albo-atrum* to grow and establish itself in water is not known; however, this species is not generally considered a water-borne fungus. As noted in Section 4.2.1, negligible environmental exposure, including aquatic exposure, are expected from the proposed use of Dutch Trig in elm trees. Also, even if *Verticillium albo-atrum* strain WCS850 would ever be accidentally released into the environment, the impact, if any, would be temporary because it has largely lost its ability to persist in the environment and to compete with wild type strains of *Verticillium albo-atrum*.

Based on all the available data and information on the effects of *Verticillium albo-atrum* strain WCS850 to aquatic organisms, there is reasonable certainty that no harm will be caused to non-target aquatic organisms from the use of Dutch Trig. As a precaution, standard label statements will prohibit handlers from contaminating aquatic habitats during application and routine maintenance of the injection system.

5.0 Value

5.1 Effectiveness Against Pests

5.1.1 Acceptable Efficacy Claims

Greenhouse trials were conducted at the University of Wisconsin in 2001. Two year old cloned ramets (grown in a greenhouse) with differing levels of natural resistance were tested. Treatment with Dutch Trig prior to Dutch elm disease infection showed less discoloration under the bark, less discoloration on the cross sections and no wilting in the leaves when compared to the Dutch elm disease control treatments. There seems to be positive correlation between the tree's natural level of resistance and the induced level of protection after treatment. Therefore, the clones with a high level of natural resistance benefit most from the Dutch Trig treatment.

Field trials were conducted in the Netherlands between 1990 and 2000. Mature trees of the same species, cultivar, size and location were included in the study. Application timing varied to determine the optimum timing of treatment, duration of treatment effects and activity of Dutch Trig against multiple infections in a single season (to simulate multiple beetle infestations). Dutch Trig was found to reduce disease incidence throughout the season and activity was independent of the timing or number of Dutch elm disease infections. The effects of the treatment were less effective in the second year. Therefore, applications should be made each spring. Applications made too early in the season failed to protect against infection, therefore treatment must occur when the trees start to leaf out. Dutch Trig treatment was effective against both Dutch elm disease pathogens (*Ophiostoma ulmi*, *Ophiostoma novo-ulmi*).

5.2 Phytotoxicity to Host/Non-Target Plants

Phytotoxicity trials were conducted at the University of Washington in spring 2001. Three different elm species were tested as well as a variety of mixed tree species (including three additional elm species). Trees were monitored for signs of phytopathogenic effects throughout the summer. No wilting symptoms occurred on any of the treated tree species, even among *Verticillium* susceptible trees (for example, maple).

Two incidents of wilting in suckers were reported in the United States (Denver in 1999 on 17 trees and Chicago in 2001 on two trees) during commercial applications. No wilt was observed in the canopy of the trees. In both cases, wilting occurred 14 days after application with Dutch Trig. Refoliation occurred two weeks later. The cause of the wilting is unknown and only happened once in each city.

5.3 Economics

Denver Parks and Recreation, Forestry Division have implemented a three-pronged Dutch elm disease program consisting of a sanitation and plant health care program, experimentation with fungicides and implementation of immune system augmentation. Fewer new infections were observed over six years of treatment with Dutch Trig. The efforts of the Forestry Division to minimize elm losses due to Dutch elm disease have led to a financial benefit. By adding the Dutch Trig prevention method to a comprehensive management program, the costs for sanitation (removal and destruction of infected trees) can be greatly reduced. Beyond the expense of removing infected trees, there is a value loss for each tree removed due to the loss of aesthetic, environmental, psychological and economic values that urban trees provide to the people that live around them.

5.4 Sustainability

5.4.1 Survey of Alternatives

Thiabendazole and carbendazim are Group 1 fungicides used in both curative and preventative application through stem or root injections, often used in conjunction with eradication pruning to remove infected parts of the tree. Basal spraying of tree trunks with the Group 1B insecticide chloropyrifos is used to control overwintering bark beetles. The soil fumigant metam (Group M) is used to isolate healthy trees from infected trees by disrupting root graft infections. A summary of alternative chemical controls can be found in Appendix I, Table 3.

5.4.2 Compatibility with Current Management Practices Including Integrated Pest Management

Current methods employed to control Dutch elm disease include inventory and monitoring, sanitation, fungicide injections and the use of insecticides to control the beetle responsible for vectoring the pathogen. The use of Dutch Trig will compliment a comprehensive management program with annual treatment by reducing the incidence of the disease to as low as 1% per year. Thus the costs for the sanitation part of a control program will eventually be reduced because fewer trees will need to be removed. Furthermore, Dutch Trig is unique in that it is the only method that acts on the natural defences of the tree rather than acting on the fungus that causes the disease or its insect vectors. For this reason, Dutch Trig compliments an existing program by enhancing the resistance of the trees while the other methods seek to reduce the likelihood that the trees can be infected by the disease. It should be noted that Dutch Trig cannot be used in combination with fungicides, as these products will kill the *Verticillium* active ingredient.

5.4.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

SAR is the activation of plant defences in uninfected parts of the plant which makes the entire plant more resistant to secondary infection. The effect is long lasting and often confers broad-based resistance to different pathogens. Development of pathogen resistance to plant defences has not been documented. Although the possibility of resistance developing with sole reliance on this product is not impossible, it would involve complex mechanisms within the pathogen population.

5.4.4 Contribution to Risk Reduction and Sustainability

Treatment with Dutch Trig is performed using a self contained application system and requires no mixing or diluting, thus reducing exposure to the applicator. In addition, there are no adverse effects to non-target organisms including the beetle vector or other tree species.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The management of toxic substances is guided by the federal government's Toxic Substances Management Policy (TSMP), which puts forward a preventive and precautionary approach to deal with substances that enter the environment and could harm the environment or human health. The policy provides decision makers with direction and sets out a science-based management framework to ensure that federal programs are consistent with its objectives. One of the key management objectives is virtual elimination from the environment of toxic substances that result predominantly from human activity and that are persistent and bioaccumulative. These substances are referred to in the policy as Track 1 substances.

In its review, the PMRA took into account the federal Toxic Substances Management Policy and followed its Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*. Substances associated with its use were also considered, including microcontaminants in the technical product, *Verticillium* isolate WCS850, and formulants in the end-use product, Dutch Trig. The PMRA has reached the following conclusions.

Verticillium isolate WCS850 does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. There are also no formulants, contaminants or impurities present in the end-use product that would meet the TSMF Track 1 criteria. Therefore, the use of *Verticillium* isolate WCS850 and Dutch Trig is not expected to result in the entry of Track 1 substances into the environment.

6.2 Formulants and Contaminants of Health or Environmental Concern

The technical grade active ingredient, *Verticillium* isolate WCS850, does not contain any contaminants of health or environmental concern identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641–2643: List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern.

The end-use product, Dutch Trig, does not contain any contaminants of health or environmental concern identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641–2643: List of Pest Control Product Formulants of Health or Environmental Concern.

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for *Verticillium albo-atrum* strain WCS850 and Dutch Trig were judged to be adequate to assess their potential human health and environmental risks. The technical grade active ingredient was fully characterized and the specifications were supported by the analyses of a sufficient number of batches. Storage stability data were sufficient to support a shelf life of 10 weeks at 4°C.

7.2 Human Health and Safety

The acute infectivity study and other relevant information submitted in support of *Verticillium albo-atrum* strain WCS850 were determined to be sufficiently complete to permit a decision on registration. Spores of *Verticillium albo-atrum* strain WCS850 were not pathogenic or infective in the rat via intraperitoneal injection exposure route. Requests to waive all other health requirements were accepted based on the biological properties of the MPCA, the nature of the end-use formulation and the proposed use pattern of Dutch Trig.

Although *Verticillium albo-atrum* is not known to express any specific sensitizing compounds, the PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, and exposure to allergens, including *Verticillium albo-atrum* strain WCS850, may cause allergies following repeated exposures.

When handled according to the label instructions, negligible worker exposure to *Verticillium albo-atrum* strain WCS850 is expected. However, worker exposure to *Verticillium albo-atrum* strain WCS850, may occur via the dermal route during routine maintenance of the injection equipment. Exposure to workers during routine maintenance is expected to be low. To prevent exposure during application and during maintenance of the injection equipment, the label will specify that anyone applying or maintaining injection equipment must wear waterproof gloves, long-sleeved shirts, long pants, and shoes plus socks.

While *Verticillium albo-atrum* strain WCS850 has the potential to be a sensitizing agent, no additional label restrictions or risk mitigation measures are necessary to protect workers. Repeated exposures to this MPCA are not likely to occur since the product is injected into elm trees by trained personnel using a closed injection system.

The label does allow applications to elm trees in residential or recreational areas, but non-occupational exposure and risks to adults, infants and children are low. As the injected material is contained within the treated trees, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

Dutch Trig will only be injected into elm trees. Negligible to no risk is expected for the general population, including infants and children, or animals because there are no direct applications of Dutch Trig to food or feed crops. Therefore, there is no concern for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

7.3 Environmental Risk

A non-target study on various deciduous tree species, scientific rationales and supporting published scientific literature submitted in support of *Verticillium albo-atrum* strain WCS850 were determined to be sufficiently complete to permit a decision on registration.

Data and waiver rationales were submitted to address the hazards of *Verticillium albo-atrum* strain WCS850 to non-target organisms. These data and other published information showed that the use of Dutch Trig containing *Verticillium albo-atrum* strain WCS850 does not pose a significant risk to birds, mammals, arthropods (including honeybees), fish, non-arthropod invertebrates, plants, or algae.

No additional studies are required to address the environmental fate and behaviour of *Verticillium albo-atrum* strain WCS850. Environmental fate data (Tier II/III) are not normally required in the absence of significant toxicological effects in non-target organisms in Tier I testing. Environmental exposure to *Verticillium albo-atrum* strain WCS850 is expected to be negligible and the potential for *Verticillium albo-atrum* strain WCS850 to proliferate in the environment is extremely low due to its inability to compete and produce resting structures.

As a precaution, standard label statements will prohibit handlers from contaminating aquatic habitats during application and routine maintenance of the injection system.

7.4 Value

Field data showed consistent control of Dutch elm disease with properly timed yearly applications at the proposed rate or lower. No phytotoxicity was observed in the trials, even when twice the rate was used. Laboratory data indicated that no phytopathogenic effects occurred when Dutch Trig is applied to various species of elm trees or other tree species. Dutch Trig has been used successfully in the United States on American elm trees (*Ulmus americana*). It provides year long protection against multiple infection events, but yearly applications are required. The timing of application is very important to providing optimum efficacy. It is important to apply Dutch Trig as soon as leaves have started to transpire after foliage has been established to allow *Verticillium albo-atrum* strain WCS850 to spread within the tree as early in the season as possible.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Verticillium* isolate WCS850 and Dutch Trig, containing the microbial pest control agent *Verticillium albo-atrum* strain WCS850 to prevent Dutch elm disease in uninfected elm trees.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

°C	degree(s) Celsius
CABRI	European Union's Common Access to Biological Resources and Information Consortium
CBS	Central Bureau for Fungi (Centraalbureau voor Schimmelcultures)
CFU	colony forming unit
cm	centimetres
DNA	deoxyribonucleic acid
GLP	good laboratory practices
Kg	kilogram(s)
L	litre
mL	millilitre
MPCA	microbial pest control agent
MRL	maximum residue limit
PCR	polymerase chain reaction
PMRA	Pest Management Regulatory Agency
ppm	parts per million
SAR	system acquired resistance
TSMP	Toxic Substances Management Policy
USEPA	United States Environmental Protection Agency

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *Verticillium albo-atrum* strain WCS850

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of <i>Verticillium</i> Isolate WCS850 (technical grade active ingredient)				
Acute Oral Toxicity and Infectivity	A data waiver was requested based on the biological properties of <i>Verticillium albo-atrum</i> strain WCS850 and the proposed use pattern of Dutch Trig. The MPCA does not grow at temperatures above 30°C and few adverse effects to <i>Verticillium</i> spp. were reported in published scientific literature despite intensive research. Furthermore, negligible exposure is expected from the proposed use of Dutch Trig to prevent Dutch elm disease in elm trees.	WAIVER ACCEPTED		PMRA 1583116 1583124 1583131 1583132 1583173 1583179 1583180 1583181 1583182 1583183 1583184
Acute Pulmonary Toxicity and Infectivity	A data waiver was requested based on the biological properties of <i>Verticillium albo-atrum</i> strain WCS850 and the proposed use pattern of Dutch Trig. The MPCA does not grow at temperatures above 30°C and few adverse effects to <i>Verticillium</i> spp. were reported in published scientific literature despite intensive research. Furthermore, negligible exposure is expected from the proposed use of Dutch Trig to prevent Dutch elm disease in elm trees.	WAIVER ACCEPTED		PMRA 1583116 1583124 1583131 1583132 1583173 1583179 1583180 1583181 1583182 1583183 1583184
Intraperitoneal Injection Infectivity	Rat–Sprague Dawley 3/sex dosed with 2 mL Dutch Trig containing <i>Verticillium albo-atrum</i> strain WCS850 at $\sim 9.1 \times 10^6$ CFU/mL 2/sex untreated control	- no observable effects	- no mortalities - no significant toxicity - With the exception of one untreated female on Day 8, all animals gained body weight throughout the study period. The observed weight loss was not considered to be significant. - no abnormal findings at necropsy NOT PATHOGENIC	PMRA 1583133

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Dermal Toxicity and Irritation		<p>A data waiver was requested based on the biological properties of <i>Verticillium albo-atrum</i> strain WCS850 and the proposed use pattern of Dutch Trig. The MPCA does not grow at temperatures above 30°C and few adverse effects to <i>Verticillium</i> spp. were reported in published scientific literature despite intensive research. Furthermore, negligible exposure is expected from the proposed use of Dutch Trig to prevent Dutch elm disease in elm trees.</p> <p style="text-align: center;">WAIVER ACCEPTED</p>		PMRA 1583116 1583124 1583131 1583132 1583173 1583179 1583180 1583181 1583182 1583183 1583184
Acute Toxicity of Dutch Trig (end-use product)				
All Acute Toxicity Testing		<p>A data waiver was requested based on the negligible exposure that is expected from the proposed use of Dutch Trig, and the nature and concentration of the formulation ingredients present in the end-use product.</p> <p style="text-align: center;">WAIVER ACCEPTED</p>		PMRA 1583116 1583124 1583131 1583132 1583173 1583179 1583180 1583181 1583182 1583183 1583184

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Organisms				
Vertebrates				
Birds	Oral		A waiver was submitted based on the biological and ecological properties of the MPCA, the limited potential for exposure from the use of Dutch Trig, and the nature of the end-use formulation. The MPCA does not grow at temperatures above 30°C and no adverse effects to <i>Verticillium albo-atrum</i> were reported in published scientific literature. Also, negligible exposure is expected from the proposed use of Dutch Trig to prevent Dutch elm disease in elm trees.	PMRA
	Pulmonary			
WAIVER ACCEPTED				
Wild Mammals	A waiver was submitted based on the biological and ecological properties of the MPCA, the limited potential for exposure from the use of Dutch Trig, and the nature of the end-use formulation. The MPCA does not grow at temperatures above 30°C and no adverse effects to <i>Verticillium albo-atrum</i> were reported in published scientific literature. Also, negligible exposure is expected from the proposed use of Dutch Trig to prevent Dutch elm disease in elm trees.			PMRA
WAIVER ACCEPTED				
Invertebrates				
Arthropods				
Terrestrial Arthropods	A waiver was submitted based on the biological and ecological properties of the MPCA, the limited potential for exposure from the use of Dutch Trig, and the nature of the end-use formulation. No adverse effects to <i>Verticillium albo-atrum</i> were reported in published scientific literature. Also, negligible exposure is expected from the proposed use of Dutch Trig to prevent Dutch elm disease in elm trees.			PMRA
WAIVER ACCEPTED				
Non-arthropods				
Terrestrial Non-Arthropod Invertebrates	A waiver was submitted based on the biological and ecological properties of the MPCA, the limited potential for exposure from the use of Dutch Trig, and the nature of the end-use formulation. No adverse effects to <i>Verticillium albo-atrum</i> were reported in published scientific literature. Also, negligible exposure is expected from the proposed use of Dutch Trig to prevent Dutch elm disease in elm trees.			PMRA
WAIVER ACCEPTED				

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Plants				
Plants	Injection into various tree species, <i>Acer macrophyllum</i> , <i>Acer saccharum</i> , <i>Aesculus hippocastanum</i> , <i>Betula papyrifera</i> , <i>Castanea</i> spp., <i>Cornus nuttallii</i> , <i>Crataegus monogyna</i> , <i>Fagus</i> spp., <i>Magnolia</i> spp., <i>Malus</i> spp., <i>Prunus lusitanica</i> , <i>Prunus serrata</i> , <i>Quercus rubra</i> , <i>Rhododendron</i> spp., <i>Sorbus aucuparia</i> , <i>Ulmus carpinifolia</i> , <i>Ulmus leavis</i> , <i>Ulmus pumila</i> , and <i>Viburnum</i> spp.	Dutch Trig containing spores of <i>Verticillium albo-atrum</i> strain WCS850 Nominal: 1×10^7 CFU/mL Observation period: 16 weeks	No phytopathogenic (wilt) or phytotoxic effects were observed throughout the growing season. No replacement study is required due to negligible environmental exposure. SUPPLEMENTAL	PMRA 1583126
Aquatic Organisms				
Vertebrates				
Fish	A waiver was submitted based on the biological and ecological properties of the MPCA, the limited potential for exposure from the use of Dutch Trig, and the nature of the end-use formulation. No adverse effects to <i>Verticillium albo-atrum</i> were reported in published scientific literature. Also, negligible exposure is expected from the proposed use of Dutch Trig to prevent Dutch elm disease in elm trees.			PMRA 1583112 1583114 1583116 1583173 1583179 1583180 WAIVER ACCEPTED 1583181 1583182 1583183 1583184

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Invertebrates				
Arthropods				
Aquatic Arthropods and Non-Arthropod Invertebrates	A waiver was submitted based on the biological and ecological properties of the MPCA, the limited potential for exposure from the use of Dutch Trig, and the nature of the end-use formulation. No adverse effects to <i>Verticillium albo-atrum</i> were reported in published scientific literature. Also, negligible exposure is expected from the proposed use of Dutch Trig to prevent Dutch elm disease in elm trees.	WAIVER ACCEPTED		PMRA 1583112 1583114 1583116 1583173 1583179 1583180 1583181 1583182 1583183 1583184
Plants				
Aquatic Plants	A waiver was submitted based on the biological and ecological properties of the MPCA, the limited potential for exposure from the use of Dutch Trig, and the nature of the end-use formulation. No adverse effects to <i>Verticillium albo-atrum</i> were reported in published scientific literature. Also, negligible exposure is expected from the proposed use of Dutch Trig to prevent Dutch elm disease in elm trees.	WAIVER ACCEPTED		PMRA 1583112 1583114 1583116 1583173 1583179 1583180 1583181 1583182 1583183 1583184

Table 3 Alternative Active Ingredients Registered to Control Dutch Elm Disease

Active Ingredient	Classification	Fungicide/Insecticide	Mode of Action
Thiabendazole	1	Fungicide	Injected through drill holes in trunk for preventative and therapeutic treatment of Dutch elm disease.
Carbendazim	1	Fungicide	Injected through drill holes in trunk to protect from disease or arrest disease at earliest stages of infection.
Metam	M	Fungicide	Soil fumigant to prevent root graft transmission of Dutch elm disease.
Chlorpyrifos	1B	Insecticide	Sprayed on base of trunk to prevent adult beetle from overwintering in uninfected trees and to reduce beetle populations in disease free areas.

Table 4 Use (label) Claims Proposed by Applicant and Whether Acceptable or Unsupported

Proposed Use Claim	Supported / Unsupported
For control of Dutch elm disease on American elm (<i>Ulmus americana</i>), inject one drop of Dutch Trig from a 40 ml vial every 10 cm of tree circumference with the Dutch Trig tree inoculation tool. Apply in early spring after bud break before full leaf (at 50% leaf expansion). Make one application per year. Only inject healthy elm trees which show no signs of Dutch elm disease.	Supported

References

A. List of Studies/Information Submitted by Registrant

1.0 Chemistry and Human and Animal Health

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PMRA Document Number: 1583110

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PMRA Document Number: 1583111

Reference: 1998, Manipulation of *V. dahliae*, DACO: M1.2,M2.7.1,M7.0, M8.2.2, M9.6

PMRA Document Number: 1583112

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PMRA Document Number: 1583114

Reference: Hawksworth, D.L. 1970, *Verticillium albo-atrum*, Academic Press. 1980, DACO: M1.2,M2.7.2,M4.9,M5.0,M7.0,M8.2.1

PMRA Document Number: 1583115

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PMRA Document Number: 1583122

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Reference: 2000, Assessing the life span of Dutch Trig in *Ulmus americana*, DACO: M1.2,M2.7.2,M7.0,M8.2.2,M9.2

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Reference: 1997, Expert Letter concerning *Verticillium*, DACO: M1.2,M2.7.2,M5.0,M7.0, M8.2.2, M9.2

PMRA Document Number: 1583126

Reference: 2002, Dutch Trig non-target host range study, DACO: M1.2,M10.3.1,M7.0, M8.2.2, M9.8.1

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