CONCLUSION ON PESTICIDE PEER REVIEW

Conclusion on the peer review of the pesticide risk assessment of the active substance *Lecanicillium muscarium* strain Ve6 notified as *Verticillium lecanii*[1]

European Food Safety Authority[2]

European Food Safety Authority (EFSA), Parma, Italy

SUMMARY

Lecanicillium muscarium, notified as *Verticillium lecanii*, is one of the 295 substances of the fourth stage of the review programme covered by Commission Regulation (EC) No 2229/2004[3].

Lecanicillium muscarium strain Ve6 was included in Annex I to Directive 91/414/EEC on 1 May 2009 pursuant to Article 24b of the Regulation (EC) No 2229/2004 (hereinafter referred to as 'the Regulation'). In accordance with Article 25a of the Regulation the European Food Safety Authority (EFSA) is required to deliver by 31 December 2010 its view on the draft review report submitted by the Commission of the European Communities (hereinafter referred to as 'the Commission') in accordance with Article 25(1) of the Regulation. This review report has been established as a result of the initial evaluation provided by the designated rapporteur Member State in the Draft Assessment Report (DAR). The EFSA therefore organised a peer review of the DAR. The conclusions of the peer review are set out in this report.

The Netherlands being the designated rapporteur Member State submitted the DAR on *Lecanicillium muscarium* strain Ve6 in accordance with the provisions of Article 22(1) of the Regulation (EC) No 2229/2004, which was received by the EFSA on 5 July 2007. The peer review was initiated on 7 May 2008 by dispatching the DAR for consultation to the sole notifier Koppert Beheer B.V., and on 21 January 2009 to the Member States. Subsequently, the comments received on the DAR were examined and responded by the rapporteur Member State in the reporting table. This table was evaluated by the EFSA to identify the remaining issues. The identified issues as well as further information made available by the notifier upon request were evaluated in a scientific meeting with Member State experts in June 2009.

A final discussion of the outcome of the consultation of experts took place during a written procedure with the Member States in November 2009 leading to the conclusions as laid down in this report.

The conclusion was reached on the basis of the evaluation of the representative uses on cucumber, tomato, sweet pepper, strawberry and ornamentals for the control of whitefly and thrips. The

1 On request from the European Commission, Question No EFSA-Q-2009-00255, issued on 18 December 2009.
2 Correspondence: praper@efsa.europa.eu
3 OJ L379, 24.12.2004, p.13, as amended by Regulation (EC) No 1095/2007 (OJ L246, 21.9.2007, p.19).

Suggested citation: European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance *Lecanicillium muscarium* strain Ve6, notified as *Verticillium lecanii*. EFSA Journal 2010; 8(1):1446. [45 pp.]. doi:10.2903/j.efsa.2010.1446. Available online: www.efsa.europa.eu

© European Food Safety Authority, 2009

formulation is applied as a spray to the crops. Full details of the application rate and timings can be found in the list of end points attached at appendix A to this report.

The representative formulated product for the evaluation was 'Mycotal', a wettable powder formulation (WP).

The available data indicate that the micro-organism is not competitive in the environment unless the host species are present, it is not pathogenic to humans, nor toxic or infective and does not produce any known toxicologically significant secondary metabolites, therefore methods of analysis for monitoring are not required. However, it should be noted that some methods are given in the DAR although there are no validation data.

Sufficient test methods and data relating to physical, chemical and technical properties are available. Methods for the unequivocal identification of the strain could not be concluded on, as the new information has not been peer-reviewed. The acceptability of the methods for microbial contaminants was questioned as they appeared not to be ISO methods. After the experts' meeting the notifier confirmed that ISO methods were used. Further validation is needed for the whitefly assay. It was considered that the level of contaminating pathogens was in some cases too high when compared to internationally available proposed levels for pathogenic microbial contaminants, and this is considered by EFSA as a critical area of concern.

In acute toxicity studies, *Lecanicillium muscarium* strain Ve6 did not induce signs of toxicity, pathogenicity or infectivity. The human/rat body temperature would mean that the micro-organism would not remain viable in these warm-blooded species. Clinical cases of systemic infection were described in the open literature in immuno-compromised patients, either receiving chemo/radiotherapy, or treated with intraperitoneal antibiotics; very rare cases of keratitis were caused by *Lecanicillium* species.

No potential for skin sensitisation was found in a Magnusson & Kligman test. Sensitisation studies with micro-organisms are considered to be of limited value, as reactions to foreign proteins (most micro-organisms) can be anticipated. Therefore, all micro-organisms should be regarded as potential sensitisers (in contact with skin and by inhalation). The following phrase was agreed by the experts "**Micro-organisms may have the potential to provoke sensitising reactions**", since labelling with risk phrases applicable to chemicals (according to Directive 67/548/EEC[4] and Directive 1999/45/EC[5]) is not appropriate for micro-organisms.

In a 28-day study by inhalation in rats conducted with the representative formulation 'Mycotal', the no observed adverse effect level (NOAEL) was found at 1 mg/m^3, based on macro- and microscopic changes in the lungs, nasal cavity and mediastinal lymph nodes most pronounced at 10 and 100 mg/m^3 groups. Although these changes were indicative of a local immune reaction rather than a toxicological effect, this could not be confirmed due to the lack of a control group in the study. Short-term toxicity is not expected, because accumulation of *Lecanicillium muscarium* strain Ve6 has not been demonstrated in the body. No potential for genotoxicity was found *in vitro*. As no known toxicologically significant metabolite was identified, the PRAPeR M3 meeting of experts agreed that no further data would be required for the genotoxicity end point.

No reference values were set and none were needed as the micro-organism is not pathogenic or infective and does not produce toxins.

[4] Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, OJ P 196, 16.08.1967, pp. 1 - 98

[5] Directive 1999/45/EC of the European Parliament and of the Council of 31 May 1999 concerning the approximation of the laws, regulations and administrative provisions of the Member States relating to the classification, packaging and labelling of dangerous preparations, OJ L 200, 30 July 1999, pp. 1-68

Low risk was anticipated for operators if adequate personal protective equipment (PPE) and respiratory protective equipment (RPE) are worn. Workers are also required to use PPE to lower dermal exposure. No bystanders should be allowed during greenhouse applications. A concern was raised by the experts over outdoor bystander exposure by inhalation due to the potential sensitisation by inhalation and no protection possible for bystanders. The rapporteur Member State provided a risk characterisation for bystanders after the experts' meeting, concluding that bystander exposure is low when compared to the amount of micro-organisms that are inhaled daily as a background value. Nevertheless, a concern over outdoor bystander inhalation remains. Furthermore, for outdoor uses, as there is an outstanding issue with the specification of the product with regard to some pathogenic contaminants, the exposure risk assessment of bystanders could not be finalized.

Lecanicillium muscarium strain Ve6 does not colonise the plant surface, and thus, the micro-organism is considered not likely to grow and multiply on plant material. Dietary exposure from use of *Lecanicillium muscarium* strain Ve6 is likely to be minimal. Moreover, any potentially occurring residual deposits on the treated crops are not relevant, as no human health concerns have been identified due to the toxicological profile of this strain. This assessment is subject to a final specification for microbial contamination of the plant protection product.

The available data on the fate and behaviour in the environment indicated that the only component that required environmental exposure and risk assessment was the colony forming units of *Lecanicillium muscarium* strain Ve6. Data on the competitiveness and persistence of added *Lecanicillium muscarium* to soil and natural surface water indicated that the organism was not very competitive, and that following addition of *Lecanicillium muscarium* to the soil environment or common artificial plant substrate, or spray drift exposure to surface water, levels would be expected to decline. However, information on the natural background concentrations in soil was not presented and no specific data on the influence of UV light on persistence and multiplication in water were available. There was sufficient evidence to show that *Lecanicillium muscarium* strain Ve6 does not produce any known secondary metabolites of toxicological or environmental concern.

A general consideration to address the risk to non-target organisms was the very narrow 'natural' host or target range of *Lecanicillium muscarium,* in addition to the apparent lack of evidence that birds, aquatic organisms, bees, non-target arthropods, earthworms and terrestrial plants are among the 'natural' target or host range of *Lecanicillium muscarium.* Furthermore, it was considered that the occurrence of 'natural' epizootics in the field could impose the same 'risks' to non-target organisms as epizootics induced by products with *Lecanicillium muscarium.*

No acute toxic, infective or pathogenic effects were identified in any of the studies on birds, aquatic organisms, bees or earthworms based on the data available.

However, data gaps were identified during the peer review to address the potential infectivity in fish and the potential toxicity to aquatic invertebrates, both being relevant for outdoor uses only.

Many studies indicated no or only minimal effects on different non-target arthropod species exposed to *Lecanicillium muscarium.* There were however concerns among Member State experts about the strains and formulations used in the studies, and also concerns about the test conditions. Some effects were in fact seen on the species *Encarsia formosa*. A data gap was identified to further address the risk to non-target arthropods.

KEY WORDS

Lecanicillium muscarium strain Ve6, *Verticillium lecanii* Ve6, peer review, risk assessment, pesticide, bio-control agent, insecticide

TABLE OF CONTENTS

BACKGROUND

Commission Regulation (EC) No 2229/2004[6] laying down the detailed rules for the implementation of the fourth stage of the work programme referred to in Article 8(2) of Council Directive 91/414/EEC, regulates for the European Food Safety Authority (EFSA) the procedure of evaluation of the Draft Assessment Reports (DAR) provided by the designated rapporteur Member State.

Lecanicillium muscarium notified as *Verticillium lecanii* is one of the 295 substances of the fourth stage of the review programme covered by Commission Regulation (EC) No 2229/2004.

Lecanicillium muscarium strain Ve6 was included in Annex I to Directive 91/414/EEC on 1 May 2009 pursuant to Article 24b of the Regulation (EC) No 2229/2004 (hereinafter referred to as 'the Regulation'). In accordance with Article 25a of the Regulation the European Food Safety Authority (EFSA) is required to deliver by 31 December 2010 its view on the draft review report submitted by the Commission of the European Communities (hereinafter referred to as 'the Commission') in accordance with Article 25(1) of the Regulation. This review report has been established as a result of the initial evaluation provided by the designated rapporteur Member State in the DAR. The EFSA therefore organised a peer review of the DAR. The conclusions of the peer review are set out in this report.

In accordance with the provisions of Article 22(1) of the Regulation, The Netherlands submitted the DAR (The Netherlands, 2007) on *Lecanicillium muscarium* strain Ve6, which was received by the EFSA on 5 July 2007. In accordance with Article 24(2) of the Regulation (EC) the DAR was distributed for consultation on 7 May 2008 to the sole notifier Koppert Beheer B.V., as identified by the rapporteur Member State, and on 21 January 2009 to the Member States.

The comments received on the DAR were evaluated and addressed by the rapporteur Member State. Based on this evaluation, the EFSA identified and agreed on lacking information to be addressed by the notifier as well as issues for further detailed discussion at expert level.

Taking into account the requested information received from the notifier, a scientific discussion took place in an expert meeting in June 2009. The report of this meeting has been made available to the Member States electronically.

A final discussion of the outcome of the consultation of experts took place during a written procedure with the Member States in November 2009.

During the peer review of the DAR and the consultation of technical experts no critical issues were identified for consultation of the Scientific Panel on Plant Protection Products and their Residues (PPR).

This conclusion summarises the results of the peer review on the active substance and the representative formulation evaluated as finalised at the end of the examination period provided for by the same Article. A list of the relevant end points for the active substance as well as the formulation is provided in Appendix A to this report.

The documentation developed during the peer review was compiled as a Peer Review Report (EFSA, 2010) comprising of the documents summarising and addressing the comments received on the initial evaluation provided in the rapporteur Member State's DAR:

- the comments received,
- the resulting reporting table (revision 1-1; 4 May 2009),

6 OJ L379, 24.12.2004, p.13, as amended by Regulation (EC) No 1095/2007 (OJ L246, 21.9.2007, p.19).

as well as the documents summarising the follow-up of the issues identified as not finalised at the end of the commenting period:

- the reports of the scientific expert consultation,
- the evaluation table (revision 2-1; 7 December 2009).

Given the importance of the DAR including its addendum (compiled version of November 2009 containing all individually submitted addenda) (The Netherlands, 2009) and the Peer Review Report with respect to the examination of the active substance, both documents are considered respectively as background documents A and B to this conclusion.

THE IDENTITY OF THE MICRO-ORGANISM AND THE PROPERTIES OF THE FORMULATED PRODUCT

It should be noted that *Lecanicillium muscarium* strain Ve6 was called *Verticillium lecanii* strain Ve6. Due to a recent reclassification the fungus *Verticillium lecanii* strain Ve6 has been renamed to the new species *Lecanicillium muscarium* strain Ve6.

In the text of all sections, where parts of the DAR are quoted and where it is not clear that the micro-organism tested was *Lecanicillium muscarium* strain Ve6, then the name of the micro-organism remains as it was presented in the DAR.

The micro-organism is *Lecanicillium muscarium* strain Ve6. The strain is deposited in CABI Genetic Resource Collection, Surrey, UK (=IMI) 268317, Centraal bureau Schimmelcultures (CBS), Baarn, The Netherlands CBS 102071 and The Agricultural Research Service Collection of Entomopathogenic Fungi (ARSEF) USDA-ARS Plant Protection Research Unit, Ithaca, USA, ARSEF 5128. *Lecanicillium muscarium* is a mitosporic fungus that belongs to the phylum of Deuteromycotina and the order of Hyphomycetes (or Moniliales). Hyphomycetes produce mitosporic asexual structures (called conidia) directly from the vegetative state or hyphae. *Lecanicillium muscarium* produces conidia as aggregates in slimy heads.

Lecanicillium muscarium strain Ve6 is used to control whitefly and thrips. It infects adults and larvae using both physical forces as well as enzyme action. Strong hyphal growth is observed before penetration of the host. The insects die within 7-10 days of infection.

The representative formulated product for the evaluation was 'Mycotal', a wettable powder formulation (WP).

The evaluated representative uses were on cucumber, tomato, sweet pepper, strawberry and ornamentals for the control of whitefly and thrips. The formulation is applied as a spray to the crops. Full details of the application rate and timings can be found in the list of end points attached at Appendix A.

SPECIFIC CONCLUSIONS OF THE EVALUATION

1. Identity of the micro-organism/biological properties/physical and technical properties and methods of analysis.

The wettable powder formulation is produced directly from the fermentation product. The resulting formulation will contain a minimum *Lecanicillium muscarium* strain Ve6 content of 1 x 10^{10} CFU/g. At the moment a method that will unequivocally identify the strain has not been concluded on, although it is noted that a new method was provided in an addendum to B1-B2 dated June 2009 of the Final Addendum (The Netherlands, 2009), but as this was a new study it could not be considered in the peer review. A finalised specification for microbial contaminants can not be concluded on. The PRAPeR M3 meeting of experts concluded that the proposed levels are higher than given in the draft OECD document[7]. The experts agreed that at least the levels for coliforms, *Staphylococcus aureus* and streptococci should be lowered. Given that all internationally available proposed levels for pathogenic microbial contaminates have been taken into consideration and in some cases these levels are exceeded, EFSA consider this as a critical area of concern. The proposed levels were on the basis

[7] Draft OECD Issue Paper "Discussion on Microbial Contaminant Limits for Microbial Pest Control Products", Version 2 Prepared on 23 March 2009, D. Rochon, L. Heikkilä and B. Belliveau, Health Evaluation Directorate, PMRA, Health Canada, Ottawa, Ontario, Canada

of the analysis of material with standardised ISO methods. As the levels of these organisms should be reduced, a new specification with supporting batch analysis will be needed.

There is currently no FAO specification for *Lecanicillium muscarium* strain Ve6.

The entomopathogenic fungus *Lecanicillium muscarium* has a worldwide geographic distribution on many different substrates: as a soil pathogen (on other fungi), as a hyperparasite on rust fungi, and on plant material. *Lecanicillium muscarium* has also been found as a natural infestation of several greenhouse pests; whitefly on cucumber and chrysanthemum, and has been described to decimate greenhouse populations of aphids and scales.

The mode of action of *Lecanicillium muscarium* strain Ve6 is described as follows. The infection of whitefly with *Verticillium lecanii Zimm.* was microscopically investigated (via light and electron microscopy). Different stages of infection were observed; spore germination, growth on cuticle, penetration and parasitisation of the interior, and the release of new infection units. Spores of *Verticillium lecanii* germinate on the insects' cuticle within 12-48 hours. Strong hyphal growth on the cuticle is observed before penetration of the host. The cuticle is penetrated, and the tissue is affected within 48 hours after infection. Once in the host, *Verticillium lecanii* 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 large number of hyphal bodies have been formed inside the body cavity. For a better understanding of the development of the fungus on the host, histological studies were performed after different stages of infection. Histological section studies of whitefly killed and fixed after different exposure times indicate fungus penetration and invasion (destruction of the inner organs) of the tissues as the cause of death. Thrips are probably killed as a result of multiple lesions of the cuticle by enzymatic degradation, as no fungal material was found in the haemolymph of the insects at the time of death. In addition, *Verticillium lecanii* has also been described to secrete lytic enzymes that play a major role in penetrating the cyst wall of *Heterodera schachtii*.

It was concluded in the DAR that from the information available the infectivity of *Lecanicillium muscarium* strain Ve6 is confined to whitefly and thrips, and there is no evidence or indication from available data on this strain that other organisms may be adversely affected, including beneficial insects. The conclusion of the peer review is that there is insufficient strain-specific information to conclude on this point (see section 5).

It has been demonstrated that *Lecanicillium muscarium* strain Ve6 is not pathogenic to humans. In fact no *Lecanicillium* are known to be human pathogens. It has also been demonstrated that it is not a plant pathogen. The genetic stability of the strain has also been shown to be acceptable. There is no evidence to suggest that this strain of fungus could produce antibiotics that could interfere with the use of antibiotics in human or veterinary medicine.

Sufficient test methods and data relating to physical, chemical and technical properties are available. Also adequate analytical methods are available for the quantification of *Lecanicillium muscarium* strain Ve6. Methods for the unequivocal identification of the strain could not be concluded on, as the new information has not been peer-reviewed. The whitefly assay test for the potency of the material (trading standard for marketed material) is currently not accepted for the following reasons. The quality criteria set by the manufacturer is 80 - 100% mortality under the whitefly test conditions. From the validation data supplied for this method it is shown that out of 6 assays 50% failed the criteria of 80 - 100% mortality. Whether this is because the batches are out of specification or the method does not work is unclear. The acceptability of the methods for microbial contaminants was questioned, as they appeared not to be ISO methods. After the experts' meeting the notifier confirmed that ISO methods were used.

As the micro-organism is not competitive in the environment unless the host species are present, it is not pathogenic to humans and does not produce any known toxicologically significant secondary metabolites, methods of analysis for monitoring are not required.

2. Mammalian toxicity

Verticillium spp were renamed to different *Lecanicillium* spp (refer to section '*The identity of the micro-organism and the properties of the formulated product'*), however references to the open literature were kept with its original name, unless the strain Ve6 was specifically identified.

In several publications *Verticillium lecanii* have been reported to produce a different combination of metabolites. Fungal metabolite production appeared to be dependent on the culture conditions and strain. Extracts from cultures grown under laboratory-scale still liquid conditions of *Lecanicillium muscarium* strain Ve6 contained the so-called destruxins A, B and E. Destruxins are cyclic peptides that elicit a range of biological responses including cytotoxicity and inhibition of gene expression. Destruxins were not produced under conditions as practised in commercial situations (solid-state fermentation and in aerated (shaken) liquid fermentation – the latter is not yet used at this stage); they were also not detected in the formulated product 'Mycotal' or its non-formulated spores.

During the PRAPeR M3 meeting the experts agreed with the conclusion of the rapporteur Member State that toxins are unlikely to be important for the mode of action.

No specification of the batches used in the toxicological studies is available. It could be concluded that these are not necessary, provided that adequate quality control is undertaken on the batches produced, certifying that toxicologically relevant metabolites and contaminants are kept below agreed levels of significance. As the levels of some contaminating pathogens were found to be too high when compared to internationally available proposed levels for pathogenic microbial contaminants (refer to section 1), a critical area of concern was raised by EFSA for the human exposure to these levels of pathogenic microbes.

Most Tier I and Tier II studies were performed with *Lecanicillium muscarium* strain Ve6, basic studies were conducted with the representative formulation 'Mycotal', however, skin- and eye irritation studies were carried out with an unknown strain of *Verticillium lecanii*. Overall, the toxicological database was regarded as sufficient to draw a conclusion.

2.1. Medical data

Medical surveillance

A report from 1982 (Volume 3, B.6.1.1.1, The Netherlands, 2007) describes the prick testing of healthy subjects who were exposed to *Verticillium* material for 6 months to 4 years. All subjects were negative in the prick tests and all respiratory examinations (respiratory function, Forced Vital Capacity and Forced Expiratory Volume in one second), and physical examinations were regarded normal.

In a prick testing programme from 1986 with *Verticillium lecanii* strain Ve6 (*Lecanicillium muscarium* strain Ve6) and strain Ve2, run on two sites, where employees had been exposed to *Verticillium lecanii* for periods for up to 10 years, positive reaction (wealing response) was noted in 4/116 and 4/31 subjects, respectively. The positive reactions at both sites were noted for the strain Ve2 or the combination of strains Ve2 and Ve6, no reaction was scored when tested with strain Ve6 alone. Eighty-five employees out of 116, including the four skin positive ones, were also subjected to a medical programme on several haematological, hepatic and renal parameters, lung function and immunoglobulins. These results revealed no abnormalities, indicative of a non-toxic reaction.

In a 2003 report (Volume 3, B.6.1.1.1, The Netherlands, 2007), symptoms and work conditions of 316 persons working in greenhouses using microbiological pesticides were obtained by interview at annual examinations during two years. Spirometry, bronchial challenge and skin prick test with standard allergens were also measured. The use of *Verticillium* was considered as not related to any symptoms of sensitisation and inflammatory lung diseases among greenhouse workers, whereas the incidence and prevalence of respiratory symptoms and eye irritancy for *Bacillus thuringiensis* and *Trichoderma harzianum* were relatively higher.

A published study from 2004 (Volume 3, B.6.1.1.1, The Netherlands, 2007) showed the results of IgE serology testing at baseline and up to three years of follow-up in greenhouse employees. Nine to 21 percent of the sera tested were positive to *Verticillium lecanii*, however, a work-related assessment of symptoms was not conducted to allow a conclusion on the sensitisation properties of *Lecanicillium muscarium* strain Ve6.

Direct observations, e.g. clinical cases

Clinical cases were reported in the open literature describing human infection by fungi from the genus *Verticillium.* One patient, in which *Verticillium* was isolated from a swelling on the arm, had several underlying diseases (one kidney was removed and he received radiotherapy and chemotherapy). The lesion on the arm responded to antifungal therapy and the swelling disappeared gradually.

From seven patients that suffered from a fungal peritonitis, one case was identified as caused by a *Verticillium* species. All these patients had been treated for bacterial peritonitis and were treated by intraperitoneal antibiotics in the previous two months. Patients were cured by removal of the catheter and by antifungal therapy.

A report from 2002 (Volume 3, B.6.1.1.4, The Netherlands, 2007) describes a case of infectious keratitis caused by a *Verticillium* species, without history of trauma. The patient recovered after antifungal therapy. The author emphasized that *Verticillium* species are very rare causes of keratitis.

First treatment

In case of human infection by *Lecanicillium muscarium* strain Ve6, the treatment relies on antifungal therapeutic agents, such as fluconazole and amphotericin B. *Lecanicillium muscarium* is not known to be resistant to antibiotics or anti-microbial agents used in human or veterinary medicine.

2.2. Sensitisation

Sensitisation studies with micro-organisms are considered to be of limited value, as reactions to foreign proteins (most micro-organisms) can be anticipated. Therefore, all micro-organisms should be regarded as potential sensitisers. Nevertheless, a study conducted with *Verticillium lecanii* spp. according to the Magnusson & Kligman method was submitted and considered acceptable by the rapporteur Member State; no potential for sensitisation was found under the conditions of this study.

The consensus of the PRAPeR M3 meeting of experts was to use the following labelling phrase for all micro-organisms: "**Micro-organisms may have the potential to provoke sensitising reactions**".

2.3. Acute toxicity, pathogenicity, infectiveness

Acute toxicity, pathogenicity and infectiveness were investigated on *Lecanicillium muscarium* strain Ve6 in single dose toxicity tests in rats, by the oral, intravenous, inhalation and intraperitoneal routes of exposure; mice were tested intraperitoneally. All studies except the intravenous one were considered as supplementary by the rapporteur Member State, because the method of homogenisation of the organs was not described; the adequacy of the method to release any fungus from the cells for

microbial examination could not be assessed. Therefore, the pathogenicity and infectiveness information was not addressed adequately in these individual studies, but the toxicity evaluation was accepted. The levels tested ranged from 6.9 x 10^6 spores/animal (nominal dose) to 3.0 x 10^8 spores/animal (actual dose measured).

The experts discussed the reliability of the acute studies. The intravenous study was accepted by the rapporteur Member State and considered reliable for pathogenicity and infectiveness; the human/rat body temperature would mean that the micro-organism would not remain viable in these warm-blooded species. Therefore, it was agreed that the available studies could be relied on when taken all together, and in combination with the information available on the temperature range for survival of the micro-organism.

Acute oral

No mortality or abnormal clinical signs were noted in the oral studies, body weights were not affected by the treatment, no pathology finding was evidenced, and no fungus was detected in the organs and faeces samples. *Lecanicillium muscarium* strain Ve6 did not induce signs of toxicity, infectivity or pathogenicity by the oral route, the acute oral LD_{50} was > 3.0 x 10^8 spores/animal.

Acute intravenous

Upon intravenous exposure, no mortality, clinical signs or pathology findings were observed; it was shown that *Lecanicillium muscarium* strain Ve6 did not colonise and was not infective, as no viable fungus was recovered from the tissues, except immediately after dosing. The acute intravenous LD_{50} was > 1.2 x 10^7 spores/animal (nominal concentration).

Acute inhalation

Inhalation of *Lecanicillium muscarium* strain Ve6 did not cause mortality. Transient body weight and food consumption decreases were observed, as well as poor coating conditions and slight hypoactivity in exposed males. There were no macro- or microscopic pathology findings and no test microbe was isolated from any organ. The dose to which the rats were exposed was poorly defined as the "maximum practicable dose"; therefore no LD_{50} could be defined.

Acute intraperitoneal

When administered by intraperitoneal route in rats, both viable (1.2 x 10^8 spores/rat) and autoclaved spores of *Lecanicillium muscarium* strain Ve6 produced mortality (5/30 and 4/30 animals, respectively) within two days of dosing, probably due to acute peritonitis. Other effects seen in both groups included decreased body weight and food consumption, altered haematological and biochemical parameters, and adhesion of several abdominal organs. Histopathological examination revealed peritonitis with abscess formation involving many organs in the abdominal cavity. No fungus was isolated from the organs. As signs of peritonitis were seen for the micro-organism in both its viable and inactivated form, it could not be concluded that the effects were caused by *Lecanicillium muscarium* strain Ve6; it was considered that these would rather represent an immune-related reaction. Signs of peritonitis were reproduced in a further test on rats, without producing mortality. The acute intraperitoneal LD_{50} was > 1.2 x 10^8 spores/animal.

Two studies conducted via intraperitoneal route in mice did not cause mortality, but signs of peritonitis were induced by the viable spores, inactivated spores and carrier material (without the spores).

2.4. Genotoxicity

Lecanicillium muscarium strain Ve6 has been evaluated *in vitro* for point mutations in an Ames test with *S. typhimurium*. The test was accepted by the rapporteur Member State and gave negative results both with and without metabolic activation.

Information on a Vitotox test carried out on a crude extract of *Verticillium lecanii* was provided by the notifier. The Vitotox test can simultaneously determine cytotoxicity as well as genotoxicity. The authors concluded that the crude extract did not show any mutagenicity or genotoxicity in the Ames or the Vitotox assay.

As part of the EU RAFBCA-project[8], genotoxicity of *Lecanicillium muscarium* was studied on a number of different *Salmonella typhimurium* strains and on *E. coli* strains with polar and non-polar extracts of unformulated spores of 'Mycotal' and extracts from the preparation 'Mycotal'. No mutagenic effects were found with any of these crude extracts, which would contain all possible metabolites. A statement was provided indicating that pure metabolites were also tested including destruxins A and that no mutagenic effects were found.

An *in vivo* micronucleus test in rat was submitted by the notifier but no conclusion was drawn by the rapporteur Member State, as no evidence was provided that the compound had reached the target cells. The study was not considered acceptable.

As no *in vitro* clastogenicity study was performed, the experts discussed the need for further genotoxicity testing, together with the potential metabolites/toxin production of *Lecanicillium muscarium* strain Ve6. It was agreed that the production of metabolites in the presence of the target organism was sufficiently addressed, as none were identified as being involved in the mode of action. As no known toxicologically significant secondary metabolite was identified, it could be agreed that no further data would be required for the genotoxicity end point.

2.5. Short-term toxicity and pathogenicity

A 28-day inhalation study was performed in rats with the preparation 'Mycotal' (containing *Lecanicillium muscarium* strain Ve6, but also a large quantity of other proteinaceous material known to be respiratory sensitiser). The study was found acceptable by the rapporteur Member State, although infectivity was examined only in the lungs. No mortality or clinical signs were observed up to 100 mg/m^3. Macro- and microscopical examination revealed changes in the lungs, nasal cavity and mediastinal lymph nodes most pronounced in the 10 and 100 mg/m^3 (indexed as very slight for the 1 mg/m^3) groups. These changes were indicative of a local immune reaction rather than a toxicological effect, but this could not be confirmed, as no control was included in the study. The no observed adverse effect level (NOAEL) for 'Mycotal' was set at 1 mg/m^3 (1.08 x 10^7 spores/m^3).

Short-term toxicity is not expected because accumulation of *Lecanicillium muscarium* strain Ve6 in the body has not been demonstrated; acute studies did not reveal signs of toxicity, infectivity or pathogenicity; no further study was required.

2.6. Other studies (tier II)

No further Tier II study was provided. According to the results of the Tier I studies, no further studies were required.

[8] Risk Assessment of Fungal Biological Control Agents

2.7. Reference values

Lecanicillium muscarium has a worldwide geographic distribution in soils, other fungi and plant material. The Ve6 strain has been shown not to produce any destruxins or other metabolite of concern. Spores of *Lecanicillium muscarium* strain Ve6 germinate and grow between 5°C and 30°C. At 37°C germination of some spores was obtained, but no further growth occurred, indicating its inability to colonise warm-blooded animals. The micro-organism did not show signs of infectivity or pathogenicity in rats, signs of toxicity were most probably derived from sensitisation reactions upon intraperitoneal or repeated inhalation exposure.

It is generally accepted that no reference values (acceptable daily intake –ADI, acute reference dose – ARfD or acceptable operator exposure level –AOEL) are needed in cases where the micro-organism is not pathogenic or infective and does not produce toxins.

2.8. Exposure assessment to operators, workers and bystanders

Operator exposure

It is recognised that the exposure models used for the risk assessment of chemical active substances are not easily applied to microbial pest control agents (MCPAs); therefore a qualitative assessment has been considered for operator exposure.

As no known toxicologically relevant metabolite is present in the formulation, only exposure to spores via the dermal and inhalation routes was considered. *Lecanicillium muscarium* strain Ve6 did not show toxicity, infectivity or pathogenicity to warm-blooded animals; clinical cases of systemic infection were described in the open literature in immuno-compromised patients (either receiving chemo/radiotherapy or treated with intraperitoneal antibiotics).

Because all micro-organisms are regarded as potential sensitisers (via the dermal and inhalation routes), personal protective equipment (PPE), as gloves and protective clothing, and respiratory protective equipment (RPE) as P3 filter, should always be used when handling the product and during spray applications. Low risk is anticipated for operators when 'Mycotal' is applied according to the representative uses with adequate personal protective equipment (PPE and RPE). In view of the fact that proper personal protective equipment should be used, the outstanding issue of pathogenic contaminants with regard to the proposed specification is not considered critical for operators.

Worker exposure

No risk characterisation was presented for workers without the use of PPE in the DAR. Therefore the experts agreed that PPE (as gloves, long-sleeved shirt and long trousers) needs to be recommended for use by workers re-entering crops treated with 'Mycotal'. In view of the fact that proper personal protective equipment should be considered, the outstanding issue of pathogenic contaminants with regard to the proposed specification is not regarded critical for workers.

Bystander exposure

During spraying operations in greenhouses, there should be no bystander present; therefore no bystander exposure is expected.

For outdoor use, as no protection can be provided for bystanders, and as a concern was raised during the PRAPeR M3 experts' meeting for bystander exposure via the inhalatory route, the rapporteur Member State was requested to carry out a risk assessment for this scenario.

The rapporteur Member State provided in the revised addendum to Volume 3, B.6 (The Netherlands, 2009) an indication of the estimated exposure to 'Mycotal' using the EUROPOEM II model for

unprotected bystanders. According to this model, the inhalation exposure is estimated to be 0.006 mg a.s./day, corresponding to 3.7 x 10^5 CFU/day. The rapporteur Member State expressed the view that this amount is extremely low when compared to the amount of micro-organisms that are inhaled daily as a background value, stressing that it is unknown whether the default values used in the model are applicable to micro-organisms.

Assuming a rat respiration rate of 45 L/kg bw/hour and a rat body weight of 200 g, the rat exposure at the NOAEL level of 1 mg/m^3 obtained in the 28-day inhalation study in rats with the formulation would be 0.054 mg a.s./day. In comparison to the inhalation exposure of 0.006 mg a.s./day obtained in the EUROPOEM II model, the Margin of Exposure (MoE) is about 10.

EFSA note: The MoE for bystanders of pesticide applications should be at least 100 (standard assessment factor for derivation of the AOEL for intra-individual and interspecies variability). Therefore the concern identified during the experts' meeting over the sensitisation potential of micro-organisms by inhalation could not be ruled out. On the other hand, it has to be acknowledged that the model used has not been validated for micro-organism applications, leading to many uncertainties in such an exposure risk assessment. Taking a conservative precautionary approach, with which the rapporteur Member State expressed a strong disagreement, a point of concern remains, as identified during the experts' meeting over the sensitisation potential of micro-organisms by inhalation for outdoor uses. Furthermore, for outdoor uses, as there is an outstanding issue with the specification of the product with regard to some pathogenic contaminants, the exposure risk assessment of bystanders could not be finalized.

3. Residues

3.1. Nature and magnitude of residues in plant

Lecanicillium muscarium strain Ve6 is not known to generate toxins or other metabolites with undesirable properties (see chapters 1 and 2 of this document). Therefore the investigation of the metabolism of *Lecanicillium muscarium* strain Ve6 on plant surfaces has not been necessary.

Residue trials with *Lecanicillium muscarium* strain Ve6 which would investigate the persistence and actual levels of the micro-organism on crops were not submitted. Some studies on persistence were supplied that showed that this micro-organism does not persist and multiply on the plant surface. However, residue trials are not considered necessary based on the human toxicology assessment. The evaluation of persistence and actual residue levels in the case of non-pathogenic/non-toxic micro-organisms is not considered relevant.

3.2. Nature and magnitude of residues in livestock

Not applicable, as potential intake of *Lecanicillium muscarium* strain Ve6 through feed will not lead to any residue in food of animal origin.

3.3. Consumer risk assessment

Humans and animals can be commonly exposed to *Lecanicillium muscarium* strain Ve6, an organism found in many environmental compartments. No toxicological reference values were identified for *Lecanicillium muscarium* strain Ve6, as explained in chapter 2 of this document.

Dietary exposure from the use of *Lecanicillium muscarium* strain Ve6 is likely to be minimal. Any potentially remaining fungal spores on harvested crop parts are not likely to germinate and grow, and moreover will be exposed to unfavourable conditions. Furthermore, residues of the microbial

pesticide are likely to be removed from the treated food by washing and processing. Thus, the amount of residues the consumer will be exposed to, if any, is likely to be very low.

Even if residues are not removed, it is believed that dietary exposure to the microbial agent will result in negligible risk to consumers, as in view of the toxicological profile of this strain, no hazard to human health has been identified. Because of the low toxicity and the low exposure of *Lecanicillium muscarium* strain Ve6 expected from the proposed uses, there is no concern for acute and chronic risks for the general population or sensitive subpopulations, such as infants and children.

However, as there is an outstanding issue with the specification of the product with regard to microbial contaminants, the risk to the consumer can not be finalized and the issue has been regarded by EFSA as a critical area of concern. This is because the material being applied to edible crops has higher levels of pathogenic micro-organisms compared to current internationally available proposed levels for pathogenic microbial contaminants. In addition to this, the fate of these pathogens on the harvested commodities is not known and it may be possible that at least some of them will multiply.

3.4. Proposed MRLs

Based on the risk assessment for the consumer it was concluded that MRLs for *Lecanicillium muscarium* strain Ve6 on food commodities are not required. Thus, *Lecanicillium muscarium* strain Ve6 is considered eligible for inclusion in the Annex VI of Regulation 396/2005[9].

4. Environmental fate and behaviour

4.1. Fate and behaviour in soil

4.1.1. Persistence and multiplication in soil of the micro-organism

Available data on persistence of *Lecanicillium muscarium* strain Ve6 in soil (experiments on 2 soils, at 22°C and 40% maximum water holding capacity (MWHC)) evaluated in the DAR and in the addendum to Volume 3 B8 of June 2009 (The Netherlands, 2009) indicated that *Lecanicillium muscarium* strain Ve6 counts fell to 30-40% of the initial level following incubation for four days. As no studies were submitted on the natural background levels of *Lecanicillium muscarium* in soil, the PRAPeR M3 meeting of experts identified a data gap for natural background levels in soil for this micro-organism.
Two additional studies with a different strain, V24, were submitted. These studies showed that the soil parameters (soil moisture, soil temperature and % organic matter) play an important role in the persistence of spores. Persistence was highest at 20°C (tested: 20, 25, and 30°C), 7% MWHC soil moisture (tested: 7, 33, 60 and 98% MWHC) and a high content of organic matter (tested: 19 and 36% OM).
Information on multiplication and persistence in mineral wool was provided in the addendum to Volume 3 B8: a decline of *Lecanicillium muscarium* in this common artificial plant substrate was comparable to that in soil.
The predicted initial concentration in soil (PIEC) were recalculated by the rapporteur Member State (addendum to Volume 3 B8, June 2009) assuming 12 applications with a dose rate of $2x10^{13}$ CFU/ha, a soil depth of 5 cm, a soil bulk density of 1500 kg/m^3, no crop interception and no degradation between applications.

[9] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC, OJ L 70, 16.3.2005, p1.

4.1.2. Persistence in soil of any relevant metabolite formed by the micro-organism under relevant environmental conditions

The peer review agreed with the conclusion in the DAR that there was sufficient evidence that *Lecanicillium muscarium* strain Ve6 does not produce any known secondary metabolites of toxicological or environmental concern.

4.2. Fate and behaviour in water

4.2.1. Persistence and multiplication in water of the micro-organism

The available data on the behaviour of *Lecanicillium muscarium* strain Ve6 spores in water showed that conidiospores do not germinate in a non-aerated situation and remain viable for 2-3 days only, while in stirred aerated water they persist for more than 95% after 7 days. No specific data on the influence of UV light on the persistence and multiplication in water were submitted in the dossiers, and therefore a data gap was identified in the PRAPeR M3 meeting. Some information on the susceptibility of *Lecanicillium muscarium* conidia to UV-B radiation and general information on UV light penetration in greenhouses were reported in the addendum to Volume 3 B8 of November 2009 (The Netherlands, 2009). However, in view of the restrictions concerning the acceptance of new (i.e. newly submitted) studies after the submission of the DAR to EFSA, as laid down in Commission Regulation (EC) No 1095/2007, this new information could not be considered in the peer review.

4.2.2. Persistence in water of any relevant metabolite formed by the micro-organism under relevant environmental conditions

The peer review agreed with the conclusion in the DAR that there was sufficient evidence that *Lecanicillium muscarium* strain Ve6 does not produce any known secondary metabolites of toxicological or environmental concern.

4.3. Mobility

4.3.1. Mobility of the micro-organism

The peer review agreed that transport of the micro-organism away from the target treated field or glasshouse soil will be negligible, although the mechanisms of spread of *Lecanicillium muscarium* are not exactly known. Spores are not spread by air, naturally, and are not released from conidiophores without water contact. Passive spread can occur by means of splashing, and probably by mechanical transfer by other arthropoda present in greenhouses.

4.3.2. Mobility in soil of any relevant metabolite formed by the micro-organism under relevant environmental conditions

The peer review agreed with the conclusion in the DAR that there was sufficient evidence that *Lecanicillium muscarium* strain Ve6 does not produce any known secondary metabolites of toxicological or environmental concern.

4.4. Considerations for concentration of the micro-organism in air

The amount of fungal spores in the air, and the time these spores are detectable in the air after application, was investigated in an experimental study in greenhouse. The peer review agreed with the conclusion in the DAR that transport of spores will not occur through the air. If spores are released in the air after an application, these spores will settle after 22 hours.

5. Ecotoxicology

Lecanicillium muscarium[10] was discussed in the PRAPeR M3 meeting of experts in June 2009. As effects on non-target organisms were based on infectivity and pathogenicity studies, the 'toxicity over exposure approach' usually applied for chemical active substances was considered less relevant. Weight of evidence from other information was considered more relevant for the risk assessment, e.g. optimum temperature range for growth of the micro-organism or mode of action.

Member State experts did not accept the argument that the risk from the intended uses could be addressed by lack of field records indicating a (potential) risk for non-target organisms, in spite of existing use in various countries. Such an argumentation would require that the comparability of the intended uses with existing use practise should be confirmed.

5.1. Risk to terrestrial vertebrates

Whereas birds were not considered to be exposed to *Lecanicillium muscarium* from the intended uses in greenhouses, exposure could not be excluded for the repeated outdoor applications in strawberries.

Based on the experimental end point value, the microbiology, the high body temperature of birds, and the 'natural' target or host range of *Lecanicillium muscarium*, there were no indications to support the assumption that the filamentous fungus *Lecanicillium muscarium* would have any acute or short-term effects on birds, even if exposed. In addition, it was the consensus of Member State experts that cytotoxins in infected insects, if present, would be likely to be present at negligible levels and that the risk to birds was likely to be low. The limited data did not indicate any infectivity or pathogenicity to birds.

5.2. Risk to aquatic organisms

Toxicity to algae was considered very unlikely, given the natural target or host range of *Lecanicillium muscarium*. Acute toxicity studies provided for fish and *Daphnia* indicated no effects from exposure to spores of *Lecanicillium muscarium.* The rapporteur Member State noted that the exposure to fish and *Daphnia* was limited due to the low dispersability of spores in water.

Although aquatic organisms may be exposed to *Lecanicillium muscarium* strain Ve6, the rapporteur Member State considered the risk as low, based on the facts that no aquatic organisms had been identified as natural target or host of *Lecanicillium muscarium*, and that it was considered to be a soil inhabitant. Additionally, the slight water dispersability and apparent lack of toxicity to aquatic organisms indicated a low risk.

It was however the consensus of Member State experts that the available *Daphnia* study could not sufficiently address the risk to aquatic invertebrates due to (1) the short study duration compared to the expected time of effects (given the mode of action) and (2) the limited exposure due to the low dispersability of spores. Member State experts considered chronic studies with *Daphnia* or *Chironomus* to be more appropriate. Furthermore, the Member State experts identified a data gap to address the potential infectivity in fish, as no information was provided to assess this in the acute fish toxicity study. These two data gaps should be addressed before the risk to aquatic organisms could be finalised. Both data gaps are relevant to outdoor uses only.

[10] See section '*The identity of the micro-organism and the properties of the formulated product'* regarding species and strain name

5.3. Risk to bees

Oral and contact toxicity studies indicated a low toxicity to bees, and there were no signs of infectivity or pathogenicity. Neither laboratory tests to bumblebees (less well-documented), nor field test with honeybees sprayed with 'Mycotal' indicated any effects. Furthermore, it was considered that the occurrence of 'natural' epizootics in the field could impose the same 'risks' to bees as 'induced' epizootics by products with *Lecanicillium muscarium.* On this basis the risk to bees was assessed as low.

5.4. Risk to other arthropod species

The available data largely demonstrate no effects on adults of many arthropod species[11], though under particular conditions effects on *Encarsia formosa* were identified. Generally, however, there were some uncertainties in the open literature available regarding the strains used, the formulation or the test conditions, such as temperature and humidity (important for the infestation success).

The risk to non-target arthropods was assessed as low by the rapporteur Member State based on the narrow 'natural' host or target range of *Lecanicillium muscarium*, and the apparent lack of significant toxic, infective or pathogenic effects in various laboratory studies following direct exposure or exposure to dried residues on natural substrates with *Lecanicillium muscarium* containing products. In addition, a less well-documented field test in an orchard indicated slightly harmful effects on *T. pyri* (effect percentages 25-50%). Furthermore, the rapporteur Member State argued that 'natural' epizootics in the field could impose the same 'risks' to non-target arthropods as epizootics 'induced' by products with *Lecanicillium muscarium.*

For the outdoor field use the Member State experts concluded that the available information was weak for non-target arthropods. The experts agreed on a data gap for further information to address the risk to non-target arthropods including arthropods living on the soil surface. This information should be pertinent for the correct preparation, strain and relevant test conditions.

5.5. Risk to earthworms

An acceptable acute toxicity study with earthworms revealed no harmful effects, and no signs of infective or pathogenic effects were identified. The long-term risk to earthworms was considered as low since long-term exposure of earthworms was considered unlikely. Furthermore, earthworms were not considered to be among the 'natural' target or host range of *Lecanicillium muscarium*. The risk to earthworms for the intended uses was assessed as low.

5.6. Risk to soil non-target micro-organisms

The application of *Lecanicillium muscarium* was regarded as having effects on the ecology of the soil micro-organism ecosystem. The scale of impact, both in time and in space, was however difficult to assess. However, no scientific indications of adverse effects due to such applications were identified. Also, in case soil micro-organisms were exposed, risks may not differ from 'natural' epizootics, particularly taking into account the relatively persistent character of conidia. Overall, the risk to soil non-target micro-organisms was assessed as low.

[11] *Encarsia formosa*, adult *Phytoseiulus persimilis*, 3rd instar caterpillars *Pieris brassicae*, *Agonum dorsale*, *Bembidion lampros*, *B. obtusum*, *Demetrias atricapillus*, *Harpales rufipes*, *Pterostichus cupreus*, *Trechus quadristriatus*, and the staphylinid beetle *Tachyporus hypnorum*), Collembola (*Folsomia candida*), Hymenoptera (*Lasius niger*), Diptera larvae (*Episyrphus balteatus*), Neuroptera larvae (*Chrysoperla carnea*), Dermaptera (*Forficula auricularia*), a spider *Erigone* spp. and a woodlouse *Oniscus* ssp. and others.

5.7. Risk to other non-target-organisms (flora and fauna)

Terrestrial plants were not considered to be among the 'natural' target or host range of *Lecanicillium muscarium* and the occurrence of 'natural' epizootics in the field could impose the same 'risks' to terrestrial plants as 'induced' epizootics by products with *Lecanicillium muscarium.* Based on these considerations and the low likelihood of long-term exposure, the risk to terrestrial plants was assessed as low for the intended uses of *Lecanicillium muscarium.*

6. Residue definitions

6.1. Soil

Definition for risk assessment:	Colony forming units of *Lecanicillium muscarium* strain Ve6
Definition for monitoring:	The peer review considered that due to the hazard profile a monitoring definition was not necessary.

6.2. Water

6.2.1. Ground water

Definition for exposure assessment:	Colony forming units of *Lecanicillium muscarium* strain Ve6
Definition for monitoring:	The peer review considered that due to the hazard profile a monitoring definition was not necessary.

6.2.2. Surface water

Definition for risk assessment

in surface water:	Colony forming units of *Lecanicillium muscarium* strain Ve6
in sediment:	Colony forming units of *Lecanicillium muscarium* strain Ve6
Definition for monitoring:	The peer review considered that due to the hazard profile a monitoring definition was not necessary.

6.3. Air

Definition for risk assessment:	Colony forming units of *Lecanicillium muscarium* strain Ve6
Definition for monitoring:	The peer review considered that due to the hazard profile a monitoring definition was not necessary.

6.4. Food of plant origin

Definition for risk assessment:	not allocated, no hazard identified
Definition for monitoring:	not required

6.5. Food of animal origin

Definition for risk assessment:	not required

Definition for monitoring: not required

6.6. Overview of the risk assessment of compounds listed in residue definitions for the environmental compartments

Not applicable.

LIST OF STUDIES TO BE GENERATED, STILL ONGOING OR AVAILABLE BUT NOT PEER REVIEWED

- Method for the unequivocal identification of the strain (relevant for all representative uses evaluated; already available and evaluated in the addendum to B1-B2 dated June 2009, however, in view of the restrictions concerning the acceptance of new (i.e. newly submitted) studies after the submission of the DAR to EFSA, as laid down in Commission Regulation (EC) No 1095/2007, this new study could not be considered in the peer review; see section 1)
- Revised specification with supporting batch analysis data using validated methods for the microbial contaminants (relevant for all representative uses evaluated; submission date unknown; see section 1)
- Further validation or explanation for the validation data supplied for the whitefly bioassay (relevant for all representative uses evaluated; submission date unknown, see section 1)
- The reference Walter et al. (1988) should be provided in English (relevant for all representative uses evaluated; submission date unknown). This is a data gap for procedural reasons as all studies should be translated into English.
- Natural background concentrations in soil (relevant for all representative uses evaluated; submission date unknown; see section 4.1.1)
- Information on the influence of UV light on persistence and multiplication in water (relevant for the representative uses evaluated; some information on the susceptibility of *Lecanicillium muscarium* conidia to UV-B radiation and general information on UV light penetration in greenhouses were submitted and reported in the addendum of November 2009, however, in view of the restrictions concerning the acceptance of new (i.e. newly submitted) studies after the submission of the DAR to EFSA, as laid down in Commission Regulation (EC) No 1095/2007, this new information was not considered in the peer review; see section 4.2.1)
- Comparability of the intended uses with the existing use practise should be assessed, if the lack of field records on effects on non-target organisms from existing uses should be used to address the risk from the representative uses (relevant for outdoor uses; submission date unknown; see section 5)
- Further information to address the potential risk to aquatic invertebrates (relevant for outdoor uses; submission date unknown; see section 5.2)
- Further information to address the potential infectivity in fish (relevant for outdoor uses; submission date unknown; see section 5.2)
- Further information to address the risk to non-target arthropods, including arthropods living on the soil surface (relevant for outdoor uses; submission date unknown; see section 5.4).

CONCLUSIONS AND RECOMMENDATIONS

OVERALL CONCLUSIONS

The conclusion was reached on the basis of the evaluation of the representative uses on cucumber, tomato, sweet pepper, strawberry and ornamentals for the control of whitefly and thrips. The formulation is applied as a spray to the crops. Full details of the application rate and timings can be found in the list of end points attached at Appendix A.

The representative formulated product for the evaluation was ‘Mycotal’, a wettable powder formulation (WP).

The available data indicate that the micro-organism is not competitive in the environment unless the host species are present, it is not pathogenic to humans, nor toxic or infective and does not produce any known toxicologically significant secondary metabolites, therefore methods of analysis for monitoring are not required. However, it should be noted that some methods are given in the DAR although there are no validation data.

Sufficient test methods and data relating to physical, chemical and technical properties are available. Methods for the unequivocal identification of the strain could not be concluded on, as the new information has not been peer-reviewed. The acceptability of the methods for microbial contaminants was questioned as they appeared not to be ISO methods. After the experts' meeting the notifier confirmed that ISO methods were used. Further validation is needed for the whitefly assay. It was considered that the level of contaminating pathogens was in some cases too high when compared to internationally available proposed levels for pathogenic microbial contaminants, and this is considered by EFSA as a critical area of concern.

In acute toxicity studies, *Lecanicillium muscarium* strain Ve6 did not induce signs of toxicity, pathogenicity or infectivity. The human/rat body temperature would mean that the micro-organism would not remain viable in these warm-blooded species. Clinical cases of systemic infection were described in the open literature in immuno-compromised patients, either receiving chemo/radiotherapy or treated with intraperitoneal antibiotics; very rare cases of keratitis were caused by *Lecanicillium* species.

No potential for skin sensitisation was found in a Magnusson & Kligman test. Sensitisation studies with micro-organisms are considered to be of limited value, as reactions to foreign proteins (most micro-organisms) can be anticipated. Therefore, all micro-organisms should be regarded as potential sensitisers (in contact with skin and by inhalation) and the following phrase was agreed by the experts "**Micro-organisms may have the potential to provoke sensitising reactions**".

In a 28-day study by inhalation in rats conducted with the representative formulation 'Mycotal', the NOAEL was found at 1 mg/m^3 based on macro- and microscopic changes in the lungs, nasal cavity and mediastinal lymph nodes most pronounced at 10 and 100 mg/m^3 groups. Although these changes were indicative of a local immune reaction rather than a toxicological effect, this could not be confirmed due to the lack of a control group in the study. Short-term toxicity is not expected, because accumulation of *Lecanicillium muscarium* strain Ve6 has not been demonstrated in the body. No potential for genotoxicity was found *in vitro*. As no known toxicologically significant metabolite was identified, the experts agreed that no further data would be required for the genotoxicity end point.

No reference values were set and none were needed as the micro-organism is not pathogenic or infective and does not produce toxins.

Low risk was anticipated for operators and workers if adequate personal protective equipment (PPE) and respiratory protective equipment (RPE) for operators are worn. A concern was raised by the experts over outdoor bystander exposure by inhalation due to the potential sensitisation by inhalation and no protection possible for bystanders. A risk characterisation for bystanders was provided by the rapporteur Member State after the experts' meeting, concluding that bystander exposure is low when compared to the amount of micro-organisms that are inhaled daily as a background value. Nevertheless, a concern over outdoor bystander inhalation remains. Furthermore, for outdoor uses, as there is an outstanding issue with the specification of the product with regard to some pathogenic contaminants, the exposure risk assessment of bystanders could not be finalized.

Lecanicillium muscarium strain Ve6 does not colonise the plant surface, and thus, the micro-organism is considered not likely to grow and multiply on plant material. Dietary exposure from the use of *Lecanicillium muscarium* strain Ve6 is likely to be minimal. Moreover, any potentially occurring residual deposits on the treated crops are not relevant, as no human health concerns have been

identified due to the toxicological profile of this strain. This assessment is subject to a final specification for microbial contamination of the plant protection product.

The available data on the fate and behaviour in the environment indicated that the only component that required environmental exposure and risk assessment was the colony forming units of *Lecanicillium muscarium* strain Ve6. Data on the competitiveness and persistence of added *Lecanicillium muscarium* to soil and natural surface water indicated that the organism was not very competitive, and that following addition of *Lecanicillium muscarium* to the soil environment or common artificial plant substrate, or spray drift exposure to surface water, levels would be expected to decline. However, information on the natural background concentrations in soil was not presented and no specific data on the influence of UV light on persistence and multiplication in water were available. There was sufficient evidence to show that *Lecanicillium muscarium* strain Ve6 does not produce any known secondary metabolites of toxicological or environmental concern.

A general consideration to address the risk to non-target organisms was the very narrow 'natural' host or target range of *Lecanicillium muscarium,* in addition to the apparent lack of evidence that birds, aquatic organisms, bees, non-target arthropods, earthworms and terrestrial plants are among the 'natural' target or host range of *Lecanicillium muscarium.* Furthermore, it was considered that the occurrence of 'natural' epizootics in the field could impose the same 'risks' to non-target organisms as epizootics induced by products with *Lecanicillium muscarium.*

No acute toxic, infective or pathogenic effects were identified in any of the studies on birds, aquatic organisms, bees or earthworms based on the data available.

However, data gaps were identified during the peer review to address the potential infectivity in fish and the potential toxicity to aquatic invertebrates. Both data gaps are relevant to outdoor uses only.

Many studies indicated no or only minimal effects on different non-target arthropod species exposed to *Lecanicillium muscarium*. There were however concerns among Member State experts about the strains and formulations used in the studies, and also concerns about the test conditions. Some effects were in fact seen on the species *Encarsia formosa*. A data gap was identified to further address the risk to non-target arthropods.

PARTICULAR CONDITIONS PROPOSED TO BE TAKEN INTO ACCOUNT TO MANAGE THE RISK(S) IDENTIFIED

- Low risk is anticipated for operators if personal protective equipment (gloves and protective clothing) and respiratory protective equipment are worn (see section 2.8).
- Low risk is anticipated for workers if personal protective equipment is worn (see section 2.8).

ISSUES THAT COULD NOT BE FINALIZED

- Specification for microbial contaminants (see section 1).
- Consumer risk assessment as well as bystander exposure assessment for the outdoor use cannot be finalized as there is an outstanding issue with the proposed specification with regard to the level of pathogenic contaminants (see sections 2.8 and 3.3).
- Micro-organisms are potential sensitisers in contact with skin and by inhalation; sensitisation by inhalation to unprotected bystanders was not addressed for field use (see section 2.8).
- The risk to aquatic organisms could not be fully addressed on basis of the data available (see section 5.2).

- The risk to non-target arthropods could not be fully addressed based on the data available (see section 5.4).

CRITICAL AREAS OF CONCERN

- The levels of coliforms, *Staphylococcus aureus* and streptococci, which are regarded as pathogenic contaminants, are too high when compared to internationally available proposed levels for pathogenic microbial contaminants. As this is the case, and given that these are human pathogens that may contaminate edible crops, it is appropriate for EFSA to identify this as a critical area of concern.

REFERENCES

EFSA (European Food Safety Authority), 2010. Peer Review Report to the conclusion regarding the peer review of the pesticide risk assessment of the active substance *Lecanicillium muscarium* strain Ve6. EFSA Journal 2010; 8(1):1446. [45 pp.]. doi:10.2903/j.efsa.2010.1446.

The Netherlands, 2007. Draft Assessment Report (DAR) on the active substance *Lecanicillium muscarium* strain Ve6 prepared by the rapporteur Member State the Netherlands in the framework of Directive 91/414/EEC, June 2007.

The Netherlands, 2009. Final Addendum to the Draft Assessment Report on *Lecanicillium muscarium* strain Ve6, compiled by EFSA, November 2009.

APPENDICES

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

Chapter 1 Identity, Biological properties, Details of Uses, Further Information

Active micro-organism	*Lecanicillium muscarium* Ve6 (*Verticillium lecanii* Ve6)
Function (e.g. control of fungi)	Control of whitefly and thrips
Rapporteur Member State	The Netherlands

Identity of the micro-organism (Annex IIM 1)

Name of the organism	*Lecanicillium muscarium* Ve6 (former name*: Verticillium lecanii* Ve6)	
Taxonomy	Kingdom: Fungi, Phylum: Deuteromycotina Order: Hyphomycetes (syn. Moniliales) Genus: Lecanicillium Species: muscarium	Kingdom: Fungi, Phylum: Deuteromycotina Order: Hyphomycetes (syn. Moniliales) Genus: Verticillium Species: lecanii
Species, subspecies, strain:	Strain: Ve6	Strain: Ve6
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). Open for a method to unequivocally identify this strain.	
Culture collection	CABI (=IMI) 268317, CBS 102071, ARSEF 5128	
Minimum and maximum concentration of the micro-organism used for manufacturing of the formulated product (cfu/g; cfu/L, etc.):	The material used for solid medium manufacturing of formulated product contains ± $1x10^{11}$ spores per gram technical spore powder (97-99%, dried conidiospores, 1-3 % media remnants). The material used for liquid medium manufacturing of formulated product contains ± $2\text{-}5x10^{10}$ spores per gram	

	technical spore powder (49-52%, dried blastospores, 48-51% additives).
Identity and content of relevant impurities in the technical grade micro-organism:	No relevant human/mammalian metabolites or toxins present in product or being produced by *Lecanicillium muscarium* Ve6. Contaminating micro-organisms: Open, subject to new specification and supporting batch analysis.
Is the MCPA genetically modified; if so provide type of modification	No

Biological properties of the micro-organism (Annex IIM 2)

Origin and natural occurrence, background level	Natural habitat: soil pathogen, hyper parasite on rusts, parasite on cyst-nematodes, saprophyte on ripening grain and various insects, especially on aphids and scales. Whiteflies are also parasitized by *V. lecanii* by nature in greenhouses. Inoculum is often available in the soil. The entomopathogenic fungus *V. lecanii* occurs worldwide.
Target organism(s)	Whiteflies (*Bemisia tabaci, Trialeurodes vaporariorum*) and thrips (*Frankliniella occidentalis*).
Mode of action	The insect dies after formation of a great number of hyphal bodies inside the body cavity. The mode of action has not been completely elucidated.
Host specificity	*Verticillium lecanii* has, under fungus favourable conditions, a broad spectrum, but especially affects Homoptera. Especially aphids and scales are affected. Rusts are also affected. Large differences between isolates exist. *V. lecanii* has never been observed as a pathogen on plants or warm-blooded animals.
Life cycle	The fungus reproduces asexually forming conidia (spores) directly from the vegetative state.
Infectivity, dispersal and colonisation ability	Spores of *Verticillium lecanii* strain Ve6 germinate and grow radially between 5 and 30°C. *V. lecanii* will not multiply on crops when its nutrient supply is limited to that with which it is applied. The mechanism of dispersal is not exactly known. It has been speculated that insects and soil organisms take spores with them from the soil to the leaves, after which other insects can be infected too. Spores are not spread by air.
Relationships to known pathogens	*Verticillium lecanii* is not closely related to known plant or human pathogens.
Genetic stability	*Verticillium lecanii* strain V6 is stored at -85°C and cultured in such a way that the strain is still. The genetic stability of the strain has been shown to be acceptable.

Production of relevant metabolites/toxins	Destruxins are produced in small quantities by the fungus in a laboratory scale production process (liquid still culture), not used commercially. No destruxins were identified in 'Mycotal' nor in 'Mycotal'-treated crops.
Resistance/sensitivity to antibiotics/anti-microbial agents used in human or veterinary medicine	*Verticillium* spp. can be treated with several antibiotics. *V. lecanii* is not known to be resistant to any of these antibiotics.

Classification and proposed labelling

with regard to the micro-organism:	The micro-organism should be classified as potentially sensitising by inhalation and skin contact. The following phrase should be used: "Micro-organisms may have the potential to provoke sensitising reactions" No classification and labelling for the micro-organism regarding the environment is proposed.

Summary of representative uses evaluated (*Lecanicillium muscarium* Ve6)*

Crop and/ or situation	Product name	F G or I	Pests or Group of pests controlled	Formulation		Application				Application rate per treatment			PHI days	Remarks:
				Type	Conc. of as CFU/g (g/kg)	method kind	growth stage & season	number min max	interval between applications (min)	Kg as/hL (CFU/hL) min max	water L/ha min max	Kg as/ha (CFU/ha) min max		
Cucumber	Mycotal	G	Whitefly, thrips	WP	$1x10^{10}$ (161)	Spray application	Nymphs, all year round	2 12*	7	0.0161 ($1x10^{12}$) 0.0161 ($1x10^{12}$)	2000	0.322 ($2x10^{13}$)	0**	[1]
Tomato	Mycotal	G	Whitefly,	WP	$1x10^{10}$ (161)	Spray application	Nymphs, all year round	2 12*	7	0.0161 ($1x10^{12}$) 0.0161 ($1x10^{12}$)	2000	0.322 ($2x10^{13}$)	0**	[1]
Sweet pepper	Mycotal	G	Whitefly, thrips	WP	$1x10^{10}$ (161)	Spray application	Nymphs, all year round	2 12*	7	0.0161 ($1x10^{12}$) 0.0161 ($1x10^{12}$)	2000	0.322 ($2x10^{13}$)	0**	[1]
Strawberry	Mycotal	G	Whitefly, thrips	WP	$1x10^{10}$ (161)	Spray application	Nymphs, all year round	2 12*	7	0.0161 ($1x10^{12}$) 0.0161 ($1x10^{12}$)	1000	0.161 ($1x10^{13}$)	0**	[1]
Strawberry	Mycotal	F	Whitefly, thrips	WP	$1x10^{10}$ (161)	Spray application	Nymphs, all year round	2 12*	7	0.0161 ($1x10^{12}$) 0.0161 ($1x10^{12}$)	1000	0.161 ($1x10^{13}$)	0**	[1][2]
Ornamentals	Mycotal	G	Whitefly, thrips	WP	$1x10^{10}$ (161)	Spray application	Nymphs, all year round	2 12*	7	0.0161 ($1x10^{12}$) 0.0161 ($1x10^{12}$)	1000 2000	0b 0.322 ($2x10^{13}$)	0**	[1]

∗For uses where the column "Remarks" is marked in grey further consideration is necessary.
Uses should be crossed out when the notifier no longer supports this use(s).

[1] The level of pathogenic microbial contamination in the product is higher compared to current, internationally available proposed levels for pathogenic microbial contaminants.
[2] For outdoor uses, considering the potential sensitisation by inhalation for unprotected bystanders as well as the outstanding issue with the specification of the product with regard to some pathogenic contaminants, the exposure risk assessment of bystanders could not be finalized.

*: For good control of the whitefly population it is recommended to apply MYCOTAL two till four times with an interval of seven days. In case new infections occur later in the season, full treatment with MYCOTAL (two till four applications per treatment) can be repeated.; **: MYCOTAL does not have a pre-harvest interval. However, according to good agricultural practise the product should not be sprayed on the crop on the day of harvest before harvesting.

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989

(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated
(i) Cfu=colony forming units and g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

Chapter 2 Analytical Methods

Analytical methods for the micro-organism (Annex IIM 4.2; 4.3; IIIM 5.4)

Manufactured micro-organism (principle of method)	Two methods of production of spores of *Lecanicillium muscarium* strain Ve6 are presented: solid fermentation (conidiospores) and liquid shaken fermentation (blastospores).
Impurities and contaminating micro-organisms in manufactured material (principle of method)	ISO methods were used.
Microbial plant protection product (principle of method)	Viable spore count method available, but open for the whitefly assay.

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

of the active micro-organism (principle of method)	Residues of the active micro-organism are determined by plating samples onto malt agar extract or selective medium (Rose bengal chloramphenicol agar). Colonies can be identified by morphological identification methods. The methods were not validated, however, this is not an issue as monitoring methods are not required.
of relevant metabolites/toxins (principle of method)	No method is required since no known toxins were detected on the crop.

Chapter 3 Impact on human health (Annex IIM 5; IIIM 7)

Medical data, surveillance and observations	Based on the total toxicological package of the active substance *Verticillium lecanii*, it is concluded that the active substance and its products do not exhibit infectivity or pathogenicity. *Verticillium lecanii* strain Ve6 is not indicated to be significantly allergenic, even to a more highly allergic population than average. The strains do not appear to be toxic.
Sensitisation (experience in humans and study results: type of study)	No evidence was found that exposure under pilot plant conditions had resulted in sensitisation of any sort in these subjects who were all healthy and without symptoms. The use of *Verticillium* was not related to any symptoms of sensitization or inflammatory lung diseases among greenhouse workers. Exposure to microbial biopesticides containing *Bacillus thuringiensis* or *Verticillium lecanii* may confer a risk of IgE-mediated sensitization. In future research there is a need to identify allergenic components in the preparations, perform studies on non-exposed controls and analyze the relation between sensitization and health parameters. *V. lecanii spp* (strain not indicated) was negative in a Maximisation test.
Toxicity	
after acute oral exposure:	No adverse effects. Rat oral LD_{50} >3.0 x 10^8 spores/animal
after acute inhalation exposure:	No adverse effects. Rat inhalation LD_{50} >maximal practical dose (not further specified)
after acute intraperitoneal/subcutaneous exposure:	Mortality, clinical signs, changes in clinical pathology (rat, high dose (ca. 10^8 CFU/animal)), body weight loss, lesion in the abdominal cavity (rat and mouse, low and high dose (ca. 10^6-10^7 or ca. 10^8 CFU/animal)), considered to be an acute immune-reaction rather than a toxicity reaction. Rat intraperitoneal LD_{50} >1.2 x 10^8 spores/animal
after acute intravenous exposure:	No adverse effects.

	Rat intravenous LD_{50} >1.2 x 10^7 spores/animal (nominal concentration)
Infectivity	
after acute oral exposure:	No indication of infectivity
after acute inhalation exposure:	No indication of infectivity
after acute intraperitoneal/subcutaneous exposure:	No indication of infectivity
after acute intravenous exposure:	Not infective
Pathogenicity	
after acute oral exposure:	No indication of pathogenicity
after acute inhalation exposure:	No indication of pathogenicity
after acute intraperitoneal/subcutaneous exposure:	No indication of pathogenicity
after acute intravenous exposure:	Not pathogenic
Genotoxicity	No genotoxic potential
Cell culture study	No data - not required
Short term toxicity/pathogenicity	**Mycotal:** By inhalation: Local effects were noted in the lungs, possibly immune (irritation) related, since the formulation was tested, which contains - in addition to *V. lecanii* Ve6 - a rather large quantity of a known respiratory sensitiser as a co-formulant. NOAEL: 1 mg/m^3 Mycotal.
Specific toxicity, pathogenicity and infectiveness studies	No data - no further testing required.

ADI	Not applicable, lack of adverse effects due to *V. lecanii* Ve6 in studies performed (low toxicity, no infectivity or pathogenicity) and lack of known toxicologically relevant metabolites.
AOEL	Not applicable, lack of adverse effects due to *V. lecanii* Ve6 in studies performed (low toxicity, no infectivity or pathogenicity) and lack of known toxicologically relevant metabolites.
ARfD	Not applicable, lack of adverse effects due to *V. lecanii* Ve6 in studies performed (low toxicity, no infectivity or pathogenicity) and lack of known toxicologically relevant metabolites.

Exposure scenarios (including method of calculation)

Application method	Spray application
Operator	Low risk when personal protective equipment (PPE) as gloves and protective clothing and respiratory protective equipment (RPE) are used.
Workers	Low risk when proper PPE (as gloves, long-sleeved shirt and long trousers) is used.
Bystanders	Low risk, however, for outdoor uses considering the potential sensitisation by inhalation for unprotected bystanders as well as the outstanding issue with the specification of the product with regard to some pathogenic contaminants, the exposure risk assessment could not be finalized.

Chapter 4 Residues

Residues on Treated Products, Food and Feed (Annex IIM 6; IIIM 8)

Non-viable residues:	No risk for the consumer is expected, since no toxins are expected to occur during and after application of 'Mycotal'.
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. However, the consumer risk assessment is subject to a final specification for microbial contamination of the plant protection product.

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

Persistence and multiplication

in soil:	The $PEC_{soil,max}$ of *L. muscarium* is 3.2 x 10^8 CFU/g soil considering 12 applications and no degradation as agreed in PRAPeR M2. Based on available data the half-life of *L. muscarium* is estimated to be 4-5 days.
in water:	Due to the slight water dispersability of spores in water the PEC_{water} cannot be calculated. There is some evidence that multiplication in water might be expected to be limited.
in air:	Amount of released spores of *L. muscarium* after application decreased to almost the level of before application after 22 hours.

Mobility	The mechanisms of spread of *L. muscarium* are not exactly known. Aphids may carry spores from the soil to the leaves, causing infection in other insects. Spores are not spread by air, naturally, and are not released from conidiophores without water contact. Passive spread can occur by means of splashing, and probably by mechanic transfer by other Arthropoda present in the greenhouse. Mobility of spores through leaching to the groundwater does not occur.

Chapter 6 Effects on Non-target Species (Annex IIM 8; IIIM 10)

Effects on terrestrial vertebrates

Effects on birds:	No evidence of pathogenicity or replication of strain Ve6 of *Lecanicillium muscarium* in birds. 5-d LC_{50} of 19 mg a.s./kg bw/day (equals 1.2 x 10^9 CFU/kg bw/day)

Effects on aquatic organisms

Effects on fish:	96-h EC_{50} > 97 mg a.s./L or > 6.2×10^9 CFU/L
Effects on invertebrates:	24-h EC_{50} > 6.0 mg a.s./L or > 3.8×10^8 CFU/L*
Effect on algae:	No studies submitted; not required
Effect on aquatic plants:	No studies submitted; not required
	* Study duration considered too short to address the full risk to aquatic invertebrates, given the mode of action. Data gap for study to further address risk to aquatic invertebrates. Due to the low dispersability a chronic study to *Chironomus* may even be more appropriate.

Effects on arthropods

Effects on bees	Oral LD_{50}: > 112 µg a.s./bee or > 7.1×10^6 CFU/bee Contact LD_{50}: > 100 µg a.s./bee or > 6.3×10^6 CFU/bee Oral and contact NOED c. 8.0 mg a.s./bumblebee or 5.0×10^8 CFU/bumblebee No signs of toxicity, infectivity or pathogenicity.
Effects on other arthropods than bees	No effects of contact exposure in laboratory experiments with an isolate of *Lecanicillium* ssp. at 1.0 x 10^7 spores/mL, tested on 20 different non-target arthropod species. There were indications of effects on *E. formosa* parasitizing on whitefly exposed to *Lecanicillium* ssp. in a laboratory study. There were concerns regarding the test conditions, strains and formulations used in all laboratory studies. No effects of dried residues of the product Micro Germin Plus (amongst other, *L. muscarium*),

	at a rate of 4 kg product/ha in laboratory and semi-field tests. Slightly harmful (25-50% effects) in a field study at a rate of 4 kg product/ha against *T. pyri*. The field study was less documented.

Effects on soil organisms

Effects on earthworms	No signs of infectivity or pathogenicity to earthworms of *Lecanicillium muscarium* at concentration ≤ 1000 mg a.s./kg soil dwt. or 6.3×10^{10} CFU/kg soil dwt.
Effects on non-target soil micro-organisms	No studies submitted; not required
Additional studies	No studies submitted; not required

APPENDIX B – USED COMPOUNDS CODES

Not applicable.

ABBREVIATIONS

1/n	slope of Freundlich isotherm
ε	decadic molar extinction coefficient
°C	degree Celsius (centigrade)
µg	microgram
µm	micrometer (micron)
a.s.	active substance
AChE	acetylcholinesterase
ADE	actual dermal exposure
ADI	acceptable daily intake
AF	assessment factor
AOEL	acceptable operator exposure level
AP	alkaline phosphatase
AR	applied radioactivity
ARfD	acute reference dose
AST	aspartate aminotransferase (SGOT)
AV	avoidance factor
BCF	bioconcentration factor
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstract Service
CFU	colony forming units
ChE	cholinesterase
CI	confidence interval
CIPAC	Collaborative International Pesticide Analytical Council Limited
CL	confidence limits
d	day
DAA	days after application
DAR	draft assessment report
DAT	days after treatment
DM	dry matter
DT_{50}	period required for 50 percent disappearance (define method of estimation)
DT_{90}	period required for 90 percent disappearance (define method of estimation)
dw	dry weight
EbC_{50}	effective concentration (biomass)
EC_{50}	effective concentration
ECHA	European Chemical Agency
EEC	European Economic Community
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINKS	European List of New Chemical Substances
EMDI	estimated maximum daily intake
ER_{50}	emergence rate/effective rate, median
ErC_{50}	effective concentration (growth rate)
EU	European Union
EUROPOEM	European Predictive Operator Exposure Model
f(twa)	time weighted average factor
FAO	Food and Agriculture Organisation of the United Nations
FIR	Food intake rate
FOB	functional observation battery
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
g	gram
GAP	good agricultural practice

GC	gas chromatography
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GGT	gamma glutamyl transferase
GM	geometric mean
GS	growth stage
GSH	glutathion
h	hour(s)
ha	hectare
Hb	haemoglobin
Hct	haematocrit
hL	hectolitre
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	high pressure liquid chromatography – mass spectrometry
HQ	hazard quotient
IEDI	international estimated daily intake
IESTI	international estimated short-term intake
IPM	integrated pest management
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint Meeting on the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
K_{doc}	organic carbon linear adsorption coefficient
kg	kilogram
K_{Foc}	Freundlich organic carbon adsorption coefficient
K_{Fom}	Freundlich organic matter adsorption coefficient
L	litre
LC	liquid chromatography
LC_{50}	lethal concentration, median
LC-MS	liquid chromatography-mass spectrometry
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, median; dosis letalis media
LDH	lactate dehydrogenase
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOQ	limit of quantification (determination)
m	metre
M/L	mixing and loading
MAF	multiple application factor
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCPA	microbial pest control agent
MCV	mean corpuscular volume
mg	milligram
mL	millilitre
mm	millimetre
MoE	margin of exposure
MRL	maximum residue limit or level
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
MWHC	maximum water holding capacity

NESTI	national estimated short-term intake
ng	nanogram
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
OM	organic matter content
Pa	Pascal
PD	proportion of different food types
PEC	predicted environmental concentration
PEC_{air}	predicted environmental concentration in air
PEC_{gw}	predicted environmental concentration in ground water
PEC_{sed}	predicted environmental concentration in sediment
PEC_{soil}	predicted environmental concentration in soil
PEC_{sw}	predicted environmental concentration in surface water
$PIEC_{soil}$	predicted initial environmental concentration in soil
pH	pH-value
PHED	pesticide handler's exposure data
PHI	pre-harvest interval
PIE	potential inhalation exposure
pK_a	negative logarithm (to the base 10) of the dissociation constant
P_{ow}	partition coefficient between *n*-octanol and water
PPE	personal protective equipment
ppm	parts per million (10^{-6})
ppp	plant protection product
PT	proportion of diet obtained in the treated area
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r^2	coefficient of determination
RAFBCA	Risk Assessment of Fungal Biological Control Agents
RMS	rapporteur Member State
RPE	respiratory protective equipment
RUD	residue per unit dose
SC	suspension concentrate
SD	standard deviation
SFO	single first-order
spp	subspecies
SSD	species sensitivity distribution
STMR	supervised trials median residue
$t_{1/2}$	half-life (define method of estimation)
TER	toxicity exposure ratio
TER_A	toxicity exposure ratio for acute exposure
TER_{LT}	toxicity exposure ratio following chronic exposure
TER_{ST}	toxicity exposure ratio following repeated exposure
TK	technical concentrate
TLV	threshold limit value
TMDI	theoretical maximum daily intake
TRR	total radioactive residue
TSH	thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
UDS	unscheduled DNA synthesis
UV	ultraviolet
W/S	water/sediment

w/v	weight per volume
w/w	weight per weight
WBC	white blood cell
WP	wettable powder
WHO	World Health Organisation
wk	week
yr	year