

Conclusion regarding the peer review of the pesticide risk assessment of the active substance

***Paecilomyces lilacinus* strain 251**

finalised: 13 June 2007

SUMMARY

Paecilomyces lilacinus strain 251 is a new active substance for which in accordance with Article 6 (2) of Council Directive 91/414/EEC¹ Belgium received an application from Prophyta for inclusion in Annex I to Directive 91/414/EEC. Complying with Article 6 of Directive 91/414/EEC, the completeness of the dossier was evaluated and confirmed by Commission Decision 2003/305/EC².

Following the agreement between the EU-Commission and the EFSA for the EFSA to organise a peer review of those new active substances for which the decision on the completeness of the dossier had been published after June 2002, the designated rapporteur Member State Belgium made the report of its initial evaluation of the dossier on *P. lilacinus* strain 251, hereafter referred to as the draft assessment report (DAR), available on 8 December 2004. This draft assessment report was distributed for consultation to the Member States and the notifier on 9 November 2004.

The peer review was initiated on 9 November 2004 by dispatching the draft assessment report for consultation of the Member States and the notifier. Subsequently, the comments received on the DAR were examined by the rapporteur Member State and the need for additional data was agreed in an evaluation meeting in September 2005. Remaining issues as well as further data made available by the notifier upon request were evaluated in a series of scientific meetings with Member State experts in January 2007.

A final discussion of the outcome of the consultation of experts took place with representatives from the Member States on 25 April 2007 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 potato, tomato, tobacco, lettuce, celery, cucurbits, carrots, grapes and ornamentals for the control of nematodes. The formulation is usually applied as a soil drench but can also be used as a dip for young plants. Full details of the application rate and timings can be found in the attached end points.

The representative formulated product for the evaluation was BioAct WG a wettable granule formulation (WG).

¹ OJ No L 230, 19.8.1991, p.1 as last amended by OJ L 106, 24.4.2007, p.14

² OJ No L 112, 6.5.2003, p.10

As the available data indicate the organism is not competitive in the environment unless the host species are present, it is not pathogenic to humans, toxic or infective and does not produce any known toxicologically significant secondary metabolites, methods of analysis for monitoring are not required. However, it should be noted that some methods for soil and water are given in the DAR. Sufficient analytical methods as well as methods and data relating to physical, chemical and technical properties are available to ensure that quality control measurements of the plant protection product are possible.

In acute toxicity studies, *P. lilacinus* strain 251 did not induce signs of toxicity, pathogenicity and infectivity. Literature shows that fungal infection by *P. lilacinus* occurs rarely, mainly in immunodepressed subjects. Several investigators have described *P. lilacinus* endophthalmitis, in some cases with severe morbidity. Dermatologic infections constitute a large group of published reports. An epidemic with *P. lilacinus* was described with direct inoculation of the microorganism onto the skin with secondary haematogenous spread in one case. Infection manifested as cutaneous lesions without evidence of dissemination. Skin lesions erupted insidiously without fever and varied greatly. Additional accounts of *P. lilacinus* infection include reports of chronic maxillary sinusitis and a report of pulmonary mycosis. There is no evidence for any infection, toxicity and pathogenicity of this strain in manufacturing workers. The formulation Bioact was found positive in a Bühler assay. The classification as Xn, R43 ("May cause sensitisation by skin contact") was proposed, together with classification as R42 ("May cause sensitisation by inhalation"). *P. lilacinus* extract of strain 251 does not have any clastogenic effect under experimental conditions. No ADI is proposed (the strain 251 has been shown not to produce any paecylotoxin and the formulated product contains purified spores). The AOEL is not deemed necessary since the microorganisms is not pathogenic or infective and does not produce toxins. No exposure assessment is therefore needed.

P. lilacinus strain 251 does not colonise the root surface and thus the micro-organism is considered not likely to grow and multiply on plant material. Dietary exposure from use *P. lilacinus* strain 251 is likely to be minimal. Moreover, any potentially occurring residual deposits on the treated crops are not relevant as a human health concern due to the toxicological profile of this strain.

The available data indicated that the only component that required an environmental exposure and risk assessment was colony forming units of *P. lilacinus* Strain 251. Data on competitiveness and persistence of added *P. lilacinus* Strain 251 to soil and natural surface water, indicated that the organism was not very competitive and that following addition of *P. lilacinus* Strain 251 to the soil environment or spray drift exposure to surface water levels would be expected to decline. As a consequence of the method of application (incorporation in the soil or watering in or transplant drench) the organism would not be expected to move significantly out of the target treated area (upper soil layers). Consequently groundwater exposure to *P. lilacinus* Strain 251 would not be expected.

P. lilacinus strain 251 does not grow above 33°C and it does not produce toxic metabolites. In the DAR it was explained that the product PBP-01001-I is applied as a pre-planting soil treatment

followed by incorporation in soil and/or transplant drench. These use patterns suggest low likelihood of direct exposure and a low risk to birds and mammals. However the GAP table lists also uses with treatments all over the growing season and all sprayer types. For this uses direct exposure via contaminated food items is likely to occur. No risk assessment was conducted for birds and mammals from direct exposure and some uncertainty remains on the risk to bird and mammals for uses where direct exposure occurs.

A study on the persistence and survival of conidia in pond water showed that the potential of proliferation of *P. lilacinus* in surface water is low. Toxicity studies showed that the formulated product has no adverse effects up to the highest tested dose of 100 mg/L. Effects on algal growth were observed in the high test concentrations. The cause of the effects was not clear but if the endpoint from the study is compared to maximum PEC_{sw} the resulting TER of >10 indicates a low risk to algae. Overall it is concluded that the risk to aquatic organisms is low. Tests with non-target arthropods showed that the leaf dwelling species *Aphidius rhopalosiphi* reacted more sensitive than the other species tested (e.g. *Typhlodromus pyri* and the soil dwelling species *Poecilus cupreus*) indicating a potential high risk to leaf dwelling arthropods. The risk to non-target arthropods was discussed in the PRAPeR experts' meeting. It was concluded that the risk needs to be addressed further for uses where exposure of leaf dwelling arthropods is likely. For these uses risk mitigation measures should be applied like irrigating the plants, placing the sprayer nozzles close to the ground and using nozzles producing big droplets.

Tests with beneficial nematodes gave some indication that non-target species related to the group of the target organisms would not be efficiently parasitized by *P. lilacinus*. However the reliability of test results is uncertain since no positive control or a susceptible nematode species were included in the tests.

The risk to bees, earthworms, soil non-target micro-organisms and biological methods of sewage treatment plants was considered as low.

Key words: *Paecilomyces lilacinus* strain 251 peer review, risk assessment, pesticide, control of nematodes

Table of Contents

Summary	1
Background	5
The Identity of the micro-organism and the properties of the formulated product	6
Specific Conclusions of the Evaluation	7
1. Identity of the micro-organism/biological properties/physical and technical properties and methods of analysis.	7
2. Mammalian infectivity, pathogenicity and toxicology	9
2.1. Medical data	9
2.2. Sensitisation	10
2.3. Acute toxicity, pathogenicity, infectiveness	10
2.4. Genotoxicity	11
2.5. Short term toxicity and pathogenicity	11
2.6. Other studies (tier II)	11
2.7. Reference values	12
2.8. Exposure assessment	12
3. Residues	13
3.1. Nature and magnitude of residues in plant	13
3.2. Nature and magnitude of residues in livestock	13
3.3. Consumer risk assessment	13
3.4. Proposed MRLs	14
4. Environmental fate and behaviour	14
4.1. Fate and behaviour in soil	14
4.1.1. Persistence and multiplication in soil of the micro-organism	14
4.1.2. Persistence in soil of any relevant metabolite formed by the micro-organism under relevant environmental conditions	14
4.2. Fate and behaviour in water	14
4.2.1. Persistence and multiplication in water of the micro-organism	14
4.2.2. Persistence in water of any relevant metabolite formed by the micro-organism under relevant environmental conditions	14
4.3. Mobility	15
4.3.1. Mobility of the micro-organism	15
4.3.2. Mobility in soil of any relevant metabolite formed by the micro-organism under relevant environmental conditions	15
4.4. Considerations for concentration of the micro-organism in air	15
5. Ecotoxicology	15
5.1. Risk to terrestrial vertebrates	15
5.2. Risk to aquatic organisms	16
5.3. Risk to bees	16
5.4. Risk to other arthropod species	16
5.5. Risk to earthworms	17
5.6. Risk to other soil non-target organisms	17
5.7. Risk to soil non-target micro-organisms	17
5.8. Risk to other non-target-organisms (flora and fauna)	17
5.9. Risk to biological methods of sewage treatment	18
6. Residue definitions	18
List of studies to be generated, still ongoing or available but not peer reviewed	19
Conclusions and Recommendations	19
Critical areas of concern	20
Appendix 1 – List of endpoints for the active substance and the representative formulation	21
Appendix 2 – Abbreviations used in the list of endpoints	34

BACKGROUND

In accordance with Article 6 (2) of Council Directive 91/414/EEC Belgium received an application from Prophyta for inclusion of the active substance *Paecilomyces lilacinus* strain 251 in Annex I to Directive 91/414/EEC. Complying with Article 6 of Directive 91/414/EEC, the completeness of the dossier was evaluated and confirmed by Commission Decision 2003/305/EC.

Following the agreement between the EU-Commission and EFSA for EFSA to organise a peer review of those new active substances for which the completeness of the dossier had been officially confirmed after June 2002, the designated rapporteur Member State Belgium submitted the report of its initial evaluation of the dossier on *P. lilacinus* strain 251, hereafter referred to as the draft assessment report (DAR), to EFSA on 8 December 2004. This draft assessment report was distributed for consultation to the Member States and the notifier on 9 November 2004.

The comments received on the draft assessment report were evaluated and addressed by the rapporteur Member State. Based on this evaluation, representatives from Member States identified and agreed in an evaluation meeting on 28 September 2005 on data requirements to be addressed by the notifier as well as issues for further detailed discussion at expert level. A representative of the notifier was attending this meeting.

Taking into account the information received from the notifier addressing the request for further data, a scientific discussion of the identified data requirements and/or issues took place in a meeting of Member State experts organised by EFSA in January 2007. The reports of these meetings have been made available to the Member States electronically.

A final discussion of the outcome of the consultation of experts took place with representatives from Member States on 25 April 2007 leading to the conclusions as laid down in this report.

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

Following the agreement between the EU Commission and EFSA regarding the peer review of new active substances, 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. A list of the relevant end points for the active substance as well as the formulation is provided in appendix 1.

The documentation developed during the peer review was compiled as a **peer review report** comprising of the documents summarising and addressing the comments received on the initial evaluation provided in the rapporteur Member State's draft assessment report:

- the comments received,
- the resulting reporting table (rev. 1-1 of 10 October 2005),
- the consultation report

as well as the documents summarising the follow-up of the issues identified as finalised at the end of the commenting period:

- the reports of the scientific expert consultation,
- the evaluation table (rev. 2-0 of 19 April 2007).

Given the importance of the draft assessment report including its addendum (compiled version of July 2006 containing all individually submitted addenda) 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

The micro-organism is *Paecilomyces lilacinus* strain 251. The strain is deposited in the Australian Government Analytical Laboratories and it has been given the accession number 89/030550. *P. lilacinus* strain 251 is a soil micro-organism and it grows on insects and nematodes. It is a filamentous fungi which is closely related to *Penicillium*. The genus *Paecilomyces* is restricted to species with verticillate conidiophores bearing divergent whorls of branches and phialides.

P. lilacinus strain 251 is used to control nematodes. It infects nematode eggs using both physical forces as well as enzyme action. The fungus may also be able to infect other stages of the nematodes life-cycle but this is likely not to be significant in the field as the other stages live within the plant tissue and will therefore be protected from the fungi.

The representative formulated product for the evaluation was BioAct WG a wettable granule formulation (WG).

The evaluated representative uses were on potato, tomato, tobacco, lettuce celery, cucurbits, carrots, grapes and ornamentals for the control of nematodes. The formulation is usually applied as a soil drench but can also be used as a dip for young plants. Full details of the application rate and timings can be found in the attached endpoints.

SPECIFIC CONCLUSIONS OF THE EVALUATION

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

The wettable granule formulation is produced directly from the fermentation product. The resulting formulation will contain a minimum *P. lilacinus* strain 251 content of 2×10^9 CFU/g. Any material when plated out is found to have a micro-organism contamination level $>0.001\%$ of the *P. lilacinus* strain 251 content can not be used and must be destroyed. *P. lilacinus* strain 251 is not known to produce any relevant secondary metabolites. As the method to determine the micro-organism contamination level was incorrectly placed in a confidential addendum the details are presented here for transparency.

Two random samples of 100 g each are taken from a product batch of 200 kg and every single sample is examined in two fold replicates. 10 g of each sample are suspended in 1000 ml sterile NaCl solution (0.8%) for 30 min and stirred with a IKAMAG RCT magnet stirrer until the granules are fully dissolved and the conidia are suspended. 3×0.1 ml of the resulting conidia suspension are dispersed on Petri dishes containing potato dextrose agar (PDA, MERCK No.: 105398, pH 6.5) using a Drygalski spatula. The Petri dishes are incubated for 64-96 hours at 25°C. Developing bacterial and fungal colonies morphologically different from *P. lilacinus* strain 251 are identified and counted and their total density is calculated according to the relevant dilution factor. For each 200 kg product batch 12 individual results are obtained.

There is currently no FAO specification for *P. lilacinus* strain 251.

This nematophagous fungus is a common soil hyphomycete especially in the tropics.

P. lilacinus strain 251 was isolated from a Meloidogyne egg mass in Los Banos, Philippines. The natural occurrence of the species is ubiquitous. However, it is unlikely that this exact strain is indigenous to Europe.

The principle mode of action is as follows, before infecting a nematode egg, *P. lilacinus* strain 251 flattens against the egg surface and becomes closely associated with it. *P. lilacinus* strain 251 produces simple appressoria; anywhere on the nematode eggshell either after a few hyphae grow along the egg surface, or after a network of hyphae form on the egg. The presence of appressoria appears to indicate that the egg is, or is about to be infected. In either case, the appressorium appears the same, as a simple swelling at the end of a hypha, closely appressed to the eggshell.

Penetration of egg-shells involves both physical forces and chemical action:

P. lilacinus strain 251 has been shown to produce proteases and also red-yeast cell-wall lytic enzymes. Since red-yeast cell walls contain chitin, as do *M. javanica* eggs the same enzyme could be involved in chitin degradation. Lipase activity has also been detected in *P. lilacinus* strain 251. A serine protease has been isolated from *P. lilacinus* and was induced by vitellin, an eggshell component, and by intact eggs of *M. hapla*. Grooves left by dislodged hyphae have probably been

formed by enzyme action. Enzymes could thus break down the eggshell by attacking the protein layer itself, proteins that cross link the chitin layer, the eggshell lipids or the chitin, to enable a narrow infection peg to push through. Pressure is clearly involved in the infection of *M.javanica* eggs by *P. lilacinus* as the eggshell can clearly be seen pushed away from the penetrant hypha.

After growing out of the egg, *P. lilacinus* strain 251 would need to move nutrients derived from the nematode embryo to the developing conidiophore. *P. lilacinus* strain 251 is able to recognize and infect other sedentary life stages of *M. javanica*. This may not be significant in the field as these stages live within the plant tissue and are protected from the fungus.

From the information available the infectivity of *P. lilacinus* strain 251 is confined to plant-parasitic nematodes and there is no evidence or indication from available data on this strain that other soil organisms may be adversely affected, including beneficial nematodes or earthworms. In a few cases *P. lilacinus* isolates have been found to parasitise soil inhabiting insects, but the reported abundance of insects infested with this fungus and/or the infestation level were low. Some mycoparasitic property of *P. lilacinus* is reported and *P. lilacinus* was found to partly colonize sclerotia and conidia of *Aspergillus flavus*, which is acting as a plant damaging soil fungus.

It has been demonstrated that *P. lilacinus* strain 251 is not pathogenic to humans. There are however, pathogenic strains of the fungus but they differ from strain 251 at the LDH-A locus. 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 anti-biotics that could interfere with the use of antibiotics in human or veterinary medicine.

The assessment of the data package revealed no issues that need to be included as critical areas of concern with respect to the identity, biological properties, physical, chemical and technical properties of the respective formulation.

Sufficient test methods and data relating to physical, chemical and technical properties are available. Also adequate analytical methods are available for the identification and quantification *P. lilacinus* strain 251 in the representative formulation as well as for the quantification of any micro-biological contaminants. Therefore, enough data are available to ensure that quality control measurements of the plant protection product are possible.

As the 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 infectivity, pathogenicity and toxicology

General considerations

Different isolates of *P. lilacinus* have been reported to produce the so-called paecilotoxins, a group of neutral straight peptides with high oral toxicity, found to be associated with clinical isolates of *P. lilacinus* infections (Mikami et al., 1989). Strain 251 of *P. lilacinus* has been shown not to produce any paecilotoxin. The formulated product contains purified spores and is free of metabolites potentially formed during the cultivation.

Some studies are performed with the pure strain 251, some others with the formulation, and some studies are performed with both. Overall, the toxicological database was regarded as sufficient to draw a conclusion.

2.1. MEDICAL DATA

Literature shows that fungal infection by *P. lilacinus* occurs rarely. Protracted neutropenia, graft-versus-host disease, therapy for graft-versus-host disease, severe mycosis's, skin toxicity after chemoradiotherapy, broad-spectrum antimicrobial prophylaxis, parenteral nutrition, and central venous access devices all predispose patients to invasive infection and fungemia.

Infection with moulds other than *Aspergilli*, such as *Paecilomyces* species, is rare.

Eye infections: several investigations describe that *P. lilacinus* infections involve the eye (endophthalmitis). Twelve patients became infected after implantation of contaminated intraocular lenses. *P. lilacinus* caused severe morbidity, as the majority of eyes involved were not salvageable; furthermore it was found to survive commercial sterilization methods. It has morphologic characteristics similar to *Penicillium* sp. making initial diagnosis difficult.

Epidemic post cataract fungal endophthalmitis with this species are reported. There are a number of reports of endophthalmitis by this pathogen after cataract extraction. *P. lilacinus* infection was also reported to involve an immuno-compromised patient, patients treated with topical corticosteroid, and extended contact lens wearers. Treatment relied on antifungal agents that could be administered via ocular and systemic routes

Dermatologic infections constitute a large group of published reports. Since 1977, nearly 25 cases of cutaneous infection due to *Paecilomyces* species have been reported. The source of such skin infection may be the contamination of implantable or semi-implantable surgical prosthetic devices with this ubiquitous mold. A recent outbreak of nosocomial cutaneous *P. lilacinus* infection in highly compromised individuals was traced to a contaminated topical moisturizing agent. (Safdar, 2002)

An epidemic with *P. lilacinus* was described that was traced to a rare mode of transmission- direct inoculation of the microorganism onto the skin- with secondary haematogenous spread in one case. Infection manifested as cutaneous lesions without evidence of dissemination. Skin lesions erupted insidiously without fever and varied greatly. *P. lilacinus* was apparently resistant in vitro to amphotericin B, itraconazole, fluconazole and miconazole. Recovery from myelo-suppression was crucial for clinical resolution. Skin lesions resolved rapidly after the recovery of neutrophil count in all patients except those with graft-versus-host disease.

Additional accounts of *P. lilacinus* infection include reports of chronic maxillary sinusitis, cured with surgical debridement, and a report of pulmonary mycosis, which required Amphotericin B therapy.

The strain 251 is not pathogenic. No data were found in the literature suggesting some pathogenicity of this specific strain.

Manufacturing plant personnel

Despite long-term exposure of the personnel of the applicant, there is no evidence for any infection, toxicity and pathogenicity of this strain. Statements on the health of personnel exposed to strain 251 of *P. lilacinus* are available from the applicant, as well as from an Australian company. From 1999 to 2002 no health problems, fungal infections or symptoms of pathogenicity occurred. The production process for BioAct WG ensures that only purified spores of the bio-control strain are found in the end-use product.

First treatment

Treatment of human *P. lilacinus* infections relies on antifungal therapeutic agents.

P. lilacinus often shows variable resistance to amphotericin B, flucytosine, and the triazole-based drugs. In most cases, treatment is difficult, and the in vitro susceptibility of *P. lilacinus* or currently available alternative agents, such as itraconazole and capsosfungin is uncertain. Treatment with ketoconazole and/or terbinafine has produced a durable response in patients with disease that is refractory to amphotericin B. In a recent report, voriconazole has been shown to have fungicidal activity against *P. lilacinus*, a finding that is in contrast to the fungistatic effect of amphotericin B and itraconazole.

Sensitisation, allergenicity observations

The personnel involved in the development of the product and workers at the manufacturing plant have not shown any allergic reactions upon repeated exposure to the active ingredient *P. lilacinus* strain 251 since the beginning of handling this strain, in November 1999. In the Australian company no allergies have been reported among those who have been exposed to *P. lilacinus* strain 251 since 2002.

Direct observations, e.g. clinical cases

There is no evidence for any infection, toxicity and pathogenicity of this strain.

2.2. SENSITISATION

The formulation Bioact was a sensitizer when tested in a Bühler assay. The classification as Xn, R43 was proposed.

The RMS proposed also a “default” classification as R42 (“May cause sensitisation by inhalation”). With regard to this point the experts decided to leave this classification proposal and ask the Commission to solve the general problem with classification of microorganisms.

2.3. ACUTE TOXICITY, PATHOGENICITY, INFECTIVENESS

Acute toxicity, infectivity and pathogenicity were investigated in single dose toxicity tests in rats.

No mortality or abnormal clinical signs were reported. Transient body weight decreases were sometimes reported with complete recovery at the end of observation period. Clearance of strain 251 was investigated after intra-tracheal instillation and after intra-peritoneal administration. The fungus disappeared completely between day 8 and day 18 after challenge. A second study was realized by intra-tracheal instillation. Conidia were detected in lung and in pulmonary associated lymph nodes and in caecum. The presence of the microorganism in these tissues is probably related to a defence mechanism, inducing stimulation of the phagocyte defences to inhibit or kill the fungus. There was no fungal multiplication in the lymph nodes. After acute dermal application of the strain or the product, erythema and oedema was observed on treated area from day 2 to day 7 with the pure active ingredient in 4/10 animals and in 1/10 with the formulated product.

In summary, *P. lilacinus* strain 251 did not induce signs of toxicity, infectivity or pathogenicity ($LD_{50} > 2000$ mg/kg bw). The acute intraperitoneal LD_{50} is > 2000 mg/kg bw ($= 9 \times 10^9$ CFU/kg). Under these conditions, it is concluded that BioAct WG is not toxic, pathogenic nor infective.

2.4. GENOTOXICITY

Both with and without metabolic activation in two independent assays, no biologically significant increase in the number of revertants was noted in four *Salmonella typhimurium* strains.

P. lilacinus extract of strain 251 does not have any clastogenic effect under experimental conditions.

2.5. SHORT TERM TOXICITY AND PATHOGENICITY

Potential role of lytic enzymes in pathogenicity was considered in an addendum. It is shown that *P. lilacinus* strain 251 produces enzymes which are specific for the degradation of the skin of nematode eggs. It was considered unlikely that such highly specific enzymes can be pathogenic to mammals.

The micro-organism was considered not toxic, pathogenic and infective in the acute studies.

It was agreed to waive the requirement for a repeat dose study for this strain. Some MSs asked to have more certainty that the fungus does not grow above 37°C. The experts agreed to set the maximum temperature limit at 35 °C to cover the concerns that the microorganism cannot survive under the temperature regime of mammals and is not infective to human beings: *P. lilacinus* strain 251 is able to germinate only at short exposure periods under elevated temperature (36°C) but germ cells are deformed and subject to lysis. No formation of mycelium or production of conidia occurs. Under prolonged exposure at 36°C the fungus dies.

2.6. OTHER STUDIES (TIER II)

The significance of the isolation of fungal spores in well-perfused organs of rats exposed by intratracheal instillation was discussed in the experts' meeting. The experts agreed that that spores were the consequence of small lesions in the trachea mucosa and subsequent contamination of damaged blood vessels with instilled microorganisms.

2.7. REFERENCE VALUES

Acceptable Daily intake (ADI) and Acute reference dose (ARfD)

Paecilomyces lilacinus is a typically soil-born fungus geographically widespread. This nematophagous fungus differs genetically from pathogenic fungi. The strain 251 has been shown not to produce any paecylotoxin and the formulated product contains purified spores. No target organ, or dose-effect relationship or NOAEL can be determined. Therefore, no ADI and ARfD are proposed.

Acceptable Operator Exposure Level (AOEL)

The AOEL was discussed in the experts' meeting. It was concluded that an AOEL is not needed in those cases the microorganisms is not pathogenic or infective and does not produce toxins.

2.8 EXPOSURE ASSESSMENT

PPE was discussed in the meeting. At least inhalation protective equipment is needed as well as skin protective equipment and gloves. This will as well be re-discussed under the general part of the meeting.

At least it was proposed to maintain the R and S phrases that are presented in the box "irritation, sensitisation" since they follow the conservative approach recommended in the directive.

Dermal absorption

For micro-organisms it was proposed not to allocate a value for dermal absorption.

Exposure to operators, workers and bystanders

The maximum dose rate of BioAct WG is 20 kg/ha, equivalent to 0.25 kg active substance, or 4 x 10¹³ CFU applied up to 4 times per growing season.

BioAct WG is formulated as water dispersible granules, which is dust-free and therefore impose hardly any inhalation exposure.

The preparation is to be applied as a spray directly onto soil surface or as a soil drench, pre- or post-planting, and subsequently either is incorporated into the top layer of the soil or drained into the soil by watering.

Operator exposure

BioAct WG is a water dispersible granule formulation (WG) containing 12.5 g/kg *P. lilacinus* strain 251 and is recommended for drenching and spray application in different crops.

Since no adverse effects were obtained in any study on toxicity, pathogenicity or infectiveness, the experts agreed that calculations on the operator exposure/risk are not needed: no target organ exists and no dose-effect response (LOAEL) can be determined. Moreover, due to the mode of application of BioAct WG, i.e. drip irrigation, the exposure of the operator is confined to mixing and loading and, therefore, minimal (the water dispersible granules are dust-free).

Bystander exposure

Not relevant (no hazard identified).

Worker exposure

Not relevant (no hazard identified, unlikely to be exposed).

3. Residues

3.1. NATURE AND MAGNITUDE OF RESIDUES IN PLANT

Paecilomyces lilacinus strain 251 is not known to generate toxins or other metabolites with undesirable properties (see chapter 1 and 2 of this document). Therefore the investigation of the metabolism of *P. lilacinus* strain 251 on plant surfaces has not been necessary.

Residue trials with *P. lilacinus* strain 251 which would investigate the persistence and actual levels of the micro-organism on crops were not submitted, but 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-organism is not considered relevant.

However, in a study (reported in public literature) it was investigated whether *P. lilacinus* strain 251 can colonise roots. When eight crop plant species (tomato, cotton, wheat, barley, pineapple, potato, banana and capsicum) were challenged with *P. lilacinus* strain 251, fungal hyphae were never detected within roots of these crops, though occasionally colonies arose from the root surface. In conclusion, *P. lilacinus* strain 251 does not behave like a parasitic fungus on a root surface, i.e. it does not colonise the root surface to a significant extent and thus the micro-organism is considered not likely to grow and multiply on plant material.

3.2. NATURE AND MAGNITUDE OF RESIDUES IN LIVESTOCK

Not applicable as potential intake of *P. lilacinus* strain 251 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 *P. lilacinus* strain 251, an organism found in soil. No toxicological or pathological endpoints were identified for *P. lilacinus* strain 251, as demonstrated in chapter 2 of this document.

Dietary exposure from use *P. lilacinus* strain 251 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 treated food by washing and processing. Thus, the amount of residues the consumer will be exposed, if any, is likely to be very low.

Even if residues are not removed, however, 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

P. lilacinus strain 251 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.

3.4. PROPOSED MRLs

Based on the risk assessment for the consumer it was concluded that MRLs for *P. lilacinus* strain 251 on food commodities are not required. Thus, *P. lilacinus* strain 251 is considered eligible for inclusion in the Annex VI of Regulation 396/2005.

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 *P. lilacinus* strain 251 (experiments on 3 soils under outdoor conditions in Sydney, Australia, a further trial in a sugar beet field at an unreported location and trials under greenhouse conditions) evaluated in the DAR and addendum of July 2006 supports and suggests that its use would not result in increase of *P. lilacinus* strain 251 in soil over that at which it was originally applied. Levels of CFU would decline to background levels over about three to six months.

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 *P. lilacinus* strain 251 does not produce any 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

Available data on *P. lilacinus* strain 251 (experiments on a natural surface water at 20°C in the laboratory) evaluated in the addendum of July 2006 supports and suggests, that should viable *P. lilacinus* strain 251 reach surface water, for example as a consequence of spray drift, its level will decline relatively rapidly (declined to 1-2% of the dosed level after 14 days).

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 *P. lilacinus* strain 251 does not produce any 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 as a consequence of the method of application including incorporation in the soil or watering in. A pot experiment evaluated in the addendum of July 2006 indicated vertical movement of conidia below 20cm would not be expected even under intense irrigation / rainfall conditions.

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 *P. lilacinus* strain 251 does not produce any secondary metabolites of toxicological or environmental concern.

4.4. CONSIDERATIONS FOR CONCENTRATION OF THE MICRO-ORGANISM IN AIR

As there were no particular concerns identified for operator, worker or bystander exposure to *P. lilacinus* strain 251, the peer review agreed that information on potential concentrations in air were not required.

5. Ecotoxicology

Paecilomyces lilacinus was discussed at the PRAPeR experts' meeting on microorganisms in January 2007.

All ecotoxicological studies were performed with the formulated product. This is considered acceptable since potential adverse effects of co-formulants would be covered by the test results and would not lead to an underestimation of the risk.

5.1. RISK TO TERRESTRIAL VERTEBRATES

The acute oral toxicity and a toxicity study with intraperitoneal administration of the test substance were conducted with rats showing low toxicity to rats ($LD_{50} > 2000$ mg/kg bw, NOAEL = 2000 mg/kg bw). No toxicity data with birds were made available. The optimum growth temperature profile of *P. lilacinus* strain 251 suggests no growth above 33°C. Therefore it is considered unlikely that *P. lilacinus* would cause systemic mycosis in birds or mammals. Uncertainty remains with regard to other terrestrial vertebrates like amphibians and reptiles which have lower body temperatures than birds and mammals. *Paecilomyces lilacinus* does not produce any metabolites of ecotoxicological concern. In the DAR it was explained that the product PBP-01001-I is applied as a pre-planting soil treatment followed by incorporation in soil and/or transplant drench. These use patterns suggest low likelihood of direct exposure and a low risk to birds and mammals. However the GAP table lists also uses with treatments all over the growing season and all sprayer types. For this uses direct exposure via contaminated food items is likely to occur. No risk assessment was conducted for birds and mammals from direct exposure and some uncertainty remains on the risk to bird and mammals for

uses where direct exposure occurs. However the exposure of birds and mammals will be minimised by the application method and suggested risk mitigation for non-target arthropods (see point 5.4).

5.2. RISK TO AQUATIC ORGANISMS

The formulation PBP-01002-I was tested with fish, daphnids and algae indicating a low toxicity to aquatic organisms ($LC_{50}/EC_{50} > 100$ mg formulation/L and $EbC_{50} = 71.77$ mg formulation/L). The resulting TER values were well above the trigger of 100 and 10. The meeting discussed the effects observed in the algae study which were explained in the study report as indirect effects of competition for nutrients between the algae and the fungi while on the contrary persistence and growth of the fungi in surface water was assumed to be unlikely. The applicant explained that the effects would be most likely due to the composition of the test medium and the formulation. The explanations were considered not fully conclusive by the meeting. A new study on the persistence and survival of conidia in pond water suggest that the potency to proliferate in natural surface water is low and since the TER value based on the endpoint from the algae study is well above the trigger the risk to algae is considered to be low.

5.3. RISK TO BEES

No information on potential adverse effects of *P. lilacinus* on bees was submitted. The product PBP-01001-I is applied as a pre-planting soil treatment followed by incorporation in soil and/or transplant drench. These use patterns suggest low likelihood of exposure of bees and hence the risk to bees is considered to be low.

5.4. RISK TO OTHER ARTHROPOD SPECIES

Tests were conducted with *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Poecilus cupreus* and *Aleochara bilineata*. The effects were $< 30\%$ at a treatment rate of 3 kg formulation/ha for *T. pyri* and also for the soil dwelling arthropods *P. cupreus* and *A. bilineata* at a concentration of 6×10^9 CFU/kg dry soil. Worst case exposure of soil dwelling arthropods was calculated as 9.3×10^7 CFU/kg dry soil indicating that the risk to soil dwelling arthropods is low. Concerns were raised during the experts meeting with regard to the risk to *Aphidius rhopalosiphi* where mortality of 37% and a reduction in beneficial capacity of 74.4% were observed at a treatment rate of 3 kg formulation/ha. The meeting agreed that it is not necessary to address the potential high risk to leaf dwelling arthropods if exposure is negligible for the in-crop and off-crop area. The RMS explained that the application is on soil and with special application technique only. However the GAP table includes some inconsistencies e.g. listing “all sprayer types” for some uses. Therefore it was not possible during the meeting to agree whether exposure of leaf dwelling arthropods is negligible. Some experts wanted to have more data on soil dwelling arthropods but the majority of the meeting considered the risk to soil dwelling arthropods as sufficiently addressed based on the results from the tests with two soil dwelling insect species.

Overall it is concluded that the risk to soil dwelling arthropods is assumed to be low. A potential high risk to sensitive groups of leaf dwelling arthropods cannot be excluded for the uses where exposure is possible.

The RMS received further clarification of the applicant with regard to the application of the product. The applicant stated that contamination of above ground parts of the plants is possible from the representative uses. However the treatment is against soil dwelling nematodes and the treatment of plant parts is not intended. Therefore it is proposed that plants are irrigated after the treatment to ensure washing the spores from the plant surface to reach the soil. The risk of drift to off-field areas can be mitigated by placing the nozzles as close to the ground as possible and the use of nozzles producing big droplets (drift reduction nozzles). In order to mitigate the potential high risk to sensitive groups of leaf dwelling arthropods the above mentioned measures should be indicated in the labelling of the product.

5.5. RISK TO EARTHWORMS

No significant effects on body weight or number of juveniles were observed in a long-term study with the formulation and *Eisenia foetida* at concentrations of 133 and 400 mg formulation/kg dry soil corresponding to 2 and 6×10^9 CFU/kg dry soil which clearly exceeds the calculated worst case exposure in soil of 9.3×10^7 CFU/kg dry soil. The long-term TER was calculated as 64.5 indicating a low risk to earthworms.

5.6. RISK TO OTHER SOIL NON-TARGET ORGANISMS

The risk to earthworms and soil micro-organisms was assessed as low. Therefore no risk assessment for other soil non-target organisms is considered necessary.

5.7. RISK TO SOIL NON-TARGET MICRO-ORGANISMS

No effects of $>25\%$ on soil respiration and nitrification were observed at a dose of 71 mg formulation/kg dry soil corresponding to 1.6×10^8 CFU/kg dry soil. The test concentration exceeded the worst case exposure calculated as 9.3×10^7 CFU/kg dry soil indicating a low risk to soil non-target micro-organisms.

5.8. RISK TO OTHER NON-TARGET-ORGANISMS (FLORA AND FAUNA)

Additional studies were submitted on effects of *P. lilacinus* on non-target nematode species. No significant levels of parasitism were observed in the 5 nematode species tested (*Caenorhabditis elegans* and four entomopathogenic species). The RMS considered the results as difficult to interpret since no positive controls or susceptible plant parasitic nematodes were included in the test.

5.9. RISK TO BIOLOGICAL METHODS OF SEWAGE TREATMENT

No information was made available. However due to the application pattern it is unlikely that *P. lilacinus* would enter sewage treatment plants in significant amounts and considering its biology is unlikely that it would pose a high risk to biological methods of sewage treatment.

6. Residue definitions

Soil

Definitions for risk assessment: Colony forming units of *P. lilacinus* Strain 251

Definitions for monitoring: The peer review considered that due to the hazard profile a monitoring definition was not necessary.

Water

Ground water

Definitions for exposure assessment: Colony forming units of *P. lilacinus* Strain 251

Definitions for monitoring: The peer review considered that due to the hazard profile a monitoring definition was not necessary.

Surface water

Definitions for risk assessment: Colony forming units of *P. lilacinus* Strain 251

Definitions for monitoring: The peer review considered that due to the hazard profile a monitoring definition was not necessary.

Air

Definitions for risk assessment: Colony forming units of *P. lilacinus* Strain 251

Definitions for monitoring: The peer review considered that due to the hazard profile a monitoring definition was not necessary.

Food of plant origin

Definitions for risk assessment: not allocated, no hazard identified

Definitions for monitoring: not required

Food of animal origin

Definitions for risk assessment: not required

Definitions for monitoring: not required

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

- The risk to leaf dwelling arthropods needs to be addressed further. (relevant for the uses where in-field and/or off field exposure of leaf dwelling non-target arthropods is likely to occur, data gap identified in the PRAPeR experts' meeting in January 2007; no submission date proposed by the applicant; a statement on the application was received by the RMS; refer to point 5.4)

CONCLUSIONS AND RECOMMENDATIONS

Overall conclusions

The conclusion was reached on the basis of the evaluation of the representative uses on potato, tomato, tobacco, lettuce, celery, cucurbits, carrots, grapes and ornamentals for the control of nematodes. The formulation is usually applied as a soil drench but can also be used as a dip for young plants. Full details of the application rate and timings can be found in the attached end points.

The representative formulated product for the evaluation was BioAct WG a wettable granule formulation (WG).

As the 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. However, it should be noted that some methods for soil and water are given in the DAR.

Sufficient analytical methods as well as methods and data relating to physical, chemical and technical properties are available to ensure that quality control measurements of the plant protection product are possible.

P. lilacinus strain 251 did not induce signs of toxicity, pathogenicity and infectivity. The formulation Bioact was found positive in a Bühler assay. The classification as Xn, R43 ("May cause sensitisation by skin contact") was proposed, together with classification as R42 ("May cause sensitisation by inhalation"). *P. lilacinus* extract of strain 251 does not have any clastogenic effect under experimental conditions. No ADI is proposed (the strain 251 has been shown not to produce any paecylotoxin and the formulated product contains purified spores). The AOEL is not deemed necessary since the microorganisms is not pathogenic or infective and does not produce toxins. No exposure assessment is therefore needed.

P. lilacinus strain 251 does not colonise the root surface and thus the micro-organism is considered not likely to grow and multiply on plant material. Dietary exposure from use *P. lilacinus* strain 251 is likely to be minimal. Moreover, any potentially occurring residual deposits on the treated crops are not relevant as a human health concern due to the toxicological profile of this strain.

The available data indicated that the only component that required an environmental exposure and risk assessment was colony forming units of *P. lilacinus* Strain 251. Data on competitiveness and persistence of added *P. lilacinus* Strain 251 to soil and natural surface water, indicated that the organism was not very competitive and that following addition of *P. lilacinus* Strain 251 to the soil environment or spray drift exposure to surface water levels would be expected to decline. As a consequence of the method of application (incorporation in the soil or watering in or transplant drench) the organism would not be expected to move significantly out of the target treated area (upper soil layers). Consequently groundwater exposure to *P. lilacinus* Strain 251 would not be expected.

The risk to non-target organisms was generally considered to be low. Severe adverse effects were observed in a test with the formulation and the leaf dwelling insect *Aphidius rhopalosiphi*. The risk to leaf dwelling arthropods needs to be addressed further for uses where exposure of leaf dwelling arthropods is likely to occur. Risk mitigation measures like irrigation of plants after treatment, placing sprayer nozzles close to the ground and the use of drift reducing nozzles are suggested for uses where exposure of leaf dwelling arthropods is likely.

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

- In order to mitigate the potential high risk to sensitive groups of leaf dwelling arthropods the following measures should be indicated in the labelling of the product: irrigation of plants after treatment to wash spores from the plant, placing sprayer nozzles close to the ground and the use of drift reducing nozzles.

Critical areas of concern

- None

APPENDIX 1 – LIST OF ENDPOINTS FOR THE ACTIVE SUBSTANCE AND THE REPRESENTATIVE FORMULATION

(Abbreviations used in this list are explained in appendix 2)

Microorganism (ISO Common Name)	<i>Paecilomyces lilacinus</i> strain 251
Function (e.g. fungicide)	Control of nematodes
Rapporteur Member State	Belgium

Appendix 1.1: Identity and biological properties of the micro-organism

Known or new organism	<i>Paecilomyces lilacinus</i> (Thom) Samson 1974 strain 251 (AGAL: n°89/030550)
Taxonomy:	Fungus imperfectus, hyphomycetales (Moniliales), <i>Paecilomyces</i>
Species, subspecies, strain	<i>Paecilomyces lilacinus</i> strain 251
Identification/detection	Morphological criteria, molecular fingerprint
Methods of analysis	Plating on agar for morphological identification Physiological criteria (UV effects, effects of temperature on growth and germination) DNA analysis and enzyme electrophoresis for molecular fingerprint
Mode of action	Oviparasitic (containing well formed juveniles and earlier eggs), and endoparasitic to various growth stages of nematodes. Penetration of egg-shells involving both physical forces and chemical action: <i>P. lilacinus</i> produces a serine protease, cell-wall lytic enzymes and lipase. Enzymes could break down eggshell by attacking the protein layer itself, proteins that cross link the chitin layer, the eggshell lipids or chitin, to enable a narrow infection peg to push through. Pressure is involved in infection. Once an egg is infected the nutrients available for the fungus stimulate proliferation of the hyphae on that egg, enabling subsequent growth to adjacent eggs. Occasionally, the fungus parasitises egg laying females by penetration through anal or vulval opening destroying eggs before laying process

Appendix 1 – List of end points for the active substance and the representative formulation

Life cycle	Asexual life cycle; opportunistic fungus and saprophyte; survival not dependent on its host; may survive in soil; growth enhanced in rhizosphere. Colonize eggs and kills them by parasitism, cysts and sometimes female reproductive organs
Host specificity	Attack and killing several plant parasitic nematodes species (Heteroderid) at different developmental stages. Mycoparasitic property sometimes reported Infective dose: 1×10^7 spores/g soil for nursery application 1×10^7 - 1×10^8 spores/ml for field application as drench to soil adjacent to plants
Known opportunist	Strain 251 was not isolated from human or animals. Other isolates of the species act as opportunistic pathogens in humans.
Toxin production	Strain 251 does not produce toxins
Resistance	Stable phenotype maintained through standard procedures; no indication of decreasing efficacy against plant-parasitic nematodes
Resting stages	Conidiospores
Production control	The occurrence of contaminants is prevented by use of pure stock cultures; fermentation in closed system, using sterile growth medium. Fermentation yields a suspension of spores and only spores are transferred by filtration before formulation step. Before and after fermentation, quality control is employed. Purity of each batch is determined by plating onto non selective medium. Bacterial and fungal contaminants are detected by the naked eye. A microbial contamination at a level exceeding 0.001% of the content of an a.i, equivalent to 2×10^4 CFU/g product is not acceptable any batch that exceeds this level must be destroyed. Each batch is simultaneously controlled for the number of spores of <i>P. lilacinus</i> in the formulated preparation.

Appendix 1.2: Hazard to humans

Pathogenicity	Non pathogenic upon different routes of exposure
Infectivity	Non infectious via intra-tracheal and intraperitoneal route

Appendix 1 – List of end points for the active substance and the representative formulation

Toxicity	<p>Rat LD₅₀ oral > 4 x 10⁹ CFU/kg bw (2000 mg/kg bw)</p> <p>Rat acute intra-tracheal instillation LD₅₀ >1.3x10⁸ spores /rat</p> <p>Rat acute percutaneous toxicity LD₅₀>2000 mg/kg bw(4x10⁹ CFU/kg bw)</p>
Irritation, sensitization	Not skin irritant
Genotoxicity	Not genotoxic <i>in vitro</i> and <i>in vivo</i>
Medical reports	Limited database. No adverse health effects observed among personnel involved in laboratory investigations
Formulation	<p>Rat LD₅₀ oral >2000 mg/kg bw (= 4x10⁹ CFU/kg)</p> <p>Rat acute intra-tracheal instillation LD₅₀ >2.5x10⁸ spores /rat</p> <p>Rat, intra-peritoneal single dose: LD₅₀>2000 mg/kg bw (9x10⁹ CFU/kg bw)</p> <p>Rat acute percutaneous toxicity LD₅₀>2000 mg/kg bw (4x10⁹ CFU/kg bw)</p>
Irritation, sensitization	<p>Not eye irritant but labelled S25</p> <p>Skin sensitizer, Xi, R43</p> <p>As a general rule, potential respiratory sensitiser, R42</p> <p>S36/37, S23, S45</p>

Appendix 1.3: Hazard to the environment

Impact on non-targets	<p>No effects on birds and mammals are expected as <i>P. lilacinus</i> Strain 251 does not grow above 35°C.</p> <p><i>Onchorhynchus mykiss</i>: LC₅₀ > 100 mg formulation/L (or 4.5 x 10⁸ CFU/ L)</p> <p><i>Daphnia magna</i> : EC₅₀ > 100 mg formulation/L (or 4.5 x 10⁸ CFU/ L)</p> <p><i>Desmodesmus subspicatus</i>: E_bC₅₀= 71.77 mg product/L (or 3.2 x 10⁸ CFU/ L)</p> <p>No effects on bees are expected.</p> <p><i>Aphidius rhopalosiphi</i>: E = 74.4% (reduction of beneficial capacity at 1.36 x 10¹³ CFU/ ha)</p> <p><i>Typhlodromus pyri</i>: E = 5.13% (reduction of beneficial capacity at 1.36 x 10¹³ CFU/ ha)</p> <p><i>Poecilus cupreus</i> larvae: E = 7.9% (mortality at 6.0 x 10⁹ CFU/ kg dry soil)</p>
-----------------------	--

Formulation	<p><i>Aleochara bilineata</i>: E = 10.4% (reduction of reproduction at 6.0×10^9 CFU/ kg dry soil)</p> <p><i>Eisenia foetida</i>: no effect on mortality, body weight, reproduction of at 2.0 and 6.0×10^9 CFU/ kg dry soil)</p> <p>soil respiration and nitrification: effect less than 25% after 28 days at 3.2×10^8 CFU/ kg dry soil</p>
	<p>Risk is considered acceptable</p> <p>Based on the results of the static toxicity test with <i>Desmodesmus subspicatus</i> the formulation should be labelled R52. But as the observed effects might be attributed to nutrient competition between the a.s. and the algae, no labelling for environmental effects is proposed.</p>

Appendix 1.4: Operator exposure

Application method	Spraying pre-planting, transplant drenching (band treatment)
Operator exposure:	Not relevant, since an AOEL was not necessary (not pathogenic, not infective, no toxic metabolites to vertebrates)
Worker exposure	Not relevant
Bystander exposure	Not relevant

Appendix 1.5: Exposure of the environment

Natural occurrence, background level:	On species level global and ubiquitous distribution mainly in soils (common saprophytic fungus)
Application method	<ul style="list-style-type: none"> - Pre-planting soil treatment: spraying onto soil surface, with subsequent incorporation into top soil layer - At/post planting: band treatment as soil drench or watering individual plants - Dipping of tubers and seedlings
Post release control	Not necessary, population density independent of applied dose and subject to regulation by various environmental factors.

Appendix 1.6: Consumer exposure and risk assessment

Residues

No residue data required as absence of human infectivity, pathogenicity and toxicity upon exposure to the fungus shown by the toxicity studies as well as by the observations of persons which were in contact with the fungus.
Based on available information consumer exposure expected to be minimal to non existent.
Dietary risk to consumers is negligible

Appendix 1 – List of end points for the active substance and the representative formulation

Summary of representative uses evaluated

Crop and/or situation	Member State or Country	Product name	F G or I	Pests or Group of pest controlled	Formulation		Application				Application rate per treatment			PHI (days)	Remarks (m)
					Type	Conc. of as	method kind	growth stage & season	Number (max)	interval between applications	Kg as/hL	Water L/ha	Kg as/ha		
(a)			(b)	(c)	(d-f)	(i)	(f-h)	(j)	(k)		(n)			(l)	(m)
Potato (SOLTU)	N/S	PBP-01001-I	F: outdoor or field use	Root lesion nematodes (<i>Pratylenchus spp.</i>) and potato cyst nematode (<i>Globodera spp.</i>)	Water dispersible granule	2 x 10 ⁹ CFU/g (minimum)	a) spraying/dipping of tubers into product suspension (at 0.1g product/tuber) or b) preplanting soil treatment: motor -, knapsack- or hand sprayer or pouring by hand, followed by soil incorporation and c) soil drench band treatment (spraying or pouring)	Pre-planting (tuber or soil) and throughout the season	4	8 weeks	0.010 to 0.025	200-500 L/ha	0.050	no PHI required	b) Spray in a band along the planting line and incorporate to a 10-15 cm depth in moist soil using a rotary hoe. Do not allow soil to dry out before incorporation
Tomato (I) (LYPES)	N/S	PBP-01001-I	G: glasshouse application	Root knot nematode (<i>Meloidogyne spp.</i>)	Water dispersible granule	2 x 10 ⁹ CFU/g (minimum)	a) immersion or spraying of seedling trays with subsequent watering b) see tomato II	a) Seedlings, 1 day before transplanting; b) if glasshouse culture: throughout the season	a) 1 b) 4	a) not applicable b) 6-8 weeks	0.125 to 0.313	200-500 L/ha	0.625	no PHI required	Treatment of seedlings always followed by either pre-planting soil treatment or transplant drench (see tomato II)

‡ Endpoints identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Appendix 1 – List of end points for the active substance and the representative formulation

Crop and/or situation	Member State or Country	Product name	F G or I	Pests or Group of pest controlled	Formulation		Application				Application rate per treatment			PHI (days)	Remarks (m)
					Type	Conc. of as	method kind	growth stage & season	Number (max)	interval between applications	Kg as/hL	Water L/ha	Kg as/ha		
(a)			(b)	(c)	(d-f)	(i)	(f-h)	(j)	(k)		(n)			(l)	(m)
Tomato (II) (LYPES)		PBP-01001-I	F: outdoor or field use	Root knot nematode (<i>Meloidogyne spp.</i>)	Water dispersible granule	2 x 10 ⁹ CFU/g (minimum)	pre-planting soil treatment of a) whole area or b) planting line: all sprayer types or pouring by hand, followed by soil incorporation transplant drench: spray or pour	Pre-planting 7-10 days before planting, or at planting, and throughout the season	4	6-8 weeks	0.01 to 0.125	200-1000 L/ha	a) 0.25 or b) band treatment: 0.063	No PHI required	Pre-planting: Spray whole area or in a band along the planting line and incorporate to a 10-15 cm depth in moist soil using a rotary hoe. Do not allow soil to dry out before application Transplant drench (band treatments): add product to water tank of planting machine calibrated to supply 200 mL/plant or pour 200 mL product suspension by hand around the base of each plant after planting, followed by watering
Tobacco (I) (NIOTA)	Austria, France, Germany, Greece, Italy, Portugal, Spain, UK	PBP-01001-I	G: glasshouse application	Root knot nematode (<i>Meloidogyne spp.</i>)	Water dispersible granule	2 x 10 ⁹ CFU/g (minimum)	immersion or spraying of speed-ling trays with subsequent watering	Seedlings, 1 day before transplanting	1	n.a.	0.125 to 0.313	200-500 L/ha	0.625	no PHI required	Treatment of seedlings always followed by either pre-planting soil treatment or transplant drench (see Tobacco (II))

‡ Endpoints identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Appendix 1 – List of end points for the active substance and the representative formulation

Crop and/or situation	Member State or Country	Product name	F G or I	Pests or Group of pest controlled	Formulation		Application				Application rate per treatment			PHI (days)	Remarks (m)
					Type	Conc. of as	method kind	growth stage & season	Number (max)	interval between applications	Kg as/hL	Water L/ha	Kg as/ha		
(a)			(b)	(c)	(d-f)	(i)	(f-h)	(j)	(k)		(n)			(l)	(m)
Tobacco (II) (NIOTA)	Austria, France, Germany, Greece, Italy, Portugal, Spain, UK	PBP-01001-I	F: outdoor or field use	Root knot nematode (<i>Meloidogyne spp.</i>)	Water dispersible granule	2 x 10 ⁹ CFU/g (minimum)	pre-planting soil treatment of a) whole area or b) band: all sprayer types or pouring by hand, followed by soil incorporation transplant drench: spray or pour	Pre-planting or at planting and throughout the growing season	4	6-8 weeks	0.01 to 0.125	200-1000 L/ha	a) 0.25 or b) band treatment: 0.063	no PHI required	Pre-planting: Spray whole area or in a band along the planting line and incorporate to a 10-15 cm depth in moist soil using a rotary hoe. Do not allow soil to dry out before application Transplant drench (band treatments): add product to water tank of planting machine calibrated to supply 200 mL/plant or pour 200 mL product suspension by hand around the base of each plant after planting, followed by watering
Lettuce (I) (LACIC)	N/S,	PBP-01001-I	G: glasshouse application	Root knot nematode (<i>Meloidogyne spp.</i>)	Water dispersible granule	2 x 10 ⁹ CFU/g (minimum)	a) immersion or spraying of speed-ling trays with subsequent watering b) see lettuce II	a) Seedlings, 1 day before transplanting; b) if glasshouse culture: throughout the season	a) 1 b) 4	a) not applicable b) 6-8 weeks	0.125 to 0.313	200-500 L/ha	0.625	no PHI required	Treatment of seedlings always followed by either pre-planting soil treatment or transplant drench (see Lettuce (II))

‡ Endpoints identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Appendix 1 – List of end points for the active substance and the representative formulation

Crop and/or situation	Member State or Country	Product name	F G or I	Pests or Group of pest controlled	Formulation		Application				Application rate per treatment			PHI (days)	Remarks (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	Number (max) (k)	interval between applications	Kg as/hL (n)	Water L/ha	Kg as/ha		
Lettuce (II) (LACIC)	N/S	PBP-01001-I	F: outdoor or field use	Root knot nematode (<i>Meloidogyne spp.</i>)	Water disper-sible granule	2 x 10 ⁹ CFU/g (minimum)	pre-planting soil treatment of a) whole area or b) band: all sprayer types or pouring by hand, followed by soil incorporation transplant drench: spray or pour	Pre-planting or at planting and throughout the season	4	6-8 weeks	0.01 to 0.125	200-1000 L/ha	a) 0.25 or b) band treatment: 0.063	no PHI required	Pre-planting: Spray whole area or in a band along the planting line and incorporate to a 10-15 cm depth in moist soil using a rotary hoe. Do not allow soil to dry out before application Transplant drench (band treatments): add product to water tank of planting machine calibrated to supply 200 mL/plant or pour 200 mL product suspension by hand around the base of each plant after planting, followed by watering
Celery (I) (APUGV)	N/S	PBP-01001-I	G: glasshouse application	Root knot nematode (<i>Meloidogyne spp.</i>)	Water dispersible granule	2 x 10 ⁹ CFU/g (minimum)	a) immersion or spraying of speed-ling trays with subsequent watering b) see celery II	a) Seedlings, 1 day before transplanting; b) if glasshouse culture: throughout the season	a) 1 b) 4	a) not applicable b) 6-8 weeks	0.125 to 0.313	200-500 L/ha	0.625	no PHI required	Treatment of seedlings always followed by either pre-planting soil treatment or transplant drench (see Celery (II))

‡ Endpoints identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Appendix 1 – List of end points for the active substance and the representative formulation

Crop and/or situation	Member State or Country	Product name	F G or I	Pests or Group of pest controlled	Formulation		Application				Application rate per treatment			PHI (days)	Remarks (m)
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	Number (max) (k)	interval between applications	Kg as/hL (n)	Water L/ha	Kg as/ha		
Celery (II) (APUGV)	N/S	PBP-01001-I	F: outdoor or field use	Root knot nematode (<i>Meloidogyne spp.</i>)	Water disper-sible granule	2 x 10 ⁹ CFU/g (minimum)	pre-planting soil treatment of a) whole area or b) band: all sprayer types or pouring by hand, followed by soil incorporation transplant drench: spray or pour	Pre-planting or at planting and throughout the season	4	6-8 weeks	0.01 to 0.125	200-1000 L/ha	a) 0.25 or b) band treatment: 0.063	no PHI required	Pre-planting: Spray whole area or in a band along the planting line and incorporate to a 10-15 cm depth in moist soil using a rotary hoe. Do not allow soil to dry out before application Transplant drench (band treatments): add product to water tank of planting machine calibrated to supply 200 mL/plant or pour 200 mL product suspension by hand around the base of each plant after planting, followed by watering
Cucurbit (I) (CUUPE)	N/S	PBP-01001-I	G: glasshouse application	Root knot nematode (<i>Meloidogyne spp.</i>)	Water dispersible granule	2 x 10 ⁹ CFU/g (minimum)	immersion or spraying of seedling trays with subsequent watering	Seedlings, 1 day before transplanting	1	n.a.	0.125 to 0.313	200-500 L/ha	0.625	no PHI required	Treatment of seedlings always followed by either pre-planting soil treatment or transplant drench (see Cucurbit (II))

‡ Endpoints identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Appendix 1 – List of end points for the active substance and the representative formulation

Crop and/or situation	Member State or Country	Product name	F G or I	Pests or Group of pest controlled	Formulation		Application				Application rate per treatment			PHI (days)	Remarks (m)
					Type	Conc. of as	method kind	growth stage & season	Number (max)	interval between applications	Kg as/hL	Water L/ha	Kg as/ha		
(a)			(b)	(c)	(d-f)	(i)	(f-h)	(j)	(k)		(n)			(l)	(m)
Cucurbit (II) (CUUPE)	N/S	PBP-01001-I	F: outdoor or field use	Root knot nematode (<i>Meloidogyne spp.</i>)	Water disper-sible granule	2 x 10 ⁹ CFU/g (minimum)	pre-planting soil treatment of a) whole area or b) band: all sprayer types or pouring by hand, followed by soil incorporation transplant drench: spray or pour	Pre-planting or at planting and throughout the season	4	6-8 weeks	0.01 to 0.065	200-1000 L/ha	a) 0.13 or b) band treatment: 0.063	no PHI required	Pre-planting: Spray whole area or in a band along the planting line and incorporate to a 10-15 cm depth in moist soil using a rotary hoe. Do not allow soil to dry out before application Transplant drench (band treatments): add product to water tank of planting machine calibrated to supply 200 mL/plant or pour 200 mL product suspension by hand around the base of each plant after planting, followed by watering

‡ Endpoints identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Appendix 1 – List of end points for the active substance and the representative formulation

Crop and/or situation	Member State or Country	Product name	F G or I	Pests or Group of pest controlled	Formulation		Application				Application rate per treatment			PHI (days)	Remarks (m)
					Type	Conc. of as	method kind	growth stage & season	Number (max)	interval between applications	Kg as/hL	Water L/ha	Kg as/ha		
(a)			(b)	(c)	(d-f)	(i)	(f-h)	(j)	(k)		(n)			(l)	(m)
Carrots (DAUCA)	N/S	PBP-01001-I	G: glasshouse application or F: outdoor or field use	Root knot nematode (<i>Meloidogyne spp.</i>)	Water dispersible granule	2 x 10 ⁹ CFU/g (minimum)	pre-planting soil treatment of a) whole area or b) band: all sprayer types or pouring by hand, followed by soil incorporation transplant drench: spray or pour	Pre-planting or at sowing and throughout the growing season	4	6-8 weeks	0.01 to 0.125	200-1000 L/ha	a) 0.25 or b) band treatment: 0.063	no PHI required	Pre-planting: Spray whole area or in a band along the sowing line and incorporate to a 10-15 cm depth in moist soil using a rotary hoe. Do not allow soil to dry out before application Transplant drench (band treatments): add product to water tank of planting machine calibrated to supply 200 mL/plant or pour 200 mL product suspension by hand around the base of each plant, followed by watering
Ornamentals (I) (NNNZZ)	N/S	PBP-01001-I	G: glasshouse application	Root knot nematode (<i>Meloidogyne spp.</i>)	Water dispersible granule	2 x 10 ⁹ CFU/g (minimum)	a) immersion or spraying of speed-ling trays with subsequent watering b) see ornamentals II	Seedlings, 1 day before transplanting	1	n.a.	0.125 to 0.313	200-500 L/ha	0.625	no PHI required	Treatment of seedlings always followed by either pre-planting soil treatment or transplant drench (see ornamentals II)

‡ Endpoints identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Appendix 1 – List of end points for the active substance and the representative formulation

Crop and/or situation	Member State or Country	Product name	F G or I	Pests or Group of pest controlled	Formulation		Application				Application rate per treatment			PHI (days)	Remarks (m)
					Type	Conc. of as	method kind	growth stage & season	Number (max)	interval between applications	Kg as/hL	Water L/ha	Kg as/ha		
(a)			(b)	(c)	(d-f)	(i)	(f-h)	(j)	(k)		(n)			(l)	(m)
Grapevine (VITVI)	Austria, France, Germany, Greece, Italy, Portugal, Spain	PBP-01001-I	F: outdoor or field use	Root knot nematode (<i>Meloidogyne spp.</i>)	Water disper-sible granule	2 x 10 ⁹ CFU/g (minimum)	Soil drench		At bud set and 3 weeks before harvest	Dependent on development stage, roughly a few months	0.025 to 0.125	200-1000 L/ha	0.250	3 weeks	Apply 300 mL around the vine in a 50 cm ark. Use 2 g/plant (100 g in 15L water for 50 plants)

(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) 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

‡ Endpoints identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

APPENDIX 2 – ABBREVIATIONS USED IN THE LIST OF ENDPOINTS

ADI	acceptable daily intake
AOEL	acceptable operator exposure level
ARfD	acute reference dose
a.s.	active substance
bw	body weight
CA	Chemical Abstract
CAS	Chemical Abstract Service
CIPAC	Collaborative International Pesticide Analytical Council Limited
CFU	colony forming units
d	day
DAR	draft assessment report
DM	dry matter
DT ₅₀	period required for 50 percent dissipation (define method of estimation)
DT ₉₀	period required for 90 percent dissipation (define method of estimation)
dw	dry weight
ϵ	decadic molar extinction coefficient
EC ₅₀	effective concentration
EEC	European Economic Community
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINKS	European List of New Chemical Substances
EMDI	estimated maximum daily intake
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
GAP	good agricultural practice
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GS	growth stage
h	hour(s)
ha	hectare
hL	hectolitre
HPLC	high pressure liquid chromatography or high performance liquid chromatography
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
ip	intraperitoneal
iv	intravenous
K _{oc}	organic carbon adsorption coefficient
L	litre

LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LC ₅₀	lethal concentration, median
LD ₅₀	lethal dose, median; dosis letalis media
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOQ	limit of quantification (determination)
µg	microgram
mN	milli-Newton
MRL	maximum residue limit or level
MS	mass spectrometry
NESTI	national estimated short term intake
NIR	near-infrared-(spectroscopy)
nm	nanometer
NOAEL	no observed adverse effect level
NOEL	no observed effect level
PEC	predicted environmental concentration
PEC _A	predicted environmental concentration in air
PEC _S	predicted environmental concentration in soil
PEC _{SW}	predicted environmental concentration in surface water
PEC _{GW}	predicted environmental concentration in ground water
PHI	pre-harvest interval
pK _a	negative logarithm (to the base 10) of the dissociation constant
PPE	personal protective equipment
ppm	parts per million (10 ⁻⁶)
ppp	plant protection product
r ²	coefficient of determination
RPE	respiratory protective equipment
STMR	supervised trials median residue
TER	toxicity exposure ratio
TMDI	theoretical maximum daily intake
UV	ultraviolet
WHO	World Health Organisation
WG	water dispersible granule
yr	year