Appendix to:
EFSA (European Food Safety Authority), 2020. Conclusion on the peer review of the pesticide risk assessment of the active substance *Purpureocillium lilacinum* strain 251 EFSA Journal 2020;18(9):6238, 13 pp. doi:10.2903/j.efsa.2020.6238
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**Appendix A – List of end points for the active substance and the representative formulation**

**Identity, Biological properties, Details of uses, Further information, and Proposed Classification and Labelling**

| Active microorganism: | *Purpureocillium lilacinum* strain 251 |
|-----------------------|---------------------------------------|
| Function (e.g. control of fungi): | Biological nematicide |

| Rapporteur Member State: | Hungary |
|--------------------------|---------|
| Co-rapporteur Member State: | The Netherlands |

**Identity of the Microbial or Viral Agent used in plant protection / Active Substance** (Regulation (EU) No 283/2013, Annex Part B, point 1; OECD IIM Point 1)

| Name of the organism: | *Purpureocillium lilacinum* strain 251 |
|-----------------------|---------------------------------------|
| Taxonomy:             |                                       |
| Kingdom:              | Fungi                                 |
| Division:             | Ascomycota                            |
| Class:                | Sordariomycetes                       |
| Order:                | Hypocreales                           |
| Family:               | Ophiocordycipitaceae                  |
| Genus:                | *Purpureocillium*                     |

| Species, subspecies, strain: | Species: *Purpureocillium lilacinum* |
|-------------------------------|--------------------------------------|
| Strain                        | *Purpureocillium lilacinum* 251      |

| Identification / detection: | *Purpureocillium lilacinum* can be identified by its phenotypic characterization. The genetic characterization of the strain is done by LP-RAPD analysis. |
|-----------------------------|---------------------------------------------------------------------------------|

| Culture collection:         | The strain 251 is deposited at the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) in Braunschweig, Germany (Deposit. No.: DSM23289). Australian Government Analytical Laboratories (AGAL), in Pymble, Australia, under access number 89/030550 |
|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

| Minimum and maximum concentration of the MPCA used for manufacturing of the formulated product (cfu; g/kg): | The technical grade of MPCA is only a hypothetical stage in the continuous production process of end use product with *Purpureocillium lilacinum* strain 251 as active substance. The MPCA content in the representative product formulation BioAct WG: min. 1 x 10^{13} CFU/kg (max. 2.2 x 10^{13} CFU/kg) (60 g/kg) |
|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|


| Identity and content of relevant impurities, additives, contaminating organisms in the technical grade of MPCA: | No relevant impurities or contaminating organisms were established in the available 5-batch analysis |
|---|---|
| Is the MPCA genetically modified; if so provide type of modification | *Purpureocillium lilacinum* strain 251 is not genetically modified or mutant |
**Biological properties of the microorganism** (Regulation (EU) N° 283/2013, Annex Part B, point 2; OECD IIM Point 2)

| Origin and natural occurrence, Background level: | *P. lilacinum* is a widely distributed saprophyte. It has been isolated from soils on a global scale, in e.g. Ghana, Liberia, New York State, Israel, the Netherlands and Germany, and is reported to further occur in India, Pakistan, Sri Lanka, Hong Kong, Japan, Hawaii, North and South America, Australia, Central Africa, South Africa, Somalia, Egypt, Turkey, France and Italy, and seems to be most frequent in warmer regions. The **natural occurrence** of this species is ubiquitous, it has been found in desert soil, sand dunes, and estuarine sediments. *P. lilacinum* occurs in different types of soil (loess, podzol, rendzina, chernozem and solonchak) whether uncultivated or cultivated, and under a wide range of crops, and is inhabiting the rhizosphere of cultivated plants. Strain 251 of *P. lilacinum* originates from infested egg-masses of a plant-parasitic nematode (*Meloidogyne* spp.) in the Philippines. |
| --- | --- |
| Target organism(s): | The envisaged target organisms are plant parasitic nematodes, such as root-knot nematodes (e.g. *Meloidogyne* sp.), burrowing or cyst nematodes (e.g. *Heterodera* sp, *Globodera* sp.) and root lesion nematodes (*Pratylenchus* sp.). |
| 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: *P. lilacinum* 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. |
| Host specificity: | Based on the mode of action the host range of *P. lilacinum* 251 is not restricted only to specific plant pathogenic nematodes. The strain 251 is specified on plant-parasitic nematodes of different genera at different developmental stages. There is no mammalian infectivity or pathogenicity (no growth at 36°C). The strain is not toxic for tested non-target organisms. |
| Life cycle: | No known sexual state, mycelial growth, production of conidiospores. The survival is not dependent on its host. *P. lilacinum* may survive in soil; growth is enhanced in rhizosphere. |
| Infectivity, dispersal and colonisation ability: | *P. lilacinum* strain 251 is non-infectious via the intratracheal and intraperitoneal route. |
| Relationships to known plant, animal or human pathogens: | Other strains of *Paecilomyces lilacinus* or *Purpureocillium lilacinum*, have been reported to cause mycosis infections. These relatively rare and uncommon infections were almost always associated with immuno-compromised patients or due to contamination within a surgery. |
| Genetic stability: | The genetic stability of traits of fungi is not relevant, since gene exchange in fungi is a rare event. Thus, no transfer of genetically coded properties can take place among fungi or between fungi and other micro-organisms. |
| Information on the production of relevant metabolites (especially toxins): | Some strains of *P. lilacinum* were reported to produce paecilotoxins. For *P. lilacinum* 251 it was demonstrated that this strain does not produce this toxin. |
|Resistance/sensitivity to antibiotics/anti-microbial agents used in human or veterinary medicine: | *P. lilacinum* 251 cannot grow at temperatures >36°C. Resistance study is currently ongoing.|
Summary of uses supported by available data (Regulation (EU) Nº 283/2013, Annex Part B, point 3; OECD IIM Point 3)

| Crop and/or situation | Zone | Product code | F/G or I | Pests or Group of pests controlled | Formulation | Application | Application rate per treatment | PHI (days) | Remarks: |
|-----------------------|------|--------------|---------|-----------------------------------|-------------|------------|--------------------------------|------------|----------|
|                       |      |              | (a)     |                                   |             | (d-f)      | (i)                             | (j)        | (k)      | (l)       | (m) |
| “Soil treatment, against Meloidogyne” | Central and Southern Europe | Bioact WG | F/G* | Meloidogyne spp. | WG | 1st application: Drip irrigation or soil drench which may be followed by mechanical incorporation | Pre-transplant | 1 | -/ | 0.024 - 0.12 (2.4 × 10^{11} - 1.2 × 10^{12} spores/hL) | 200 - 1,000 | 0.24 kg /ha (4 × 10^{11} spores/ha) 4 kg product/ha | 0 |
| Tomato               |      |              |         |                                   |             |           |                                 |            |          |            |    |
|                       |      |              | F/G*   | Meloidogyne spp. | WG | Dipping (of seedlings) Drip irrigation Or Soil drench | At transplant | 1 | -/ | 0.024 - 0.12 (2.4 × 10^{11} - 1.2 × 10^{12} spores/hL) | 200 - 1,000 | 0.24 kg /ha (4 × 10^{11} spores/ha) 4 kg product/ha | 0 |
|                       |      |              | F/G*   | Meloidogyne spp. | WG | Drip irrigation or Soil drench | Post-transplant | 5 (4-6 weeks) | 4 – 8 weeks | 0.024 - 0.12 (2.4 × 10^{11} - 1.2 × 10^{12} spores/hL) | 200 - 1,000 | 0.24 kg /ha (4 × 10^{11} spores/ha) 4 kg product/ha | 0 |
| Crop and/or situation | Zone | Product code | FG or I | Pests or Group of pests controlled | Formulation | Application | Application rate per treatment | PHI (days) | Remarks: |
|-----------------------|------|--------------|--------|-----------------------------------|-------------|------------|-------------------------------|------------|---------|
| "Soil treatment, against Meloidogyne" | Central and Southern Europe | BioAct WG F/G | Meloidogyne spp. | 60 g/kg (1x10^13 spores/kg) | Pre-transplant | 1 | 0.024 - 0.12 (2.4 x 10^11 - 1.2 x 10^12 spores/hL) | 200 - 1,000 | 0.24 kg /ha (4 x 10^13 spores/ha) 4 kg product/ha |
| Cucumber | F/G | Meloidogyne spp. | Dipping (of seedlings) Drip irrigation or Soil drench | At transplant | 1 | 0.012 - 0.12 (1.2 x 10^11 - 1.2 x 10^12 spores/hL) | 200 - 1,000 | 0.12 - 0.24 kg /ha (2 - 4 x 10^13 spores/ha) 2 - 4 kg product/ha |
| F/G | Meloidogyne spp. | Drip irrigation or Soil drench | Post-transplant 5 (4-6 weeks) 4 – 8 weeks | 0.024 - 0.12 (2.4 x 10^11 - 1.2 x 10^12 spores/hL) | 200 - 1,000 | 0.24 kg /ha (4 x 10^13 spores/ha) 4 kg product/ha |

Although the GAP might be presented differently in principle no changes of the GAP is needed. The notifier recommends higher water amount, but this is obviously compensated with more drench water. For the transplant treatment: Low rate is sufficient under normal conditions; use high rate only if seedlings cannot be treated after planting and dilution effects occur in soil and/or if pre-plant treatment was not done! Water is used simply as the carrier, first for the soil directed application and second for delivery of the fungal spores into the root system (BioAct remaining just on the soil surface are of no use!). This may happen in two steps or in one combined step, e.g. with drip irrigation. The product needs to be properly distributed around and through the root system of treated plants. If application is
with little water, then the watering-in of the spores into the soil needs to happen with additional (irrigation) water. Alternative rates are only proposed for the seedling treatments, whereby the low rate is emphasised for tray. The high rate is only to compensate dilutions (e.g. if applied immediately after planting in the field.)

* - Greenhouse is considered to be as a permanent structure.

** Remarks:**

(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), greenhouse application (G) or indoor application (I)

(c) e.g. biting and sucking 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 plants - 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) The minimum and maximum number of application possible under practical conditions of use must be provided

(l) PHI - minimum pre-harvest interval

(m) Remarks may include: Extent of use/economic importance/restrictions

n.r not relevant

CFU Colony Forming Unit
Classification and proposed labelling (Symbol, Indication of danger, Risk phrases, Safety phrases)

| Category                              | Classification |
|---------------------------------------|----------------|
| with regard to physical/chemical data | No classification |
| with regard to toxicological data     | No classification |
|                                       | ‘Micro-organisms may have the potential to provoke sensitising reactions’ |
| with regard to fate and behaviour     | No classification |
| with regard to ecotoxicological data  | No classification |

Methods of analysis (Regulation (EU) N° 283/2013, Annex Part B, point 4 and Regulation (EU) N° 284/2013, Annex Part B, point 5)

Analytical methods for the microorganism (MA 4.1 & MP 5.1; OECD IIM 4.3 & IIIM 5.1)

| Category                                                      | Method                                                                                           |
|---------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Manufactured microorganism (principle of method)              | Determination of the total number of spores and the spore viability by counting.                  |
| Impurities and contaminating microorganisms in manufactured material (principle of method) | Regular quality checks for purity and presence of contaminants by internationally recognized methods |
| Microbial Pest Control Product (principle of method)          | Specified viability and microbiological purity are the main quality criteria for the end-product: |
|                                                              | The product is checked for product concentration, germination, viability of spores and biological purity and contamination. |

Analytical methods for residues (viable and non-viable) in exposed compartments and organisms (MA 4.2 & MP 5.2; OECD IIM 4.5 & IIIM 5.2)

| Category                                                      | Method                                                                                           |
|---------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| of the active microorganism (principle of method)             | No residue definition/MRL is set or expected, therefore no methods are required.                  |
| of relevant metabolites (principle of method)                 | No residue definition/MRL is set or expected, so no methods are required.                         |
**Impact on Human and Animal Health** (Regulation (EU) No 283/2013, Annex Part B, point 5 and Regulation (EU) No 284/2013, Annex Part B, point 7)

| Medical data: (including medical surveillance on manufacturing plant personnel) | There is no direct observation suggesting that exposure to *Purpureocillium lilacinum* strain 251 could be related to harmful effects in humans. There is no information suggesting the occurrence of poisoning incidents which could be related to an exposure to *Purpureocillium lilacinum* strain 251. *Purpureocillium lilacinum* strain 251 is part of the natural environment and was never reported to be a human pathogen and, therefore, a possible impact on human health was not subjected to more detailed investigations. Moreover, since November 1999 personnel of the applicant’s manufacturing plant and of the developmental laboratories in Germany has been exposed to this fungus without having shown any health problems (including allergic reactions), fungal infections or symptoms of pathogenicity. |
|---|---|
| Sensitisation: | The study on skin sensitization potential of BioAct did not reveal any sensitisation reactions. However, there are no validated tests for the identification of skin sensitization potential of microorganisms. Therefore, the use of the standard precautionary phrase “Microorganisms may have the potential to provoke sensitizing reactions” is suggested. |
| (MA 5.2.1 & MP 7.2.3; OECD IIM 5.2 & IIIM 7.1.6) | |
| Acute oral infectivity, toxicity and pathogenicity: | There is no evidence of toxicity or infectivity/pathogenicity to rats following a single oral administration of $1.8 \times 10^9$ spores/g *Purpureocillium lilacinum* strain 251. Acute oral LD$_{50}$ > 2000 mg/kg (<3.6 x $10^8$ spores of *Purpureocillium lilacinum* lilacinum strain 251/rat). |
| (MA 5.2.2.1 & MP 7.1.1; OECD IIM 5.3.2 & IIIM 7.1.1) | |
| Acute intratracheal/inhalation infectivity, toxicity and pathogenicity: | There is no evidence of toxicity, infectivity/pathogenicity to rats following a single intratracheal injection of $2.5 \times 10^8$ spores CFU/rat *Purpureocillium lilacinum* strain 251 formulated as WG. |
| (MA 5.2.2.2 & MP 7.1.2; OECD IIM 5.3.3 & IIIM 7.1.3) | |
| Acute intravenous/intraperitoneal infectivity: | There is no evidence of infectivity to rats following a single intraperitoneal administration of $1 \times 10^{10}$ spores/rat *Purpureocillium lilacinum* strain 251 formulated as WG. Clearance occurred within 3 weeks after intraperitoneal treatment. |
| (MA 5.2.2.3; OECD IIM 5.3.4) | |
| Genotoxicity: | There is no indication of toxin production by *Purpureocillium lilacinum* strain 251. |
| (MA 5.2.3; OECD IIM 5.3.5) | |
| Cell culture study: | No data; not required |
| (MA 5.2.4; OECD IIM 5.3.6) | |
| Information on short-term toxicity and pathogenicity: | No data; not required |
| (MA 5.2.5; OECD IIM 5.3.7) | |
| Dermal toxicity: | Rat: LD$_{50}$ > 2000 mg/kg bw; no mortality, no signs of pathogenicity and infectivity. |
| (MP 7.1.3; OECD IIM 7.1.2) | |
| Specific toxicity, pathogenicity and infectivity: | No data, not required |
| (MA 5.3; OECD IIM 5.5) | |
| Genotoxicity – *in vivo* studies in germ cells: | No data, not required |
| (MA 5.5; OECD IIM 5.5.3) | |

**Reference values**
AOEL/AOEL/ ADI/ARfD: Not needed.

Exposure (operator, workers, bystander, consumer): (MA 6.1 & MP 7.3, 8.0; OECD IIM 5.6 & IIIM 7.2, 7.3) Not conducted, not needed.

Residues (Regulation (EU) Nº 283/2013, Annex Part B, point 6 and Regulation (EU) Nº 284/2013, Annex Part B, point 8; OECD IIM Point 6 & IIIM Point 8)

Viable residues: No residue data required as absence of human infectivity, pathogenicity and toxicity upon exposure to the fungus shown by the toxicity data.

Non-viable residues: No residue data required due to the absence of toxins or any toxicologically relevant secondary metabolites.

Fate and Behaviour in the Environment (Regulation (EU) Nº 283/2013, Annex Part B, point 7 and Regulation (EU) Nº 284/2013, Annex Part B, point 9; OECD IIM Point 7 & IIIM Point 9)

Persistence and multiplication (competitiveness) in soil, water and air:

| Persistence and multiplication (competitiveness) in soil, water and air: |
|---------------------------------------------------------------|
| **P. lilacinum** strain 251 is naturally occurring in soil, where its multiplication and persistence are restricted by natural competitors and unfavourable conditions. Thus, the number of **P. lilacinum** spores decline with the time after application. For **P. lilacinum** strain 251 a decrease of viable spores in soil within the first three months after application has been shown. |
| Worst case PECsoil calculations of 4 kg formulated product (FP) for 7 treatments per season considering no interception. |
| PECsoil initial | mg a.s./kg | CFU/kg | mg FP/kg |
| single application | 0.320 | 6.45x10⁷ | 5.333 |
| multiple application | 2.24 | 4.52x10⁸ | - |

Due to the nature of the WG preparation and the intended use and application method by soil drip irrigation, or drench, exposure to surface water is considered negligible.

Dispersal of spores via aerosols is not anticipated due to the nature of this preparation and the intended application by soil drip irrigation, or drench, according to Good Agricultural Practice.

Mobility:
The intended application by soil drip irrigation or drench, excludes that spores of **P. lilacinum** strain 251 may be transported by aerosols through air. A vertical translocation of the spores to deeper soil profiles is not expected.

Effects on non-target organisms (Regulation (EU) Nº 283/2013, Annex Part B, point 8 and Regulation (EU) Nº 284/2013, Annex Part B, point 10; OECD IIM Point 8 & IIIM Point 10).

Additional studies from the published literature were submitted and considered as supportive information. Complete summaries are available in the RAR Vol 3 B9.

Effects on birds and other terrestrial vertebrates (MA 8.1 & MP 10.1; OECD IIM 8.1 & IIIM 10.1)
**Peer review of the pesticide risk assessment of the active substance P**

*purpureocillium lilacinum strain 251*

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**Dosage**

| Test substance | Category (e.g. insectivorous bird) and species | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|----------------|-----------------------------------------------|------------|---------------------------------------------------------------------------------|
| **LD₅₀ > 2000 mg/kg b.w. (> 2.42 x 10¹⁰ CFU/kg b.w.)*** | Purpureocillium lilacinum 251 WG (6%) preparation | 1 d dosing and 14 d observation period | No mortalities, no signs of toxicity |
| **LD₅₀ > 2000 mg/kg b.w. (> 3.6 x 10⁹ CFU/kg b.w.)** | BioAct WG preparation | 14 d | No mortalities, no signs of toxicity and pathogenicity |

*The RMS has recalculated the endpoints based on the number of viable spores (CFU) as stated in the certificate of analysis.

**Effects on aquatic organisms** (MA 8.2 & 10.2; OECD IIM 8.2, 8.3 & IIIM 10.2)

| Group | Test substance | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-------|----------------|------------|---------------------------------------------------------------------------------|
| **Fish species (specify)** | | | |
| *Oncorhynchus mykiss* | PBP01001-IWG preparation | 4 d | 4 d LC₅₀: > 100 mg/L (corresponding to > 4.53 x 10⁸ CFU/L (nom))  NOEC (based on mortality and sublethal effects): 100 mg/L (corresponding to 4.53 x 10⁸ CFU/L (nom))¹  The study cannot provide appropriate information on infectiveness and pathogenicity of *P. lilacinum* strain 251. Further information is required. |
| **Invertebrate species (specify)** | | | |
| *Daphnia magna* | PBP01001-IWG preparation | 2 d | 2 d EC₅₀: > 100 mg/L (corresponding to > 4.53 x 10⁸ CFU/L (nom))  NOEC (based on immobility and sublethal effects): 100 mg/L (corresponding to 4.53 x 10⁸ CFU/L (nom))¹  The study cannot provide appropriate information on infectiveness and pathogenicity of *P. lilacinum* strain 251. Further information is required. |

¹ nominal value, without analytical verification

**Effects on algae:** (species, growth, growth rate, capacity to recover) (MA 8.2.3 & MP 10.2; OECD IIM 8.4 & IIIM 10.2)

| Group | Test substance | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-------|----------------|------------|---------------------------------------------------------------------------------|
| | | | No valid data available, not required |

**Effects on aquatic plants:** (species, growth, growth rate, capacity to recover) (MA 8.2.4 & MP 10.2; OECD IIM 8.5 & IIIM 10.2)

| Group | Test substance | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-------|----------------|------------|---------------------------------------------------------------------------------|
| | | | No data, not required |
**Effects on bees** (MA 8.3 & MP 10.3; OECD IIM 8.7 & IIIM 10.3)

| Species                        | Test Substance                  | Route/timescale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-------------------------------|---------------------------------|-----------------|------------------------------------------------------------------------------------------|
| *Apis mellifera*              | *Purpureocillium lilacinum* strain 251 | oral 48h        | Not tested, not required                                                                 |
|                               |                                  | contact 48h     | Not tested, not required                                                                 |

**Effects on terrestrial arthropods other than bees** (MA 8.4 & MP 10.4; OECD IIM 8.8 & IIIM 10.4)

| Species                        | Stage                            | Test Substance                  | Dose                                          | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-------------------------------|----------------------------------|---------------------------------|-----------------------------------------------|------------------------------------------------------------------------------------------|
| *Aphidius rhopalosiphi*        | adult                            | PBP01001-IWG preparation        | 3.0 kg/ha (1.36 x 10\(^{13}\) CFU/ha)          | Significant effects on the survival and reproduction at the tested concentration              |
|                               |                                  |                                 |                                               | Mortality: 37.5%                                                                           |
|                               |                                  |                                 |                                               | Reduction in reproduction: 59.05%                                                           |
|                               |                                  |                                 |                                               | Reduction in beneficial capacity: 74.4%                                                      |
|                               |                                  |                                 |                                               | The study cannot provide appropriate information on infectiveness and pathogenicity of *P. lilacinum* strain 251. |
| *Typhlodromus pyri*           | 24 hour old protonymphs          | PBP01001-IWG preparation        | 3.0 kg/ha (1.36 x 10\(^{13}\) CFU/ha)          | No significant effects on the survival and reproduction at the tested concentration           |
|                               |                                  |                                 |                                               | Mortality: 6%                                                                              |
|                               |                                  |                                 |                                               | Reduction in reproduction: -0.9%*                                                           |
|                               |                                  |                                 |                                               | Reduction in beneficial capacity: 5.13%                                                      |
|                               |                                  |                                 |                                               | No records were taken of any visual inspections on the surviving mites for symptoms of infections and pathogenicity. |
| *Aleochara bilineata*         | 2 - 6 day old adults             | BioAct WG preparation           | 400 mg/kg soil d.w. (6 x 10\(^{9}\) CFU/ha)     | No significant effects on the reproduction at the tested concentration                          |
|                               |                                  |                                 |                                               | Reduction in reproduction: 10.4%                                                            |
|                               |                                  |                                 |                                               | No records were taken of any visual inspections on the surviving beetles for symptoms of infections and pathogenicity. |
| *Poecilus cupreus*            | 12 - 48 hour old larvae          | BioAct WG preparation           | 400 mg/kg soil d.w. (6 x 10\(^{9}\) CFU/ha)     | No significant effects on the mortality, developmental time and hatching weight at the tested concentration |
|                               |                                  |                                 |                                               | Mortality: 7.9%                                                                            |
|                               |                                  |                                 |                                               | No records were taken of any visual inspections on the surviving beetles                     |
Effects on other terrestrial invertebrates (MA 8.5 & MP 10.5; OECD IIM 8.9.1 and IIM 8.9.2 & IIIM 10.5)

Toxicity, infectivity and pathogenicity: (endpoint, value or other description of effects)

| **Eisenia fetida:** | 56 d NOEC (based on mortality, body weight and reproduction) 400 mg BioAct WG/kg soil d.w. (equivalent to $6 \times 10^9$ CFU/kg soil d.w.)* |
|---------------------|----------------------------------------------------------------------------------------------------------------------------------|
|                     | No adverse, pathogenic or toxic effects of *P. lilacinus* strain 251 to earthworms were demonstrated.                           |
| **Folsomia candida:** | 28 d NOEC (based on mortality and reproduction) 562 mg *Purpureocillium lilacinum* 251 WG/kg soil d.w. (equivalent to $6.8 \times 10^9$ CFU/kg soil d.w.) |
|                     | 28 d EC$_{10}$ (reproduction) 604 mg *Purpureocillium lilacinum* 251 WG/kg soil d.w. (equivalent to $7.3 \times 10^9$ CFU/kg soil d.w.) |
|                     | 28 d EC$_{20}$ (reproduction) 765 mg *Purpureocillium lilacinum* 251 WG/kg soil d.w. (equivalent to $9.3 \times 10^9$ CFU/kg soil d.w.)* |
|                     | The study cannot provide appropriate information on infectiveness and pathogenicity of *P. lilacinum* strain 251. Further information is required. |

Further information: Available information from open published literature was available and considered supportive. Complete summaries are available in the RAR Vol 3 B9.

* the test item was mixed into the soil

Effects on soil microorganisms (MA 8.6 & MP 10.6; OECD IIM 8.10 & IIIM 10.6)

No long-term effects on the soil nitrogen transformation (measured as NO$_3$-N production) and soil C-transformation (measured as oxygen consumption) tested up to a concentration of 80 mg PBP01001-l/kg dry soil (corresponding to $3.62 \times 10^9$ CFU/kg soil d.w.)

Additional studies (MA 8.7 & MP 10.7; OECD IIM 8.11 & IIIM 10.7)

Available information from open literature suggests that *Purpureocillium* lilacinum could be regarded as an opportunistic fungal pathogen acting in both immune-compromised and immune-competent terrestrial cold blood organisms.