BioAct WG, 1 X 10^{10} spores/gram (60 g/kg)

of *Purpureocillium lilacinum (syn. Paecilomyces lilacinus)* 251

Microbial pest control product against plant parasitic 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 IIIM, Section 6

Point IIIM 10: Effects of the Microbial Pest Control Product on non-target organisms

Date: January 2016

Applicant

Bayer CropScience AG

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OWNERSHIP STATEMENT

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.

Due to the product history studies were conducted with different formulations, as described for every study. The composition of these is confidential and described in detail in Document J, Point IIIM 1.7.2.2. These formulations and the new representative formulation are all comparable for their effects on non target organisms.

A summary of the GAP table is presented in **Table IIIM 10-1** below.

[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

Table IIIM 6-1 Summary of critical Good Agricultural Practice for BioAct WG

Crop and/ or situation (crop destination / purpose of crop)	F G or I	Pests or Group of pests controlled	Application			Application rate			PHI (days)
			Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications)	kg as/hL min max	water L/ha min max	kg as/ha min max	
Vegetables (tomatoes, cucurbits), soil decontamination against *Meloidogyne*	F/G	*Meloidogyne* spp.	1st application: Drip irrigation or Soil drench or Mechnical incorpo-ration or Dipping (of seedlings)	Pre-transplant	1	0.012 - 0.24 (4×10^{12} - 2×10^{13} spores/hL)	200 - 1,000	0.24 kg /ha (4×10^{13} spores/ha) 4 kg product/ ha	0
			Drip irrigation or Soil drench	At transplant	1	0.006 - 0.24 (2×10^{12} - 2×10^{13} spores/hL)	200 - 1,000	0.12 - 0.24 kg /ha (4×10^{13} spores/ha) 4 kg product/ ha	0
			Drip irrigation or Soil drench	Post-transplant	4 (4-6 weeks)	0.012 - 0.24 (4×10^{12} - 2×10^{13} spores/hL)	200 - 1,000	0.24 kg /ha (4×10^{13} spores/ha) 4 kg product/ ha	0

Please note:
Population development of microorganisms applied to soil is very difficult to predict, as it depends on the soil type, abiotic factors like humidity, temperature or availability of organic matter, and on the crop. Models used for chemical active ingredients are not appropriate for a prediction of microbial populations. As worst case, the maximum number of applications is considered for the risk assessment within the frame of the risk envelope approach. In addition, the risk assessment is calculated with single application rates.

IIIM 10 Rationale to waive additional testing, based on adequacy of information provided for MPCA, to permit an assessment of the impact of the MPCP on non-target organisms

IIIM 10.1 Effects on birds and mammals

Birds

An acute oral toxicity study on birds with *P. lilacinum* 251 WG specification 102000028478-02 was conducted and the results are summarized in the table below. Reasoning for providing this study in document MII section 8.1 is provided within the study report and also in the summary below.

Table 10.2-1 Ecotoxicological endpoints for birds

Study type	Test substance	Species	Endpoint		Reference
Acute oral	*P. lilacinum* 251 (1.21 x 10^{10} spores/g prod.)	Bobwhite quail (*Colinus virginianus*)	LD50	>2000 mg test item/kg bw >3.38 x 10^{10} spores/kg bw	[redacted], L; [redacted]-[redacted], M.; 2015; M-534859-01-1

Report:	KIIM 8.1; [redacted], J. and [redacted]-[redacted], M., 2015
Title:	Acute oral LIMIT-test toxicity of *Purpureocillium lilacinum* to Bobwhite quail (*Colinus virginianus*)
Report No:	M2902363-5
Document No:	M-534859-01-1
Guidelines:	OECD Guideline 223 US EPA OCSPP 850
GLP:	Yes

Materials and Methods:

The study was conducted during the period 26.05.2015 to 02.07.2015, by the facility Environmental Safety - Testing of Bayer CropScience AG, Development, [redacted], Germany.

Native spores of *Purpureocillium lilacinum* are extremely small, light-weight and electrostatically charged and thus cannot be handled in an open system. This makes weighing the spores into gelatine capsules in the testing facility technically impossible. Therefore for the purpose of application a vehicle was used consisting to 99.8% of easily digestibly carbohydrates, proteins and lipids. The test item (vehicle plus spores denominated as "*Purpureocillium lilacinum* 251 WG 6 (6 %)"; TOX20047-00; Supplier Batch ID: EBMX000282; Specification no.: 102000028478) contained 1.69 x 10^{10} total spores /g (1.21 x 10^{10} viable spores/g).

As test animals adult female and male Bobwhite quails (*Colinus virginianus*) were used. The birds were housed individually and acclimated to laboratory conditions for 21 days. After this period they were orally dosed one-time with gelatine capsules filled with the test item. The limit dose group of 5 quails was dosed with 2000 mg test item per kg body weight. Additionally, 10 control quails were administered with capsules containing the vehicle only at the same amount per unit body weight that was given to the birds dosed with the test item. After dosing, all quails were continuously observed for a time period of 14 days. All quails were identified by numbered and coloured leg bands. Each cage was identified by the study number, cage no. and test concentration. The individual test item amounts were calculated based on the body weights of the quails, one day prior to dosing (day -1). The quails were starved for 16 hours prior to dosing. Afterwards they had free access to feed. During the whole test period, the control quails were held under the same conditions as the dosed quails. The test units were maintained at a mean temperature of 21.8 °C, a mean relative humidity of 52.3 % and a 8 hour light/16 hour dark cycle. Mortality and signs of intoxication were observed continuously during the first two hours and hourly on the day of dosing and at least once daily throughout the 14 days observation period. Body weights were recorded at day -1 (one day before dosing), on study days 3 and 7, and on day 14 (termination of the study). Feed consumption was measured daily until day 3 after dosing and afterwards for the time periods of days 3 – 7 and 7 – 14. On study days 1, 2, 3, 7 and 14 all remaining feed was replaced by fresh feed after cleaning of the feeding container. At the end of the study all surviving quails were sacrificed by CO2 asphyxiation and afterwards gross necropsies were carried out on all the sacrificed quails.

Findings:
No mortality was observed. During the whole experimental phase (0-14 days), all quails showed a good and healthy condition. No symptoms were visible, only one bird had signs of transient (1h) diarrhea after 4 hours after application of the test item. Throughout the study conduct feed consumption was similar between dosed and control birds. There was no considerable difference in body weights during the course of the study between dosed and control quails. Neither in the quails administered with the spores nor in the control quails signs of intoxication were found.

Conclusions:
The acute LD50 for Bobwhite quail, orally dosed with Purpureocillium lilacinum, was >2000 mg test item/kg bw equivalent to > 3.38 x 10^{10} total spores/kg bw.
The non-lethal dose (NLD) accounted for ≥ 2000 mg test item /kg bw equivalent to > 3.38 x 10^{10} total spores/kg bw.

Exposure

Following Good Agricultural Practice (see Doc. D-1) *P. lilacinum* 251 will be applied directly onto the soil surface by soil irrigation (drip or drench) or by tray drench/dipping following watering to assure full incorporation into the soil. Therefore, the risk for exposure of birds to *P. lilacinum* 251 is not expected. In addition, this strain of *P. lilacinum* is not pathogenic or infectious for vertebrates, as indicated by the submitted toxicological studies (see Annex II, Doc IIM, Section 3 and below). Moreover, it has to be considered that birds are endothermic animals with an average body temperature of 37.7-43.5°C. The optimum growth temperature profiles of *P. lilacinum* 251 show that it did not grow above 33°C. Mycosis in birds is therefore not expected. In conclusion studies on toxicity, pathogenicity and infectivity towards non-exposed vertebrates, such as birds, are not required.

Mammals

An acute oral toxicity study on rats with the active substance *P. lilacinum* 251 was conducted (Bolt, 1997; please refer to Annex II, Doc IIM, Section 3, Point IIM 5.3.3 for the study summary). The test substance was administered as a 10% w/w homogenous suspension in water at a dose of 20 mL/kg (equivalent to 2000 mg/kg *P. lilacinum* 251). No abnormal clinical signs were observed. Therefore, the acute oral LD50 of *P. lilacinum* 251 was found to be greater than 2000 mg/kg in rat.

Table 10.2-2 Ecotoxicological endpoints for mammals

Test item	EU agreed endpoints	Test species	Reference
Mammals			
P. lilacinum 251	LD_{50} oral > 2000 mg/kg bw	Sprague Dawley rat	[redacted],; 1997; M-476459-02 **(please refer also to Annex II, Doc IIM, Section 3, Point IIM 5.3.2)**

Exposure
Following Good Agricultural Practice (see Doc. D-1) *P. lilacinum* 251 will be applied directly onto the soil surface by soil irrigation (drip or drench) or by tray drench/dipping following watering to assure full incorporation into the soil. Therefore, the risk for exposure of mammals to *P. lilacinum* 251 is not expected. In addition, this strain of *P. lilacinum* is not pathogenic or infectious for vertebrates, as indicated by the submitted toxicological studies (see Annex II, Doc IIM, Section 3 and below).

IIIM 10.2 Effects on aquatic organisms

No new studies are submitted assessing the effect of BioAct WG on aquatic organisms. Instead already evaluated studies on the formulated product PBP-01001-I, containing *P. lilacinum* 251 spores (nominal 2.7 × 10^9 spores/g), are considered for the evaluation of risk of BioAct WG (please

see Table 10.2-1). Moreover, no relevant literature was found to inform the risk assessment of *P. lilacinum* 251 to aquatic organisms. Please refer to Annex II, Doc IIM, Section 6 for the results from the latest literature search.

Table 10.2-1 Ecotoxicological endpoints for aquatic organisms

Test item	EU agreed endpoints	Test species	Reference
Fish			
PBP-01001-I (4.53×10^9 spores/g)	**LC_{50} (96 h)> 100 mg formulation/L** **NOEC (96 h) = 100 mg formulation/L** **(= 4.5×10^8 CFU/L)**	***Onchorhynchus mykiss***	**[redacted], 2001a** **(please refer also to Annex II, Doc IIM, Section 6, Point IIM 8.2)** M-467660-01-1
Aquatic invertebrates			
PBP-01001-I (4.53×10^9 spores/g)	**EC_{50} (48 h) > 100 mg formulation/L** **NOEC (48 h) = 100 mg formulation/L** **(= 4.5×10^8 CFU/L)**	***Daphnia magna***	**[redacted], 2001b** **(please refer also to Annex II, Doc IIM, Section 6, Point IIM 8.3)** M-467656-01-1
Algae			
PBP-01001-I (4.53×10^9 spores/g)	**E_bC_{50} (72 h) = 31.77 mg formulation/L** **(3.2×10^8 CFU/L)** **E_rC_{50} (72 h) = 256.43 mg formulation/L** **($= 1.16 \times 10^9$ CFU/L)** **NOEC (72 h) = 28.9 mg formulation/L** **($= 1.61 \times 10^8$ CFU/L)**	***Desmodesmus subspicatus***	**[redacted], 2011** **(please refer also to Annex II, Doc IIM, Section 6, Point IIM 8.4)** M-467680-01-1

Fish

In 2001 an acute toxicity study on rainbow trout was conducted with PBP-01001-I, lilac tan solid granules comprising *P. lilacinum* 251 formulated as WG. This combined range finding and limit test was complying with OECD guideline 203, respectively EEC directive C.1 ([redacted], 200,1M-467660-01-1). A range of 6 concentrations up to the limit concentration of 100 mg/L was tested, employing the suggested 1g animals/concentration to achieve a 99.9% probability level. Test concentrations were 0.001, 0.01, 0.1, 1.0, 10 and 100 mg/L. Mortality and clinical signs were assessed at 3, 6, 24, 48, 72 and 96 h after test start to determine the median effective concentration (EC_{50}). Temperature, pH value and dissolved oxygen concentration were monitored daily, at 24h intervals throughout the study performance.

All criteria for validity were met in this test. Body size and body weight were not adversely affected by exposure to the test substance. All fish survived the 96 h exposure to the test substance up to 100 mg/L, and no fish exhibited signs of toxicity or behavioural changes during the course of this study. Therefore, the NOEC (No Observable Effect Concentration) for PBP-01001-I was 100 mg/L, and the 96h EC_{50} was estimated to exceed the maximum concentration tested, i.e. 100 mg/L of test substance, with a probability of 99.9%. The NOEC of 100 mg a.s./L is equivalent to nominal 2×10^8 CFU of *P. lilacinum* 251 and was 4.5×10^8/L, based on the analytical certificate of the tested batch for the formulated product PBP-01001-I.

Since the formulated Product BioAct WG is comparable to PBP-10001-I (please refer to the introduction), the TER values were calculated in consideration of the formulation BioAct WG and the spore number of the active substance *P. lilacinum* 251.

Aquatic invertebrates

Toxic effects of PBP-01001-I on *Daphnia magna* STRAUS were assessed using the 48 h acute immobilization test according to OECD guideline 202, and EEC directive C.2 ([redacted], 2001, M-467656-01-1). In a combined range finding and limit test a range of 6 concentrations up to the limit concentration of 100 mg/L was tested under conditions of a limit test design. Under static conditions 5 freshly hatched daphnids per treatment group, in 4 replicates, were exposed to PBP-01001 at 0.001, 0.01, 0.1, 1.0, 10 and 100 mg/L (equivalent to nominal 2×10^3 to 2×10^8 CFU/L). The negative control consisted of the test medium water, and a positive control comprised two concentrations of the toxic reference potassium dichromate (0.9 or 1.9 mg/L), respectively. Immobilized daphnids were enumerated at 24 and 48 h following study start to determine the median effective concentration (EC_{50}). Temperature, pH value and dissolved oxygen concentration were monitored initially and at 24 and 48 h of the study performance.

No mortalities or effects were observed in the test substance groups up to a concentration of 100 mg/L, which therefore represents the NOEC (no Observable Effect Concentration). 100 mg a.s./L are equivalent to nominal 2×10^8 CFU/L of *P. lilacinum* 251 and actual 4.5×10^7 CFU/L, based on the analytical certificate of the employed batch for the formulation PBP-01001-I. The EC_{50} was estimated to exceed the tested maximum concentration of 100 mg/L with a probability of 99.9%. The results for the positive control potassium dichromate confirmed the validity of this test.

Since the formulated Product BioAct WG is comparable to PBP-10001-I (please refer to the introduction), the TER values were calculated in consideration of the formulation BioAct WG and the spore number of the active substance *P. lilacinum* 251.

Algae

[redacted] (2001, M-467680-01-1) tested toxic effects of PBP-01001-I (4.9×10^9 CFU/g) on the single cell green alga *Desmodesmus subspicatus*, employing OECD guideline 201 and EEC directive C.3. Exponentially growing cultures of the single cell green alga *Desmodesmus. subspicatus* (DDA1, strain no. SAG 86.81) were exposed to 6 concentrations of test substance under defined conditions in a synthetic growth medium for several generations. According to results of a range finding test the 6 concentrations were set as 0 to 141.99 mg/L, differing by a geometric factor of 1.7. The cell growth was measured 24, 48, and 72 hours after initiation of the test. The inhibition of growth was determined by calculating the E_rC, E_bC, LOEC, and NOEC (EC = effective concentration, indices **r** and **b** refer to "growth rate" and "biomass", respectively).
Significant inhibitory effects were observed from 49.13 to 141.99 mg/L after 72 h for the biomass integral and for the growth rate (calculated by Dunnett's-Test). During the test, at the three highest test substance concentrations, active growth of *P. lilacinum* (spore germination and mycelial growth) was observed.
The inhibitory effects reflect the nutrient competition between the test organism and the green alga *Desmodesmus subspicatus,* considering that fungal growth was observed at concentrations of ~50 mg/L test substance and higher, and that the employed conditions were supportive for growth of saprophytic micro-organisms. Under the conditions of this test, where conidia of *P: lilacinum* 251 were incubated at 23 to 24°C in nutrient solution on a rotary shaker, additionally offering organic substrate in the form of algal debris the observed fungal growth is a natural consequence. Under growth limiting conditions prevailing in natural waters the alga is more competitive and spores will be subject to sedimentation.

Toxicity exposure ratios

Following Good Agricultural Practice (see Doc. D-1) *P. lilacinum* 251 will be applied directly onto the soil surface by soil irrigation (drip or drench) or by tray drench/dipping following watering to assure full incorporation into the soil. Therefore, spray drift and run-off can be excluded and thus, exposure to aquatic organisms.

IIIM 10.3 Effects on bees

Following Good Agricultural Practice (see Doc. D-1) *P. lilacinum* 251 will be applied directly onto the soil surface by soil irrigation (drip or drench) or by tray drench/dipping, with subsequent incorporation into the soil by watering. Therefore, exposure of honeybees to the active substance or the formulated product is not expected and studies on honey bees are not required. Moreover, no adverse effects of *P. lilacinum* on bees were reported in the peer reviewed open literature [redacted]; 2015; M-542804-01 . Effects on bees due to an application of BioAct WG are therefore unlikely.

IIIM 10.4 Effects on non-target arthropods other than bees

Effects on foliage dwelling non-target arthropods

No new studies are submitted assessing the effect of formulated product BioAct WG on foliage dwelling arthropods other than bees. Previously submitted studies, evaluated for the risk assessment of the *P. lilacinum* 251 formulation PBP-01001-I and BioAct WG on foliage dwelling arthropods other than bees, are presented below.
Moreover, *P. lilacinum* was discussed at the PRAPeR experts' meeting on microorganisms in January 2007. According to the EFSA Scientific Report (2007)[2] it was 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". Data on Collembola as representative of soil dwelling arthropods are presented below.

Further data are available from [redacted], 2000; M-490114-01), who tested *P. lilacinum* strain 251 towards several arthropod species within the scope of her thesis.

Table 10.4-1 Ecotoxicological endpoints for arthropods other than bees

Test item	Ecotoxicological endpoints	Test species	Reference
PB-01001-I (4.53×10^9 spores/g)	E = 14.4% (reduction of beneficial capacity at 1.36×10^{13} CFU/ha)	*Aphidius rhopalosiphi*	[redacted], 2001 M-467682-01-1 (please refer to Annex II, Doc IIIM, Section 6, point IIM 8.8)
PB-01001-I (4.53×10^9 spores/g)	E = 0.13% (reduction of beneficial capacity at 1.36×10^{13} CFU/ha)	*Typhlodromus lyri*	[redacted], 2001M-467670-01-1 (please refer to Annex II, Doc IIIM, Section 6, point IIM 8.8)
BioAct® WG 102000028478-01 (1.5×10^{10} spores/g)	E = 3.9% (mortality at 6×10^9 CFU/kg d.w. soil)	*Poecilus cupreus*	[redacted], 2004a M-467505-01-1 (please refer to Annex II, Doc IIIM, Section 6, Point IIM 8.8)
BioAct® WG 102000028478-01 (1.5×10^{10} spores/g)	E = 10.4% (reduction of reproduction capacity at 6×10^9 CFU/kg d.w. soil)	*Aleochara bilineata*	[redacted], 2004b M-467522-01-1 (please refer to Annex II, Doc IIIM, Section 6, Point IIM 8.8)

Acute toxicity to the Aphid parasitoid *Aphidius rhopalosiphi*

The acute toxicity of PBP-01001-I to the Aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) was determined in a laboratory study using worst case exposure conditions ([redacted], 2001, M-467682-01-1). The study was performed in accordance with the ESCORT guidance document for regulatory testing procedures for pesticides with non-target arthropods ([redacted] et al., 1994) and the draft guideline of the ring testing group (Mead-Briggs et al., 2000). Dimethoate was

[2] EFSA (European Food Safety Authority), 2007. Conclusion regarding the peer review of the pesticide risk assessment of the active substance Paecilomyces lilacinus. 35 pp. doi:10.2903/j.efsa.2007.103r

used as positive control, at an application rate of 0.30 mL/ha. The test substance PBP-01001-I was applied at a field dose rate of 30 kg/ha in 2000 L water/ha, equivalent to 3 kg/ha in a 200 L/ha water volume (1.5%). The negative control was distilled water applied at 200 L/ha.
Each variant included 4 replicates containing 10 adults (five male and five females) introduced into the exposure units with an aspirator. Test organisms were exposed to the test substance on glass plates assembled to the exposure unit after the test substance had been sprayed and allowed to dry. At ½, 2, 24 and 48 hours of exposure mortality was assessed and 15 surviving females per group were transferred individually for testing fertility. After 24h the condition of the employed females was recorded and the number of parasitized aphids was counted after 11 days.

Mortality: After 48 hours the mortality in the group exposed to PBP-01001-I was 37.5% compared to 0% in the control, and 100% in the group exposed to the toxic standard. The mortality in the toxic standard and in the test substance group was statistically significantly increased compared to the control. The mortality values needed not be corrected for mortality in control since there was 0% mortality in untreated.

Fecundity: The total number of mummies developed within 11 days was 107 in the control group, corresponding to 7.13 mummies per female. In the test substance group a total of 35 mummies were produced, corresponding to 2.92 mummies per female. These values are statistically significantly different from the numbers found in the control group. The resultant reduction in reproduction was calculated as 59.05%. The reproduction factor was calculated to be 0.41 compared to the control.

In adults of *A. rhopalosiphi* PBP-01001-I applied at a dose corresponding to 30 kg/ha caused 37.50% mortality, which is below the applicable trigger value of 50%, as suggested within discussions in the ESCORT II working group. In the fertility test a reduction in reproduction rate was determined (reproduction factor 0.41), but there was high variation among the 15 individual females in both control and test substance group, ranging from 0 to 23 in control and 0 to 19 in treated animals. Compared to previous control data both results were regarded to be in the range of normal variability as found in this test system. Investigations of the Expert's group of *Aphidius rhopalosiphi* showed that a 50% treatment effect on fecundity of treated insects can only be determined with at least 80% confidence.
Considering the current discussions within the Expert's group, it was concluded that PBP-01001-I will cause no detrimental effect on the mortality of *A. rhopalosiphi*, even when applied at a rate of 30 kg/ha in 2000 L water/ha (1.5%), equivalent 8.1×10^{14} spores/ha.

Although the spore concentration of the new formulation BioAct WG is higher (1×10^{10} spores/g) in comparison to the previous formulation PBP-01001-I (4.3×10^{9} spores/g), the maximum accumulated application rate of BioAct WG (2.4×10^{14} CFU/ha) is still below the highest tested spore concentration. Moreover, it needs to be considered that estimated mortality was below 50% and only reduction of beneficial capacity was observed.

Based on the acute toxicity study described above and taking into account the current discussions within the Expert's group it was assumed that dried residues of PBP-01001-I will cause no unacceptable adverse effects on *Aphidius rhopalosiphi* under conditions of field use, and that therefore, the requirements of Directive 1107/2009 are fulfilled. These findings are transferrable to the current formulation BioAct WG.
Further, this highly sensitive species will not be exposed to BioAct WG under the proposed conditions of field use (see Doc M-1).

Acute toxicity to *Typhlodromus pyri*

The toxicity of PBP-01001-I to the predatory mite, *Typhlodromus pyri* Scheuten (Acari, *Phytoseiidae*) was assessed using a laboratory test ([redacted], 2001, M-467670-01-1).
The principles of this study were based on the guidance document for regulatory testing procedures of pesticides with non-target arthropods ([redacted] et al., 1994), the guidelines of [redacted] (1988) and [redacted] (1988), and improvements of the ring test group (Blümel et al., 2000).
Dimethoate was used as positive control, applied at 0.015 L/ha in a 200 L/ha water volume. PBP-01001-I was tested at a field dose rate of 30 kg/ha in 2000 L water/ha, equivalent to 3 kg/ha in a 200 L/ha water volume (1.5%). The negative control was deionized water applied at a rate of 200 L/ha.

Each variant included 5 replicates containing 20 adults (protonymphs) each. Protonymphs were exposed to a freshly applied dry layer of the test substance on glass cover slides for 7 days. Mortality was assessed after 3 and 7 days. The fecundity of treated and control mites was assessed at day 10, 13 and 14 following exposure, by enumerating eggs and juveniles and determining the cumulative number of eggs per female.

The mean mortality of *Typhlodromus pyri* after 7 days exposure to glass plates treated with PBP-01001-I was 6.0 % compared to 0.0 % in the control group and 85% in the group exposed to the toxic standard. The corrected mortality for the PBP-01001-I treated and toxic standard group were the same, since no mortality was observed in the untreated group. Significant effects on the mortality of *T. pyri* were observed in the toxic standard treatment (Fisher's Exact Test: $p < 0.05$). During the 7 day egg-laying period the mean cumulative number of offspring per female in the PBP-01001-I treated group was 10.8 compared to 10.8 in the control group. The reproduction of mites exposed to the test substance was not reduced.

In conclusion, under the simulated worst case exposure conditions dried residues of PBP-01001-I did not cause adverse effects on survival or reproduction of the test species and can be regarded to be non harmful to *T. pyri* up to a dose rate of 60 kg/ha applied in 200 L/ha (1.5%), equivalent to 8.1×10^{14} spores/ha.

Since the spore concentration of the new formulation BioAct WG is higher (1×10^{10} spores/g) in comparison to the previous formulation PBP-01001-I containing 4.53×10^{9} spores/g, the maximum accumulated application rate of BioAct WG (2.4×10^{14} CFU/ha) is slightly higher than the highest tested spore concentration. However, it needs to be considered that estimated mortality was below 50% and only a reduction of beneficial capacity was observed.

In the above described acute toxicity test on *Typhlodromus pyri* the effect caused by PBP-01001-I was smaller than 30% under the simulated worst case exposure conditions. Therefore it can be assumed that dried residues of BioAct WG will cause no detrimental effects on *T. pyri* under the proposed conditions of field use, employing a maximum accumulated dose rate of 24 kg/ha, and subsequent soil incorporation.

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Under worst case conditions of exposure to PBP-01001-I testing of *Aphidius rhopalosiphi* and *Typhlodromus pyri* as sensitive representative species for beneficial arthropods, did not indicate an unacceptable impact under conditions of field use. Therefore, no further studies regarding assessment of side-effects on non-target arthropods are required for *P. lilacinum* 251.

Larval toxicity to *Poecilus cupreus*

A toxicity study of BioAct® WG on larvae of the ground beetle *Poecilus cupreus* was conducted ([redacted], 2004, M-467505-01-1, please refer to Annex II, Doc IIM, Section 6, Point IIM 8.8 for the study summary). *P. cupreus* larvae were exposed to 400 mg BioAct WG 102000028478-01, containing 6×10^{9} viable spores (analysed) of *P. lilacinum*/kg d.w. soil. The test substance was compared to the toxic reference item Perfekthion (400 g dimethoate/L) and the tap water control. At each feeding time old food was removed and observations for larvae and any effects were made. 28 days after application, test units where no larvae or moulting holes could be observed during the previous two to three weeks were examined and searched for *Poecilus* larva or pupa. Where none were found, the introduced larva was counted as dead. One week after the first pupa in the whole experiment was observed, the hatching of adults was checked daily and un-deformed hatched beetles were weighed.

No significant difference between the test substance and control treatment groups was observed in terms of mortality, days until hatching or weight of hatched beetles. These parameters in the toxic reference item were statistically significantly different to the control. Toxicity of BioAct WG was 0.0% in the treated modality, and 18.6% in the water control

BioAct WG did not show adverse effects on the ground beetle *P. cupreus* at an application rate of 400 mg/kg d.w. soil, corresponding to 6×10^{9} spores/kg d.w. soil.

Since in the above stated study the effect caused by BioAct® WG on *P. cupreus* the LC_{50} was above the highest tested application rate of 400 mg/kg d.w. soil, the active substance *P. lilacinum* can be considered as save at a spore concentration above 6×10^9 spores/kg d.w. soil.

Effects on *Aleochara bilineata*

The effects of BioAct® WG on the rove beetle *Aleochara bilineata* was tested during a 28-day exposure study ([redacted], 2004, M-467522-01-1; please refer to Annex II, Doc IIM, Section 6, Point IIM 8.8 for the study summary). The test substance BioAct WG 102000028478-01 was incorporated into the soil of test vessels at a rate of 400 mg BioAct WG/kg. d.w. soil. Immediately afterwards, ten pairs (ten males and ten females) of beetles were released into the test vessels. An untreated water control and a toxic reference item, Dursban 480, 480g chlorpyrifos/L, were run in parallel. Each treatment group was replicated four times.
28 days after application, all beetles were removed from the test vessels. The vessels were kept under test conditions for one further week at which time the fly pupae were removed from the soil and the number of parasitised pupae and hatched *Aleochara* was recorded for a further approx. 35 days.
It was shown that the reproduction of *Aleochara bilineata* in the test substance treatment group was reduced by 10.4 % compared to the control. Reproduction in the toxic reference item was reduced by 99.8 %.
No reduction in parasitic capacity of *Aleochara bilineata* compared to the control was observed after exposure to BioAct® WG, active ingredient *P. lilacinum* 251 at an application rate of 400 mg per kg dry soil, corresponding to 6×10^9 viable spores (analysed) of *P. lilacinum* per kg dry soil.

The findings from studies conducted on non-target arthropods demonstrate that *P. lilacinum* 251 is not harmful to *Typhlodromus pyri* and *Aphidius rhopalosiphi*, considered the most sensitive species for testing of pesticides ([redacted]; 2003; M-542628-01-1). The reproduction effects on *A. rhopalosiphi* are lower compared to the values stated in the monograph by the Rapporteur. Soil dwelling species are not affected by the micro-organism. The micro-organism does not produce any harmful toxins. Thus, the possible effects from *P. lilacinum 251* on non-target arthropods other than bees are sufficiently reported in the dossier by the present statement. There were no harmful effects found and there is no need to conduct any additional extended laboratory or semi-field studies on other non-target arthropod species.

Toxicity exposure ratios for foliage dwelling non-target arthropods

Following Good Agricultural Practice (see Doc. D-1) *P. lilacinum* 251 will be applied directly onto the soil surface by soil irrigation (drip or drench) or by tray drench/dipping, with subsequent incorporation into the soil by watering. Therefore, exposure to leaf dwelling arthropods like to *P. lilacinum* 251 is not expected.
Following Good Agricultural Practice (see Doc. D-1) *P. lilacinum* 251 will be applied directly onto the soil surface by soil irrigation (drip or drench) or by tray drench/dipping followed by watering to assure full incorporation into the soil. Therefore, spray drift and run-off can be excluded and thus,no exposure to aquatic organismsis expected. After application, spores will colonise plant roots: therefore, no drainage towards ground or surface waters is expected.

Effects on soil dwelling non-target arthropods

A reproduction study in artificial soil on the collembolan *Folsomia candida* with *P. lilacinum* 251 was conducted and the results are summarized in the table below. Reasoning for providing this study in Annex II, Doc IIM, Section 6, Point IIM 8.8 is provided within the study report and also in the summary below.

Table 10.4-1 Ecotoxicological endpoints for soil dwelling arthropods

Test item	Endpoint	Test species	Reference
P. lilacinum 251 102000028478-02 (1.21 x 10^{10} spores/g prod.)	NOEC 562 mg /kg soil corresponding to 6.8 x 10^{9} spores/kg d.w. soil	*Folsomia candida*	[redacted], S., 2015, M-542556-01-1

Report: KIIM 8.8; [redacted], S., 2015
Title: *Purpureocillium lilacinum*: Effects on the reproduction of the collembolan *Folsomia candida* in artificial soil
Report No: 15 10 48 255 S
Document No: M-542556-01-1
Guidelines: OECD Guideline 232
GLP: Yes

Material and methods

The influence on the reproduction of the collembolan species *Folsomia candida* of the test item BioAct WG 102000028478-02 was tested in artificial soil ([redacted], S.; 2015; M-542556-01-1). 10 collembolans (10-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated) and vehicle control, 18 - 32 - 56 - 100 - 178 – 316 – 562 - 1000 mg test item/kg artificial soil dry weight at 20 ± 2°C, 400 - 800 lux, 16 h light : 8 h dark. Native spores of *Purpureocillium lilacinum* are extremely small, light-weight and electrostatically charged and thus cannot be handled in an open system. This makes weighing the spores and dissolving them in water technically impossible. Therefore, for the purpose of application a vehicle was used consisting to 99.8% of easily digestibly carbohydrates, proteins and lipids. The test item (spores plus vehicle) is denominated as "*Purpureocillium lilacinum* 251 WG 6 W.
During the study, they were fed with granulated dry yeast. Mortality and reproduction were determined after 28 days. The tested reference item was 44, 67, 100, 150 and 225 mg Boric acid/kg d.w. artificial soil. The study was valid.

Findings

Significant differences were measured between the control and the treatment regarding mortality and reproduction at the dose of 1000 mg test item/kg d.w. artificial soil. Thus, the No-Observed-Effect-Concentration (NOEC) for mortality and reproduction is 562 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 1000 mg test item/kg artificial soil dry weight.

Summary

Effects on reproduction and mortality to the soil arthropod Folsomia candida were assessed during a study with the formulation BioAct WG. The study of [redacted] (2015; M-542556-01-1) revealed a No-Observed-Effect-Concentration (NOEC) for mortality and reproduction of 562 mg test item/kg artificial soil dry weight.

Toxicity exposure ratios for soil dwelling non-target arthropods

To calculate the risk for the exposure of Collembola, the risk assessment is carried out by comparing the predicted environmental concentration of the product BioAct WG in soil (PEC_{soil}) with the endpoints obtained from the study performed with the product BioAct WG ([redacted], S.; 2015; M-542556-01-1). Furthermore, the PEC_{soil} in terms of spores is compared to the endpoints of the product converted to amounts of spores actually present in the test. The toxicity/exposure ratio (TER) is derived from the No-Observed-Effect-Concentration (NOEC) and was calculated according to the formula:

$$TER = \frac{NOEC\,(mg/kg\,soil)}{PEC_{soil}\,(mg/kg\,soil)}$$

Table 10.4-2 TER values for soil dwelling arthropods

Test organism	Test substance	NOEC	PEC_{Soil}	TER	Trigger value
Folsomia candida	BioAct WG 102000028478-02	562 mg prod./kg d.w. soil	Single application: 5.33 mg product/kg d.w. soil	105	5
			Multiple applications*: 32 mg/kg product/kg d.w. soil	17.6	
		6.8 x 10^7 spores/kg d.w. soil	Single application: 5.3 × 10^7 CFU/kg d.w. soil	128	
			Multiple applications*: 3.2 × 10^8 CFU/kg d.w. soil	21.3	

* Even in case 6 consecutive applications as described in the GAP for Bioact WG (see section Table IIIM 6-1) are applied, no significant accumulation is expected. To demonstrate that there is even no risk indicated under the unrealistic worst assumption that all six applications would completely accumulate, also a risk assessment for the six-fold PEC_{soil} value is provided.

The calculated TER values for the formulation and for the spores are above the Annex VI trigger value of 5, indicating that GAP directed use of BioAct WG poses no risk to soil dwelling non-target arthropods.

Risk mitigation

No risk mitigation measures are required.

IIIM 10.5 Effects on earthworms

No new studies are submitted assessing the effect of formulated product BioAct WG on earthworms. The study already evaluated is presented below. Additionally no relevant scientific papers were identified, presenting any toxic effects of *P. lilacinum* on earthworms (please refer to Annex II; Doc IIM, Section 6, Point IIM 8.9.1).

Table 10.5-1 Ecotoxicological endpoints for earthworms

Test item	EU agreed endpoints (SANCO/10184/2003 - rev. final – 14/07/2006)	Test species	Reference
BioAct® WG 102000028478-02 (60 × 10^9 spores/g)	no effect on mortality, body weight, reproduction at 2 × 10^9 and 6 × 10^9 CFU/kg d.w. soil	*Eisenia foetida*	[redacted], 2004, M-467522-01-1 (please refer to Annex II, Doc IIM, Section 6, Point IIM 8.9.1)

Results of an experiment to test the effect of *P. lilacinum* 251 on earthworms are available from the registration process of Bioact® WG in Australia ([redacted], 1998c). No specific guideline was stated. The test substance contained *P. lilacinum* 251 at 3 × 10^9 CFU/g. Earthworms, sized 3 to 15cm, were collected from a compost heap. 24 earthworms per treatment were exposed to 2 dose rates of *P. lilacinum*: 0.5 and 5 g per 2 litres. The untreated control comprised 24 earthworms in the same volume of soil. All tretament groups were incubated at 21°C for 2 weeks. At study end and after 5 and 9 weeks mortality was assessed by counting, and infectivity towards adults and eggs was determined by visual inspection.

At the different assessment dates there was no mortality, but reproduction of earthworms in all treatments and at all assessment dates, as indicated by the recovery of additional very small (<1cm) earthworms. At study end the number of hatchlings was clearly greater in the treatment groups compared to untreated control. No infected worms were found and eggs apparently were healthy also, as indicated by the continued hatching observed in the test substance group within the 7 weeks post-exposure period.
This study has not been performed in compliance with directive OECD 207, and the species were not identified and not obtained from cultures but of natural origin, the soil substrate was not specified and there were no replicates. Still, the design of this study is appropriate to conclude that this strain lacks an infection potential towards earthworms and will not be a risk for natural populations of earthworms.

In addition a sublethal toxicity study with BioAct WG 1020000284178-01 on earthworm (*Eisenia foetida*) in artificial soil was conducted. This study was conducted under GLP and according to OECD 222 ([redacted], 2004 (M-467522-01-1). The sublethal toxicity of BioAct WG to E. foetida was evaluated during an eight week exposure period. BioAct WG was tested at two treatment rates: 133 mg and 400 mg BioAct WG/kg d.w. soil, corresponding to 2 × 10^9 and 6 × 10^9 viable spores (analysed). Ten adult earthworms (between two and twelve months old, with clitellum) that had been acclimatised for one day in test soil were rinsed, blotted dry, weighed and placed onto the soil within half an hour of application. A water control group was tested in parallel with four replicates of each treatment group. As a positive control, Derosal flüssig 32.4% carbendazim was tested at a rate of 12.6 mg/kg d.w. soil.
Following the exposure to BioAct® WG (active ingredient *P. lilacinum* 251, at application rates of 133 and 400 mg per kg dry soil, corresponding to 2 × 10^9 and 6.0 × 10^9 viable spores (analysed) of *P. lilacinum* 251 per kg dry soil, no effects on mortality, mean body weight change and reproduction were observed.

Toxicity exposure ratios for earthworms

To calculate the risk for the exposure of earthworms, the risk assessment is carried out by comparing the predicted environmental concentration of the product BioAct WG in soil (PEC_{soil}) with the endpoints obtained from the already available study performed with the product BioAct WG ([redacted] J.; 2004; M-467522-01-1). Furthermore, the PEC_{soil} in terms of spores is compared to the endpoints of the product converted to amounts of spores actually present in the test. The toxicity/exposure ratio (TER) is derived from the No-Observed-Effect-Concentration (NOEC) and was calculated according to the formula:

$$TER = \frac{NOEC\ (mg/kg\ soil)}{PEC_{soil}\ (mg/kg\ soil)}$$

Table 10.5-2 TER values for earthworms

Test organism	Test substance	NOEC	PEC_{Soil}	TER	Trigger value
Eisenia fetida	BioAct WG 102000028478-01	≥400 mg prod./kg d.w. soil	Single application: 5.33 mg product/kg d.w. soil	≥75	5
			Multiple applications*: 32 mg/kg product/kg d.w. soil	≥12.5	
		≥6 x 10^9 spores/kg d.w. soil	Single application: 5.3 × 10^7 CFU/kg d.w. soil	≥113	
			Multiple applications*: 3.2 × 10^8 CFU/kg d.w. soil	≥18.8	

* Even in case 6 consecutive applications as described in the GAP for Bioact WG (see section Table IIIM 6-1) are applied, no significant accumulation is expected. To demonstrate that there is even no risk indicated under the unrealistic worst assumption that all six applications would completely accumulate, also a risk assessment for the six-fold PEC_{soil} value is provided.

The calculated TER values for the formulation and for the spores are above the Annex VI trigger value of 5, indicating that GAP-directed use of BioAct WG poses no risk to earthworms.

Furthermore, it is generally accepted that earthworms do not have any microbial pathogen. Therefore, pathogenicity or infectivity of *P. lilacinum* 251 to earthworms can be excluded.
Therefore, under conditions of field use no adverse effects on natural populations of earthworms are expected following application of BioAct WG, and it can be concluded that the product fulfils the criteria for the authorisation of preparations according to EU directive 1107/2009.

Risk mitigation

No risk mitigation measures are required.

IIIM 10.6 Effects on soil micro-organisms

Potential side effects of PBP-01001-I (*P. lilacinum* 251 formulated as WG) on the activity of the soil microflora was assessed according to the SETAC guideline dates March 1995 ([redacted], 2002 M-467720-01-1). Soil of stated origin, low in organic carbon and high in sand content (loamy sand) was employed as worst case, with maximum availability of active substance. Soil characteristics were determined and the soil was sieved to <2mm particle size. A dose rate of 2× the maximum field dose rate of PBP-01001-I = 60 kg/ha was applied as a stock solution to 6 kg of test soil, resulting in calculated 80.0 mg product/kg soil. PBP-01001-I treated and deionized water treated control received Lucerne flour in addition. Soils were thoroughly mixed and sub-divided into 3 replicates à 2 kg soil each, placed in 2 l glass bottles for incubation at 20 ± 2°C in the dark under constant humidity conditions. Samples were taken after 6h, 14 days and 28 days to determine soil dry weight, pH, ammonium-N, nitrate-N, nitrite-N (changes in the content of different nitrogen forms indicate the nitrogen turnover). In addition a test for short-term respiration was performed on 200g sub-samples according to the OxiTop System®, to assess the carbon mineralization capacity.

The incubation of soils was terminated at day 28, since the deviation in nitrogen mineralization of control soil and test substance treated soil did not reach the trigger value of 25% defined by the SETAC guideline. The deviation in the nitrate content of PBP-01001-I treated soil compared to control was -16.74%.
Regarding carbon mineralization there was no significant deviation in short-term respiration among the different treatments at study end, 28 days after treatment. The observed difference of +6.49% for the PBP-01001-I treated soil is in the range of normal variability

In conclusion, the effects of *P. lilacinum* 251 formulated as WG on the nitrogen turnover and the impact on soil respiration are considered to be negligible under the envisaged conditions of field use of PBP-01001-I.

For additional information, please refer to Annex II, Section 6, Doc IIM, Point IIM 8.10.

Risk assessment for soil microorganisms

To calculate the risk for the exposure of soil microorganisms, the risk assessment is carried out by comparing the recommended application rate of the product BioAct WG to soil with the endpoints obtained from the already available study performed with the product PBP-01001-I ([redacted], U., 2002; M-467720-01-1). Furthermore, the predicted environmental concentration (PEC_{soil}) in terms of spores for BioAct WG is compared to the endpoints of the product PBP-01001-I converted to amounts of spores actually present in the test. The toxicity/exposure ratio (TER) is derived from the No-Observed-Effect-Application Rate (NOEAR) and was calculated according to the formula:

$$TER = \frac{NOEAR\ (mg/kg\ soil)}{PEC_{soil}\ (mg/kg\ soil)}$$

Table 10.6-1 TER values for soil microorganisms

Test organism	Test substance	NOEAR[1)]	PEC_{Soil}	TER	Trigger value
Soil micro-organisms	BioAct WG PBP-1001-I	60 kg prod./ha ≙80 mg prod./kg d.w. soil	Single application: 5.33 mg product/kg d.w. soil	≥15	1
			Multiple applications*: 32 mg/kg product/kg d.w. soil	≥2.5	
		≥3.62 x 10^8 spores/kg d.w. soil	Single application: 5.3 × 10^7 CFU/kg d.w. soil	≥6.8	
			Multiple applications*: 3.2 × 10^8 CFU/kg d.w. soil	≥1.1	

1) Assumptions for conversion: The test substance is uniformly distributed within the top 5 cm of the soil, the soil bulk density is 1.5 g dry weight of soil/cm^3

* Even in case 6 consecutive applications as described in the GAP for Bioact WG (see section Table IIIM 6-1) are applied, no significant accumulation is expected. To demonstrate that there is even no risk indicated under the unrealistic worst assumption that all six applications would completely accumulate, also a risk assessment for the six-fold PEC_{soil} value is provided.

Risk assessment

According to current regulatory requirements the risk for soil microorganisms is acceptable, if the effect of the recommended application rate of a compound/product on nitrogen or carbon mineralisation is < 25% at the end of the study, typically 28 days after application. This calculation is equivalent to a TER value of 1 when comparing the calculated PEC_{soil} with the no effect application rate (NOEAR).

The study by [redacted] (2002; M-467720-01-1) was performed with an application rate of 60 kg of the product PBP-1001-I. PBP-1001-I is also a WG formulation of *P.lilacinum* Strain 251, however with a lower concentration of spores than the current BioAct WG. In order to convert the results by Yo(0iz to the current BioAct WG, the numbers of spores applied in the study were calculated and compared to the application rates of spores resulting from GAP directed use of the current BioAct WG formulation (Table 10.6-1). The calculated TER values for the formulation and for the spores exceed the trigger value of 1, indicating that GAP directed use of BioAct WG poses no risk to soil microorganisms.

Overall, within the soil microorganism tests in no case, deviations from the control exceeded 25% after 28 days, indicating low risk to soil microorganisms.
Thus, no unacceptable risks to soil non-target micro-organisms is to be expected from the use of BioAct WG, if the product is used according to the recommended use pattern.

Risk mitigation

No risk mitigation measures are required.

IIIM 10.7 Additional studies

No new additional studies were conducted with the formulation BioAct WG. One additional study on the risk on beneficial nematodes was already evaluated (Anonymous, 1992, Please refer to Annex II, Doc IIM, Section 6, Point IIM 8.9.2 for the study summary).

Test species were entomopathogenic nematodes of all representatives for potential biological insecticides, including *Heterorhabditis bacteriophora* strain NC1; *Steinernema feltiae* (Filipjev); *S. carpocapseae* (Weiser); *S. glaseri* (Steiner), which were employed as unsheathed third stage juveniles, and in addition the common free living nematode *Caenorhabditis elegans* was tested as a mixed population of juveniles and adults.
P. lilacinus conidia were harvested from sporulating cultures and transferred to the Petri dishes using a needle to coat the drop of water containing the nematodes with 3×10^6 conidia per treatment. Within 8h the conidia were absorbed by the agar and came into contact with the nematodes. After 3 days incubation period, mortality was assessed. Dead nematodes were examined under the light microscope (200×) for evidence of fungal growth.. The percentage of dead nematodes was <3% in any treatment. There was no difference between test substance treated and control nematodes for both, the % recovery and % mortality.
In conclusion, mortality under the test conditions employed was very low and not related to the treatment. This positive test result is regarded as worst case relative to field applications, due to the employed exposure conditions with unrealistically high numbers of conidia coated directly on the nematode cuticle. Further, for entomopathogenic nematodes the tested juvenile stage 3 is an appropriate stage to test side-effects since this is the only stage found outside the insect host, which could be exposed to the fungus.

Since no side effects on beneficial nematodes were shown under extreme test conditions, no effects are expected under field conditions as well.

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
KIIIM 10.1 /01	[redacted], J.	1997	Acute oral toxicity of Bioact (Paecilomyces lilacinus) in the rat Pharmatox, Round Corner N.S.W., Australia Bayer CropScience, Report No.: Pharamtox 21953Arpt4 Edition Number: M-476459-02-1 Date: 1997-05-23 **Amended: 1997-05-30** GLP/GEP: yes, unpublished **...also filed as IIIM 7.1.1 /01**	Yes	Bayer CropScience
KIIIM 10.1 /02	[redacted], J.; [redacted]-[redacted], M.	2015	Acute oral LIMIT test toxicity of of Purpureocillium lilacinum to bobwhite quail (Colinus virginianus) (in accordance with OECD 223 and US EPA OCSPP 850) Bayer CropScience, Report No.: M-502363-5, Edition Number: M-534859-01-1 Date: 2015-09-21 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIIM 10.2 /01	[redacted], A.	2001	Acute toxicity testing of PBP-01001-1 (Paecilomyces lilacinus, Strain 251, formulated as WG) in rainbow trout (Oncorhynchus mykiss) (Teleostei, Salmonidae) [redacted], Germany Bayer CropScience, Report No.: 20011290/01-AAOm, Edition Number: M-467660-01-1 Date: 2001-12-18 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIIM 10.2 /02	[redacted], A.	2001	Assessment of toxic effects of PBP-01001-1 (Paecilomyces lilacinus, Strain 251, formulated as WG) on Daphnia magnus using the 48 h acute immobilisation test [redacted], Germany Bayer CropScience, Report No.: 20011290/01-AADm, Edition Number: M-467656-01-1 Date: 2001-12-18 GLP/GEP: yes, unpublished	Yes	Bayer CropScience

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
KIIIM 10.2 /03	[redacted], D.	2001	Testing of toxic effects of PBP-1001-l (Paecilomyces lilacinus Strain 251 formulated as WG) on the Single Cell Green Alga Desmodesmus subspicat [redacted], Germany Bayer CropScience, Report No.: 20011290/01-AADs, Edition Number: M-467680-01-1 Date: 2001-11-12 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIIM 10.3 /01	[redacted], I.	2015	Literature review on Purpureocillium lilacinum strain 251 and metabolites: Section 6: Effects on non-target organisms [redacted] Germany Bayer CropScience, Report No.: M-542804-01-1, Edition Number: M-542804-01-1 Date: 2015-11-23 GLP/GEP: n.a., unpublished	Yes	Bayer CropScience
KIIIM 10.4 /01	[redacted], R. J.	2000	Paecilomyces lilacinus as a biocontrol agent - Chapter 6 - The effect of Paecilomyces lilacinus on non-target invertebrates Report No.: M-490114-01-1, Edition Number: M-490114-01-1 GLP/GEP: n.a., published	No	
KIIIM 10.4 /02	[redacted], M.	2001	PBP-01001-l: Acute toxicity to the Aphid Parasitoid, Aphidius rhopalosiphi (Hymenoptera, Braconidae) in the laboratory Arbeitsgemeinschaft [redacted], Germany Bayer CropScience, Report No.: 20011290/01-NLAp, Edition Number: M-467682-01-1 Date: 2001-10-10 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIIM 10.4 /03	[redacted], K. L.; [redacted], N.; [redacted], E. G.; [redacted] S.; [redacted], P.	1994	Guidance document on regulatory testing procedures for pesticides with non-target arthropods Publisher:Society Environmental Toxicology and Chemistry-Europe, Location:Netherland, Pages:1-52, Year:1994, Report No.: Lit. 6841, Edition Number: M-001914-01-1 GLP/GEP: n.a., published	No	

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
KIIIM 10.4 /04	[redacted], H.	2001	PBP-01001-l: Toxicity to the Predatory Mite, Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the laboratory [redacted], Germany Bayer CropScience, Report No.: 20011290/01-NLTp, Edition Number: M-467670-01-1 Date: 2001-11-26 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIIM 10.4 /05	[redacted], J.	2004	Toxicity to larvae of the ground beetle poecilus cupreus L. (Coleoptera, Carabidae) in the laboratory - BioAct WG [redacted] Germany Bayer CropScience, Report No.: 20041022/01-NEPc, Edition Number: M-467505-01-1 Date: 2004-08-12 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIIM 10.4 /06	[redacted], J.	2004	BioAct WG: Sublethal toxicity to the earthworm Eisenia fetida in artificial soil [redacted], Germany Bayer CropScience, Report No.: 20041022/01-NREf, Edition Number: M-467522-01-1 Date: 2004-06-04 GLP/GEP: yes, unpublished **...also filed: KIIIM 10.5 /01**	Yes	Bayer CropScience
KIIIM 10.4 /07	[redacted], W.	2003	Paecilomyces lilacinus strain 251 - PBP-01001-I - Annex IIB, point 8 resp. annex IIIB, point 10: Effects on non-target organisms - Statement to the draft monograph (DAR) [redacted], Germany Bayer CropScience, Report No.: M-542628-01-1, Edition Number: M-542628-01-1 Date: 2003-11-01 GLP/GEP: n.a., unpublished	Yes	Bayer CropScience

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**
KIIIM 10.4 /08	[redacted], S.	2015	Purpureocillium lilacinum: Effects on the reproduction of the collembolan Folsomia candida in artificial soil [redacted], Germany Report No.: 15 10 48 255 S, Edition Number: M-542536-01-1 Date: 2015-12-15 GLP/GEP: yes, unpublished	Yes	
KIIIM 10.5 /01	[redacted], J.	2004	BioAct WG: Sublethal toxicity to the earthworm Eisenia fetida in artificial soil [redacted], Germany Bayer CropScience, Report No.: 20041122/01-AREf, Edition Number: M-467522-01-1 Date: 2004-06-04 GLP/GEP: yes, unpublished **...also filed: KIIIM 10.4 /06**	Yes	Bayer CropScience
KIIIM 10.6 /01	[redacted], U.	2003	Assessment of the side effects of PBP-1001-l (Paecilomyces lilacinus sTrain 251 formulated as WG) on the activity of the soil Microflora Arbeitsgemeinschaft [redacted], Germany Bayer CropScience, Report No.: 20021290/U-ABMF, Edition Number: M-467720-01-1 Date: 2002-11-09 GLP/GEP: yes, unpublished	Yes	Bayer CropScience
KIIIM 10.7 /01	[redacted]	1992	Tests of the active ingredient of the bionematicide nemacheck on some beneficial nematodes Year:1992, Report No.: M-489347-01-1, Edition Number: M-489347-01-1 GLP/GEP: n.a., published	No	