Peer review of the pesticide risk assessment of the active substance Akanthomyces muscarius strain Ve6, formerly Lecanicillium muscarium strain Ve6

European Food Safety Authority (EFSA),

Maria Anastassiadou, Maria Arena, Domenica Auteri, Alba Brancato, Laszlo Bura, Luis Carrasco Cabrera, Eugenia Chaideftou, Arianna Chiusolo, Daniele Court Marques, Federica Crivellente, Chloe De Lentdecker, Mark Egsmose, Gabriella Fait, Luna Greco, Carla Huizing, Alessio Ippolito, Frederique Istace, Samira Jarrah, Dimitra Kardassi, Renata Leuschner, Alfonso Lostia, Christopher Lythgo, Oriol Magrans, Iris Mangas, Ileana Miron, Tunde Molnar, Laura Padovani, Juan Manuel Parra Morte, Ragnor Pedersen, Hermine Reich, Miguel Santos, Rositsa Serafimova, Rachel Sharp, Alois Stanek, Juergen Sturma, Csaba Szentes, Andrea Terron, Manuela Tiramani, Benedicte Vagenende and Laura Villamar-Bouza

Abstract

The conclusions of the European Food Safety Authority (EFSA) following the peer review of the initial risk assessments carried out by the competent authorities of the rapporteur Member State, the Netherlands, and co-rapporteur Member State, France, for the pesticide active substance Akanthomyces muscarius strain Ve6 formerly Lecanicillium muscarium strain Ve6 are reported. The context of the peer review was that required by Commission Implementing Regulation (EU) No 844/2012 as amended by Commission Implementing Regulation (EU) No 2018/1659. The conclusions were reached on the basis of the evaluation of the representative uses of Akanthomyces muscarius strain Ve6 as an insecticide on fruiting vegetables of cucurbitaceae with edible and inedible peel, fruiting vegetables of solanaceae, strawberries, floriculture crops (except roses), cut roses and tree nursery. The reliable end points, appropriate for use in regulatory risk assessment, are presented. Missing information identified as being required by the regulatory framework is listed. Concerns are identified.

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Correspondence: pesticides.peerreview@efsa.europa.eu
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Summary

Commission Implementing Regulation (EU) No 844/2012\textsuperscript{1}, as amended by Commission Implementing Regulation (EU) No 2018/1659\textsuperscript{2}, lays down the procedure for the renewal of the approval of active substances submitted under Article 14 of Regulation (EC) No 1107/2009. The list of those substances is established in Commission Implementing Regulation (EU) No 686/2012, as amended by Commission Implementing Regulation (EU) 2016/183. *Lecanicillium muscarium* strain Ve6 is one of the active substances listed in Regulation (EU) No 2016/183. The species has been re-named to *Akanthomyces muscarius* during the peer review process. Already in 2001, the species was reclassified from *Verticillium lecanii* to *Lecanicillium muscarium*.

In accordance with Article 1 of Regulation (EU) No 844/2012, the rapporteur Member State (RMS), the Netherlands, and co-rapporteur Member State (co-RMS), France, received an application from Koppert B.V. for the renewal of approval of the active substance *Lecanicillium muscarium* strain Ve6, which is now named *Akanthomyces muscarius* strain Ve6.

An initial evaluation of the dossier on *Akanthomyces muscarius* strain Ve6 was provided by the RMS in the renewal assessment report (RAR) and subsequently, a peer review of the pesticide risk assessment on the RMS evaluation was conducted by EFSA in accordance with Article 13 of Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659. The following conclusions are derived.

The uses of *Akanthomyces muscarius* strain Ve6 according to the representative uses as an insecticide on fruiting vegetables of cucurbitaceae with edible and inedible peel, fruiting vegetables of solanaceae, strawberries, floriculture crops (except roses), cut roses and tree nursery, as proposed at European Union (EU) level result in a sufficient insecticidal efficacy against the target organisms.

In the mammalian toxicology section, no concern or data gap has been identified with regard to the microorganism itself. It has been shown that the strain under evaluation would not produce destruxins, either in the formulated product or after application. However, such information could not be retrieved for the other metabolites potentially produced by *Akanthomyces* spp. A data gap has been identified for information on the toxicity of these secondary metabolites, should they be produced in the formulated product or after application.

Based on the available information, it cannot be excluded that exposure of humans to viable residues (both spores and mycelium) may occur. However, this can be considered acceptable based on the available information that residual spores and cells have no adverse health effects. However, a data gap was identified regarding toxicity of unidentified potentially produced secondary metabolites and consequently their consumer relevance cannot be assessed.

The information and evidence provided was considered insufficient to conclude on the likely competitiveness, persistence and multiplication of *Akanthomyces muscarius* Ve6 in soil and surface water.

For the representative use in high technology (permanent) greenhouses, the assessment of the potential for infectivity and pathogenicity in aquatic organisms could not be finalised. For the representative use to strawberries in walk-in tunnels, the assessment of the potential for infectivity and pathogenicity in aquatic organisms, bees, non-target arthropods and the assessment to soil microorganism communities could not be finalised. As the identification of secondary metabolites produced in the environment was not finalised, the risk to non-target organisms from such metabolites could also not be finalised.

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\textsuperscript{1} Commission Implementing Regulation (EU) No 844/2012 of 18 September 2012 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 252, 19.9.2012, p. 26–32.

\textsuperscript{2} Commission implementing Regulation (EU) 2018/1659 of 7 November 2018 amending Implementing Regulation (EU) No 844/2012 in view of the scientific criteria for the determination of endocrine disrupting properties introduced by Regulation (EU) 2018/605. OJ L 278, 8.11.2018, p 3–6.
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Background

Commission Implementing Regulation (EU) No 844/2012 as amended by Commission Implementing Regulation (EU) No 2018/1659 (hereinafter referred to as ‘the Regulation’), lays down the provisions for the procedure of the renewal of the approval of active substances, submitted under Article 14 of Regulation (EC) No 1107/2009. This regulates for the European Food Safety Authority (EFSA) the procedure for organising the consultation of Member States, the applicant(s) and the public on the initial evaluation provided by the rapporteur Member State (RMS) and/or co-rapporteur Member State (co-RMS) in the renewal assessment report (RAR), and the organisation of an expert consultation where appropriate.

In accordance with Article 13 of the Regulation, unless formally informed by the European Commission that a conclusion is not necessary, EFSA is required to adopt a conclusion on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 within 5 months from the end of the period provided for the submission of written comments, subject to an extension of up to 3 months where additional information is required to be submitted by the applicant(s) in accordance with Article 13(3).

In accordance with Article 1 of the Regulation, the RMS, the Netherlands, and co-RMS, France, received an application from Koppert B.V. for the renewal of approval of the active substance Akanthomyces muscarius strain Ve6. The species was previously named Lecanicillium muscarium. Complying with Article 8 of the Regulation, the RMS checked the completeness of the dossier and informed the applicant, the co-RMS (France), the European Commission and EFSA about the admissibility.

The RMS provided its initial evaluation of the dossier on Akanthomyces muscarius Ve6 in the RAR, which was received by EFSA on 30 January 2018 (Netherlands, 2018a).

In accordance with Article 12 of the Regulation, EFSA distributed the RAR to the Member States and the applicant, Koppert B.V., for consultation and comments on 15 May 2018. EFSA also provided comments. In addition, EFSA conducted a public consultation on the RAR. EFSA collated and forwarded all comments received to the European Commission on 16 July 2018. At the same time, the collated comments were forwarded to the RMS for compilation and evaluation in the format of reporting table. In addition, the applicant was invited to respond to the comments received. The applicant responded to the comments in column 3 of the reporting table. The comments and the applicant’s response were evaluated by the RMS in column 3.

The need for expert consultation and the necessity for additional information to be submitted by the applicant in accordance with Article 13(3) of the Regulation were considered in a telephone conference between EFSA, the RMS on 17 August 2018. On the basis of the comments received, the applicant’s response to the comments and the RMS’s evaluation thereof, it was concluded that additional information should be requested from the applicant and that there was no need to conduct an expert consultation.

The outcome of the telephone conference, together with EFSA’s further consideration of the comments, is reflected in the conclusions set out in column 4 of the reporting table. All points that were identified as unresolved at the end of the comment evaluation phase and which required further consideration, were compiled by EFSA in the format of an evaluation table.

The conclusions arising from the consideration by EFSA, and as appropriate by the RMS, of the points identified in the evaluation table and the written consultation on the assessment of additional information, where these took place, were reported in the final column of the evaluation table.

A final consultation on the conclusions arising from the peer review of the risk assessment took place with Member States via a written procedure in March 2019.

This conclusion report summarises the outcome of the peer review of the risk assessment of the active substance and the representative formulation, evaluated on the basis of the representative uses of Akanthomyces muscarius strain Ve6 as an insecticide on fruiting vegetables of cucurbitaceae with edible and inedible peel, fruiting vegetables of solanaceae, strawberries, floriculture crops (except roses), cut roses and tree nursery, as proposed by the applicant. In accordance with Article 12(2) of Regulation (EC) No 1107/2009, risk mitigation options identified in the RAR and considered during the peer review are presented in the conclusion. A list of the relevant end points for the active substance and the formulation is provided in Appendix A.

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3 Regulation (EC) No 1107/2009 of 21 October 2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.
In addition, a key supporting document to this conclusion is the peer review report (EFSA, 2019), which is a compilation of the documentation developed to evaluate and address all issues raised in the peer review, from the initial commenting phase to the conclusion. The peer review report comprises the following documents, in which all views expressed during the course of the peer review, including minority views, where applicable, can be found:

- the comments received on the RAR;
- the reporting table (17 August 2018);
- the evaluation table (28 February 2019);
- the comments received on the assessment of the additional information;
- the comments received on the draft EFSA conclusion.

Given the importance of the RAR, including its revisions (Netherlands, 2018b), and the peer review report, both documents are considered as background documents to this conclusion and thus are made publicly available.

It is recommended that this conclusion report and its background documents would not be accepted to support any registration outside the EU for which the applicant has not demonstrated that it has regulatory access to the information on which this conclusion report is based.

The identity of the microorganism and the properties of the formulated product

*Lecanicillium muscarium* isolate 19-79 (strain Ve6) is a fungus deposited to the CBS Filamentous Fungi database4 under accession number CBS 102071, to the ARS Collection of Entomopathogenic Fungal Cultures (ARSEF) under accession number ARSEF 5128. The strain has been formerly classified as *Verticillium lecanii* Ve6. (CABI Genetic Resource Collection, CABI(=IMI) 268317). The current taxonomy of the species *Lecanicillium muscarium* has been revised and *L. muscarium* is classified now as *Akanthomyces muscarius*. Sequences of internal transcribed spacer (ITS) region and three mitochondrial genes of *Akanthomyces muscarius* strain Ve6 are deposited at the GeneBank (National Center for Biotechnology Information, USA) under accession numbers EF512971 and EF513028, respectively. *Akanthomyces muscarius* strain Ve6 is worldwide occurring fungus, first isolated in 1979 from the glasshouse whitefly Troilieurodes vaporariorum.

The representative formulated product for the evaluation was ‘MYCOTAL WG’, a water dispersible granule (WG) containing 1 × 10^{13} spores/kg (declared range 9.5 × 10^{12} to 1.2 × 10^{13} spores/kg and 5.3 × 10^{12} to 8.9 × 10^{12} CFU/kg, respectively) or 48 g/kg *Akanthomyces muscarius* strain Ve6. An FAO specification does not exist for this product.

The representative uses evaluated as insecticide against thrips and whitefly comprise spray applications in fruiting vegetables of cucurbitaceae with edible and inedible peel, fruiting vegetables of solanaceae, strawberries, floriculture crops (except roses), cut roses and tree nursery. Full details of the Good Agricultural Practices (GAPs) can be found in the list of end points in Appendix A.

Data were submitted to conclude that the uses of *Akanthomyces muscarius* strain Ve6 according to the representative uses proposed at EU level result in a sufficient efficacy as an insecticide against whitefly and thrips, following the guidance document SANCO/2012/11251-rev. 4 (European Commission, 2014).

Conclusions of the evaluation

1. **Identity of the microorganism/biological properties/physical and technical properties and methods of analysis**

   The following guidance documents were followed in the production of this conclusion: European Commission, 2012 and EFSA, 2012.

   The technical grade microbial pest control agent (MPCA) is only a hypothetical stage in the continuous production process of the end use product (MPCP). As a consequence, the specification is given only for the end-use product ‘MYCOTAL WG’, containing minimum 5.3 × 10^{12} CFU/kg, (9.5 × 10^{12} spores/kg) of *Akanthomyces muscarius* strain Ve6.

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4 The Centraal bureau Schimmelcultures (CBS) has been renamed to Westerdijk Fungal Biodiversity Institute and is currently located in Utrecht, The Netherlands.
Mitochondrial restriction fragment length polymorphism (RFLP) was shown to distinguish *Akanthomyces muscarius* strain Ve6 from strains of the same species. The sequences of internal transcribed spacer 1, 5.8 S ribosomal RNA gene, internal transcribed spacer 2 (complete sequence) and small subunit of ribosomal RNA (rns) gene (partial sequence; mitochondrial) are used for *Akanthomyces muscarius* Ve6 identification on DNA level.

The analysis of contaminating microorganisms in commercially produced batches complies with the requirements of the Working Document on Microbial Contaminant Limits (European Commission, 2012).

*Akanthomyces muscarius* is not known to be related to any human or animal pathogen, or to fungi with adverse effects to the environment.

The possible production of destruxin A, B and E were evaluated for *Akanthomyces muscarius* strain Ve6 in liquid cultures, in rice cultures and solid agar medium, using high-performance liquid chromatography (HPLC) analyses and mass spectroscopy. Destruxins were not detected neither in the non-formulated spores nor in the product MYCOTAL WG. Destruxins were not found on tomato and cucumber plants after a foliar application of MYCOTAL at a dose of 10^8 spores/mL of spray (2 × 10^14 spores/ha, i.e. 10 times the recommended dose, three applications at one week interval).

*Akanthomyces muscarius* strain Ve6 can grow between 5 and 30°C with the optimal growth temperature of 25°C. There was no growth seen above 37°C.

Specific information addressing the issue of resistance/sensitivity to antibiotics and other antimicrobial agents of *Akanthomyces muscarius* strain Ve6 has not been presented; as a consequence, a data gap was identified for further assessment. It should be noted that the RMS disagreed with the setting of a data gap.

Acceptable methods are available for the determination of the microorganism content in the formulation. A data gap was identified for the storage stability of MYCOTAL in aluminium bags.

A residue definition was not applicable for *Akanthomyces muscarius* strain Ve6; therefore, post-registration monitoring methods are not needed.

### 2. Mammalian toxicity

#### General data

*Akanthomyces muscarius* strain Ve6 was renamed from *Lecanicillium muscarium* Ve6 or *Lecanicillium lecanii* Ve6 (formerly *Verticillium lecanii* Ve6). These references were kept for the literature search that was updated considering also the relevant metabolites potentially formed by the microbial control agent (MCA). The microorganism has a worldwide geographic distribution in soils, other fungi and plant material. It is not a known human or mammalian pathogen. Spores of *Akanthomyces muscarius* Ve6 germinate and grow between 5°C and 30°C. At 37°C, germination of some spores was obtained, but no further growth occurred, indicating its inability to colonise warm-blooded animals. Several papers describe clinical cases of human infection related to immunocompromised people or after trauma. Sensitisation potential was suspected by serology testing; however, an assessment of the symptoms was either not conducted to allow a conclusion on the sensitisation properties of the MCA or there were no indications of sensitisation or inflammatory lung diseases under pilot conditions and among greenhouse workers.

#### Toxicity studies

Based on acute toxicity studies by oral, intravenous, inhalation and intraperitoneal routes, the MCA did not exhibit infectivity or pathogenicity potential; however, signs of toxicity were observed after intraperitoneal administration, most probably unspecific local effects derived from intraperitoneal injection of proteinaceous material. Local effects were also observed in the lungs after short-term administration of the formulation by inhalation. Genotoxicity tests were provided on the MCA (Ames and Vitotox tests) and formulated product (Ames test) giving negative results. *In vitro* clastogenicity was not investigated, which would only be needed if strain-specific relevant metabolites would be identified (see paragraph below).

#### Secondary metabolites

The possible metabolites identified for the species *A. muscarius* comprise bassianolide, vertilecanins, vermalin A and B, lecanindole, lecanicillone A-C, lecanicilloid, destruxin and verticilide as assessed from peer-reviewed literature on the biological properties. Destruxins have been shown to be relevant secondary metabolites based on a broad spectrum of references evaluating possible effects of...
destruxins, or destruxin B in particular, to apoptosis in human carcinoma cells. As part of the EU RAFBCA-project\(^5\) (final report submitted with the dossier), genotoxicity of *Akanthomyces muscarius* was studied on a number of different Salmonella Typhimurium strains and on *E. coli* strains with polar and non-polar extracts of unformulated spores of ‘Mycotal’ and extracts from the preparation ‘Mycotal’. No mutagenic effects were found with any of these crude extracts, which would contain all possible metabolites. A statement was provided indicating that pure metabolites were also tested including destruxins A and that no mutagenic effects were found.

It has been shown that the strain under evaluation would not produce destruxins, either in the formulated product or after application. Such information could, however, not be retrieved for the other metabolites. In the environmental fate section of the RAR, it is stated that adequate information to address the potential for *Akanthomyces muscarius* Ve6 to produce secondary metabolites/toxins was not available. Accordingly a data gap has been identified for information on the toxicity of secondary metabolites/toxins such as bassianolide, vertilecanins, vermalin A and B, lecanindole, lecanicillione A-C, lecanicillloid and verticilide should they be produced by *Akanthomyces muscarius* Ve6. The RMS did not agree with the setting of this data gap, considering that the microorganism is commonly found in the environment and has been used for many years as plant protection product, taking into consideration that testing of the crude extract did not raise concern and that the literature search did not reveal information relevant to human health (or the environment), the RMS concluded that enough information is available to address the toxicological relevance of metabolites.

**Reference values and non-dietary exposure**

It is generally accepted that no reference values (acceptable daily intake – ADI, acute reference dose – ARfD, acceptable operator exposure level – AOEL or acute acceptable operator exposure level – AAOEL) are needed in cases where the microorganism is not pathogenic or infective and does not produce toxins (which remains to be confirmed). Accordingly, no exposure risk assessment would be needed. It is noted that operators and workers are recommended to use personal protective equipment (PPE) because all microorganisms are regarded as potential sensitisers (via the dermal and inhalation routes). Considering the data gap identified for secondary metabolites/toxins except destruxins, operator and worker exposure could not be finalised – as mentioned above, the RMS considered this issue sufficiently addressed. Assuming that the product is used in high technology (permanent) greenhouses or tunnels that are closed during application and 12 hours afterwards, bystanders and residents are unlikely to be exposed to the MCA.

3. **Residues**

*Akanthomyces muscarius* Ve6 is intended to be used on cucumber, tomato, sweet pepper, strawberries and ornamentals, the latter are not relevant for dietary consumer exposure. A preharvest interval (PHI) of one day is included in the GAP. Recently updated information on the active strain *Akanthomyces muscarius* Ve6 indicates that it does not produce destruxins. However, notably not all potentially relevant metabolites which the strain might be able to produce based on information on the respective species were investigated.

There is an indication that viable spores of the strain *Akanthomyces muscarius* Ve6 survive for a limited time on leaves and fruits and that soon after application, a decline of spore counts is observed. Therefore, in the GAP, it is recommended to repeat foliar treatments within one week to ensure high enough viable counts of *Akanthomyces muscarius* Ve6 for insecticidal control of plant diseases.

It cannot be excluded that exposure of humans to viable residues (both spores and mycelium) may occur, but this can be considered acceptable since residual spores and cells have no adverse health effects. However, it was noted that not all potentially relevant secondary metabolites were investigated as to whether they were produced by *Akanthomyces muscarius* Ve6 and a data gap was identified with regard to their toxicity. Therefore, since this information on potential secondary metabolites is not available, a consumer risk assessment cannot be completed considering the current state of available information further to earlier assessments (EFSA, 2016).

4. **Environmental fate and behaviour**

Satisfactory information has been provided in relation to potential interference of *Akanthomyces muscarius* Ve6 with the analytical systems for the control of the quality of drinking water provided for

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\(^5\) Risk Assessment of Fungal Biological Control Agents.
in Directive 98/83/EC\(^6\) (see specific Annex VI decision-making criteria in Part II Commission Regulation (EU) No 546/2011\(^7\)). As these methods utilise chromogenic agents to which *Akanthomyces muscarius* does not give a response, it was considered unlikely that *Akanthomyces muscarius* Ve6 would interfere with the methodologies used for such determinations.

As *Akanthomyces muscarius* Ve6 is a ‘wild type’, there are no marker genes in the strain which would permit analysis of a frequency of genetic exchange. As the genetic diversity and drift in the wild-type population have not been ascertained, it would not be possible to distinguish any genetic drift from that in the wild population based on the information provided. Though it is acknowledged that the possibility and effects of transfer of genetic material are no different for *Akanthomyces muscarius* Ve6 than for other naturally occurring *Akanthomyces muscarius* strains, transfer of genetic material by *Akanthomyces muscarius* Ve6 after application is possible and could not be excluded based on the information in the dossier.

4.1. **Fate and behaviour in the environment of the microorganism**

Information was derived from published literature on the species of *Akanthomyces muscarius* and studies on *Akanthomyces muscarius* Ve6 in relation to its persistence and multiplication in soil. The studies on *Akanthomyces muscarius* Ve6 in soil were of limited duration so the information and evidence provided were considered insufficient to conclude on the likely competitiveness, persistence and multiplication of *Akanthomyces muscarius* Ve6 in field soil. This conclusion is also applicable regarding soil and other growing media used in protected crop production systems. Consequently, EFSA concluded that it is unclear if the strain will respect the uniform principles criterion of not being expected to persist and multiply in soil- or plant-growing media in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years. This led to a data gap (see Section 7) and an issue that could not be finalised (see Section 9).

With respect to the persistence and multiplication in surface water, unpublished studies were available for a formulation containing *Akanthomyces muscarius*. The studies provided information on the persistence of *Akanthomyces muscarius* in water. The information on the persistence/multiplication/germination of *Akanthomyces muscarius* in natural surface water was considered not sufficient to demonstrate that *Akanthomyces muscarius* Ve6 is likely to decline in surface water; therefore, a data gap (see Section 7) was identified together with an issue that could not be finalised (see Section 9). PEC surface water for field use and high technology (permanent) greenhouses have been calculated considering the spray drift and run-off routes of exposure (see Appendix A).

The literature search according to the EFSA guidance (EFSA, 2011) on *Akanthomyces muscarius* did not provide any information on occurrence or behaviour in air.

Regarding mobility in general, vertical distribution of the microbial organism through soil is unlikely to happen based on information available in the submitted published scientific paper on *Akanthomyces muscarius*.

4.2. **Fate and behaviour in the environment of any relevant metabolite formed by the microorganism under relevant environmental conditions**

According to scientific papers retrieved through the literature search the species *Akanthomyces muscarius* and presented in the Volume 3 B.8 of the RAR, it is able to produce secondary metabolites such as destruxins, glucanolytic and chitinolytic enzymes. Some of these appear to be related to the mycoparasitic process. It has been shown that the strain under evaluation would not produce destruxins, based on the data submitted for this section.

It is not known to what extent *Akanthomyces muscarius* Ve6 will produce any other metabolites following its application once the spores reach the soil, should they grow. Adequate information to address the potential for *Akanthomyces muscarius* Ve6 to produce secondary metabolites/toxins was not available. Therefore, a data gap was identified. Consequently, it is not clear if such metabolites

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\(^6\) Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.98, p. 32-54.

\(^7\) Commission Regulation (EU) 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127–175.
might fulfil the criteria according to Part B Section 7 (iv) of Commission Regulation (EU) 283/20138 namely:

- the relevant metabolite is stable outside the microorganism;
- a toxic effect of the relevant metabolite is independent of the presence of the microorganism;
- the relevant metabolite is expected to occur in the environment in concentrations considerably higher than under natural conditions.

Therefore, data on the potential for Akanthomyces muscarius Ve6 to produce metabolites in relation to these criteria are necessary to assess if the further data requirements and the corresponding risk assessment according to Commission Regulation (EU) No 283/2013, part A, Section 7 (standard data requirements and assessment mandatory for chemical plant protections active substances) are triggered. Consequently, this resulted in a data gap (see Section 7) and issue that could not be finalised (see Section 9).

5. Ecotoxicology

The applicant confirmed that the representative uses to cucumber, tomatoes, sweet peppers, strawberries and floriculture crops in glasshouses were only intended for use in high technology (permanent) greenhouses.9 The risk assessment for non-target organisms was therefore performed accordingly. The exposure assessment for a second representative use to strawberries in walk-in tunnels was appropriately assumed to be comparable to applications in open fields; however, it is worth noting that the GAP specifies that the tunnels should be closed for 12 h after application which may reduce the potential for exposure to non-target organisms.

As discussed in Section 4, the identification and exposure assessment for secondary metabolites in the environment could not be finalised. Consequently, the risk to non-target organisms from such metabolites cannot be assessed and a data gap is identified. This is relevant for all groups of non-target organisms for the representative use to strawberries in walk-in tunnels. For the representative uses in high technology (permanent) greenhouses, the data gap is relevant for aquatic organisms and soil organisms (only in the case that the secondary metabolites are persistent and when the high technology (permanent) greenhouses is removed). The RMS does not agree with the conclusion regarding the need for a risk assessment for secondary metabolites in the environment for the representative uses of Akanthomyces muscarius Ve6. The RMS is of the opinion that, as the mode of action is not via toxins, any secondary metabolites which are produced will be of insignificant amounts for non-target organisms. Furthermore, literature searches performed by the applicant did not indicate a concern for non-target organisms from potential secondary metabolites.

As concluded in Section 2, Akanthomyces muscarius Ve6 is not expected to be infectious or pathogenic in mammals. Consequently, a low risk to wild mammals from the microorganism is concluded. A study investigating the toxicity, infectivity and pathogenicity of Akanthomyces muscarius Ve6 to birds was available and did not indicate any adverse effects. Consequently, a low risk to birds was concluded. Furthermore, no exposure to birds and mammals is anticipated for the representative uses to high technology (permanent) greenhouses.

Only acute studies for fish and aquatic invertebrates were available. It was shown that the concentrations used in these studies resulted in a margin of safety above that expected for the representative uses of Akanthomyces muscarius Ve6. Nevertheless, owing to the short exposure and observation period in the available studies, this risk assessment cannot be considered to provide evidence regarding the potential for infectivity and pathogenicity. A report of Verticillium lecanii (the previous name for Akanthomyces muscarius Ve6) infecting the swim bladder of Baltic salmon was included in a literature study investigating the reason for mortality in a fish farm. The RMS noted a number of reliability concerns related to the study and furthermore concluded that the presence of Verticillium lecanii in the swim bladder could not be concluded to be the cause of the mortality as several other microorganisms were also detected. Owing to the reliability issues, EFSA agreed with the RMS’s conclusion that this study did not confirm infectivity of Akanthomyces muscarius Ve6 in fish. It was argued that the representative use of Akanthomyces muscarius Ve6 is unlikely to result in the

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8 Commission Regulation (EU) 283/2013 of 1 March 2013 setting out the data requirements for active substances in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1-94.

9 Please refer to the applicant response in column 3 of 9(36) of the reporting table. Consequently, EFSA added a clarification in the GAP table presented in Appendix 1.
infectivity or pathogenicity of fish and aquatic invertebrates for several reasons such as low exposure, natural occurrence in aquatic water bodies, lack of evidence that aquatic organisms are in the host range and lack of reliable studies reporting infectivity in the scientific literature. However, as no substantial evidence has been provided, overall, a low risk to fish and aquatic invertebrates from infectivity and pathogenicity cannot be concluded. A data gap and issue not finalised is therefore concluded for all representative uses given that exposure of Akanthomyces muscarius Ve6 to surface water is anticipated for all uses. The RMS does not agree with the need for further information and considers that the risk to aquatic organisms is low.

Reliable acute toxicity data were available for honeybees. Whilst the data did not show that Akanthomyces muscarius Ve6 is toxic to honeybees the data were not considered sufficient to investigate the potential for infectivity and pathogenicity. Although the RMS included several arguments to suggest that Akanthomyces muscarius Ve6 is unlikely to be infectious or pathogenic to honeybees, these arguments were not considered to provide substantial evidence to exclude the potential for infectivity or pathogenicity. Furthermore, it is noted that the Akanthomyces muscarius Ve6 is targeted towards the nymph stage of pests but no data on honeybee larvae were available. Therefore, a data gap was identified to further address the potential for infectivity and pathogenicity to honeybees (relevant for the representative walk-in tunnel use to strawberries). Owing to the lack of exposure, the risk to honeybees and wild bees from the representative uses in permanent greenhouses is low. The RMS also included arguments to exclude a risk to pollinators used in greenhouses. However, no reliable data was provided to support the arguments, and therefore, a risk to pollinators introduced to greenhouses cannot be excluded.

Several papers were available which indicated that the Akanthomyces muscarius Ve6 is not infectious or pathogenic to several species of non-target arthropods. However, information was also presented that demonstrated that Akanthomyces muscarius Ve6 has the capacity to infect and cause mortality of the predatory mite *P. persimilis*. Although the key study was not considered to be totally reliable, EFSA considers that the effects should not be disregarded. Therefore, a data gap to address the risk to non-target arthropods is identified (relevant for the representative walk-in tunnel use to strawberries). Owing to the lack of exposure, the risk to non-target arthropods from the representative uses in permanent greenhouses is low.

Suitable data were available and indicated that Akanthomyces muscarius Ve6 is unlikely to be infectious or pathogenic to earthworms. No specific data were available demonstrating the effects of Akanthomyces muscarius Ve6 on soil microorganism communities; therefore, a data gap was concluded (relevant for the representative walk-in tunnel use to strawberries). The RMS is of the opinion that the use of microbial pesticides in general poses a low risk to soil microorganism communities which was supported by a reference to a literature study where a meta-analysis showed that microbial antagonists could have a short-term effect on the abundance of the fungal communities in soils but with recovery within 70 days. Owing to the lack of exposure, the risk to soil microorganisms from the representative uses in permanent greenhouses is low.

### 6. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments (Tables 1–4)

#### Table 1: Soil

| Compound (name and/or code) | Persistence | Ecotoxicology |
|-----------------------------|-------------|---------------|
| Akanthomyces muscarius Ve6  | Data gap.   | Data gap for soil microorganisms (relevant only for use to strawberries in walk-in tunnels) |
| Toxins/secondary metabolites such as destruxins, glucanolytic and chitinolytic enzymes | Data gap, pending identification |
This is a list of data gaps identified during the peer review process, including those areas in which a study may have been made available during the peer review process but not considered for procedural reasons (without prejudice to the provisions of Article 56 of Regulation (EC) No 1107/2009 concerning information on potentially harmful effects).

- Specific information addressing the issue of resistance/sensitivity to antibiotics and other antimicrobial agents of *Akanthomyces muscarii* strain Ve6 (relevant for all representative uses evaluated; see Sections 1 and 2).
- Storage stability of MYCOTAL WG in aluminium bags (relevant for all representative uses evaluated; see Section 1).
- Adequate information to address the uniform principles criterion of the strain not being expected to persist and multiply in soil or plant growing media and in surface water in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years was not available (relevant for all representative uses evaluated; see Section 4).
- Information on the toxicity of secondary metabolites/toxins such as destruxins, glucanolytic and chitinolytic enzymes is missing and would be required should they be produced by *Akanthomyces muscarii* Ve6, since evidence for non-production currently lacking (relevant for all representative uses evaluated; see Sections 2, 3, 4 and 5).
- Further data are needed to address the potential of *Akanthomyces muscarii* Ve6 for infectivity and pathogenicity to fish and aquatic organisms (relevant for all representative uses; see Section 5).
- Further data are needed to address the potential of *Akanthomyces muscarii* Ve6 for infectivity and pathogenicity to bees (relevant only for use to strawberries in walk-in tunnels; see Section 5).
Further information is needed to address the potential of *Akanthomyces muscarius* Ve6 to be infectious or pathogenic to non-target arthropods (relevant only for use to strawberries in walk-in tunnels; see Section 5).

Further information investigating the effects of *Akanthomyces muscarius* Ve6 on soil microorganism communities is needed (relevant only for use to strawberries in walk-in tunnels; see Section 5).

### 8. Particular conditions proposed to be taken into account to manage the risk(s) identified

- None identified.

### 9. Concerns

#### 9.1. Issues that could not be finalised

An issue is listed as ‘could not be finalised’ if there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the uniform principles in accordance with Article 29(6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011 and if the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

An issue is also listed as ‘could not be finalised’ if the available information is considered insufficient to conclude on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009.

1) The production of relevant toxins/secondary metabolites of potential concern for humans and the environment cannot be excluded. Therefore, the risk assessment cannot be finalised for human and animal health and the environment including the assessment of potential groundwater exposure (see Sections 2, 3, 4 and 5).

2) The information on the persistence/multiplication/germination of *Akanthomyces muscarium* in soil, natural surface water was considered not sufficient to demonstrate that *Akanthomyces muscarium* Ve6 is likely to decline in soil and in surface water, therefore a data gap was identified.

3) The assessment could not be finalised for fish, aquatic invertebrates, bees, non-target arthropods and soil microorganisms (fish and aquatic invertebrates are relevant for all representative uses whereas bees, non-target arthropods and soil microorganisms are only relevant for the representative walk-in tunnel use to strawberries) (see Section 5).

#### 9.2. Critical areas of concern

An issue is listed as a critical area of concern if there is enough information available to perform an assessment for the representative uses in line with the uniform principles in accordance with Article 29(6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011, and if this assessment does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater, or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if the assessment at a higher tier level could not be finalised due to lack of information, and if the assessment performed at the lower tier level does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater, or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if, in the light of current scientific and technical knowledge using guidance documents available at the time of application, the active substance is not expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009.

- None identified.
9.2.1. Overview of the concerns identified for each representative use considered (Table 5)

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in Section 8, has been evaluated as being effective, then ‘risk identified’ is not indicated in Table 5.)

| Table 5: Overview of concerns |
|--------------------------------|
| Representative use | Cucumber high technology (permanent) greenhouses | Tomato high technology (permanent) greenhouses | Sweet pepper high technology (permanent) greenhouses | Strawberries high technology (permanent) greenhouses | Strawberries Walk-in tunnels |
| Operator risk | Risk identified | | | | |
| Assessment not finalised | | | | | |
| Worker risk | Risk identified | | | | |
| Assessment not finalised | | | | | |
| Resident/bystander risk | Risk identified | | | | |
| Assessment not finalised | | | | | |
| Consumer risk | Risk identified | | | | |
| Assessment not finalised | | | | | |
| Risk to wild non-target terrestrial vertebrates | Risk identified | | | | |
| Assessment not finalised | | | | | |
| Risk to wild non-target terrestrial organisms other than vertebrates | Risk identified | | | | |
| Assessment not finalised | | | | | |
| Risk to aquatic organisms | Risk identified | | | | |
| Assessment not finalised | | | | | |
| Groundwater exposure to active substance | Legal parametric value breached | | | | |
| Assessment not finalised | | | | | |
| Groundwater exposure to metabolites | Legal parametric value breached(a) | | | | |
| Parametric value of 10 μg/L(b) breached | | | | | |
| Assessment not finalised | | | | | |

The superscript numbers relate to the numbered points indicated in Section 9.1. Where there is no superscript number, see Sections 2-6 for further information.

(a): When the consideration for classification made in the context of this evaluation under Regulation (EC) No 1107/2009 is confirmed under Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008.

(b): Value for non-relevant metabolites prescribed in SANCO/221/2000-rev. 10 final, European Commission, 2003.

(c): The risk assessment for non-target organisms from potential secondary metabolites is not finalised. In the case of the representative uses in high technology permanent greenhouses, potential for exposure to non-target organisms is only anticipated in the case that identified metabolites are persistent and the greenhouse is removed.
| Representative use                                      | Floriculture crops, except cut roses high technology (permanent) greenhouses |
|--------------------------------------------------------|-----------------------------------------------------------------------------|
| **Operator risk**                                      | Risk identified                                                             |
|                                                        | Assessment not finalised                                                      |
| **Worker risk**                                        | Risk identified                                                             |
|                                                        | Assessment not finalised                                                      |
| **Resident/bystander risk**                            | Risk identified                                                             |
|                                                        | Assessment not finalised                                                      |
| **Consumer risk**                                      | Risk identified                                                             |
|                                                        | Assessment not finalised                                                      |
| **Risk to wild non-target terrestrial vertebrates**    | Risk identified                                                             |
|                                                        | Assessment not finalised                                                      |
| **Risk to wild non-target terrestrial organisms other than vertebrates** | Risk identified                                                             |
|                                                        | Assessment not finalised                                                      |
| **Risk to aquatic organisms**                         | Risk identified                                                             |
|                                                        | Assessment not finalised                                                      |
| **Groundwater exposure to active substance**           | Legal parametric value breached                                             |
|                                                        | Assessment not finalised                                                      |
| **Groundwater exposure to metabolites**                | Legal parametric value breached\(^{(a)}\)                                  |
|                                                        | Parametric value of 10 μg/L\(^{(b)}\) breached                             |
|                                                        | Assessment not finalised                                                      |

Columns are grey if no safe use can be identified. The superscript numbers relate to the numbered points indicated in Section 9.1. Where there is no superscript number, see Sections 2–6 for further information.

(a): When the consideration for classification made in the context of this evaluation under Regulation (EC) No 1107/2009 is confirmed under Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008.

(b): Value for non-relevant metabolites prescribed in SANCO/221/2000-rev. 10 final, European Commission, 2003.

(c): The risk assessment for non-target organisms from potential secondary metabolites is not finalised. In the case of the representative uses in high technology permanent greenhouses, potential for exposure to non-target organisms is only anticipated in the case that identified metabolites are persistent and the greenhouse is removed.

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**Abbreviations**

| Symbol | Description |
|--------|-------------|
| 1/n    | slope of Freundlich isotherm |
| λ      | wavelength |
| ε      | decadic molar extinction coefficient |
| a.s.   | active substance |
| AAOEL  | acute acceptable operator exposure level |
| ADI    | acceptable daily intake |
| AOEL   | acceptable operator exposure level |
| AR     | applied radioactivity |
| AR    | androgen receptor |
| ARfD   | acute reference dose |
| ARSEF  | ARS Collection of Entomopathogenic Fungal Cultures |
| CABI   | Centre for Agricultural Bioscience International |
| CAS    | Chemical Abstracts Service |
| CBS    | Centraal bureau Schimmelcultures |
| CFU    | colony-forming units |
| CHO    | Chinese hamster ovary cells |
| CI     | confidence interval |
| DAR    | draft assessment report |
| DAT    | days after treatment |
| DM     | dry matter |
| DNA    | deoxyribonucleic acid |
| FAO    | Food and Agriculture Organization of the United Nations |
| FOCUS  | Forum for the Co-ordination of Pesticide Fate Models and their Use |
| GAP    | Good Agricultural Practice |
| CBS    | Filamentous Fungi database |
| HPLC   | high-pressure liquid chromatography |
| IMI    | International Mycological Institute |
| ISO    | International Organization for Standardization |
| ITS    | internal transcribed spacer |
| iv     | intravenous |
| LD₅₀   | lethal dose, median; dosis letalis media |
| mm     | millimetre (also used for mean measured concentrations) |
| MOA    | mode of action |
| MPCA   | active agent of the microbial pest control product |
| MPCP   | microbial pest control product |
| Pa     | pascal |
| PHI    | preharvest interval |
| P_{ow} | partition coefficient between n-octanol and water |
| PPE    | personal protective equipment |
| ppm    | parts per million (10⁻⁶) |
| r²     | coefficient of determination |
| RAR    | Renewal Assessment Report |
| RFLP   | restriction fragment length polymorphism |
| RMS    | rapporteur Member State |
| SD     | standard deviation |
| RNA    | ribonucleic acid |
| w/v    | weight per unit volume |
| w/w    | weight per unit weight |
| WBC    | white blood cell |
| WG     | water-dispersible granule |
| WHO    | World Health Organization |
Appendix A – List of end points for the active substance and the representative formulation

Appendix A can be found in the online version of this output ('Supporting information' section):
https://doi.org/10.2903/j.efsa.2020.6121