European Commission

List of Endpoints

- *Beauveria bassiana* PRRI 5339 -

Rapporteur Member State: The Netherlands

December 2016

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Rapporteur Member State	Month and year	Microbial or Viral Agent (Name)
The Netherlands	December 2016	*Beauveria bassiana* PPRI 5339

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

Active microorganism:	*Beauveria bassiana* strain PPRI 5339
Function (*e.g.* control of fungi):	Insecticide
Rapporteur Member State:	Netherlands
Co-rapporteur Member State:	-
Identity of the Microbial or Viral Agent used in plant protection / Active Substance) (Regulation (EU) N° 283/2013, Annex Part B, point 1; OECD IIM Point 1)	
Name of the organism:	*Beauveria bassiana* strain PPRI 5339
Taxonomy:	Phylum: Deuteromycota, Class: Hyphomycetes, Order: Moniliales, Family: Moniliaceae, Genus: Beauveria
Species, subspecies, strain:	Species: *Beauveria bassiana* Strain: strain PPRI 5339
Identification / detection:	Identification of strain PPRI 5339 was based on 18S, ITS1, 5.8S and ITS2 rRNA gene sequencing.
Culture collection:	Deposited at Agricultural Research Culture Collection (NRRL) International Depositary Authority under accession number: NRRL 50757
Minimum and maximum concentration of the MPCA used for manufacturing of the formulated product (cfu; g/kg):	$>1.0 \times 10^{11}$ CFU/g
Identity and content of relevant impurities, additives, contaminating organisms in the technical grade of MPCA:	No relevant impurities, additives or contaminating organisms.
Is the MPCA genetically modified; if so provide type of modification	Not genetically modified.

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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:	*Beauveria bassiana* strain PPRI 5339 is an entomopathogenic fungus which is a common pathogen of many insect species. It is a ubiquitous and cosmopolitan fungus, found in a variety of soils from a broad range of climatic conditions in most countries.
Target organism(s):	Whiteflies *(Aleurodidae)* and thrips *(Thysanoptera)*
Mode of action:	The conidia of *Beauveria bassiana* strain PPRI 5339 adhere to the insect cuticle by means of hydrophobic interaction between the spore wall and epicuticle lipids. The conidia germinate, and the germ tube penetrates the cuticle, using a specific series of enzymes, which in turn degrade the lipids, protein and chitin in the insect cuticle. In the insect body, the fungus multiplies in the haemocœl as a blastospore, or yeast-like cell, and enzymes begin to destroy the internal structures of the host insect causing morbidity within 36 - 72 hours. Reduced feeding and immobility are rapidly evident, and the insect dies within between 4 to 10 days post-infection. The time to death will depend on the insect species, age and conidial dose. After death, the blastospores transform into mycelia, which emerge through the cuticle and form spores. These cover the cadaver as a characteristic white growth. Sporulation occurs only in conditions of high humidity.
Host specificity:	*Beauveria bassiana* is specific to insects. Not pathogenic to humans or plants.
Life cycle:	The conidia adhere to the insect cuticle, germinate and penetrate in the insect body, where they replicate as yeast- like cells (blastospores) and destroy the internal structures, causing morbidity within 36 - 72 hours. After death of the insect, the blastospores transform into mycelia, which emerge through the cuticle and form spores.
Infectivity, dispersal and colonisation ability:	*Beauveria bassiana* strain PPRI 5339 has an optimal growth temperature range between 22 °C and 28 °C. The conidia are not able to germinate or grow at ≥32 °C and are sensitive to UV light. *Beauveria bassiana* strain PPRI 5339 was shown to be unstable stable in sterile and non-sterile tap and in both light and darkness. Under natural conditions, the conidia of *Beauveria bassiana* germinate and die in the absence of a suitable host insect within two days, and in aqueous environments. The conidia survive naturally in sheltered habitats and require specific environmental conditions of moderate temperature, high humidity and high insect population density for epizootic spread and dispersal.
Relationships to known plant, animal or human pathogens:	*Beauveria bassiana* strain PPRI 5339 is not related to any known human or plant pathogen.
Genetic stability:	Fungi possess low genome plasticity due to the narrower scope of mechanisms available to eukaryotic cells for the incorporation of DNA. In line with this, Horizontal Gene Transfer (HGT) is still considered to be anecdotal in fungi and to date has not been shown to occur in *Beauveria bassiana*.

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Information on the production of relevant metabolites (especially toxins):	Beauvericin: max <0.5 mg/kg
Resistance/ sensitivity to antibiotics / anti-microbial agents used in human or veterinary medicine:	*Beauveria bassiana* is an opportunistic pathogen that has very rarely caused infections in humans and is not related to any known human pathogen. No specific information addressing the issue of resistance/sensitivity to antibiotics and other antimicrobial agents has been found in the open literature. Spread of resistance (genes) to antimyotic agents between fungal species is not of concern (as for bacteria) with regard to antibiotics.

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Summary of uses supported by available data (Regulation (EU) N° 283/2013, Annex Part B, point 3; OECD IIM Point 3)

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**			**Application rate**			**PHI** (days)	**Remarks:**
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
1	EU (Protected crops)	Solanaceae Tomato *Solanum lycopersicum* (LYPES) Sweet pepper *Capsicum annuum* (CPSAN) Aubergine *Solanum melongena* (SOLME) Solanaceae Edible and processed fruits	G	Thrips e.g. *Frankliniella occidentalis* (FRANOC) and *Thrips tabaci* (THRITB) Whitefly e.g. *Trialeurodes vaporariorum* (TRIAVA), *Bemisia tabaci* (BEMITA) and *Bemisia argentifolii* (BEMIAR)	Spraying SP	BBCH 11-89 (jan-dec)	a) 122 (3 days) b) 122	a) 2.5 b) 305	a) 100 ($1*10^{13}$ CFU /ha) b) 12200 ($1.22*10^{15}$ CFU/ha)	500-2500	0	Dose rate is 0.1% (0.5L product per 500 L water/2.5L product per 2500 L water) ; 100 mL product per 100 L of water on label. Water volume used depends on development stage of crop (low volume – early crops stage, high volume – late crop stage).
2	EU (Protected crops)	Cucurbitaceae Cucumber *Cucumis sativus* (CUMSA) Edible and processed fruits	G	Thrips e.g. *Frankliniella occidentalis* (FRANOC) and *Thrips tabaci* (THRITB) Whitefly e.g. *Trialeurodes vaporariorum* (TRIAVA), *Bemisia tabaci* (BEMITA) and *Bemisia argentifolii* (BEMIAR)	Spraying SP	BBCH 11-89 (jan-dec)	a) 122 (3 days) b) 122	a) 2.5 b) 305	a) 100($1*10^{13}$ CFU /ha) b) 12200 ($1.22*10^{15}$ CFU/ha)	500-2500	0	Dose rate is 0.1% (0.5L product per 500 L water/2.5L product per 2500 L water) ; 100 mL product per 100 L of water on label. Water volume used depends on development stage of crop (low volume – early crops stage, high volume – late crop stage).

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3	EU (Protected crops)	Ornamentals (e.g. CHYIN, CHYHO, GEBSS roses) Non-edible/Non-food crop	G	Thrips e.g. *Frankliniella occidentalis* (FRANOC) and *Thrips tabaci* (THRITB) Whitefly e.g. *Trialeurodes vaporariorum* (TRIAVA), *Bemisia tabaci* (BEMITA) and *Bemisia argentifolii* (BEMIAR)	Spraying SP	BBCH 11-99 (jan-dec)	a) 122 (3 days) b) 122	a) 2.5 b) 305	a) 100 b) 12200	500-2500	0	Dose rate is 0.1% (0.5L product per 500 L water/2.5L product per 2500 L water) ; 100 mL product per 100 L of water on label. Water volume used depends on development stage of crop (low volume – early crops stage, high volume – late crop stage).

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

with regard to physical/chemical data:	Unclassified
with regard to toxicological data:	Unclassified
with regard to fate and behaviour:	Unclassified
with regard to ecotoxicological data:	Unclassified

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)

Manufactured microorganism (principle of method):	Analytical methods for the identification of the technical *Beauveria bassiana* strain PPRI 5339 include the following: • Characterisation by microscopic examination of the visual morphology • Sequencing of the ITS ribosomal region including the 3' end of the 18S region, the ITS1 region, the 5.8S region and the ITS2 region. Determination of fungal spore-count and spore viability by haemocytometer counts and *in vitro* germination assay.
Impurities and contaminating microorganisms in manufactured material (principle of method):	Enumeration of microbial contaminants (bacteria and fungi), determining the presence/absence of enteric bacteria such as *Shigella, Salmonella* and *Vibrio* spp. by plating out on selective media.
Microbial Pest Control Product (principle of method):	Analytical methods for the formulation are as used for the active substance.

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)

of the active microorganism (principle of method):	Not required
of relevant metabolites (principle of method):	None relevant

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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; OECD IIM 5.1)	No adverse effects in employees who had been in occupational contact with strain PPRI 5339. For *Beauveria bassiana* in general, few cases of local (eye) and systemic infections were reported with the latter occurring only in immunocompromised or severely ill patients. Evidence of allergenicity by respiratory or skin contact.
Sensitisation: (MA 5.2.1 & MP 7.2.3; OECD IIM 5.2 & IIIM 7.1.6)	Microorganisms may have the potential to provoke sensitising reactions. Evidence of allergenicity by inhalation in laboratory animals and humans and indications of allergic skin reactions in humans. Buehler test in Guinea pigs negative for the micro-organism but positive for the formulation Broadband® /BAS 408 00 I.
Acute oral infectivity, toxicity and pathogenicity: (MA 5.2.2.1 & MP 7.1.1; OECD IIM 5.3.2 & IIIM 7.1.1)	LD_{50} > 2000 mg/kg bw in rats; No evidence of toxicity, pathogenicity or infectivity; rapid clearance (by day 3 post dosing) with other strain (LF14) at 1×10^{8} CFU/animal
Acute intratracheal/inhalation infectivity, toxicity and pathogenicity: (MA 5.2.2.2 & MP 7.1.2; OECD IIM 5.3.3 & IIIM 7.1.3)	LC_{50} >7.7×10^{7} CFU/animal in rats after intratracheal application; no evidence of infectivity but local effects on the lungs (inflammation/immune reaction, organ weight increase), transient reduction in body weight gain; clearance complete by day 7 post dosing.
Acute intravenous/intraperitonealinfectivity: (MA 5.2.2.3; OECD IIM 5.3.4)	LD_{50} > 7.8×10^{7} CFU in rats; no evidence of toxicity, or pathogenicity
Genotoxicity: (MA 5.2.3; OECD IIM 5.3.5)	No concern (negative in the Ames test)
Cell culture study: (MA 5.2.4; OECD IIM 5.3.6)	Not required
Information on short-term toxicity and pathogenicity: (MA 5.2.5; OECD IIM 5.3.7)	Not required
Dermal toxicity: (MP 7.1.3; OECD IIIM 7.1.2)	Rabbit: LD50 > 5050 mg/kg; no systemic effects, slightly irritating to the skin (no classification needed).
Specific toxicity, pathogenicity and infectivity: (MA 5.3; OECD IIM 5.5)	Irritating to eyes Slightly irritating to skin (no classification needed)
Genotoxicity – *in vivo* studies in germ cells: (MA 5.5; OECD IIM 5.5.3)	No data – not required.

Reference values

***Beauveria bassiana* PPRI 5339**	
AOEL:	Not established, not needed
ADI:	Not established, not needed
ARfD:	Not established, not needed
Beauvericin	
AOEL:	0.0025 µg/kg bw/day

Exposure (operator, workers, bystander,	Based on the risk assessment, it can be concluded

consumer): (MA 6.1 & MP 7.3, 8.0; OECD IIM 5.6 & IIIM 7.2, 7.3)	that no adverse health effects are expected for the protected operator, the unprotected worker, bystanders and residents after dermal and respiratory exposure to *Beauveria bassiana* strain PPRI 5339 GHA as a result of the application of BAS 480 000 I in glasshouses.

Residues (Regulation (EU) N° 283/2013, Annex Part B, point 6 and Regulation (EU) N° 284/2013, Annex Part B, point 8; OECD IIM Point 6 & IIIM Point 8)

Viable residues:	*Beauveria bassiana* breaks down under UV light and concentrations on leaves decline rapidly. Multiplication in or on food or feedingstuff is not expected to occur.
Non-viable residues:	None.

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; OECD IIM Point 7 & IIIM Point 9)

Persistence and multiplication (competitiveness) in soil, water and air:	*Beauveria bassiana* PPRI 5339 and its conidia are not persistent and is unlikely that they proliferate in water and air. The micro-organisms have the natural habitat in soil therefore proliferation in soil is possible.
Mobility:	*Beauveria bassiana* PPRI 5339 and its conidia are not considered mobile.

Effects on non-target organisms (Regulation (EU) N° 283/2013, Annex Part B, point 8 and Regulation (EU) N° 284/2013, Annex Part B, point 10; OECD IIM Point 8 & IIIM Point 10)

Effects on birds and other terrestrial vertebrates (MA 8.1 & MP 10.1; OECD IIM 8.1 & IIIM 10.1)

Species	**strain tested**	**Test duration**	**Dose range**	**Results/ Endpoint**	**Observations**
TOXICITY					
American kestrel (*Falco sparvensis)*	GHA	50 days dietary field test in nestlings and fledglings	5×10^6 spores/g body weight	$>5 \times 10^6$ spores/g body weight	No adverse effects on growth or body mass and behaviour

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Species	strain tested	Test duration	Dose range	Results/ Endpoint	Observations
Ring-necked pheasants (*Phasianus colchicus*)	GHA	25 d (2 days of feeding, 4 day interval , 16 day observation)	1–1.5 × 10^5 viable conidia per insect, 4 – 6 × 10^5 conidia/bird/day	>4 –- 6 × 10^5 conidia/bird/day	No mortality. No significantly different weight gain differences in behaviour among the treatment groups
Chicken (*Gallus domesticus*)	UNIOESTE 4	5-day dietary toxicity, 28 d observation	10^9 conidia /g food	>10^9 conidia /g food	No adverse effects on mortality, behaviour or weight
INFECTIVENESS					
Ring-necked pheasants (*Phasianus colchicus*)	GHA	25 d (2 days of feeding)	5 × 10^6 spores/g body weight	>5 × 10^6 spores/g body weight	no detectable histopathological changes, no clinical signs of infection
Chicken (*Gallus domesticus*)	UNIOESTE 4	5-day dietary toxicity, 28 d observation	10^9 conidia /g food	>10^9 conidia /g food	no visible histopathological changes
PATHOGENICITY					
Ring-necked pheasants (*Phasianus colchicus*)	GHA	25 d (2 days of feeding 4 day interval , 16 day observation)	5 × 10^6 spores/g body weight	>5 × 10^6 spores/g body weight	no pathogenicity observed
Chicken (*Gallus domesticus*)	UNIOESTE 4	5-day dietary toxicity, 28 d observation	10^9 conidia /g food	>10^9 conidia /g food	no signs of pathogenicity

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

Species	Test duration	Dose range	Results/Endpoint	Observations
TOXICITY				
Inland Silverside Fish (*Menidia beryllina*)	9 days	8.3 x 10^3 – 1.2 x 10^7 spores/ml	No dose-response effect, larval hatch and thus embryo death was significantly affected at 8.3 10^4 and 8.3 x 10^5 spores/ml	mortality, embryo rupture
Grass shrimp (*Palaemonetes pugio)*	12 days (embryos) 3 days (larva) 7 days(adult)	10^4 – 10^6 spores/ml	LD50 > 1.6 x 10^6 spores/ml (embryos) LD50 < 5 x10^5 spores/organism	mortality
Fresh green algae (*P. subcapitata*)	96 h	3.0 x 10^7 CFU/L (im)	E_y/E_rC50 > 7.38 x 10^5 CFU/L	Growth rate and yield
INFECTIVENESS				
Inland Silverside Fish (*Menidia beryllina*)	9 days	8.3 x 10^3 – 1.2 x 10^7 spores/ml	No dose-response effect	Advanced hyphal growth on chorion surface, ruptured embryos
Grass shrimp *Palaemonetes pugio*	12 days (embryos) 3 days (larva) 7 days(adult)	10^4 – 10^6 spores/ml		Mycelial proliferation throughout the tissue of 2 out of 75 embryos. No infection of larvae. No infection of adults exposed via water. Infection of the adults exposed throughintrahemocoelic injections.
Fresh green algae (*P. subcapitata*)	96 h	3.0 x 10^6 CFU/L (im)	E_y/E_rC50 > 7.38 x 10^5 CFU/L	No infectivity

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Species	Test duration	Dose range	Results/Endpoint	Observations
PATHOGENICITY				
Inland Silverside Fish (*Menidia beryllina*)	9 days	8.3×10^3 – 1.2×10^7 spores/ml	No dose-response effect	
Grass shrimp *Palaemonetes pugio*	12 days (embryos) 3 days (larva) 7 days(adult)	10^4 – 10^6 spores/ml		Infection resulted in dead of the 2 embryos and 95% of the adults exposed through intrahemocoelic injections

Effects on bees (MA 8.3 & MP 10.3; OECD IIM 8.7 & IIIM 10.3)

Species	Test type	MCPA	Endpoints and observations
Bumble bee (*Bombus terrestris*)	10-day contact exposure	*Beauveria bassiana* strain PPRI 5339	LD_{50} >3.1×10^9 cfu/L No signs of infectivity or pathogenicity were detected upon autopsy and no overt signs of infections after post-mortem incubation
	10-day oral exposure		LD_{50} >2.9×10^{11} cfu/L No signs of infectivity or pathogenicity were detected upon autopsy and no overt signs of infections after post-mortem incubation
Honey bee	10-day contact exposure		LD_{50} >2.9×10^9 cfu/L At the MHC, *Beauveria bassiana* strain PPRI 5339 exhibited limited infectivity to honey bees
	10-day oral exposure		LD_{50} > 2.9×10^{11} cfu/L Nine bees out of 30 died of MPCA treatment during the study, of which five showed overt signs of infection in the form of profuse fluffy white fungal growth over the cadaver post-mortem incubation. This was subsequently identified as *Beauveria bassiana* after culturing
	Field study – whole colony exposure with test	*Beauveria bassiana* isolate 05002	No adverse effects on colony health at treatment rates ranging from 1.72×10^{10} cfu/g to 3.70 ×

	substance added directly to the hive		10^{10} cfu/g.
	Field study – whole colony exposure with test substance added directly to the hive	*Beauveria bassiana* isolate 05002	No adverse effects on colony health at treatment rates ranging from 6.33 x 10^{9} cfu/g to 1.96 × 10^{10} cfu/g.
Bumble bee *(Bombus terrestris)*	Oral and topical exposure	*Beauveria bassiana GHA* (Botanigard®)	Oral: no adverse effects at 2.5 × 10^{10} cfu/L Topical: adverse effects at 2.5 × 10^{10} cfu/L
Common eastern b umble bee *(Bombus impatiens)*		*Beauveria bassiana* (Botanigard®ES)	Topical and oral application of *Beauveria bassiana* at five concentrations ranging from of 2.33 × 10^{9} to 4.66 × 10^{10} CFU/L did not have any significant effects on bumblebee workers and colonies

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

Species	Test type	Test substance	Endpoints and observations
Aphidius rhopalosiphi	Tier I	Broadband (BAS 480 00 I)	LT > 10 days, 10-day 50% effect > 3.7 × 10^{8} cfu/mL for mortality and fecundity
Orius laevigatus			LT > 10 days, 10-day 50% effect > 4.1 × 10^{8} cfu/mL for mortality and fecundity
Grassland arthropod community	Field	*Beauveria bassiana*	No reduction in numbers, diversity or species richness of soil dwelling invertebrate communities at 10^{12} spores/ha
			No unacceptable effects occurred on non-target arthropods communities with an application of 10^{12} spores *Beauveria bassiana*/ha.
Thanasimus formicarius	Laboratory	*Beauveria bassiana*	50% effect >1 × 10^{7} spores/mL

Effects on other terrestrial invertebrates (MA 8.5 & MP 10.5; OECD IIM 8.9.1 and IIM 8.9.2 & IIIM 10.5)

Species	Test type	Test substance	Endpoints and observations
Lumbricus terrestris	Laboratory	*B. bassiana*	5 d LD50 > 2.77 x 10^{16} CFU/ha Exposure of earthworms to 2.17 x 10^{15} CFU/ha and 2.77 x 10^{16} CFU/ha for 7 and 14 d, and 5 d, respectively did not in mortality

			of the earthworms. The fungus was carried through soil by the earthworm; conidia recovered from the earthworm casts remained viable

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

Species	Test type	Test substance	Endpoints
Soil micro-organisms	Field study	*Beauveria bassiana*	No adverse effects at 3 × 10^{13} conidia/50 m^2

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

Reference was made to the review by Zimmermann (2007) A fungal suspension of *Beauveria bassiana* was fed to the leopard frog, *Rana pipiens* via gastric incubation. The dosage was 9.8 x 10^8 conidia. No mortality or fungus recovery was recorded in any of the tissues other than the stomach where recoveries are expected because of the oral intake. The viscera were free of any fungal elements. Viability of spores was established in fecal washings of pellets indicating that the spores are inactivated in the gut, but remain viable. A fungus attributed to be *Beauveria bassiana* was observed to cause infections in a captive American alligator. The reptiles were in captivity and under temperature stress which may explain their susceptibility to the fungus