Renewal Assessment Report

Lecanicillium muscarium Ve6

Volume 1

January 2018

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: France

Version history

When	What
January 2018	Initial RAR

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Level 1

Lecanicillium muscarium Ve6

1 Statement of subject matter and purpose for which this report has been prepared and background information on the application

1.1 Context in which the renewal assessment report was prepared

1.1.1 Purpose for which the renewal assessment report was prepared

This Renewal Assessment Report (RAR) is prepared for the renewal of the approval of the active substance *Lecanicillium muscarium* Ve6. *Lecanicillium muscarium* Ve6 is part of the AIR4 renewal programme for active substances (Commission Implementing Regulation (EU) No 844/2012).

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

The Netherlands (as Rapporteur Member State) conducted the full evaluation and prepared the RAR for the active substance *Lecanicillium muscarium* Ve6 and the RAR was peer reviewed by the Co-Rapporteur Member State France before submission to Commission and EFSA.

1.1.3 EU Regulatory history for use in plant protection products

Lecanicillium muscarium Ve6 was evaluated as an existing active substance by the Rapporteur Member State The Netherlands. The main data submitter at that time was Koppert B.V.
Lecanicillium muscarium Ve6 is approved since 1 May 2009 (Commission Directive 2008/113/EC of 8 December 2008). The current expiry date is 30 April 2019.
The review Report - SANCO/1861/08 – rev. 3 is dated 3 June 2008
The review Report - SANCO/1861/08 – rev. 5 is dated 11 July 2014, considering new information available.
The EFSA conclusion is published on 29 January 2010 (EFSA Journal 2010; 8(1):1446, Conclusion on the peer review of the pesticide risk assessment of the active substance *Lecanicillium muscarium* strain Ve6 notified as Verticillium lecanii.

1.1.4 Evaluations carried out under other regulatory contexts

No information.

1.2 Applicant information

1.2.1 Name and address of applicant(s) for approval of the active substance

Name	Koppert B.V.
Address	Veilingweg 14 P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands
Contact person	[redacted]
Phone	[redacted]
Fax	[redacted]
Email	[redacted]

1.2.2 Producer or producers of the active substance

Name	Koppert B.V.
Address	Veilingweg 14 P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands
Contact person	[redacted]
Phone	[redacted]
Fax	[redacted]
Email	[redacted]

1.2.3 Information relating to the collective provision of dossiers

Not applicable. Koppert B.V. is the sole data submitter.

1.3 Identity of the micro-organism

1.3.1 Name and species description, strain characterisation	*Lecanicillium muscarium* Ve6
1.3.1.1 Composition of material used for manufacturing of the formulated product	
Reference to Volume 4	

1.3.1.2 Accession number in culture collection	CABI (=IMI) 268317, Westerdijk Fungal Biodiversity Institute (previous CBS) 102071, ARSEF 5128
1.3.1.3 Scientific name and taxonomic grouping, i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the micro-organism	
Taxonomy	Kingdom: Fungi, Phylum: Deuteromycotina Order: Hyphomycetes (syn. Moniliales) Genus: Lecanicillium Species: muscarium Strain : Ve6
Indigenous or non-indigenous	indigenous
Wild type	*Lecanicillium muscarium* Ve6
Spontaneous or induced mutant*	Not applicable
Genetically modified according to Directive 2001/18/EC*	No
* All known differences between the modified micro-organism and the parent wild strain must be provided	
1.3.1.4 Test procedures and criteria used for identification	
Morphological identification: Colony size 18-22 mm, white or pale yellow, cotton wool like, hyphae rarely in bundles (10 days at 20°C, Malt Extract Agar. Colony underside colourless, yellow or ochraceous. Phialids detached or in few whorls on conidiophores or slightly differentiated hyphae from the aerial mycelium, needle form, high variability in size, 12-40 * 0.8-3 µm. Conidia one-celled in heads, often parallel to phialide tip, cylindrical with both ends well rounded or ellipse, 2.3-10 * 1.0-2.6 µm. Chlamydospores absent. Spore sizes of the Mycotal-strain 4.2±0.9 µm - 1.6±0.2 µm. (6 days at 23°C, Saboureaud Dextrose agar).	
1.3.1.5 Common name or alternative and superseded names and code names used during the development	

Code no.	Used by
19-79, GCRI-79 HRI 19-79	R. Hall
Ve6	Tate and Lyle, MRL
KBV 10-88 KBV 88-M01 KV01 3*	Koppert B.V.
Verticillium lecanii Ve6 has been reclassified as *Lecanicillium muscarium* by Zare and Gams (2001).	

1.3.1.6	**Relationship to known pathogens**	*Verticillium muscarium* is not closely related to known plant or human pathogens
1.3.1.7	**Method of manufacture (synthesis pathway) of the active substance**	Reference to Volume 4
1.3.2	**Specification of the material used for manufacturing of formulated products**	Reference to Volume 4
1.3.3	**Content of the micro-organism**	The nominal content of *L. muscarium* Ve6 is 1×10^{10} spores/g.
1.3.4	**Identity and content of impurities, additives, contaminating micro-organisms**	
1.3.4.1	Significant impurities	No relevant human/mammalian metabolites or toxins present in product or being produced by *Lecanicillium muscarium* Ve6.
1.3.4.2	Relevant impurities	No relevant human/mammalian metabolites or toxins present in product or being produced by *Lecanicillium muscarium* Ve6.
1.3.4.3	Additives	No relevant human/mammalian metabolites or toxins present in product or being produced by *Lecanicillium muscarium* Ve6.
1.3.4.4	Contaminating micro-organisms	Contaminating micro-organisms < $5x10^5$ *Escherichia Coli, Salmonella* and *Staphylococcus aureus* are not detected in the batches. Aerobic Plate Count 37°C presented content from 1000 to 53000 CFU/g.
1.3.5	**Analytical profile of batches**	Reference to Volume 4

1.4 Information on the plant protection product

1.4.1 Applicant	Koppert B.V.
1.4.2 Producer of the plant protection product	Koppert B.V.
1.4.3 Current, former and proposed trade names and development code numbers	
Trade Name	MYCOTAL
Code Numbers	19-79, GCRI-79, HRI 19-79 (R. Hall) Ve 6 (Tate & Lyle, MRL) KBV 10-88, KBV 88-M01, KV01 3* (Koppert B.V.)

1.4.4	**Detailed quantitative and qualitative information on the composition of the plant protection product**	
1.4.4.1	Composition of the plant protection product	Reference to Volume 4
1.4.4.2	Information on the active substances	48 g/kg; 1×10^{13} spores/kg
1.4.4.3	Information on safeners, synergists and co-formulants	Reference to Volume 4
1.4.5	**Type and code of the plant protection product**	Water dispersible granule (WG-formulation)
1.4.6	**Function**	Insecticide
1.4.7	**Field of use envisaged**	Target organisms of *Verticillium lecanii* strain Ve 6 are whiteflies (*Bemisia tabaci, Trialeurodes vaporariorum*) and thrips (*Frankliniella occidentalis*) in cultivation of protected crops
1.4.8	**Effects on harmful organisms**	The insect dies after formation of a great number of hyphal bodies inside the body cavity. The mode of action is based on direct infection.

1.5 Detailed uses of the plant protection product

1.5.1 Details of representative uses

PPP (product name/code):	MYCOTAL	Formulation type:	WG
Active Substance:	*Lecanicillium muscarium* Ve6 (19-79)	Conc. of a.s.:	48 g/kg; 1×10^{13} spores/kg
Applicant:	Koppert B.V.	professional use	☒
Zone(s):	EU	non professional use	☐
Safener:	-	Conc. of safener:	-
Synergist:	-	Conc. of synergist:	-
Verified by RMS:	yes		

1	2	3	4	5	6	7	8	9	10	11	12	13
Use-No.	Member state(s)	Crop and/or situation[a] (crop destination / purpose of crop)	F G or I	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application			Application rate			PHI (days)	Remarks: [b]
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg product / ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha (spores/ha) a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		e.g. g safener/synergist per ha
1	EU	Fruiting vegetables of Cucurbitaceae with edible peeland inedible peel	G	Nymphs of whitefly and thrips: *Bemisia tabaci*; *Trialeurodes vaporariorum*; *Frankliniella occidentalis*; *Thrips tabaci*	Spray application	At first sign of infestation Jan-Dec., BBCH 0-99	a) 12 (7) b) 36 (7)	a) 2 b) 72	a) 96 (2×10^{13}) b) 3456 (7.2×10^{14})	1000/2000	1	3 cycles/year

2	EU	Fruiting vegetables of Solanaceae,	G	Nymphs of whitefly and thrips: *Bemisia tabaci; Trialeurodes vaporariorum; Frankliniella occidentalis; Thrips tabaci*	Spray application	At first sign of infestation Jan-Dec., BBCH 0-99	a)12 (7) b) 12 (7)	a) 2 b) 24	a) 96 (2×10^{13}) b) 1152 (2.4×10^{14})	1000/2000	1	1 cycle/year
3	EU	Strawberry	G	Nymphs of whitefly and thrips: *Bemisia tabaci*; *Trialeurodes vaporariorum*; *Frankliniella occidentalis*; *Thrips tabaci*	Spray application	At first sign of infestation Jan-Dec., BBCH 0-99	a) 12 (7) b) 24 (7)	a) 1 b) 24	a) 48 (1×10^{13}) b) 1152 (2.4×10^{14})	1000	1	2 cycles/year
4	EU	Strawberry	F	Nymphs of whitefly and thrips: Bemisia tabaci; Trialeurodes vaporariorum; Frankliniella occidentalis; Thrips tabaci	Spray application	At first sign of infestation Jan-Dec., BBCH 0-99	a) 12 (7) b) 24 (7)	a) 1 b) 24	a) 48 (1 × 1013) b) 1152 (2.4 × 1014	1000	1	2 cycles/year; to be applied in closed plastic tunnels
5	EU	Floriculture crops, except cut roses	G	Nymphs of whitefly and thrips: *Bemisia tabaci*; *Trialeurodes vaporariorum*; *Frankliniella occidentalis*; *Thrips tabaci*	Spray application	At first sign of infestation Jan-Dec., BBCH 0-99	a) 4 (7) b) 24 (7)	a) 2 b) 48	a) 96 (2×10^{13}) b) 2304 (4.8×10^{14})	1000/2000	1	1-7 cycles/year; max. number of applications per year: 24
6	EU	Cut roses	G	Nymphs of whitefly and thrips: *Bemisia tabaci*; *Trialeurodes vaporariorum*; *Frankliniella occidentalis*; *Thrips tabaci*	Spray application	At first sign of infestation Jan-Dec., BBCH 0-99	a) 24 (7) b) 24 (7)	a) 3 b) 72	a) 144 (3×10^{13}) b) 3456 (7.2×10^{14})	1000/3000	0	per 12 month

7	EU	Tree nursery	G	Nymphs of whitefly and thrips: *Bemisia tabaci*; *Trialeurodes vaporariorum*; *Frankliniella occidentalis*; *Thrips tabaci*	Spray application	At first sign of infestation Jan-Dec., BBCH 0-99	a) 24 (7) b) 24 (7)	a) 2 b) 48	a) 96 (2×10^{13}) b) 2304 (4.8×10^{14})	1000/2000	0	per 12 month

1.5.2 Further information on representative uses

The representative formulation, Mycotal, is sprayed on plants at first sign of infestation. The number and timing of application depends on the crop and ranges between 4- 24 applications with 7 days interval. Please see section 1.5.1 GAP table for details on the application frequency.

Products based on *Lecanicillium muscarium* are already authorised, and have been evaluated according uniform principles in the past. No undesirable or unintended side-effects have been observed. No necessary waiting period or other precautions are needed to avoid phytotoxic effects on succeeding crops;

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

No data on other uses submitted.

1.5.4 Overview on authorisations in EU Member States

Product / Code	Crop F/P	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/crop	Active substance total dose/ha (max)
Mycotal	Cucumber, tomato, sweet pepper P	Denmark	677-1	2 kg/ha	0.096 kg/ha	12	1.152 kg/ha
Mycotal	Strawberry P	Denmark	677-1	1 kg/ha	0.048 kg/ha	12	0.576 kg/ha
Mycotal	Ornamentals P	Denmark	677-1	2 kg/ha	0.096 kg/ha	12	1.152 kg/ha
Mycotal	Roses P	France	2040354	2 kg/ha	0.096 kg/ha	12	1.152 kg/ha
Mycotal	Tomato P	France	2040354	2 kg/ha	0.096 kg/ha	12	1.152 kg/ha
Mycotal	Cucumber P	France	2040354	2 kg/ha	0.096 kg/ha	12	1.152 kg/ha
Mycotal	Flowers, ornamentals P	France	2040354	2 kg/ha	0.096 kg/ha	12	1.152 kg/ha
Mycotal	Strawberry P	France	2040354	1 kg/ha	0.048 kg/ha	12	0.576 kg/ha
Mycotal	Sweet pepper P	France	2040354	2 kg/ha	0.096 kg/ha	12	1.152 kg/ha
Mycotal	Tomato P	Italy	015436	2 kg/ha	0.096 kg/ha	2 - 8 per crop cycle (or 4 - 16 per 12 months)	0.768 kg/ha
Mycotal	Sweet pepper P	Italy	015436	2 kg/ha	0.096 kg/ha		0.768 kg/ha
Mycotal	Eggplant P	Italy	015436	2 kg/ha	0.096 kg/ha		0.768 kg/ha
Mycotal	Cucumber P	Italy	015436	2 kg/ha	0.096 kg/ha		0.768 kg/ha
Mycotal	Water melon P	Italy	015436	2 kg/ha	0.096 kg/ha		0.768 kg/ha
Mycotal	Melon P	Italy	015436	2 kg/ha	0.096 kg/ha		0.768 kg/ha
Mycotal	Courgette P	Italy	015436	2 kg/ha	0.096 kg/ha		0.768 kg/ha
Mycotal	Strawberry P	Italy	015436	1 kg/ha	0.048 kg/ha		0.384 kg/ha

Product / Code	Crop F/P	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/crop	Active substance total dose/ha (max)
Mycotal	Bean, green bean P	Italy	015436	2 kg/ha	0.096 kg/ha		0.768 kg/ha
Mycotal	Lettuce P	Italy	015436	2 kg/ha	0.096 kg/ha		0.768 kg/ha
Mycotal	Onion P	Italy	015436	2 kg/ha	0.096 kg/ha		0.768 kg/ha
Mycotal	Leek P	Italy	015436	2 kg/ha	0.096 kg/ha		0.768 kg/ha
Mycotal	Rose and other flowers and ornamentals P	Italy	015436	2 kg/ha	0.096 kg/ha		0.768 kg/ha
Mycotal	Strawberry P	Netherlands	10980	1 kg/ha	0.048 kg/ha	12 per crop cycle	0.576 kg/ha
Mycotal	Fruiting vegetables of Cucurbitaceae with edible peel P	Netherlands	10980	2 kg/ha	0.096 kg/ha	12 per crop cycle	1.152 kg/ha
Mycotal	Melon F	Netherlands	10980	2 kg/ha	0.096 kg/ha	12 per crop cycle	1.152 kg/ha
Mycotal	Fruiting vegetables of Solanaceae P	Netherlands	10980	2 kg/ha	0.096 kg/ha	12 per crop cycle	1.152 kg/ha
Mycotal	Floriculture crops, except cut roses P	Netherlands	10980	2 kg/ha	0.096 kg/ha	24 per 12 months	2.304 kg/ha
Mycotal	Cut roses P	Netherlands	10980	3 kg/ha	0.144 kg/ha	24 per 12 months	3.456 kg/ha
Mycotal	Tree nursery crops P	Netherlands	10980	2 kg/ha	0.096 kg/ha	24 per 12 months	2.304 kg/ha
Mycotal	Sweet pepper P	Spain	24435	3 kg/ha	0.144 kg/ha	3 per season	0.432 kg/ha
Mycotal	Tomato P	Spain	24435	3 kg/ha	0.144 kg/ha	3 per season	0.432 kg/ha
Mycotal	Cucumber	UK	MAPP16644	2 kg/ha	0.096 kg/ha	8	0.768 kg/ha

Product / Code	Crop F/P	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/crop	Active substance total dose/ha (max)
	P						
Mycotal	Ornamental plant production P	UK	MAPP16644	2 kg/ha	0.096 kg/ha	8	0.768 kg/ha
Mycotal	Pepper P	UK	MAPP16644	2 kg/ha	0.096 kg/ha	8	0.768 kg/ha
Mycotal	Strawberry P	UK	MAPP16644	2 kg/ha	0.096 kg/ha	8	0.768 kg/ha
Mycotal	Tomato P	UK	MAPP16644	2 kg/ha	0.096 kg/ha	8	0.768 kg/ha

Level 2

Lecanicillium muscarium Ve6

2 Summary of active substance hazard and of product risk assessment

2.1 Identity

L. muscarium Ve6 can be identified morphologically upon species level: Colony size 18-22 mm, white or pale yellow, cotton wool like, hyphae rarely in bundles (10 days at 20°C, Malt Extract Agar. Colony underside is colorless, yellow or ochraceous. Phialids detached or in few whorls on conidiophores or slightly differentiated hyphae from the aerial mycelium, needle form, high variability in size, 12-40 * 0.8-3 µm. Conidia one-celled in heads, often parallel to phialide tip, cylindrical with both ends well rounded or ellipse, 2.3-10 * 1.0-2.6 µm.
Chlamydospores absent. Spore sizes of *L. muscarium* Ve6 4.2±0.9 µm - 1.6±0.2 µm. (6 days at 23°C, Saboureaud Dextrose agar).

Additionally, molecular techniques as ITS sequence analysis and RFLP patterns, can be used for identification on strain level.

It can be concluded that the provided information are sufficient to identify *L. muscarium* Ve6 at strain level.
MYCOTAL is a water dispersible granules [WG] microbial plant protection. The production of MYCOTAL is a continuous process. Therefore a technical material does in fact not exist. Hence, data on the technical material of *L. muscarium* Ve6 are not relevant. Therefore, for the renewal of the active substance, a new 5-batch analysis of the formulated product is submitted. The content of *Lecanicillium muscarium* Ve6 ranges from 9.5×10^9 to 1.2×10^{10} spores/g and 5.3×10^9 to 8.9×10^9 CFU/g, respectively. The nominal content of *L. muscarium* Ve6 is 1×10^{10} spores/g.

MYCOTAL is used for the biological control of insects.

2.2 Biological properties

2.2.1 Summary of biological properties of the active substance

Lecanicillium muscarium Ve6 is active against the plant-pests whitefly and thrips. *Lecanicillium muscarium* Ve6 germinate on the whitefly cuticle within 12-48 hours, followed by hyphal growth on the cuticle before penetration of the host. The cuticula is penetrated, and tissue is affected within 48 hours after infection. Once in the host, *Lecanicillium muscarium* Ve6 forms blastospores which spread

through the haemolymph of the arthropod host and lead to further infection. The insect dies within 7-10 days, when a great number of hyphal bodies has been formed inside the body cavity.

Thrips are probably killed as a result of multiple lesions of the cuticle by enzymatic degradation (no fungal material was found in the haemolymph of the insect at the time of death).

2.2.2 Summary of physical, chemical and technical properties of the plant protection product

The product MYCOTAL is a light ivory solid formulation with particle size between 0.075 – 0.5 mm and essentially non-dusty. The formulation is not explosive or oxidizing nor is it flammable or auto-flammable. The formulation does not have any corrosion characteristics as the pH of a 1% dilution is 7.2. The pour density is 0.499 g/mL and the tap density is 0.515 g/mL. All physical, chemical properties indicate that no particular problems are to be expected when used and stored as recommended on the label. The technical properties were acceptable, however the flowability was not within specs as after 20 liftings ~16% remained on the sieve. Therefore the label should include the phrase “agitate spray solution when applying the formulation” to reduce solids in the spray solution. The shelf-life study for 6 months at 2-6˚C is executed with a different (‘old’ WP-formulation). However, it can be concluded that this significant composition change has no major impact on the phys-chem characteristics of the new applied for WG-formulation, and therefore the formulations can be considered equivalent and extrapolation of the results is acceptable.

Results from the DAR, August 2008: Volume 3, Annex B, Point B.2.2 from the Annex I inclusion of Lecanicillium muscarium Ve6 (Mycotal) are considered still relevant in support for the renewal.

A storage stability study (6 months of storage at 4 ± 2°C) for MYCOTAL in aluminium bags is ongoing and is required.

Additional note: only data to support an in-use concentration of 0.1% was provided. Based on the GAP, it appears 0.3% concentrations are also possible. Considering the product will need to be agitated during use, only the foam persistence at 0.3% may still need to be addressed.

2.3 Data on application and efficacy

2.3.1 Summary of effectiveness

L. muscarium Ve6 is effective against whitefly (*Bemisia tabaci*) and thrips (*Trialeurodes vaporariorum*; *Frankliniella occidentalis*; *Thrips tabaci*) in in protected crops: Fruiting vegetables of Cucurbitaceae with edible skin, melon, fruiting vegetables of Solanaceae, bean with pod, strawberry, floriculture crops and cut roses, tree nursery. No new effectivity studies were presented for the renewal of the active substance.

2.3.2 Summary of information on the development of resistance

Resistance of whitefly or thrips against *L. muscarium* Ve6 has not been reported since introduction of this strain as microbial insecticide in the 80s.

Lenacillium muscarium does not have an IRAC classification (Insecticide Resistance Action Committee).

For this renewal dossier a literature search was performed to find all recent (from 2006 onwards) references relevant for development of resistance. One review was identified in the latest literature search reporting on development of resistance against *L. muscarium* Ve6 or the product MYCOTAL in insect pests. This review outlines the current state of knowledge on the development of insecticide resistance in insect pests and discusses strategies for manipulation of certain important fungal entomopathogens as potential microbial tool in the insecticide resistance management programme for sustainable pest management systems.

2.3.3 Summary of adverse effects on treated crops

Considering that the substance is approved and that the extant authorizations of plant protection products containing *Lecanicillium muscarium* have already been evaluated according to the Uniform Principles, no other information on adverse effects on treated crops is considered to be necessary at this time.

2.3.4 Summary of observations on other undesirable or unintended side-effects

Considering that the substance is approved and that the extant authorizations of plant protection products containing *Lecanicillium muscarium* have already been evaluated according to the Uniform Principles, no other information on undesirable side-effects on treated crops is considered to be necessary at this time.

2.4 Further information

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

SDS of MYCOTAL (Anonymous 2016)

Information on safe handling:	Prevent the formation of dust. Do not place in a warm environment or in direct sunlight.
Preventing fire and explosion:	No special measures required. The product is non-flammable.
Storage:	Storage temperature: 4 - 8°C: Keep dry in robust, closed packaging. Do not freeze.
Information relating to shared storage:	Keep away from food, drink and animal feed.
Transport:	Not dangerous for transport according to ADR/RID, IMO and IATA FIRE-FIGHTING MEASURES
Suitable extinguishing agents:	CO_2 foam or water jet
Unsuitable extinguishing agents:	Not applicable

2.4.2 Summary of procedures for destruction or decontamination

Remove according to local authority recommendations, e.g. convey to a licensed incinerator.
European waste catalogue: 02 01 99: wastes not otherwise specified.

2.4.3 Summary of emergency measures in case of an accident

Personal precautions:

Wear protective clothing.

Respiratory Protection: In case of opening of the packing, blending/loading and spraying, wear preferably a filtermask with filtertype P3.

Hand protection: Wear safety gloves (butylrubber, PVC)

Eye Protection: Wear closed eye protection (monogoggles)

Other Protective Measures: Wear protective clothing. The usual precautions for handling chemicals should be considered.

Environmental precautions:

No specific recommendations.

Cleaning methods:

Cover up spillages of the product before carefully vacuuming it up. If necessary, remove the last residues with water. Dispense of rinsing water via the sewerage system.

2.5 Analytical methods

The micro-organism was identified morphologically as *Lecanicillium muscarium.* According to the notifier major changes in characteristics of *L. muscarium* Ve6 will result in a change in germinability and effectiveness in bioassay, hence a change in characteristics can be detected in the viable spore content test and the qualitative bio-assay.

The amount of spores in formulated product is determined using a Bürker-Turk counting chamber.

Twice a year a randomly chosen cryoampule with the mother stock is checked for contaminants and identity. Genetic fingerprinting (RFLP), ITS and mtDNA were used for identification on strain level.

Microbial contaminants are to be analyzed with the standard ISO methods

2.6 Impact on human and animal health

2.6.1 Effects having relevance to human and animal health arising from exposure to the micro-organism or to impurities, additives, contaminating micro-organisms contained in the material used for manufacturing of formulated products

The studies were performed with *Verticillium lecanii* Ve6. *Verticillium lecanii* Ve6 has been reclassified to the new species *Lecanicillium muscarium* (Zare and Gams, 2001). Therefore, the conclusions drawn for *Verticillium lecanii* Ve6 are considered valid for *Lecanicillium muscarium* Ve6.

The oral toxicity study was considered acceptable for evaluation of the oral LD50 of *Lecanicillium muscarium* Ve6 and was considered to be > 3.0×10^8 spores/animal. A second study (nominal dose 1.2×10^8 spores/animal) confirmed the non-toxicity seen in the first study.

In the acute i.v. toxicity study *Lecanicillium muscarium* Ve6 was proven non-toxic, and not colonising or infective. The study provided acceptable results as immediately after dosing *Lecanicillium muscarium* Ve6 was detected in the organs.

In the acute inhalation study *Lecanicillium muscarium* Ve6 was considered not toxic, no LC_{50} can be determined, as the dose used is defined as maximal practical dose, and the measurements of the aerosol concentrations are not elucidated.

Four acute i.p. studies were submitted by the notifier: two rat studies (1.2×10^8 and 3.4×10^7 spores/animal) and 2 mice studies (4.5×10^7 and 6.9×10^6 spores/animal). These studies were acceptable for the evaluation of toxicity, but not for infectivity/pathogenicity.
In the highest dose for rats mortality was seen, preceded with hypoactivity, hunched posture and piloerection. In all studies, macroscopic examination revealed adhesion in the abdominal cavity involving many organs. Microscopic examination revealed granulomateous peritonitis with abscesses. These findings were noted for both the viable spores (SSP) and inactivated autoclaved spores (ASSP). This indicates a non-specific immune response to foreign material, consisting of a suspension of (autoclaved heat-inactivated) spores that was injected into the peritoneum. This is supported by the haematological data, as on day 15 increased white blood cells and increased neutrophils were noted, that ceased by day 30. Overall these (local) effects ceased by day 30, and were considered an acute immune-reaction rather than a toxicity reaction.

Overall on the subject of infectivity of *Lecanicillium muscarium* Ve6 the conducted studies could not

completely elucidate whether the fungus is infective/pathogenic or not. In these studies spores were administered, and no fungus was obtained by culturing the homogenates of the organs. The conditions within the mammalian body are considered not optimal for germination of the spores, as the fungi germinate on host pests with conditions different from vertebrates. Furthermore, *Lecanicillium muscarium* Ve6 was not infective when administered i.v. Data from open literature indicate that when *Lecanicillium muscarium* Ve6 was administered i.p., no infectivity was observed. In addition, the literature submitted on clinical cases of (systemic) infections of *Lecanicillium muscarium* Ve6 described immunocompromised cases (either having received chemo/radio-therapy or being treated with intraperitoneal antibiotics). Taken together the RMS agrees with the notifier that *Lecanicillium muscarium* Ve6 is not infective and thus not pathogenic.

Genotoxicity

No destruxins, considered to be the important toxins produced by *L. muscarium* Ve6 in laboratory cultures, could be detected in the product MYCOTAL, in the unformulated spores or in tomatoes or cucumbers after application of MYCOTAL (10 times the recommended dose). Moreover, the mode of action of *Lecanicillium muscarium* Ve6 is not considered to be based on toxins. Therefore, it can be concluded that any possible exposure to toxins is expected to be negligible, and no genotoxicity test are considered necessary. Moreover, the submitted reports indicate no genotoxic potential of spore- or MYCOTAL extracts, destruxins, or crude extracts.

Cell culture

Conditions within the body are considered not optimal for germination of the spores, as the fungi germinate on host pests conditions completely different from those occurring in vertebrates (germination conditions are not completely elucidated). Furthermore, *L. muscarium* Ve6 was not infective in the studies submitted. Therefore, a cell culture study for *L. muscarium* Ve6 is considered not relevant.

Short toxicity and pathogenicity

A 28-day inhalation study was conducted in which rats were exposed to 0, 1, 10 and 100 mg/m^3 of the formulation MYCOTAL (containing *L. muscarium* Ve6, but also a large quantity of other proteinaceous material, known to be a (respiratory) sensitiser). Based on the very slight changes noted at microscopic examination in the nasal cavity, lungs and the mediastinal lymph nodes the NOAEL was set at 1 mg/m^3.

MYCOTAL was the representative formulation for the EU review of *Lecanicillium muscarium* Ve6. The product contains *Lecanicillium muscarium* Ve6 at 48 g/kg (equivalent to1 × 10^{13} spores/kg). It is intended for use as insecticide on cucurbits, Solanaceae and ornamentals in protected uses (greenhouses and tunnels).

The LC_{50} for MYCOTAL in the acute inhalation study was set at > 893 mg/m^3.
The dermal toxicity study was considered unacceptable, as the dose used was far below the required 2 g/kg. However, according to the RMS there is no need to submit an additional acute dermal toxicity study, as no acute toxicity is expected by dermal routing, the sensitisation study did not indicate any skin reaction, the product is considered not acute toxic following dermal exposure, and the non-active ingredients are considered non-toxic.

In the eye and skin irritation studies the active ingredient was used instead of the product. As the additives have no toxic or irritating potential, the RMS accepts both studies. In addition, although the *L. muscarium* strain was not indicated, the RMS accepted these studies on a species level as any irritancy is not expected to be strain-specific. Therefore, it can be concluded that MYCOTAL does not need to be classified for eye or skin irritation.

2.6.2 Impact on human health arising from exposure to the micro-organims or to impurities, additives, contaminating micro-organisms contained in the material used for manufacturing of formulated products

L. muscarium is not (systemically) infective and thus not pathogenic. Therefore, no risk is foreseen for the operator when MYCOTAL is applied according to the intended use making use of proper personal protective equipment. Furthermore, it can be concluded that no risk is foreseen for the re-entry worker when MYCOTAL is applied according to the intended use making use of proper **dermal** personal protective equipment. During spraying operations there should be no bystanders present in the greenhouse. No exposure to bystanders is therefore expected.

2.6.3 Summary of product exposure and risk assessment

Exposure of operators, workers, and bystanders to *L. muscarium* Ve6, if even occurring, can be considered safe even with the overly conservative approach, please see 2.6.2.

2.7 Residues in or on treated products, food and feed

2.7.1 Persistence and likelihood of multiplication in or on crops, feedstuffs or foodstuffs

With regard to the persistence and stability of metabolites on (edible parts of) crops, it can be conclud-

ed that the destruxins itself, when applied as such, were still present on tomato leaves after two weeks under greenhouse conditions. However, no toxins are expected to occur during and after application of MYCOTAL. Destruxins could not be detected in the extracts. The amounts found are very low, and since the mode of action of *L. muscarium* Ve6 is not considered to be based on toxins, the relevance of these unidentified compounds is questioned. It was concluded that investigations into the occurrence of any possible metabolite produced by *L. muscarium* Ve6 should be considered irrelevant for the risk assessment.

Although it was shown, that *L. muscarium* may colonize plants, residues of *L. muscarium* Ve6 were never detected in food or feed.

2.7.2 Further information required

No specific new data is required.

2.7.3 Non-viable residues

A method to determine non-viable residues is not necessary, since *L. muscarium* Ve6 does not produce metabolites of toxicological concern.

2.7.4 Viable residues

Active micro-organism remaining on treated plants are determined by plating samples onto malt agar extract or selective medium (Rose bengal chloramphenicol agar). Colonies can be identified by morphological identification methods.

2.7.5 Summary of residue behavior resulting

With regard to the persistence and stability of metabolites on (edible parts of) crops, it can be concluded that the destruxins itself, when applied as such, were still present on tomato leaves after two weeks under greenhouse conditions. However, no toxins are expected to occur during and after application of MYCOTAL. Destruxins could not be detected in the extracts. The amounts found are very low, and since the mode of action of *L. muscarium* Ve6 is not considered to be based on toxins, the relevance of these unidentified compounds is questioned. It was concluded that investigations into the occurrence of any possible metabolite produced by *L. muscarium* Ve6 should be considered irrelevant for the risk assessment.

Although it was shown, that *L. muscarium* may colonize plants, residues of *L. muscarium* Ve6 were never detected in food or feed.

It can therefore be concluded that no risk for the consumer due to the exposure to *L. muscarium* Ve6 is expected.

2.8 Fate and behaviour in the environment

The species *Lecanicillium muscarium* has a global natural distribution. The persistence of *L. muscarium* Ve6 in the environment upon application will depend among others things on the presence of suitable substrates and on competition with the *in situ* microbial community. It is expected that numbers of *L. muscarium* Ve6 will decline during a time course after application to eventually return to background numbers.
The CFU numbers per square millimeter of related strain *Lecanicillium muscarium* Ve2 on leaf surfaces of Chrysanthemum decreased from 146 to 0 within a period of 14 days upon applicaton by spraying on the leaf surface (Gardner et al., 1984).

2.8.1 Summary of fate and behaviour in soil

Lecanicillium muscarium has a global natural distribution in soil. As natural component of the soil food web, it is expected that the high densities of *Lecanicillium muscarium* Ve6 that are caused by application will return to their natural levels.
Upon application, *Lecanicillium muscarium* Ve6 can persist at least 40 days in soil (Hollingsworth, 1983). No data are available on the long-term persistence of this strain in soil. Field studies with related species *Lecanicillium lecanii* show that this species can persist for more than a year in soil, but after 1.5 years *L. lecanii* CFUs were not detected anymore.
The highest predicted environmental density in soil of *Lecanicillium muscarium* Ve6 upon application is 9.6 x 10^8 CFU/kg soil in the top 5 cm of soil (corresponding to 4.61 mg a.s./kg soil), which is based on the use on fruiting vegetables of Cucurbitaceae (2 x 10^{13} CFU or 96 g a.s. per hectare per application, 36 applications per season), assuming no growth or degradation between treatments.

2.8.2 Summary of fate and behaviour in water

No information is available on the natural occurrence and abundance of *Lecanicillium muscarium* Ve6 in aquatic systems. As *Lecanicillium muscarium* occurs globally in soils, it is expected that dispersal of this microorganism to aquatic systems will also occur under natural conditions. It is expected that the numbers of *Lecanicillium muscarium* Ve6 will return to background levels upon application either due to the inability of the microorganism to survive in aquatic systems, or due to competition with the

in situ microbial community.

CFU numbers of *Lecanicillium muscarium* Ve6 remained stable in short-term incubations in sterile demi-water and in water with fish (7 and 6-day incubation period, respectively). No data is available on the long-term population dynamics of *Lecanicillium muscarium* Ve6 in natural aquatic systems.

The highest predicted initial environmental density in surface water upon application of the representative formulation is 1.71 x 10^6 CFU/L (corresponding to 8.23 x 10^{-3} mg a.s./L), which is based on the field use on strawberries (1 x 10^{13} CFU or 48 g a.s. per hectare per application, 24 applications per season), assuming no growth or degradaton between treatments and using Rautmann drift values and the parameterisation of the TOXSWA standard ditch.

No interference of *Lecanicillium muscarium* Ve6 with the analytical systems for the control of the quality of drinking water is expected.

2.8.3 Summary of fate and behaviour in air

Lecanicillium muscarium Ve6 can become airborne during application. However, viable numbers are expected to rapidly decline in air, either due to settling out or to death of the microorganism due to dessication or UV-radiation. Experimental data from a greenhouse environment show that the number of spores detected in air were back to background levels within a day after greenhouse application (Samson, 1990).

2.8.4 Summary of mobility

In three soils tested (sand, loamy sand, sandy loam), no leaching of *Lecanicillium muscarium* Ve6 was detected (Hollingsworth, 1983).

Short-range dispersal of *Lecanicillium muscarium* is likely to occur through rain-splash. In addition, dispersal can occur by arthropods (Down et al., 2009).

Long-range transport through air is not expected to be an important factor in the dispersal of *Lecanicillium muscarium* Ve6, as numbers of spores detected in air are back to background levels within a day after greenhouse application (Samson, 1990). Long-range transport may occur in free-flowing aquatic systems, as CFU numbers of *Lecanicillium muscarium* Ve6 were shown to be stable in water during at least 6 days.

2.9 Effects on non-target species

2.9.1 Summary of effects on birds (and other terrestrial vertebrates)

The table below shows a summary of the studies on effects on birds treated with MPCA.

Table 2.9.1-1: Summary of the studies on effects on birds treated with MPCA

	Test sub-stance	Test duration	Dose range	Observa-tions	Results/ Endpoint	Refer-ence
TOXICITY	*L. muscariu m* (Mycotal TGAI)	30 d (5 d exposure)	19 mg a.s./kg bw (correspondin g with 1.2 × 10^9 CFU/kg bw),	mortalities or signs of toxicity	No mortalities, signs of toxicity or clinical signs LD_{50} > 19 mg a.s./kg bw (correspondin g with 1.2 × 10^9 CFU/kg bw)	[redacted] (1998)
INFECTIVE-NESS		30 d (5 d exposure)	19 mg a.s./kg bw (correspondin g with 1.2 × 10^9 CFU/kg bw),	viable spores in the organs	Tissues, faeces and blood samples showed no occurrence of the a.s.. Therefore no infectivity or pathogenicity was indicated	[redacted] (1998)
PATHO-GENICITY		30 d (5 d exposure)	19 mg a.s./kg bw (correspondin g with 1.2 × 10^9 CFU/kg bw),	gross pathogenicity following necropsy		[redacted] (1998)

2.9.2 Summary of effects on aquatic organisms

Protocolled laboratory, non-GLP tests indicated no adverse acute effects (mortality, abnormal behaviour, infectivity, pathogenicity) on:

A. young rainbow trouts (*Oncorhynchus mykiss*) at an actual application rate of ≤ 97 mg a.s./L (≤ 6.1×10^9 CFU/L),
B. young daphnids at an application rate of ≤ 6.0 mg a.s./L (≤ 3.8×10^8 CFU/L).

Both the above endpoint values are based on actual concentrations from less reliable laboratory tests. They were based on 'maximised' concentrations, without using a solvent for the hydrophobic conidia, causing a limited exposure. Indeed, the recoveries of *L. muscarium* in the test water were 0.95-11% for the rainbow trout and 0.29-0.66% for the daphnid tests. Gross clinical signs of infection or pathogenicity were not observed for the fish and Agar plate counts of dead daphnids did not show the presence of *L. muscarium*. However, the test duration may have been too short to determine pathogenicity/infectivity effects. According to OPPTS 885.4200 the study duration should be 30 days.

A fish kill case in a Finnish fish farm in 1986 with Atlantic salmon (*Salmo salar*) is reported in the published literature. This fish kill was attributed to *Lecanicillium* ssp, so it may have been caused by

L. longisporum rather than by *L. muscarium*, the micro-organism under review. The high fish densities in fish farms may have played a role in this fish kill as well.

There are no unambiguous records of *L. muscarium* having affected aquatic organisms in the field, whereas this filamentous fungus has been used in various countries (though predominantly in greenhouses, thus not substantially contaminating nearby surface water)[1].

Various sources do not indicate adverse toxicological, infective and pathogenic effects on aquatic organisms following exposure to *L. muscarium* (Burges, 1981;Copping, 2004).

In summary, no clear treatment- or dose-related toxic, infective or pathogenic effects to aquatic organisms have been established in an aqueous environment after exposure to conidia of *L. muscarium.* There are no indications of the potential intrinsic toxicity of toxins or other relevant metabolites of *L. muscarium.* There are no tests available with the product under review but with the a.s., which is acceptable.

Table 2.9.2-1: Summary of the studies on effects on aquatic organisms treated with the MPCA

Species	Test substance	Test duration	Dose range	Observations	Results/ Endpoint	Reference
TOXICITY						
Fish:	Unknown product with *L. muscarium*, VE6-58 SSP, white powder, 8.8×10^9 CFU/g	96 h	10, 100 and 1000 mg a.s./L (nominal), corresponding with 6.3 $\times 10^8$, 6.3 $\times 10^9$ and 6.3 $\times 10^{10}$ CFU/L	Mortality, clinical igns, abnorml behaviour	96 h LC_{50} > 97 mg a.s./kg bw/day (corresponding with > $6.2x10^9$ CFU/L (average highest initial concentrations over the two top-dose replicates and the top-dose without fish)	[redacted] (1983) (DAR 2007)
Invertebrates	MYCOTAL, as dry powder with *L. muscarium*	24 h	62, 125, 250, 500 and 1000 mg a.s./L	Mortality, immobilisation	24-h EC_{50} of > 6.0 mg a.s./L (> 3.8 $\times 10^8$ CFU/L)	Quinlan (1983) (DAR 2007)

[1] Mycotal with *L. muscarium* has been registrated in *e.g.* Finland, Italy, Japan, Netherlands, Norway, Switzerland, Turkey, UK (OECD, 2005).

Species	Test substance	Test duration	Dose range	Observations	Results/ Endpoint	Reference
			(nominal), corresponding with 3.9 × 10^9, 7.9 × 10^9, 1.6 × 10^{10}, 3.2 × 10^{10} and 6.3 × 10^{10} CFU/L		(actual conc) [b]	
INFECTIVENESS/PATHOGENICITY						
Fish					No signs of infectivity or pathogenicity of *L. muscarium* for fish under laboratory conditions after single dosages of ≤ 97 mg a.s./L (equalling ≤ 6.1 × 10^9 CFU/L; see Table B.9.2.1.1.a). Study is considered as supporting information for r.a.. [a]	[redacted] (1983) (DAR 2007)
Invertebrates					No signs of daphnid infectivity or pathogenicity under laboratory conditions at single actual dosages of ≤ 6.0 mg a.s./L (equalling ≤ 3.8 × 10^8 CFU/L; see study 8.2.2/01, Table B.9.2.2.1.a). Study is considered as	Quinlan (1983) (DAR 2007)

Species	Test substance	Test duration	Dose range	Observations	Results/ Endpoint	Reference
					supporting information for r.a..[a]	

[a]: endpoint considered as supporting information due to short study duration
[b]: probably limited exposure due to the low dispersability of the test substance (recoveries of 0.29-0.66% of nominal)

Public literature:

Test species	*Daphnia magna*	Reference
Toxicity	No adverse effects at 1.7×10^9 CFU/mL[a]	Gradila (2013) (submitted for renewal)
Infectivity / Pathogenicity	Not determined	

[a] Less reliable, due to absence of (reporting of) analytical measurements. Also the test strain is unknown.

2.9.3 Summary of effects on bees

The table below shows a summary of the studies on effects on bees treated with the MPCA.

Table 2.9.3-1: Summary of the studies on effects on bees treated with the MPCA

Species	Test substance	Test duration	Method	Results/ Endpoint	Observations	Reference
Apis mellifera	*L. muscarium*, Mycotal technical grade, white powder, actual concentration 9.4×10^{10} CFU/g (c. 98% w/w)	4 d exposure	Topical application	LR_{50} >100 µg a.s./bee (corresponding with $>6.3 \times 10^6$ CFU/bee)	Mortality, infectivity, pathogenicity*	Kling (2000)
	L. muscarium, Mycotal technical grade white powder, actual	4 d exposure	Oral dosage	LR_{50} >112 µg a.s./bee[1](corresponding with $>7.1 \times 10^6$ CFU/bee)	Mortality, infectivity, pathogenicity*	Kling (2000)

Species	Test substance	Test duration	Method	Results/ Endpoint	Observations	Reference
	concentration 9.4×10^{10} CFU/g (c. 98% w/w)					

[1] : intake, based on the actual feed consumption (nominal application rate was 100 µg a.s./bee)

*The study duration according to OPPTS 885.4380 should be 30 days, as in general for microbial a.s. a longer test duration is required in order to establish possible effects. This study was performed according to the chemical EPPO guideline. Therefore, with regard to infectivity and pathogenicity the studies are considered as less reliable, and supporting information only for the risk assessment.

Public literature

In the DAR (2007), some references from the pubic literature were discussed. It appears that bees are not among the 'natural' target or host range, although the available data are not conclusive in this respect. No indications of adverse effects were monitored in tests with bumblebees (*Bombus terrestris*), apart perhaps a slightly earlier production of young queens following dusting or oral feeding with MYCOTAL. However, the differences were small and no statistical analysis had been performed. Based on these bumblebee contact tests, the contact NOED was established at c. 8.0 mg a.s./bee. The lack of effects on bumblebee and honeybee following contact may be explained by the difficulties of conidia to pass the arthropod's hairs. Bumblebees were also orally exposed to a product with *L. muscarium* mixed with pollen. This dietary exposure did not show adverse effects to bumblebees as well (oral 18-d NOED: c. 8.0 mg a.s./bee). No *L. muscarium* was found in the nestboxes, on brood or on adults. Although these tests did not indicate toxic, infective or pathogenic effects, there are indications of a higher susceptibility of bees to MYCOTAL at high application rates under laboratory conditions. However, test results were difficult to evaluate as temperature and RH were generally not reported. One field test with the product MYCOTAL did not indicate hazardous effects to active colonies with honeybees, when sprayed with a 1.5×10^6 CFU/mL spore suspension. The lack of treatment-related mortalities was probably due to the lack of growth of *L. muscarium* at 35ºC, the average temperature within beehives.

From the recent literature search submitted for the purpose of renewal, no relevant references were identified reporting on effects of *L. muscarium* on bees or indicating that bees are affected by *L. muscarium* or that the fungus is known as a bee pathogen, respectively.

2.9.4 Summary of effects on arthropods other than bees

The available information from the different studies is summarised in Table 2.9.4-1 below:

Table 2.9.4-1: Summary of the studies on effects non-target arthropods treated with the MPCA (public literature)

Species	Test substance	Maximum exposure in study	Author / summary provided in	RMS conclusion
Trichogramma cacoecidae *Encarsia formosa* *Aphidius matricariae* *Phytseiulus persimilis* *Typhlodromus pyri* *Chrysoperla carnea* *Forficula auricularia* *Semiadalia notata*	Micro Germin (a.s. *Verticillium lecanii*)	0.4% product 4 kg product/ha; laboratory (RMS: content of a.s. unknown)	Vol.3, MA B.9, Section 8.4 (Sterk *et al.*, 1999) (DAR 2007)	**No toxic, infective or pathogenic effects to NTAs were observed.** **Not useful for quantitative r.a. since exposure unknown, but considered as supporting info.**
Agonum dorsale *Bembidion lampros* *Bembidion obtusum* *Demetrias atricapillus* *Harpalus rufipes* *Pterostichus cupreus* *Trechus quadristriatus* *Tachyporus hypnorum* *Folsomia candida* *Lasius niger* *Episyrphum balteatus* (larvae) *Chrysoperla carnea* *Forficula auricularia* *Erigone sp.* *Oniscus sp.*	In fact two isolates of the former V. lecanii were used: C3, isolated from the aphid Macrosiphonionella sanborni or 170.76, isolated from Cydia pomonella. Whereas in view of the target arthropod the C3 isolate probably refers to L. longisporum, the muscarium/longisporum option is more difficult to determine for the 170.66 isolate. As both isolates suppressed six hemipteran species, amongst them various aphids, both isolates were probably L. longisporum. Therefore it is difficult to extrapolate these test results to L. muscarium.	1×10^7 spores/mL in spray solution 3 mL applied on 80 × 50 mm; laboratory	Vol.3, MA B.9, Section 8.4 (Sitch and Jackson, 1997) (DAR 2007)	**No toxic, infective or pathogenic effects to NTAs were observed.** **Less useful for r.a. due to the use of different strains/species than *L. Muscarium*.**
Encarsia formosa	*Verticillium lecanii*	3.6×10^7 spores/mL	Vol.3, MA B.9, Section 8.4 (Flexner et al, 1986) (DAR 2007)	**No or slight mortality to adult parasitoid *E. Formosa*. Test methodology and results are difficult to verify, therefore less useful for r.a., but considered as supporting info.**

Phytoseiulus persimilis	*L. muscarium* Ve24	Spray residues on leaves using spraying solutions with $2x10^6$ and $2x10^7$ spores/mL	Vol.3, MA B.9, study 8.4/02 (Donka et al, 2008) (submitted for purpose of renewal)	**A low risk is indicated for predatory mites exposed to spray residues on leaves using spraying solutions with $2x10^6$ and $2x10^7$ spores/mL.** **From the study, the target organisms of Ve24 appear to be comparable with Ve6. However, no further information is available to support extrapolation between these strains.**
Encarsia formosa *Macrolophus caliginosus*	MYCOTAL	3.9×10^4 spores *L. muscarium* Ve6/cm² leaf surface	Vol.3, MA B.9, study 8.4/03 (Hamdi et al, 2011) (submitted for the purpose of renewal)	**Although there may be a slight reduction in parasitisation efficacy by *E. formosa*, the study indicates that *L. muscarium* (product Mycotal) poses a low risk for natural enemies (bugs (*M. caliginosus*) and parasitic wasps (*E. formosa*)) at the recommended dose rate (0.1%, corr. to $3.9x10^4$ CFU/cm², corr to $3.9x10^{12}$ CFU/ha).**
Encarsia formosa *Phytoseiulus persimilis* *Macrolophus caliginosus*	MYCOTAL & Addit	MYCOTAL & Addit was applied at the recommended dosages: 1 g/L (= $1x10^{10}$CFU/L) and 0.25%, respectively three times with an interval of a week	Vol.3, MA B.9, study 8.4/04 (Anonymous, 2006c) (submitted for the purpose of renewal)	**<25% adverse effect according to authors/applicant. RMS noted reduction in parasitisation for *E. formosa* may be >50%. However, the report was very concise, therefore no firm conclusions can be drawn, although RMS considers that the company would not benefit if their product would adversely affect beneficials whilst stating that this is not the case to the users. The report is considered as supporting information.**
Aphidius colemani *Harmonia axyridis*	MYCOTAL	dose rates of 0.0, 2.5×10^{10}, 5×10^{10} and 10×10^{10} conidia/L	Vol.3, MA B.9, study 8.4/06 (Aqueel, 2013) (submitted for the pur-	**Reduced reproductive succes of *A. colemani* (ca. 5% and 15% reduction in parasitisation and emergence resp. (visual assessment RMS)).** **RMS notes that it was unclear in the article for which treatment rates the**

			pose of re-newal)	**results were presented. Therefore the study is less useful for quantitative r.a., but considered as supporting info.**

2.9.5 Summary of effects on earthworms and other soil non-target macro-organisms

The table below shows a summary of the studies on effects on earthworms treated with the MPCA.

Table 2.9.5-1: Summary of the studies on effects on earthworms treated with the MPCA

Species	Test substance	Test duration	Method	Observations	Results/ Endpoint	Reference
Eisenia fetida	*L. muscarium*, Mycotal technical grade white powder, actual concentration 9.5×10^{10} CFU/g (98% w/w)	14 d exposure	Mixing through artificial soil (10% OM)	Mortality, infectivity, pathogenicity	LC_{50} 1000 mg a.s./kg soil dw, corresponding with 6.3×10^{10} CFU/kg soil dw	Study KMA 8.5/01 Wachter (2000)

Public literature:

No adverse effects were found on *E. fetida* at 1.7×10^9 UFM/mL dispersed in artificial soil. Infectivity and pathogenicity was not investigated. The tested strain was not reported. The test is considered useful as indicative information in a weight of evidence approach with regard to results on toxicity, due to the unknown test concentration in the soil and the unknown strain.

2.9.6 Summary of effects on soil micro-organisms

No data available, no relevant articles were found in the literature search.

2.9.7 Summary of effects on other non target (flora and fauna)

No data available, no relevant articles were found in the literature search.

Data on terrestrial plants are not required according to the data requirements for micro-organisms in Com. Regulation (EU) No 283/2013.

2.9.8 Summary of effects on biological methods for sewage treatment

No data available, not considered necessary.

2.9.9 Summary of product exposure and risk assessment

2.9.9.1 Birds

No unacceptable adverse effects to birds are expected due to:

(a) the apparent lack of evidence of birds being among the 'natural' target or host range of *L. muscarium* in general: *V. lecanii* has never been observed as a pathogen on warm-blooded animals (see section on Identity/biological properties). This may be explained by the temperature preferences of *L. muscarium.* which are *in vitro* generally below an average bird body temperature of 41 ºC;

(b) the lack of toxic, infective or pathogenic effects in a protocolled laboratory test (see Vol.3, MA B.9, KMA8.1/01);

(c) the lack of field records indicating a (potential) risk for birds, in spite of use in various countries;

(d) the occurrence of natural epizootics in the field could impose the same risk to birds as epizootics introduced by products with *L. muscarium* (i.e. in case of birds eating the same number of infected insects).

2.9.9.2 Aquatic organisms

Based on the available data the acute margin of safety (MOS) values of fish and *Daphnia* for *L. muscarium* were calculated. It should be noted that TER-trigger values for chemical a.s. are not validated for microbial a.s. and therefore are not applicable.

Two worst-case exposure scenarios were chosen that assume complete accumulation of spores following:

- 24 applications at 1 x 10^{13} CFU/ha in strawberry tunnels

- 36 applications at 2 x 10^{13} CFU/ha in fruiting vegetables of Cucurbitaceae

Based on the predicted environmental density (PED_{SW}), calculated as 1.71×10^{6} spores/L, the margin of safety (MOS) for fish is derived from the EC_{50} value according to the formula:

$$MOS = \frac{EC_{50}[CFU/L]}{PED_{SW}[spores/L]}$$

Margin of safety (MOS) for fish and *Daphnia* exposed to *L. muscarium* Ve6 after use of MYCOTAL with 48 applications at 1 x 10^{13} CFU/ha in strawberry tunnels

Use pattern	Test organism	PED_{SW} [a)]	EC_{50}	MOS
24 × 1x10^{13} CFU/ha in tunnels (strawberry)	*Oncorhynchus mykiss*	1.71 × 10^6 CFU/L	> 6.2 × 10^9 CFU/L	>3626
	Daphnia		> 3.8 × 10^8 CFU/L	>222
36 x 2 x 10^{13} CFU/ha in fruiting vegetables of Cucurbitaceae (G)	*Oncorhynchus mykiss*	3.43 × 10^5 CFU/L	> 6.2 × 10^9 CFU/L	>18076
	Daphnia		> 3.8 × 10^8 CFU/L	>1108

a) Based on drift from accumulated applications, assuming no degradation between applications

The calculated margin of safety values are high, indicating an acceptable acute risk to fish and aquatic invertebrates after application of MYCOTAL at the maximum recommended use rate.

Although theoretically acute and short-term risks to aquatic organisms via indirect exposure (drainwater with conidia, runoff, erosion of soil particles with sorbed conidia) cannot be excluded, the actual risks via these routes are considered acceptable in view of:

(a) the margin of safety calculated above;
(b) the apparent lack of evidence of aquatic organisms being among the natural target or host range of *L. muscarium* in general, and a narrow natural host or target range (*L. muscarium* especially affects homoptera) specifically,
(c) the preference of *L. muscarium* for soil environments rather than aqueous environments, also clearly expressed by the natural target or host range, in which no aquatic organisms are listed,
(d) the slight water dispersability of spores in water (it should be noted in this respect that, theoretically, co-formulants may be added to a product to overcome this limitation),
(e) the lack of toxic, infective or pathogenic effects to fish and daphnids in protocolled laboratory tests with maximum hazard concentrations (see in Vol.3, MA B.9);
(f) the lack of field records indicating a (potential) risk for aquatic organisms, in spite of Mycotal use in various countries.

2.9.9.3 Bees

For the risk assessment the maximum single application rate of 144 g a.s./ha, corresponding to 3 × 10^{13} CFU/ha is considered. Since the trigger value of 50 for the hazard quotient approach for chemical a.s. does not apply, a different approach is followed by calculating the margin of safety between spraying liquid and test solution.

Exposure and margin of safety assessment for honey bees

Crop scenario	AR [a)]	Minimum Water	Maximum field concentration	Exposure	Concentration in test solution	MOS[b)] test / field
Greenhouse	0.144 kg a.s./ha	1000 L/ha	0.144g a.s./L	oral	5.0 g a.s./L	34.7
				contact	25 g a.s./L	174

a) Maximum single application rate
b) Margin of safety; concentration in test solution/ maximum field concentration

In comparison of tested concentration, at which no treatment-related mortality was indicated and no clinical signs of toxicity or abnormal behaviour were observed, and the maximum single application rate (AR), the margin of safety is 34.7 and 174 for the oral and contact exposure of MYCOTAL, respectively, which can be considered as sufficiently high to exclude a risk on bees.

Moreover, MYCOTAL application is only recommended under high humidity conditions. This can only be realized when tunnels/greenhouses are closed (for at least 12 hrs after application is recommended) and when application is done in the evening, when evaporation is low, i.e. after bee flight. Therefore, it can also be concluded that the exposure is generally to be considered as low.

Concluding, in view of:

(a) the apparent lack of evidence that bees are the target or host range of *L. muscarium*, but instead an apparently narrow host or target range, specifically whitefly and thrips,
(b) the lack of toxic, infective or pathogenic effects of *L. muscarium* to honeybees in protocolled and well documented laboratory tests (see Tables 9.3.1.a and 9.3.1.b in Vol.3, MA B.9), *idem* to bumblebees in less well documented laboratory tests; the lack of treatment-related effects in a field test with honeybees sprayed with Mycotal (1.5×10^6 CFU/mL),
(c) the lack of field records indicating a (potential) risk for honeybees and bumblebees, in spite of Mycotal use in various countries,
(d) the margin of safety taking the maximally recommended application rate into account do not indicate unacceptable risks to honeybees either via direct contact or via oral exposure to *L. muscarium* (see Table B.9.3.1-1),
(e) the occurrence of natural epizootics in the field could impose the same risks to bees as introduced epizootics by products with *L. muscarium*,

the risk to bees is generally considered acceptable. However, as there are no infectivity and pathogenicity data under moist air conditions (RH $\geq$ 90%, the optimal humidity for optimal efficacy of the a.s. in greenhouses), the risks for bumblebees under such conditions in greenhouses cannot be excluded. Notwithstanding these specific potential risks, such data for bumblebees are strictly not required,

and data for honeybees generally suffice. In conclusion, the Uniform Principle criteria are considered to be met.

2.9.9.4 Non-target arthropods other than bees

A summary of available data is given in section 2.9.4. For comparison of tested rates:

- The proposed spraying solution concentration is: 1-3 x 10^{10} CFU/L
- The max. proposed single dose rate is: 3x10^{13} CFU/ha

In principle, adverse effects to NTAs (bees excl) cannot be excluded at the proposed conditions of use in view of the mode of action (contact arthropod pathogenicity) and the microbiological properties, and some slight effects noted on parasitoid wasps (*E. formosa*) in the available articles. However, in view of:

(a) the apparent lack of evidence that NTAs (bees excl) in general are the target or host range of *L. muscarium*, but instead the strain has an apparently narrow host or target range, specifically whitefly and thrips,
(b) the apparent lack of toxic, infective or pathogenic effects in various laboratory studies,
(c) the lack of greenhouse or field records indicating a (potential) risk for NTAs (bees excl), in spite of use in various countries,
(d) natural epizootics in the field could impose the same risks to NTAs (bees excl), as epizootics introduced by products with *L. muscarium*,

the risk to NTAs is considered acceptable. In strawberry fields, the occurrence of high RH values and temperatures for an optimal efficacy — estimated ≥ 80% and ≥ 18 ºC, respectively — may be less frequent than in well-controlled greenhouses, possibly dependent on the extent of the (semi-) protection with *e.g.* plastic or other materials. Therefore outdoor effects may be limited as well. In conclusion, the Uniform Principle criteria are considered to be met, this in view of circumstantial evidence rather than on TER outcomes.

2.9.9.5 Earthworms

Based on the predicted environmental density in soil (PEDsoil) calculated inVol.3, MA B.8.1.1 (Table 8.1.1-4), the margin of safety (MOS) for earthworms is derived in the table below from the LC_{50} according to the following formula:

$$MOS = \frac{LC_{50}[\text{spores/kgsoil dw}]}{PED_{soil}[\text{spores/kgsoil dw}]}$$

It should be noted that TER-trigger values for chemcial a.s. are not validated for microbial a.s. and therefore are not applicable.

For the use in greenhouses a common practice is that the soil is sterilised on a regular basis and therefore the risk to earthworms is less relevant. However, this may not be applicable for all MS and for completeness of the risk assessment the worst case greenhouse use is included in the table below.

Risk assessment for earthworms exposed to *L. muscarium* Ve6 after use of MYCOTAL

Use pattern	Test organism	LC_{50}	$PED_{soil,\ max\ initial}$ [a)]	MOS
$24 \times 1x10^{13}$ CFU/ha in strawberry tunnels (F)	*Eisenia fetida*	6.3×10^{10} spores/kg soil dw	3.2×10^{8} CFU/kg soil dw	197
$36 \times 2x10^{13}$ CFU/ha in fruiting vegetables of *Cucurbitaceae* (G)	*Eisenia fetida*	6.3×10^{10} spores/kg soil dw	9.6×10^{8} CFU/kg soil dw	66

a) Total per year

The calculated MOS value is high, indicating an acceptable acute risk to earthworms after application of MYCOTAL at the maximum recommended use rates.

This is further confirmed by a study from the public literature (Gradila, 2013; KMA 8.5/02) with *E. fetida*. With regard to toxicity, the study determined no adverse effects at 1.7×10^{9} UFM/mL dispersed in artificial soil. The results are considered as supportive information only, due to the unknown concentration of test item in the soil, and also due to the unknown test strain. In the test infectivity and pathogenicity was not investigated.

In view of:

(a) the margin of safety calculated above,
(b) the apparent lack of evidence of earthworms being among the natural target or host range of *L. muscarium* in general,
(c) the lack of acute toxic, infective or pathogenic effects to *Eisenia fetida* in a protocolled, GLP laboratory test following artificial soil mixing with technical grade *L. muscarium*,
(d) the lack of greenhouse or field records indicating a (potential) risk for earthworms, in spite of use in various countries
(e) the natural presence of *L. muscarium* in soil;

the acute risk to earthworms is considered acceptable. Long-term risks with respect to e.g. reproduction as a result of the intended uses are considered unlikely.

2.9.9.6 Non-target soil micro-organisms

Toxic or competitive effects of the generally occurring filamentous soil fungus *L. muscarium* to other soil micro-organisms probably exist. These effects, however, should be seen in the context of the intricate and largely unknown population dynamics of a terrestrial ecosystem. However, there are no scientific, empirical indications for any adverse effect rather than on a theoretical level (no relevant articles were found in the literature search, see B.9.8). It was considered outside the scope of this monograph to evaluate all tests and studies on interactions with other micro-organisms. Whereas these tests and studies obviously exist, they are generally laboratory studies of which the test results are difficult to extrapolate to field conditions. This is due to the complexity of the soil compartment, the intricate interrelations between biotic and abiotic factors and the still limited knowledge on soil microbial ecology in general. It cannot be excluded that the application of *L. muscarium* will have a certain effect on the ecology of the soil ecosystem, as would have any intervention. The scale of possible effects due to the application of *L. muscarium*, both in time and in space, however, is difficult to analyse. There seem to be no scientific indications for obvious adverse effects due to such applications. It may be expected that the spraying will be followed by interactions with local micro-flora and fauna in case the a.s. will contact the soil. It is also noted however that, in case soil micro-organisms are exposed, risks may not differ from 'natural' epizootics, particularly taking the relatively persistent character of conidia into account. In conclusion, the Uniform Principle criteria are assumed to be met in a weight of evidence approach.

2.9.9.7 Additional studies

No data available, not considered necessary.

2.10 Classification and labelling

2.10.1 Classification and Labelling of the active substance

Classification and labelling of chemical substances based on the criteria according to Regulation (EC) No 1272/2008 and Directive 67/548/EEC are not applicable to micro-organisms.
However micro-organisms should be regarded as potential sensitisers and the following hazard statement has to be applied:
Micro-organisms may have the potential to provoke sensitising reactions.

2.10.2 Classification and Labelling of the plant protection product

Labelling:

Signal word: -

Hazard statements: -

Precautionary statements: P280

Proposed notes assigned to an entry:

Notes in accordance with CLP Regulation, Annex VI, Section 1.1.3

Labelling: Micro-organisms may have the potency to provoke sensitization reaction.

This labelling phrase implies that PPE have to be worn when handling the product or applying the product:

- Gloves (butylrubber, PVC)
- Closed eye protection (monogoogles)
- Protective clothing.

In case of opening of the packing, blending/loading and spraying, wear preferably a filtermask with filtertype P3.

2.11 Relevance of metabolites in groundwater

No relevant human/mammalian metabolites or toxins present in product or being produced by *Lecanicillium muscarium* Ve6.

2.12 Consideration of isomeric composition in the risk assessment

No information is required as micro-organisms do not have isomers.

2.13 Residue definitions

2.13.1 Definition of residues for exposure/risk assessment

Not applicable.

2.13.2 Definition of residues for monitoring

Not applicable.

Level 3

Lecanicillium muscarium Ve6

3 Proposed decision with respect to the application

3.1 Background to the proposed decision

3.1.1 Proposal on acceptability against the decision making criteria – Article 4 and annex II of regulation (EC) No 1107/2009

3.1.1.1	Article 4			
		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		There are still some questions for the applicant to be answered (see under 3.1.4). However, these questions are not considered major data gaps and therefore it is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with.
3.1.1.2	Submission of further information			
		Yes	No	
i)	It is considered that a complete dossier has been submitted	X		
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			Not applicable
3.1.1.3	Restrictions on approval			
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.		X	

3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		No need for ADI, AOEL and/or ARfD since lack of adverse effects due to *L. muscarium* Ve6 in studies performed.
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.	X		Viable residues: No risk for the consumer is expected since an increase of spore numbers or mycelium on leaves and fruits is deemed not to occur under practical conditions and spore numbers decrease quickly over time. Non-viable residues: No risk for the consumer is expected, since no toxins are expected to occur during and after application of Mycotal. No need for a residue definition.
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		Considering that the substance is approved and that the extant authorizations of plant protection products containing *Lecanicillium muscarium* have already been evaluated according to the Uniform Principles, no other efficacy information is considered to be necessary at this time.

Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.	X		No relevant human/mammalian metabolites or toxins present in product or being produced by *Lecanicillium muscarium* Ve6.
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.	X		
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.			Not applicable for micro-organisms.
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted			Not applicable for micro-organisms.
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.	X		
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.			Not applicable for micro-organisms.
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of	X		

	Regulation 1107/2009.			
Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		No need for ADI, AOEL and/or ARfD since lack of adverse effects due to *L. muscarium* Ve6 in studies performed.
Impact on human health - proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	Not applicable for micro-organisms.
Impact on human health - proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**		X	Not applicable for micro-organisms.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			

Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**		X	Not applicable for micro-organisms.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Impact on human health - proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **carcinogenic category 2 and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties**		X	Not applicable for micro-organisms.
ii)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **toxic for reproduction category 2 and** in addition the RMS considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**		X	Not applicable for micro-organisms.
iii)	Linked to either i) or ii) immediately above. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is			

	used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	Not applicable for micro-organisms.
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	Not applicable to micro-organisms. (*Lecanicillium muscarium* is not toxic and does not produce any relevant metabolites.)
Very persistent and very bioaccumulative substance (vPvB)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	Not applicable for micro-organisms.
Ecotoxicology				
		Yes	No	
	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.	X		See section 2.9.9 for risk assessment summaries.
	It is considered that, on the basis of the assessment of Communi-		X	Not applicable for micro-organisms.

	ty or internationally agreed test guidelines, the substance **HAS** endocrine disrupting properties that may cause adverse effects on non-target organisms.			
	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.			
	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.	X		Yes for all intended uses. See section 2.9.9 for risk assessment summaries.
Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.			Not applicable for micro-organisms.
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		

3.1.2 Proposal – Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		X	

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	An active substance which is a micro-organism may be considered as being of low-risk unless at strain level it has demonstrated multiple resistance to anti-microbials used in human or veterinary medicine.			Inconclusive: Insufficient information is available to fully exclude anti-biotic resistance (see Volume 3, B.2.8).

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

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 Identity of the active substance or formulation				
Clarification is needed on the relationship to known pathogens. For more details see Volume 3MA, B.1.3.5.	All uses	X		
Applicant please provide us with a proposal on information on any specific agricultural, plant health or environmental conditions under which the preparation may or may not be used. For more details see Volume 3MP, B.4.3.2.	All uses	X		
More clarification is needed on the method of identification. For more details see Volume 3MA, B.5.1.1.	All uses	X		
Clarification is needed on the morphological identification. For more details see Volume 3MA, B.5.1.5.	All uses	X		
The address of the plant location of the technical microbial active substance should be provided (reference to Volume 4).	All uses	X		
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
A shelf-life study in the commercial packag-	All uses			Study indicated

ing at the proposed storage conditions, including information on contaminants.				available, but not yet submitted
The in-use concentrations need to be clarified as the GAP suggests a concentration range of 0.1 – 0.3%, whereas the studies with regard to technical properties of the plant protection product only cover concentrations of up to 0.1%.	All uses	X		
3.1.4.3 Data on uses and efficacy				
None				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
None				
3.1.4.5 Methods of analysis				
None				
3.1.4.6 Toxicology and metabolism				
The applicant should perform a new literature search including the metabolites only or in combination with toxicology (not only the combination of metabolites and the microorganism) (see Volume 3, B.6 for more details).	All uses	X		
3.1.4.7 Residue data				
None				
3.1.4.8 Environmental fate and behaviour				
None				
3.1.4.9 Ecotoxicology				
The applicant is requested to submit a comprehensive search of the published literature	All uses	X		

with the aim to find all references regarding the production of toxins or metabolites of ecotoxicological concern.				

3.1.5 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, as laid out in Commission Regulation (EU) No 546/2011, 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).

Area of the risk assessment that could not be finalised on the basis of the available data	**Relevance in relation to representative use(s)**
None	

3.1.6 Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 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 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.

Critical area of concern identified	**Relevance in relation to representative use(s)**
None	

3.1.7 Overview table 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 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)

Representative use		Cucur-bitaceae	Solana-ceae	Straw-berry	Straw-berry	Flori-culture	Cut roses
Operator risk	Risk identified						
	Assessment not finalised						
Worker risk	Risk identified						
	Assessment not finalised						
Bystander risk	Risk identified						
	Assessment not finalised						
Consumer risk	Risk identified						
	Assessment not finalised						
Risk to wild non target terrestrial vertebrates	Risk identified						
	Assessment not finalised						
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified						
	Assessment not finalised						
Risk to aquatic organisms	Risk identified						
	Assessment not finalised						
Groundwater exposure active substance	Legal parametric value breached						
	Assessment not finalised						
Groundwater exposure metabolites	Legal parametric value breached						
	Parametric value of 10 µg/L(a) breached						
	Assessment not finalised						
Comments/Remarks							

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.

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

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	**Justification**
None	

3.1.9 Critical issues on which the Co RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur Member State. Only the points relevant for the decision making process should be listed.

Issue on which Co-RMS disagrees with RMS	Opinion of Co-RMS	Opinion of RMS
None		

3.2 Proposed decision

[redacted]

3.3 Rational for the conditions and restrictions to be associated with the approval or authorisation(s), as appropriate

3.3.1 Particular conditions proposed to be taken into account to manage the risk identified

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
[redacted]	

3.4 Appendices

3.4.1 Guidance documents used in this assessment

Guidances applicable at the time of submission of the additional dossier were used in this assessment.

3.5 Reference list

Not applicable.