Appendix to:

EFSA (European Food Safety Authority), 2019. Conclusion on the peer review of the pesticide risk assessment of the active substance *Verticillium albo-atrum* strain WCS850. EFSA Journal 2019;17(1):5575, 21 pp. doi:10.2903/j.efsa.2019.5575

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### Appendix A – List of end points for the active substance and the representative formulation

#### Identity, Biological properties, Details of uses, Proposed Classification and Labelling

| Active micro-organism | *Verticillium albo-atrum* strain WCS850 |
|-----------------------|----------------------------------------|
| Function (e.g. fungicide) | Preventive treatment (induction of systemic acquired resistance) of Dutch elm disease |

#### Rapporteur Member State

Sweden

#### Co-rapporteur Member State

The Netherlands

#### Identity of the Microbial or Viral Agent used in plant protection / Active Substance

(Regulation (EU) N° 283/2013, Annex Part B, point 1; OECD IIM Point 1)

| Name of the organism | *Verticillium albo-atrum* strain WCS850 |
|----------------------|----------------------------------------|
| Taxonomy             | Kingdom: Fungi<br>Division: Ascomycota<br>Class: Sordariomycetes<br>Subclass: Hypocreomycetidae<br>Order: Glomerellales<br>Family: Plectosphaerellaceae<br>Genus: Verticillium<br>Species: V. albo-atrum<br>Strain: WCS850 |

| Species, subspecies, strain | *Verticillium albo-atrum* strain WCS850 |
|-----------------------------|----------------------------------------|
| Identification / Detection  | Identification to strain level performed using whole genome sequencing (AFLP) and ITS-PCR, which can distinguish *Verticillium albo-atrum* strain WCS850 from other *Verticillium* species. However, this method does not allow to distinguish *Verticillium albo-atrum* strain WCS850 from other strain of *V. albo-atrum* and a method for this is being developed. |

| Culture collection | CBS 276.92 |
|--------------------|------------|
| Minimum and maximum concentration of the MPCA used for manufacturing of the formulated product (cfu; g/kg) | 0.7-1.5 x 10^7 CFU/ml distilled water |
| Identity and content of relevant impurities, additives, contaminating organisms in the technical grade of MPCA (cfu; g/kg) | Not applicable. The MPCA is a pure suspension of conidiospores in distilled water, no impurities or additives are present. The level of contaminating micro-organisms are <1 CFU/ml |
| Is the MPCA genetically modified; if so provide type of modification | No |
**Biological properties of the microorganism**  
*(Regulation (EU) N° 283/2013, Annex Part B, point 2; OECD IIM Point 2)*

| **Origin and natural occurrence** | **V. albo-atrum** WCS850 originates from a potato field in the Netherlands. |
|-------------------------------|---------------------------------------------------------------------|
| **Background level** | The species *V. albo-atrum* has a wide-spread geographical distribution in soil, but occurs mainly in temperate regions. It has a rather northern distribution in Europe and US.  
There is no information on background level of *V. albo-atrum*. |
| **Target organism(s)** | There is no specific target organism; *Verticillium albo-atrum* WCS850 is used as a protective agent, to prevent Dutch elm tree disease. |
| **Mode of action** | Preventative treatment on elms. This treatment induces natural resistance in elm trees and is based on the principle of systemic acquired resistance (SAR). |
| **Host specificity** | N/A; because of the mode of action and no interaction between MPCP and the target organism. |
| **Life cycle** | Conidiospores are injected into the xylem where they germinate and remain close to the site of injection in the trunk. No dispersal throughout the tree. No transmissibility will occur after application. |
| **Infectivity, dispersal and colonisation ability** | Known plant pathogen. Not a known human or mammalian pathogen, however there are reports on human *Verticillium* sp. infections in immuno-compromised patients.  
The optimum temperature for growth is 20-24°C, the minimum temperature is 10°C. The fungus does not grow at 30°C. It infects other plants via the roots that come into contact with it. In the absence of suitable host plants *V. albo-atrum* rapidly decreases. |
| **Relationships to known plant, animal or human pathogens** | The information submitted regarding production of metabolites (especially toxins) is limited. *Verticillium albo-atrum* WCS850 is derived from a parent strain that is known to produce phytotoxins; e.g. causing wilting. Hyaline strains of *Verticillium* are generally regarded as less pathogenic. No relevant articles (up until 2005) were identified that indicate the human/mammalian pathogenicity of the fungus. |
| **Genetic stability** | *Verticillium* WCS850 is a natural hyaline mutant of the parent *Verticillium albo-atrum*. This hyaline mutant has arisen spontaneously and is stable in its growth. No further information on the mutation is known.  
For production, a new sample is taken every year from the original stock culture. |
| **Information on the production of relevant metabolites (especially toxins)** | The information submitted regarding the production of metabolites (especially toxins) is limited. No reports found in search for *Verticillium dahliae* and *Verticillium albo-atrum* on possible metabolites of concern for human health and/or the environment. |
| **Resistance/ sensitivity to antibiotics / anti-microbial agents used in human or veterinary medicine** | No reports on resistance or sensitivity to anti-microbial agents.  
Anti-microbial agents used against *Verticillium* sp. infection include; amphotericin B, caspofungin, voriconazole and itraconazole. |
### Summary of uses supported by available data

(Regulation (EU) N° 283/2013, Annex Part B, point 3)

| Crop and/or situation (a) | Zone or Member State | Product name/code | F, G or I (b) | Pests or Group of pests controlled (c) | Formulation type (d-f) | conc. of as (i) | Application method kind (f-h) | growth stage & season (j) | number min-max (k) | interval between applications (min) (days) | PHI (days) | Remarks: |
|---------------------------|----------------------|-------------------|--------------|-------------------------------|----------------------|----------------|-------------------------------|-------------------------|-----------------|----------------------------------|-----------|---------|
| Elm trees (Ulmus spec.)   | Central              | Dutch Trig        | F            | Vascular fungus: Ophiostoma novo-ulmi | SU                   | 1.0 x 10⁷ spores/ml | Direct trunk injection       | Bud break in spring (May) | 1               | Not applicable                   | n.a.      | The formulation of the MPCP used for injection is exactly the same as the formulation of the MPCA (1 x 10⁷ spores/ml H₂O). There is no dilution of the MPCA to obtain the MPCP at any stage. It is not possible to convert the application rate to kg MPCA per ha or spores per ha, because of the nature of the treatment and application, being direct tree trunk injection of ornamental elm trees in public green spaces and in private environments such as ornamental gardens, alongside driveways etc. One direct-trunk-injection is done every 10 cm. of trunk circumference. One injection is 0.10 ml of MPCP (= 0.10 ml of MPCA). Every injection contains therefore 1 x 10⁶ spores. |

(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure) (b) Outdoor or field use (F), greenhouse application (G) or indoor application (I) (c) e.g. biting and sucking insects, soil born insects, foliar fungi, weeds (d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR) (e) GCPF Codes - GIFAP Technical Monograph No 2, 1989 (f) All abbreviations used must be explained (g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench (h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated (i) g/kg or g/L (j) Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application (k) The minimum and maximum number of applications possible under practical conditions of use must be provided (l) PHI - minimum pre-harvest interval (m) Remarks may include: Extent of use/economic importance/restrictions
**Classification and proposed labelling (Symbol, Indication of danger, Risk phrases, Safety phrases)**

| Classification and proposed labelling | Not applicable |
|---------------------------------------|----------------|
| with regard to physical/chemical data | As for other microorganisms based products, the warning phrase ‘Microorganisms may have the potential to provoke sensitising reactions’, can be applied taking into account that hazard statements applicable to chemicals (according to Regulation (EC) No 1272/2008) are not appropriate for microorganisms. |
| with regard to toxicological data | Not applicable |
| with regard to fate and behaviour | Not applicable |
| with regard to ecotoxicological data | Not applicable |

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1 Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, 1-1355.
Peer review of the pesticide risk assessment of the active substance *Verticillium albo-atrum* strain WCS8

Method of analysis

(Regulation (EU) N° 283/2013, Annex Part B, point 4 and Regulation (EU) N° 284/2013, Annex Part B, point 5)

**Analytical methods for the micro-organism** (MA 4.1 & MP 5.1; OECD IIM 4.3 & IIIM 5.1)

| Manufactured microorganism (principle of method) | Manufacturing process: Confidential information, See Volume 4 for details. Identification and quantification are performed by microscopy and plating on standard laboratory media. |
| --- | --- |
| Impurities and contaminating microorganisms in manufactured material (principle of method) | Microscopically and by plating on standard laboratory media, see Volume 4 for details. |
| Microbial Pest Control Product (principle of method) | Manufacturing process: Confidential information, See Volume 4 for details. Identification and quantification are performed by microscopy and plating on standard laboratory media. |

**Analytical methods for residues (viable and non-viable) in exposed compartments and organisms**

(MA 4.2 & MP 5.2; OECD IIM 4.5 & IIIM 5.2)

| Analysis of the active microorganism (principle of method) | Not required since there is no dietary consumer exposure expected. |
| --- | --- |
| Analysis of relevant metabolites (principle of method) | Not required since there is no dietary consumer exposure expected. |
Impact on Human and Animal health

(Regulation (EU) N° 283/2013, Annex Part A, point 5 and Regulation (EU) N° 284/2013, Annex Part A, point 7)

Medical data (including medical surveillance on manufacturing plant personnel)
(MA 5.1.1; OECD IIM 5.1)
Sensitisation
(MA 5.2.1 & MP 7.2.3; OECD IIM 5.2 & IIM 7.1.6)
Acute oral infectivity, toxicity and pathogenicity
(MA 5.2.2.1 & MP 7.1.1; OECD IIM 5.3.2 & IIM 7.1.1)
Acute intratracheal/inhalation infectivity, toxicity and pathogenicity
(MA 5.2.2.2 & MP 7.1.2; OECD IIM 5.3.3 & IIM 7.1.3)
Acute intravenous/intraperitoneal infectivity
(MA 5.2.2.3; OECD 5.3.4)
Genotoxicity
(MA 5.2.3; OECD IIM 5.3.5)
Cell culture study
(MA 5.2.4; OECD IIM 5.3.6)
Information on short-term toxicity and pathogenicity
(MA 5.2.5; OECD IIM 5.3.7)
Dermal toxicity
(MP 7.1.3; OECD IIM 7.1.2)
Specific toxicity, pathogenicity and infectivity
(MA 5.3; OECD IIM 5.5)
Genotoxicity – in vivo studies in germ cells
(MA 5.5; OECD IIM 5.5.3)

| Impact on Human and Animal health |
|----------------------------------|
| No health effects have been reported in manufacturing plant personnel or green house workers. |
| Considering that all microbials should be regarded as potential sensitizers, the agreed warning phrase is: “Microorganisms may have the potential to provoke sensitising reactions”. |
| No data available; not required. |
| No data available; not required. |
| Rat NOAEL = 1.7 x 10^7 CFU/animal |
| No treatment-related findings. |
| No data available; not required. |
| Not applicable |
| No data available; not required. |
| No data available; not required. |
| Not applicable |
| No data available; not required. |

Reference values

| AOEL |
|------|
| Not applicable. Based on the intended use, only a negligible human exposure is expected. |

| ADI |
|------|
| Not applicable. Based on the intended use no consumer exposure is expected. |

| ARfD |
|------|
| Not applicable. Based on the intended use no consumer exposure is expected. |

Exposure

Exposure (operator, workers, bystander, consumer)
(MA 6.1 & MP 7.3, 8.0; OECD IIM 5.6 & IIM 7.2, 7.3)

| Exposure (operator, workers, bystander, consumer) |
|-----------------------------------------------|
| Operator/worker: The closed injection tool prevents spills of the formulated product into the environment as well as onto hands and clothes of the users. A negligible exposure of operators is expected when opening and connecting the vial to the injection tool. The re-entry workers are not exposed to Verticillium WCS850. Furthermore, only trained personnel apply the product, which is not sold to the general public. |
| Bystander: No exposure for this representative use. |
| Consumer: No exposure for this representative use. |

| Exposure (operator, workers, bystander, consumer) |
|-----------------------------------------------|
| Not applicable. Based on the intended use, only a negligible human exposure is expected. |

| Exposed |
|--------|
| Not applicable. Based on the intended use no consumer exposure is expected. |
| Not applicable. Based on the intended use no consumer exposure is expected. |
Residues

(Regulation (EU) N° 283/2013, Annex Part B, point 6 and Regulation (EU) N° 284/2013, Annex Part B, point 8; OECD IIM Point 6 & IIIM Point 8)

| Type of Residues                  | Description                                                                 |
|----------------------------------|-----------------------------------------------------------------------------|
| Viable residues                  | Not applicable. Viable residues on edible plants parts and/or on succeeding crops are not expected. Therefore, a dietary consumer risk assessment is not required. |
| Non-viable residues              | Not applicable. Non-viable residues on edible plants parts and/or on succeeding crops are not expected. Therefore, a dietary consumer risk assessment is not required. |
Fate and behaviour in the environment

(Regulation (EU) N° 283/2013, Annex Part B, point 7 and Regulation (EU) N° 284/2013, Annex Part B, point 9; OECD IIM Point 7 & IIIM Point 9)

Persistence and multiplication (competitiveness) in soil, water and air

Verticillium albo-atrum WCS850 is not expected to reach the leaves or roots after vaccination is administered. Verticillium albo-atrum WCS850 is expected to be confined to the site of injection after vaccination. Persistence and multiplication in soil, water and air is therefore expected to be negligible.

Mobility

Mobility is expected to be negligible.
Effects on non-target organisms

(Regulation (EU) N° 283/2013, Annex Part B, point 8 and Regulation (EU) N° 284/2013, Annex Part B, point 10; OECD IIM Point 8 & IIIM Point 10)

### Effects on birds and other terrestrial vertebrates (MA 8.1 & MP 10.1; OECD IIM 8.1 & IIIM 10.1)

| Application rate (kg MPCA/ha) | Test substance | Crop | Category (e.g. insectivorous bird) and species | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-------------------------------|----------------|------|-----------------------------------------------|------------|-------------------------------------------------------------------------------------|
| Studies on birds and other terrestrial vertebrates are not available, nor required. |

### Effects on aquatic organisms (MA 8.2 & MP 10.2; OECD IIM 8.2, 8.3 & IIIM 10.2)

| Group | Test substance | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-------|----------------|------------|-------------------------------------------------------------------------------------|
| Laboratory tests: Fish species (specify): |
| Studies on fish are not available, nor required. |
| Invertebrates species (specify): |
| Studies on aquatic invertebrates are not available, nor required. |

### Effects on algae: (species, growth, growth rate, capacity to recover) ( MA 8.2.3 & MP 10.2; OECD IIM 8.4 & IIIM 10.2)

| Studies on algae are not available, nor required. |

### Effects on aquatic plants: (species, growth, growth rate, capacity to recover) ( MA 8.2.4 & MP 10.2; OECD IIM 8.5 & IIIM 10.2)

| Studies on aquatic plants are not available, nor required. |

### Effects on bees (MA 8.3 & MP 10.3; OECD IIM 8.7 & IIM 10.3)

| Species | Crop | Test substance | Route/time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|---------|------|---------------|-----------------|-------------------------------------------------------------------------------------|
| Studies on bees are not available, nor required. |

### Effects on terrestrial arthropods other than bees (MA 8.4 & MP 10.4; OECD IIM 8.8 & IIIM 10.4)

| Species | Stage | Test substance | Dose (kg MPCA/ha) | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|---------|-------|----------------|------------------|-------------------------------------------------------------------------------------|
| Studies on arthropods other than bees are not available, nor required. |

### Effects on other terrestrial invertebrates (MA 8.5 & MP 10.5; OECD IIM 8.9.1 & IIM 8.9.2 & IIIM 10.5)

Studies on other terrestrial invertebrates are not available, nor required.

### Effects on soil microorganisms (MA 8.6 & MP 10.6; OECD IIM 8.10 & IIIM 10.6)

Studies on soil micro-organisms are not available, nor required.

### Additional studies (MA 8.7 & MP 10.7; OECD 8.11 & IIIM 10.7)

Additional studies are not available, nor required.