Peer review of the pesticide risk assessment of the active substance *Ampelomyces quisqualis* strain AQ10

European Food Safety Authority (EFSA),
Maria Arena, Domenica Auteri, Stefania Barmaz, Giulia Bellisai, Alba Brancato, Daniela Brocca, Laszlo Bura, Harry Byers, Arianna Chiusolo, Daniele Court Marques, Federica Crivellente, Chloe De Lentdecker, Mark Egsmose, Zoltan Erdos, Gabriella Falt, Lucien Ferreira, Marina Goumenou, Luna Greco, Alessio Ippolito, Frederique Istace, Samira Jarrah, Dimitra Kardassi, Renata Leuschner, Christopher Lythgo, Jose Oriol Magrans, Paula Medina, Ileana Miron, Tunde Molnar, Alexandre Nougadere, Laura Padovani, Juan Manuel Parra Morte, Ragnor Pedersen, Hermine Reich, Angela Sacchi, Miguel Santos, Rositsa Serafi
mova, Rachel Sharp, Alois Stanek, Franz Streissl, Juergen Sturma, Csaba Szentes, Jose Tarazona, Andrea Terron, Anne Theobald, Benedicte Vagenende, Alessia Verani and Laura Villamar-Bouza

Abstract

The conclusions of EFSA following the peer review of the initial risk assessments carried out by the competent authorities of the rapporteur Member State, France, and the co-rapporteur Member State, Germany, for the pesticide active substance *Ampelomyces quisqualis* strain AQ10 are reported. The context of the peer review was that required by Commission Implementing Regulation (EU) No 844/2012. The conclusions were reached on the basis of the evaluation of the representative uses of *Ampelomyces quisqualis* strain AQ10 as a fungicide by spraying against powdery mildew in grapes and spray applications in tomato, pepper and aubergine grown under protection or in the open field. 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.

© 2017 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

**Keywords:** *Ampelomyces quisqualis* strain AQ10, peer review, risk assessment, pesticide, fungicide

**Requestor:** European Commission

**Question number:** EFSA-Q-2015-00021

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

Commission Implementing Regulation (EU) No 844/2012 (hereinafter referred to as ‘the Regulation’) 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. *Ampelomyces quisqualis* strain AQ10 is one of the active substances listed in Regulation (EU) No 686/2012.

In accordance with Article 1 of the Regulation, the rapporteur Member State (RMS), France, and co-rapporteur Member State (co-RMS), Germany, received an application from CBC (Europe) S.r.l. for the renewal of approval of the active substance *Ampelomyces quisqualis* AQ10. Complying with Article 8 of the Regulation, the RMS checked the completeness of the dossier and informed the applicant, the co-RMS (Germany), the European Commission and the European Food Safety Authority (EFSA) about the admissibility.

The RMS provided its initial evaluation of the dossier on *Ampelomyces quisqualis* AQ10 in the renewal assessment report (RAR), which was received by EFSA on 25 November 2016. In accordance with Article 12 of the Regulation, EFSA distributed the RAR to the Member States and the applicant, CBC (Europe) S.r.l., for comments on 9 January 2017. 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 15 March 2017.

Following consideration of the comments received on the RAR, it was concluded that additional information should be requested from the applicant, and that EFSA should conduct an expert consultation in the area of mammalian toxicology.

In accordance with Article 13(1) of the Regulation, EFSA should adopt a conclusion on whether *Ampelomyces quisqualis* strain AQ10 can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 of the European Parliament and of the Council.

The conclusions laid down in this report were reached on the basis of the evaluation of the representative uses of *Ampelomyces quisqualis* strain AQ10 as a fungicide by spraying against powdery mildews in grapes (open field), and tomato, pepper, aubergine (grown under protected conditions or in open field) as proposed by the applicant. Full details of the representative uses can be found in Appendix A of this report.

The uses of *Ampelomyces quisqualis* strain AQ10 according to the representative uses proposed result in a sufficient fungicidal efficacy against powdery mildew.

In the section identity, physical–chemical and technical properties, and analytical methods, data gaps were identified for an experimental method using molecular tools to confirm the relevance of these markers in identifying *Ampelomyces quisqualis* strain AQ10 at strain level, for further data to confirm that strain AQ10 cannot produce relevant secondary metabolites/toxins, for testing of the antibiotics sensitivity and for the determination of microbial contaminants of the formulation before and after storage.

In the mammalian toxicology area, considering the data gap set for the identification and quantification of secondary metabolites/toxins produced by *Ampelomyces quisqualis* AQ10 in the product and after application, non-dietary exposure assessment to potential secondary metabolites/toxins or toxins could not be concluded.

It is not necessary to perform a dietary consumer risk assessment for viable cells of *Ampelomyces quisqualis* strain AQ10 on the raw agricultural commodity because they are not of concern for human health. However, because of remaining uncertainties related to the potential presence and/or formation of secondary metabolites/toxins which may be harmful to humans, a consumer risk assessment cannot be carried out. Therefore, the existing inclusion in Annex IV of Regulation (EC) No 396/2005 may need further consideration by risk managers, as the information considered in this renewal application including its review of the published literature supersedes the consideration in the relevant EFSA reasoned opinion relating to inclusion in Annex IV (EFSA, 2008).

Information/evidence to conclude on the potential for competitiveness, persistence and multiplication of *Ampelomyces quisqualis* AQ10 in water, field soil as well as soil and other growing media used in glasshouse was not available. Adequate information to address the potential for *Ampelomyces quisqualis* AQ10 to produce secondary metabolites/toxins including antibiotics was not available. Both these issues have led to data gaps and assessments not being finalised.

In the area of ecotoxicology, data gaps were identified for further information to address toxicity, infectivity and pathogenicity and the risk to aquatic organisms, honey bees, earthworms and soil microorganisms. In addition, further information is needed to address the potential effects to non-target organisms of the secondary metabolites/toxins produced by *Ampelomyces quisqualis* strain AQ10.
Table of contents

Abstract................................................................................................................................................... 1
Summary................................................................................................................................................. 3
Background ............................................................................................................................................. 5
The active substance and the formulated product....................................................................................... 6
Conclusions of the evaluation.................................................................................................................... 6
1. Identity of the microorganism/biological properties/physical and technical properties and methods of analysis ............................................................................................................................... 6
2. Mammalian toxicity............................................................................................................................ 7
3. Residues........................................................................................................................................... 8
4. Environmental fate and behaviour ...................................................................................................... 8
4.1. Fate and behaviour in the environment of the microorganism............................................................... 8
4.2. Fate and behaviour in the environment of any relevant metabolite formed by the microorganism under relevant environmental conditions ........................................................................................................... 9
5. Ecotoxicology.................................................................................................................................... 9
6. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments (Tables 1–4) ......................................................................... 11
7. Data gaps identified for the representative uses evaluated ................................................................. 12
8. Particular conditions proposed to be taken into account to manage the risk(s) identified .................. 12
9. Concerns for the representative uses evaluated ................................................................................... 12
9.1. Issues that could not be finalised ........................................................................................................ 12
9.2. Critical areas of concern..................................................................................................................... 13
9.3. Overview of the concerns identified for each representative use considered.................................. 13
References............................................................................................................................................... 14
Abbreviations ........................................................................................................................................... 15
Appendix A – List of end points for the active substance and the representative formulation................... 16
Appendix B – Used compound codes .................................................................................................... 17
Background

Commission Implementing Regulation (EU) No 844/2012¹ (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 1107/2009². 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 8 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 France and co-RMS Germany received an application from CBC (Europe) S.r.l. for the renewal of approval of the active substance Ampelomyces quisqualis AQ10. Complying with Article 8 of the Regulation, the RMS checked the completeness of the dossier and informed the applicant, the co-RMS (Germany), the European Commission and EFSA about the admissibility.

The RMS provided its initial evaluation of the dossier on Ampelomyces quisqualis AQ10 in the RAR, which was received by EFSA on 25 November 2016 (France, 2016).

In accordance with Article 12 of the Regulation, EFSA distributed the RAR to the Member States and the applicant, CBC (Europe) S.r.l., for consultation and comments on 9 January 2017 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 15 March 2017. At the same time, the collated comments were forwarded to the RMS for compilation and evaluation in the format of a reporting table. The applicant was invited to respond 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 and the RMS on 27 April 2017. 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 EFSA should conduct an expert consultation in the area of mammalian toxicology.

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, including those issues to be considered in an expert consultation, 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, together with the outcome of the expert consultation 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 October/November 2017.

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 Ampelomyces quisqualis AQ10 as a fungicide by spraying against powdery mildews in grapes (open field), and tomato, pepper, aubergine (grown under protected conditions or in open field) as proposed by the applicant. A list of the relevant end points for the active substance and the formulation is provided in Appendix A.

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.
² 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, 2017), 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 (25 April 2017);
- the evaluation table (25 October 2017);
- the report(s) of the scientific consultation with Member State experts (where relevant);
- the comments received on the assessment of the additional information (where relevant);
- the comments received on the draft EFSA conclusion.

Given the importance of the RAR, including its revisions (France, 2017), 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 European Union (EU) for which the applicant has not demonstrated that it has regulatory access to the information on which this conclusion report is based.

The active substance and the formulated product

*Ampelomyces quisqualis* strain AQ10 is a fungus deposited at the National Collection of Microorganisms Cultures (CNCM), Pasteur Institute, Paris, France, under the accession number I-807. *Ampelomyces quisqualis* strain AQ10 is a naturally occurring microscopic fungus, initially isolated from powdery mildew parasitising *Catha edulis* in Israel.

The representative formulated product for the evaluation was 'AQ10', a water-dispersible granule (WG) containing 580 g/kg (nominal $5 \times 10^{12}$ viable spores/kg, minimum content $3 \times 10^{12}$ viable spores/kg, maximum $7 \times 10^{12}$ viable spores/kg) *Ampelomyces quisqualis* strain AQ10.

The representative uses evaluated comprise applications as a fungicide by spraying against powdery mildews in grapes (open field), and tomato, pepper, aubergine (grown under protected conditions or in open field).

Data were submitted to conclude that the uses of *Ampelomyces quisqualis* strain AQ10 according to the representative uses proposed at EU level result in a sufficient fungicidal efficacy against powdery mildew, 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: SANCO/12116/2012-rev. 0 (European Commission, 2012) and Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (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 formulated product AQ-10 of min. $3 \times 10^{12}$ viable spores/kg.

Molecular methods, using several molecular markers (restriction fragment length polymorphism (RFLP) markers, actin gene, internal transcribed spacer (ITS) region of rDNA, microsatellite sequence (simple sequence repeat (SSR)) and inter-simple sequence repeat (ISSR) markers) can be used for the identification of *Ampelomyces quisqualis* strain AQ10. A data gap was, however, set for an experimental method using molecular tools to confirm the relevance of these markers in identifying *Ampelomyces quisqualis* strain AQ10 at strain level and discriminate the strain AQ10 from other closest strains of the same RFLP group or from the same lineage.

In addition, the literature search has shown that *Ampelomyces* sp. can produce other secondary metabolites such as quinones, phenolic compounds or plant growth hormones. The possibility of strain AQ10 to secrete such compounds under different conditions was not addressed; therefore, further data are considered necessary to confirm that strain AQ10 cannot produce secondary metabolites/toxins both during the manufacturing procedure and following the application of the product (see all Sections).
There is no evidence of direct relationships of *Ampelomyces quisqualis* strain AQ10 to known plant, animal or human pathogens.

The optimum temperature for spore germination and pycnidia formation by *Ampelomyces quisqualis* strain AQ10 ranged from 15°C to 25°C. *Ampelomyces quisqualis* strain AQ10 is able to grow at a large pH range (3-8) with a good growth and sporulation between pH 5.5 and 7.0. Information on the microorganism’s resistance or sensitivity to antibiotics was not determined, as a consequence a data gap was identified.

The assessment of the data package revealed no issues that need to be included as critical areas of concern with respect to the identity, physical and technical properties of the representative formulation; however, a data gap was identified for the determination of microbial contaminants according to European Commission (2012) before and after storage. The preparation must be stored at a temperature below 30°C for maximum 1 year.

Acceptable methods are available for the determination of the microorganism in the formulation and for the determination of the content of contaminating microorganisms.

2. **Mammalian toxicity**

*Ampelomyces quisqualis* strain AQ10 was discussed during the Pesticides Peer Review teleconference 149.

No adverse health effects have been reported for workers and operators being involved in the production process, handling or applying *Ampelomyces quisqualis* strain AQ10. No clinical cases, pathogenicity or sensitisation linked to *Ampelomyces quisqualis* strain AQ10 have been found in the open literature and the microorganism is not related to human pathogens.

The EFSA Panel on Biological Hazards could not recommend *Ampelomyces quisqualis* strain AQ10 along with all the filamentous fungi used as plant protection agents for the qualified presumption of safety (QPS) list (EFSA BIOHAZ Panel, 2013).

The toxicity studies have been conducted with an old preparation derived from an old manufacturing site that, although not compliant with European Commission (2012) for several pathogen indicators, was considered for an extrapolation to the new preparation. Further information is not required provided that adequate quality control is undertaken on the batches produced, ensuring that toxicologically relevant pathogenic contaminants are kept below levels internationally recognised as safe (see Section 1).

*Ampelomyces quisqualis* strain AQ10 (and consequently also AQ10⁰, the representative formulation) showed no evidence of toxicity, pathogenicity or infectivity following acute oral, intratracheal or intraperitoneal administration as well as following 13-week consecutive dosing in rats. Acute dermal toxicity was found to be low. Equivocal results were obtained in a Magnusson and Kligman test for skin sensitisation; however, considering the limitations of such a study for microorganisms and the lack of investigation of the sensitisation by inhalation, the following warning phrase is applicable: ‘*Ampelomyces quisqualis* AQ10 may have the potential to provoke sensitising reactions’.

A dimethyl sulfoxide (DMSO) extract of *Ampelomyces quisqualis* strain AQ10 was not mutagenic in an Ames test with *Salmonella* Typhimurium or genotoxic in an *in vitro* mammalian chromosome aberration assay either in the presence or in the absence of metabolic activation. In addition, no clastogenic potential was observed in a mammalian erythrocyte micronucleus test recognised of limited validity since there was no proof of bone marrow exposure to the test substance.

In the open literature, *Ampelomyces quisqualis* was shown to produce many lytic enzymes including β-N-acetylglucosaminidase, β-glucosidase, β-1,3- and α-1,4-glucanase, ribonuclease, chitinase and acid phosphatase or hydrolytic enzymes including lipases and endonuclease. The literature search has shown that *Ampelomyces* sp. can produce other metabolites such as quinones, phenolic compounds or plant growth hormones. In addition, a genome wide transcription profiling has been reported in the open literature during host-induced germination. This study identified genes harboured by the strain AQ10 and their upregulation in the presence of the powdery mildew. However, the actual presence of these toxins has not been investigated and could not be sufficiently substantiated. Therefore, a data gap was set for the identification and quantification of secondary metabolites/toxins produced by *Ampelomyces quisqualis* strain AQ10 in the product and after application.

The derivation of toxicological reference values for the microorganism is not considered necessary based on the lack of toxicity, infectivity or pathogenicity in the available studies. Accordingly, non-
dietary exposure estimates are not necessary with regard to the exposure to the microorganism. Considering the data gap for identification and quantification of secondary metabolites/toxins produced by *Ampelomyces quisqualis* AQ10 in the product and after application, non-dietary exposure assessment to potential secondary metabolites/toxins could not be concluded.

### 3. Residues

*Ampelomyces quisqualis* strain AQ10 is naturally occurring and a hyperparasite of the Erysiphaceae family. The strain is not able to grow and to proliferate outside its host in nutrient-poor situations such as in the crop canopy (phyloplane). It does, however, grow on nutrient enriched growth media used in the manufacturing process. The strain colonises, germinates, penetrates host cells and forms pycnidia (fruiting bodies) from which conidia of *Ampelomyces quisqualis* strain AQ10 can be released after rupture of the pycnidial wall.

It is not necessary to perform a dietary consumer risk assessment for the remaining viable cells of *Ampelomyces quisqualis* strain AQ10 on the raw agricultural commodity because the latter did not show any adverse human health effects (see Section 2).

With regard to potential secondary metabolites/toxins, it has to be noted that the possibility of *Ampelomyces quisqualis* strain AQ10 to produce such compounds under different conditions (presence or absence of natural host), during germination, and during the fermentation process in the medium has not been addressed (see Sections 1 and 2). Therefore, a data gap was identified to confirm that *Ampelomyces quisqualis* strain AQ10 cannot produce (secondary) metabolites/toxins. In the absence of information on the nature of potential metabolites and their toxicological properties, a consumer risk assessment cannot be performed.

Taking into account the remaining uncertainties related to the presence of potential secondary metabolites/toxins which may be harmful to humans which resulted in the consumer risk assessment being not finalised, inclusion in Annex IV of Regulation (EC) No 396/2005\(^3\) may need further consideration by risk managers. The information considered in this renewal application including its review of the published literature, supersedes the previous consideration in the relevant EFSA reasoned opinion relating to inclusion in Annex IV (EFSA, 2008).

### 4. Environmental fate and behaviour

Satisfactory information has been provided in relation to potential interference of *Ampelomyces quisqualis* strain AQ10 with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC\(^4\) (see specific Annex VI decision making criteria in Part II Commission Regulation (EU) No 546/2011\(^5\)). As these methods require pathogenic bacteria to be identified and confirmed as absent, it was considered unlikely that a filamentous fungus or their pycnidia/conidia would interfere with methodologies used for such determinations.

The strain *Ampelomyces quisqualis* strain AQ10 is a naturally occurring fungus that parasitises hyphae, conidiophores and cleistothecia of powdery mildew diseases. It is a not genetically modified hyperparasite that parasitises hyphae, conidiophores and cleistothecia of powdery mildew diseases. Since *Ampelomyces quisqualis* is naturally occurring, 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 has not been ascertained, it would not be possible to distinguish any genetic drift from that in the wild population.

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

Some information was derived from published literature on the species *A. quisqualis* in relation to its **persistence and multiplication in soil.** The information and evidence provided is not sufficient to conclude on the potential for competitiveness, persistence and multiplication of *Ampelomyces quisqualis*.
strain AQ10 in field soil as well as soil and other growing media used in glasshouse production systems, where nutrient availability is higher than on leaves in the crop canopy. A data gap was identified for further information on this as the data are considered insufficient to conclude that *Ampelomyces quisqualis* strain AQ10 will respect the uniform principles criterion of not being expected to persist in soil or plant growing media in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years. Predicted environmental concentrations (PEC) in soil have been calculated (see Appendix A).

With respect to the **persistence and multiplication in water** only published peer reviewed literature was available. The scientific papers provided where not specifically designed to investigate the persistence of *Ampelomyces quisqualis* strain AQ10 in water. A data gap was identified as the knowledge on the persistence/multiplication/germination of *Ampelomyces quisqualis* strain AQ10 in natural surface water was insufficient, though it is acknowledged nutrient availability will be lower than in soil or other growing media. PEC surface water have been calculated considering the spray drift and runoff routes of exposure (see Appendix A).

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

Regarding **mobility** generally, vertical distribution of pycnidia/conidia through soil is unlikely to happen. Local dispersal through aerosol particles is possible.

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

*Ampelomyces* sp. are able to produce secondary metabolites such as quinones and or phenolic compounds with *A. quisqualis* also known to produce lytic enzymes. Some of these are inhibitory to fungi or bacteria, including human pathogens, i.e. have antibiotic potential.

It is not known to what extent *Ampelomyces quisqualis* strain AQ10 will produce any metabolites following its application once the spores reach soil, should they grow. As already discussed in Section 2, a paper indicated that for strain AQ10, transcripts coding for proteins related to the biosynthesis of toxic metabolites are present. It is not known whether the possible associated toxins were produced as this was not investigated. Therefore, a data gap was identified. A data gap has also been identified as it is not clear whether metabolites may be present in the product (see Section 1). It is not clear if such metabolites might fulfil the criteria according to Part B Section 7 (iv) of Commission Regulation (EU) 283/20136 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 *Ampelomyces quisqualis* strain AQ10 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 assessments being not finalised, (see Section 9).

### 5. Ecotoxicology

Signs of toxicity, infectivity and pathogenicity of *Ampelomyces quisqualis* strain AQ10 were not observed in the available studies on **birds** and **mammals**. Therefore, a low risk to birds and wild mammals was concluded for all the representative uses.

Regarding **aquatic organisms**, the available information on fish and aquatic invertebrates (acute toxicities studies) may address the toxicity of *Ampelomyces quisqualis* strain AQ10 but was not considered sufficient to address the infectivity and the pathogenicity of *A. quisqualis* strain AQ10; therefore, a data gap was identified. Sufficient information was available to address the effects on algae. The available studies did not address the effects of *Ampelomyces quisqualis* strain AQ10 to

6 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. 1–84.
aquatic plants; however, since *A. quisqualis* has no herbicidal activity and no phytotoxicity was observed in the efficacy trials, a low risk to algae and aquatic plants was concluded for all the representative uses.

A study addressing the toxicity and the pathogenicity of *Ampelomyces quisqualis* strain AQ10 to honeybees was available; however, during the peer review, this study was considered not reliable and a data gap was identified. It is noted that in the literature a study on *Bombus terrestris*, was reported, where no effects up to the highest tested dose were observed. However, it cannot be ensured that the exposure in this study would cover the representative uses since the tested doses were too low.

The available information was not sufficient to address the toxicity, infectivity and pathogenicity to non-target arthropods for *Ampelomyces quisqualis* strain AQ10 for all representative uses. Literature data were available demonstrating the absence of effects in non-target arthropods, considering these data a low risk could be concluded for non-target arthropods. Suitable studies addressing the toxicity, infectivity and pathogenicity of *Ampelomyces quisqualis* strain AQ10 to earthworms and on soil microorganisms were not available leading to a data gap.

Further information is needed to address the potential effects to non-target organisms of the secondary metabolites/toxins should they be produced by *Ampelomyces quisqualis* strain AQ10 (data gap, see also Section 4).
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                                                                 |
|---------------------------------------------|-----------------------------------------------------------------------------|------------------------------------------------------------------------------|
| *Ampelomyces quisqualis* strain AQ10        | Data gap. Information/evidence to conclude on the potential for competitiveness, persistence and multiplication of *Ampelomyces quisqualis* strain AQ10 in field soil as well as soil and other growing media used in glasshouse production systems was not available | Data gap                                                                     |
| Toxins/secondary metabolites such as quinone and or phenolic compounds | Data gap. Adequate information to address the potential for *Ampelomyces quisqualis* strain AQ10 to produce secondary metabolites/toxins including antibiotics was not available | Data gap                                                                     |

Table 2: Groundwater

| Compound (name and/or code)                  | Mobility in soil | > 0.1 µg/L at 1 m depth for the representative uses\(^{(a)}\) | Pesticidal activity | Toxicological relevance |
|---------------------------------------------|------------------|-------------------------------------------------------------|--------------------|------------------------|
| Toxins/secondary metabolites such as quinone and or phenolic compounds | Data gap pending on their identification and quantification | Data gap pending on their identification and quantification | Open                | Open                   |

\(^{(a)}\): FOCUS scenarios or a relevant lysimeter.

Table 3: Surface water and sediment

| Compound (name and/or code)                  | Ecotoxicology    |
|---------------------------------------------|------------------|
| *Ampelomyces quisqualis* strain AQ10        | Data gap         |
| Toxins/secondary metabolites such as quinone and or phenolic compounds | Data gap         |

Table 4: Air

| Compound (name and/or code)                  | Toxicology                                                                 |
|---------------------------------------------|-----------------------------------------------------------------------------|
| *Ampelomyces quisqualis* strain AQ10        | In two intratracheal studies at dose of \(7.4 \times 10^5\) CFU/animal (male) and \(7.5 \times 10^5\) CFU/animal (female) (first study) or \(3.5 \times 10^7\) CFU/animal (second study), some mortality was observed after dosing in the first study and associated to inflammation in the second study. No sign of pathogenicity or infectivity were observed. The clearance was complete from all tissues analysed 3 days after administration |
| Toxins/secondary metabolites such as quinone and or phenolic compounds | Open |

CFU: colony forming units.
7. **Data gaps identified for the representative uses evaluated**

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).

- Experimental method using molecular tools to confirm the relevance of the proposed markers in identifying *Ampelomyces quisqualis* strain AQ10 at strain level and discriminate the strain AQ10 from other closest strains of the same RFLP group or from the same lineage (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 1).
- Adequate information to address the potential for *Ampelomyces quisqualis* strain AQ10 to produce secondary metabolites/toxins including antibiotics in the product and after application was not available. (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see all Sections).
- Determination of microbial contaminants of the formulation according to SANCO/12116/2012-rev.0 before and after storage (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 1).
- Information on the microorganism’s resistance or sensitivity to antibiotics (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Sections 1 and 2).
- Information on the toxicity of secondary metabolites/toxins is missing and would be required should they be produced (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 2).
- Information/evidence to conclude on the potential for competitiveness, persistence and multiplication of *Ampelomyces quisqualis* AQ10 in field soil was not available (relevant for all representative field uses evaluated; submission date proposed by the applicant: unknown; see Section 4).
- Information/evidence to conclude on the potential for competitiveness, persistence and multiplication of *Ampelomyces quisqualis* strain AQ10 in soil and other growing media used in glasshouse production systems was not available (relevant for all representative protected uses evaluated; submission date proposed by the applicant: unknown; see Section 4).
- Information/evidence to conclude on the potential for competitiveness, persistence and multiplication of *Ampelomyces quisqualis* strain AQ10 in water was not available (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 4).
- Further information to address the infectiveness and the pathogenicity to fish and aquatic invertebrates for *Ampelomyces quisqualis* strain AQ10 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).
- Further information to address the toxicity, infectiveness and the pathogenicity to honeybees, earthworms and soil microorganisms for *Ampelomyces quisqualis* strain AQ10 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).
- Further information is needed to address the potential effects of secondary metabolites/toxins should they be produced by *Ampelomyces quisqualis* strain AQ10 to non-target organisms (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Section 5).

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

No particular conditions are proposed for the representative uses evaluated.

9. **Concerns for the representative uses evaluated**

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 information available was insufficient to demonstrate that *Ampelomyces quisqualis* strain AQ10 would respect the uniform principles criterion of not being expected to persist in soil other plant growing media or natural water systems in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years and should this not be the case, satisfy the uniform principles associated unless clause (see Section 4).

2) The production of toxins/secondary metabolites cannot be excluded. Therefore, the risk assessment cannot be finalised for operators, workers, residents, bystander, consumers and the environment including the assessment of potential groundwater exposure. (see Sections 2, 3, 4 and 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 proposed for the representative uses assessed.

9.3. Overview of the concerns identified for each representative use considered

(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.)

All columns are grey, as for the time being no safe use could be identified.

---

7 Commission Regulation (EU) No 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.
Table 5: Overview of concerns

| Representative use                              | Grapes (grape-vine and table grapes) | Field tomato, pepper, aubergine | Protected tomato, pepper, aubergine |
|--------------------------------------------------|--------------------------------------|---------------------------------|-------------------------------------|
| **Operator risk**                                | Risk identified                      | X²                              | X²                                 |
|                                                  | Assessment not finalised             |                                 | X²                                 |
| **Worker risk**                                  | Risk identified                      | X²                              | X²                                 |
|                                                  | Assessment not finalised             |                                 | X²                                 |
| **Resident/bystander risk**                      | Risk identified                      | X²                              | X²                                 |
|                                                  | Assessment not finalised             |                                 | X²                                 |
| **Consumer risk**                                | Risk identified                      | X²                              | X²                                 |
|                                                  | Assessment not finalised             |                                 | X²                                 |
| **Risk to wild non-target terrestrial vertebrates** | Risk identified                      | X²                              | X²                                 |
|                                                  | Assessment not finalised             |                                 | X²                                 |
| **Risk to wild non-target terrestrial organisms other than vertebrates** | Risk identified                      | X²                              | X²                                 |
|                                                  | Assessment not finalised             |                                 | X²                                 |
| **Risk to aquatic organisms**                    | Risk identified                      | X²                              | X²                                 |
|                                                  | Assessment not finalised             |                                 | X²                                 |
| **Groundwater exposure to active substance**     | Legal parametric value breached      |                                 |                                     |
|                                                  | Assessment not finalised             |                                 |                                     |
| **Groundwater exposure to metabolites**          | Legal parametric value breached      |                                 |                                     |
|                                                  | Parametric value of 10 µg/L breached |                                 |                                     |
|                                                  | Assessment not finalised             |                                 |                                     |

Columns are grey if no safe use can be identified. The superscript numbers relate to the numbered points indicated in Sections 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.

References

EFSA (European Food Safety Authority), 2008. Reasoned opinion first establishment of Annex IV of Regulation (EC) 396/2005. EFSA Journal 2008;6(1):115r, 161 pp. https://doi.org/10.2903/j.efsa.2008.115r

EFSA (European Food Safety Authority), 2011. Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092, 49 pp. https://doi.org/10.2903/j.efsa.2011.2092

EFSA (European Food Safety Authority), 2012. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740, 10 pp. https://doi.org/10.2903/j.efsa.2012.2740

EFSA (European Food Safety Authority), 2017. Peer review report to the conclusion regarding the peer review of the pesticide risk assessment of the active substance *Ampelomyces quisqualis* strain AQ10. Available online: www.efsa.europa.eu

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013. QPS 2013 update. EFSA Journal 2013;11(11):3449, 106 pp. https://doi.org/10.2903/j.efsa.2013.3449

European Commission, 2004. Review report for the active substance *Ampelomyces quisqualis*. Finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 8 October 2004 in view of the inclusion of *Ampelomyces quisqualis* in Annex I of Council Directive 91/414/EEC. 4205/VC/98 - final, 7 October 2005, 3 pp.

European Commission, 2012. Working document on microbial contaminant limits for microbial pest control products. SANCO/12116/2012-rev. 0, September 2012.

European Commission, 2014. Guidance document on the renewal of approval of active substances to be assessed in compliance with Regulation (EU) No 844/2012. SANCO/2012/11251-rev. 4, 12 December 2014.
European Commission, 2015. Guidance document on criteria for the inclusion of active substances into Annex IV of Regulation (EC) No 396/2005. SANCO/11188/2013-rev. 2, 14 September 2015.

France, 2016. Renewal Assessment Report (RAR) on the active substance *Ampelomyces quisqualis* strain AQ10 prepared by the rapporteur Member State France, in the framework of Commission Implementing Regulation (EU) No 844/2012, November 2016. Available online: www.efsa.europa.eu

France, 2017. Revised Renewal Assessment Report (RAR) on *Ampelomyces quisqualis* strain AQ10 prepared by the rapporteur Member State France in the framework of Regulation (EC) No 844/2012, September 2017. Available online: www.efsa.europa.eu

**Abbreviations**

| Abbreviation | Description                          |
|--------------|--------------------------------------|
| CFU          | colony-forming units                 |
| CNCM         | Collection Nationale de Cultures de Micro-organismes |
| DMSO         | dimethyl sulfoxide                    |
| EEC          | European Economic Community           |
| FOCUS        | Forum for the Co-ordination of Pesticide Fate Models and their Use |
| ISSR         | inter-simple sequence repeat          |
| ITS          | internal transcribed spacer           |
| MPCA         | active agent of the microbial pest control product |
| MPCCP        | microbial pest control product        |
| PEC          | predicted environmental concentration |
| PEC_{soil}   | predicted environmental concentration in soil |
| PEC_{sw}     | predicted environmental concentration in surface water |
| RAR          | Renewal Assessment Report             |
| RFLP         | restriction fragment length polymorphism |
| RMS          | rapporteur Member State               |
| SSR          | simple sequence repeat                |
| WG           | water-dispersible granule             |

Peer review of the pesticide risk assessment of the active substance *Ampelomyces quisqualis* strain AQ10
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.2017.5078
### Appendix B – Used compound codes

| Code/trivial name(a) | Chemical name/SMILES notation | Structural formula |
|---------------------|-------------------------------|--------------------|
| —                   | —                             | —                  |