European Commission

VOLUME 3 – Annex B

- *Beauveria bassiana* PPRI 5339 -

B.7 Residue data

Rapporteur Member State: The Netherlands

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B.7 Residues in or on treated products, food and feed (Annex IIM 6 and IIIB 8)

B.7.1 Persistence and likelihood of multiplication in or on crops, feedingstuffs or foodstuffs (Annex IIB 6.3 and Annex IIIB 8)

The mode of action of *Beauveria bassiana* involves destruction of its host by enzymatic action. Adherence to epicuticle surfaces of insects stimulates the infectious conidiospores to germinate. Infection commences by penetration of the germ tube producing a series of enzymes which degrade macro-molecular structures of the cuticle (chitin, lipids, proteins). Inside the host the products of further lysis processes are used by the entomopathogen for biosynthesis of fungal structures (blastospores, mycelium).

This infection mechanisms of *B. bassiana* is highly specific and strictly restricted to certain insects. Since the fungus is not capable to replicate outside its host any multiplication in or on food or feedingstuff is not expected to occur.

Beauveria bassiana does not persist in or on crops. It has been shown that growth of *B. bassiana* strain PPRI 5339 does not occur at temperatures of $\geq$32 °C (Neethling and Brown, 2012). In addition the conidiospores of *B. bassiana* PPRI 5339 are sensitive to sunlight radiation. To this end concentrations on the surface of leaves exposed to sunlight were found to decline swiftly (Neethling *et al.*, 2014). The studies on the influence of UV light and temperature are summarized in section B.2.1.5 and more details can be found there.

In the absence of favorable environmental conditions and a viable host *B. bassiana* can neither thrive nor replicate. Hence the MPCA is unlikely to persist in or on crops, food and feeding stuffs in significant concentrations, i.e. concentrations which are considerably higher than under natural conditions.

B.7.2 Exposure to consumers (Annex IIB 6.2 and Annex IIIB 8))

B.7.2.1 Non-viable residues

In the case of *B. bassiana* strain PPRI 5339, fungal multiplication and enzymes cause disruption of functioning of the host and destroy the internal organs. Strasser et al. (2000) reviewed the secondary metabolites producted by *Beauveria, Metarhizium* and *Tolypocladium.* The quantities of secondary metabolites produced by these fungi *in vivo* are usually much less those secreted in nutrient rich liquid media. They concluded that the use of fungal insecticides would not result in harmful toxin levels in the environment and posed no serious risk to humans.

No residue trials have been carried out for this crop, due to the fact that no residue definition is required for *Beauveria bassiana* strain PPRI 5339.

Therefore, in order to carry out an acute risk assessment for this product, the toxicity of the potential metabolite beauvericin to the end consumer was considered.

Analysis of the production batches of *Beauveria bassiana* PPRI 5339 concentrate has demonstrated no measureable residues of beauvericin above the limit of quantification of 0.5 mg/kg (Cheng, KM, 2013, see Volume 4 for more detail). The notifier provided an exposure assessment based on the TTC concept which is indicated below. It is noted by the RMS that for the currently approved *B. bassiana* strain a maximum level of 5 mg/kg was set for beauvericin. The proposed specification of strain PPRI 5339 is far below that concentration.

Due to the nature of the active substance, only one application is considered in the dietary risk assessment, with no pre-harvest interval.

Typical commercial yields of glasshouse grown tomatoes, cucumbers, sweet peppers and aubergines have been used to calculate a worst case residue scenario, based on the assumption that all applied active substance reaches fruit, and is not intercepted by leaves and the crop is harvested immediately after the a single application.

A recent publication by EFSA (EFSA Journal 2014;12(8):3802) has indicated that there are insufficient data to propose an acceptable daily intake (ADI) or acute reference dose (ARfD) for beauvericin, and has used the toxic threshold of concern (TTC) approach to carry out a consumer risk assessment for beauvericin in food and feed. Using this approach for this submission leads to a chronic dietary exposure limit of 0.0025 µg/kg bw/day (based on a TTC value for compounds with a structural alert for genotoxicity.

The combination of the estimation of the presence of metabolites and the TTC value of 0.0025 µg/kg bw/day will give rise to a worst case estimation of the dietary risk to the consumer from beauvericin present within the formulation as applied.

The calculated concentrations of residues of beauvericin in the proposed crops arising from the use proposed use of BAS 480 00 1 is shown in Table 7.2.1-1.

Table 7.2.1-1: Worst case calculations of residues of beauvericin arising from the use of BAS 480 00 1

Crop	***Beauveria bassiana* application rate (kg/ha)**	**Beauvericin concentration (mg/ha)***	**Typical commercial yield (kg/m^2)**	**Final residue of Beauvericin (µg/kg crop)**
Cherry Tomatoes	0.1	0.05	26	0.00019
Round Tomatoes	0.1	0.05	65	0.00008
Cucumbers	0.1	0.05	60	0.00017

Sweet Peppers	0.1	0.05	30	0.00008
Aubergines	0.1	0.05	40	0.00013

* Worst case estimation based on the LOQ since no beauvericin was detected in the product.

Using this approach, it can be seen that consumption of crop treated with BAS 480 00 1 at a rate of 0.1 kg/ha is unlikely to cause any ill health effects to consumers.

B.7.2.2 Viable residues

Beauveria bassiana is a ubiquitous entomogenous fungus which can be isolated from infected insects, soils or plants in a wide range of habitats. It may be considered as indigenous to temperate and tropical zones. The infectious mechanisms of *B. bassiana* are highly specific to insects, thus the fungus is not regarded as a plant or vertebrate pathogen.

The conidiospores of *B. bassiana* 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. The natural background level of *B. bassiana* was estimated (Sheepmaker and Butt 2010) by calculating the 95th percentile of the geometric mean measured CFU/g soil reported in 6 studies. The resulting value of 830 CFU/g soil was considered a reasonable representation of the upper natural background levels of *B bassiana,* as by using the 95th percentile, very high peaks were excluded. *Beauveria bassiana* was found to be susceptible to elevated temperatures (Fargues *et al.*, 1997) and UV-light (Krieg *et al*, 1981). Optimal growth temperatures of the MPCA range between 25 °C and 28 °C (Neething and Brown, 2012) (At temperatures above 36 °C the conidiospores are not able to germinate or grow, with inhibition of growth commencing at temperatures of 32 °C and higher. To this end *B. bassiana* PPRI 5339 is not expected to grow and multiply at mammalian or avian body temperatures.

In addition water availability is a critical parameter determining infection. At least 95 % relative humidity at the insect surface is necessary for spore germination, germ tube extension and infection to occur (Hallsworth *et al.*, 1999). Under natural conditions of aqueous environments, the conidiospores of *B. bassiana* germinating in the absence of a suitable host insect, die within a short time. Greenhouse studies indicate that *B. bassiana* is effective for about 3 days. The study by Neethling *et al.,* 2014 provides evidence that *B. bassiana* strain PPRI 5339 is highly susceptible to damage from UV light. Similarly, Edgington *et al.*, 2000 showed that viable *B. bassiana* conidia were almost completely inactivated by exposure to 60 minutes of direct sunlight or 20 seconds of UV light at a wavelength of 302 nm.

Due to its fast degradation on plant surfaces and in soils, it is unlikely that viable residues of *B. bassiana* will occur in concentrations considerably higher than under natural conditions on the harvested food and feed.

Further studies on viable residues of *B. bassiana* strain PPRI 5339 are not considered necessary.

B.7.3 Summary and evaluation of residue behaviour (Annex IIM6.5 and Annex IIIB 8))

Beauveria bassiana is an ubiquitously occurring fungus in natural soils. Its infection mechanism is strictly restricted to insects. Consequently the fungus is not regarded to persist in the environment. Infection involves destruction of the insect host by enzymatic lysis of cellular structures. Due to this specific mode of action the fungus is not regarded as a plant or vertebrate pathogen.

The conidia of *B. bassiana* are particularly sensitive to sunlight (UV radiation) and higher temperatures. Growth of the MPCA is inhibited at temperatures exceeding 32 °C. For that reason growth in living mammalian and avian tissues is untenable. Any residues on crops are short-lived due to susceptibility to unfavourable environmental conditions.

As the occurrence of significant residues in or on crops, feedingstuff or food is deemed unlikely, no MRL or import tolerances have been set for *B. bassiana* strain PPRI 5339, and no residue studies have been conducted.

Strains of *B. bassiana* have the potential to produce metabolites such as beauvericin, but the amount of beauvericin or other metabolites which might be expected from the intended use is not likely to pose an acute or chronic risk for consumers.

Taking all available information into consideration, it can be concluded that the use of *B. bassiana* strain PPRI 5339 is unlikely to exert direct adverse effects on vertebrate or plant health and it is unlikely to occur in or on crops, feedingstuffs or foodstuffs in concentrations higher than those present under natural conditions. Metabolites of *B. bassiana* such as beauvericin may occur in the technical material and thus may be applied to plants. Due to the low residues in treated commodities no risk to consumers is expected from the use of *Beauveria bassiana* PPRI 5339.

B.7.4 References relies on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
Annex II Data and Information							
MA 6.1/01 MA 6.2.2/01 MA 2.4/01	Fargues, J, Goettel, MS, Smits, N, Ouedrago, A, Rougier, M	1997	Effect of temperature on vegetative growth of Beauveria bassiana isolates from different origins. Mycologia, 89(8), 383-392 Non GLP Published	N	N	N/A	Public

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
MA 6.1/02 MA 6.2.2/03	Neethling, D, Brown, A	2012	The effect of temperature on growth of *Beauveria bassiana* strain PPRI 5339. Becker Underwood, Mahogany Ridge, South Africa. Project Number: LE 639. Report date: 21.11.12 Non-GLP Unpublished	N	Y	New strain specific GLP study	BASF
MA 6.1/03 MA 7.1.3/01	Neethling, D, Neethling, K, Brown, A	2014	Effect of ultra-violet light on viability of *Beauveria bassiana* strain PPRI 5339. Report No: R&D061401 Non GLP Unpublished	N	Y	New strain specific GLP study	BASF
MA 6.2.1/01 MA 2.7/01	Strasser, H, Vey, A, Butt, TM	2000	Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of Metarhizium, Tlypocladium and Beauveria species? Biocontrol Science and Technology, 10, 717-735 Non GLP Published	N	N	N/A	Public
MA 6.2.2/01 MA 6.1/01	Fargues, J, Goettel, MS, Smits, N, Ouedrago, A, Rougier, M	1997	Effect of temperature on vegetative growth of Beauveria bassiana isolates from different origins. Mycologia, 89(8), 383-392 Non GLP Published	N	N	N/A	Public
MA 6.2.2/03 MA 6.1/02	Neethling, D, Brown, A	2012	The effect of temperature on growth of *Beauveria bassiana* strain PPRI 5339. Becker Underwood, Mahogany Ridge, South Africa. Project Number: LE 639. Report date: 21.11.12 Non-GLP Unpublished	N	Y	New strain specific GLP study	BASF
MA	Hallsworth,	1999	Water and temperature	N	N	N/A	Public

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
6.2.2/04 MA 2.4/02	JE, Magan, N		relations of growth of the entomogeneous fungi *Beauveria bassiana* and *Metarhizium anisopliae* and *Paecilomyces farinosus*. J Invertebr Pathol. 74, 261-266 Non GLP Published				
MA 6.2.2/05 MA 7.1.3/02	Edgington, S, Segura, H, De L Rosa, W, Williams, T	2000	Photoprotection of Beauveria bassiana: testing simple formulations for control of the coffee beerry borer. International Journal of Pest Management 43(3) 169-176 Non-GLP Published	N	N	N/A	Public
Annex III Data and Information							
			No studies submitted				