Purpureocillium lilacinum 251

Microbial pest control agent against plant pathogenic nematodes

Dossier according to OECD guidance for industry data submissions for microbial pest control products and their microbial pest control agents – August 2006

Summary documentation, Tier II

Annex IIM, Section 4

Point IIM 6: Metabolism and residue studies

Date: January 2016

Applicant

Bayer CropScience AG

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Introduction

The company Bayer CropScience AG is submitting a dossier for the re-approval of the microorganism *Purpureocillium lilacinum* 251 as an active substance under regulation (EC) 1107/2009.

The Microbial Pest Control Agent *Paecilomyces lilacinus* strain 251 was included into Annex I of Directive 91/414/EEC on 01/08/2008 (Commission Directive 2008/44/EC) and then approved according to the Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011, implementing Regulation (EC) No 1107/2009 of the European Parliament [1)]. *P. lilacinus* strain 251 was notified and defended by Prophyta GmbH. The active ingredient has been evaluated in Belgium according to Uniform Principles. The representative formulated product for the initial evaluation was the experimental formulation PBP-01001-I, containing 2 × 10^9 spores/g. PBP-01001-I, is comparable to the commercial formulation BioAct WG, containing 1 × 10^{10} spores/g, and the only changes between both formulations were slight adjustments of the content of two co-formulants, without any impact on the performance or physical properties of the formulated product. The recommended rate in terms of spores per hectare remained exactly the same. The data on PBP-01001-I can therefore be extrapolated to the formulated product BioAct WG, a wettable granule formulation (WG), the representative formulation in the present application for the renewal.

In 2013 Bayer CropScience AG acquired Prophyta Biologischer Pflanzenschutz GmbH, now named Bayer CropScience Biologics GmbH. Bayer CropScience AG is the notifier for the renewal of *P. lilacinus* strain 251 in the procedure of AIR 3.

The microorganism has been previously classified as *Paecilomyces lilacinus* until 18S rRNA gene, internal transcribed spacer (ITS) and partial translation elongation factor 1-α (TEF) sequencing revealed that *P. lilacinus* is not related to *Paecilomyces.* The new genus name *Purpureocillium* has been proposed for *P. lilacinus* and the new species name was assigned: *Purpureocillium lilacinum.* Therefore the strain is now identified as *Purpureocillium lilacinum*. In this dossier *Paecilomyces lilacinus* 251 and *Purpureocillium lilacinum* 251 are used as synonyms: *Paecilomyces lilacinus = Purpureocillium lilacinum*.

It has to be taken into account that data on *Paecilomyces lilacinus* from the open literature stated before 2011 may not necessarily provide reliable information due to insufficient classification methods used in these studies, especially, if the strain identification is not provided and/or identification methods used were based solely on morphological characteristics. However, they may provide relevant information transferrable to *Purpureocillium lilacinum*.

Purpureocillium lilacinum 251 is a ubiquitous, saprobic filamentous fungus commonly isolated from soil, decaying vegetation, insects and nematodes. Strains of *P. lilacinum* are used in plant protection products due to their nematicide activity. The mode of action against plant pathogenic nematodes of *P. lilacinum* strain 251 is principally based upon parasitism of nematode eggs as well as the vermiform stages of the nematodes, leading eventually to their death. With regard to the results of toxicity and ecotoxicity studies of the active substance *P. lilacinum* strain 251, it can be concluded that *P. lilacinum* strain 251 shows no risk for exposed humans, animals and environment.

P. lilacinum 251 is intended to be used in plant protection products to control plant pathogenic nematodes. The representative use presented in this dossier comprises applications of the formulation BioAct WG in protected and non-protected vegetable crops to control root know nematode, *Meloidogyne* spp.

Here we submit data that were previously evaluated by RMS Belgium as well as new data and information based on literature searches and studies.

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[1] OJEU L94/13 Commission Directive 2008/44/EC of 4 April 2008 amending Council Directive 91/414/EEC to include benthiavalicarb, boscalid, carvone, fluoxastrobin, *Paecilomyces lilacinus* and prothioconazole as active substances

IIM 6 Metabolism and residues studies on the microbial pest control agent

New Data 2015

A literature search was conducted to identify scientific peer-reviewed open literature on the active substance *Purpureocillium lilacinum* 251 which may affect the assessment on human health, animal health and/or the environment, with the special consideration of residues in or on treated products ([redacted], 2015, M-542619-01-1). Therefore references relevant for residues of *Paecilomyces lilacinus*, *Penicillium lilacinum* or *Purpureocillium lilacinum* analysed on products, food and feed or plants were considered. The search performed by use of the STN database and comprised searches in Agricola, BIOSIS, MEDLINE, CAB Abstracts, SCISEARCH and Chemmical Abstracts, DRUGU, EMBASE, Esbiobase, IPA, Pascal, POSciTech, Toxcenter and FSTA databases. Keywords considered in the search were *Paecilomyces lilacinus, Penicillium lilacinum or Purpureocillium lilacinum,* soil, earth, ground, land, terrestrial, water, aquatic, air, aerial, sky, skies, heaven, atmosphere, residue, crop, consumer, food, feed, risk, metabolic, as well as related terms. In total 174 references were evaluated basing on their title and abstracts, whether they contain relevant information. Nine references were evaluated in detail, basing on their full texts. Basing on the full text evaluation, only three reports were identified as relevant for this section.

Cited references (abstracts):

Report: KIIM 6/01 – [redacted], I. (2015), Literature review on active substance Purpureocillium lilacinum strain 251 and metabolites: Residues in or on treated products, food and feed
Not published.

Abstract: The review was made in order to identify scientific peer-reviewed open literature on the active substance *Purpureocillium lilacinum* 251 which may affect the assessment on human health, animal health and/or the environment, with the special consideration of residues in or on treated products.
The literature research was conducted on the STN database and comprised searches in Agricola, BIOSIS, MEDLINE, CAB Abstracts, SCISEARCH and Chemmical Abstracts, DRUGU, EMBASE, Esbiobase, IPA, Pascal, POSciTech, Toxcenter and FSTA databases. Search strategy aimed to find all recent (from 2005 onwards) references that are of relevance.
The criteria for relevance used are summarised below:

- Property investigated was relevant for data requirements of Regulation (EC) 1107/2009
- Subject relevant for residues of *Paecilomyces lilacinus*, *Penicillium lilacinum* or *Purpureocillium lilacinum* analysed on products, food and feed
- Subject relevant for residues of *Paecilomyces lilacinus*, *Penicillium lilacinum* or *Purpureocillium lilacinum* occurrence on plants
- Test species/system relevant to the residues on products, food and feed
- Application on crops and consumer risk
- Relevant crop / trial location

In total, 174 records were evaluated basing on title and abstracts. Of these, 9 reports were identified for the full text evaluation, 3 reports were identified as relevant and supportive for Section 4.

IIM 6.1 Rationale for waiver of residue data based on information showing that MPCA is not hazardous to mammals, i.e. lack of potential for a known mammalian toxin and negative result from the acute oral toxicity test

EU-Dossier: Doc M-IIB, Point 6

The applicant applies for a waiver for performing residue trials with PBP-01001-I, based on the following considerations.

The nature of the product and its active substance are not adequately described and assessed by applying the term 'residue', or by quantifying 'residues', since this definition commonly implies a toxicological concern of the residual deposit of a plant protection product, which is not attributable to PBP-01001-I and *P. lilacinum* 251, for following reasons:

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- *P. lilacinus* is a wide-spread, ubiquitous and common soil-born fungus, living mainly on decay of organic matter. *P. lilacinum* 251 is of natural origin, and is not genetically modified. Despite natural long-term exposure of the human population in the Philippines and the exposed personnel of the applicant there is no evidence for any infectivity, toxicity and pathogenicity of this strain.
- This strain is not an opportunistic human pathogen. Lack of infectivity, toxicity and pathogenicity is confirmed by results of acute toxicological studies, showing 100% clearance of spores from all tissues and body fluids, and no treatment related adverse effects in test animals signs at a single oral dose of 2000 mg/kg b.w. upon different routes of exposure (see Annex II, Doc IIM, Section 3).
- Infectivity of *P. lilacinum* 251 is ruled out by the inability of this strain to grow at temperatures of the human body (>36 °C no growth was recorded, see Annex II, Doc IIM, Section 1, Point IIM 2.8; EU-Dossier: Doc. M-IIB, Section 1, Point 2.5).
- Further, *P. lilacinum* 251 does not act via toxins in nematode control, and does not produce the well-known paecilotoxin, or secondary metabolites of toxicological concern, as evidenced by its extremely low acute toxicity (see Annex II, Doc IIM, Section 1, Point IIM 2.3.2 and 2.6, and Section 3; EU-Dossier: Doc. M-IIB, Section 1, Point 2.2.2 and 2.8, and Section 3, respectively).
- The production process for PBP-01001-I ensures that no secondary metabolites but only purified spores of the biocontrol strain are found in the end-use product.

In summary, the lack of infectivity and a treatment related effect upon exposure to *P. lilacinum* 251 indicate that residual deposits of this fungus will not impose a health risk for consumers. In this case, there is no need and no scientifically justified value to define an Acceptable Daily Intake (ADI). Therefore, calculation of the potential exposure of consumers in terms of the Theoretical Maximum Daily Intake (TMDI) and its relation to the ADI is not relevant, and conclusively a Maximum Residue Level (MRL) need not be proposed.

Within the pending registration process for the preparation Bioact® WG, in its composition similar to PBP-01001-I, Australia has granted a Certificate of an exemption for an active constituent (National Registration Authority, Australia 1998, M-492010-01-1).

EU-Dossier: Doc M-IIB, Point 2.2.2

Risks for uptake of the micro-organism:

Considering the intended field of use for the water dispersible granule the only potential way of entry of the micro-organism into humans is accidental ingestion of material upon mixing or applying the preparation, or handling the treated soil. This can easily be avoided by cautious and thorough handling according to the instructions for use.

Upon access to the resources of the plant this saprophytic fungus would act like a plant pathogen, but the opposite effect of improving plant growth and yield has been confirmed by field experiences worldwide. Therefore it is clear that *P. lilacinum* is not able to enter plant tissue and cannot be translocated in plants.

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New Data 2015

A literature search was conducted to identify scientific peer-reviewed open literature on the active substance *Purpureocillium lilacinum* 251 regarding residues in or on treated products (please refer to the literature review report submitted in Point IIIM 6).

[redacted]-[redacted] et al. (2011, M-534512-01-1) compared clinical isolates with strains isolated from soil, insects and nematodes baising on sequence analysis of 18S rRNA, ITS and TEF sequences. The authors showed that *P. lilacinus* is not related to the type strain of *Paecilomyces vaiotii*, which is thermophilic and often pathogenic. As a consequence a new designation as *Purpureocillium lilacinum* was made (please refer to Annex II, Doc IIM, Point IIM 1.3.1). However, the majority of

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studied *P. lilacinum* strains from soil, indoor environment, insect larvae, nematodes and decaying vegetation were located in ohne cluster together with strains originating from clinical specimens and hospital environments. Unfortunately, *P. lilacinum* 251 was not considered in this study. The authors discussed therefore, that it is could be possible that isolates of *P. lilacinum* used as biological control agents could form mycoses in humans or vertebrates as well. However, it has to be considered that sequence similarities of genes not related with virulence factors do not predict anything about the ability to cause mycosis. Regarding *P. lilacinum* 251, toxicity studies proved clearly that this strain is not toxic to humans or vertebrates (please refer to Annex III, Section 3). Moreover, it has been shown, that *P. lilacinum* 251 does not grow at temperatures of the human body:

[redacted] (2006, M-534354-01-1) studied the effect of temperature on the growth, germination, germ-tube extension and survival on *P. lilacinum* 251 on different growth media. It was shown, that *P. lilacinum* 251 did not grow at 36 °C. The germination at 36 °C was significantly lower in comparison to the germination at incubation temperatures of 24-33 °C, for the first 24 h. Germination at 36 °C was comparable to the other temperatures after 48 h. Thus, germination at 36 °C was delayed. However, no further germ-tube extension was found after exposure for 80-95 h.

In particular with regard to the statement of [redacted] et al. (2011, M-534512-01-1) on the possibility of mycoses it is very unlikely, that *P. lilacinum* 251 is hazardorous on mammals since it does not grow at temperatures above 36 °C. Moreover, safety of *P. lilacinum* 251 was clearly confirmed by toxicity studies.

<u>Cited references (abstracts):</u>

Report: KIIM 6.1/02 – [redacted], J.; [redacted], L.; [redacted], T.; [redacted], S.-B.; [redacted], A.M.; [redacted], N.L.; [redacted], R.A. (2011), *Purpureocillium*, a new genus for the medically important *Paecilomyces lilacinus*
Published report. FEMS Microbiology Letters, 321(2), 141-149
Abstract: *Paecilomyces lilacinus* was described more than a century ago and is a commonly occurring fungus in soil. However, in the last decade this fungus has been increasingly found as the causal agent of infections in man and other vertebrates. Most cases of disease are described from patients with compromised immune systems or intraocular lens implants. In this study, we compared clin. isolates with strains isolated from soil, insects and nematodes using 18S rRNA gene, internal transcribed spacer (ITS) and partial translation elongation factor 1-.alpha. (TEF) sequences. Our data show that *P. lilacinus* is not related to *Paecilomyces*, represented by the well-known thermophilic and often pathogenic *Paecilomyces variotii*. The new genus name *Purpureocillium* is proposed for *P. lilacinus* and the new combination *Purpureocillium lilacinum* is made here. Furthermore, the examd. *Purpureocillium lilacinum* isolated grouped in two clades based on ITS and partial TEF sequences. The ITS and TEF sequences of the *Purpureocillium lilacinum* isolates used for biocontrol of nematode pests are identical to those causing infections in (immunocompromised) humans. The use of high concns. of *Purpureocillium lilacinum* spores for biocontrol poses a health risk in immunocompromised humans and more research is needed to det. the pathogenicity factors of *Purpureocillium lilacinum* .

Report: KIIM 6.1/03 – [redacted], S. (2006), Effect of temperature on growth, germination, germ-tube extension and survival of *Paecilomyces lilacinus* strain 251.
Published report. Biocontrol science and technology, 16, 535-546
Abstract: Summary: *Paecilomyces lilacinus* and in particular the commercial strain 251 has been intensively tested for biological control of plant parasitic nematodes. Since this species has been mentioned in a number of reports concerning infection of humans, the human health risk for *Paecilomyces lilacinus* strain 251 was investigated. The effects of time, temperature and growth medium on radial colony growth and germination were determined. Additionally, exposure to 36 degrees C and its effect on germ-tube extension and on survival of conidia was evaluated. Radial growth was significantly affected by temperature, growth medium and their interaction. Optimum temperatures were between 24 and 30 degrees C, but no growth was found at 36 degrees C. Germination rate was significantly influenced by time, medium, temperature and their interactions. The optimum temperature range for germination was between 28 and 30 degrees C. Formulated conidia were capable of germinating at 36 degrees C. However, studies on germ-tube extension conducted at 36 degrees C showed a delay in development for 28-49 h and no further germ-tube

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extension was found after exposure for 80-95 h. Slopes of survival curves were significantly influenced by the type of conidia tested. In general, conidia did not survive exposure to 36 degrees C for 168 h. These experiments indicate the temperature conditions where the strain is likely to be active and provide supporting data for full environmental and health risk assessments of biocontrol fungi.

IIM 6.2 Rationale for waiver based on a substantiated estimation that MPCA is unlikely to occur on treated food/feed stuffs in concentrations considerably higher than under natural conditions

EU-Dossier: Doc M-IIB, Point 6

The applicant applies for a waiver for performing residue trials with PBP-01001-I, based on the following considerations.

- The inert ingredients of PBP-01001-I are natural organic compounds, used in human food, which present no health risk for consumers either.
- In most of the crops envisaged for use of PBP-01001-I no deposit is likely to occur, since soil drench applications rule out a direct contact between the applied product and the fruit. This applies to all crops with above ground harvest, such as grapes, tomato, and tobacco.
- After harvest any remaining fungal spores on potato, celery and carrots will be exposed to unfavourable conditions (e.g. dryness), and are not likely to germinate and grow on the harvested crop.
- Any potentially occurring residual deposits on these crops are not relevant as a human health concern in view of the toxicological profile of this strain and likely to be minimal in amount due to the low environmental concentration in soil predicted from maximum field use of PBP-01001-I (PEC_{soil} = 8 × 10^4 CFU per mL soil in top 5 cm, see calculation in Annex II, Section 6; EU-Dossier: Doc. M-IIB, Section 6, Point 9).

P. lilacinus is not able to enter plants and infest them, as evidenced from its beneficial effect on plant health and growth. As a saprophytic fungus it would use the resources of the plant host in case access was possible.

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New Data 2015

[redacted] & [redacted] (2006, M-534361-01-1) studied the persistence of *P. lilacinum* 251 in soil in dependency of the planted crop. Moreover, endophytic colonization of *P. lilacinum* 251 on plant roots of different crops 14-15 weeks after soil application was examined. It was shown, that P. *lilacinum* 251 was detected at low densities in barley roots (9.6-16.0 × 10^2 CFU/g root), and at very low densities in roots of banana, corn, wheat and cabbage (0.03-0.9 × 10^2 CFU/g root). In roots of beans, cucumber, eggplant, pepper and tomato, no *P. lilacinum* 251 was detected. However *P. lilacinum* 251 was shown to persist in rhizosphere and to maintain a high density of sufficient biocontrol, it was never found to proliferate or to establish in the soil and rhizosphere. Occurrence or proliferation on plants, food or feeding stuff is therefore very unlikely.

Cited references (abstracts):

Report: KIIM 6.2/01 – [redacted], C.I.; [redacted], S. (2006), Effect of plant species on persistence of *Paecilomyces lilacinus* strain 251 in soil and on root colonization by the fungus
Published report. Plant and Soil, 283(1-2), 25-31

Abstract: The effect of 12 plant species on the persistence of *Paecilomyces lilacinus* strain 251 in soil was investigated. After incorporating formulated conidia into non-sterile soil followed by transplanting different test plants, the population dynamic of the fungus was detd. over 100 days. At termination of the expt., the fungal population in the planted soil was compared to the d. of *P. lilacinus* in the rhizosphere and the percent increase or decrease was calcd. for each crop. In addn., the potential of *P. lilacinus* strain 251 to colonize roots endophytically was investigated. Comparison of the slopes describing the population dynamics of the fungus showed no significant differences between soil without plants and soil from the root zone of the majority of the test plants. Bean was the only plant species consistently exerting a neg. effect on the persistence of *P.*

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lilacinus strain 251 in the soil. For the first time, *P. lilacinus* strain 251 was isolated in significant nos. from healthy root tissue of barley plants.

IIM 6.3 Persistence and likelihood of multiplication in or on crops, feedingstuffs or foodstuffs

P. lilacinum 251 is to be applied directly on soil. Although it has been shown, that this strain persist in the rhizosphere of plants, it does not proliferate in soil. *P. lilacinum* may colonize roots of plants at very low concentrations after application. Since natural habitat of *P. lilacinum* is soil and not plants itself, colonization of plants or food- and feedingstuffs is very unlikely.

IIM 6.4 Further information required

Not relevant.

IIM 6.4.1 Non-viable residues

Production of secondary metabolites is a part of the mode of action of *P. lilacinum* against pathogenic nematodes. Therefore, mainly enzymes relevant for the perforation of the egg-shell are involved (please refer to Annex II, Doc IIM, Section 1 (Point IIM 2.3.2). Since *P. lilacinum* 251 is to be applied directly onto soil, no accumulation of these substances is expected on food or feed.

IIM 6.4.2 Viable residues

Due to the fact that the active ingredient is a viable micro-organism of ubiquitous occurrence and predominance in the soil-microflora the term residue is not applicable to this preparation.

IIM 6.5 Summary of residue behaviour and overall evaluation

P. lilacinum is an ubiquitous fungus, typically colonizing the plant rhizosphere. Therefore, its application on the soil just means a fluctuation of the natural population. *P. lilacinum* 251 has been shown to persist in the rhizosphere, which is essential for its activity against pathogenic nematodes. However, it is not known to proliferate in soil. Due to soil application, and since it does not colonize plants, *P. lilacinum* 251 is not expected to persist on plants, food- or feedingstuffs.

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References

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 6 /02	[redacted], I.	2015	Literature review on effects on human health of Purpureocillium lilacinum strain 251 and its metabolites: Residues in or on treated products, food and feed [redacted], Germany Bayer CropScience, Report No.: 1011296-CA-06-01, Edition Number: M-542619-01-1 Date: 2015-09-30 GLP/GEP: n.a., unpublished	Yes	Bayer CropScience
KIIM 6.1 /01	[redacted], P.	1998	Certificate of exemption for an active constituent National Registration Authority, Kingston, Australia Bayer CropScience, Report No.: M-492010-01-1, Edition Number: M-492010-01-1 Date: 1998-10-23 GLP/GEP: n.a., unpublished **...also filed: KIIM 6 /01**	Yes	Bayer CropScience
KIIM 6.1 /02	[redacted], J.; [redacted], K.; [redacted], S.; [redacted], A. M.; [redacted], N. L.; [redacted], R. A.	2011	Purpureocillium, a new genus for the medically important Paecilomyces lilacinus Year:2011, Report No.: M-534512-01-1, Edition Number: M-534512-01-1 Date: 2011-12-31 GLP/GEP: no, published **...also filed: KIIM 1.3.1 /07** **...also filed: KIIM 1.3.3 /05** **...also filed: KIIM 2.7.1 /18** **...also filed: KIIM 5.2.4 /03** **...also filed: KIIM 7.1 /01**	No	
KIIM 6.1 /03	[redacted], S.	2006	Effect of temperature on growth, germination, germ-tube extension and survival of Paecilomyces lilacinus strain 251 . Journal:Biocontrol science and technology (2006) , Year:2006, Report No.: M-534354-01-1, Edition Number: M-534354-01-1 Date: 2006-12-31 GLP/GEP: no, published	No	

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Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP/GEP status *(where relevant)*, published or not	Data protect. claimed	Owner
KIIM 6.1 /04	[redacted], C. I.; [redacted], S.	2006	Effect of plant species on persistence of Paecilomyces lilacinus strain 251 in soil and on root colonization by the fungus Year:2006, Report No.: M-534361-01-1, Edition Number: M-534361-01-1 Date: 2006-12-31, GLP/GEP: no, published **...also filed: KIIM 7.1.1 /16**	No	

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