European Commission

VOLUME 3 – Annex B

- *Beauveria bassiana* PPRI 5339 -

B.6 Effects on Human Health

Rapporteur Member State: The Netherlands

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Draft Assessment Report and Proposed decision of the Netherlands prepared in the context of the possible approval of Beauveria bassiana PPRI 5339 under Regulation (EC) 1107/2009

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B.6 Effects on human health (Annex IIM 5/IIIM 7)

B.6.1 Tier I – the active micro-organism (Annex IIM 5)

B.6.1.1 Basic information

PPRI 5339 is a naturally occurring, non-modified strain of *B. bassiana* strain, originally isolated by Dr Schalk Schoeman from the larva of a tortoise beetle, *Conchyloctenia punctata* (Coleoptera: Cassidinae) collected in Escombe, Queensburgh, KwaZulu Natal, South Africa. This fungal species is not closely related to any known microorganism that has to be regarded as a regular human or mammalian pathogen. A further, more general argument against infectivity and pathogenicity to man is that most *B. bassiana* strains do not survive and replicate at temperatures higher than 35°C. *Beauveria bassiana* strain PPRI 5339 was shown not to grow at temperatures of 32 ^{o}C and above (see B.2.1.5).

At the time of writing, two *B. bassiana* strain have been approved (ATCC 74040 and GHA) and two more are pending (147 and NPP111B005). Strain PPRI 5339 was found to be 98-99% similar to the two approved strains, following ribosomal sequencing, and therefore strain PPRI 5339 can be considered essentially identical to strains ATCC74040 and GHA. Specific studies for infectivity, pathogenicity and toxicity have been performed with strain PPRI 5339 in laboratory animals and are reported under B.6.1.2, supplemented with other relevant data where applicable.

B.6.1.1.1 Medical data (Annex IIM 5.2)

No epidemiological studies on the presence of *B. bassiana* in humans or on a possible relationship with diseases in humans have been performed. Thus, experience in humans is based on medical surveillance during manufacturing, a few anecdotal reports from the open literature on the isolation of *B. bassiana* from patients suffering from different diseases and a small number of studies on the occurrence of this micro-organism in neonates and on allergenicity in humans.

B.6.1.1.2 Medical surveillance on manufacturing plant personnel (Annex IIM 5.2.1/5.2.2/5.2.3)

No evidence of adverse health effects have been observed in any of the employees involved in the production, handling and application of *B. bassiana* strain PPRI 5339. However, microbial products may have the potential to be skin sensitisers, due to the high protein content. It is therefore important that employees, workers and operators handling *B. bassiana* strain PPRI 5339 wear appropriate protective equipment.

Report: Neethling, K (2013) Memorandum: Exposure to, and adverse health effects from, exposure to *Beauveria bassiana* strain PPRI 5339 during manufacturing. BASF Agricultural Specialities (PTY) Ltd., 91 Clifton Caanyon Drive, Clifton Farm Stockville Quarry, Mahogany Ridge, KwaZulu Natal, South Africa. 11th November 2013.

Summary

BASF Agricultural Specialities (PTY) Ltd (formerly Becker Underwood South Africa (Pty) Ltd. and before that Biological Control Products (Pty) Ltd.) manufactures *B. bassiana* strain PPRI 5339. The company started producing this strain in October 2002 and to date has made 1.01 tons of technical grade material (23,580 liters of formulated product).

During this time, no evidence of any adverse health effects have been observed by those individuals producing, processing, packaging, analyzing, handling, applying or otherwise exposed to *B. bassiana* strain PPRI 5339 technical grade material or formulated products.

B.6.1.1.3 Sensitisation/allergenicity observations, if appropriate (Annex IIM 5.2.1/5.2.2)

Westwood et al. (2005) used crude extracts of *B. bassiana* strain ATCC 90517 for immunoblotting with pooled as well as individual human sera and found IgE binding of a number of potential allergens present in the extracts. The authors reported evidence of *B. bassiana*-specific proteins that might cause allergies as well as indication for cross-sensitisation because of certain reactive epitopes that this microorganism has in common with other fungi such as *Alternaria alternata* or *Aspergillus fumigatus*. In the *in vivo* part of this study, ten people were intradermally injected 0.1 mL of a dialysed (against 0.15 N NaCl) and filtered crude *B. bassiana* extract and monitored for 15 - 30 min for the development of allergenic reactions. Control persons received saline and histamine. Seven out of the 10 participants treated with the extract displayed skin reactions. Although this may be not considered entirely convincing since it is sometimes difficult to distinguish between skin reaction because of sensitisation and irritation, it must be seriously taken into account that four of five people who had reported previously occupational exposure to *B. bassiana* showed a positive skin reaction as well as bands suggesting reactivity in the Western blot.

In a study in the Netherlands, Beaumont *et al.* (1985a & 1985b) examined patients with recurrent allergic bronchial obstruction and identified *B. bassiana* (strain not specified) as an allergen by means of intracutaneous challenge with strong reactions in 5 of 73 test persons (6.8%). This percentage was higher than seen with most other fungal species included in this investigation although the presence of *B. bassiana* spores in air samples was low.

Henke et al. (2002) reported allergic alveolitis in an immunocompromised patient suffering from disseminated infection with *Beauveria spp.* (see section B.6.1.1.4 for more information on this study).

Semalulu et al. (1992) cited references that claimed occurrence of moderate or even severe allergenic reactions among scientists working with *B. bassiana* but also mentioned papers suggesting that there were no deleterious effects in workers handling this fungus.

Taking into consideration the presented information from the open literature and observations in laboratory animals presented herein, there is enough evidence to consider *B. bassiana*, irrespective of the strain, a potential human allergen by both skin and inhalatory contact.

B.6.1.1.4 Direct observation, e.g. clinical cases (Annex IIM 5.2.4)

There is evidence from the open literature that *B. bassiana* (strain never specified) was isolated in few cases from people who suffered from various diseases. The study reports involve abnormally favorable circumstances as infections occur in a few profoundly immune-compromised patients and eye infections after surgery or trauma. It is not likely that such persons will get into close contact with *Beauveria bassiana* when used for plant protection purposes.

Eye infections

Several cases of eye infections have been reported in literature (see below). In summary, these ophthalmological case reports do not point to a special risk of *B. bassiana* strain PPRI 5339 to cause eye infections in operators since adverse effects were only seen after damage to the cornea that allowed invasion of the fungus into the eye. There are some reports on keratitis or other ocular disease in humans with intact cornea, but these events are rare. The findings suggest a possible affinity of *B. bassiana* to ocular tissues once the cornea had been penetrated.

Report:	Figueira, L., Pinheiro, D., Moreira, R., Pinto, E., Simões, J., Camisa, E., Torrão, L., Palmares, J., Falcão-Reis, F. (2012) *Beauveria bassiana* keratitis in bullous keratopathy: antifungal sensitivity testing and management. Eur J Ophthalmol 2012; 22 (5): 814-818.
Guidelines:	Not applicable
GLP:	Not applicable

Summary

The authors reported the first case of *B. bassiana* keratitis in a patient with aphakic bullous keratopathy. The fungal keratitis proved to be highly resistant to topical clotrimazole. Species identification was based on DNA sequence analysis. The minimal inhibitory concentrations (MIC) obtained were 2 µg/mL for voriconazole, 0.250 µg/mL for posaconazole, and >128 µg/mL for fluconazole; amphotericin B MIC was >16 µg/mL. In the absence of clinical improvement, a penetrating keratoplasty (PK) was performed. The patient was discharged on topical and systemic voriconazole and prednisolone 40 mg PO/day. The eye remained calm with a transparent cornea and clear anterior chamber.

B. bassiana keratitis is extremely rare, with only a few cases reported. Its risk factors are still unclear. This study reported the first case in a patient with aphakic bullous keratopathy, which proved highly resistant to antifungal therapy (antifungal susceptibility results are presented). A PK was necessary for clinical improvement.

Report: Sonoyama H, raki-Sasaki K, Kazama S, Kawasaki T, Ideta H, Sunada A, Asari S, Inoue Y, Hayashi K (2008) The characteristics of keratomycosis by Beauveria bassiana and its successful treatment with antimycotic agents. *Clin. Ophthalmol.*, 2(3): 675-678.

Guidelines: Not applicable

GLP: Not applicable

Summary

An 80-year-old woman struck her left eye with the frame of her glasses. Due to continuing ocular pain and hyperemia, she was referred to the hospital 9 days after the original injury. The patient had a long history of recurrent diabetic iritis and continuously used topical antibiotics and corticosteroids. At the time of her first visit to the hospital, there was ulceration of the corneal epithelium at the 5 o'clock region along with slight superficial infiltration, slight oedema, and ciliary injection. No endothelial plaques or any immune rings were noted. Although a part of the Descemet's membrane was folded, there was only slight anterior chamber inflammation. A corneal scraping smear indicated the presence of Gram-positive fungal septate hyphae with budding, which led to a diagnosis of keratomycosis by filamentous fungus. Positive staining by Fungiflora Y also supported the keratomycosis diagnosis. From the culture of the corneal scraping, the authors were able to isolate a whitish yellow colony, with the fungus exhibiting zigzag rachis and oval conidia, which are characteristics of *B. bassiana* in slide cultures. Two different microbiology laboratories, the Department of the Laboratory for Clinical Investigation at Osaka University Hospital and the Chiba University Research Center for Pathogenic Fungi and Microbial Toxicoses, independently confirmed the presence of the organism. Topical voriconazole with miconazole, pimaricin and oral itraconazole were effective and the lesion disappeared leaving only a mild scar at 2 months.

Report: Tu EY and Park AJ (2007) Recalcitrant Beauveria bassiana keratitis: confocal microscopy findings and treatment with posaconazole (Noxafil). *Cornea*, 26(8): 1008-1010.

Guidelines: Not applicable

GLP: Not applicable

Summary

A 58-year-old woman was referred to the University of Illinois Eye and Ear Infirmary (IEEI) for evaluation of a recalcitrant central corneal ulcer in the right eye for the past month. She was an Acuvue II soft contact lens (Johnson & Johnson, Jacksonville, FL) wearer and reported good lens hygiene habits with Opti-Free solutions (Alcon, Fort Worth, TX). There was no history of trauma, but the patient kept a rose garden and lived near several golf courses. Before presentation, various combinations of Vigamox, Tobradex, prednisolone acetate, and subsequently, trifluridine and scopolamine, were tried without improvement. On presentation to the Wheaton Eye Clinic, corneal cultures were obtained, and fortified topical vancomycin 50 mg/mL and tobramycin 14 mg/mL were initiated hourly for 3 days without improvement. Cultures and smears returned negative, and she was referred to the IEEI for further evaluation. The patient was extremely uncomfortable, with severe photophobia and a best-corrected vision of 20/400 and 20/40 in the right and left eyes, respectively. The left eye appeared normal. The right eye exhibited a shallow 3 x 4 mm central ulcer, minimally necrotic, with a clear corneal periphery and no hypopyon. Confocal microscopy (Confoscan 3, Nidek, Japan) and corneal scrapings for Diff- Quick smear (DIFCO, Detroit, MI) and culture were obtained.

Confocal microscopy revealed extensive filamentary forms consistent with a filamentous keratitis. A corneal scraping was performed for culture and Diff-Quick stain, which confirmed the presence of a filamentous organism. Natamycin 5% was added hourly with a discontinuation of antibacterial medications. Fluconazole 200 mg daily was added 3 days later with no change in clinical appearance or symptoms over the next 8 days. Because fungal cultures remained negative, topical Bactrim was added for the next week to cover possible Nocardia keratitis. Topical voriconazole 1% was added when fungal cultures revealed a *Beauveria* species after progressive worsening on a regimen of topical natamycin/oral fluconazole for 18 days. For the next 4 days, the patient had worsening pain on the 3-drug regimen, necessitating the addition of oral posaconazole 200 mg 4 times daily and discontinuation of the fluconazole. The *Beauveria* sp. was later identified as *B. bassiana* resistant to amphotericin B.

Report:	Ishibashi J, Matsumoto Y, Takei K (1984) The effects of intravenous miconazole on fungal keratitis. *American Journal of Ophthalmology* 98:433-437
Guidelines:	Not applicable
GLP:	Not applicable

Summary

A 44-year old man got a particle of dust in his left eye. The eye became irritated after a few days and he was referred to an ophthalmologist who prescribed antibiotic eye drops for a corneal ulcer. The lesion progressed and the patient was treated with antibiotics and corticosteroids before being referred to the Department of Ophthalmology, Institute of Clinical Medicine, University of Tsukuba, Ibaraki, Japan. Potassium hydroxide and india ink preparation of the corneal stroma showed septate hyphae.

Two cultures of the corneal stroma grew *Beauveria bassiana*. The infection was successfully treated with intravenously infused miconazole.

Systemic disease

A few studies reported systemic disease in immunocompromised patients which were (potentially) linked to *Beauveria bassiana*. The available published information suggests that disseminated infections have been observed only in people under immunosuppression following exposure to chemotherapeutic drugs or in individuals who were severely ill. It is not likely that such persons will get into close contact with *Beauveria bassiana* when used for plant protection purposes. Health risks for operators, bystanders or workers can be rather expected to arise from the sensitising properties of this micro-organism.

Report: Henke MO, de Hoog GS, Gross U, Zimmerman G, Kraemer D, Weigh M (2002) Human deep tissue infection with an entomopathogenic *Beauveria* Species. *Journal of Clinical Microbiology* 40(7): 2698-2702

Guidelines: Not applicable

GLP: Not applicable

Summary

The authors reported disseminated infection with *Beauveria spp.* in a 38-year-old woman who had been diagnosed to have extramedullary acute myeloid leukaemia and was successfully treated by chemotherapy. Two weeks after discharge from hospital, the patient was readmitted because of severe dyspnea, dry cough, pain in the right upper abdomen, and fever. Lung function was impaired and a CT scan of the thorax revealed a discrete interstitial infiltrate. Allergic alveolitis was histologically confirmed by transbronchial lung biopsy. Ultrasonography of the abdomen revealed multiple lesions in the liver and spleen, suggesting systemic fungal infection. In liver biopsy samples, extensive focal necrosis was recorded. Samples from these specimens, verified cultures of *Beauveria bassiana* and of the related species *Beauveria brongniartii* were cultured, compared, and gave similar results, but genotyping (sequencing) pointed rather to the latter one. The woman patient was treated with steroids and, following microbiological and pathological findings of fungal infection, antifungal therapy was initiated with itraconazole. This was successful and, three weeks later, the patient recovered. Seven weeks later no fungal lesions could be detected by CT scan and ultrasound.

Report: Tucker DL, Beresford CH, Sigler L, Rogers K. (2004) Disseminated *Beauveria bassian* Infection in a patient with acute lymphoblastic leukemia. *Journal of Clinical Microbiology* 41(11): 5412-5414

Guidelines: Not applicable

GLP: Not applicable

Summary

A systemic infection with *Beauveria bassiana* in a 44-year-old woman from a rural area who underwent chemotherapy for treatment of acute lymphoblastic leukemia was reported. First signs of infection (fever, neutropenia) appeared on day 15 of cytostatic treatment. Few days later, small (< 1 cm) purple macula "cigarette burn"- like lesions were noted on the left upper arm. Skin lesions progressed, involving the patient's arms, legs, buttocks, and face, and became necrotic and exudative. Histopathological examination of skin biopsy specimen revealed sharply demarcated areas of necrosis with lack of cellular reaction at the interface. The necrotic tissue was heavily permeated by fungal hyphae, which also invaded the local blood vessels. The isolate was identified as *Beauveria bassiana* due to morphological, physiological, and growth characteristics. This finding was confirmed by gene sequencing.

Four days after the skin lesions, the patient complained of symptoms of sinusitis, headache, and facial pain and had percussion tenderness over her maxillary sinuses. Later on, she developed a persistent haemorrhagic left-sided pleural effusion and there was evidence of lung necrosis. Serum transaminases were elevated from day 21 but abdominal ultrasound scan was normal.

She received first fluconazole and then intravenous amphotericin in combination with itraconazole for a further 25 days. Antifungal therapy was continued for the duration of her neutropenia. According to the report, treatment was successful and the skin lesions continued to heal over several months with some scarring.

Report: Gürcan S, Tuğrul M, Yöruk Y, Özer B, Tatman-Otkun M, Otkum M. (2006) *Mycoses* 49: 246-248

Guidelines: Not applicable

GLP: Not applicable

Summary

The authors reported isolation of *Beauveria bassiana* from a 51-year- old man who suffered from lung adenocarcinoma with penetration of the thoracic wall. In the third week after operation, sample was taken to diagnose empyema because of increased and turbid pleural fluid occurred. A pleural fluid sample contained 1600 leucocytes/µL (50 % of them were polymorphonuclear cells). Additionally, blastospores and hyphae were seen in Gram and Giemsa stained smears. Fluid was cultured and *Beauveria bassiana* identified. A second operation was performed and empyema regressed within one week. Antifungal therapy was not applied. Thus, in this case, it seems not entirely clear whether the symptoms and pathological findings were actually due to the infection.

Further information on occurrence in humans and medical use of *B. bassiana*:

B. bassiana was found in the nasal mucus of neonates (Lackner et al. 2003). None of the newborns showed clinical symptoms of nasal fungal colonization and it was therefore not considered to be a pathological finding by the study author.

Report:	Lackner A, Freudenschuss K, Buzina W, Stammberger, Panzitt T, Schosteritsch St, Braun H. (2003) *Laryngo-Rhino-Otol* 83: 117-121 (German with English abstract)
Guidelines:	Not applicable
GLP:	Not applicable

Summary

The authors assessed the time period after birth after which fungal spores of different species could be detected in human nasal mucus. Therefore, nasal mucus samples were taken from 30 neonates immediately after birth, on the first and fourth day *post partum*, and (so far available) after two and four months. The samples were obtained with sterile cotton swabs and cultured on Sabouraud glucose agar plates at 25 °C. Fungal cultures were identified either by conventional microscopy or by molecular techniques. In order to show whether fungi in nasal mucus of newborns were acquired by contamination during birth, mucus of the maternal vagina was examined as well.

All newborns and their mothers did not show or report clinical signs of fungal disease. Just after birth, in 6 of 30 neonates fungal cultures were detected in nasal mucus. In three of them, *C. albicans* was found, probably due to contamination when passing the maternal vagina as cultures of vaginal mucus of their mothers were positive for *C. albicans* too. Another three neonates showed *Penicillium* sp., and one of these also *B. bassiana*. Positive fungal cultures were obtained in 2 of 29 or in 4 of 26 neonates on the second or fifth day of life, respectively. In all instances, fungal presence in nasal mucus was limited to one day only. After the second month of life, examination of nasal mucus yielded positive fungal cultures in 8 of 11, after four months in 17 of 18 babies with a wide array of different species. The authors considered the finding, *i.e.,* fungal positive cultures from almost all nasal mucus, after four months of life as similar to the situation in adults, and hence fungal spores should be considered a normal content of nasal mucus, which alone would not be a pathological finding. These results support the assumption of ubiquitious occurrence of *Beauveria bassiana*.

Overall conclusion:

The available data indicate that *Beauveria bassiana* is a rare human pathogen. It was isolated in very few cases from eye infection or from disseminated infection in immunocompromised patients. It is not known whether these properties can be attributed to certain strains since such information is lacking in the case report. However, eye infections were observed only after mechanical damage to the cornea and systemic disease was confined to severely immunocompromised people. It is not likely that such

persons will get into close contact with *Beauveria bassiana* when used for plant protection purposes. Therefore, the effects are of limited relevance for human health risk of strain PPRI 5339 with regard to its intended use in plant protection.

B.6.1.2 Basic studies (Annex IIB 5.3)

B.6.1.2.1 Sensitisation (Annex IIM 5.3.1)

Following the PRAPeR M2 meeting on 16-18th February 2009 in Parma Italy, a skin sensitisation study is no longer required for a European registration of a microorganism active substance. All microorganisms are considered to be potential allergens and the phrase 'Contains *Beauveria bassiana* strain PPRI 5339. Micro-organisms may have the potential to provoke a sensitising reaction' must appear on the label. Nonetheless, a single study on the skin sensitisation of *B. bassiana* was submitted by the notifier and is summarized below.

Report:	[redacted] (2007b) Skin sensitisation of Bb spore concentrate in Guinea Pigs by Magnusson and Kligman. [redacted] Unpublished Report No. 06/338-104T.
Guidelines:	OECD Guidelines for the Testing of Chemicals No.: 406
GLP:	Yes
Deviations:	The batch number of sunflower oil used was different to that outlined in the study plan. This deviation did not affect the validity of the study.

Aim of the Study

The aim of the study was to evaluate the skin sensitisation potential of Bb (*B. bassiana* strain PPRI 5339) spore concentrate in the guinea pig using the Magnusson and Kligman method.

Materials and methods

Start of Experiment: 27 February 2007

End of Experiment: 23 March 2007

Test Item

Name:	Bb spore concentrate
Batch No.:	F1096
Physical State:	Powder
Colour:	Uniform cream powder
Purity:	Not reported
Storage conditions:	In a cool (5°C), dry and dark place.

Male Dunkin Hartley guinea pigs (provided by [redacted]) in acceptable health condition were used for the study. Before exposure, an area of approximately 5 x 5 cm on the scapular region was clipped free of hair and shaven close with care.

Before the preliminary dose range finding study, a test item formulation evaluation was performed which concluded that a concentration of 30 % (in vehicle) was the maximum practical formulation that would be prepared and practically applied and that intradermal concentrations should be ≤ 1 %. Based on the results intra-dermal treatment the test item was applied at concentration of 0.01 % (w/v). Dermal induction treatment was conducted at a concentration of 20 % (w/v). For the challenge exposure, all animals of the treatment and control group were treated with concentrations of 5 % (w/v) test item in vehicle. The time of exposure was 24 hours (for the intra-dermal and challenge doses) and 48 hours (for the dermal induction dose). Symptoms were examined and scored 1, 24, 48 and 72 hours after exposure.

For intra-dermal induction row of 3 injections (6 in all) was made on each side of the test animal. Two injections were made with 0.1 mL of Freund's complete adjuvant (FCA) mixed with physiological saline solution (1:1). Two injections were made with 0.1 ml of the test item (0.01 %) homogenized in vehicle (95% sunflower oil and 5 % cremophor). The final two injections were made with 0.1 ml of test item (0.01 %) mixed with physiological saline solution and homogenized in Freund's complete adjuvant (1:1). The control animals were treated similarly as the test group but the vehicle, without the test item, was used for injections.

For dermal induction, seven days after the intra-dermal induction, 0.5 mL of the test item (20 %, w/v) was spread on the surface prepared previously and covered with a standard (5x5 cm) porous gauze patch for 48 hours. Control animals were treated with vehicle only.

For the challenge exposure, animals were exposed using a closed patch. Left shaved flank areas of the animals (both the test and the control) were treated with 0.5 ml of the test item (at concentration of 5 %). The right shaved flank areas were treated with 0.5 ml of vehicle (95% sunflower oil and 5 % cremophor), in all cases.

Dermal irritation scores were evaluated in accordance with the scoring system by Draize (1959).

The main study design is summarised in the following table.

Table B.6.1.2.1-1: Study design used in main skin sensitisation test.

	Intra-dermal Induction Exposure (Main Study 1)		**Dermal Induction Exposure (Main Study II)**		**Challenge Exposure (Main Study III)**	
Dose Group	Control	Test	Control	Test	Control	Test
No. of animals	5	10	5	10	5	10
Test Item Concentration (%)	-	0.01	-	20	5	5
Day of Treatment	1	1	8	8	22	22
Time of Exposure (h)	24	24	48	48	24	24
Time of	24 hours	24 hours	1, 24, 48	1, 24, 48	24 and 48	24 and 48

Observations	after the treatment	after the treatment	and 72 hours after the patch removal	and 72 hours after the patch removal	hours after the patch removal	hours after the patch removal

Findings

Test Group

A positive response was seen in eight out of ten animals in the test group (80 %). The mean Draize response scores were 1.6 and 1.2 at 24 and 48 hours respectively. The dermal scores observed on the skin surface of previously sensitised guinea pigs represented patchy (score 1) to confluent (score 2) erythema. On the opposite (right) side treated with vehicle no reaction was found.

The dermal responses observed following challenge with the test item in the main study are presented in the following tables.

Table B.6.1.2.1-2: Dermal symptoms of guinea pigs challenged with the test item (test group)

Animal Number Sex: male	**Scores of Dermal Reaction**	
	24 hours (after patch removal)	**24 hours (after patch removal)**
104	2	1
106	2	1
107	2	2
108	0	0
109	2	2
111	2	1
112	0	0
113	2	2
114	2	2
115	2	1
Mean of Scores	1.60	1.20
% Positive	80	80

Control Group

No visible changes to the skin of the control animals was observed throughout the study. The positive control (2-mercaptobenzothiazole) showed positive reaction in six out of ten animals 24 hours after patch removal.

Body Weight

There were no notable differences between the test animal group and the control group.

Conclusions

The results of the study indicate that Bb concentrate is a skin sensitiser. Whilst classification under Regulation (EC) No 1272/2008 is not necessary for microorganisms, all microorganisms are considered to be potential allergens and the phrase "Contains *Beauveria bassiana* strain PPRI 5339. Micro-organisms may have the potential to provoke a sensitising reaction' must appear on the label.

B.6.1.2.2 Acute toxicity, pathogenicity and infectiveness

B.6.1.2.2.1 Acute oral toxicity, pathogenicity and infectiveness (Annex IIM 5.3.2)

STUDY 1

Report:	[redacted] (2011d), Acute oral toxicity study (UDP) in rats. [redacted]
	Unpublished Report No. 15367-11
Guidelines:	Guidelines for the Testing of Chemicals No.: 425.
	US EPA OCSPP 870.1100
	FIFRA 7 USC 136, OECD
GLP:	Yes
Deviations:	The provided Certificate of Analysis was not accompanied by a GLP compliance statement.
	Stability information was not provided to the testing facility.
	Mixture analysis was not performed.
	Relative humidity was at times outside protocol range.
	The deviations listed did not affect the outcome of the study.

Aim of the study

The objective of this study was to assess the acute oral toxicity potential of the test substance when administered by gavage to rats.

Materials and methods

Day of Treatment:	23 August 2011
End of Experiment:	15 September 2011

Test Item

Name:	*Beauveria bassiana* strain PPRI 5339
Batch No.:	S627 C2
Physical State:	Light cream powder
Purity:	2.06 x 10^{11} spores/g (per provided information)
Storage conditions:	Room temperature

Albino Sprague-Dawley rats (provided by [redacted] in acceptable health condition were used for the study.

Three animals (all female) were selected for the study. The test substance was mixed with water to produce a 40% w/v concentration. An individual dose was calculated based on its fasted body weight and administered by gavage at a volume of 12.5 mL/kg. Each dose was administered using an

appropriately sized syringe and stainless steel ball-tipped intubation needle.
Observations for mortality and clinical/behavioural signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Day 7 and 14.
On day 14, after dosing, each animal was euthanized by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

Findings

Mortality
There was no mortality during the study. The estimated acute oral LD_{50}, as indicated by the data, was determined to be greater than 5000 mg/kg.

Clinical symptoms
The only clinical sign presented was activity decrease in one animal on the day of dosing.

Body weight and body weight gain
All individual animals exhibited weekly weight gain during the study.

Necropsy
Gross necropsy conducted at the termination of the study revealed no observable abnormalities.

Conclusions
The test substance, *B. bassiana* strain PPRI 5339, was evaluated for its acute oral toxicity potential when administered to albino rats. The acute oral LD_{50} is estimated to be >5000 mg/kg bw in females.

STUDY 2

Report:	[redacted] (2007b), Acute oral toxicity study (acute toxic class method) of test item Bb spore concentrate in rats. [redacted]. Unpublished Report No. 06/338-001P
Guidelines:	OECD Guidelines for the Testing of Chemicals No.: 423 EU Method B.1. US EPAOPPTS 870.1100
GLP:	Yes
Deviations:	None

Aim of the Study

An acute oral toxicity study was performed to assess the acute toxicity of test item Bb (*B. bassiana* strain PPRI 5339) spore concentrate in rats. The results of the study allowed the test item to be ranked according to the classification systems, currently in use.

Materials and methods

Day of Treatment: 01 and 02 February 2007

End of Experiment: 15 and 16 February 2007

Test Item

Name:	Bb spore concentrate
Batch No.:	F1096
Physical State:	Uniform cream powder
Expiry Date:	June 2007
Storage conditions:	Store away from light in a cool (5 °C), dry dark place

Female CRL(WI)BR Wistar rats (provided by [redacted] in acceptable health condition were used for the study.

A single oral treatment was carried out by gavage for each animal after an overnight food withdrawal. The animals were treated at a dose level of 2000 mg/kg bw with a Bb spore concentrate of 200 mg/mL prepared with mixture of 95% (v/v) sunflower oil and 5% (v/v) cremophor. The dose rate corresponded to a treatment volume of 10 mL/kg bw.

Clinical observations were performed for all animals 30 minutes, 1, 2, 3, 4 and 6 hours after dosing and daily for 14 days thereafter. Food was made available again 3 hours after the treatment. Body weight was measured weekly. Gross necropsy was performed on all animals at termination of examination (day 14).

Results

Mortality

No mortality occurred after a single oral administration of Bb spore concentrate at 2000 mg/kg bw dose level in female CRL:(WI) BR rats.

Clinical symptoms

No animals showed clinical symptoms on the day of the treatment and the following 14-day observation period in either groups. The physical condition and behaviour of animals were normal in all animals during the following 14-day observation period.

Body weight and body weight gain

The mean body weight and the body weight gain of animals were in the normal range during the two weeks observation period, similar to the expected values in untreated animals of the same age and strain.

Necropsy

No macroscopic alterations related to the toxic effect Bb spore concentrate were found.

Conclusions

The acute oral LD_{50} value of the test item Bb spore concentrate was >2000 mg/kg bw in female CRL:(WI) BR rats.

STUDY 3 (public literature)

Reference: Acosta, ME, Perfetti, DC, Eduarte, G, Moreno, PM (2012) Oral pathogenicity of an autochthonous isolate of the entomopathogenic fungus *Beauveria bassiana* in mice (*Mus musculus*, strain NMRI). Boletin de Malariologia Salud Ambiental, (2012) 52(1): 79-86

Guidelines: None

GLP: Not applicable

Remarks RMS: *The original study is in Spanish, but an English translation was submitted by the applicant.*

Summary

This study evaluated the pathogenicity of native isolate LF14 of *B. bassiana* (Fungi: Ascomyocota) following oral administration to white mice (NMRI strain), both male and female (12 animals per sex). A suspension of 1 x 10^8 conidia/mL was added to the food of the animals (amount not specified) and was consumed for five days (food and treatment was renewed daily). Control animals were fed on diet treated with only distilled water. Daily observations included clinical behaviour, body weight and assessment of physiological clearance of *B. bassiana* by analysis of faeces. Clinical observations included signs of irritation in skin, fur, eyes and mucous membranes, overall assessment of health and behaviour. For clearance, direct examination of faeces were made mounting faecal samples on slides and staining cotton blue and viewing under a light microscope. Faecal samples were also cultured on solid medium for 7-14 days at 26 °C and >90 % relative humidity. The number of germinated conidia (%) was estimated after 24, 48 and 72 hours. Following the incubation period sporulation (conidia/mL) was assessed using a haemocytometer.

Each animal was subject to complete necropsy on days 3, 7 and 14 after treatment, including infectivity analysis of sampled tissues, which were cultured on solid medium. For histopathology, a section of the tissue samples were fixed in 10% formaldehyde and embedded in paraffin for histological sections 5-7 µm. The sections were stained with haematoxylin - eosin (H & E) and

evaluated and photographed under a light microscope. Organs assessed were skin, kidney, liver, lung, spleen, stomach, small bowel, intestine heart and trachea.

Statistical analysis was conducted to determine any significant differences between mean body weights, % germination of conidia and sporulation. Analysis was conducted using the statistical package MINTIAB version 13.2.

Animals showed no discernible pathological changes or death and had 100% active-normal behaviour. In all the mice groups, including control ones, there was an increase in the mean weight gained through time, with statistically significance difference between the sexes ($p<0.0000$). Viable conidia were observed in faeces until 7 days after consuming *B. bassiana*. No anatomopathological changes or germinated conidia were detected in analysed organs, with normal tissue reactions, suggesting no evidence for fungal multiplication.

Conclusions

On the bases of this study it was concluded that isolate LF14 of *B. bassiana* orally administered is non-pathogenic in white mice (NMRI strain).

B.6.1.2.2.2 Acute inhalation toxicity, pathogenicity and infectiveness (Annex IIM 5.3.3)

STUDY 1

Report:	[redacted] (2013), *Beauveria bassiana* strain PPRI 5339 Acute Pulmonary Toxicity/Pathogenicity in Rats. [redacted] Unpublished Report No. 16701-12
Guidelines:	OCSPP 885.3150
GLP:	Yes
Deviations:	Necropsy on one animal was not performed in a timely manner after discovery of death. The deviation listed did not affect the outcome of the study.

Aim of the study

The aim of the study was to assess the acute toxicity and pathogenicity of *Beauveria bassiana* strain PPRI 5339 following intratracheal exposure to the rat.

Materials and methods

Test Item

Name:	*Beauveria bassiana* strain PPRI 5339 Spore Concentrate
Batch No.:	S627 C2

Physical State:	Light cream powder
Purity:	2.06 x 10^{11} spores/g (per provided information)
Storage conditions:	Refrigerated

Albino Sprague Dawley rats (provided by [redacted] in acceptable health condition were used for the study. Thirty six male and 36 female rats (nulliparous and not pregnant) plus three validation animals were selected for the study. The three validation animals were used to assess recovery efficiency from test animals during the study. In the validation group tissues were spike to contain approximately 10^6 CFU.

A 1g sample of the test item was serially diluted with sterile phosphate buffer saline (PBS). A portion of 12 mL was rendered nonviable through autoclaving for 45 min. The test substance, or inactivated test substance, was administered into the trachea of each animal in a single 0.1 mL dose of the dilution prepared in PBS. A group of 6 animals per sex were used for the untreated control (Group I), 4 animals per sex for the shelf control (Group II), 8 animals per sex for the inactive test item (Group III) and 18 animals per sex for the viable test item.

Body weights were recorded pre-dose on day 0 and weekly thereafter. Observations for mortality and signs of pharmacological and/or toxicological effects were made three times following dosing and daily thereafter for 21 days. Observations included, but were not limited to the skin, fur, eyes and mucous membranes; respiratory circulatory, autonomic and central nervous systems; somatomotor activity and behaviour patterns. Particular attention was given to tremors, convulsions, diarrhea, lethargy, salivation, sleep and coma.

On Day 0, three animals per sex from the viable test item group were sacrificed, and 3 animals per sex were sacrificed weekly thereafter. Two animals per sex from the untreated control, 1 animal per sex from the shelf control and three animals per sex from the inactive test item were sacrificed on Day 0 after dosing. All other animals were sacrificed at study termination. A gross necropsy was conducted on each animal at the interim time of sacrifice or at the time of discovery of death. The gross necropsy included gross observations of external surfaces, all orifices and thoracic, abdominal and pelvic cavities. Organ weights were recorded for all animals sacrificed.

Quantification of the test microbe was performed on tissues removed at each interim sacrifice to determine the infectivity/pathogenicity and estimate clearance of the test item after dosing. On Day 0, lungs were the only tissues assessed. At other time points, tissues/fluid removed from each animal included: kidneys, brain, liver, lungs, spleen, blood, representative lymph nodes and cecum contents. Histopathological evaluation was only conducted on abnormal tissues. All tissues and fluid samples removed for quantification were cultured to provide qualitative and quantitative measurements of the test microbe.

One-way parametric analysis of variance (ANOVA) was performed on the recorded body weights, body weight change and relative organ weights as a percentage of body weights.

Findings

Dose verification of the test substance demonstrated a count of $7.7x10^8$ CFU/ml or $7.7x10^7$ CFU per rat.

The only mortality during the study was a single animal from the inactive test item group on Day 19. No abnormalities were noted during clinical observations. No observable abnormalities were noted except in the animal that was found dead from the non-viable test item group, in this animal the organs were black. Clearance of test item from the lungs was achieved by day 14 as quantification results from plated tissues yielded two consecutive counts that were below the clearance threshold. The test item was not observed in any blood, brain, lungs, liver, spleen, kidneys, mesenteric lymph nodes and cecum contents by Day 7. A summary of the quantification of test item in observed tissues is presented in table B.6.1.2.2-1.

Table B.6.1.2.2.2-1: Summary of quantification of organisms in tissues

	Blood CFU/mL	Brain CFU/g	Lungs CFU/g	Liver CFU/g	Spleen CFU/g	Kidneys CFU/g	MLN CFU/g	Cecum Contents CFU/g
Day 0	NA	NA	0	NA	NA	NA	NA	NA
Day 3	0/<100	0<100	1.1×10^2	0/<100	0/<100	0/<100	9.4×10^1	0
Day 7	0	0	0	0	0/<100	0	0	0
Day 14	0	0	0	0	0	0	0	0

NA – Not applicable, only lungs were collected from animals sacrificed on Day 0
MLN – Mesenteric Lymph Nodes
0/<100 – all values were either less than 100 or 0

Overall, with males and females combined, the relative lung weights for the MPCA group were significantly greater than the control group.

Table B.6.1.2.2.2-2: Summary of organ weights

	Males		Females	
	Absolute	Relative	Absolute	Relative
I Untreated	2.1588	0.6240	1.8400	0.7509
II Shelf	2.1066	0.6061	1.6680	0.7266
Control*	2.1379	0.6139	1.7712	0.7412
III Inactive	2.2643	0.6329	1.8802	0.7245
IV MPCA	2.4386	0.6962	1.9358	0.8871

*Control group was Group I&II combined.

Conclusion

With no plated organisms seen and no observed abnormalities during general health observations at necropsy, there is no evidence for toxic or pathogenic effects caused by the test item. The test item was concluded to be non-toxic to rats when administered by tracheal injection in a single dose of 7.7×10^8 CFU/mL or 7.7×10^7 CFU/rat.

STUDY 2

Report:	[redacted] (2012a), Acute inhalation toxicity in rats. [redacted]
	Unpublished Report No. 15687-11
Guidelines:	US EPA OCSPP 870.1300
GLP:	No

Aim of the study

The objective of this study was to evaluate the test substance for its acute inhalation toxicity potential in male albino rats (Sprague Dawley).

Materials and methods

Day of Treatment:	26 October 2011
End of Experiment:	28 October 2011

Test Item

Name:	*Beauveria bassiana* strain PPRI 5339
Batch No.:	S627 C2
Physical State:	Light cream powder
Purity:	2.06 x 10^{11} spores/g (per Sponsor Certificate of Analysis)
Storage conditions:	Room temperature

Albino Sprague-Dawley rats [redacted] in acceptable health condition were used for the study.

Two male rats were selected for testing and were exposed to an aerosol generated from the undiluted test substance (fine powder) for a period of four hours. When 99% concentration (t-99) was attained, the animals were inserted into a ~ 15 L stained steel nose-only inhalation chamber for the specified exposure period. At the termination of the exposure period, the animals were returned to their stock laboratory cages.

The concentration of the test substance in the exposure atmosphere was determined gravimetrically twice per hour and nominally at the end of the exposure. The nominal concentration was determined by dividing the loss in weight of the test substance after the exposure by the total volume of air passed through the chamber.

Particle size was determined twice during the exposure, using a cascade impactor. The MMAD and particle size distributions are calculated from these data using probit analysis.

Observation for mortality and signs of pharmacological and/or toxicological effects were made frequently on the day of exposure (Day 0) and at least once daily thereafter. Individual body weights were recorded just prior to the inhalation exposure and at the time of discovery after death.

Both animals were subjected to gross necropsy and all normalities recorded. Sections of each lung of both animals were preserved in 10% neutral buffered formalin for histopathological examination by Colorado Histo-Prep (see STUDY 3).

Results

Mortality/Estimated LC_{50} values

Both animals were found dead on Day 2 of the study. The acute inhalation LC_{50} for *Beauveria bassiana* is < 5.39 mg/L in male albino rats.

Body weights

Individual body weights are presented in the following table.

Table B.6.1.2.2.2-3: Body weights, time of death and gross necropsy findings for acute inhalation toxicity of *Beauveria bassiana* strain PPRI 5339 in rats

Animal Number	Body Weights (g)			Time of Death	Gross Necropsy Findings
	Day 0	Day 7	Final		
31-M	270	-	234	Day 2	Red crust around mouth; lungs & liver mottled
32-M	262	-	228	Day 2	Red crust around mouth; lungs & liver mottled

Indicates time of discovery after death

Pharmacologic and/or Toxicological Signs

All observations are presented in the following tables.

Table B.6.1.2.2.2-4: Pharmacologic and /or toxicologic signs in acute inhalation toxicity study of *Beauveria bassiana* in albino rats up to 6 hours after exposure.

Reaction and severity	Hours after exposure begins				
	0.5	1.0	2.5	4.5	6.0
Piloerection (m)	0	0	0	2	2
Activity increase (m)	0	0	0	2	2

v – very slight, s – slight, m – moderate, e – extreme

Note: Digits indicate number of animals exhibiting reaction.

Table B.6.1.2.2.2-5: Pharmacological and /or toxicological signs in acute inhalation toxicity study of *Beauveria bassiana* in albino rats up to 14 days after exposure.

Reaction and severity	Days after exposure	
	1	2
Piloerection (e)	2	
Activity decrease (e)	2	
Respiratory gurgle	2	
Body tremors	2	
Death	0	2

v – very slight, s – slight, m – moderate, e – extreme

Note: Digits indicate number of animals exhibiting reaction. If discovery was between scheduled observations, death is presented under next observation time.

Necropsy findings

Individual necropsy findings are presented in Table B.6.1.2.2-3. The gross necropsy conducted on each animal revealed abnormal findings of red rust at the mouth and mottled lungs and liver.

Conclusion

Beauveria bassiana was evaluated for its acute inhalation toxicity potential in albino rats. The acute inhalation LC_{50} is < 5.39 mg/L in males.

STUDY 3

Report: [redacted] (2011) Pilot inhalation toxicity study in rats. [redacted]
Unpublished Report No. CHP 076Y ([redacted] 15687-11)

Guidelines: None

GLP: No

Deviations: None

Aim of the Study

The aim of this study was to conduct histopathological evaluation of the lungs from both animals used in [redacted] 2012a (see STUDY 2).

Method

The test substance was administered to 2 male rats at 5 mg/L for 4 hours.

The lungs from both animals were submitted to Colorado Histo-Prep for histopathological evaluation by a board-certified veterinary pathologist.

The samples were trimmed, processed, sectioned and stained. Two trims from the largest lobes were submitted and one trim of each of the other 4 lobes was submitted. Histopathology of the tissues was conducted on slides stained with hematoxylin and eosin.

Results

There were moderate to severe lung lesions in both rats. In Rat No. 31M there was necrosis of the lining epithelium of secondary bronchi, terminal bronchioles and alveolar ducts. There was acute inflammation in the walls of these airways that often extended into adjacent alveoli. Many of these adjacent alveoli contained free inflammatory cells and cellular debris. There was diffuse congestion of blood vessels and alveolar walls, and many free blood cells (hemorrhage) in alveoli. The lesions were present throughout the entire lobe with essentially the same severity in all lobes submitted.

In Rat No. 32M, the lung lesions were similar to those of Rat No. 31M except not as extensive. There was necrosis of the lining epithelium in secondary bronchi, terminal bronchioles and alveolar duct with acute inflammation in the walls of these airways. The inflammation did not extend into adjacent airways as was observed in Rat No. 31M and adjacent alveoli did not have as many inflammatory cells or cellular debris. A few large bacteria were observed in the airways suggesting the rat may have had a considerable interval between death and necropsy or potentially died on study. This would account for very similar distribution of lesions, but less extensive or severe than those of Rat No. 31M. Diffuse congestion and hemorrhage was similar to that of Rat No. 31M.

Conclusion

An effect due to the test substance was present in the lungs of both rats in this study. The lesions consisted of necrosis of the lining epithelium of secondary bronchi, terminal bronchioles and alveolar ducts. There was acute inflammation in the walls of these airways and adjacent alveoli. Diffuse congestion and haemorrhage was present in all lobes. The same lesions were present in Rat No. 32M, but were less extensive and appeared as they were perhaps of shorter duration. A few bacteria were present in the tissues suggesting this rat may have died on study or had a longer period between death and necropsy. There was essentially no difference in the lesions among the different lobes in respective rats.

STUDY 4

Report:	[redacted] (2012b), Acute inhalation toxicity study in rats. [redacted]
	Unpublished Report No. 15369-11
Guidelines:	OECD Guidelines for the Testing of Chemicals No.: 403
	US EPA OCSPP 870.1300
	FIFRA 7 USC 136
GLP:	Yes
Deviations:	The provided Certificate of Analysis was not accompanied by a GLP compliance statement.
	Stability information was not provided to the testing facility.
	Relative humidity was at times outside protocol range.
	The deviations listed did not affect the outcome of the study.

Aim of the study

The objective of this study was to assess the acute inhalation toxicity potential of the test substance in albino rats.

Materials and methods

Day of Treatment:	18 August and 14 September 2011
End of Experiment:	1 September and 28 September 2011

Test Item

Name:	*Beauveria bassiana* strain PPRI 5339
Batch No.:	5627 C2
Physical State:	Light cream powder
Purity:	2.06×10^{11} spores/g (per provided information)

Storage conditions: Room temperature

Albino Sprague-Dawley rats (provided by [redacted] in acceptable health condition were used for the study.

Twenty rats (5 male and 5 female per each of two exposure levels) were selected for testing and were exposed to an aerosol generated from the undiluted test substance (fine powder) for a period of four hours at concentrations of either 5.28 mg/L or 2.59 mg/L. When 99% concentration (t-99) was attained, the animals were inserted into an ~ 15 L stained steel nose-only inhalation chamber for the specified exposure period. At the termination of the exposure period, the animals were returned to their stock laboratory cages.

The concentration of the test substance in the exposure atmosphere was determined gravimetrically twice per hour and nominally at the end of the exposure. The nominal concentration was determined by dividing the loss in weight of the test substance after the exposure by the total volume of air passed through the chamber. Particle size was determined twice during the exposure, using a cascade impactor. The MMAD and particle size distributions are calculated from these data using probit analysis.

Observation for mortality and signs of pharmacological and/or toxicological effects were made frequently on the day of exposure (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to the inhalation exposure and at Days 7 and 14, or at the time of discovery after death.

On Day 14 after exposure, each surviving animal was euthanized. All study animals, whether dying during the study or euthanized were subjected to gross necropsy and all abnormalities recorded.

Results

Mortality/estimated LC_{50} values

Mortality data is presented in the following table.

Table B.6.1.2.2.2-6: Mortality data for acute inhalation study of *Beauveria bassiana* on albino rats

Test Substance Conc. (mg/L)	Number dead/Number treated		
	Males	Females	Combination
5.28	3/5	3/5	6/10
2.59	4/5	3/5	7/10

The acute inhalation LC_{50} of *Beauveria bassiana* strain PPRI 5339 is < 2.59 mg/L to albino rats.

Body weights

Animals surviving to termination exhibited weekly weight gain during the study except in one male and one female that lost weight between Days 0 and 7.

Clinical signs

Clinical signs are presented in the following tables. Prominent in-life observations included activity decrease and piloerection, which were clear in surviving animals by Day 3. Respiratory gurgle was only observed in animals that died during the study.

Table B.6.1.2.2.2-7: Pharmacological and /or toxicological signs in acute inhalation toxicity study of *Beauveria bassiana* in albino rats up to 6 hours after exposure at a concentration of 5.28 mg/L.

Reaction and severity	Hours after exposure begins				
	0.5	1.0	2.5	4.5	6.0
Males					
Piloerection (m)	0	0	0	5	5
Activity increase (m)	0	0	0	5	5
Females					
Piloerection (m)	0	0	0	5	5
Activity increase (m)	0	0	0	5	5

v – very slight, s – slight, m – moderate, e – extreme
Note: Digits indicate number of animals exhibiting reaction.

Table B.6.1.2.2.2-8: Pharmacological and /or toxicological signs in acute inhalation toxicity study of *Beauveria bassiana* in albino rats up to 14 days after exposure at a concentration of 5.28 mg/L.

Reaction and severity	Days after exposure													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Males														
Piloerection (m)	5	3	0	0	0	0	0	0	0	0	0	0	0	0
Activity decrease (m)	5	3	0	0	0	0	0	0	0	0	0	0	0	0
Death	0	2	1	0	0	0	0	0	0	0	0	0	0	0
Females														
Piloerection (me)	5	4	0	0	0	0	0	0	0	0	0	0	0	0
Activity decrease (m)	5	4	0	0	0	0	0	0	0	0	0	0	0	0
Death	0	1	2	0	0	0	0	0	0	0	0	0	0	0

v – very slight, s – slight, m – moderate, e – extreme
Note: Digits indicate number of animals exhibiting reaction. If discovery was between scheduled observations, death is presented under next observation time. Time of death indicates time of discovery after death. If discovery was between scheduled observations, death is presented under next observation time.

Table B.6.1.2.2.2-9: Pharmacological and /or toxicological signs in acute inhalation toxicity study of *Beauveria bassiana* in albino rats up to 6 hours after exposure at an exposure concentration of 2.59 mg/L.

Reaction and severity	Hours after exposure begins				
	0.5	1.0	2.5	4.5	6.0
Males					
Piloerection (m)	0	0	0	5	5
Activity increase (m)	0	0	0	5	5
Females					
Piloerection (m)	0	0	0	5	5
Activity increase (m)	0	0	0	5	5

v – very slight, s – slight, m – moderate, e – extreme
Note: Digits indicate number of animals exhibiting reaction.

Table B.6.1.2.2.2-10: Pharmacological and /or toxicological signs in acute inhalation toxicity study of *Beauveria bassiana* in albino rats up to 14 days after exposure at a concentration of 2.59 mg/L.

Reaction and severity	Days after exposure													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Males														
Piloerection (s-e)	5	5	1	0	0	0	0	0	0	0	0	0	0	0
Activity decrease (s-e)	5	5	2	0	0	0	0	0	0	0	0	0	0	0
Respiratory gurgle	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Death	0	0	2	2	0	0	0	0	0	0	0	0	0	0
Females														
Piloerection (s-e)	5	5	1	0	0	0	0	0	0	0	0	0	0	0
Activity decrease (s-e)	5	5	1	0	0	0	0	0	0	0	0	0	0	0
Respiratory gurgle	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Death	0	0	2	1	0	0	0	0	0	0	0	0	0	0

v – very slight, s – slight, m – moderate, e – extreme

Note: Digits indicate number of animals exhibiting reaction. If discovery was between scheduled observations, death is presented under next observation time. Time of death indicates time of discovery after death. If discovery was between scheduled observations, death is presented under next observation time.

Necropsy

Abnormal necropsy findings that occurred in animals that died on test pertained to crusted muzzle, strained/matted genital fur, discoloured lungs/liver/kidneys and empty gastrointestinal tract. The gross necropsy on the seven animals surviving to termination of the study revealed no observable abnormalities except discoloured lungs in one male and two females.

Conclusions

Beauveria bassiana strain PPRI 5339 was evaluated for its acute inhalation toxicity potential in albino rats. The acute inhalation LC_{50} is < 2.59 mg/L in males and females.

B.6.1.2.2.3 Intraperitoneal/subcutaneous single dose (Annex IIM 5.3.4)

Report: [redacted] (2012c) *Beauveria bassiana* PPRI 5339 Spore Concentrate. Acute intraperitoneal injection toxicity/pathogenicity study in rats. [redacted]

Unpublished Report No. 16448-12

Guidelines: US EPA OSCPP 885.3200

FIFRA 7 USC 136

GLP: Yes

Deviations: Characterization and stability information was not provided to the testing facility.

The relative humidity was outside protocol range.
Air change is outside protocol range.
Body weights for 23 males and 2 females were outside the protocol range.
The deviations listed did not adversely affect the outcome of the study.

Aim of the study

This study was conducted to evaluate the intraperitoneal (IP) toxicity, infectivity and pathogenicity *Beauveria bassiana* strain PPRI 5339 spore concentrate a single high dose exposure and an adequate post-exposure period.

Materials and methods

Day of Treatment: 27 July 2012
End of Experiment: 17 August 2012

Test Item

Name:	*Beauveria bassiana* strain PPRI 5339
Batch No.:	S627 C2
Physical State:	Light cream powder
Storage conditions:	Refrigerated

Albino Sprague-Dawley rats (provided by [redacted]) in acceptable health condition were used for the study. Fifty rats (25 male and 25 female) were selected for this study.
Three groups of animals were used for this study:
Group I: Untreated control (5 animals per sex)
Group II: Inactive test substance (15 animals per sex)
Group III: Active test substance (5 animals per sex)

A 1:100 dilution of the test substance was made in a 3 step process. An aliquot of this test substance solution was autoclaved for 60 minutes at 121 °C in order to make the test substance inactivated for Group II. Following dosing to Group III, a sample of the dosing solution was used to enumerate the organisms on agar plates for dose verification.
After sterilising the injection site with alcohol, the animals were dosed with active test item (Day 0) by IP injection at a level of 7.8×10^7 CFU/rat (15 animals per sex) or inactive (autoclaved) test substance (5 animals per sex). A treated control group was conducted concurrently.

Body weights were recorded on Day 0 (prior to dosing) and on Days 7, 14 and 21.
Observations for mortality and signs of pharmacological and/or toxicological effects were conducted three times on Day 0 and after dosing and daily thereafter. Observations included but were not limited to; skin and fur, eyes and mucus membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour patterns.
Organ weights were taken from all animals sacrificed or dying on test day except any on Day 0.

Six animals (3 per sex) from Group III were sacrificed one hour after dosing, and the peritoneal cavity from each animal was washed with 3 mL of sterile saline. The wash was recovered and plated for dosing verification and enumeration. All surviving animals were sacrificed on Day 21. A gross necropsy was conducted on each sacrificed animal at the interim times and at the termination of the study or at the time of discover after death. The gross necropsy included the following: gross observations of external surfaces, all orifices, thoracic, abdominal and pelvic cavities. Organ weights (mesenteric lymph nodes, lungs, liver, kidneys, spleen and brain) were recorded for all animals sacrificed or found dead after Day 0.

A one-way parametric analysis of variance (ANOVA) with Tukey's Multiple Comparisons Test was performed on the recorded body weights, body weight gains and the relative organ weights as a percentage of body weights.

Results

Mortality

There was no mortality in any group during the study.

Body Weights

There were no significant differences between body weights or body weight gain apart from Day 0 where male body weights for Group II were significantly greater than Group I and Group III (p=0.0070). This was not considered to be an effect of the test item.

Observations

There were no abnormalities observed during the course of the study.

Peritoneal Wash

For the six animals sacrificed one hour after dosing, the peritoneal cavity wash samples yielded an average colony forming unit (CFU) count of 3.0×10^5.

Necropsy findings

The gross necropsy revealed no observable abnormalities in any group.

Organ Weights

Absolute and relative mean organ weights are presented in the following tables:

Table B.6.1.2.2.3-1: Absolute mean organ weights (g)

Group	Sex	MLN	Lungs	Liver	Kidney	Spleen	Brain
I Untreated	Males	0.1661	2.4189	18.1134	3.6460	0.9002	1.3591
II Inactive	Males	0.1783	2.3515	17.4857	3.6158	0.9846	1.4100
III Test Item	Males	0.1161	2.5636	16.8617	3.4776	1.2043	1.6191
I Untreated	Females	0.0789	1.7641	10.2321	2.1634	0.7300	1.2488
II Inactive	Females	0.1078	1.8704	10.7953	2.2160	0.7113	1.0257
III Test Item	Females	0.1036	2.1092	10.1722	2.3342	0.8731	1.2389

MLN – Mesenteric Lymph Nodes

Table B.6.1.2.2.3-2: Relative mean organ weights (%)

Group	Sex	MLN	Lungs	Liver	Kidney	Spleen	Brain
I Untreated	Males	0.0495	0.7213	5.3998	1.0870	0.2684	0.4059
II Inactive	Males	0.0535	0.7034	5.2279	1.0821	0.2959	0.4219
III Test Item	Males	0.0346	0.7650	5.0304	1.0377	0.3609	0.4841
I Untreated	Females	0.0365	0.8238	4.7887	1.0141	0.3395	0.5792
II Inactive	Females	0.0513	0.8891	5.1608	1.0614	0.3369	0.4940
III Test Item	Females	0.0467	0.9461	4.5550	1.0454	0.3918	0.5486

MLN – Mesenteric Lymph Nodes

There were some significant differences in the relative organ weights. For males, Group III relative brain weights were significantly greater than Group I (p=0.0230). Group III relative spleen weights were significantly greater than Group I relative spleen weights (p=0.0234). Group II relative MLN weights were significantly greater than Group III MLN weights (p=0.0209). For females, Group II relative liver weights were significantly greater than Group II relative liver weights (p=0.0027). Combined spleen relative organ weights in Group III were significantly greater than Group I spleen relative organ weights (p=0.0150). There were no other significant differences in organ weights between groups.

Conclusions

There was no mortality in any group during the study. During observations for clinical signs, all animals appeared normal for the duration of the study. The gross necropsy conducted at termination of the study revealed no observable abnormalities.

The test substance *Beauveria bassiana* PPRI 5339 was determined to be non-toxic and non-pathogenic to rats when administered by IP injections at a single dose of 7.8 x 10^8 CFU/ml) or 7.8 x 10^7 CFU/rat.

B.6.1.2.2.4 Acute dermal toxicity study

STUDY 1

Report:	[redacted] (2011c), Acute dermal toxicity study in rats. [redacted]
	Unpublished Report No. 15368-11
Guidelines:	US EPA OCSPP 870.1200
	FIFRA 7 USC 136
	OECD Guidelines for the Testing of Chemicals No.: 402
GLP:	Yes
Deviations:	The provided Certificate of Analysis was not accompanied by a GLP compliance statement.
	Stability information was not provided to the testing facility.
	Relative humidity was at times outside the protocol range. The deviations did not affect the outcome of the study.

Aim of the Study

The aim of the study was to assess the systemic toxicity potential and relative skin irritancy of the test substance when administered to rats.

Materials and methods

Day of Treatment:	25 August 2011
End of Experiment:	8 September 2011

Test Item

Name:	*Beauveria bassiana* strain PPRI 5339
Batch No.:	S627 C2
Physical State:	Light cream powder
Purity:	2.06 x 10^{11} spores/g (per provided information)
Storage conditions:	Room temperature

Albino Sprague-Dawley rats (provided by [redacted] in acceptable health condition were used for the study. Ten animals (5 male, 5 female) were selected for the study. A test site of at least 10% of the total body surface area was prepared on the dorsal surface of the trunk the day prior to treatment. Each animal was treated with 5050 mg/kg of the test item. Each dose was moistened with a sufficient amount of DI water (1 ml/g test substance) and covered with a surgical gauze patch. The test substance was maintained in contact with the skin for 24 hours. After 24 hours the test sites were gently washed.

Observations for mortality and clinical/behavioural signs of toxicity were made at least three times on the day of dosing (Day 0) and at least once daily thereafter for 14 days. Individual body weights were recorded just prior to dosing and on Days 7 and 14.

Observations for evidence of dermal irritation were made at approximately 60 minutes after removal of wrappings and on Days 4, 7, 11 and 14.

On day 14, after dosing, animals were euthanized and subjected to gross necropsy and all anomalies recorded.

Findings

Mortality

There was no mortality during the study. The estimated acute dermal LD_{50} was determined to be >5050 mg/kg body weight.

Clinical symptoms

All animals appeared normal for the duration of the study. There were no signs of dermal irritation at any observation during the study.

Body weight and body weight gain

The individual animals exhibited weekly weight gain during the study, with the exception of one female that lost 4g between Days 0 and 7.

Necropsy

Gross necropsy conducted at the termination of the study revealed no observable abnormalities.

Conclusions

The test substance, *B. bassiana* strain PPRI 5339, was evaluated for its acute dermal toxicity potential and relative skin irritancy when administered to albino rats. The acute dermal LD_{50}, as indicated by the data, is > 5050 mg/kg in males and females. There were no signs of dermal irritation.

STUDY 2

Report:	[redacted] (2007a) Acute dermal toxicity study of test item Bb spore concentrate in rats. [redacted] Unpublished Report No. 06/338-002P
Guidelines:	OECD Guidelines for Testing of Chemicals No.: 402 EU Method B.3 OPPTS 870.1200
GLP:	Yes
Deviations:	None

Aim of the Study

The acute dermal toxicity of test item Bb (*B. bassiana* strain PPRI 5339) spore concentrate was assessed in RL(WI) BR rats to provide information on health hazards likely to arise from 24 hour exposure by the dermal route.

Materials and methods

Day of Treatment: 01 February 2007

End of Experiment: 15 February 2007

Test Item

Name:	Bb spore concentrate
Batch No.:	F1096
Physical State:	Uniform cream powder
Expiry Date:	June 2007
Storage conditions:	Store away from light in a cool (5 °C), dry dark place

CRL(WI)BR Wistar rats (provided by [redacted] in acceptable health condition were used for the study.

Five male and five female animals were treated with Bb (*B. bassiana* strain PPRI 5339) spore concentrate by a single 24-hour dermal exposure in its original form at a dose level of 2000 mg/kg bw. 24 hours prior to the start of the treatment period the trunk of each animal was shaven. The test item was applied as a single dose uniformly over an area of approximately 10 per cent of the total body surface for a 24-hour exposure period. Sterile gauze pads were placed over the skin of each animal where the test item was applied. The gauzes were kept in contact with the skin by a patch with an adhesive hypoallergenic plaster.

Clinical examinations were made on the day of treatment 1 hour and 5 hours after the application of the test item, and once each day for 14 days thereafter. The body weights of animals were recorded on day 0 and weekly thereafter. A gross necropsy was performed in all animals at the end of observation period.

Findings

Mortality

No mortality occurred during the study.

Clinical symptoms

There were no behavioural changes or general signs of toxicity in male or female animals treated at 2000 mg/kg bw.

Dermal alterations were observed in one female animal. Erythema and mild oedema were noted on the treated skin from day 1, which recovered between days 2 and 4 furthermore epithelization was registered between days 2 and 8.

Body weight and body weight gain

The body weight development was undisturbed in male and female animals during the 14 day observation period, i.e. similar to that expected in untreated animals of the same age and strain.

Necropsy

No macroscopic alterations related to the toxic effect of the test item were seen during the necropsy.

Conclusions

Under these experimental conditions, the acute dermal LD_{50} value of the test item Bb spore concentrate was greater than 2000 mg/kg in male and female CRL:(WI) BR rats.

B.6.1.2.3 Genotoxicity testing (Annex IIM 5.3.5)

B.6.1.2.3.1 *In vitro* studies (Annex IIM 5.3.5)

PPRI 5339:

Report:	[redacted] (2014) *Beauveria bassiana* PPRI 5339: Bacterial reverse mutation assay. [redacted] Unpublished Report No. 8295690
Guidelines:	OECD Guidelines for the Testing of Chemicals No.: 471
GLP:	Yes
Deviations:	In order to achieve 2300g the centrifuge used was set at 3290 instead of the 300 rpm (2300 g) stated in the protocol.

Aim of the study

The objective of this study was to evaluate the potential mutagenic activity of *Beauveria bassiana* PPRI 5339 by examining its ability to revert five hisitidine-requiring strains of *Salmonella typhimurium* in the absence and presence of a rat liver metabolising system.

Materials and methods

Start of Experiment: 24 January 2014

End of Experiment: 17 February 2014

Test Items

Name: *Beauveria bassiana* strain PPRI 5339

Batch No.: S826

Physical State: Fine off-white powder

Storage conditions: Refrigerated

Purity: 1 x 10^{11} cfu/g (nominal)

Expiry Date: 08 August 2014

The bacterial strains listed in Table B.6.1.2.3.1-1 were used in this study:

Table B.6.1.2.3.1-1: Bacterial strains used in the evaluation of the potential mutagenic activity of *Beauveria bassiana* PPRI 5339

Organism	Strain	Type of Mutation	Mutant Gene
S. typhimurium	TA98	frame-shift	histidine
S. typhimurium	TA100	base-pair substitution	histidine
S. typhimurium	TA1535	base-pair substitution	histidine
S. typhimurium	TA1537	frame-shift	histidine
S. typhimurium	TA102	base-pair substitution	histidine

Test article stock formulations were formulated as a suspension under subdued lighting in DMSO. This was ultrasonicated for 5 minutes and allowed to cool prior to centrifugation at 3290 (2300g) for 5 minutes. The supernatant was membrane filter-sterilised and subsequent dilutions made with DMSO. All test article formulations were protected from light and used with approximately 4 hours of initial formulation. The concentrations listed in Table B.6.1.2.3.1-2 were tested. 0.1 mL volume additions of test article solutions were used for all plate-incorporation treatments, 0.05 mL volume additions were used for all pre-incubation treatments.

Table B.6.1.2.3.1-2: Concentrations of *Beauveria bassiana* PPRI 5339 tested for mutagenic activity

Experiment	S-9	Concentration of Treatment Formulation (mg/ml)	Nominal Final Concentration (µg/plate)
Range-Finder and Experiment I	- and +	0.05	5
		0.16	16
		0.5	50
		1.6	160
		5	500
		16	1600
		50	5000
Experiment 2	- and +	1.6*	160
		3*	300
		6.25*	625
		12.5*	1250
		25*	2500
		50*	5000

*Concentration of treatment formulations used for the Experiment 2 pre-incubation treatments were twice that stated above, in order to permit treatments at the final concentrations stated, whilst volume additions were reduced to 0.05 mL.

Control treatments were performed using the same addition volumes per plate as the test article treatments. Vehicle controls comprised treatments in quintuplicate with the vehicle DMSO, and

positive controls were included in triplicate without and with S-9. The positive control chemicals were used according to Table B.6.1.2.3.1-3.

Table B.6.1.2.3.1-3: Positive controls used to determine the mutagenicity of *Beauveria bassiana* PPRI 5339

Chemical	Stock Concentration (µg/mL)	Final Concentration (µg/plate)	Strain(s)	S-9
2-nitrofluorene (2NF)	50	5	TA98	-
Sodium azide (NaN_3)	20	2	TA100, TA1535	-
9-=aminoacridine (AAC)	500	50	TA1537	-
Mitomycin C (MMC)	2	0.2	TA102	-
Benzo[a]pyrene (B[a]P)	100*	10	TA98	+
2-aminoanthracene (AAN)	50*	5	TA100, TA1535, TA1537	+
	200*	20	TA102	+

*Concentration of treatment formulations used for the Experiment 2 pre-incubation treatments were twice that stated above, in order to permit treatments at the final concentrations stated, whilst volume additions were reduced to 0.05 mL.

As the results of Experiment 1 were negative, treatments in the presence of S-9 in Experiment 2 included a pre-incubation step. Quantities of test article or control solution (reduced to 0.05 mL) were mixed together an incubated for 20 minutes at 37 ± 1°C protected from light for 3 days. Following incubation, these plates were examined for evidence of toxicity to the background lawn, and where possible, revertant colonies were counted.

Individual plate counts were recorded separately and the mean and standard deviation of the plate counts for each treatment were determined. Data were considered acceptable if the vehicle control counts fell within the laboratory's historical control ranges.

The presence, or otherwise, of a concentration response was checked by non-statistical analysis, up to limiting levels (e.g. toxicity or 500 µg/plate).

Results

An initial Range-Finder Experiment was used for toxicity assessment only. The background bacterial lawn was considered to be normal with no indications of a feeding effect.

Experiments 1 and 2 demonstrated no evidence of toxicity. Following *Beauveria bassiana* PPRI 5339 treatments of all the test strains in the absence and presence of S-9, no increases in revertant numbers were observed ≥1.5-fold (in strain TA102), ≥2-fold (in strains TA98 and TA100) or ≥3-fold (in strains TA1535 and TA1537) the concurrent vehicle control. This study was considered therefore to have provided no evidence of any *Beauveria bassiana* PPRI 5339 mutagenic activity in this assay system.

Table B.6.1.2.3.1-4: Number of revertant colonies

Conc.	TA98		TA 100		TA1535		TA1537		TA102	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Experiment 1										
DMSO	18.6	5.3	84.4	98.4	17.0	12.4	10.2	13.0	280.2	234.2

5	18.3	4.5	93.0	87.3	19.3	12.7	9.0	10.0	265.3	215.0
16	17.7	1.2	97.3	80.7	17.0	10.3	4.7	9.0	296.3	262.7
50	16.0	7.0	92.0	78.7	18.3	12.0	7.7	13.0	278.7	262.3
160	21.0	4.5	115.7	102.0	18.0	12.3	9.7	13.3	283.7	267.0
500	18.3	6.7	106.3	105.3	15.7	16.3	8.7	8.3	247.3	276.0
1600	16.7	5.0	99.3	107.0	16.0	12.3	6.7	8.0	259.0	223.3
5000	13.0	1.0	90.7	88.0	22.0	16.7	6.0	7.7	272.0	220.7
pos. control	814.3	340.3	658.3	870.0	438.7	201.0	105.3	130.0	872.0	1463.0
Experiment 2										
DMSO	16.8	32.2	97.2	131.6	21.8	13.8	5.4	13.4	247.2	196.8
160	20.3	37.3	96.3	143.0	21.3	15.3	5.3	10.7	276.3	213.3
300	13.7	30.3	105.0	143.7	21.7	17.0	3.7	15.3	266.3	202.3
625	17.0	34.0	96.7	154.7	21.0	19.0	7.3	14.0	261.0	191.7
1250	16.7	38.7	98.7	138.7	19.0	18.0	10.0	14.0	281.0	200.0
2500	13.3	30.3	91.3	153.7	20.0	17.7	8.7	15.3	298.0	179.0
5000	20.7	29.7	111.7	143.3	32.7	12.7	4.7	10.7	259.7	190.3
pos. control	587.3	340.7	636.7	976.7	475.7	214.3	136.7	208.3	706.7	765.3

Conclusions

It was concluded that *Beauveria bassiana* PPRI 5339 did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested under the conditions of this study. These conditions included treatments at concentrations up to 5000 µg/plate (the maximum recommended concentration according to current regulatory guidelines), in the absence and presence of a rat liver metabolic activation system (S-9).

Genotoxicity of beauverecin:

The genotoxic potential of beauvericin was investigated in vitro in several short-term tests. Metabolic activation with S9 was not used except in the bacterial mutagenicity assays.

A summary of the results and conclusions drawn from the in vitro genotoxicity tests on beauvericin is presented in the following table.

Table B.6.1.2.3.1-5: Summary of in vitro genotoxicity studies for beauvericin

Test	Cell System	Dose	Metabolic Activation	Exposure Time (h)	Result	Reference
Reverse Gene Mutation Assay (AMES)	*S. typhimurium* T A97, T A98, T A100, T A102, T A1535	0.2, 2, 20 and 500 µg/plate	With and without S9	N/A	Negative	Fotso and Smith (2003)

Chromosomal aberrations	Human lymphocytes	1.25, 2.5, 5 and 10 µM	Without S9	48	Positive from 2.5 µM	Çelik et al. (2010)
Micronucleus test	Human lymphocytes	1.25, 2.5, 5 and 10 µM	Without S9	48	Positive from 5µM	Çelik et al. (2010)
Micronucleus test	Porcine kidney PK15 cells	0.064, 0.64 and 6.4 µM	Without S9	24 and 48	Positive at 0. 64 and 6.4 µM (24 hours) and at 6.4 µM (48 hours)	Klarić et al. (2008a)
Sister chromatid exchanges	Human lymphocytes	1.25, 2.5, 5 and 10 µM	Without S9	48	Positive at all doses	Çelik et al. (2010)
Comet assay (alkaline)	Porcine kidney PK15 cells	0.1 and 0.5 µM	Without S9	1 and 24	Positive at 0.5 µM (24 hours)	Klarić et al. (2010)
Comet assay (alkaline)	Human leucocytes	0.1 and 0.5 µM	Without S9	1 and 24	Slightly positive at 0.5 µM (24 hours)	Klarić et al. (2010)

The bacterial reverse mutation test was carried out using 5 *S. typhimurium* strains (TA97, TA98, TA100, TA102 and TA1535). In the spot test a single beauvericin concentration of 2 µg/plate was used. A range of concentrations of beauvericin (0.2 to 500 µg/plate), both with and without S9, was used in the plate incorporation test. Three positive controls (2-aminofluorene, sodium azide and dexon) and a negative control were included in the assays. Beauvericin was non-mutagenic to all *S. typhimurium* strains, either with or without metabolic activation (Fotso and Smith, 2003).

The genotoxic potential of beauvericin was investigated *in vitro* in cultures of human lymphocytes with the chromosome aberration, sister-chromatid exchange and micronucleus assays. Cytotoxicity was measured by mitotic, proliferative and nuclear division indices. A significant concentration- dependent increase was caused by beauvericin in the number of chromosomal aberrations per cell (from 1.25 µM beauvericin), sister chromatid exchanges (from 1.25 µM), and micronuclei (from 5 µM). A significant decrease the mitotic index was observed at the two highest concentrations studied (5 and 10 µM). A decrease in the proliferative and replication indices was also observed but was not significant. The authors concluded that the results indicated that beauvericin is genotoxic to human lymphocytes *in vitro* (Çelik et al., 2010). The EFSA Panel on Contaminants in the Food Chain (CONTAM) noted that the effects of cytotoxicity in all these assays, particularly at the highest concentrations of beauvericin used (5 and 10 µM), cannot be excluded[1]. However, although the conditions of the micronucleus assay were not according to recommended guidelines, micronuclei increased in a concentration-dependent manner in human lymphocytes following beauvericin treatment.

[1] EFSA Scientific Opinion on the Risks to Human and Animal Health Related to the Presence of Beauvericin and Enniantins in Food and Feed. EFSA Journal 2014; 12(8):3802.

After 24 and 48 hour treatment with 0.05, 0.5 and 5 µg/ml of beauvericin, Giemsa-stained binucleated Porcine Kidney PK15 cells were scored for the presence of micronuclei, nuclear buds (NBs) and nucleoplasmatic bridges. Beauvericin induced micronuclei and nucleoplasmatic bridges in a dose-dependent manner, showing predominantly clastogenic effects. A dose-dependent increase in NBs was observed after 24-hour exposure. Prolonged treatment with 5 µg/mL of beauvericin resulted in a decrease in NBs, which according to the authors, could be related to the extrusion of micronuclei and/or saturation of the genotoxic effect (Klarić et al., 2008a). The authors suggested that the effects observed may be due to oxidative stress. The CONTAM Panel noted that this would imply a threshold mode of action[1]. The CONTAM Panel also noted that the study did not give any details on cytotoxicity. However, Klarić also reported in other papers that a significant decrease in cell viability preceded an increase in LDH activity observed in porcine kidney PK15 cells after treatment with beauvericin for 48 hours at a concentration of 5 µg/mL (Klarić et al., 2006), and that the IC_{50} using the MTT reduction assay was 5.0 ± 0.6 µM (Klarić et al., 2010). Thus the genotoxicity results at the beauvericin concentration of 6.4 µM should be interpreted with caution.

The genotoxic potential of beauvericin was investigated in porcine kidney epithelial PK15 cells and human leukocytes using the alkaline Comet assay. Genotoxicity to PK15 cells was time- and concentration dependent. Beauvericin (0.5 µM) evoked statistically significant DNA damage in PK15 cells, considering all Comet tail parameters (tail length, tail intensity, tail moment, and abnormal sized tails (AST)) measured after 24 hours of treatment, but no significant effects were seen after treatment with beauvericin (0.1 µM), or after one hour treatment with either 0.1 µM or 0.5 µM beauvericin. The effect of beauvericin on human leukocytes was slightly concentration dependent but showed little time dependence. Tail momentum, but not tail length and tail intensity, was significantly higher in human leukocytes treated with beauvericin (0.5 µM) than in control cells after 24 hours of exposure. After one hour exposure of human leukocytes to beauvericin (0.5 µM), the changes that were observed in tail length, tail intensity and tail moment were not statistically different from control cells. No effects on comet tail parameters were seen after treatment of human leukocytes with beauvericin (0.1 µM). DNA damage changes were less pronounced in human leukocytes than in PK15 cells.

Based on the available data on beauvericin, it cannot be ruled out that the substance is genotoxic. Whilst there is no *in vivo* study available on the substance to make a sufficient conclusion on genotoxicity, the registrant proposes the value of 0.15 µg/person per day, corresponding to 0.0025 µg/kg bw/day, be used as the basis for a reference value for human health risk assessment. This value is the threshold of toxicological concern for compounds with a structural alert for genotoxicity based on the Cramer decision tree for Class III substances. This TTC value for beauvericin was derived by applying a safety factor of 10 to the standard 1.5 µg/kg bw/day TTC recommended for a Cramer Class III substance.

No beauvericin was detected in the active substance with a limit of detection (LOD) for analysis of the active substance of 0.1 mg/kg beauvericin (Volume 4 C.1.2.3). For previously approved *Beauveria*

bassiana strain a maximum of 5 mg/kg was set for beauvericin. *B. bassiana* PPRI 5339 is far below this level. Therefore, no particular toxicological concern is expected.

B.6.1.2.4 Cell culture study (Annex IIM 5.3.6)

No study required.

B.6.1.2.5 Information on short-term toxicity and pathogenicity (Annex IIM 5.3.7/ Annex IIM 5.3.7.1)

B.6.1.2.5.1 Health effects after repeated inhalatory exposure (Annex IIM 5.3.7.2)

Not required for this submission. No evidence of intrinsic toxicity or pathogenicity was observed in acute exposure studies, no further testing of toxicity is required.

B.6.1.2.6 Proposed treatment: first aid measures, medical treatment (Annex IIM 5.2.5)

There are no specific first aid measures for *B. bassiana*. According to the available data concerning infectivity, pathogenicity and toxicity and taking into account the rather low exposure potential, adverse health effects other than sensitisation (primarily by inhalation) upon occasional contact are unlikely. Nonetheless, standard hygienic practices and precautions should be maintained.

Inhalation, swallowing and eye contact should be avoided. Vomiting after swallowing may be dangerous due to potential aspiration.

In humans, eye, lung and disseminated infection with *B. bassiana* have been occasionally reported in the public literature. In the unlikely case of such events following contact with strain PPRI 5339, specialist´s expertise and treatment with anti-mycotic drugs will be needed.

B.6.1.3 Summary and conclusions of Tier I studies

Based on original studies presented on *B. bassiana* strain PPRI 5339 and supporting publications on acute effects irrespective of the *B. bassiana* strain used, it can be concluded that strain PPRI 5339, as well as the species as a whole, is not acutely toxic to mammals, nor does it display any pathogenic or infective properties that would be a concern to humans exposed as a result of using this active substance as a plant protection product. This has been proven in experiments employing different routes of exposure such as oral, inhalative (intratracheal), intrapertitoneal and topical dermal application.

In contrast, adverse effects were noted following inhalatory (aerosol) exposure to rats. Several studies are submitted showing significant mortality of animals following aerosol exposure of *B. bassiana* for 4 hours [redacted] 2012a & 2012b, see B.6.1.2.2, study 2 and 4). Subsequent histopathological analysis of lungs from these studies [redacted] 2011, see B.6.1.2.2, study 3) showed lungs contained moderate to severe lesions, including necrosis of the lining of the epithelium of secondary bronchi, terminal

bronchioles and alveolar ducts. These effects were also accompanied by acute inflammation of the walls of these airways that extended to adjacent alveoli in one animal. Many of these adjacent alveoli contained free inflammatory cells and cellular debris. There was diffuse congestion of blood vessels and alveolar walls, and many free blood cells (hemorrhage) in alveoli.

The submitted studies are in contrast to acute intratracheal toxicity studies conducted for other strains of *B. bassiana* (ATCC 74040 & GHA), where no mortality was observed, but local effects of inflammation/immune reaction and organ weight increase were observed in the lungs in addition to accumulation of cellular debris. These studies were conducted using an intratracheal application method, where the test item is applied directly onto the trachea. The mortality seen in studies by [redacted] 2012a & 2012b, is considered to be an artefact of the test method employed, i.e. aerosol exposure for a period of 4 hours. Such an exposure regime would induce a much more extreme inflammation/immune reaction than that seen in the intratracheal exposure, due to the sheer amount of the test item filling the lungs for 4 hours. An intake of 2.59 mg/L and taking into account 2.06×10^{11} CFU/g, an inhalation rate of 45 L/kg bw/h (see AOEL guidance document SANCO 7531, rev 10) and a mean body weight of 186 g in females results in an exposure of 1.79×10^{10} CFU/4 h, which is 3-orders of magnitude higher than in the intratracheal study. Build-up of cellular debris may also have contributed to the observed mortality in the 4 hour exposure. The pathological observations in the lungs support asphyxiation as a cause of death in the animals due to inflammation and necrosis as a result of an autoimmune reaction in the lung tissue. Where a similar application method to that of the cited strains ATCC 74040 and GHA was used (i.e. intratracheal), [redacted] 2013 (B.6.1.2.2.2, study 1) showed no mortality with active PPRI 5339.

The apparent immunological response seen in animals exposed via the aerosol inhalatory route is supported by the positive result seen for skin sensitisation [redacted] 2007, see B.6.1.2.1), although given the extreme response seen following inhalation studies this route is considered to be more critical than sensitisation elicited by dermal exposure.

Microorganisms are generally thought to have the potential to provoke sensitising reactions. The primary and supporting information submitted indicate that *B. bassiana* has the potential to induce inhalatory allergenicity and allergic skin reactions in laboratory animals and humans. Data from other *Beauveria bassiana* strains (ATCC 74040 & GHA) indicate that this effect is not strain specific and is a mechanism for the species in general and the phrase 'Contains *Beauveria bassiana* strain PPRI 5339. Micro-organisms may have the potential to provoke a sensitising reaction' must appear on the label for this active substance.

Genotoxic potential of *B. bassiana* strain PPRI 5339 was assessed using the bacterial reverse mutation assay ([redacted] 2014, see B.6.1.2.3.1), which was negative. However, there is a suite of *in vitro* genotoxicity studies conducted on the metabolite beauvericin (see B.6.1.2.3.1) indicating positive genotoxic potential. No beauvericin was detected in the active substance with a limit of detection

(LOD) for analysis of the active substance of 0.1 mg/kg beauvericin (Volume 4 C.1.2.3). For previously approved *Beauveria bassiana* strain a maximum of 5 mg/kg was set for beauvericin. *B. bassiana* PPRI 5339 is far below this level. Therefore, no particular toxicological concern is expected.

No further toxicity studies were conducted on *B. bassiana* strain PPRI 5339 and no further studies were deemed necessary. Whilst Regulation (EC) No 283/2013 indicates that a short-term study (minimum 28 days) is generally required for this substance, the registrant concluded that this study should be waived because no evidence of pathogenicity or infectivity was observed in the comprehensive acute data-set submitted. Additionally, the inhalation route is considered the most critical in relation to the leading health effect (sensitisation) and further long-term studies would not provide any further information to aid risk assessment and facilitate appropriate risk management measures for operators and workers.

Study/Route/Method	**Species**	**Dose per Animal**	**Results**	**Conclusion**	**Reference**
Skin Sensitisation OECD 406	Guinea Pigs	0.01% (w/v) intra-dermal, 20% (w/v) dermal induction. 5% (w/v) challenge	Positive	Sensitising	[redacted] (2007b)
Acute Oral Toxicity OECD 425	Rat	5000 mg/kg	No mortality observed during study No other significant observations	LD_{50} = >5000 mg/kg	[redacted] (2011d)
Acute Oral Toxicity OECD 423	Rat	2000 mg/kg	No mortality observed during study No other significant observations	LD_{50} = >2000 mg/kg	[redacted] (2007b)
Oral pathogenicity following exposure via feed	Mouse	Conidia suspension of 1 x 10^8 conidia/mL added to animal's feed	No evidence of pathogenicity or other pathological changes. Clearance of conidia in faeces after 7 days.	Not pathogenic	Acosta, ME *et al.* (2012)
Acute Pulmonary Toxicity/ Pathogenicity	Rat	7.7 x 10^7 CFU/rat	A single animal died during the study. No other signs of toxicity or pathogenicity	Non-toxic and non-pathogenic to rats	[redacted] (2013)
Acute Inhalation Toxicity	Rat	5.39 mg/L (aerosol)	2/2 Animals dosed were	LC_{50} = <5.39 mg/L	[redacted] (2012a)

		for 4 hours	found dead on Day 2 of the study		
Acute Inhalation Toxicity OECD 403	Rat	2.59 & 5.28 mg/L (aerosol) for 4 hours	Mortality by day 14: 6/10 for 5.28 mg/L 7/10 for 2.59 mg/L	LC_{50} = <2.59 mg/L	[redacted] (2012)
Histopathological evaluation of the lungs from both animals used in [redacted] 2012a	Rat	N/A	Lesions – necrosis of epithelial lining. acute inflammation, alveolar cellular debris, haemorrhaging in all lobes.	Mortality in [redacted] (2012a) was due to local effects of inflammation and not systemic toxicity	[redacted] (2011)
Acute Intraperitoneal Toxicity/Pathogenicity	Rat	7.8×10^7 CFU/rat	No mortality or abnormal observations	Non-toxic and non-pathogenic to rat	[redacted]
Dermal Pathogenicity-Topical administration semi-occlusive.	Mouse	1×10^9 conidia/mL	No adverse effects observed	Non-toxic and non-pathogenic to rats following	Acosta-Quinetero, ME (2011)
Acute Dermal Toxicity OECD 402	Rat	5050 mg/kg – semi-occlusive	No evidence of toxicity.	LD_{50} = >5050 mg/kg	[redacted] (2011c)
Acute Dermal Toxicity OECD 402	Rat	2000 mg/kg – semi occlusive	No mortality observed. Erythema and mild oedema noted day 1, which recovered between days 2 and 4. Epithelisation was registered between days 2 and 8.	LD_{50} = >2000 mg/kg	[redacted] (2007a)
Bacterial Reverse Mutation (AMES) OECD 471	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA102		No evidence of mutagenicity, with or without metabolic activation	Negative	[redacted] (2014)

B.6.2 Tier II – the active micro-organism (Annex IIM 5.5)

B.6.2.1 Specific toxicity, pathogenicity and infectiveness studies (Annex IIM 5.5.1)

B.6.2.1.1 Eye irritation

STUDY 1

Report:	[redacted] (2011a), Acute eye irritation study in rabbits. [redacted]
	Unpublished Report No. 15370-11
Guidelines:	US EPA OCSPP 870.2400
	FIFRA 7 USC 136
	OECD Guidelines for the Testing of Chemicals No.: 405.
GLP:	Yes
Deviations:	The provided Certificate of Analysis was not accompanied by a GLP compliance statement.
	Stability information was not provided to the testing facility.
	Relative humidity was at times outside the protocol range. The deviations did not affect the outcome of the study.

Aim of the Study

The aim of the study was to assess the relative level of eye irritation following a single exposure of the test substance to rabbits.

Materials and methods

Day of Treatment:	22 August 2011
End of Experiment:	29 August 2011

Test Item

Name:	*Beauveria bassiana* strain PPRI 5339
Batch No.:	S627 C2
Physical State:	Powder
Colour:	Light cream powder
Purity:	2.06 x 10^{11} spores/g (per provided information)
Storage conditions:	Store away from light in a cool (5 °C), dry dark place

Three albino New Zealand White rabbits (provided by [redacted] in acceptable health condition were used for the study. Both eyes of each animal were examined 24 hours prior to the start of the study. Only those animals without eye defects or irritation were selected for testing.

A single dose of 100mg of the test item was placed into the conjunctival sac of the right eye of each animal. The lids were held closed gently for one second to prevent the loss of the test item. The untreated left eye served as a control.

The eyes were examined at 1, 24, 48 and 72 hours then 4 and 7 days after treatment and the grades of ocular reaction recorded. The corneas of all treated eyes were examined immediately after the 24 hour observation with a fluorescein sodium ophthalmic solution. Any of the corneas that exhibited fluorescein staining at the 24 hour observation were examined in this manner at each consecutive observation until fluorescein staining of the cornea no longer occurred. All treated eyes were washed with water for one minute immediately after recording the 24 hour observation.
The irritation of the cornea was recorded using a four point grading scale Draize *et al.* (1944).

Findings

A summary of the irritation scores is presented in the following table.

Table B.6.2.1.1-1: Average (n=3) values assigned to ocular reactions after treatment of rabbits with *Beauveria bassiana* strain PPRI 5339

	Hours after treatment				**Days after treatment**	
I. Cornea	**1**	**24**	**48**	**72**	**4**	**7**
A. Opacity	0.0	1.0	1.0	1.0	0.0	0.0
B. Area	0.0	2.0	2.0	2.0	1.7	0.0
C. Fluorescein Staining	-	PA/PB	PA/PB	PA/PB	0.0	-
D. Stippling	0.0	0.0	0.0	0.0	0.0	0.0
II. Iris						
A. Grade	0.0	0.0	0.0	0.0	0.0	0.0
III. Conjunctiva						
A. Redness	1t	2.0	2.3	2.3	0.7	0.0
B. Chemosis	0.7	2.3	2.3	2.3	0.7	0.0
C. Discharge	1.0	2.0	2.0	2.0	0.0	0.0
D. Necrosis/Ulceration	0.0	0.0	0.0	0.0	0.0	0.0

P – Positive fluorescein staining (A – One quarter or less; B – Greater than one quarter but less than half), t – Trace of test substance in eye.

Conclusions

Due to the reversible effects observed related to conjuctival redness and conjunctival oedema (chemosis) - the average score for these values at 24, 48 and 72 hours post exposure is ≥2 - the study indicates that the test item is irritating to the eye. No classification is necessary based on this study as Regulation (EC) No 1272/2008 does not apply to microorganisms.

STUDY 2

Report: [redacted] (2007a), Acute eye irritation study of test item Bb Spore Concentrate in rabbits. [redacted]

[redacted]

Unpublished Report No. 06/338-005N

Guidelines: OECD Guidelines for Testing of Chemicals No.: 405
EU Method B.5
US EPA OPPTS 870.2400

GLP: Yes

Deviations None

Aim of the Study

The objective of the study was to determine the acute eye irritation effect of the test item Bb (*B. bassiana* strain PPRI 5339) spore concentrate in New Zealand White Rabbits.

Materials and methods

Day of Treatment: 18 January 2007
End of Experiment: 25 January 2007

Test Item

Name:	Bb spore concentrate
Batch No.:	F1096
Physical State:	Powder
Colour:	Uniform cream powder
Expiry Date:	June 2007
Storage conditions:	Store away from light in a cool (5 °C), dry dark place

New Zealand White rabbits (provided by [redacted] in acceptable health condition were used for the study. Both eyes of each animal provisionally selected for testing were examined 24 hours prior to the start of the study. Animals showing eye irritation, ocular defects or pre-existing corneal injury were not used.

A single dose of 0.1g of the test item Bb (*B. bassiana* strain PPRI 5339) spore concentrate was administered to 3 male rabbits by instillation into the conjuctival sac of the left eye. The eyelids were held closed gently for several seconds to prevent the loss of the test item. The contralateral eye served as a control. The eyes of the test animals were not washed out after the application of the test item.

The eyes were examined at 1, 24, 48 and 72 hours then one week after the treatment. The duration of the observation period was sufficient for the statement of reversibility or irreversibility of changes. Any clinical signs of toxicity or signs of ill-health during the study were recorded.

Findings

One hour after the single application of test item Bb spore concentrate into the eyes of the rabbits slight to moderate redness, slight chemosis and severely increased discharge were observed in the eye of the test animals.
There were no effects observed in the cornea and iris during the study period.
48 hours after the treatment in one animal only slight redness was seen. The other two animals had moderate redness, slight chemosis and slightly increased discharge.
72 hours after the treatment slight to moderate redness occurred. One week after the treatment every animal was symptom-free.
At one week after treatment the study was terminated, with all animals free of symptoms of irritation.
During the study the control eyes of animals were symptom-free. The general state and behaviour of the animals were normal throughout the study period. There were no notable body weight changes during the contact and observation period.

The animals' individual mean scores (considering readings at 24, 48 and 72 hours after the treatment) are listed in Table B.6.2.1.1-2.

Table B.6.2.1.1-2: Mean scores of symptoms after treatment of rabbits with Bb spore concentrate

Symptom	Individual mean scores		
	24 hours after treatment	48 hours after treatment	72 hours after treatment
Cornea opacity	0.00	0.00	0.00
Iris	0.00	0.00	0.00
Redness	1.33	1.66	2.00
Chemosis	0.33	0.66	1.33
Discharge	0.33	1.33	1.33

Conclusion

In conclusion, test item Bb spore concentrate applied to the rabbits' eye mucosa, caused slight to severe conjunctival irritant effects, fully reversible within 1 week. No classification is necessary based on this study as Regulation (EC) No 1272/2008 does not apply to microorganisms.

B.6.2.1.2 Skin irritation

Report: [redacted] (2011b), Acute dermal irritation study in rabbits. [redacted]
Unpublished Report No. 15371-11

Guidelines: US EPA OCSPP 870.2500, FIFRA 7 U.S.C. 136
OECD Guidelines for the Testing of Chemicals No.: 404

GLP: Yes

Deviations: The provided Certificate of Analysis was not accompanied by a GLP compliance statement.

Stability information was not provided to the testing facility.
Relative humidity was at times outside the protocol range. The deviations did not affect the outcome of the study.

Aim of the Study

The aim of the study was to assess the relative primary skin irritation level of the test substance *B. bassiana* strain PPRI 5339 on rabbits.

Materials and methods

Day of Treatment: 23 August 2011
End of Experiment: 26 August 2011

Test Item

Name: *Beauveria bassiana* strain PPRI 5339
Batch No.: S627 C2
Physical State: Light cream powder
Purity: 2.06 x 10^{11} spores/g provided information
Storage conditions: Room temperature

Albino New Zealand White rabbits (provided by [redacted] in acceptable health condition were used for the study.
Three animals (2 male, 1 female) were selected for the study. A test site of at least 8cm x 8cm was prepared on the dorsal area of the trunk the day prior to treatment. Each test site was treated with 500 mg test item moistened with 0.5 ml DI water and covered with a semi-permeable dressing. The test substance was maintained in contact with the skin for 4 hours.
Observations for erythrema and edema formation and any other dermal defects or irritation were made a 1, 24, 48 and 72 hours after removal of the dressings. Dermal irritation was scored on a four point scale (Draize technique).

Findings

The scores for this study are presented in the following table.

Table B.6.2.1.2-1: Results of acute dermal irritation study in rabbits for *Beauveria bassiana* strain PPRI 5339.

Animal Number	Erythema				Oedema				Primary irritation scores
	Hours				Hours				
	1	24	48	72	1	24	48	72	
0502-M	0	0	0	0	0	0	0	0	0.00
0504-M	1	1	0	0	0	0	0	0	0.50
0545-F	1	0	0	0	0	0	0	0	0.25
Primary irritation index									0.3

M – male, F – female

Conclusions

The primary irritation index of 0.3 out of a possible 8 was obtained from the observations at 1, 24, 48 and 72 hours and was used to give *B. bassiana* strain PPRI 5339 a descriptive rating of slightly irritating. No assessment of classification is necessary based on this study as Regulation (EC) No 1272/2008 does not apply to microorganisms.

B.6.2.2 *In vivo* studies in somatic cells (Annex IIM 5.5.2)

Based on the results of the studies and data from tier 1, no further study is required.

B.6.2.3 Genotoxicity – In vivo studies in germ cells (Annex IIM 5.5.3)

Based on the results of the studies and data from tier 1, no further study is required.

B.6.2.4 Summary and conclusions of Tier II studies

A summary of the studies submitted is presented in the following table. The data submitted indicates that *B. bassiana* is potentially irritating to the eye, but not irritating to the skin of laboratory animals.

Acute Eye Irritation OECD 405	Rabbit	100 mg/eye	Positive	Irritating to eye	[redacted] (2011a)
Acute Eye Irritation OECD 405	Rabbit	100 mg/eye	Negative	Not irritating to eye	[redacted] (2007a)
Acute Dermal Irritation OECD 404	Rabbit	500 mg per animal – semi-occlusive	Negative	Not irritating	[redacted] (2011b)

B.6.3 Summary of mammalian toxicity, pathogenicity and effectiveness and overall evaluation of the active micro-organism (Annex IIM 5.6)

In public literature it was reported that *B. bassiana* was isolated in a few cases from people suffering from eye infection. It is not known whether these pathogenic properties can be attributed to certain strains since such information is usually lacking in the case reports. Disseminated infections have been observed only in people under immunosuppression following exposure to chemotherapeutic drugs or in individuals who were severely ill. It is not likely that such persons will get into close contact with *Beauveria bassiana* when used for plant protection purposes.

Open literature reported evidence of *B. bassiana*-proteins that might cause allergies as well as indication for cross-sensitisation because of certain reactive epitopes that this microorganism has in common with other fungi such as *Alternaria alternata* or *Aspergillus fumigatus*. Based on the information *B. bassiana* should be regarded as a potential human allergen to both skin and inhalatory contact.

Based on original studies presented on *B. bassiana* strain PPRI 5339 and supporting publications on acute effects irrespective of the *B. bassiana* strain used, it can be concluded that strain PPRI 5339, as well as the species as a whole, is not acutely toxic to mammals, nor does it display any pathogenic or infective properties that would be a concern to humans exposed as a result of using this active substance as a plant protection product.

Severe reactions were seen in the lungs of rats during acute inhalation toxicity studies submitted under ([redacted] 2012a & 2012b, see B.6.1.2.2, study 2 and 4) when exposure was conducted using aerosols generated from the powdered test item. Histopathological investigation of the lungs of rats exposed to a 5.39 mg/L aerosol of the test item for 4 hours revealed moderate to severe lesions, including necrosis of the lining of the epithelium of secondary bronchi, terminal bronchioles and alveolar ducts. These effects were also accompanied by acute inflammation of the walls of these airways that extended to adjacent alveoli in one animal. Many of these adjacent alveoli contained free inflammatory cells and cellular debris. There was diffuse congestion of blood vessels and alveolar walls, and many free blood cells (hemorrhage) in alveoli. The effects seen in the acute inhalation studies are consistent with an allergic reaction to aerosol exposure and mortality was believed to have been caused by asphyxiation due to inflammation of the lungs.
The mortality seen in the studies by [redacted], 2012a & 2012b, is considered to be an artefact of the test method employed, i.e. aerosol exposure for a period of 4 hours. Such an exposure regime would induce a much more extreme inflammation/immune reaction than that seen in the intratracheal exposure, due to the sheer amount of the test item filling the lungs for 4 hours. The build-up of cellular debris may have also contributed to asphyxiation. When *B. bassiana* strain PPRI 5339 was tested with a similar acute intratracheal challenge [redacted] 2013, B.6.1.2.2.2, study 1) as reported in the DAR of strains ATCC 74040 and GHA, similar results to that of ATCC 74040 and GHA were seen with no test animal mortality as a result of treatment with active PPRI 5339.

Microorganisms are generally thought to have the potential to provoke sensitising reactions. The primary and supporting information submitted indicate that *B. bassiana* has the potential to induce inhalatory allergenicity and allergic skin reactions in laboratory animals and humans. Data from other *Beauveria bassiana* strains (ATCC 74040 & GHA) indicate that this effect is not strain specific and is a mechanism for the species in general and the phrase 'Contains *Beauveria bassiana* strain PPRI 5339. Micro-organisms may have the potential to provoke a sensitising reaction' must appear on the label for this active substance.

Genotoxic potential of *B. bassiana* strain PPRI 5339 was assessed using the bacterial reverse mutation assay [redacted] 2014, see B.6.1.2.3.1), which was negative. The genotoxic potential of the metabolite beauvericin was investigated in *in vitro* studies in public literature indicating a positive genotoxic potential. No beauvericin was detected in the active substance with a limit of detection (LOD) for analysis of the active substance of 0.1 mg/kg beauvericin (Volume 4 C.1.2.3). For previously

approved *Beauveria bassiana* strain a maximum of 5 mg/kg was set for beauvericin. *B. bassiana* PPRI 5339 is well below this level. Therefore, no particular toxicological concern is expected.

B.6.4 Effects on human health – the preparation (Annex IIIM 7)

B.6.4.1 Basic acute toxicity studies – the preparation (Annex IIIM 7.1)

BAS 480 00 I is an OD formulation containing 4.0 × 10^{12} CFU/L, equivalent to 40 g *Beauveria bassiana* strain PPRI 5339/L.

No studies were carried out with the formulation. The notifier indicated that the formulants do not influence the toxicity of the formulation. This statement was checked by the RMS and based on the composition of the formulation and the toxicological properties of the co-formulants (see Volume 4) no classification and labelling is required.

However, based on the sensitising potential of the active substance the phrase 'Contains *Beauveria bassiana* strain PPRI 5339. Micro-organisms may have the potential to provoke a sensitising reaction' must appear on the label.

B.6.4.2 Data on exposure – the preparation (Annex IIIM 7.2)

The intended uses are limited to glasshouse use only, which will be applied with a knapsack sprayer, and cover:

1. Edible and processed fruits; and
2. Ornamentals

Local and systemic effects following inhalation (aerosol) exposure, elucidated by studies on laboratory animals utilising aerosol methods of dosing (see M-MA 5.2.2.2) should be considered in the risk assessment. The leading health effects following inhalation exposure are related to inflammation of the lungs as a result of an immune reaction to the microorganism, which was proven to result in mortality through prolonged exposure to aerosols containing the active substance.

In the intratracheal study by [redacted] 2013, no evidence of toxicity was observed in laboratory animals at 7.7×10^{7} CFU/rat.

Estimation of operator exposure to *Beauveria bassian* PPRI 5339

Operator exposure estimates were calculated using the German model and the Dutch greenhouse model:

1. The German model for the protection of operators (BBA 1992)
   Uniform principles for assuring the protection of operators' health when using plant protection

products (uniform principles for operator protection), Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Heft 277, Berlin 1992 (in German).

2. Van Golstein Brouwers, Y.G.C., Marquart, J. and Van Hemmen, J.J. (1996). Assessment of occupational exposure to pesticides in agriculture. Part IV. Protocol for the use of generic exposure data. TNO Nutrition and Food Research Institute, The Netherlands. TNO Report V 96.120 "

The operator exposure has been calculated utilising personal protective equipment expected of operators applying the product BAS 480 00 I.

The following assumptions are made for the estimation of operator exposure.

Table 6.4.2-1: Input variables used for operator exposure estimation using the German model and Dutch greenhouse model

Intended use	High Crops, Hand Held (HCHH) * Manual spraying in greenhouses ^
Type of preparation	Liquid *
Application rate	0.1 kg a.s/ha
Treated area per day	1 ha
Dermal absorption	75% for concentrate and spray dilution (EFSA default values, 40 g/L formulation)
Inhalation absorption	100 %
Body weight	70 kg
Inhalation PPE Factors/Reduction Factors	0.08 (particle filtering half mask - mixing/loading & application) * 2 (non-powered mask filter type 2) ^
Dermal PPE Factors/Reduction Factors	0.01 (hands: protective gloves – mixing & loading & application) * 0.8 (head: particle filtering half mask – application) * 0.05 (body: protective garment & sturdy footwear - application) * 0.1 (gloves & coverall) ^

* Applicable to German model only

^ Applicable to Dutch glasshouse model only

Using the above input parameters, operator exposure was estimated for mixing/loading and application, each model used incorporates mixing & loading into exposure calculations.

Estimated operator exposures for each model are presented in the following table. Since operators are required to wear respiratory protection and protective clothing based on the sensitising properties no assessment without PPE was made.

Table 6.4.2-2: Estimated exposure values based on the German operator exposure model and Dutch greenhouse model

Exposure route and type of work	German model: Estimated operator exposure (HCHH) With PPE	Dutch greenhouse model: Estimated operator exposure With PPE
Dermal exposure		
Mixing/loading	0.00220 mg/kg bw/d	-
Application	0.00557 mg/kg bw/d	-
Total (dermal)	0.00776 mg/kg bw/d	0.0214 mg/kg bw/d
Inhalation exposure		
Mixing/loading	5.71×10^{-6} mg/kg bw/d	-
Application	3.43×10^{-5} mg/kg bw/d	-
Total (inhalation)	4.0×10^{-5} mg/kg bw/d	1.4×10^{-4} mg/kg bw/d

The maximum inhalation exposure is 1.4×10^{-4} mg/kg bw/d. The formulation Broadband consist of 4.0×10^{12} CFU/L (equivalent to 40 g *Beauveria bassiana* strain PPRI 5339/L). Therefore, the exposure of 1.4×10^{-4} mg/kg bw/d corresponds to 14000 CFU/kg bw or 9.8×10^{5} CFU/person. This is 1.3% of the dose applied in the intratracheal study (7.7×10^{7} CFU/rat) were no evidence of toxicity was observed.

In conclusion, there is low risk to operators when applying the product BAS 480 00 I in accordance with the representative uses.

Consideration of worker exposure to *Beauveria bassian* PPRI 5339

The greatest potential for worker exposure following re-entry into glasshouses will be via dermal contact. Inhalation exposure is generally confined to a brief period after application, while the product is drying. Since inhalation exposure is the route of concern the risk for the worker is expected to be low.

Consideration of bystander/worker exposure to *Beauveria bassian* PPRI 5339

The representative uses of Broadband are all conducted in glasshouses. No bystander or resident exposure is foreseen.

Consideration of operator/worker/bystander exposure to metabolites

The applicant provided an estimation of operator exposure to beauvericin taking into account a maximum level of beauvericin of 5 mg/kg as specified for the two approved strains of *Beauveria bassiana*. The applicant proposed to use the Threshold of Toxicological Concern approach as already mentioned in the DAR of the approved strains. Taking into account that beauvericin in *Beauveria bassiana* strain PPRI 5339 could not be detected above the LOQ of 0.5 mg/kg, far below the maximum content of 5 mg/kg set for the currently approved *Beauveria bassiana* strains, it is expected that there is no risk for operators. This is also shown based on the operator risk assessment. The

maximum exposure of 2.15×10^{-8} kg a.s./kg bw/d corresponds to 1.077×10^{-5} µg beauvericin/kg bw/d or 0.43% of the proposed reference value of 0.0025 µg/kg bw/day.

B.6.4.3 Available toxicological data relating to non-active substances – the preparation (Annex IIIM 7.4)

The co-formulants are not expected to affect the overall toxicity of the preparation. See volume 4 for more information on the composition of the formulation.

B.6.4.4 Supplementary studies for combinations of plant protection products (Annex IIIM 7.5)

No tank mix is proposed therefore no further information is required.

B.6.5 Summary and evaluation of health effects – the preparation (Annex IIIM 7.6)

No toxicity studies were carried out with the formulation. Based on the composition of the formulation and the toxicological properties of the co-formulants (see Volume 4) no classification and labelling is required.

However, due to the sensitising potential of the active substance the phrase 'Contains *Beauveria bassian* strain PPRI 5339. Micro-organisms may have the potential to provoke a sensitising reaction' must appear on the label and respiratory protection and protective clothing should be used when applying the product.

Estimated exposures from appropriate exposure models for operators, and workers re-entering glasshouses after application showed a low risk for operators and workers. No assessment of risk to residents and bystanders was conducted, as the representative uses are glasshouse only uses.

B.6.6 References relied on

Literature search

A literature search was carried out by the notifier (M document, section 9). The overall conduct of the literature search is agreed with by the RMS and is summarized below.

The search terms were included both the microorganism as well as potential metabolites:

Beauveria bassiana
Tenellin
Vivotoxins
Bassianolide
Bassianin
Beauvericin.

Search engines used were Thomson Innovation (which covers Web of Science), TOXLINE and Pubmed. A justification for the chosen search engines was provided and were agreed with by the RMS. The search was carried out in June 2014 and covered the 10 year period prior to the search date.

As it was expected to obtain a relatively large number of papers, a first selection (referred to *rapid assessment* in the EFSA guidance) was carried out on the basis of the title and abstract, excluding only the papers which were obviously irrelevant. In the second step (referred to *detailed assessment* in the EFSA guidance), the full-text documents were obtained for the remaining papers and a second selection was made. Papers were compared to key qualifying criteria, all which must be have been met for selection, selected papers were then scored for their reliability.

The following relevance criteria were used.

Table 6.6-1: Toxicological and metabolism studies

Key qualifying criteria	
1.	Well-defined test material
2.	Effects are directly related to the test material in question
3.	Applicable test species
4.	Control health/mortality reported
5	Relevant endpoint data
Assessment level qualifying criteria (*each conforming point will score 1, each non-conforming point will receive 0*)	
1.	Material application/ dosing to test system
2.	Concordance with guidelines and study length appropriate
3.	Appropriately designed for statistical robustness

4.	Test material information to justify exposure regime
5.	Acceptable statistical methods and/ or singular data sets

[a] For trade name products details may be verified independently

Table 6.6-2:Human case studies

Key qualifying criteria[a]	
1.	Patient exposed to relevant substance
2.	Detailed description of circumstances and pathology
3.	Medical history of the patient available
4.	Effects directly attributable to exposure

[a] Due to the nature of this type of information, no assessment level qualifying criteria are assignable. Literature conforming to all key qualifying criteria are considered relevant without restriction.

The summary results of the literature search are presented in Tables 6.6-3.

Table 6.6-3: An overview of the results of the literature search for *Beauveria bassiana* and its major metabolites

Data requirements	**Number of references**
Total number of summary records retrieved after all searches of peer-reviewed literature (excluding duplicates)	2494
Number of summary records excluded from search after first tier assessment (*rapid assessment*) of relevance [a]	2428
Total number of full-text documents assessed in detail (*detailed assessment*)	80
Number of studies excluded from further consideration after second tier assessment of relevance	40
Number of studies not excluded for relevance after second tier assessment (i.e. relevant studies and studies of unclear relevance)[b]	40

[a] Most duplicates excluded by the search engine

[b] For all these studies a final and detailed assessment for relevance was made alongside the assessment of methodological quality of the studies based on the full text

Reference list

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 5/01	Zimmermann, G.	2007	Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Science*	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
			and Technology, 17 (5/6): 553-596 Not GLP Published **Not summarised, review paper**				
MA 5.1/01	Neethling, K.	2013	Memorandum: Exposure to, and adverse health effects from, exposure to *Beauveria bassiana* strain PPRI 5339 during manufacturing. BASF Agricultural Specialities (PTY) Ltd., 91 Clifton Caanyon Drive, Clifton Farm Stockville Quarry, Mahogany Ridge, KwaZulu Natal, South Africa. 11th November 2013. Not GLP Unpublished	N	N	Confidential internal company memorandum	BASF
MA 5.1.3	Westwood, GS, Huang, SW, Keyhani, NO	2005	Allergens of the entomopathogenic fungus *Beauveria bassiana*. Clin Mol Allergy 3, 1 - 8 Non GLP Published	N	N	N/A	Public
MA 5.1.3	Beaumont, F, Kauffmann, HF, Demonchy, JGR, Sluiter, HJ, Devries, K	1985 a	Volumetric aerobiological Survey of Conidial Fungi in the Northeast Netherlands. 2. Comparison of Aerobiological data and Skin- Tests with Mold Extracts in an Asthmatic Population. Allergy	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
			40, 181-186 GLP: O, published: J 1304084 / TOX2007-470 Non GLP Published				
MA 5.1.3	Beaumont, F, Kauffmann, HF, Vandermark, TH, Sluiter, HJ, Devries, K	1985 b	Volumetric aerobiological Survey of Conidial Fungi in the Northeast Netherlands. 1. Seasonal Patterns and the Influence of Meteorological Variables. Allergy 40, 173-180 Non GLP Published	N	N	N/A	Public
MA 5.1.3 MA 5.1.4	Henke, MO, de Hoog, GS, Gross, U., Zimmermann, G, Kraemer, D, Weig, M	2002	Human deep tissue infection with an entomopathogenic Beauveria species. J Clin Microbiol 40, 2698-2702 Non GLP Published	Y	N	N/A	Public
MA 5.1.3	Semalulu, S.S., MacPherson, J.M., Schiefer, H.B., Khachatourians, G.G	1992	Pathogenicity of Beauveria bassiana in mice. Journal of Veterinary Medicins. Series B, 39, 81-90 Non GLP Published	Y	N	N/A	Public
5.1.4	Sachs, SW, Baum, J, Mies, C	1985	*Beauveria bassiana* keratitis Brit. J. Ophthalmol. 69, 548-550 Non GLP Published	Y	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
5.1.4	Ishibashi, Y, Matsumoto, Y, Takei, K	1984	The effects of intravenous miconazole on fungal keratitis. Am. J. Ophthalmol. 98, 433-437 Non GLP Published	Y	N	N/A	Public
MA 5.1.4/01	Figueira, L., Pinheiro, D., Moreira, R., Pinto, E., Simões, J., Camisa, E., Torrão, L., Palmares, J., Falcão-Reis, F.	2012	*Beauveria bassiana* keratitis in bullous keratopathy: antifungal sensitivity testing and management. Eur J Ophthalmol 2012; 22 (5): 814-818. Not GLP Published	N	N	N/A	Public
MA 5.1.4/02	Sonoyama, H., raki-Sasaki, K., Kazama, S., Kawasaki,T., Ideta, H., Sunada, A., Asari, S., Inoue, Y., Hayashi, K.	2008	The characteristics of keratomycosis by *Beauveria bassiana* and its successful treatment with antimycotic agents. *Clin Ophthalmol.,* 2(3): 675-678. Not GLP Published	N	N	N/A	Public
MA 5.1.4/03	Tu, E.Y., and Park, A.J.	2007	Recalcitrant *Beauveria bassiana* keratitis: confocal microscopy findings and treatment with posaconazole (Noxafil). *Cornea*, 26(8): 1008-1010. Not GLP Published	N	N	N/A	Public
5.1.4 5.1.3	Henke, MO, de Hoog, GS, Gross, U., Zimmermann, G, Kraemer, D,	2002	Human deep tissue infection with an entomopathogenic Beauveria species. J Clin Microbiol 40,	Y	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
	Weig, M		2698-2702 Non GLP Published				
5.1.4	Tucker, DL, Beresford, CH, Sigler, L, Rogers, K	2004	Disseminated *Beauveria bassiana* infection in a patient with acute lymphoblastic leukemia. J Clin Microbiol. 42, 5412-5414 Non GLP Published	Y	N	N/A	Public
5.1.4	Gürcan, S, Tugrul, HM, Yörük, Y, Özer, B, Tatman-Otkun, M, Otkun, M	2006	First case report of empyema caused by *Beauveria bassiana*. Mycoses 49, 246-248 Non GLP Published	Y	N	N/A	Public
MA 5.2/01	[redacted]	2011 a	Acute eye irritation study in rabbits. [redacted] Unpublished Report No. 15370-11 GLP Unpublished	Y	Y	Strain-specific new GLP laboratory study	BASF
MA 5.2/02	[redacted]	2007 a	Acute eye irritation study of test item Bb Spore Concentrate in rabbits. [redacted] Unpublished Report	Y	Y	Strain-specific new GLP laboratory study	BASF

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
			No. 06/338-005N GLP Unpublished				
MA 5.2/03	[redacted]	2011b	Acute dermal irritation study in rabbits. [redacted]. Unpublished Report No. 15371-11 GLP Unpublished	Y	Y	Strain-specific new GLP laboratory study	BASF
MA 5.2/04	[redacted]	2011c	Acute dermal toxicity study in rats. [redacted] Unpublished Report No. 15368-11 GLP Unpublished	Y	Y	Strain-specific new GLP laboratory study	BASF
MA 5.2/05	[redacted]	2007a	Acute dermal toxicity study of test item Bb spore concentrate in rats. [redacted]. Unpublished Report No. 06/338-002P GLP Unpublished	Y	Y	Strain-specific new GLP laboratory study	BASF
MA 5.2.1/01	Anonymous	2009	REPORT OF PRAPeR EXPERT MEETING M2	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
			Peer Review Programme under Directive 91/414/EEC Meeting to discuss the approach for assessment of plant protection products containing micro-organisms as the active substance Date: 16 – 18 February 2009 Venue: Parma (Italy) **Source not summarised, used as supporting information only.**				
MA 5.2.1/02	Anonymous	2012	Minutes from 355th Committee on Pesticides (ACP), 15th May 2012. **Source not summarised, used as supporting information only.**				
MA 5.2.1/03	[redacted]	2007b	Skin sensitisation of Bb spore concentrate in Guinea Pigs by Magnusson and Kligman. [redacted]. Unpublished Report No. 06/338-104T. GLP Unpublished	Y	Y	Strain-specific new GLP laboratory study	BASF
MA 5.2.2.1/01	[redacted]	2011d	Acute oral toxicity study (UDP) in rats. [redacted]	Y	Y	Strain-specific new GLP laboratory	BASF

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
			[redacted] Unpublished Report No. 15367-11 GLP Unpublished			study	
MA 5.2.2.1/02	[redacted]	2007b	Acute oral toxicity study (acute toxic class method) of test item Bb spore concentrate in rats. [redacted] Unpublished Report No. 06/338-001P GLP Unpublished	Y	Y	Strain-specific new GLP laboratory study	BASF
MA 5.2.2.1/03	Acosta, M.E., Perfetti, D.C., Eduarte, G., Moreno, PM.	2012	Oral pathogenicity of an autochthonous isolate of the entomopathogenic fungus *Beauveria bassiana* in mice (Mus musculus, strain NMRI). Boletin de Malariologia Salud Ambiental, (2012) 52(1): 79-86. Not GLP Published	Y	N	N/A	Public
MA 5.2.2.2/01	[redacted]	2013	*Beauveria bassiana* strain PPRI 5339 Acute Pulmonary Toxicity/Pathogenicity in Rats. [redacted]	Y	Y	Strain-specific new GLP laboratory study	BASF

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
			Unpublished Report No. 16701-12 GLP Unpublished				
MA 5.2.2.2/02	[redacted]	2012a	Acute inhalation toxicity in rats. [redacted] Unpublished Report No. 15687-11 Not GLP Unpublished	Y	Y	Strain-specific new laboratory study	BASF
MA 5.2.2.2/03	[redacted]	2012b	Acute inhalation toxicity study in rats. [redacted] Unpublished Report No. 15369-11. GLP Unpublished	Y	Y	Strain-specific new GLP laboratory study	BASF
MA 5.2.2.2/04	[redacted]	2011	Pilot inhalation toxicity study in rats. [redacted] Unpublished Report No. CHP 076Y ([redacted] 15687-11) Not GLP Unpublished	Y	Y	Strain-specific new laboratory study	BASF
MA 5.2.2.3/01	[redacted]	2012c	*Beauveria bassiana* PPRI 5339 Spore Concentrate. Acute intraperitoneal	Y	Y	Strain-specific new GLP laboratory	BASF

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
			injection toxicity/pathogenicity study in rats. [redacted] Unpublished Report No. 16448-12 GLP Unpublished			study	
5.2.3.1	Fotso J and Smith, JS	2003	Evaluation of beauvericin toxicity with the bacterial bioluminescence assay and the Ames mutagenicity bioassay. Journal of Food Science, 68, 1938–1941. Non GLP Published	N	N	N/A	Public
5.2.1.3	Çelike M, Aksoy, H Yilmaz, S	2010	Evaluation of Beauvericin genotoxicity with the chromosomal aberrations, sister-chromatid exchanges and micronucleus assays. Ecotoxicology and Environmental Safety, 73, 1553–1557. Non GLP Published	N	N	N/A	Public
5.2.3.1	Klarić, MS, Pepeljnjak, S, Rumora, L,	2006	Cytotoxic and apoptotic effects of fumonisin B-1, beauvericin and	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
	Ljubanovic, D		ochratoxin A on porcine kidney PK-15 cells. Toxicology Letters, 164, S59–S60. Non GLP Published				
5.2.3.1	Klarić, MS, Pepeljnjak, S, Rozgaj, R	2008 a	Genotoxicity of fumonisin B1, beauvericin and ochratoxin A in porcine kidney PK15 cells: effects of individual and combined treatment. Croatica Chemica Acta, 81, 139–146. Non GLP Published	N	N	N/A	Public
5.2.3.1	Klarić, MS, Rumora, L, Ljbnnovic, D Pepeljnjak	2008 b	Cytotoxicity and apoptosis induced by fumonisin B1, beauvericin and ochratoxin A in porcine kidney PK15 cells: effects of individual and combined treatment. Archives of Toxicology, 82, 247–255. Non GLP Published	N	N	N/A	Public
5.2.3.1	Klarić, MS, Daraboš, D, Rozgaj, R, Kašub, V Pepeljnjak, S	2010	Beauvericin and ochratoxin A genotoxicity evaluated using the alkaline comet assay: single and combined genotoxic action. Archives of Toxicology, 84, 641–650. 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 5.2.3.1/01	[redacted]	2014	*Beauveria bassiana* PPRI 5339: Bacterial reverse mutation assay. [redacted] Unpublished Report No. 8295690. GLP Unpublished	Y	Y	Strain-specific new GLP laboratory study	GLP
MA 5.3/01	Acosta-Quintero, M.E., Cazorla-Perfetti, D.J., Eduarte-Pereira, G., Morales-Moreno, P.	2011	Dermal Pathogenicity of an Autochthonous Isolate of Entomopathogenic Fungus *Beauveria bassiana* in Mice. Revisita Centifica-Facultada de Ciencias Veterinarias, (2011) 21(6): 477-483. Not GLP Published	Y	N	N/A	Public
Annex III Data and Information							
			No studies submitted				

Appendix 1-1: Operator exposure assessment – German model

Estimation of operator exposure (acc. to the German model)

Active substance (a.s.)	***Beauveria bassiana* PPRI 5339**	
Product	**Broadband**	
Intended use(s)	**e.g. cereals**	
	High Crops, Hand Held (HCHH)	
Type of preparation	**Liquid**	
Application rate (AR)	**0.1**	**kg a.s./ha**
Treated area per day (A)	**1**	**ha/d**
Systemic AOEL		**mg/kg bw/d**
Dermal absorption (DA)	**75**	**% for mixing/loading (m/l)**
	75	**% for application (appl.)**
Inhalation absorption (IA)	**100**	**%**
Body weight (BW)	**70**	**kg**

Personal protective equipment:	**BVL code**	**Reduction factor**	**to lower:**	
Particle filtering half mask (m/l) [1]	ST1102	0.08	I_M	☑
Half mask with combined filter (m/l) [1]	ST2102	0.02	I_M	☐
Particle filtering half mask (appl.) [1]	ST1203	0.08	I_A	☑
		0.8	$D_{A(C)}$	
Half mask with combined filter (appl.) [1]	ST2202	0.02	I_A	☐
		0.8	$D_{A(C)}$	
Protective gloves (m/l) [2]	SS110	0.01	$D_{M(H)}$	☑
Protective gloves (appl.) [2]	SS120	0.01	$D_{A(H)}$	☑
Protective garment + sturdy footwear (appl.) [2]	SS2202	0.05	$D_{A(B)}$	☑
Broad-brimmed headgear (appl.) [2]	SS420	0.5	$D_{A(C)}$	☐
Hood and visor (appl.) [2]	SS520	0.05	$D_{A(C)}$	☐

[1] DIN EN 149 (2001), [2] BVL (2006) Guidelines for requirements concerning personal protective equipment in plant protection

Estimated inhalation exposure:	**Personal protective equipment (PPE)**	**Factor**
I_M	ST1102	0.08
I_A	ST1203	0.08

Estimated dermal exposure:	**Personal protective equipment (PPE)**	**Factor**
$D_{M(H)}$	SS110	0.01
$D_{A(H)}$	SS120	0.01
$D_{A(C)}$	ST1203	0.8
$D_{A(B)}$	SS2202	0.05

Estimation of operator exposure: German model

Input parameters considered for the estimation of operator exposure:

Formulation type:	Liquid		**Application technique:**	High Crops, Hand Held (HCHH)	
Application rate (AR):	0.1	kg			
Area treated per day (A):	1	ha	**Dermal hands m/l ($D_{M(H)}$):**	205	mg/person/kg a.s.
Dermal absorption (DA):	75	% (concentr.)	**Dermal hands appl. ($D_{A(H)}$):**	10.6	mg/person/kg a.s.
	75	% (dilution)	**Dermal body appl. ($D_{A(B)}$):**	25	mg/person/kg a.s.
Inhalation absorption (IA):	100	%	**Dermal head appl. ($D_{A(C)}$):**	4.8	mg/person/kg a.s.
Body weight (BW):	70	kg/person	**Inhalation m/l (I_M):**	0.05	mg/person/kg a.s.
AOEL		mg/kg bw/d	**Inhalation appl. (I_A):**	0.3	mg/person/kg a.s.

Operator exposure towards Beauveria bassiana PPRI 5339					
Without PPE			**With PPE**		
Operators: Systemic dermal exposure after application in e.g. cereals					
Dermal exposure during mixing/loading					
Hands			Hands		
$SDE_{OM(H)} = (D_{M(H)} \times AR \times A \times DA) / BW$			$SDE_{OM(H)} = (D_{M(H)} \times AR \times A \times PPE^{1)} \times DA) / BW$		
(205 x 0.1 x 1 x 75%) / 70			(205 x 0.1 x 1 x 0.01 x 75%) / 70		
External dermal exposure	20.5	mg/person	External dermal exposure	0.205	mg/person
External dermal exposure	0.2928571	mg/kg bw/d	External dermal exposure	0.0029286	mg/kg bw/d
Systemic dermal exposure	**0.219643**	**mg/kg bw/d**	**Systemic dermal exposure**	**0.002196**	**mg/kg bw/d**
Dermal exposure during application					
Hands			Hands		
$SDE_{OA(H)} = (D_{A(H)} \times AR \times A \times DA) / BW$			$SDE_{OA(H)} = (D_{A(H)} \times AR \times A \times PPE^{1)} \times DA) / BW$		
(10.6 x 0.1 x 1 x 75%) / 70			(10.6 x 0.1 x 1 x 0.01 x 75%) / 70		
External dermal exposure	1.06	mg/person	External dermal exposure	0.0106	mg/person
External dermal exposure	0.0151429	mg/kg bw/d	External dermal exposure	0.0001514	mg/kg bw/d
Systemic dermal exposure	**0.011357**	**mg/kg bw/d**	**Systemic dermal exposure**	**0.000114**	**mg/kg bw/d**
Body			Body		
$SDE_{OA(B)} = (D_{A(B)} \times AR \times A \times DA) / BW$			$SDE_{OA(B)} = (D_{A(B)} \times AR \times A \times PPE^{2)} \times DA) / BW$		
(25 x 0.1 x 1 x 75%) / 70			(25 x 0.1 x 1 x 0.05 x 75%) / 70		
External dermal exposure	2.5	mg/person	External dermal exposure	0.125	mg/person
External dermal exposure	0.0357143	mg/kg bw/d	External dermal exposure	0.0017857	mg/kg bw/d
Systemic dermal exposure	**0.026786**	**mg/kg bw/d**	**Systemic dermal exposure**	**0.001339**	**mg/kg bw/d**
Head			Head		
$SDE_{OA(C)} = (D_{A(C)} \times AR \times A \times DA) / BW$			$SDE_{OA(C)} = (D_{A(C)} \times AR \times A \times PPE^{3)} \times DA) / BW$		
(4.8 x 0.1 x 1 x 75%) / 70			(4.8 x 0.1 x 1 x 0.8 x 75%) / 70		
External dermal exposure	0.48	mg/person	External dermal exposure	0.384	mg/person
External dermal exposure	0.0068571	mg/kg bw/d	External dermal exposure	0.0054857	mg/kg bw/d
Systemic dermal exposure	**0.005143**	**mg/kg bw/d**	**Systemic dermal exposure**	**0.004114**	**mg/kg bw/d**
Total systemic dermal exposure: $SDE_O = SDE_{OM(H)} + SDE_{OA(H)} + SDE_{OA(B)} + SDE_{OA(C)}$			Total systemic dermal exposure: $SDE_O = SDE_{OM(H)} + SDE_{OA(H)} + SDE_{OA(B)} + SDE_{OA(C)}$		
Total external dermal exposure	24.54	mg/person	Total external dermal exposure	0.7246	mg/person
Total external dermal exposure	0.3505714	mg/kg bw/d	Total external dermal exposure	0.0103514	mg/kg bw/d
Total systemic dermal exposure	**0.26293**	**mg/kg bw/d**	**Total systemic dermal exposure**	**0.00776**	**mg/kg bw/d**

Operators: Systemic inhalation exposure after application in e.g. cereals					
Inhalation exposure during mixing/loading					
$SIE_{OM} = (I_M \times AR \times A \times IA) / BW$			$SIE_{OM} = (I_M \times AR \times A \times PPE^{4)} \times IA) / BW$		
(0.05 x 0.1 x 1 x 100%) / 70			(0.05 x 0.1 x 1 x 0.08 x 100%) / 70		
External inhalation exposure	0.005	mg/person	External inhalation exposure	0.0004	mg/person
External inhalation exposure	7.143E-05	mg/kg bw/d	External inhalation exposure	5.714E-06	mg/kg bw/d
Systemic inhalation exposure	**0.000071**	**mg/kg bw/d**	**Systemic inhalation exposure**	**0.000006**	**mg/kg bw/d**
Inhalation exposure during application					
$SIE_{OA} = (I_A \times AR \times A \times IA) / BW$			$SIE_{OA} = (I_A \times AR \times A \times PPE^{4)} \times IA) / BW$		
(0.3 x 0.1 x 1 x 100%) / 70			(0.3 x 0.1 x 1 x 0.08 x 100%) / 70		
External inhalation exposure	0.03	mg/person	External inhalation exposure	0.0024	mg/person
External inhalation exposure	0.0004286	mg/kg bw/d	External inhalation exposure	3.429E-05	mg/kg bw/d
Systemic inhalation exposure	**0.000429**	**mg/kg bw/d**	**Systemic inhalation exposure**	**0.000034**	**mg/kg bw/d**
Total systemic inhalation exposure: $SIE_O = SIE_{OM} + SIE_{OA}$			Total systemic inhalation exposure: $SIE_O = SIE_{OM} + SIE_{OA}$		
Total external inhalation exposure	0.035000	mg/person	Total external inhalation exposure	0.002800	mg/person
Total external inhalation exposure	0.000500	mg/kg bw/d	Total external inhalation exposure	0.000040	mg/kg bw/d
Total systemic inhalation exposure	**0.000500**	**mg/kg bw/d**	**Total systemic inhalation exposure**	**0.000040**	**mg/kg bw/d**
Total systemic exposure: $SE_O = SDE_O + SIE_O$			Total systemic exposure: $SE_O = SDE_O + SIE_O$		
Total systemic exposure	18.44000	mg/person	Total systemic exposure	0.54625	mg/person
Total systemic exposure	**0.263429**	**mg/kg bw/d**	**Total systemic exposure**	**0.007804**	**mg/kg bw/d**
% of AOEL		**%**	**% of AOEL**		**%**

[1)] reduction factor for gloves is 0.01 (professional applications) and 0.5 (home/allotment garden applications), resp.

[2)] reduction factor for protective garment is 0.05 (prof. appl.) and 0.5 (workwear, home/allotment garden appl.), resp.

[3)] reduction factor for broad brimmed headgear and hood and visor is 0.5 and 0.05, respectively (professional appl.)

[4)] reduction factor for RPE is 0.08 (particle filter) and 0.02 (combined vapour and particle filter), resp. (prof. appl.)

Appendix 1-2: Operator exposure assessment – Dutch greenhouse model

OPERATOR EXPOSURE			DUTCH GREENHOUSE MODEL	
form	Broadband		Application including mixing and loading	
a.s.	Beauveria bassiana PPRI 5339			
Parameter		**Value**	**Unit**	**References, comments**
MANUAL SPRAYING in greenhouses				
AR	Application rate	0.1	kg a.s./ha	summary of intended uses
A	Area treated	1	ha/ day	Dutch model
Inhalation Exposure				without PPE
SV	Surrogate Exposure Value	1	mg a.s./ kg a.s.	For dusting see note* (Dutch model)
Inhalation Exposure (without PPE)		**0.1**	mg a.s./ day	IE = SV x AR x A
Inhalation Exposure (with PPE)				with PPE
	PPE-factor	10		Non-powered mask filtertype 2 (most conservative): 10; more advanced RPE: see note** (Dutch model)
Inhalation Exposure (with PPE)		**0.01**	mg a.s./ day	IE(PPE) = (1/PPE factor) x IE
Dermal Exposure				without PPE
SV	Surrogate Exposure Value	200	mg a.s./ kg a.s.	For dusting see note* (Dutch model)
Dermal Exposure		**20**	mg a.s./ day	DE = SV x AR x A
Dermal Exposure (with PPE)				with PPE
	PPE-factor	10		Gloves + coverall: 10 (Dutch model)
Dermal Exposure (with PPE)		**2**	mg a.s./ day	DE(PPE) = (1/PPE-factor) x DE
Internal exposure				
IA	Inhalation Absorption	100	%	
DA	Dermal Absorption	75	%	
	AOEL		mg a.s./ day	based on 70 kg bw
		Without PPE	**With PPE**	
	Internal exposure	[mg a.s. / day]	[mg a.s. / day]	
	Inhalation	0.1000	0.0100	IE(int) = IE x (IA/100)
	Dermal	15.0000	1.5000	DE(int) = DE x (DA/100)
	Total	**15.1000**	**1.5100**	**sum**

Appendix 1-3: Worker exposure assessment – EUROPOEM II & DUTCH model

WORKER EXPOSURE			EUROPOEM II & DUTCH MODEL	
form	Broadband		Re-entry in greenhouses	
a.s.	Beauveria bassiana PPRI 5339			
Parameter		**Value**	**Unit**	**References, comments**
Re-entry activities in greenhouses				
AR	Application rate	0.1	kg a.s./ha	summary of intended uses
Worker				
Duration				
Tc	Cutting	3	hours / day	default: 3 h (Dutch model)
Tsb	Sorting/ bundling	3	hours / day	default: 3 h (Dutch model)
Tt	Total duration	6	hours / day	default: 6 h (Europoem II)
Inhalation Exposure				without PPE
Task Specific Factor				
	Surrogate value (indicative)			
TF	Cutting	0.1	(mg a.s./h)/ (kg/ha)	Dutch model
TF	Sorting/ bundling	0.01	(mg a.s./h)/ (kg/ha)	Dutch model
Inhalation Exposure				
	Cutting	0.03	mg a.s./ day	IE = AR x Tc x TF
	Sorting/ bundling	0.003	mg a.s./ day	IE = AR x Tsb x TF
	Total	**0.033**	mg a.s./ day	sum
Dermal Exposure				
DFR	Dislodgeable foliar residue	30	mg a.s./m2/kg a.s./ha	default (Europoem II)
TC	Transfer coefficient	0.5	m2/ hour	vegetable (field): 0.25; ornamentals: 0.5; small fruit: 0.3; large fruit: 0.45 (Europoem II)
Dermal Exposure		**9**	mg a.s./ day	DE = DFR x AR x TC x Tt
Internal exposure				
IA	Inhalation Absorption	100	%	
DA	Dermal Absorption	75	%	
	PPE-factor inhalation	10		reduction factor*
	PPE-factor dermal	5		gloves**
	AOEL		mg a.s./ day	based on 70 kg bw
		Without PPE	**With PPE**	
	Internal exposure	[mg a.s./ day]	[mg a.s./ day]	
	Inhalation	0.033	0.003	IE(int) = IE x (IA100)
	Dermal	6.750	1.350	DE(int) = DE x (DA/100)
	Total	**6.783**	**1.353**	**sum**