Appendix to:
EFSA (European Food Safety Authority), 2020. Conclusion on the peer review of the pesticide risk assessment of the active substance *Metarhizium brunneum* BIPESCO 5/F52. EFSA Journal 2020;18(10):6274, 29 pp. doi:10.2903/j.efsa.2020.6274
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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:** | *Metarhizium brunneum* BIPESCO 5/F52 (formerly *Metarhizium anisopliae* var. *anisopliae* BIPESCO 5/F52) |
| **Function (e.g. control of fungi):** | Insecticide and acaricide |
| **Rapporteur Member State:** | The Netherlands |
| **Co-rapporteur Member State:** | France |

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

| Name of the organism: | *Metarhizium brunneum* BIPESCO 5/F52 |
|-----------------------|-------------------------------------|

**Taxonomy:**

- Kingdom: Fungi
- Sub-Kingdom: Neomycota
- Phylum: Ascomycota
- Sub-Phylum: Euascomycotina
- Class: Pyrenomycetes
- Order: Hypocreales
- Family: Clavicipitaceae
- Genus: *Metarhizium*
- Species: *brunneum*
- Strain: BIPESCO 5/F52

**Species, subspecies, strain:**

*Brunneum*, BIPESCO 5/F52

**Identification / detection:**

EF1α sequence analysis and subsequent comparison with sequences of references strains and simple sequence repeat (SSR, microsatellite) marker analysis to discriminate genotypes within individual *Metarhizium* species.

**Culture collection:**

- BBA, Germany: M.a. 43 (acronyms Z43);
- BIPESCO project, EU: BIPESCO 5
- CBS number 123710 (BIPESCO 5)
- DSM number 21978 (BIPESCO 5)
- Novozymes, Earth BioScience, F52 (F-52)
- HRI, UK: 275-86 (acronyms V275 or KVL 275 or HRI 27586);
- KVL Denmark: KVL 99-112 (Ma 275 or V 275);
- Bayer, Germany: DSM 3884; BIO1020
- ATCC, USA: ATCC 90448;
- USDA, Ithaca, USA: ARSEF 1095.

| Minimum and maximum concentration of the MPCA used for manufacturing of the formulated product (cfu; g/kg): | BIPESCO 5 (TGAI and MPCP are considered to be the same. Below specification also applies to the product GranMet GR):
Min: $7.24 \times 10^7$ CFU/g ($7.24 \times 10^{10}$ CFU/kg)
Max: $9.91 \times 10^7$ CFU/g ($9.91 \times 10^{10}$ CFU/kg)

F52: 996 g spores/kg (min. $9 \times 10^{12}$ CFU/kg, max. $6 \times 10^{13}$ CFU/kg) data gap for 5-batch data |

| Identity and content of relevant impurities, additives, contaminating organisms in the technical grade of MPCA: | There are no relevant impurities or additives.
Toxicologically relevant metabolite: swainsonine
Contaminating organisms: complies with SANCO/12116/2012 (European Commission, 2012) |

| Is the MPCA genetically modified; if so provide type of modification | No |
### Biological properties of the microorganism (Regulation (EU) N° 283/2013, Annex Part B, point 2)

| Origin and natural occurrence, Metarhizium brunneum BIPESCO 5/F52 | Metarhizium brunneum BIPESCO 5/F52 is a subculture from the isolate Ma43 that was isolated from a codling moth (*Cydia pomonella*) in Austria. *Metarhizium brunneum* is a natural occurring, rhizosphere competent fungus. |
|---|---|
| Background level: | The natural background levels show a large variation, with concentrations measured from 0 to 1 x 10⁶ CFU/ g dry soil. |
| Target organism(s): | Insect pests susceptible to *M. brunneum* include aphids, thrips, whitefly, scarabs (Coleoptera, Melolonthidae), weevils (Coleoptera: Curculionidae), mites, and gnats. |
| Mode of action: | Stages of host infection: adhesion of infection propagules (e.g. conidium and blastospore) to the surface of the host cuticle, differentiation of infection structures (appressoria and penetration pegs), penetration of the cuticle using enzymes and mechanical force, colonization of the hemocoel and emergence of the conidiophores for external sporulation on the cadaver. |
| Host specificity: | Species of Coleoptera, Isoptera, Hemiptera, Lepidoptera (Noctuidae, including *Galleria mellonella* and *Cydia pomonella*), Diptera, Elateridae (click beetles: the larvae in particular, also named wireworms) and Acarina (including *Boophilus microplus*, *Ixodes scapularum* and *Rhipicephalus sanguineus*). |
| Life cycle: | *Metarhizium brunneum* is an anamorphic entomopathogenic fungus. No sexual reproduction has so far been observed in nature. The life cycle includes the following stages: Adhesion of infection propagules (e.g. conidium and blastospore) to the surface of the host cuticle, differentiation of infection structures (appressoria and penetration pegs), penetration of the cuticle using enzymes and mechanical force, colonization of the hemocoel and emergence of the conidiophores for external sporulation on the cadaver. |
| Infectivity, dispersal and colonisation ability: | Infectivity: Human and animal infection is highly unlikely as sporulation of *M. brunneum* occurs only between 25 – 30 °C and not at human body temperature. Dispersal: *Metarhizium* spores can be dispersed by a wide range of organisms such as adult cockchafer, earthworms, phoretic mites, acari, collembola, dipteran and coleopteran larvae. Colonisation ability: Despite wide distribution, it does not compete significantly with other fungi or bacteria as a saprophyte in soil. Colonisation is affected by biotic and abiotic factors such as presence of host, temperature, humidity and solar radiation. |
| Relationships to known plant, animal or human pathogens: | There are no records of the relationship to known plant or animal or human pathogens. |
| Genetic stability: | Stable strain, retains stability on the substrate Sabouraud dextrose agar. It has not been modified by genetic techniques and there are no known
| **Information on the production of relevant metabolites (especially toxins):** | The main metabolites produced by *M. brunneum* are destruxin A, B, D, and E, cytochalasin C, D and E, and swainsonine. The production of these metabolites is dependent on the strain, specific environment and nutritional conditions and is considered limited under field conditions. The toxicological relevance of destruxins and cytochalasins is not concluded upon. Swainsonine is considered to be toxicologically relevant. |
| **Resistance/ sensitivity to antibiotics / anti-microbial agents used in human or veterinary medicine:** | Inhibited by voraconazole and posaconazole. Reduction of growth or no spore formulation detected for itraconazole, micafungin and caspofungin. |
Summary of uses supported by available data (Regulation (EU) N° 283/2013, Annex Part B, point 3)

GranMetGR

| Crop and/or situation (a)                                                                 | Member State or Country | Product name | F || G || I (b) | Pests or Group of pests controlled (c) | Preparation | Application | Application rate per treatment | PHI (days) (m) | Remarks |
|-------------------------------------------------------------------------------------------|-------------------------|--------------|--------------|-----------------|-------------|-------------|---------------------------------|----------------|---------|
| Meadows, Grassland and Sport fields Golf courses Football grounds                         | EU-member states        | GranMet° GR  | F            | Popillia japonica (POPIJA) (Japanese beetle) White grubs Phyllopertha horticola, (PHPHIO) (Garden chafer) Amphimallon solstitialis (AMHISO) (Summer chafer) Amphimallon majalis (AMHMJ) (European chafer) | GR (Colonized barley grain) | Overall drilling* | BBCH 0-99 (including pre emergence) | 1-2 n.a. n.a. one application/year a) 1500 g biomass (dry wt.)/ha 5 x 10^{13} CFU/ha b) 1500 g biomass (dry wt.)/ha 5 x 10^{13} CFU/ha two applications/year a) 750 g biomass (dry wt.)/ha 2.5 x 10^{13} CFU/ha | *Manual application on small areas: After cutting the turf at 5-10 cm depth with suitable tools (spade, sod cutters or hand-held lawn edgers), GRANMET° GR should be spread evenly by hand into the soil slits which have to be closed after application. Or using aerification digging forks in order to pierce the soil surface (ca. 15 wholes/per m²). After application of GranMet°GR the wholes shall be filled and closed with soil. Mechanical application: |
Crop and/or situation (a) | Member State or Country | Product name | F G or I (b) | Pests or Group of pests controlled (c) | Preparation | Application | Application rate per treatment | PHI (days) (m) | Remarks |
|-----------------------------|-------------------------|--------------|--------------|------------------------------------------|-------------|------------|-------------------------------|----------------|---------|
|                             |                         |              |              |                                          |             |            |                               |                |         |
|                             |                         |              |              |                                          |             |            |                               |                |         |
|                             |                         |              |              |                                          |             |            |                               |                |         |

**Preparation**
- Type (d-f)
- Conc. a.s. (i)
- method kind (f-h)
- range of growth stages & season (j)
- number min-max (k)
- Interval between application (min)

**Application**
- g a.s./L min-max (l)
- Water L/ha min-max (l)

**Application rate per treatment**
- g/(kg) a.s./ha min-max (l)

**Remarks**
- b) 1500 g biomass (dry wt.)/ha
- 5 x 10^{13} CFU/ha

After deep grass cutting, aeration of the lawn and application of the granules and scattering them into the aeration holes.

Mechanical application at reseeding: GranMet°GR is incorporated to the ground with a mechanical slit seeder or seed drill machine (as seed grain seeders or cereal seeders). The seed machine must be provided with disks or coulters to incorporate the granules in the soil. The product should be applied into the soils cuts which have to be sealed with a roller.
| Crop and/or situation (a) | Member State or Country | Product name | F G or I (b) | Pests or Group of pests controlled (c) | Preparation | Application | Application rate per treatment | PHI (days) (m) | Remarks |
|--------------------------|--------------------------|--------------|--------------|--------------------------------------|-------------|------------|-------------------------------|----------------|---------|
| Maize                    | EU-member states         | GranMet°GR   | F            | Diabrotica virgifera (DIABVI) (Western corn rootworm) | GR (Colonized barley grain) | 9.91 x 10^{10} CFU/kg (30 g M. brunneum biomass/ kg GranMet °GR) | Overall drilling | Before sowing (April to June) | 1 | - | n.a. | n.a. | 1500 g biomass (dry wt.)/ha 5 x 10^{13} CFU/ha | Mechanical application before corn seeding: GranMet°GR is incorporated to the ground with a mechanical slit seeder or seed drill machine (as seed grain seeders or cereal seeders). The seed machine must be provided with disks or coulters to incorporate the granules in the soil. The product should be applied into the soils cuts which have to be sealed with a roller or a similar machine. Please refer to M-MP 6 for further information on the application. |
| Crop and/or situation (a) | Member State or Country | Product name | F or G or I (b) | Pests or Group of pests controlled (c) | Preparation | Application | Application rate per treatment | PHI (days) | Remarks |
|---------------------------|-------------------------|--------------|-----------------|--------------------------------------|-------------|-------------|---------------------------------|------------|---------|
| Wine grape and table grape | EU-member states | GranMet° GR | F Daktulosphaira vitifolae (VITEVI) | GR (Colonized barley grain) | 9.91 x 10^10 CFU/kg (30 g M. brunneum biomass/kg GranMet°GR) | Overall drilling | Spring (BBCH 01-69) | 1 | - | n.a. | n.a. | 1500 g biomass (dry wt.)/ha 5 x 10^{13} CFU/ha | GranMet°GR is applied with a sowing machine in combination with a rotary harrow for direct incorporation granules into soil. Or individual plant drilling. Please refer to M-MP 6 for further information on the application method and equipment |
| Ornamentals(floriculture, tree nurseries, and perennials) | EU-member states | GranMet° GR | G Popillia japonica (POPIJA) (Japanese beetle) | GR (Colonized barley grain) | 9.91 x 10^{10} CFU/kg (30 g M. brunneum biomass/kg GranMet°GR) | incorporation in (potting) soil | Before planting Jan-Dec | 1 | - | n.a. | n.a. | 36 kg biomass (dry wt.)/ha (1.2 x 10^{13} CFU/ha) | Application rate was calculated for a large pot of 50 cm with a soil volume of 30000 cm³/pot. Generally, application rate depends on the plant species, the pot size and soil volume per pot assuming an |
| Crop and/or situation (a) | Member State or Country | Product name | FG or I (b) | Pests or Group of pests controlled (c) | Preparation | Application | Application rate per treatment | PHI (days) (m) | Remarks |
|--------------------------|-------------------------|--------------|-------------|---------------------------------------|-------------|------------|--------------------------------|----------------|---------|
| Strawberry (FRAAN)       | EU-member states        | GranMet°GR   | G           | Otiorhynchus sp (OTISP)               | GR (Colonized barley grain) | 9.91 x 10^10 CFU/kg (30 g M. brunneum biomass/kg GranMet°GR) | incorporation in (potting) soil | Before transplanting, Jan-Dec | 1       | n.a.    | n.a.    | 13.2 kg biomass (dry wt.) /ha (4.4 x 10^{14} CFU/ha) | Application rate was calculated for a pot of 10cm with a soil volume of 400 cm^3/pot. Generally, application rate depends on pot size and soil volume per pot assuming an application rate of 1 kg/m^3 potting soil. |

(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure).
(b) Outdoor or field use (F), greenhouse use including walk-in tunnel (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) CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide.
(f) All abbreviations used must be explained.
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench.
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated.
(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypry). In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).
(j) Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application.
(k) Indicate the minimum and maximum number of applications possible under practical conditions of use.
(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha).
(m) PHI - minimum pre-harvest interval.
| Crop and/or situation (a) | Member State/Country | Product name | F G or I (b) | Pests or Group of pests controlled (c) | Preparation | Application | Application rate per treatment | PHI (days) (l) | Remarks: (m) |
|--------------------------|----------------------|--------------|-------------|----------------------------------------|-------------|------------|---------------------------------|----------------|--------------|
| Strawberry               | EU                   | Met52 granular/ Bio1020 | F          | Black vine weevil *Otiorhyncus sulcatus* | GR 2% = min 9x10¹¹ CFU/kg MIPC | Potting soil incorporation | All growth stages | Jan-Dec | 1-2 | 7 | 0.5 kg/m² (150 kg/ha) -1 kg/m³ (300 kg/ha) | 4.5x10¹¹ CFU/m³ (1.35x10¹⁴ CFU/ha) - 9x10¹¹ CFU/m³ (2.7x10¹⁴ CFU/ha) | No water | none |
| Strawberry               | EU                   | Met52 granular/ Bio1020 | F          | Black vine weevil *Otiorhyncus sulcatus* | GR 2% = min 9x10¹¹ CFU/kg MIPC | soil incorporation | All growth stages | Jan-Dec | 1-2 | 7 | 12.5 kg/ha - 25 kg/ha | 1.13x10¹² CFU/ha - 2.25x10¹³ CFU/ha | No water | none |
| Strawberry               | EU                   | Met52 granular/ Bio1020 | G          | Black vine weevil *Otiorhyncus sulcatus* | GR 2% = min 9x10¹¹ CFU/kg MIPC | Potting soil incorporation | All growth stages | Jan-Dec | 1-2 | 7 | 0.5 kg/m² (150 kg/ha) -1 kg/m³ (300 kg/ha) | 4.5x10¹¹ CFU/m³ (1.35x10¹⁴ CFU/ha) - 9x10¹¹ CFU/m³ (2.7x10¹⁴ CFU/ha) | No water | none |
| Currants and berries (raspberry, gooseberry, blueberry, and other berries) | EU | Met52 granular/ Bio1020 | F          | Black vine weevil *Otiorhyncus sulcatus* | GR 2% = min 9x10¹¹ CFU/kg MIPC | Potting soil incorporation | All growth stages | Jan-Dec | 1-2 | 7 | 0.5 kg/m² (150 kg/ha) -1 kg/m³ (300 kg/ha) | 4.5x10¹¹ CFU/m³ (1.35x10¹⁴ CFU/ha) - 9x10¹¹ CFU/m³ (2.7x10¹⁴ CFU/ha) | No water | none |

* Met52 granular is mixed with compost and then this compost is slightly incorporated in the top-soil or put as a topping on the soil.
| Crop and/or situation | Member State/Country | Product name | Pests or Group of pests controlled | Preparation | Application | Application rate per treatment | PHI (days) | Remarks: |
|-----------------------|----------------------|-------------|-----------------------------------|-------------|------------|-----------------------------|-----------|---------|
| **Currants and berries (raspberry, gooseberry, blueberry, and other berries)** | EU | Met52 granular/ Bio1020 | Black vine weevil *Otiorhynchus sulcatus* | GR | 2% = min 9x10^{11} CFU/kg MPCR | soil incorporation | All growth stages Jan-Dec | 1-2 | 7 | 12.5 kg/ha - 25 kg/ha | 1.13x10^{13} CFU/ha - 2.25x10^{13} CFU/ha | No water | none | This can be done either manually or with various mechanical equipment. Eg. Seeders or drills with mounted disks to incorporate the granules/compost in the soil. The equipment would typically also have rolls to compress the soil again.
| Crop and/or situation (a) | Member State/Country | Product name | F G or I (b) | Pests or Group of pests controlled (c) | Preparation | Application | Application rate per treatment | PHI (days) (l) | Remarks: (m) |
|--------------------------|----------------------|--------------|--------------|----------------------------------------|-------------|------------|-------------------------------|---------------|-------------|
|                          |                      |              | G            | Black vine weevil *Otiorhyncus sulcatus* |             |            |                               |               |             |
| Currants and berries (raspberry, gooseberry, blueberry, and other berries) | EU | Met52 granular/ Bio1020 | GR | 2% = min 9x10^{11} CFU/kg MPCR | Potting soil incorporation | All growth stages | 1-2 | 7 | 0.5 kg/m^3 (150 kg/ha) - 1 kg/m^3 (300 g/ha) | 4.5x10^{11} CFU/m^3 (1.35x10^{14} CFU/ha) - 9x10^{11} CFU/m^3 (2.7x10^{14} CFU/ha) | No water, none |
|                          |                      |              | G            | Black vine weevil *Otiorhyncus sulcatus* |             |            |                               |               |             |
| Floriculture and ornamental crops | EU | Met52 granular/ Bio1020 | GR | 2% = min 9x10^{11} CFU/kg MPCR | Potting soil incorporation | All growth stages | 1-2 | 7 | 0.5 kg/m^3 (150 kg/ha) - 1 kg/m^3 (300 g/ha) | 4.5x10^{11} CFU/m^3 (1.35x10^{14} CFU/ha) - 9x10^{11} CFU/m^3 (2.7x10^{14} CFU/ha) | No water, none |

mounted disks to incorporate the granules/compost in the soil. The equipment would typically also have rolls to compress the soil again.

second application of the product, it is achieved at repotting while adding the media to the existing pot/plant.

Met52 granular is mixed with compost and then this compost is slightly incorporated in the top-soil or put as a topping on the soil. This can be done either manually or with various mechanical equipment. Eg.
| Crop and/or situation (a) | Member State/Country | Product name | F G or I (b) | Pests or Group of pests controlled (c) | Preparation | Application | Application rate per treatment | PHI (days) (l) | Remarks: (m) |
|--------------------------|----------------------|--------------|-------------|----------------------------------------|-------------|------------|-------------------------------|----------------|-------------|
| Floriculture and ornamental crops | EU | Met52 granular/ Bio1020 | F | Black vine weevil *Otiorhyncus sulcatus* | GR | 2% = min 9x10¹¹ CFU/kg MPCP | soil incorporation | All growth stages Jan-Dec | 1-2 | 7 | 12.5 kg/ha - 25 kg/ha | 4.5x10¹¹ CFU/m³ (1.35x10¹² CFU/ha) - 9x10¹¹ CFU/m³ (2.7x10¹⁴ CFU/ha) | No water | none |

Seeders or drills with mounted disks to incorporate the granules/compost in the soil. The equipment would typically also have rolls to compress the soil again.

| Floriculture and ornamental crops | EU | Met52 granular/ Bio1020 | G | Black vine weevil *Otiorhyncus sulcatus* | GR | 2% = min 9x10¹¹ CFU/kg MPCP | Potting soil incorporation | All growth stages Jan-Dec | 1-2 | 7 | 0.5 kg/m³ (150 kg/ha) - 1 kg/m³ (300 kg/ha) | 4.5x10¹¹ CFU/m³ (1.35x10¹² CFU/ha) - 9x10¹¹ CFU/m³ (2.7x10¹⁴ CFU/ha) | No water | none |

second application of the product, it is achieved at repotting while adding the media to the existing pot/plant.

| Tree nursery crops and perennials | EU | Met52 granular/ Bio1020 | F | Black vine weevil *Otiorhyncus sulcatus* | GR | 2% = min 9x10¹¹ CFU/kg MPCP | Potting soil incorporation | All growth stages Jan-Dec | 1-2 | 7 | 0.5 kg/m³ (150 kg/ha) - 1 kg/m³ (300 kg/ha) | 4.5x10¹¹ CFU/m³ (1.35x10¹² CFU/ha) - 9x10¹¹ CFU/m³ (2.7x10¹⁴ CFU/ha) | No water | none |

Met52 granular is mixed with compost and then this compost is slightly incorporated in the top-soil or put as a topping on the soil.
| Crop and/or situation (a) | Member State/Country | Product name | F G or I (b) | Pests or Group of pests controlled (c) | Preparation | Application | Application rate per treatment | PHI (days) (l) | Remarks: (m) |
|--------------------------|----------------------|--------------|--------------|----------------------------------------|-------------|------------|-------------------------------|----------------|-------------|
| Tree nursery crops and perennials | EU | Met52 granular/ Bio1020 | F | Black vine weevil *Otiorhyncus sulcatus* | GR | 2% = min 9x10^{11} CFU/kg MPCP soil incorporation | All growth stages Jan-Dec | 1-2 | 7 | 12.5 kg/ha - 25 kg/ha | 1.13x10^{13} CFU/ha - 2.25x10^{13} CFU/ha | No water | none |
| Tree nursery crops and perennials | EU | Met52 granular/ Bio1020 | G | Black vine weevil *Otiorhyncus sulcatus* | GR | 2% = min 9x10^{11} CFU/kg MPCP Potting soil incorporation | All growth stages Jan-Dec | 1-2 | 7 | 0.5 kg/m^3 (150 kg/ha) - 1 kg/m^3 (300 kg/ha) | 4.5x10^{11} CFU/m^3 (1.35x10^{11} CFU/ha) - 9x10^{13} CFU/m^3 (2.7x10^{11} CFU/ha) | No water | none |
| Potato | EU | Met52 granular/ Bio1020 | F | Wireworm *Agriotes sp.* | GR | 2% = soil incorporation | All growth stages Jan-Dec | 1-2 | 7 | 12.5 kg/ha - 25 kg/ha | 1.13x10^{13} CFU/ha - 2.25x10^{13} CFU/ha | No water | none |

This can be done either manually or with various mechanical equipment. Eg. Seeders or drills with mounted disks to incorporate the granules/comp post in the soil. The equipment would typically also have rolls to compress the soil again.
| Crop and/or situation (a) | Member State/Country | Product name | F G or I (b) | Pests or Group of pests controlled (c) | Preparation | Application | Application rate per treatment | PHI (days) | Remarks: (m) |
|--------------------------|----------------------|--------------|-------------|----------------------------------------|-------------|------------|-------------------------------|-----------|--------------|
|                          |                      |              |             |                                        |             |           |                               |           |              |

*For incorporation in potting soil, the application rate per hectare depends on pot size (number of pots per hectare) and soil volume per pot

(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), greenhouse use including walk-in tunnel (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) CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant-type of equipment used must be indicated
(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypry). In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).
(j) Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of applications possible under practical conditions of use
(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha
(m) PHI - minimum pre-harvest interval

min 9x10^11 CFU/kg MPCR
| Crop and/or situation | Member State/Country | Product code | F G or I (b) | Pests or Group of pests controlled (c) | Formulation | Application | Application rate per treatment | PHI (days) | Remarks: |
|-----------------------|----------------------|--------------|--------------|----------------------------------------|-------------|-------------|-------------------------------|-----------|---------|
| **Met52 OD/ Bio1020 OD** |                      |              |              |                                        |             |             |                               |           |         |
| Strawberry            | EU                   | Met52 OD     | G            | Thrips (Frankliniella occidentalis, Thrips spp.) Mites (Tetranychus urticae) | OD 11% = min 2x10^{12} CFU/L MCP | Foliar spray | All growth stages Jan-Dec | 1-10 | 0.5-1.25 L/ha - 5-12.5 L/ha | 1.0x10^{12} - 2.5x10^{12} CFU/ha - 1.0x10^{13} - 2.5x10^{13} CFU/ha | 300/1600 | none 0.031-0.417% |
| Fruiting vegetables of Cucurbitaceae | EU                   | Met52 OD     | G            | Thrips (Frankliniella occidentalis, Thrips spp.) Whiteflies (Trialeurodes vaporariorum, Bemisia tabaci) Mites (Tetranychus urticae) | OD 11% = min 2x10^{12} CFU/L MCP | Foliar spray | All growth stages Jan-Dec | 1-10 | 0.5-1.25 L/ha - 5-12.5 L/ha | 1.0x10^{12} - 2.5x10^{12} CFU/ha - 1.0x10^{13} - 2.5x10^{13} CFU/ha | 300/1500 | none 0.033-0.417% |
| Fruiting vegetables of Solanaceae | EU                   | Met52 OD     | G            | Thrips (Frankliniella occidentalis, Thrips spp.) Whiteflies (Trialeurodes vaporariorum, Bemisia tabaci) Mites (Tetranychus urticae) | OD 11% = min 2x10^{12} CFU/L MCP | Foliar spray | All growth stages Jan-Dec | 1-10 | 0.5-1.25 L/ha - 5-12.5 L/ha | 1.0x10^{12} - 2.5x10^{12} CFU/ha - 1.0x10^{13} - 2.5x10^{13} CFU/ha | 300/1500 | none 0.033-0.417% |
| Crop and/or situation                                                                 | Member State/Country | Product code | F G or I (b) | Pests or Group of pests controlled (c) | Formulation | Application | Application rate per treatment | PHI (days) | Remarks: |
|--------------------------------------------------------------------------------------|----------------------|--------------|-------------|--------------------------------------|-------------|-------------|---------------------------------|------------|---------|
| Floriculture and ornamental crops                                                    | EU                   | Met52 OD     | G           | Whiteflies (Trialeurodes vaporariorum, Bemisia tabaci) | OD          | Foliar spray | All growth stages | 1-10  | 3 |
|                                                                                      |                      |              |             |                                                     |             | Jan-Dec      |                   |            | 0.5-1.25 L/ha - 5-12.5 L/ha | 300/1500   | none    |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 1.0x10⁻¹² - 2.5x10⁻¹² CFU/ha |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 1.0x10⁻¹³ - 2.5x10⁻¹³ CFU/ha |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 300/1500             |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | none                |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 0.033-0.417%          |
| Tree nursery crops and perennials                                                    | EU                   | Met52 OD     | G           | Whiteflies (Trialeurodes vaporariorum, Bemisia tabaci) | OD          | Foliar spray | All growth stages | 1-10  | 3 |
|                                                                                      |                      |              |             |                                                     |             | Jan-Dec      |                   |            | 0.5-1.25 L/ha - 5-12.5 L/ha | 300/1500   | none    |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 1.0x10⁻¹² - 2.5x10⁻¹² CFU/ha |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 1.0x10⁻¹³ - 2.5x10⁻¹³ CFU/ha |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 300/1500             |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | none                |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 0.033-0.417%          |
| Grape                                                                                | EU                   | Met52 OD     | F           | Thrips (Frankliniella occidentalis, Thrips spp.)    | OD          | Foliar spray | All growth stages | 1-6   | 7 |
|                                                                                      |                      |              |             |                                                     |             | Jan-Dec      |                   |            | 0.5-1.25 L/ha - 3-7.5 L/ha | 300/1500   | none    |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 1.0x10⁻¹² - 2.5x10⁻¹² CFU/ha |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 1.0x10⁻¹³ - 2.5x10⁻¹³ CFU/ha |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 300/1500             |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | none                |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 0.033-0.417%          |
| Solanaceae                                                                           | EU                   | Met52 OD     | F           | Thrips (Frankliniella occidentalis, Thrips spp.)    | OD          | Foliar spray | All growth stages | 1-6   | 7 |
|                                                                                      |                      |              |             |                                                     |             | Jan-Dec      |                   |            | 0.5-1.25 L/ha - 3-7.5 L/ha | 300/1500   | none    |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 1.0x10⁻¹² - 2.5x10⁻¹² CFU/ha |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 1.0x10⁻¹³ - 2.5x10⁻¹³ CFU/ha |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 300/1500             |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | none                |
|                                                                                      |                      |              |             |                                                     |             |             |                   |            | 0.033-0.417%          |
| Crop and/or situation | Member State/Country | Product code | F G or I | Pests or Group of pests controlled | Formulation | Application | Application rate per treatment | PHI (days) | Remarks: |
|-----------------------|----------------------|--------------|----------|-----------------------------------|------------|------------|-------------------------------|------------|---------|
| Allium vegetables     | EU                   | Met52 OD     | F        | *Thrips tabaci* *Frankliniella occidentalis* | OD 11% = min 2x10^{12} CFU/L MPCP | Foliar spray All growth stages | 0.5-1.25 L/ha - 3-7.5 L/ha | 6274      | none    |

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(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), greenhouse use including walk-in tunnel (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) CropLife International Technical Monograph no 2, 6th Edition. Revised May 2008. Catalogue of pesticide
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated
(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).
(j) Growth stage range from first to last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of applications possible under practical conditions of use
(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha
(m) PHI - minimum pre-harvest interval
Further information, Efficacy. Representative products Met52® granular/ Bio1020® and Met52® OD

| Effectiveness (Regulation (EU) N° 284/2013, Annex Part A, point 6.2) |
|---------------------------------------------------------------|
| According to the latest guidance on the preparation of     |
| dossiers for the renewal of active substances,              |
| information on efficacy is not required (SANCO/10181/2013 –  |
| rev. 2.1, 13 May 2013). The representative products have      |
| all been authorised at Member State level for > 10 years and  |
| have therefore been assessed in line with Uniform Principles.|

Adverse effects on field crops (Regulation (EU) N° 284/2013, Annex Part A, point 6.4)

| The representative products have all been authorised at      |
| Member State level for > 10 years and have therefore         |
| been assessed in line with Uniform Principles. No unacceptable |
| adverse effects are known.                                   |

Observations on other undesirable or unintended side-effects (Regulation (EU) N° 284/2013, Annex Part A, point 6.5)

| The representative products have all been authorised at      |
| Member State level for > 10 years and have therefore         |
| been assessed in line with Uniform Principles. No unacceptable |
| side effects are known.                                     |

Classification and proposed labelling (Symbol, Indication of danger, Risk phrases, Safety phrases)

| with regard to physical/chemical data: | None |
|----------------------------------------|------|
| with regard to toxicological data:     | None, precautionary warning phrase for sensitization applies. |
| with regard to fate and behaviour:     | None |
| with regard to ecotoxicological data:  | None |

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)

| Manufactured microorganism (principle of method): | EF1α sequence analysis and subsequent comparison with sequences of references strains and simple sequence repeat (SSR, microsatellite) marker analysis to discriminate genotypes within individual *Metarhizium* species |
|---------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Impurities and contaminating microorganisms in manufactured material (principle of method): | Metabolites: Destruxins from solid and liquid culture filtrates using HPLC-DAD, UHPLC-DAD combined with off-line SPE, Ultrahigh-performance super-critical fluid chromatography photodiode array detector (UHPSFC-PDA). |
Cytochalasins from dust and solid and liquid culture filtrates using a multi analytical method with liquid chromatography/tandem mass spectrometry and HPLC-UV with internal standard

Contaminating microorganisms:
- BIPESCO5/F52: Standard microbiological methods from international official standardisation body (such as ISO methods)

**Microbial Pest Control Product (principle of method):**
The same methods as described for the active microorganism apply.

### Analytical methods for residues (viable and non-viable) in exposed compartments and organisms (MA 4.2 & MP 5.2)

| of the active microorganism (principle of method): | Selective media or DNA analysis (EF1α sequence and SSR microsatellite marker analysis) |
|---------------------------------------------------|--------------------------------------------------------------------------------------------|
| of relevant metabolites (principle of method):     | Destruxins: HPLC-UV method for crops, soil, water, insect cadavers QuEChERS method with UHPLC-QTOF-MS or UHPLC–MS/MS for crops |
|                                                   | Cytochalasins: Multi-target method using UHPLC-MS/MS or LC-MS/MS |

### Impact on Human and Animal Health (Regulation (EU) N° 283/2013, Annex Part B, point 5 and Regulation (EU) N° 284/2013, Annex Part B, point 7)

| Medical data: (including medical surveillance on manufacturing plant personnel) (MA 5.1.1) | A few cases of keratitis, rhinitis and skin infection in immunocompromised or injured people. No medical incidences in manufacturing plant personnel other than hypersensitivity. |
|------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sensitisation: (MA 5.2.1 & MP 7.2.3) | No study on sensitisation is required due to the absence of proper test methods to micro-organisms. Warning sentence for microbials is applicable: Contains *Metarhizium brunneum* BIPESCO 5/F52. Microorganisms may have the potential to provoke sensitising reactions Some reports of hypersensitivity in manufacturing personnel relating to incorrect use of PPE. |
| Acute oral infectivity, toxicity and pathogenicity: (MA 5.2.2.1 & MP 7.1.1) | No adverse effects, not pathogenic, not infective LD$_{50} > 1 \times 10^8$ CFU/animal |
| Acute intratracheal/inhalation infectivity, toxicity and pathogenicity: (MA 5.2.2.2 & MP 7.1.2) | Increased lung weight likely related to immune response to the test substance. Brown, mottled pigmentation of the lungs at day 21. LD$_{50} > 1.17 \times 10^8$ CFU/animal |
| Acute intravenous/intraperitonial infectivity: (MA 5.2.2.3) | Not pathogenic, not infective. Toxicity: Increase of relative spleen weights at day 3 in males and day 7 in females. LD$_{50} > 1 \times 10^7$ CFU/animal |
| Genotoxicity: (MA 5.2.3) | Supportive information: Negative results in a combined cytotoxicity and genotoxicity test (Vitotox Tests) with crude extracts and |
pure secondary metabolites (destruxin A, B, D, E, swainsonine, cytochalasin C).

Negative results in several Ames assays (standard Ames not acceptable; one Ames from literature; two Ames MPF™ I) with *M. anisopliae* crude extracts and/or filtrates containing secondary metabolites.

| Cell culture study: | Not required as *M. brunneum* BIPESCO 5/F52 is not an intracellular replicating micro-organism. |
|--------------------|---------------------------------------------------------------------------------------------------|
| Information on short-term toxicity and pathogenicity: | Not required. |
| Dermal toxicity: | Not toxic at >2 mg/kg bw spore powder |
| Specific toxicity, pathogenicity and infectivity: | Slightly irritating to the eyes |
| Genotoxicity – *in vivo* studies in germ cells: | Not required. |

**Reference values**

| AOEL: | Not required. |
| ADI: | Not required. |
| ARfD: | Not required. |

**Exposure (operator, workers, bystander, consumer):**

| Exposure (operator, workers, bystander, consumer): | No risk for operator, bystander, resident or worker is expected due to exposure to *M. brunneum* F52/BIPESCO5 after application for field uses. |
|----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (MA 6.1 & MP 7.3, 8.0) | In the absence of a quantitative risk assessment, the use of respiratory protective equipment for the operators might be considered to reduce the exposure via inhalation. Regarding to the presence in the product and possible production after application of secondary metabolites the risk assessment of operators (for GranMet GR only), workers, bystanders (for GranMet GR only) and residents cannot be concluded for both indoor and outdoor uses. |

**Residues** (Regulation (EU) N° 283/2013, Annex Part B, point 6 and Regulation (EU) N° 284/2013, Annex Part B, point 8)

| Viable residues: | Endophytic behavior was reported in laboratory and greenhouse studies, and in root tissues of plants grown in natural habitats. While transient presence seems possible, persistence and multiplication on aerial parts of crops is considered not very likely under field conditions. However, in root tissues and for greenhouses uses, endophytic behavior, i.e. colonisation and growth on plant parts cannot be excluded. |
|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                      | As *M. brunneum* BIPESCO 5/F52 is not toxic, pathogenic or infective a risk to consumer due to viable residues is not expected. |
### Fate and Behaviour in the Environment

(Regulation (EU) N° 283/2013, Annex Part B, point 7 and Regulation (EU) N° 284/2013, Annex Part B, point 9)

| Persistence and multiplication (competitiveness) in soil | Soil |
|----------------------------------------------------------|------|
| A slow but steady decline of densities of *Metarhizium brunneum* BIPESCO 5/F52 upon application is expected. As a rough estimate, a time period of 10 years post-treatment is given for the densities to reach upper natural background levels. |

**PEC**<sub>S</sub>

For the proposed uses in permanent greenhouses and walk-in tunnels of all representative formulations uses PEC<sub>S</sub> calculated for the uses in the field cover these situations. It is estimated that it takes approximately 10 years for densities to reach the natural background level upon application. However, for microbial pest control agents it is not scientifically justified to determine plateau concentrations, as the long-term population densities are influenced by a myriad of biotic and abiotic factors (of which the host population density is assumed to be an important factor for *Metarhizium brunneum*) and cannot be predicted using kinetic models.

For field applications, PEC<sub>S</sub> values are determined based on the assumptions of no interception, no growth or degradation as follows:

\[
\text{PEC}_{\text{soil}} \text{ (CFU/ kg dry soil) } = \ AR \times n \text{ per } Y/10.000 \times d \times \rho
\]

- AR is application rate (CFU/ha; using the highest concentration of the microorganism according to the product specifications)
- n per Y is number of applications per year
- 10.000 is the conversion factor from ha to m\(^2\)
- d is the thickness of the soil layer (default of 0.05 m)
- ρ is the density of soil (default of 1500 kg/m\(^3\))
| PPP        | Method kind     | Max number applications | Application rate per application | PECs [CFU/kg soil] |
|------------|-----------------|-------------------------|----------------------------------|--------------------|
| Met52 GR   | potting soil incorporation | 2 | $1.5 \times 10^{12}$ CFU/ m³ | $2.0 \times 10^9$ |
|            | soil incorporation     | 2 | $3.75 \times 10^{13}$ CFU/ha | $1.0 \times 10^8$ |
| Met52 OD   | foliar spray       | 6 | $1.125 \times 10^{13}$ CFU/ha | $9.0 \times 10^7$ |
| GranMet GR | soil incorporation  | 1 | $5 \times 10^{13}$ CFU/ha  | $6.67 \times 10^7$ |

Persistence and multiplication (competitiveness) in water: *Metarhizium brunneum* BIPESCO 5/F52 can persist for at least six months in water. (Note the evidence for this was an unpublished study that had not been carried out in an officially recognized testing facility. Consequently there is a data gap relating to the lack of quality assurance regarding this evidence). Growth is limited amongst others by the availability of nutrients and carbon sources. No instances of population density increase of *Metarhizium brunneum* through infection of host species have been reported for aquatic systems.

**PEC\text{sw}**

Values for field applications were determined using BBA\textsuperscript{1} drift values in combination with the TOXSWA standard ditch (30 cm deep with a slope of 45 degrees and volume of 210 L/m\textsuperscript{2}) and worst-case application scenario in which no growth or degradation is assumed. For the proposed field application uses based on soil application no exposure route through drift is assumed. For permanent greenhouse uses an emission percentage of 0.1% was used (irrespective of application method). For walk-in tunnels the relevant BBA drift value for crops >50cm high\textsuperscript{1} was used.

| PPP        | Crop                                    | Use | Application method | application rate | Drift/emission [%] | PEC\text{sw} [CFU/L] |
|------------|-----------------------------------------|-----|-------------------|------------------|-------------------|---------------------|
| Met52 GR   | Strawberry, Currants and berries, Floriculture and ornamental crops, Tree nursery crops and perennials | G   | Incorporation in potting soil | $9.0 \times 10^{14}$ CFU/ha (= $3 \times 10^{12}$ CFU/ m\textsuperscript{3}) per crop/season | 0.1                | $4.29 \times 10^5$ |
| Met52 OD   | Strawberry, fruiting vegetables of Cucurbitaceae & Solanaceae, Floriculture and | G   | foliar spray       | $1.125 \times 10^{14}$ CFU/ha per crop/season | 0.1                | $5.36 \times 10^4$ |
|            |                                         |     |                   | $6.41^*$         |                   | $3.44 \times 10^6$ |

\textsuperscript{1} Ganzelmeier and Rautmann drift values according to the BBA (Federal Biological Agency of Agriculture and Forestry, Germany) 2000: Bekanntmachung des Verzeichnisses risikomindernder Anwendungsbedingungen für Nichtzielorganismen. Bundesanzeiger 100: 9878-9880.
Peer review of the pesticide risk assessment of the active substance \textit{Metarhizium brunneum} BIPESCO 5/F52

ornamental crops, Tree nursery and perennials

| Plant          | Category       | Test substance | Dose          | Persistence and multiplication (competitiveness in air) | Mobility: |
|----------------|----------------|----------------|---------------|---------------------------------------------------------|-----------|
| Grapevine, Solanaceae, Allium vegetables | F | foliar spray | 6.75 \times 10^{13} CFU/ha per crop/season | \textit{Metarhizium} cannot multiply in air due to a lack of nutrients and carbon. Fungal spores are extremely susceptible to solar radiation; numbers of viable spores in air will be quickly reduced under influence of solar radiation. | Dispersal can occur through air or by animal vectors such as arthropods or earthworms. It is not expected that \textit{Metarhizium brunneum} BIPESCO 5/F52 will be transported to deeper soil layers and affect groundwater upon application. |
| GranMe t GR | G | Incorporation in potting soil (50 cm diameter pots) | 1.2 \times 10^{15} CFU/ha | * Drift value based on the BBA drift value for use in Grapevine, late application, 3 meter distance |
| Strawberry | G | Incorporation in potting soil (10 cm diameter pots) | 4.4 \times 10^{14} CFU/ha | |

Dose rate

| Dose rate | Test substance | Category (e.g. insectivorous bird and species) | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|-----------|----------------|-----------------------------------------------|------------|-----------------------------------------------------------------------------------|
| Limit dose: 3.5 \times 10^8 CFU/kg bw/day for five days | \textit{M. anisopliae} var. anisopliae (\textit{M. brunneum}) strain F52. | Omnivorous bird (Colinus virginianus) | 5-d LC_{50} of > 3.5 \times 10^8 CFU/g bw/day No signs of toxicity. No effects on appearance, behaviour and feed consumption. No evidence of pathogenicity or replication of effects |
Limit dose: 1x10^8 CFU/animal/day to animals of ca. 0.2 kg bw, single exposure

*M. anisopliae var. anisopliae (M. brunneum) strain F52.*

Rat

Single exposure, test continued to clearance

oral LD_{50} of >5x10^8 CFU/kg bw
No death
No toxicity
No pathogenicity

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### Effects on aquatic organisms (MA 8.2 & 10.2)

| Group                  | Test substance                                                                 | Time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|------------------------|-------------------------------------------------------------------------------|------------|-----------------------------------------------------------------------------------------|
| **Laboratory tests**   |                                                                                |            |                                                                                         |
| **Fish**               |                                                                                |            |                                                                                         |
| *Oncorhynchus mykiss*  | *M. anisopliae var. anisopliae (M. brunneum) strain BIPESCO 5/F52.*           | 30 days    | Toxicity, infectiveness and pathogenicity: 30-d NOEC (mortality) is ≥ 3.7x10^8 CFU/L. Surface layer of spores visible, so actual exposure may be much lower than nominal. No infectivity and pathogenicity observed in the study |
| *Oncorhynchus mykiss*  | Met52 EC containing 3.2x10^{12} CFU/kg product of *Metarhizium anisopliae var. anisopliae F52* | 4 days     | Toxicity: 4-d LC50 (mortality) is > 100 mg product/L                                      |
| **Invertebrates**      |                                                                                |            |                                                                                         |
| *Daphnia magna*        | *M. anisopliae var. anisopliae (M. brunneum) strain BIPESCO 5/F52.*           | 21 days    | Toxicity, infectiveness and pathogenicity: 21-d NOEC (reproduction, length, weight) 3.5x10^8 CFU/L (5.0 mg a.s./L). Surface layer of spores visible, so actual exposure may be much lower than nominal and adverse effects on reproduction observed at the highest concentrations (≥ 7 x 10^8 CFU/L) possibly have been caused by physical rather than toxicological effects. No infectivity and pathogenicity observed in the study |
| **Algae**              |                                                                                |            |                                                                                         |
| *Pseudokirchneriella subcapitata* | *M. anisopliae var. anisopliae (M. brunneum) strain BIPESCO 5/F52.* | 4 days     | Toxicity, infectiveness and pathogenicity: 4-d NOEbC 4.4x10^{10} CFU/L. Surface layer of spores visible, so actual exposure may be much lower than nominal. No pathogenicity observed in the study |
| **Aquatic macrophytes**|                                                                                |            | No tests submitted. Not required. Based on literature, no adverse effects to aquatic macrophytes are expected. |
Effects on bees (MA 8.3 & MP 10.3)

| Test Substance | Route/time-scale | Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects) |
|----------------|------------------|-------------------------------------------------------------------------------------------|
| **Honeybees**  |                  |                                                                                           |
| *M. anisopliae* var. *anisopliae* F52 spore powder | oral and contact/16 days | NOER > 6000 CFU/larva (>0.086 μg a.s./bee larva). No adverse effects observed. Infectivity or pathogenicity was not assessed in the study. |
| **Bumble bees** |                  |                                                                                           |
| *M. anisopliae* var. *anisopliae* F52 spore powder | Contact/6wk | NOEC = 9.3 x 10⁶ CFU/bumblebee (from contact with powder containing 10⁷ CFU/g). Indication of infectivity and pathogenicity in the highest dose. |

Toxicity, infectivity and pathogenicity to honeybees cannot be excluded. Relevant for all the representative field and walk-in tunnel uses evaluated (data gap).

Effects on terrestrial arthropods other than bees (MA 8.4 & MP 10.4)

Studies tested with the relevant strain BIPESCO/F52 (spore powder); when a product (with the relevant strain) was tested, this is indicated in the table):
| Species | Exposed life stage | Study type | Exposure duration | Effects / LR<sub>50</sub> | Infectivity/pathogenicity |
|---------|--------------------|------------|-------------------|-----------------------------|--------------------------|
| *Aphidius rhopalosiphi* (parasitic wasp) | Adults | Laboratory glass plate | 48 hours | No increased mortality at 0.0185 × 10<sup>14</sup>, 0.0556 × 10<sup>14</sup>, 0.167 × 10<sup>14</sup>, 0.5 × 10<sup>14</sup>, and 1.5 × 10<sup>14</sup> CFU/ha. LR<sub>50</sub> > 1.5 × 10<sup>14</sup> CFU/ha 93.75 kg/ha | No toxicity. Study duration too short to properly study infectivity and pathogenicity. |
| *Typhlodromus pyri* (predatory mite) | Protonymphs | Laboratory glass plate | 7 days | No increased mortality at 0.0185 × 10<sup>14</sup>, 0.0556 × 10<sup>14</sup>, 0.167 × 10<sup>14</sup>, 0.5 × 10<sup>14</sup>, and 1.5 × 10<sup>14</sup> CFU/ha. No signs of clinical toxicity or abnormal behaviour. LR<sub>50</sub> > 1.5 × 10<sup>14</sup> CFU/ha 93.75 kg/ha | No indications for pathogenicity/infectivity. |
| *Chrysoperla carnea* (green lacewing) | Larvae | Dietary exposure (moth eggs and water meal mixed with a.s.) | 12 days | No increased mortality at 4.2×10<sup>5</sup>, 4.2×10<sup>6</sup> and 4.2×10<sup>7</sup> CFU/g feed. No signs of clinical toxicity or abnormal behaviour. | Gross observations did not show signs of infectivity or pathogenicity. |
| *Hippodamia convergens* (ladybird beetle) | Adults | Dietary exposure (honey mixed with a.s.) | 22 days | No increased mortality at 4.2×10<sup>5</sup>, 4.2×10<sup>6</sup> and 4.2×10<sup>7</sup> CFU/g feed. Almost no signs of clinical toxicity or abnormal behaviour. Gross observations did not show signs of infectivity or pathogenicity. | |
| *Nasonia vitripennis* (parasitic wasp) | Adults | Dietary exposure (honey mixed with a.s.) | 26 days | No increased mortality at 4.2×10<sup>5</sup>, 4.2×10<sup>6</sup> and 4.2×10<sup>7</sup> CFU/g feed. No clinical signs attributed to test substance. Gross observations did not show signs of infectivity or pathogenicity. | |
| *P. persimilis* (predatory mite) (Met 52 EC) | Adults | Contact exposure (overspray of 2 mL per individual, or contact with dried residues on leaf discs) | 72 h | Test concentration 5×10<sup>9</sup> CFU/L. Corrected mortality 7.74% for direct application and 11.52% for dried residues. Acute toxicity effects. Results on infectivity/pathogenicity can be used as supplemental information only, as the identity of the microorganism was not confirmed during or after the test. See Vol.3 MA B.9 | |
| Species                  | Adults | Contact exposure (overspray of 2 mL per individual, or contact with dried residues on leaf discs) | Test concentration 5x10⁹ CFU/L. Corrected mortality 2.8% for direct application and 1.21% for dried residues. | Acute toxicity effects. |
|-------------------------|--------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------|
| N. californicus         | Adults | Contact exposure (overspray of 2 mL per individual, or contact with dried residues on leaf discs) | Test concentration 5x10⁹ CFU/L. Corrected mortality 2.8% for direct application and 1.21% for dried residues. | Acute toxicity effects. |
| O. albidipennis         | Adults | Dietary exposure (inoculated thrips larvae)                                                    | Test done 0-24, 24-48 h, 48-72 h and 72-96 h after larvae inoculation                          | Not assessed            |
| Coleoptera (Carabidae)  | Field population | Application on the ground of conidial suspension, 10¹³ CFU/ha (10² - 10⁹ CFU/g soil) and 6x10¹⁵ CFU/ha (2x10⁵ CFU/g soil) | Single application of M. anisopliae BIPESCO O 5/F52, monitoring one or two months later         | Mortality: Low dose: 0-0.4%, High dose: 0-5.6% (Control: 0.9-1.1%) |
| Staphylinidae in lucerne field | Field population | Application on the ground of conidial suspension, 10¹³ CFU/ha (10² - 10⁹ CFU/g soil) and 6x10¹⁵ CFU/ha (2x10⁵ CFU/g soil) | Single application of M. anisopliae BIPESCO O 5/F52, monitoring one or two months later         | Mortality: Low dose: 0-4%, High dose: 0.7-16% (Control: 0.0-6.2%) |

Results on infectivity/pathogenicity can be used as supplemental information only, as the identity of the microorganism was not confirmed during or after the test. See Vol.3 MA B.9
| Taxa Category | Description | Application Method | Study Details | Observations |
|--------------|-------------|--------------------|---------------|--------------|
| 22 taxa of non-target ground-dwelling or emerging arthropods (most abundant taxa belonged to the Diptera, Araneae and Hemiptera) | Soil invertebrate community (soil surface and plant dwelling arthropods and arthropods emerging from soil) | Application to soil via colonised barley seeds | Field study: Single application, weekly monitoring ca. 1 to 3 months after sowing | No adverse effects at 3.5 x1013 spores/ha |
| Soil fungus and invertebrate community in field study | Application to soil via colonised barley seeds | Single application resulting in a density of 10^4 - 10^5 CFU g-1 dry soil. Abundance and diversity assessed three months later. | M. anisopliae has no adverse effects on soil fungal diversity and abundance of several soil meso- and macrofauna taxa | No adverse effects (abundance and diversity are indirect measures for adverse effects via toxicity, infectivity and pathogenicity). |

In addition to the above, several studies with non-target arthropods are included in the different Vol.3 B.9 sections (MA and MP) that were considered less reliable due to study deficiencies and/or because they were not performed with the relevant strain (or an unknown strain). These studies are therefore useful as supplemental information only.

### Effects on earthworms (MA 8.4 & MP 10.4)

No signs of toxicity, infectivity or pathogenicity to earthworms of the F52 strain of *Metarhizium anisopliae* var. *anisopliae* (brunneum) (spore powder) at concentrations \( \leq 7.0 \times 10^{10} \text{CFU/kg soil dw} \) (1000 mg a.s./kg soil dw).

### Effects on soil microorganisms (MA 8.6 & MP 10.6)

Additional information on the impact on soil microorganisms should be submitted (relevant for representative field- and walk-in tunnel uses evaluated) (data gap)

### Additional studies (MA 8.7 & MP 10.7)

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