Renewal Assessment Report

Lecanicillium muscarium Ve6
- Mycotal -

Volume 3MP – B.9 Effects on non-target organisms

January 2018

Rapporteur Member State: The Netherlands

Co-Rapporteur Member State: France

Version history

When	What
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B Summary, evaluation and assessment of the data and information

B.9 Effects on non-target organisms

Intended uses:

Table B.9-1: Intended uses of MYCOTAL

Crop and/ or situation (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** Method / Kind; Timing / Growth stage of crop & season	**Application** Max. number (min. interval between applications) a) per use b) per crop/ season	**Application rate** kg product / ha a) max. rate per appl. b) max. total rate per crop/season	**Application rate** g as/ha (spores/ha) a) max. rate per appl. b) max. total rate per crop/season	**Remarks:**[a)] e.g. g safener/synergist per ha; Water L/ha min / max
Fruiting vegetables of Cucurbitaceae with edible peel and inedible peel	G	Nymphs of whitefly and thrips: *Bemisia tabaci*; *Trialeurodes vaporariorum*; *Frankliniella ocidentalis*; *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})	3 cycles/year 1000/2000 Maximum concentration in tank mix should not exceed 0.1%
Fruiting vegetables of Solanaceae,				a)12 (7) b) 12 (7)	a) 2 b) 24	a) 96 (2×10^{13}) b) 1152 (2.4×10^{14})	1 cycle/year 1000/2000 Maximum concentration in tank mix should not exceed 0.1%
Strawberry				a) 12 (7) b) 24 (7)	a) 1 b) 24	a) 48 (1×10^{13}) b) 1152 (2.4×10^{14})	2 cycles/year 1000
Floriculture crops, except cut roses				a) 4 (7) b) 24 (7)	a) 2 b) 48	a) 96 (2×10^{13}) b) 2304 (4.8×10^{14})	1-7 cycles/year; max. number of applications per year: 24 1000/2000 Maximum concentration in tank mix should not exceed 0.1%
Cut roses				a) 24 (7) b) 24 (7)	a) 3 b) 72	a) 144 (3×10^{13}) b) 3456 (7.2×10^{14})	per 12 month 1000/3000 Maximum concentration in tank mix should not exceed 0.1%
Tree nursery				a) 24 (7) b) 24 (7)	a) 2 b) 48	a) 96 (2×10^{13}) b) 2304 (4.8×10^{14})	per 12 month 1000/2000 Maximum concentration in tank mix should not exceed 0.1%

Crop and/ or situation **(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**		**Remarks:**[a)]
			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	e.g. g safener/synergist per ha Water L/ha min / max
Strawberry	F	Nymphs of whitefly and thrips: *Bemisia tabaci*; *Trialeurodes vaporariorum*; *Frankliniella ocidentalis*; *Thrips tabaci*	Spray application At first sign of infestation Jan-Dec.,	a) 12 (7) b) 24 (7)	a) 1 b) 24	a) 48 (1×10^{13}) b) 1152 (2.4×10^{14}	2 cycles/year 1000

a) Apply the product in the evenings in greenhouses and in closed tunnel systems to provide high humidity, keep closed for at least 12 hours during and after application

Metabolites/toxins

Lecanicillium muscarium Ve6 has been shown to be able to produce destruxins (see section B.2.7). However, no destruxins were detected in spores, mycelium, colonised rice, filtrates of production-scale cultures, or the end-use product. Destruxins were also not detected in plant material treated with foliar applications of the formulated product Mycotal (at ten times the recommended dose; see reference Butt et al., 2004 in section B.2).

In the DAR (2007), the following was stated in section B.9.1.4.2:

Destruxins A, B and E were detected in culture filtrates of *L. muscarium* (see Vol 3, Annex B.1, B.2.1.8). Also subtilisins and subtilisin-related proteases may be present during growth on insect cuticulas (St Leger *et al.*, 1997). However, there are no indications of any toxicity of such mixtures of toxins, enzymes and other ingredients for birds, although tests with purified toxins or enzymes of *L. muscarium* are not available. Toxins and other metabolites of *L. muscarium* can be considered biodegradable outside micro-organisms. They are effective only during the process of hyphae growth when degrading the host or target.

According to the EFSA conclusion (EFSA Journal 2010; 8(1):1446) on *L. muscarium* Ve6, the strain does not produce any metabolites of concern.

Based on the above, no further risk assessment of metabolites is considered necessary.

B.9.1 Effects on birds

No (new) studies are submitted under this point. Since the co-formulants of the product are inert and not hazardous, data on the active substance, *L. muscarium* is regarded to be adequate for the risk assessment of MYCOTAL. Please refer to Vol.3 MA B.9.

B.9.1.1 Risk assessment for birds

Since *L. muscarium* Ve6 needs very high humidity for an effective germination, application is conducted only in greenhouses, or in tunnel systems when tunnels are closed during and at least 12 hours after application. Therefore, it can be concluded that exposure of birds to *L. muscarium* Ve6 is negligible (greenhouses) or very limited (tunnels).

L. muscarium Ve6 was never described as pathogen on birds or mammals, and the literature search did not find any reference reporting effects of *Lecanicillium* sp. on birds (Scholze, 2016),

The lack of adverse effects found in the available acute oral toxicity, infectivity, pathogenicity study assessing the oral administration of *Lecanicillium muscarium* Ve6 (see Vol.3, MA B.9, KMA8.1/01) to Japanese quails may be due to the intrinsic non-toxic nature of conidia from filamentous fungi as *L. muscarium* and the optimal growth temperature of germinated conidia in hosts below the average bird body temperature (see Vol 3, Annex B.1 Identity).

In summary, 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).

In the human toxicology section there was some discussion on the subject of infectiviy of *Lecanicillium muscarium* Ve6. Taken all information together, it was concluded by RMS that *Lecanicillium muscarium* Ve6 is not infective and thus not pathogenic.

Based on the above, the risk for birds and other non-target terrestrial vertebrates is considered acceptable.

B.9.2 Effects on aquatic organisms

No (new) studies are submitted under this point. Since the co-formulants of the product are inert and not hazardous, data on the active substance, *L. muscarium* is regarded to be adequate for the risk assessment of MYCOTAL. Please refer to Vol.3 MA B.9.

B.9.2.1 Risk assessment for aquatic organisms

Based on the available data the acute margin of safety (MOS) values of fish and *Daphnia* for *L. muscarium* were calculated (**Table 9.2.1-1**). 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:

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

Table 9.2.1-1 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.

This is further confirmed by a study from the public literature (Gradila, 2013; KMA 8.2.2/02) with *D. magna*. With regard to toxicity, the study determined a 48 h EC_{50} of > 1.7 x 10^9 CFU/mL, or 1.7x10^6 CFU/L. The results is considered as supportive information only, due to the absence of (reporting of) analytical measurements. In the test infectivity and pathogenicity was not investigated (for which the study duration was too short anyway).

In addition, the following risk assessment from the DAR (2007) still applies:

L. muscarium is not expected to show substantial toxicity, infectivity or pathogenicity to aquatic animals and plants, although in view of a fish kill in a Finnish fishfarm, infectivity and pathogenicity to Atlantic salmon cannot be excluded under extreme conditions. Aquatic organisms are not among the historical target or host range. The mode of action, propagation and ecological preferences do not indicate unacceptable risks to aquatic organisms. However, potentially toxic components of *L. muscarium* as destruxins and subtilisins may theoretically be intrinsically hazardous to arthropods as has been shown by Skrobek and Butt (2004) for purified destruxins of another filamentous fungus species. Such exposure seems unlikely *in situ*, even in case of conidia reaching surface water via spray drift, as conidia are not expected to germinate and grow in an aqueous environment, thus not being able to 'discharge' toxins. Exposure via groundwater or drain water is assumed unlikely as well, as substantial leaching of *L. muscarium* is not expected in view of the hydrophobicity of its conidia, whereas sorption to soil particles is more likely. It is, however, unclear to what extent the sorption of filamentous fungi conidia to soil particles is reversible. The product Mycotal with the a.s. *L. muscarium* is intended to be used in greenhouses, and in tunnels for strawberries which will be closed during and 12 hrs after application.

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.

B.9.3 Effects on bees

No (new) studies are submitted under this point. Since the co-formulants of the product are inert and not hazardous, data on the active substance, *L. muscarium* is regarded to be adequate for the risk assessment of MYCOTAL. Please refer to Vol.3 MA B.9.

B.9.3.1 Summary and risk assessment for bees

Honeybees and bumblebees may be exposed following outdoor applications in strawberries and following indoor applications in greenhouses, when (bumble-) bees are used for pollination purposes as may be the case in *e.g.* The Netherlands. Bees may be exposed to *L. muscarium* by direct over-spray, by contact with residues on plants whilst bees are foraging for food, or by consumption of contaminated, pollen, nectar or water. The intended use of MYCOTAL is in greenhouse, where bees might be used as pollinators in the targeted crops, or in tunnels (strawberry). Therefore, exposure can not be excluded. For the risk assessment the maximum single application rate of 144 g a.s./ha, corresponding to 3×10^{13} CFU/ha will be 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.

Table B.9.3.1-1 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) (see Vol. 3 MA, section B.9.3.1, paragraph 'Other studies on bee toxicity, infectivity and pathogenicity'),

(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 risk is considered acceptable.

B.9.4 Effects on arthropods other than bees

No (new) studies are submitted under this point. Since the co-formulants of the product are inert and not hazardous, data on the active substance, *L. muscarium* is regarded to be adequate for the risk assessment of MYCOTAL. Please refer to Vol.3 MA B.9.

B.9.4.1 Summary and risk assessment for non-target arthropod species other than bees

No studies are submitted assessing the effect of the product MYCOTAL on arthropods other than bees. Several reports and publications are presented in Vol. 3, MA B.9, section 8.4. Most investigations have been performed in relation to biological plant protection and a wide range of arthropod groups including foliar dwellers as well as soil arthropods. However, GLP studies in accordance with the relevant guidelines delivering suitable endpoints for a classical risk assessment are not available.

The most susceptible route for affecting NTAs is theoretically via direct spraying, as direct contact of the a.s. with an arthropod cuticula is the primary requirement for the a.s. efficacy. Non-target arthropods (bees excl.) may be exposed (a) via direct spraying in case of plant sprayings, (b) via indirect contact with residues on sprayed plants, (c) via ingestion of conidia. These exposure scenarios may occur following applications with *L. muscarium* in greenhouses and outdoor applications in strawberries. Greenhouse NTA populations are generally introduced as part of Integrated Pest Management.

A summary of available data is given in Volume 1, 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

Effects of *L. muscarium* Ve6 only have been observed on two species of whitefly and thrips. For all other more than 20 tested non-target species no adverse effects have been observed at relevant dose levels. This points to a high specificity of the strain.

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 risk is considered acceptable, this in view of circumstantial evidence rather than on TER outcomes.

B.9.5 Effects on earthworms

No (new) studies are submitted under this point. Since the co-formulants of the product are inert and not hazardous, data on the active substance, *L. muscarium* is regarded to be adequate for the risk assessment of MYCOTAL. Please refer to Vol.3 MA B.9.

B.9.5.1 Summary and risk assessment for 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 table 9.5.1-1 from the LC_{50} according to the following formula:

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

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 Table 9.5.1-1 below.

Table 9.5.1-1 Risk assessment for earthworms exposed to *L. muscarium* Ve6 after use of MYCO-TAL

Use pattern	Test organism	LC_{50}	$PED_{soil, max initial}$ a)	MOS
24 × $1x10^{13}$ 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 × $2x10^{13}$ 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 x 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.

B.9.6 Effects on 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 risk is considered acceptable in a weight of evidence approach.

B.9.7 Effects on terrestrial plants

There are no data available with regard to possible effects of *L. muscarium* on terrestrial plants and these data are not required according to the data requirements in Com. Regulation (EU) No 283/2013. In view of its general natural occurence, host range and mode of action, adverse effects are not expected.

B.9.8 Additional studies

No data available, not considered necessary.

B.9.9 References relied on

New data submitted for the purpose of renewal:

Data point	**Author(s)**	**Year**	**Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not**	**Vertebrate study Y/N**	**Data protection claimed Y/N**	**Justification if data protection is claimed**	**Owner**
KMA 8.1/01	Scholze, I.	2016	LITERATURE REVIEW ON LECANICILLIUM MUSCARIUM VE6 (19-79): EFFECTS ON NON-TARGET ORGANISMS Koppert, 2191392-MA-08-01 GAB Consulting GmbH, Heidelberg, Germany GLP/GEP: no Published: no	no	yes	New data for active ingredient, not previously submitted nor evaluated	KBS
KMA 8.1/02	Anonymous	2006a	EFFECTS OF VERTICILLIUM LECANII STRAIN VE6 ON REPRODUCTION OF BIRDS. Koppert, not stated Koppert Biological Systems GLP/GEP: no Published: no	no	yes	New data for existing formulation, not previously submitted nor evaluated	KBS
KMA 8.2.2/01	Anonymous	2006 b	EFFECTS OF VERTICILLIUM LECANII STRAIN VE6 ON REPRODUCTION OF DAPHNIA. Koppert, not stated Koppert Biological Systems GLP/GEP: no Published: no	no	yes	New data for existing formulation, not previously submitted nor evaluated	KBS
KMA 8.2.2/02	Gradila, M., Hera, E., Sicuia, O., Dinu, M.M., Valimareanu, D.G.	2013	EVALUATION OF ACUTE TOXICITY OF THE ENTOMOPATHOGENIC FUNGI ON BIOLOGICAL SYSTEMS , not applicable Romanian Journal of Plant Protection, 6, 1-4 GLP/GEP: no Published: yes	no	no	not protected	-
KMA 8.3/01	Kamilova, F.	2016	DECLARATION MYCOTAL Koppert, not stated Koppert B.V., Berkel en Rodenrijs, NL GLP/GEP: no Published: no	no	yes	New data for active ingredient, not previously submitted nor evaluated	KBS

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KMA 8.4/01	Shinde, S.V., Patel, K.G., Purohit, M.S., Pandya, J.R., Sabalpara, A.N.	2010	LECANICILLIUM LECANII (ZIMM.) ZARE AND GAMES AN IMPORTANT BIOCONTRAL AGENT FOR THE MANAGEMENT OF INSECT PESTS - A REVIEW , not applicable Agri. Review, 31, 235-252 GLP/GEP: no Published: yes	no	no	not protected	-
KMA 8.4/02	Donka, A., Sermann, H., Büttner, C.	2008	EFFECT OF THE ENTOMOPATHOGENIC FUNGUS LECANICILLIUM MUSCARIUM ON THE PREDATORY MITE PHYTOSEIULUS PERSIMILIS AS A NON-TARGET ORGANISM , not applicable Comm. Appl. Biol. Sci. Ghent University, 73, 395-403 GLP/GEP: no Published: yes	no	no	not protected	-
KMA 8.4/03	Hamdi, F., Fargues, J., Ridray, G., Jeannequin, B., Bonato, O.	2011	COMPATIBILITY AMONG ENTOMOPATHOGENIC HYPHOCREALES AND TWO BENEFICIAL INSECTS USED TO CONTROL TRIALEURODES VAPORARIORUM (HEMIPTERA: ALEURODIDAE) IN MEDITERRANEAN GREENHOUSES , not applicable J Invertebr Pathol, 108, 1-8 GLP/GEP: no Published: yes	no	no	not protected	-
KMA 8.4/04	Anonymous	2006c	SIDE EFFECT OF MYCOTAL & ADDIT ON ENCARSIA FORMOSA, MACROLOPHUS CALIGINOSUS AND PHYTOSEIULUS PERSIMILIS Koppert, not stated Koppert Biological Systems GLP/GEP: no Published: no	no	yes	New data for active ingredient, not previously submitted nor evaluated	KBS

Data point	Author(s)	Year	Title Owner Report No. Source (where different from owner) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KMA 8.4/05	Kamilova, F.	2016	DECLARATION MYCOTAL Koppert, not stated Koppert B.V., Berkel en Rodenrijs, NL GLP/GEP: no Published: no **Submitted in: KMA 8.3/01**	no	yes	New data for active ingredient, not previously submitted nor evaluated	KBS
KMA 8.4/06	Aqueel, M.A., Leather, S.R.	2013	VIRULENCE OF VERTICILLIUM LECANII (Z.) AGAINST CEREAL APHIDS; DOES TIMING OF INFECTION AFFECT THE PERFORMANCE OF PARASITOIDS AND PREDATORS? , not applicable Pest Management Science, 69, 493-498 GLP/GEP: no Published: yes	no	no	not protected	-

Data from previous evaluation (DAR 2007):

Annex IIM Data and Information

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
IIM 8.1	Benkerroum S Tantaoui-Elaraki A	2001	Study of toxigenic moulds and mycotoxins in poultry feeds. Revue Méd. Vét. **152** (4): 335-342	N	-
IIM 8.1	[redacted]	1998	An acute toxicity study of Mycotal TGAI administered orally to Japanese quails. [redacted] Report No.7L785	Y	KBS
IIM 8.1-8.8; IIM 8.9.1	Burges HD (ed)	1981	Microbial control of pests and plant diseases, 1970-1980. Academic Press, London, New York, Toronto	N	-

Annex point / reference number	**Author(s)**	**Year**	**Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not**	**Data Protection Claimed* Y/N**	**Owner ****
IIM 8.1-8.8; IIM 8.9.1	Copping LG (ed)	2004	The Manual of Biocontrol Agents. A world compendium. rev. 3rd edition. The British Crop Protection Council. Surrey, UK	N	-
IIM 8.1-8.8; IIM 8.9.1	Hokkanen HMT Hajek AE (eds)	2003	Environmental impacts of microbial insecticides. Needs and methods for risk assessment. Kluwer Academic Publishers, Dordrecht/Boston/London	N	-
IIM 8.1-8.8; IIM 8.9.1	OECD	2005	Directory of microbial pesticides for agricultural crops in OECD countries Revised version, Kabaluk T. and Gazdik K. (eds) Agriculture and Agri-Food Canada	N	-
IIM 8.2	[redacted]	1983	The acute toxicity of Ve6-58 SSP to rainbow trout (*Salmo gairdneri*); [redacted]. Report No. TTL 3/83415	Y	KBS
IIM 8.2-8.6	Skrobek A Butt T	2005	Toxicity testing of destruxins and crude extracts from the insect-pathogenic fungus *Metarhizium anisopliae*. FEMS Microbiology Letters **251**: 23–28	N	-
IIM 8.3	Quinlan RJ	1983	*Verticillium lecanii* acute immobilisation of *Daphnia* test. University of Reading, England. Date: 02-03-1983	Y	KBS
IIM 8.4	Verhaar HJM	2005	*Verticillium lecanii*. Toxicity to algae. ENVIRON Netherlands B.V. Doc No 77KO-MYCST-20050211	Y	KBS
IIM 8.7	Gerritsen L Cornelissen B	2006	Biologische bestrijding van varroa met behulp van schimmels. PPO Bijen http://www.wur.nl/NL/nieuwsagenda/archief/nieuws/2006/Biologische_bestrijding_varroamijt_in_bijenhouderij_is_lastig.htm (in Dutch)	N	-
IIM 8.7	Kling A	2000	Assessment of side-effects of *Verticillium lecanii* to the honeybee, *Apis mellifera* L. in the laboratory. GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Öschelbronn, Germany. Report No. 20001292/01-BLUE	Y	KBS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
IIM 8.7	van Doorn A	1998	Impact of the fungi *Verticillium* and *Trichoderma* on adult bumblebees and bumblebee brood. Research Report R&D Natupol. Koppert Biological Systems. Report No. Pr980701	Y	KBS
IIM 8.7; IIM 8.8	Schuler T Hommes M Plate HP Zimmermann G	1991	*Verticillium lecanii* (Zimmermann) Viégas (Hyphomycetales: Moniliaceae): Geschichte, systematik, Verbreitung, Biologie und Anwendung im Pflanzenschutz. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft. Heft 269. Berlin, Dahlem.	N	-
IIM 8.8	Beerling EAM van den Berg D	2003	36th Annual Meeting Program and Abstracts Society for Invertebrate Pathology 26-30 July 2003 Burlington, Vermont, US	N	-
IIM 8.8	Flexner JL Lighthart B Croft BA	1986	The effects of microbial pesticides on non-target beneficial arthropods. Agric Ecosystems Environ **16**: 203-254	N	-
IIM 8.8	Quinlan RJ Chaudhry MA	unknown	Unpublished study report: non-target insect tests for toxicity/pathogenicity. University of Reading, UK	Y	KBS
IIM 8.8	Samson RA Rombach MC	1985	Biology of the fungi *Verticillium* and *Aschersonia*. In: Biological control. The glasshouse experience. Hussey NW and Scopes N (eds). Blenford press, Dorset, UK , p. 34-42	N	-
IIM 8.8	Sitch JC Jackson CW	1997	Pre-penetration effects affecting host specificity of *Verticillium lecanii*. Mycol Res **101** (5): 535-541	N	-
IIM 8.8	St Leger RJ Joshi L Roberts DW	1997	Adaptation of proteases and carbohydrases of saprophytic, phytopathogenic and entomopathogenic fungi to the requirements of their ecological niches. Microbiology **143**: 1983-1992	N	-

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
IIM 8.8	Sterk G. et al	1999	Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS working group "Pesticides and Beneficial Organisms". BioControl (formerly Entomophaga) **44**: 99-117	N	-
IIM 8.9.1	Wachter S	2000	Acute toxicity of *Verticillium lecanii* on earthworms, *Eisenia foetida* using an artificial soil test. GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Öschelbronn, Germany. Report No. 20001292/01-NLEf	Y	-

* Protection for 5 years claimed from date of decision concerning listing in Annex I - the study report has not been submitted in any of the Member States in support of an application for authorization, or (though the study report has been submitted) has not been used in any of the Member States as the basis for decision on the initial authorization, or to maintain a given authorization, of a plant protection product before the date of submission of the dossier to Rapporteur Member State.

** Owners' code identifications and names (Code identification: KBS Name: Koppert Beheer Systems)

Annex IIIM Data and Information

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
IIIM 10.1	Benkerroum S Tantaoui-Elaraki A	2001	Study of toxigenic moulds and mycotoxins in poultry feeds. Revue Méd. Vét. **152** (4): 335-342	N	-
IIIM 10.1	[redacted]	1998	An acute toxicity study of Mycotal TGAI administered orally to Japanese quails. [redacted]. Report No.7L785	Y	KBS
IIIM 10.1; IIIM 10.2; IIIM 10.4; IIIM 10.5; IIIM 10.7	Burges HD (ed)	1981	Microbial control of pests and plant diseases, 1970-1980. Academic Press, London, New York, Toronto	N	-

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IIIM 10.1; IIIM 10.2; ;IIIM 10.3; IIIM 10.4; IIIM 10.5; IIIM 10.7	Hokkanen HMT Hajek AE (eds)	2003	Environmental impacts of microbial insecticides. Needs and methods for risk assessment. Kluwer Academic Publishers, Dordrecht/Boston/London	N	-
IIIM 10.1; IIIM 10.2; IIIM 10.3; IIIM 10.4; IIIM 10.5; IIIM 10.7	Copping LG (ed)	2004	The Manual of Biocontrol Agents. A world compendium. rev. 3rd edition. The British Crop Protection Council. Surrey, UK	N	-
IIIM 10.1; IIIM 10.2; IIIM 10.3; IIIM 10.4; IIIM 10.5; IIIM 10.7	OECD	2005	Directory of microbial pesticides for agricultural crops in OECD countries Revised version, Kabaluk T. and Gazdik K. (eds) Agriculture and Agri-Food Canada	N	-
IIIM 10.2	[redacted]	1983	The acute toxicity of Ve6-58 SSP to rainbow trout (*Salmo gairdneri*); [redacted] Report No. TTL 3/83415	Y	KBS
IIIM 10.2	Quinlan RJ	1983	*Verticillium lecanii* acute immobilisation of *Daphnia* test. University of Reading, England. Date: 02-03-1983	Y	KBS
IIIM 10.2	Skrobek A Butt T	2005	Toxicity testing of destruxins and crude extracts from the insect-pathogenic fungus *Metarhizium anisopliae*. FEMS Microbiology Letters **251**: 23–28	N	-
IIIM 10.2	Verhaar HJM	2005	*Verticillium lecanii*. Toxicity to algae. ENVIRON Netherlands B.V. Doc No 77KO-MYCST-20050211	Y	KBS
IIIM 10.3	Gerritsen L Cornelissen B	2006	Biologische bestrijding van varroa met behulp van schimmels. PPO Bij-en http://www.wur.nl/NL/nieuwsagenda/archief/nieuws/2006/Biologische_bestrijding_varroamijt_in_bijenhouderij_is_lastig.htm (in Dutch)	N	-
IIIM 10.3	Kling A	2000	Assessment of side-effects of *Verticillium lecanii* to the honeybee, *Apis mellifera* L. in the laboratory. GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Öschelbronn, Germany. Report No. 20001292/01-BLUE	Y	KBS
IIIM 10.3	van Doorn A	1998	Impact of the fungi *Verticillium* and *Trichoderma* on adult bumblebees and bumblebee brood. Research Report R&D Natupol. Koppert Biological Systems. Report No. Pr980701	Y	KBS

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed* Y/N	Owner **
IIIM 10.3; IIIM 10.4	Schuler T Hommes M Plate HP Zimmermann G	1991	*Verticillium lecanii* (Zimmermann) Viégas (Hyphomycetales: Moniliaceae): Geschichte, systematik, Verbreitung, Biologie und Anwendung im Pflanzenschutz. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft. Heft 269. Berlin, Dahlem.	N	-
IIIM 10.4	Beerling EAM van den Berg D	2003	36th Annual Meeting Program and Abstracts Society for Invertebrate Pathology 26-30 July 2003 Burlington, Vermont, US	N	-
IIIM 10.4	Flexner JL Lighthart B Croft BA	1986	The effects of microbial pesticides on non-target beneficial arthropods. Agric Ecosystems Environ **16**: 203-254	N	-
IIIM 10.4	Quinlan RJ Chaudhry MA	unknown	Unpublished study report: non-target insect tests for toxicity/pathogenicity. University of Reading, UK	Y	KBS
IIIM 10.4	Samson RA Rombach MC	1985	Biology of the fungi *Verticillium* and *Aschersonia*. In: Biological control. The glasshouse experience. Hussey NW and Scopes N (eds). Blenford press, Dorset, UK , p. 34-42	N	-
IIIM 10.4	Sitch JC Jackson CW	1997	Pre-penetration effects affecting host specificity of *Verticillium lecanii*. Mycol Res **101** (5): 535-541	N	-
IIIM 10.4	St Leger RJ Joshi L Roberts DW	1997	Adaptation of proteases and carbohydrases of saprophytic, phytopathogenic and entomopathogenic fungi to the requirements of their ecological niches. Microbiology **143**: 1983-1992	N	-
IIIM 10.4	Sterk G. et al.	1999	Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS working group "Pesticides and Beneficial Organisms". BioControl (formerly Entomophaga) **44**: 99-117	N	-
IIIM 10.5	Wachter S	2000	Acute toxicity of *Verticillium lecanii* on earthworms, *Eisenia foetida* using an artificial soil test. GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Öschelbronn, Germany. Report No. 20001292/01-NLEf	Y	-

*: Protection for 5 years claimed from date of decision concerning listing in Annex I - the study report has not been

submitted any of the Member States in support of an application for authorization, or (though the study report has

been submitted) has not been used any of the Member States as the basis for decision on the initial authorization, or to maintain a given authorization, of a plant protection product before the date of submission of the dossier to Rapporteur Member State.

**: Owners' code identifications and names (Code identification: KBS Name: Koppert Beheer Systems)