

CONCLUSION ON PESTICIDE PEER REVIEW

Conclusion on the peer review of the pesticide risk assessment of the active substance *Isaria fumosorosea* strain Apopka 97 (formerly *Paecilomyces fumosoroseus* Apopka strain 97)¹

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ABSTRACT

The conclusions of the European Food Safety Authority (EFSA) following the peer review of the initial risk assessments carried out by the competent authority of the rapporteur Member State Belgium, for the pesticide active substance *Isaria fumosorosea* strain Apopka 97 are reported. The context of the peer review was that required by Commission Regulation (EU) No 1141/2010 as amended by Commission Implementing Regulation (EU) No 380/2013. The conclusions were reached on the basis of the evaluation of the representative uses of *Isaria fumosorosea* strain Apopka 97 as an insecticide on cucumbers and tomatoes grown in glasshouses. The reliable endpoints concluded as being appropriate for use in regulatory risk assessment, derived from the available studies and literature in the dossier peer reviewed, are presented. Missing information identified as being required by the regulatory framework is listed. The production of toxins/secondary metabolites cannot be excluded and therefore the risk assessment cannot be finalised for humans and the environment. The surface water exposure assessment and consequently the risk assessment for aquatic organisms are not finalised. The risk assessment for organisms involved in biological methods for sewage treatment is not finalised.

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KEY WORDS

Isaria fumosorosea strain Apopka 97, peer review, risk assessment, pesticide, insecticide

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SUMMARY

Commission Regulation (EU) No 1141/2010 (hereinafter referred to as ‘the Regulation’), as amended by Commission Implementing Regulation (EU) No 380/2013, lays down the procedure for the renewal of the approval of a second group of active substances in Annex I to Council Directive 91/414/EEC and establishes the list of those substances. *Isaria fumosorosea* strain Apopka 97 is one of the active substances listed in the Regulation.

The RMS provided its initial evaluation of the dossier on *Isaria fumosorosea* strain Apopka 97 in the Renewal Assessment Report (RAR), which was received by the EFSA on 3 June 2013. The peer review was initiated on 11 June 2013 by dispatching the RAR for consultation of the Member States and the applicant Mitsui Agriscience.

Following consideration of the comments received on the RAR, it was concluded that there was no need to conduct an expert consultation and EFSA should adopt a conclusion on whether *Isaria fumosorosea* strain Apopka 97 can be expected to meet the conditions provided for in Article 4 of Regulation (EC) No 1107/2009 of the European Parliament and the Council.

The conclusions laid down in this report were reached on the basis of the evaluation of the representative uses of *Isaria fumosorosea* strain Apopka 97 as an insecticide on cucumbers and tomatoes grown in glasshouses, as proposed by the applicant. Full details of the representative uses can be found in Appendix A to this report.

In the area of identity, physical/chemical/technical properties and methods of analysis several data gaps were identified for the microorganism and the formulation.

In the area of mammalian toxicity a data gap resulting in an issue that could not be finalised was identified to address non-consumer risk assessment to toxins/secondary metabolites.

In the area of residues and consumer exposure a data gap resulting in an issue that could not be finalised was identified to address consumer risk assessment to toxins/secondary metabolites.

The information on fate and behaviour in the environment was not sufficient to characterise the competitiveness/persistence of *Isaria fumosorosea* strain Apopka 97 in the aquatic compartment in the context of the representative use assessed, therefore a data gap was identified. Satisfactory information to demonstrate that, under the conditions of use, any toxins/secondary metabolites produced by *I. fumosorosea* strain Apopka 97 (which may include beauverolides) will not occur in the environmental compartments in concentrations considerably higher than under natural conditions was also missing. Consequently further data on the persistence, transformation and mobility of these compounds may be needed in order to assess the potential for groundwater contamination and surface water exposure.

A data gap was identified for a risk assessment to address the risk to non-target organisms from secondary metabolites/toxins. A low risk from infectivity and pathogenicity to birds, wild mammals, wild populations of bees, non-target arthropods, earthworms, soil microorganisms and non-target plants was concluded. Data gaps were identified to address the risk to aquatic organisms, pollinators used in glasshouses and sewage treatment organisms.

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BACKGROUND

Commission Regulation (EU) No 1141/2010³ (hereinafter referred to as ‘the Regulation’), as amended by Commission Implementing Regulation (EU) No 380/2013⁴, lays down the detailed rules for the procedure of the renewal of the approval of a second group of active substances. This regulates for the European Food Safety Authority (EFSA) the procedure for organising the consultation of Member States and the applicant for comments on the initial evaluation in the Renewal Assessment Report (RAR) provided by the rapporteur Member State (RMS), and the organisation of an expert consultation, where appropriate.

In accordance with Article 16 of the Regulation, if mandated, EFSA is required to adopt a conclusion on whether the active substance is expected to meet the conditions provided for in Article 4 of Regulation (EC) No 1107/2009⁵ within 6 months from the receipt of the mandate, subject to an extension of up to 9 months where additional information is required to be submitted by the applicants in accordance with Article 16(3).

In accordance with Article 4 of the Regulation Belgium (hereinafter referred to as the ‘RMS’) received an application from Mitsui Agriscience for the renewal of approval of the active substance *Isaria fumosorosea* strain Apopka 97. Complying with Article 11 of the Regulation, the RMS checked the completeness of the dossier and informed the applicant, the Commission and EFSA about the admissibility.

The RMS provided its initial evaluation of the dossier on *Isaria fumosorosea* strain Apopka 97 in the RAR, which was received by the EFSA on 3 June 2013 (Belgium, 2013). The peer review was initiated on 11 June 2013 by dispatching the RAR to Member States and the applicant Mitsui Agriscience for consultation and comments. In addition, the EFSA conducted a public consultation on the RAR. The comments received were collated by the EFSA and 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 16(3) of the Regulation were considered in a telephone conference between the EFSA, the RMS, and the European Commission on 30 September 2013. 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 there was no need to conduct an expert consultation. According to Art. 16(2) of the Regulation COM decided to consult the EFSA. The mandate was received on 11 October 2013.

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 the additional information to be submitted by the applicant, were compiled by the EFSA in the format of an Evaluation Table.

The conclusions arising from the consideration by the EFSA, and as appropriate by the RMS, of the points identified in the Evaluation Table, were reported in the final column of the Evaluation Table.

³ Commission Regulation (EU) No 1141/2010 of 7 December 2010 laying down the procedure for the renewal of the inclusion of a second group of active substances in Annex I to Council Directive 91/414/EEC and establishing the list of those substances. OJ L 322, 8.12.2011, p. 10-19.

⁴ Commission Implementing Regulation (EU) No 380/2013 of 25 April 2013 amending Regulation (EU) No 1141/2010 as regards the submission of the supplementary complete dossier to the Authority, the other Member States and the Commission. OJ L 116, 26.4.2013, p.4.

⁵ 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.

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 – April 2014.

This conclusion report summarises the outcome of the peer review of the risk assessment on the active substance and the representative formulation evaluated on the basis of the representative uses as an insecticide on cucumbers and tomatoes grown in glasshouses, as proposed by the applicant. A list of the relevant end points for the active substance as well as the formulation is provided in Appendix A. In addition, a key supporting document to this conclusion is the Peer Review Report, 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 (EFSA, 2014) comprises the following documents, in which all views expressed during the course of the peer review, including minority views, can be found:

- the comments received on the RAR,
- the Reporting Table (1 October 2013),
- the Evaluation Table (16 April 2014),
- 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 addendum (compiled version of February 2014 containing all individually submitted addenda (Belgium, 2014)) and the Peer Review Report, both documents are considered respectively as background documents A and B to this conclusion.

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 to have regulatory access to the information on which this conclusion report is based.

THE ACTIVE SUBSTANCE AND THE FORMULATED PRODUCT

The *Isaria fumosorosea* strain under review is Apopka 97. It was isolated from mealy bug in America.

The representative formulated product for the evaluation was 'Preferal' a water dispersible granule (WG) containing 2×10^9 CFU/g.

The representative use evaluated is on tomatoes and cucumbers grown in glasshouses. Full details of the GAP can be found in the list of end points in Appendix A.

CONCLUSIONS OF THE EVALUATION

1. Identity, physical/chemical/technical properties and methods of analysis

The following guidance document was followed in the production of this conclusion: OECD Issue Paper on Microbial Contaminant Limits for Microbial Pest Control Products, Series on Pesticides No. 65, ENV/JM/MONO(2011)43 (OECD, 2011).

The strain Apopka is kept in the American Type Culture Collection under the name *Paecilomyces fumosoroseus* Apopka ATCC 20874. This name is the old outdated name for the organism.

The strain is not a human pathogen and is not related to known human pathogens. The strain is not able to grow at 35 °C and above.

The content of contaminating microorganisms was shown to comply with the OECD issue paper but the methods were not validated. Sufficient validated methods were available for identification of the microorganism but the method for quantification needs to be validated. Also the methods for the metabolites, once identified, need to be validated and a data gap was identified.

For toxins/secondary metabolites further investigation of what this specific strain produces is needed.

For the formulation, data gaps were identified for studies investigating the shelf-life, sprayability, attrition resistance, pH, dust content and also the validation of the method of analysis for the microorganism. It was also stated that the formulation is premixed with water and left for a period of time to settle and only the supernatant is added to the spray tank. Data are needed to demonstrate that the spores stay in the supernatant and do not sediment out.

Methods of analysis for residues in soil, water and air are not available and a data gap was identified.

2. Mammalian toxicity

The applicant submitted a basic set of valid acute toxicity studies to evaluate the risk of the microorganism *Isaria fumosorosea* strain Apopka 97. In these studies, there was no indication for acute toxicity of the microorganism following oral, intratracheal or intraperitoneal administration of high dose levels to rats. The microorganism did not show either the potential to invade the body of the rats and did not proliferate therein excluding the risk of infection. The microorganism is neither a skin nor eye irritant. It was not a skin sensitizer in the Buehler test; however, in the absence of a reliable test for sensitisation, microorganisms in general are considered sensitising unless there is sufficient experimental evidence that there is no concern. The Ames test with a sonicated suspension of the microorganism gave a negative response.

Based on the lack of significant toxicity, infectivity or pathogenicity in the available toxicological studies the setting of health-based reference values for the microorganism is not needed. Therefore, operator and worker exposure estimates to the microorganism are not needed.

Concerning the production of toxins/secondary metabolites the microorganism is known to be capable of producing certain toxins/secondary metabolites like beauverolides that could be present in the

product. The RMS considered beauverolides are of no concern on the basis of an Ames test (negative) and acute oral mouse toxicity study ($LD_{50} > 5000$ mg/kg bw) with a mixture of beauverolides from strain Apopka 97. However, EFSA considered that the information provided on beauverolides was not sufficient to determine the toxicological profile of these toxins/secondary metabolites, also taking into account that the available studies showed some limitations (i.e. vehicle used in the acute oral toxicity study and limited number of doses tested in the Ames test). EFSA supports the proposal that each fermentation broth should be checked to ensure that no toxins/secondary metabolites are present in the product to exclude the risk for operators. It is also unknown whether workers and consumers can be exposed to these or other toxins/secondary metabolites (see section 3 and 4). Given all these uncertainties, EFSA concluded that the human-health risk assessment to toxins/secondary metabolites produced by *Isaria fumosorosea* strain Apopka 97 cannot be finalised.

3. Residues

The microorganism itself is not pathogenic and is not related to any known pathogens. The consumer risk assessment cannot be finalised until the outstanding issues regarding the production of toxins/secondary metabolites are addressed and it is confirmed by the toxicological assessment that a quantitative consumer risk assessment is not necessary.

4. Environmental fate and behaviour

No information has been provided in relation to potential interference of *Isaria fumosorosea* strain Apopka 97 with the analytical systems for the control of the quality of drinking water provided for in Council Directive 98/83/EC⁶ (as is needed by a specific Annex VI decision making criteria in part B of Commission Regulation (EU) No 546/2011⁷). However as these methods require pathogenic bacteria to be identified and confirmed as absent, it is probably unlikely that filamentous fungi or their conidia would interfere with methodologies used for such determinations.

No information has been provided on the potential transfer of genetic material from *Isaria fumosorosea* strain Apopka 97 to other organisms (see specific Annex VI decision making criteria in part B of Commission Regulation (EU) No 546/2011⁷). Fungi, such as *I. fumosorosea* are not expected to possess plasmids in their cytoplasm (only mitochondrial plasmids are known). Consequently it might be expected that the potential for transfer of genetic material from *Isaria* sp. to other organisms is unlikely.

4.1. Fate and behaviour in the environment of the microorganism

Isaria fumosorosea is a naturally soil occurring fungus in most countries of the world. However, a quantitative assessment on the level of natural occurrence of the microorganism in soil was not precisely defined in the supplied literature.

A study on the **persistence and multiplication in soil** of the specific strain *I. fumosorosea* strain Apopka 97 in dried greenhouse soil at 26°C was evaluated in the regulatory dossier. Different soil moisture levels and application levels were tested. Results showed that the production of conidia was not related to the different levels of soil moisture and of the initial amounts of blastospores added to the soil. At the last sampling date (38 days) no viable conidia were detected by colony counts.

A data gap was identified for information on viability/population dynamics in hydroponic growing media for the use of *I. fumosorosea* strain Apopka 97.

⁶ Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.1998, p.32-54.

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. 153-175.

Little information has been provided on **persistence and multiplication in water** of *Isaria fumosorosea* strain Apopka 97. A study on the viability of *Isaria fumosorosea* strain FE 9901 blastospores in tap water showed a loss in CFU within 48 hours at room temperature. In another study the survivorship of spores of *I. fumosorosea* relative to elevated temperature (40°C), sonication and UV irradiation was investigated. The findings of this study indicated that blastospores were less stable than conidia when they were stored under dried state and stored in water, but do not address fate, survival and persistence in natural surface water. Consequently a data gap has been identified. In line with the assessment performed previously for other microorganisms, it should be noted that the use in greenhouses, does not preclude surface water exposure as greenhouses have to be ventilated, they are not closed systems.

Except at the time of spraying *I. fumosorosea* would not be expected to be present in **air**. On the basis of the information presented in the RAR, it can be assumed that viability of *I. fumosorosea* conidia in the air or epigeal habitats is mainly affected by the UV-B portion of the solar spectrum.

Regarding **mobility in soil** movement in both the horizontal and vertical direction is possible by soil arthropods. A groundwater exposure assessment is not necessary for the organism since *I. fumosorosea* is neither pathogenic nor toxic to humans.

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

If, under the conditions of use, relevant metabolites are present in or produced by the microorganism, data requirements and the corresponding risk assessment as outlined under Commission Regulation (EU) No 544/2011⁸ part A point 7 need to be fulfilled if all the following conditions are met:

- 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 *I. fumosorosea* 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 544/2011 part A point 7 (standard data requirements and assessment mandatory for chemical plant protection active substances) are triggered. Consequently a data gap was identified.

5. Ecotoxicology

A data gap was concluded for information regarding formation of toxins/secondary metabolites in the production process and the environment (see section 1 and 4). Pending on the outcome of this data gap, a further data gap was identified to provide risk assessment to address the risk to non-target organisms from metabolites and toxins.

Data were available which were sufficient to demonstrate that *Isaria fumosorosea* strain Apopka 97 is not infectious or pathogenic to birds and mammals. Furthermore, exposure of terrestrial vertebrates following the representative use in glasshouses of *Isaria fumosorosea* strain Apopka 97 is not expected. Consequently, a low risk to birds and mammals from infectivity and pathogenicity was concluded.

⁸ Commission Regulation (EU) No 544/2011 of 10 June 2011 implementing Commission Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances. OJ L 155, 11.6.2011, p. 1-66.

Acute toxicity data with fish and *Daphnia magna* were available with *Isaria fumosorosea*, however, due to the study length and the study methodology followed, these studies cannot be considered sufficient to address the possibility of infectivity and pathogenicity. No data were available for algae. Contamination of surface water following the representative use of *Isaria fumosorosea* strain Apopka 97 cannot be excluded (see section 4) and therefore a data gap was concluded for further information to address the potential of infectivity and pathogenicity to aquatic organisms.

The representative uses of *Isaria fumosorosea* strain Apopka 97 is on tomatoes and cucumbers in glasshouses, therefore, exposure to natural populations of honey bees, foliar-dwelling non-target arthropods and non-target terrestrial plants was considered to be unlikely. Therefore, a low risk to honey bees, foliar-dwelling non-target arthropods and non-target terrestrial plants was concluded.

Insufficient information was available to assess the risk to pollinators which may be introduced to glasshouses as part of integrated pest management (IPM) techniques. A data gap is therefore concluded for further information to address the risk to pollinators and other beneficial arthropods if *Isaria fumosorosea* strain Apopka 97 is to be used in glasshouses with pollinators or IPM techniques.

No data were available investigating the potential for *Isaria fumosorosea* strain Apopka 97 to be pathogenic or infectious to earthworms and other soil-dwelling organisms. However, exposure of *Isaria fumosorosea* strain Apopka 97 to soil is not expected for the representative glasshouse uses and therefore a low risk to earthworms and soil microorganisms was concluded.

No information to address the risk to organisms involved in biological methods for sewage treatment was available. The exposure to sewage treatment plants from the representative uses of *Isaria fumosorosea* strain Apopka 97 cannot be excluded; therefore, a data gap and an issue not finalised was identified to address the risk to organisms involved in biological methods for sewage treatment.

6. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments

6.1. Soil

Compound (name and/or code)	Persistence	Ecotoxicology
<i>Isaria fumosorosea</i> strain Apopka 97	In a study in dried greenhouse soil at 26°C, at the last sampling date (38 days) no viable conidia were detected by colony counts.	No data available.
Relevant toxins or secondary metabolites (including beauverolides)	No data available.	No data available.

6.2. Ground water

Compound (name and/or code)	Mobility in soil	>0.1 µg/L 1m depth for the representative uses (at least one FOCUS scenario or relevant lysimeter)	Pesticidal activity	Toxicological relevance	Ecotoxicological activity
Relevant toxins or secondary metabolites (including beauverolides)	No data available.	No data available. Data gap	No data available.	No sufficient and valid data are available to exclude the relevance of beauverolides. For other toxins/secondary metabolites no data are available.	No data available.

6.3. Surface water and sediment

Compound (name and/or code)	Ecotoxicology
<i>Isaria fumosorosea</i> strain Apopka 97	Data gap identified for information to address the potential of infectivity and pathogenicity to aquatic organisms.
Relevant toxins or secondary metabolites (including beauverolides)	No data available.

6.4. Air

Compound (name and/or code)	Toxicology
<i>Isaria fumosorosea</i> strain Apopka 97	Acute intratracheal LD ₅₀ > 10 ⁶ CFU/rat. No evidence of adverse effects, colonisation or infectivity.
Relevant toxins or secondary metabolites (including beauverolides)	No data available.

7. List of studies to be generated, still ongoing or available but not peer reviewed

This is a complete list of the data gaps identified during the peer review process, including those areas where 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 of the European Parliament and of the Council concerning information on potentially harmful effects).

- Data to address the production of toxins/secondary metabolites produced by this strain and to address the risk to humans (including operators, workers and consumers) (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see section 1, 2, 3).
- Shelf-life study (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see section 1).
- For the formulation, studies investigating: sprayability, attrition resistance, pH, dust content (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see section 1).
- Data to address the spore content of the supernatant (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see section 1).
- A validated method of analysis for the metabolites (once identified), including beauverolides, is required (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see section 1).
- Validation data for the methods for the microorganism in the technical material and the formulation as well as for contaminating microorganisms (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see section 1).
- Validated methods for soil, water and air (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see section 1).
- Information on viability/population dynamics of *Isaria fumosorosea* strain Apopka 97 in hydroponic growing media such as rockwool was not available (relevant for representative uses where crops are grown in hydroponic systems; submission date proposed by the applicant: unknown; see section 4).
- Information to address the fate, survival and persistence in natural surface water of *Isaria fumosorosea* strain Apopka 97 (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see section 4).
- Satisfactory information to demonstrate that, under the conditions of use, any toxins/secondary metabolites produced by *Isaria fumosorosea* strain Apopka 97 (which may include beauverolides) will not occur in the environmental compartments in concentrations considerably higher than under natural conditions. Further data on the persistence, transformation and mobility of these compounds may be needed in order to assess the potential for groundwater contamination, surface water exposure and the relevant ecotoxicological risk assessment (relevant for all representative uses; submission date proposed by the applicant: unknown; see sections 4 and 5).

- Further information is required to address the potential of infectivity and pathogenicity to aquatic organisms (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see section 5).
- Data to address the risk to organisms involved in biological methods for sewage treatment are required (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see section 5).
- Data to address the risk to pollinators and other beneficial arthropods is required if *Isaria fumosorosea* is to be used in glasshouses with pollinators or IPM techniques (relevant for all representative uses evaluated; submission date proposed by the applicant: first quarter of 2014; see section 5).

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

None

9. Concerns

9.1. Issues that could not be finalised

An issue is listed as an issue that could not be finalised where 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 of the European Parliament and of the Council and as set out in Commission Regulation (EU) No 546/2011⁹, and where 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).

1. The production of toxins/secondary metabolites of unknown toxicity cannot be excluded and therefore the risk assessment cannot be finalised for humans (including operators, workers and consumers) and the environment, including the assessment of potential groundwater contamination and residues in plants.
2. The surface water exposure assessment and consequently the risk assessment for aquatic organisms, is not finalised.
3. The risk assessment for organisms involved in biological methods for sewage treatment is not finalised.

9.2. Critical areas of concern

An issue is listed as a critical area of concern where 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 of the European Parliament and of the Council and as set out in Commission Regulation (EU) No 546/2011, and where this assessment does not permit to conclude 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 where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level

⁹ 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.

does not permit to conclude 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.

No critical areas of concern have been identified 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 this table.)

Representative use		Tomato/cucumber grown in glasshouses
Operator risk	Risk identified	
	Assessment not finalised	X ¹
Worker risk	Risk identified	
	Assessment not finalised	X ¹
Bystander risk	Risk identified	
	Assessment not finalised	
Consumer risk	Risk identified	
	Assessment not finalised	X ¹
Risk to wild non target terrestrial vertebrates	Risk identified	
	Assessment not finalised	X ¹
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified	
	Assessment not finalised	X ^{1, 3}
Risk to aquatic organisms	Risk identified	
	Assessment not finalised	X ^{1,2}
Groundwater exposure active substance	Legal parametric value breached	
	Assessment not finalised	
Groundwater exposure metabolites	Legal parametric value breached ^(a)	
	Parametric value of 10µg/L ^(a) breached	
	Assessment not finalised	X ¹
Comments/Remarks		

The superscript numbers in this table relate to the numbered points indicated in sections 9.1 and 9.2. Where there is no superscript number see sections 2 to 6 for further information.

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

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- OECD (Organisation for Economic Co-Operation and Development), 2011. Issue Paper on Microbial Contaminant Limits for Microbial Pest Control Products, Series on Pesticides No. 65, ENV/JM/MONO(2011)43, 12 October 2011.

APPENDICES

APPENDIX A – LIST OF END POINTS FOR THE ACTIVE SUBSTANCE AND THE REPRESENTATIVE FORMULATION

1. Identity, Biological properties, Details of uses, Further information, and Proposed Classification and Labelling

Active micro-organism:	<i>Isaria fumosorosea</i> strain Apopka 97
Function (e.g. control of fungi):	Insecticide, control of whitefly
Rapporteur Member State	Belgium
Uses:	In glasshouse, spot treatment against whitefly; 2*10 ⁹ CFU/g formulation
Known or new organisms:	Known Revision of genus <i>Paecilomyces</i> : species included into <i>Isaria</i> clade followed by change of genera name (<i>Isaria</i>) and partially also in species name (<i>fumosorosea</i> instead of <i>fumosoroseus</i>). New name <i>Isaria fumosorosea</i>
Known opportunistic:	No
Toxin production	Secondary metabolites such as beauverolides have been detected. Open for other toxins/secondary metabolites.
Resistance:	Not applicable to fungi
Resting stages:	Production of conidia or resting spores in/or on the host
Production control:	- Check of product quality - Determination of pathogenicity on whiteflies. - Absence of secondary metabolites must be checked in each fermentation broth by HPLC. The quality of the HPLC method must be checked. New method for quantification of secondary metabolite beauverolide I is provided.

Identity of the Microbial Pest control Agent / Active substance (OECD data point IIM 1)

Name of the organism:	<i>Isaria fumosorosea</i>
Taxonomy:	Genus: <i>Paecilomyces</i> Family: Deuteromycotina Section: <i>Isarioidea</i> Kingdom: Fungi Subkingdom: Dikarya Division/phylum: Ascomycota Subphylum: Pezizomycotina Class: Sordariomycetes Subclass: Hypocreomycetidae Order: Hypocreales Family: Cordycipitaceae Genus: <i>Isaria</i> Species: <i>Isaria fumosorosea</i> Wize 1904
Species, subspecies, strain	<i>Isaria fumosorosea</i> (Wize) Brown & Smith strain Apopka 97; PFR 97 or CG 170 or ATCC20874
Identification:	Morphological criteria seen by classical microscopy methods;

Culture collection:	RAPD analysis: <i>Isaria fumosorosea</i> strain PFR97 Apopka is characterised with eight 10-mer primers from a RAPD kit and six 10-mer primers from another RAPD kit. These primers revealed a total of 167 repeatable bands that were used to construct strain specific RAPD patterns.
Minimum and maximum concentration of the micro-organism used for manufacturing of the formulated product (cfu/g; cfu/L, etc.):	American Type Culture Collection (ATCC) under the name <i>Paecilomyces fumosoroseus</i> Apopka ATCC 20874.
Identity and content of relevant impurities in the technical grade micro-organism:	1.0 x 10 ⁸ CFU/ml and 2.5 x 10 ⁹ CFU/ml
Is the MCPA genetically modified; if so provide type of modification	The technical grade material does not contain relevant impurities
	No modification

Biological properties of the micro-organism (OECD data point IIM 2)

Origin and natural occurrence, background level:	<ul style="list-style-type: none"> - Isolated from <i>Phenococcus solani</i> Ferris (mealy bug), on gynura in a greenhouse located in Apopka - Widely distributed throughout the world; - Isolated from many arthropods, mainly <i>Lepidoptera</i> from the air, water, plants, and other fungi and often from soil. - Found in various soil types at very low densities
Target organism(s):	Greenhouse whitefly (<i>trialeurodes vaporariorum</i>)
Mode of action:	Hyper parasitism; production of enzymes to penetrate insect cuticle and internal growth within insect and mechanically disruption of host.
Host specificity:	Very wide range of hosts. Intended use against <i>Trialeurodes vaporariorum</i> . <i>I.fumosorosea</i> is a fungus found in soil worldwide.
Life cycle:	Asexual life cycle; infective unit is blastospore
Infectivity, dispersal and colonisation ability:	Whitefly is most susceptible to infection in the N1 and N4 nymph stage. The infection cycle is rapid and symptoms of infection are apparent within 24-48 h after the conidia contact the insect. Hyphal bodies are formed in the host hemocoel followed by mycelium formation on the dorsum of the insect body within 48 h.
Relationship to known pathogens:	The genus <i>Paecilomyces</i> belongs to the group of opportunistic pathogens. They are of inherently low virulence and produce disease when the host resistance to infection is diminished (immunosuppressed people)
Genetic stability:	Stable
Production of relevant metabolites/toxins:	Open
Resistance/sensitivity to antibiotics/anti-microbial agents used in human or veterinary medicine:	<i>Isaria fumosorosea</i> shows variable resistance to amphotericin B, flucytosine and the triazole based drugs as well as terbinafine and voriconazole.

Classification and proposed labelling (Symbol, Indication of danger, Risk phrases, Safety phrases)

with regard to toxicological data of the micro-organism:	Microorganisms may have the potential to provoke sensitising reactions.
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Summary of representative uses evaluated

Crop and/or situation	Member State or Country	Product name	Pests or group of pests controlled	Formulation		Application				Application rate per treatment			PHI (days)	Remarks
				Type	Conc. of MPCA	Method kind	Growth stage & season	Number min max	Interval between applications (min)	Kg MPCP/ha min max	water L/ha min max	Kg MPCA/ha min max		
Tomato/cucumber/glasshouse	Europe	Preferal ®	Whitefly in IPM	WG	2x10 ⁹ CFU/g = 200g/kg	spraying	first whitefly larvae	2 to 3 treatments	15 days	1-3 kg	1000 to 3000	2.10 ¹² -6.10 ¹² CFU/ha corresponding to 200-600 g/ha	Not relevant	Suitable for IPM

2. Analytical methods

Analytical methods for the micro-organism (Annex IIM 4.2, 4.3; IIIM 5.3)

Manufactured micro-organism (principle of method):	Standard Operating Procedures provided for Microbiological method Open for validation
Impurities and contaminating micro-organisms in manufactured material (principle of method):	Microbiological method using specific media and method to identify production of secondary metabolites Open for validation
Microbial plant protection product (principle of method):	Microbiological method Open for validation

Analytical methods for residues (viable and non-viable) (Annex IIM 4.5)

of the active micro-organism (principle of method):	Open
of relevant metabolites (principle of method):	Open for toxins/secondary metabolites.

3. Impact on Human and Animal Health

Effects on Human Health (Annex IIM5; IIM7)

Medical data, surveillance and observations	Not infectious – allergic diseases were reported in open literature.
Sensitisation (experience in humans and study results; type of study):	No incidence during production and testing has been reported. Sensitiser (no reliable studies available, classification based on general assumptions for microorganisms)

Acute toxicity, pathogenicity and infectiveness

Acute oral toxicity, pathogenicity and infectiveness	LD ₅₀ > 1.7 x 10 ⁶ CFU/rat. No evidence of adverse effects, colonisation or infectivity.
Acute intratracheal toxicity, pathogenicity and infectiveness:	LD ₅₀ > 10 ⁶ CFU/rat. No evidence of adverse effects, colonisation or infectivity.
Intraperitoneal single dose	LD ₅₀ > 1.6 x 10 ⁷ CFU/rat. No evidence of adverse effects, colonisation or infectivity.
Genotoxicity	Negative Ames test. Further data may be required pending on the identification/quantification of toxins/secondary metabolites.
Cell culture study:	No tests performed; not necessary.
Information on short term toxicity and pathogenicity	No data. Further data may be required pending on the identification/quantification of toxins/secondary metabolites.

Specific toxicity, pathogenicity and infectiveness studies (Annex IIM, point 5.5)

Dermal toxicity:	Transient irritation completely reversible by application of 2 g blastospores + mycelium/rabbit
Eye irritation	Not irritating at 0.1 mL blastospores and mycelium/rabbit

Reference values

AOEL:	Not necessary for the microorganism. Open for the secondary metabolites/toxins
ADI:	Not necessary for the microorganism. Open for the secondary metabolites/toxins
ARfD:	Not necessary for the microorganism. Open for the secondary metabolites/toxins

Exposure scenarios (including method of calculation)

Application method:	WG 20% a.s.; 2.10 ⁹ CFU/g; 1-3 kg formulation/ha Spray concentration: 100 g formulation/hl; 1-3 applications/season.
Operator:	Operator exposure estimates to the microorganism are not needed. Each fermentation broth should be checked to ensure that no toxins/secondary metabolites are present in the product to exclude to potential risk for operators to secondary metabolites/toxins.
Workers:	Worker exposure estimates to the microorganism are not needed Worker risk assessment to secondary metabolites/toxins cannot be finalised
Bystanders/Residents:	Since the product is intended for use only in glasshouses, the possibility of inadvertent presence of bystanders/residents can be reasonably excluded.

4. Residues

Non viable residues	Open for toxins/secondary metabolites
Viable residues	No risk for the consumer is expected from the organism itself i.e. it is not pathogenic

5. Fate and behaviour in the environment (Annex IIM 7; IIM 9)

Persistence and multiplication

in soil:	The production of conidia is not related to the different levels of soil moisture and to the initial amounts of blastospores added to the soil. At day 38 no viable conidia were detected by colony counts. Mycosis is not related to initial amounts of blastospores added to the soil. Mycosis is not dependent from the moisture level of the soil.
in water:	Data gap. The data on measurement or observation of persistence and multiplication in natural surface water provided was insufficient. <i>Isaria fumosorosea</i> strain Fe 9901 does not grow in tap water.
in air:	<i>I. fumosorosea</i> is not reported from air samples in any of the papers listed and this may be because the fungus is an insect parasite.

Mobility

It is expected that the mobility of the strain *Isaria fumosorosea* Apopka 97 is limited since the only intended uses are in greenhouses. This strain is a fungus found in soil worldwide. Blastospores are short living organisms.
The fungus *I. fumosorosea* has no self mobility.

6. Effects on Non-target Organisms (Annex IIM 8; IIM 10)

Effects on terrestrial vertebrates	
Effects on birds	NOED northern bobwhite <i>Colinus virginianus</i> = 2.5×10^9 CFU/kg for 5 days, observation period was for 30 days. No signs of pathogenicity or toxicity were observed.
Risk assessment:	The risk to birds and wild mammals is assessed as low.
Effects on aquatic organisms	
Effects on fish:	96 hour LC_{50} <i>Oncorhynchus mykiss</i> > 14.7×10^8 CFU/L 96 hour NOEC <i>Oncorhynchus mykiss</i> = 14.7×10^8 CFU/L
Risk assessment:	Data gap.
Effects on freshwater invertebrates:	48 hour EC_{50} <i>Daphnia magna</i> > 14.7×10^8 CFU/L 48 hour NOEC <i>Daphnia magna</i> = 14.7×10^8 CFU/L
Risk assessment:	Data gap.
Effects on algae:	No data were submitted.
Risk assessment:	Data gap.
Effects on aquatic plants:	No data were submitted.
Effects on bees and other arthropods	
Effects on bees:	<i>Apis mellifera</i> : 48 hour oral LD_{50} > 21.63 µg a.s./bee; 48 hour contact LD_{50} > 20 µg a.s./bee
Risk assessment:	The risk to wild populations of bees is low. Data gap for pollinators which may be used in glasshouses.
Effects on terrestrial arthropods other than bees:	Supportive data included in the RAR.
Risk assessment:	The risk to terrestrial arthropods is low.
Effects on soil organisms	
Effects on other terrestrial invertebrates:	No data available.
Effects on soil micro-organisms:	No data available.
Additional studies	No further studies provided.

ABBREVIATIONS

°C	degree Celsius (centigrade)
µg	microgram
a.s.	active substance
ADI	acceptable daily intake
AOEL	acceptable operator exposure level
ARfD	acute reference dose
ATCC	American type culture collection
bw	body weight
CFU	colony forming units
EC	European Commission
EC ₅₀	effective concentration
EFSA	European Food Safety Authority
EU	European Union
g	gram
GAP	good agricultural practice
h	hour(s)
ha	hectare
hL	hectolitre
HPLC	high pressure liquid chromatography or high performance liquid chromatography
IPM	integrated pest management
kg	kilogram
L	litre
LC ₅₀	lethal concentration, median
LD ₅₀	lethal dose, median; dosis letalis media
mg	milligram
mL	millilitre
MPCA	microbial pest control agent
MPCP	microbial pest control product
NOEC	no observed effect concentration
NOED	no observed effect dose
OECD	Organisation for Economic Co-operation and Development
pH	pH-value
PHI	pre-harvest interval
RAPD	Random Amplified Polymorphic DNA
RAR	renewal assessment report
RMS	rappporteur Member State
UV	ultraviolet
WG	water dispersible granule