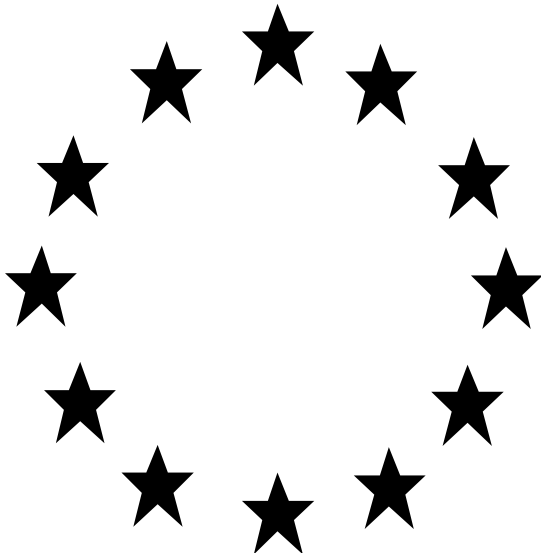


Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Germany for the existing active substance

BEAUVERIA BASSIANA GHA

of the fourth stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Volume 3, Annex B, part 1, B.1 – B.5

August 2008

Draft Assessment Report

22 November 2007

Beauveria bassiana GHA

Volume 3

Annex B

Summary, Scientific Evaluation and Assessment

Rapporteur Member State:Germany

Contents

Annex B

Beauveria bassiana GHA

B-1: Identity

B.1 Identity

B.1.1 Identity of the micro-organism (OECD IIM 1)

B.1.1.1 Name and address of applicant(s) (OECD IIM 1.1)

Applicant: Mycotech Europe Ltd.
12 Lonsdale Gardens,
Tunbridge Wells TN1 1PA,
England

Contact Point: F. J. Raveney
Product Registrtation Services Ltd.
PO Box 31
Robertsbridge TN 32 5ZL
England
Phone: +44 1580 882057
Fax: +44 1580 882057

B.1.1.2 Producer: name and address of each plant where the micro-organism is produced (OECD IIM 1.2)

Manufacturer: Laverlam International Corp.
117 South Parkmont,
Butte, Montana MT 59701,
U.S.A.

Contact Point: Dr. L. A. Mazariegos
Phone: +1 406 782 2386
Fax: +1 406 782 9912

B.1.1.3 Name and species description, strain characterisation (OECD IIM 1.3)

B.1.1.3.1 Accession number in culture collection

Beauveria bassiana strain GHA is maintained in the American Type Culture Collection under ATCC 74250.

B.1.1.3.2 Scientific name and taxonomic grouping, i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the micro-organism

Taxonomic grouping
Anamorph form:
Species: *Beauveria bassiana* (Balsamo) Vuillemin

Description:	de Hoog (1972) (BWS 2006-58)
Strain:	GHA
Genus:	Beauveria
Family:	*Moniliaceae*
Order:	Moniliales
Phylum:	Deuteromycota
Kingdom:	Fungi

Teleomorph form:	
Species:	Cordyceps spp.
Genus:	Cordyceps
Family:	Clavicipitaceae
Order:	Clavicipitales
Phylum:	Ascomycota
Kingdom:	Fungi

Beauveria bassiana (Bals.) Vuill. is a cosmopolitan, anamorph species of haploid, soil-borne Hyphomycetes. For several *B. bassiana* strains isolated in Korea and China also Cordyceps teleomorphs are described. Cordyceps is a genus of the single family Clavicipitaceae of the order Clavicipitales. New molecular studies indicate that the Hypocreales may include the Clavicipitaceae and it is in discussion to unify the orders Hypocreales and Clavicipitales. Therefore, several authors describe the teleomorph as a Hypocreales. Nevertheless, for none of the strains isolated in Europe or USA teleomorphs have been identified and only the asexually reproducing form seems to exist (Rehner & Buckley, 2005).

Morphological criteria for characterisation of the species *B. bassiana* are described by de Hoog (1972). *B. bassiana* is characterised by white, later yellowish or occasionally redish colonies and morphologically by its sympodial to whorled clusters of short-globose to flask shaped conidiogenous cells, which give rise to a succession of one-celled, hyaline, rarely yellowish, holoblastic conidia that are borne on a progressively elongating sympodial up to 20 µm long rachis. Conidia size range between (1.5-) 2 - 3 (-4) x (1.5-) 2 - 2.5 (-3) µm.

Ongoing difficulties in applying morphologically approaches to species recognition in *Beauveria* have spurred the search for additional sources of taxonomic characters and were summarised by Rehner and Buckley (2005). Alternative character systems to detect genetic variation within *Beauveria* include isoenzymes, chemo taxonomic characters, mitochondrial RFLP, immunological approaches, rRNA sequencing, RFLP, introns in the large subunit rDNA, RFLP and nucleotide sequences of ITS, SSCP analysis of taxon specific markers, RAPD markers and the combined use of morphology and RAPD markers.

B. bassiana strain GHA was originally isolated from the Southern corn rootworm, *Diabrotica undecimpunctata*, near Corvallis, Oregon, USA. *B. bassiana* strain GHA is a naturally occurring fungus that is not modified in any way during production.

Indigenous or non-indigenous: indigenous

Wild type: Yes

B.1.1.3.3 Test procedures and criteria used for identification

Morphological criteria for characterisation of the species *Beauveria bassiana* are described by de Hoog (1972). Identity at the strain level is based on a Restriction Fragment Length Polymorphism (RFLP) profile analysis of chromosomal DNA (cDNA). The DNA was extracted from mycelia and analysed using 14-restriction-enzyme - DNA-probe pairs. This experimental procedure allows at least to distinguish *B. bassiana* strain GHA from the other *B. bassiana* strain tested in this assay (Jaronski, Butler & Kahler 1993, BWS 2006-54). The active substance can be characterised by the internal SOP (Jaronski 1993, BWS 2006-70) by inoculating potato-dextrose or oatmeal agar plates with a sample of the fungal culture, and incubating at 25 ºC. Transfer a small sample of the sporulating culture to a drop of lacto-phenol-cotton blue or fuchsin and examine with a bright field or phase contrast microscope. The fungus can be characterised by reference to standard pictures (Jaronski 1993, BWS 2006-70).

Since 2003, newer updated techniques have been developed to detect *B. bassiana* strain GHA. Using random amplified polymorphic DNA (RAPD) analysis, unique fragments that distinguished GHA from other strains of *B. bassiana* have been obtained (Castrillo et al. 2003).

Further details are included in Volume 3, point B.5.1.1.

B.1.1.3.4 Common name or alternative and superseded names and code names used during the development

Synonyms: Formerly known as *Botrytis bassiana* Balsamo, *Beauveria tenella* and *Sporotrichum globuliferum*

Common name: White muscardine

CAS number: 63428-82-0

B.1.1.3.5 Relationship to known pathogens

A detailed literature search of on-line references, including MEDLINE, TOXLINE and available University sites was conducted by the notifier and no reference to the occurrence of pathogenicity to plants, other animals than insects or humans in closely related species was found.

B.1.1.4 Specification of the material used for manufacturing of formulated products (OECD IIM 1.4.1 - 1.4.2 and OECD IIM 1.4.4)

B.1.1.4.1 Content of the micro-organism

The content of the pure micro-organism, *B. bassiana* strain GHA, in the material used for manufacturing of the formulated product is:

Nominal (mean) purity: 1.37×10^{11} CFU/g
Acceptable range: 1.24×10^{11} to 1.47×10^{11} CFU/g

The technical grade material used for manufacture of end-use products contains 70 % w/w of *B. bassiana* strain GHA conidia and 30 % w/w of residual solids (insoluble starch).

B.1.1.4.2 Identity and content of impurities, additives, contaminating micro-organisms

Confidential information, see Annex C.

B.1.1.4.3 Analytical profile of batches

Confidential information, see Annex C.

B.1.1.4.4 Physical and chemical properties, of the material used for manufacturing of formulated products that is stored prior to formulation of end use products

Additional data not required according to Directive 91/414/EEC.

Data point	Comments	Guideline and Method	Test material purity and specification	Findings	Reference
Colour		Visual assessment	Purity: 1.4 x 10^{11} CFU/g	Off-white	Jaronski & Lutz 1997 CHE 2006-758
Odour		Olfactory assessment	Purity: 1.4 x 10^{11} CFU/g	Slightly musty	Jaronski & Lutz 1997 CHE 2006-758
Appearance		Visual assessment	Purity: 1.4 x 10^{11} CFU/g	Fine powder	Jaronski & Lutz 1997 CHE 2006-758
Viscosity or surface tension	Not applicable to *Beauveria bassiana* as the substance is a solid.				
Corrosive character	*B. bassiana* strain GHA will be packaged in fibreboard containers that are lined with plastic, precluding the possibility of corrosion.				
Explosivity, oxidising properties	None known or reported, and very unlikely from the physical properties of the product.				
Bulk density		ASTM D4512	Purity: 1.4 x 10^{11} CFU/g	0.183 ± 0.012 $g.cm^{-3}$ (bulk)	Jaronski & Lutz 1997 CHE 2006-758
pH (1% suspension in water)		Internal SOP.	Purity: 1.4 x 10^{11} CFU/g	pH: 5.8	Jaronski & Lutz 1997 CHE 2006-758

B.1.2 Identity of the plant protection product (OECD IIIM 1)

B.1.2.1 Current, former and proposed trade names and development code numbers (OECD IIIM 1.3)

Trade name in the EU: BotaniGard®
Trade name in the US: Mycotrol®, Mycocide GH

B.1.2.2 Applicant (OECD IIIM 1.1)

Contact person:	F. J. Raveney
Address:	Mycotech Europe Ltd., 12 Lonsdale Gardens, Tunbridge Wells TN1 1PA, England
Telephone:	+44 1580 882059
Fax:	+44 1580 882057

B.1.2.3 Manufacturer or manufacturers of the plant protection product (OECD IIIM 1.2.1)

Contact person:	Gary Chatriand
Address:	Laverlam International Corp. 117 South Parkmont, Butte, Montana MT 59701, USA
Telephone:	+1 406 782 2386
Fax:	+1 406 782 9912

B.1.2.4 Type of the preparation and code (OECD IIIM 1.5)

Wettable powder (WP)

B.1.2.5 Function (OECD IIIM 1.6)

Function:	Control of insects
Field of use:	Commercial indoor/ greenhouse use

B.1.2.6 Composition of the preparation (OECD IIIM 1.7.1 – 1.7.3)

B. bassiana strain GHA, techn.	315.0 g/kg (4.4×10^{13} CFU/kg)
B. bassiana strain GHA, pure	220.0 g/kg (4.4×10^{13} CFU/kg)

Regarding the formulants see Annex C/ Volume 4.

B.1.3 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 1.3.1 (OECD) KIIM 1.3.3	De Hoog, G. S.	1972	The genera Beauveria, Isaria, Tritirachium and Acrodontium gen. nov. Studies in Mycology 1 , 1 - 41 N/A Request Ref. No. DG 79130 GLP: N, published: J 1300919 / BWS2006-58	N	LIT
KIIM 1.3.1 (OECD)	Jaronski, S.T.; Butler,E.; Kahler, A.	1993	Beauveria bassiana strain GHA cDNA libaray construction and RFLP analysis R145-93/003 GLP: N, published: N 1300920 / BWS2006-54	Y	LAM
KIIM 1.3.3 (OECD)	Jaronski, S.T.	1993	Characterisation of Beauveria bassiana (SOP #3.5; Edition #01) and Including Sample Characterisations N/A GLP: N, published: N 1300923 / BWS2006-70	Y	LAM
KIIM 1.3.3 (OECD)	Rehner S.A., Buckley, E.	2005	A Beauveria phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs Mycologia, 97 (1), 84-98 , not applicable GLP: N, published: J 1673810 / BWS2006-33	N	LIT
KIIM 1.4.2.3 (OECD) KIIM 1.4.3	Alley,J. Rosinka, J. Jaroski, S.	1996	Contaminant Analysis of three lots of Mycotrol TGAI (Beauveria bassiana Strain GHA) 960418 GLP: N, published: N 1300925 / CHE2006-582, BWS2006-71	Y	MEU
KIIM 1.4.3 (OECD) KIIM 1.4.4	M.A.B.	1998	Analysis of TGAI production lots from 1997-1998 N/A GLP: N, published: N 1300927 / BWS2006-61, CHE2006-754	Y	LAM
KIIM 1.4.3 (OECD)	Castrillo,L.A., Vandenberg, J.D., Wraight, S.P.	2003	Strain-specific detection of introduced Beauveria bassiana in agricultural fields by use of sequence-characterized amplified region markers Journal of Invertebrate Pathology 82, 75-83 GLP: N, published: N 1675896	Y	LIT

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 1.4.3.1 (OECD)	Bradley, C.	1998	Minor Modifications to Manufacturing Process for Mycotrol TGAI and End Product Formulation N/A GLP: N, published: N 1300931 / BWS2006-60	Y	LAM
KIIM 1.4.4 (OECD)	Jaronski, S.T.	1997	Five-lot analysis of Beauveria bassiana strain GHA, technical grade active ingredient (TGAI) 97-01 GLP: J, published: N 1300932 / CHE2006-756	Y	LAM
KIIM 1.4.4 (OECD)	Jaronski, S.T.; Becarra M.; Sears, J.	1995	Analysis of Mycotrol GH TGAI (Beauveria bassiana strain GHA Conditional Powders) for the metabolites Beauvericin and Bassianolide 94-009 GLP: N, published: N 1300934 / CHE2006-752	Y	LAM
KIIM 1.4.4 (OECD)	Jaronski, S.T.	1993	Analysis of GH TGAI (Beauveria bassiana strain) for the metabolite Oosporein 93-032 GLP: N, published: N 1300935 / CHE2006-753	Y	LAM
KIIM 1.4.4 (OECD)	Chatriand, G.	2005	"No title" GLP: N, published: N 1300936 / CHE2006-794	Y	LAM
KIIM 1.4.4 (OECD)	Becerra, M.; Sears, J.	1999	Analysis of Mycotrol / BotaniGard TGAI (Beauveria bassiana strain GHA Conidial Powders) for the metabolites beauvericin and bassianolide 98-06 GLP: N, published: N 1677611 /	Y	MEU
KIIM 1.4.6 (OECD)	Jaronski, S.T.; Lutz, R.	1997	Physical and chemical properties of Beauveria bassiana srain GHA TGAI: coulor, odour, physical state, bulk densisty and pH 97-02 GLP: J, published: N 1300938 / CHE2006-758	Y	LAM
KIIM 1.4.7 (OECD)	Lubbe, L.A.J.	2005	verzoek wijziging naam toelathinghounder 20050224 WNAW. botaniGard WP 12621 N CTB (college voor de toelating van bestrijdingsmiddelen Dhr. L.A.J-Lubbe 471881 GLP: N, published: N 1300939 / BWS2006-64	Y	LAM

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIIM1 1.7.4 (OECD)	Lutz, R.; Jaronski, S.	1998	Contaminant Analysis of Three Lots of Mycotrol/BotaniGard 22WP (Beauveria bassiana Strain GHA) GLP: N, published: N 1679920 /	Y	MEU

Codes of owner

LAM Laverlam International Corporation
LIT Literature
MEU Mycotech Europe Ltd.

Annex B

Beauveria bassiana GHA

B-2: Biological properties of the organism

B.2 Biological, physical, chemical and technical properties

B.2.1 Biological properties of the micro-organism (OECD IIM 2)

Remark: A summary on the safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongiartii* has been issued by Zimmermann (2007), which includes the results of older laboratory and field studies of importance. Cited references are listed in detail at the end of this publication.

In the following chapter all references marked with * are cited in Zimmermann (2007).

B.2.1.1 History of the micro-organism and its uses. Natural occurrence and geographical distribution (OECD IIM 2.1 - 2.2)

B.2.1.1.1 Historical background

There are >700 reported strains of *Beauveria bassiana* and some have been commercialised. Most species and strains have some insecticidal properties and are pathogenic to definitive species and classes of insects (Bradley 1991, BWS 2006-63).

B.2.1.1.2 Origin and natural occurrence

The isolated strain of *Beauveria bassiana* GHA derived from a *B. bassiana* culture originally obtained from the USDA ARS Collection of Entomopathogenic Fungi as ARSEF 201. *B. bassiana* strain ARSEF 201 was originally isolated from the Southern corn rootworm, *Diabrotica undecimpunctata* on green beans on 22 October 1977, near Corvallis, Oregon, USA (ARSEF catalogue *Beauveria,* 2005). This strain was passed through laboratory infection cycles on grasshoppers (twice) and in February 1991 through migratory locusts (*Locusta migratoria*) and was then named *B. bassiana* GHA. The strain was registered at the American Type Culture Collection as number #74250 (Bradley 1991, BWS 2006-63).
In 1997 the master culture (FID# 2041) for the production strain for Botanigard products was obtained by host passage through white fly (*Bemsia argentifolii*). The currently used production slants were obtained as the first subculture of FID# 2041 in 2002.

B. bassiana, a hyphomycetous entomopathogenic fungus, is the most widely distributed species of the genus. Domsch et al. (1980*) listed the occurrence and distribution of *B. bassiana* in various countries and habitats. This fungus is generally found throughout a wide range of habitats from alpine soils to heathland, in peat bogs, soils with savannah type vegetation, and in forest and cultivated soils, in sand blows and dunes as well as in desert soils and running water, on all continents of the world.

B. bassiana can grow as saprophyte on simple carbohydrates (e.g. dextrose) and on standard media used in mycological laboratories.

Based on world-wide data, Li (1988*) listed 707 insect species as hosts of *B. bassiana*. This list comprised 521 genera and 149 families in 15 orders. In addition, 13 host species of Acarina distributed in 7 genera and 6 families are listed. Nevertheless, despite the prevalence of *B.*

bassiana on a huge number of arthropods, it is known that most isolates have a restricted host range (Goettel et al., 1990*, Vestergaard et al., 2003*).

B. bassiana has also been isolated from the surface and the interior of plants. It was isolated from bark of elm trees and *Carpinus caroliniana* (Doberski & Tribe 1980*; Bills & Polishook, 1991*) as well as from the phylloplanes of various hedgerow plants (Meyling & Eilenberg, 2006*). Wagner & Lewis (2000*) and Posada & Vega (2005*) described the occurrence of *B. bassiana* as endophyte. Airaudi & Marchisio (1996*) have isolated *B. bassiana* from the air of Turin, Italy.

The level of natural occurrence of *B. bassiana* in the soil and on foliage is not precisely defined in the supplied literature. *B. bassiana* is ubiquitous in temperate and tropical climates. USDA ARS lists over 450 isolates of *B. bassiana* from all continents (except Antarctica) (Bradley 1991, BWS 2006-63).

B.2.1.2 Information on target organism(s) (OECD IIM 2.3)

B.2.1.2.1 Description of the target organism(s) (OECD IIM 2.3.1)

Beauveria bassiana acts as an insecticide, and the primary target insects are whiteflies (*Aleurodidae*), thrips (*Thysanoptera*), and aphids (*Aphididae*). The target whitefly species are *Bemisia tabaci* and *Trialeurodes vaporariorum*.

B.2.1.2.2 Mode of action (OECD IIM 2.3.2)

Reference:
Bradley, C.(1991), Product identity and disclosure of ingredients, (BWS2006-63)
Fernandey, S. (2001), Study of conidia production and transmission of beauveria bassiana 8Balsamo) Vuill. In colorado potato beetle (*Leptinotarsa decemilineata*)

Like other entomopathogenic fungi, *B. bassiana* attacks its host insects generally percutaneous. The conidia of *B. bassiana* adhere to the insect cuticle by means of hydrophobic interaction between the spore wall and epicuticle lipids. A hydrophobin-type protein and certain enzymes assist in the attachment process. Germination of the conidia and the subsequent successful infection depend on a number of factors, e.g. susceptibility of the host and host stage, and certain environmental factors, such as optimal temperature and humidity. Before penetration, germ tubes may form so-called appressoria and infection pegs. The penetration process is by mechanical means and by the production of several enzymes, including proteases, chitinases and lipases, which degrade the insect cuticle. The penetration is followed by the invasion, which is accompanied by several host immune response activities. During the infection process, *Beauveria* spp. produces proteolytic enzymes and toxins, while the host insect responds with cellular and humoral defence reactions. In the insect body, the fungus multiplies as blastospores, or yeast-like cells, which are distributed passively in the haemolymph. Enzymes begin to destroy the internal structures of the host insect causing morbidity within 36 - 72 hours. Reduced feeding and immobility are rapidly evident, and the insect dies within 4 to 10 days post-infection. The time to death will depend on the insect species, age and conidial dose. After death of the insect, the fungus starts its saprophytic growth: blastospores transform into mycelia, which emerge through the cuticle. Aerial conidia

are formed on the surface of the insect cadaver, which build the characteristic white growth. Sporulation occurs only in conditions of high humidity.

Reference:
Document IIM:

B. bassiana strain GHA has been tested in laboratory bioassay and field trials against a number of insect species in 3 different target Orders. The strain GHA has shown infectivity at commercially practical application rates in:

Homoptera:
The strain GHA has demonstrated infectivity in whitefly (*Bemisia* spp., *Trialeurodes vaporariorum*), aphid (*Myzus persicae*, *Aphis gossypii*) and leafhopper (*Erythroneura elegantula*). In a laboratory bioassay standardised for the delivery of spores per unit area, the LC_{50} on third instar whitefly (*Bemisia argentifolii*) is about 150 spores/mm^2. In laboratory spray tower tests on adult *Aphis gossypii* using a dilution simulating commercial use rates (5×10^7 conidia/ml spray volume) infection rates were 98 % at 72 h post-spray.

Thysanoptera:
The strain GHA has been shown to infect thrips including *Frankliniella occidentalis* and *Thrips palmi*. In laboratory spray tower tests on *F. occidentalis* using a dilution simulating commercial application rates (5×10^7 conidia/ml spray volume) infection rates of adults and nymphs were 90 % at 72 h post-spray.

Coleoptera:
Data are being developed for black vine weevil, Colorado potato beetle, Apthona beetle.

Acrididae:
The strain GHA infects a broad range of grasshopper species. The LD_{50} varies according to the grasshopper stage of development. In a standardised laboratory bioassay on 4th instar *Malanoplus sanguinipes*, the LD_{50} at 10 days post infection is about 3000 conidia/insect. Other typical values may be between 1000 – 30000 conidia/insect.

Transmissibility
Beauveria bassiana is a naturally occurring fungus which can be isolated from infected insects, soil and plants in a wide range of habitats. It can grow as a saprophyte on simple carbohydrates and on standard laboratory media (Bradley, 1991). It is a contact pathogen that is transmitted either by direct contact or by spraying.

B.2.1.3 Host specificity range and effects on species other than the target harmful organism (OECD IIM 2.4)

Beauveria bassiana is not host-specific but an opportunistic entomopathogen capable of attacking insects of a wide range of different taxa.

Beauveria bassiana strain GHA acts as an insecticide, and the primary target insects are whiteflies (*Aleurodidae*), thrips (*Thysanoptera*), and aphids (*Aphididae*). Within the "*Beauveria bassiana* strain GHA (128924) Technical Document" of the U.S. Environmental Protection Agency (EPA) the following target pests are listed for strain GHA: scarab beetles,

leaf-feeding beetles (including Colorado potato beetle), whitefly, aphids, thrips, psyllids, mealybugs, leafhoppers and plant hoppers, weevils, plant bugs (including chinch, lygus and flea hoppers), borers, leaf-feeding insects, grasshoppers, locusts and Mormon crickets, stem-boring lepidoptera (including European and Southwestern corn borer).

Despite the prevalence of *B. bassiana* on a huge number of arthropods, it is known that most isolates have a restricted host range (Goettel et al., 1990*, Vestergaard et al., 2003*). Based on the wide host range, beneficial insects could be affected by *B. bassiana*. Nevertheless, Hajek and Butler (2000*) differentiate between the physiological and ecological host range, which means that non-target insects, which are infected under laboratory conditions, may not necessarily be infected in nature (confirmed by Vestergaard et al., 2003*).

It should be emphasised that infection mechanisms are highly evolved and specific only to insects. Studies show that *B. bassiana* strain GHA growth is inhibited at 33 ºC and absent at 36 ºC, thus making growth untenable in living mammalian and avian tissues. (Fargues, 1997 (BWS 2006-65), and personal communication Jaronski/Raveney 1998).

Wang et al. (2004*) monitored the fate of *B. bassiana*-strains, which had been inundatively applied against *Dendrolimus punctatus* in Southwest China. They could re-isolate the indigenous and exotic strains during one year. However, the indigenous strains were predominant in the local environment and were not displaced by the exotic ones (Wang et al., 2004). Within several papers an antagonistic effect of *B. bassiana* on plant pathogenic fungi was mentioned (Langbauer et al., 1996*; Ownley et al., 2004*; Reisenzein & Tiefenbrunner, 1997*; Vesely & Koubova, 1994*).

B.2.1.4 Development stages / life cycle of the micro-organism (OECD IIM 2.5)

The conidia adhere to the insect cuticle, germinate and penetrate in the insect body, where they replicate as yeast-like cells (blastospores) and destroy the internal structures, causing morbidity within 36-72 hours. After death of the insect, the blastospores transform into mycelia, which emerge through the cuticle and form spores.

B.2.1.5 Physiological properties (especially effects of environmental parameters on growth, infectivity, dispersal and colonisation ability) (OECD IIM 2.8)

Germination of conidia depends largely on environmental conditions including temperature, light and especially relative humidity. Most fungal entomopathogens require relative humidity above 97 % for germination and temperatures between 25 – 30 °C (Fernandez, 2001).

In general, *B. bassiana* grows in a wide temperature range from 5 to 35 °C. The optimal growth temperature for *B. bassiana* is 23 to 28 ºC, the minimum 5 - 10 °C and the maximum between 30 - 35 °C (Müller-Kögler, 1965*; Fargues et al., 1997). Conidial survival time in the environment is inversely proportional to increasing temperature. Conidia survive for weeks at 25 ºC, and days or hours at higher temperatures. (Fargues 1997, BWS 2006-65; Jaronski 1993, BWS 2006-62).
The conidia are rapidly destroyed by direct sunlight. Conidial viability decreased from > 90 % to 0.22 % after 4 hours exposure to sunlight, and the observed half-life of *B. bassiana* conidia was 2.58 hours (Jaronski 1993, BWS 2006-67).

Conidia of *B. bassiana* strain GHA remained viable in aqueous suspension at pH 5, 7, and 9 for 48 hours in a laboratory test system. After 48 hours, bacterial growth causes conidial mortality. It was shown that if *B. bassiana* strain GHA is exposed to metallic ions (Na, Ca, Cu, Fe Mg) there were no effects with the exception of copper, which is a known fungicide (Jaronski and Britton 1993, BWS 2006-68).

Under natural conditions, *B. bassiana* strain GHA conidia germinate and die within two days in the absence of a suitable host insect in aqueous environments. From the above data, it is clear that *B. bassiana* strain GHA requires particular conditions for dispersal and viability in the environment. *B. bassiana* conidia survive naturally in sheltered habitats and require specific environmental conditions of moderate temperature, high humidity and high insect population density for epizootic spread and dispersal. Although the species is ubiquitous in distribution, it does not compete significantly with other fungi, or bacteria, as a saprophyte in soil or water environments. Their are indications that *B. bassiana* strain GHA multiplies in the environment only in insect hosts, where it sporulates and disperses only under specific environmental conditions, particularly high humidity. *B. bassiana* strain GHA conidia applied as a mycoinsecticide decline to background levels due to exposure to sunlight, moisture and temperature extremes. In the absence of specific environmental conditions, the organism dies with its target host. (Personal communication, Jaronski/Raveney 1998)

B.2.1.6 Relationships to known plant or animal or human pathogens (OECD IIM 2.7)

B.2.1.6.1 Among closely related species, provide information on pathogenicity to plants, animals or humans (Annex IIM 2.7.1)

B. bassiana has no reported effects on plants. Infection mechanisms are highly evolved and specific only to insects and *B. bassiana* is not regarded as a vertebrate pathogen. A detailed literature search of on-line references, including MEDLINE, TOXLINE and available University sites was conducted by the notifier for this submission and no reference to the occurrence of pathogenicity to plants, other animals or humans in closely related species was found.

B.2.1.6.2 Among closely related species, provide information on formation of toxic metabolites: structure, stability, conditions under which they are formed, mode of action (Annex IIM 2.7.2)

Reference:

Strasser, H.; Vey, A. and Butt, T.M. (2000), Are there any Risks in using Entomopathogenic Fungi for pest Control, with Particular Reference to the Bioactive Metabolites of Metarhizium, Tolypocladium and Beauveria species? (BWS2006-66)

Bradley, C. (1993), Discussion of formation of unintentional ingredients, LAM, (BWS2006-72)

Document MII

Introduction

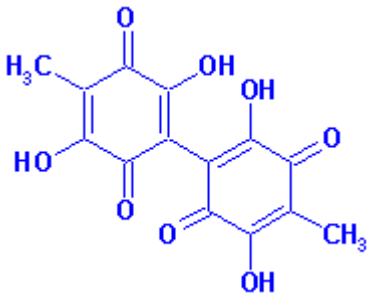
Certain entomogenous fungi belonging to the genus *Beauveria* and strains of *Beauveria bassiana* produce metabolites and endo-toxins such as beauvericin, bassianolide and oosporein.

Oosporein

Oosporein is a red-coloured dibenzoquinone produced by a large number of soil fungi. It is considered to react with proteins and amino acids through redox-reactions by changing the SH-group which results in enzyme malfunction. Oosporein will inhibit erythrocyte membrane ATPase activity in a dose-dependent manner by as much as 50 % at 200 µg/ml. These pigments have been found to inhibit Ca^{2+} -ATPases to a greater extent than Na^{+}/K^{+} -ATPase activity. (In this instance ATPase inhibitory activity for these pigments was not thought to be specific but was probably a consequence of membrane disruption, since pigments were all found to cause alterations in erythrocyte morphology and promote varying degrees of cell lysis). Oosporein is an antiviral compound that has been found to act as a competitive inhibitor of dGTP or dCTP incorporation into DNA. Oosporein has been found to be the only major secondary metabolite produced by selected commercial strains of *Beauveria brongniartii* in submerged cultures and on sterilised barley kernels. Studies on the extent of oosporein production revealed negligible levels that posed no risk to man and animals and had no phytotoxic effects to selected plant species. A theoretical prediction of 4.8 – 6.4 mg/m^2 has been proposed for the oosporein concentration that could be expected in soil. (Strasser *et al*., 2000, BWS2006-66)

Further information concerning oosporein see B.2.1.8.1:

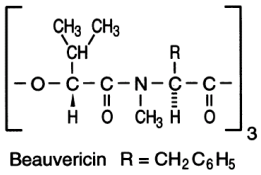
Structure:



Beauvericin

The hexadepsipeptide beauvericin is structurally related to the ionophores enniatins and differs from these only with respect to the N-methylamino acids and has been found to take two forms (A and B). Beauvericin forms Na^{+} and K^{+} complexes leading to increased permeability of natural and artificial membranes. Beauvericin shows antibiotic activity against several bacteria as well as moderate insecticidal properties. It is toxic towards several shrimp species. Its toxicity has been shown to persist in sterile seawater for at least 3 weeks. Although most investigations into the toxicity of beauvericin have been carried out on insects, it has also shown high *in vitro* toxicity towards murine and human cell lines. (Strasser *et al*., 2000, BWS2006-66)

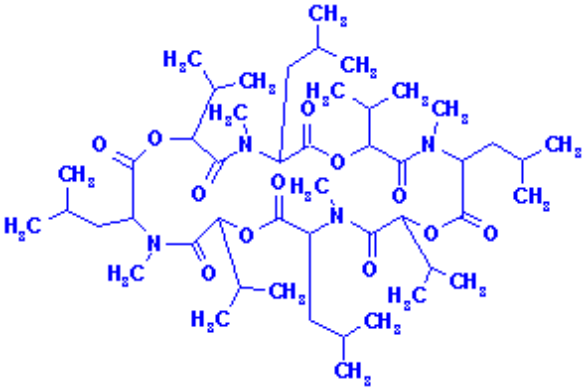
Structure:



Bassianolide

Another toxin known to be secreted by members of the genus *Beauveria*, is the cyclo-octadepsipeptide, bassianolide. This has been found to induce atonical symptoms in silkworm larvae at low doses, but was lethal at higher doses. Bassianolide, like beauvericin, is an ionophore antibiotic but differs in its reaction to different cations. Overall, little is known about the toxicity of beauvericin and bassianolide to animals and plants, but there may be a possible synergistic effect with the structurally related mycotoxin moniliformin. (Strasser *et al*., 2000, BWS2006-66)

Structure:



Conclusion

Studies carried out by Laverlam International Inc. to assess the levels of beauvericin, bassianolide and oosporein produced by *B. bassiana* strain GHA demonstrated no detectable levels (as discussed in Point B.2.1.8). Consequently it can be considered that *Beauveria bassiana* strain GHA poses no risk to man or the environment *via* the production of toxic metabolites or mycotoxins (Bradley, 1993, BWS2006-72).

B.2.1.7 Genetic stability and factors affecting it (OECD IIM 2.10)

Any organism, including *Beauveria bassiana* can undergo genetic mutation. However, characteristics of *B. bassiana* strain GHA and the Laverlam International Inc. culture maintenance and production procedures minimise the chances that a mutation would occur. Vegetative incompatibility groups reinforce genetic isolation of strains. These characteristics make genetic recombination and dispersal of mutations rare.

In commercial use, most infections are self-terminating with death of the host insect. If a mutation did occur in a commercial preparation, its spread is unlikely. Laverlam International Inc. uses culture maintenance and production procedures designed to maintain genetic stability. Starting cultures for production are maintained as replicates and not serially sub-cultured. Laverlam International Inc. uses only 4 generations from source culture (laboratory-infected insect) to final product. During more than 10 years of commercial use, and

production, no change has occurred in phenotypic characteristics or RFLP cDNA fingerprint of *B. bassiana* strain GHA (statement of the notifier).

B.2.1.8 Information on the production of metabolites (especially toxins) (OECD IIM 2.6)

B.2.1.8.1 Potential of the micro-organism to produce metabolites of concern for human health and/or the environment (Annex IIM 2.6)

Reference:
Strasser, H.; Vey, A. and Butt, T.M. (2000), Are there any Risks in using Entomopathogenic Fungi for pest Control, with Particular Reference to the Bioactive Metabolites of Metarhizium, Tolypocladium and Beauveria species? (BWS2006-66)

The applicant has stated in Document MII the following:

Introduction
Certain strains of *Beauveria bassiana* have been shown to have the potential to produce beauvericin, bassianolide and oosporein as metabolites and endo-toxins.

Beauvericin
Beauvericin is a hexadepsipeptide which has been isolated from members of the entomopathogenic fungi *Beauveria* spp. among others. Beauvericin shows antibiotic activity against several bacteria as well as moderate insecticidal properties. It is toxic towards several shrimp species and has even shown toxicity towards murine and human cell lines.

Bassianolide
Bassianolide is a cyclo-octadepsipeptide, known to be secreted by *B. bassiana*. Little is documented in relation to the toxic effects of bassianolide, but it has been shown to be potentially toxic to silkworm larvae at high doses.

Oosporein
Oosporein is a dibenzoquinone produced by a large number of soil fungi including entomogenous fungi belonging to the genus *Beauveria*. It is a known antiviral compound and an effective antibiotic against gram-positive bacteria but has no obvious effects on fungi and plants. However, oosporein has been reported to cause avian gout in broiler chicks and turkeys and it has been found that oosporein is the only major secondary metabolite produced by commercial strains of the fungus *Beauveria brongniartii* (Strasser *et al*., 2000, BWS2006-66).
A study was carried out to assess the levels of oosporein produced by *B. bassiana* Strain GHA. UV spectroscopy would typically result in a distinct peak at 550 nm, should oosporein be present. As no peaks were observed at or around the 550 nm wavelength, it can be assumed that *B. bassiana* strain GHA does not produce oosporein.

General remarks:
Although each of these compounds has been shown to produce toxic effects at different dose levels and in different organisms, studies have shown:

a) the inter- and intra-specific variation in the production of these metabolites/toxins
b) toxin quantities produced *in vivo* are usually far lower than those produced in nutrient rich liquid media; and
c) use of mycoinsecticides will not result in toxin levels rising to levels harmful to the environment (Strasser *et al.*, 2000, BWS2006-66).

Findings:
Laverlam International Inc. have analysed over 40 production lots of *B. bassiana* strain GHA and did not find either beauvericin or bassianolide in any of the batches. Analyses have also been carried out on two laboratory preparations of *B. bassiana* strain GHA and found very low levels (approx. 50 ppm) of each biochemical in one laboratory batch. To assess the contribution of these two biochemicals to the toxicity of the organism, this batch of *B. bassiana* strain GHA was tested for acute toxicity by oral, intratracheal, dermal and intraperitoneal administration to laboratory rats. There were no clinical signs of systemic toxicity in any of the animals in any of the four tests, and no mortality. Since there appears to be a strong correspondence between acute toxicity and mutagenicity for microbial genotoxins, the data from the Mycotech studies supports a conclusion that *B. bassiana* strain GHA does not secrete mutagenic biochemicals.

The applicant has given an explanation with respect to the unlikelyness of any toxin formation during the manufacturing process of *B. bassiana* strain GHA. Since this explanation contains confidential information, the wording is given in Volume 4 (C.1.2.4).
Further literature references given by the applicant are:

Oosporein:
From the physicochemical properties established for this metabolite, it can be concluded that oosporein can hardly be adsorbed by organisms. Physicochemical parameters for Oosporein have been established to allow a better understanding of the spatiotemporal distribution of this marker substance in the environment. *i*) The dependence of the oosporein solubility (C_s) from proton concentration and temperature was assessed. Oosporein is a rather strong organic acid. The basal solubility of oosporein (C_{s0} in aqueous citrate/HCl buffer at pH 1.23) was found to be 24.8±0.3 µM. The pK_a of the first deprotonation step was determined to be 2.42±0.02. A nonlinear *van't Hoff* equation was established for the temperature dependency of the oosporein solubility ($\ln C_s = a + bT^{-1} + c\ln T$, with a=-123.3, b=2678.9, and c=19.5). *ii*) A second and third deprotonation step was found in *Britton-Robinson*-buffered aqueous solution. The associated pK_a values were determined as 6.79±0.08 and 9.19±0.03. *iii*) The stability of oosporein at different pH values and temperatures was addressed. Oosporein degraded quickly under moderate alkaline conditions and with increased temperatures. The half-life dropped below 20 h at pH ≥ 8.0 and T ≥ 43°. The analysis of the *Arrhenius* plot allowed to calculate the activation energies (E_a) as 102±17 (pH 6), 100±5 (pH 8), and 91±6 kJ/mol (pH 10). *iiii*) The octanol/water partition coefficient (P_O) and its pH dependence were also determined. The distribution coefficient (D_O) for oosporein at pH 1.2 was found to be 53.7±4.1 ($\log D_O$=1.73±0.03). The partition coefficient P_O for undissociated oosporein was calculated as 56.6 ($\log P_O$=1.75) (Seger *et al.*, 2005).

Bassiacridin:

Bassiacidicin was isolated from *B. bassiana* strain EABb 90/2-Dm infecting locusts. Laboratory tests showed 90/2-Dm differes from other *B. bassiana* strains isolated from the same locust, related locust species and coleopteran and lepidopteran insects in the production of metabolites. No other strain produced this metabolite, though some were also pathogenic to locusts. These are the only available references to this metabolite. No additional information is available (Quesada-Moraga & Vey 2003, 2004).

Beauveriolides:
Dried silkworm larva infected by *B. bassiana* have been used as an analgesic and anticonvulsant in north Asian traditional medical practice. Recent chemical investigation of the crude drug led to the identification of compounds, all bearing a 4-*O*-methylglucose unit. The isolation of Beauveriolides from the culture broth of *Beauveria* species FO-6979 might prove to be pharmaceutically useful because the cyclodepsipeptides beauveriolides I and III show promising antiatherogenic activity (Isaka *et al*., 2005; Kikuchi *et al*., 2004).

Bassianin and Tenellin:
Cultures of some strains of *Beauveria bassiana* and *B. tenella* often develop a yellow color which is due to the formation of a mixture of related pigments, tenellin and bassianin. *B. bassiana* strain GHA cultures do not develop any yellow pigments (Wat *et al*., 1977.

Conclusion of the applicant:
Based on these findings, it can therefore be concluded that Beauveria bassiana strain GHA poses no risk to man or the environment via the production of toxic metabolites or mycotoxins (Bradley, 1993).

In addition,, the RMS did its own literature search regarding the possible metabolites of *B. bassiana* (beauvericin, bassianolide, bassiacridin, beauveriolides and beauverolides, bassianin and tenellin, oosporein, oxalic acid). The outcome is summarised below:

Beauvericin
Beauvericin is the most important metabolite. Beauvericin acts as specific cholesterol acyltransferase inhibitor and the substance is toxic towards *Artemia salina* larvae. It can induce programmed cell death similar to apoptosis and causes cytolysis (Logrieco et al. 1998*; Vey et al. 2001*; Pascale et al. 2002*).
Mutagenicity of beauvericin (Fotso & Smith, 2003) was tested in an Ames test with *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA1535 (provided by Molecular toxicology, Boone, NC, USA). Plate incorporation and preincubation technique were used. S9-mix from Aroclor 1254-induced male rats was used. Sodium azide, fenaminosulf, 2-aminofluorene served as positive control compounds.
In plate incorporation test with doses of up to 500 µg/plate, beauvericin was not mutatgenic with and without S9-mix. Authors stated, that the positive control compounds increased revertant frequency (data were not shown). Preincubation test showed no increase of revertant number in a concentration of 20 µg/plate (data were not shown). Toxicity was assessed in Vibrio fisheri and gave an EC_{50} for bioluminescence of approx. 70 µg/mL.

Beauvericin induced cell death in a range of various cell types: xenopus oocytes (Tang *et al.*, 2005), cultured human and rat cholangiocytes (Que *et al.*, 1997; Harnois et al., 1997), cultured human non-small cell lung cancer A549 (Lin *et al.*, 2005), HaCa cells (Lin *et al.*, 2005),

cultured human leukaemia CCRF-CEM cells (Jow *et al.*, 2004), cultured human myeloid monocytic U-937 cells (Calo *et al.*, 2004), cultured human promyeloic leukaemia HL-60 cells (Calo *et al.*, 2004), human hepatocarcinoma Hep G2 cells (Ivanova *et al.*, 2006), human fibrobast-like foetal lung MRC-5 cells (Ivanova *et al.*, 2006), isolated turkey peripheral blood lymphocytes (Dombrink-Kurtzman, 2003), cultured NIH-3T3 cells (Cook *et al.*, 1999; BVL no 1690645 , 1999, BVL no 1690648), murine tumour cells P815 (mastocytoma), Yac-1 (lymphoma) and EL-4 (thymoma) (Ojcius *et al.*, 1991), cultured porcine kidney PK15 cells (Klaric *et al.*, 2007), various human tumour cell lines [non-small-cell lung cancer, NCI-H460; pancreatic carcinoma, MIA Pa Ca-2; breast cancer, MCF-7; CNS cancer, SF268] (Zhan et al., 2007). In some of these articles induction of apoptosis was analysed and detected.

It was observed that treatment of cells with beauvericin altered intracellular Ca levels (Massini & Naf, 1980; Kouri *et al.*, 2005; Chen et al., 2006), which was attributed to an influx of extracellular Ca. Furthermore, beauvericin was found to transport Ca > K > Cs > Li > Na into liposomes (Prince *et al.*, 1974) or Rb > Ba > K > Na >> Ca >>> Li (extraction of the picrin salts from water through beauvericin-containing chloroform) (Roeske *et al.*, 1974). It was proposed, that beauvericin formed a 3:1 carrier ion complex (Benz, 1978) or pores (Kouri *et al.*, 2003).
Beauvericin might induce apoptosis by disturbing the cellular Ca levels (Ojcius *et al.*, 1991). Application of beauvericin to freshly isolated cultured myocytes from Guinea pigs or to ventricular cardiomyocytes from neonatal rats led to contraction of the cells, depolarisation of mitochondria and an acidification of cells (Kouri *et al.*, 2005).

L-type Ca current in a cultured neuronal cell line was reduced by incubation with beauvericin and mean time between spontaneous firing of action potentials was prolonged (Wu *et al.*, 2002).

Phasic and (to a higher extent the) tonic contraction of smooth muscle (from taenia coli from Guinea pigs) induced by high K levels were inhibited by beauvericin (Nakajyo *et al.*, 1987). Furthermore, contraction induced by Ca was also inhibited; inhibition by low concentrations of beauvericin (10^{-6} M) was competitive, whereas in higher concentrations ($3x10^{-6}$-10^{-5} M), it was non-competitive. Na and K content in muscle preparations were not altered by treatment with beauvericin (10^{-5} M; further experimental conditions not reported clearly).

In other studies (Krska, *et al.*, 1997; Lemmens-Gruber *et al.*, 2000), terminal ileum, papillary muscle from right ventricle and right atria were prepared from Guinea pigs and used. Contractile force of precontracted terminal ileum pieces was reduced by treatment with beauvericin. The frequency of spontaneous contraction of right atria was reduced in concentrations of 30 µM, but not below, however the force was reduced at concentrations of 0.1 µM and above (atria and papillary muscles). Action potential of papillary muscles was reversibly reduced by beauvericin.
In cultured Caco-2 cells and ventricular myocytes, Ca, Mg and Na concentration were increased after treatment with beauvericin, whereas K concentration and pH were decreased (Kamyar *et al.*, 2006).

In PK15 cells treated with beauvericin, number of viable cells was reduced, lipid peroxidation increased and GSH levels were decreased (Klaric *et al.*, 2007). In precision-cut rat liver slices, beauvericin had little influence on the sphinganine/sphingosine-ratio and was not toxic (as measured by MTT-formazan formation) to the tissue-cut slices (Norred *et al.*, 1997).

Beauvericin inhibited acylcholesterol ester formation by acyl-CoA-cholesterol acyl transferase in rat liver microsomes, cultured J774 macrophage cells and mouse peritoneal macrophages (Tomoda *et al.*, 1992).

Bassianolide

Bassianolide is a cyclodepsipeptide produced by *Beauveria bassiana* (Suzuki, 1977). Fifth instar larvae of silkworm, *Bombyx mori*, were killed when fed with artificial diet containing bassianolide at a dose of 13 ppm.

Bassianolide induced a right-shift of the dose-response curve of the norepinephrine-induced or phenylephrine-induced contraction of Guinea pig aorta (Nakajyo *et al.*, 1981; article written in Japanese, abstract and figures available in English). The maximum contraction was not altered. Bassianolide also inhibited to some extent the contraction induced by 60 mM of K, Ba or tetraethylammonium, however, contraction induced by K-free solution was not inhibited. Therefore, the authors concluded, that bassianolide inhibited the contraction mediated by drug receptors.

Treatment of preparations of Guinea pigs ilial longitudinal muscle (Nakajyo, 1982; BVL no 1690653) with bassianolide (10^{-8} - 10^{-6} M) resulted neither in contraction nor relaxation, whereas treatment with acetylcholine led to contraction, which could be inhibited with atropine. Application of bassianolide prior to atropine, histamine and prostaglandine E2 led to a right-shift of the dose-response curve [muscle contraction vs. concentration] of the respective compounds (EC_{50} values increased by a factor of approximately 10 to 100). Contraction induced by carbachol and pilocarpine were also reduced by bassianolide. Muscle contraction induced by KCl or $BaCl_2$ was not altered by bassianolide. In a similar manner, application of bassianolide to isolated vas deference led to an inhibition of contraction by norepinephrine, phenylephrine, acetylcholine, histamine. Effects of K and Ba on vas deference contraction were not altered by bassianolide.
The authors hypothesised that the action of bassianolid on ilial longitudinal muscle (alteration of compound effects exhibited through muscarinic receptor, H1 receptor, M or D receptors and acetylcholine) and on vas deference (alteration of compound effects exhibited through alpha-adrenoceptor, muscarinic receptor, H1 receptor) were result of an inhibition in the pathway between receptor binding and muscle contraction. Muscle contraction induced by K and Ba were not altered, which indicates that the contractile machinery was not altered by bassianolide.

When isolated Guinea pig ileal longitudinal muscle were treated with bassianolide (10^{-6} M), contraction induced with nicotine or acetylcholine was reduced (Nakajyo *et al.*, 1982; BVL no 1690654, 1982; BVL no 1690659). Also contraction of vas deferens of Guinea pigs induced by nicotine or acetylcholine was reduced by bassianolide (10^{-5} or $3x10^{-8}$ M, respectively). However, contraction of frog rectus abdominal muscle preparations was not altered by bassianolide when stimulated with nicotine or acetylcholine. Sartorius muscle with an attached branch of sciatic nerve form frogs and phrenic nerve with attached diaphragm muscle from mice were prepared and twiches were stimulated with electricity. Twitch strength was not altered by treatment with bassianolide.
In crude preparations of synaptosomal membrane preparations from mouse and Guinea pig brains, binding of radioactively labelled α-bungarotoxin (BTX) or of quinuclidinyl benzilate (QNB) was not altered by bassianolide (Nakajyo *et al.*, 1982; BVL no 1690659). The compounds bind to nicotinic or muscarinic acetylcholine receptors, respectively; therefore, the

authors concluded that bassianolide's inhibition of contraction had another mechanism then the binding to muscarinic receptor.

In another study, contraction of Guinea pig teania coli preparations was measured (Nakajyo, 1983). Bassianolide reduced phasic (partly) and tonic (almost completely) contraction induced by acetylcholine or K. Contraction induced by acetylcholine was reduced by a greater extent than the contraction induced by K. Cellular Na and K content and Ca uptake were not altered by bassianolide. The authors noted that the action of bassianolide was distinct from the mechanisms of verapamil or papaverine, that the mechanism does not involve changes in binding activity of acetylcholine to muscarinic receptor, changes in membrane potential induced by acetylcholine and changes in the contractile machinery of intestinal smooth muscle.

Contraction of Guinea pig hypogastric nerve-vas deferens preparations induced by electric stimulation was slightly inhibited by application of bassianolide to the nerve portion but not to the blood vessel portion (Nakajyo *et al.*, 1984; article written in Japanese, abstract and figures available in English).

Bassianolide was cytotoxic to mouse macrophages (Namatame, 1999) and showed insecticidal activity against *Artemia salina* at concentrations of more than 0.1 µM but not nematocidal activity against *C. elegans*.

Bassiacridin

Bassiacridin is a protein isolated from *in vitro* cultures of *Beauveria bassiana* stain EABb 90/2-Dm (deposited at the Spanish Collection of Culture Types, accession no. CECT 20371) (Quesada-Moraga & Vey, 2004). Isolation was directed by the toxicological properties to fourth instar *Locusta migratoria* nymphs. Toxic properties of the isolate were enriched by various chromatographic techniques. In the final extract, SDS-Page showed a single spot with a molecular wt of 60 kDa, also native PAGE and IEF showed single spots with an isoelectric point of 9.5. The isolated protein showed β-galactosidase, β-glucosidase and N-acetylglucosaminidase activity.

Peptid was digested with trypsin and sequenced, but no homology was found with standard methods. Using another strategy, a homology with a chitin binding protein of the yeast *Pichia etchellsii* was found (degree of homology could not be determined).

Cadavers of insects (fourth instar *Locusta migratoria*) injected with the fractions showed melanised dark spots on tracheae, air sacs and melanised nodules in the fat body in contact with the cuticle. Epithelial cells of the integument showed dense autolytic cytoplasmic inclusion bodies. Nodules showed accumulation of necrotic haemocytes, which were surrounded by healthy cells forming a thin envelope. Melanisation spread initially around taenidium and then melanisation and necrosis process extended to the full tracheal epithelial cell.

When bassiacridin was injected into selected insects (dose: 2.8 µg/g), varying impacts were shown. Bassiacridin was not toxic to lepidopteran *Spodoptera littoralis* (fifth instar larvae) and coleopteran *Tenebrio molitor* (fifth instar larvae), whereas it was slightly toxic to lepidopteran *Galleria mellonella* (fifth instar larvae, 16.6 % mortality). In contrast, it was equally toxic to migratory locusts *Locusta migratoria* (fourth instar nymphs) and desert locusts *Schistocera gregaria* (fourth instar nymphs) causing 42.5 % and 38.3 % mortality, respectively and slightly more toxic to Moroccan locust *Dociostaurus marroccanus* (fourth instar nymphs, 49.2 % mortality).

Treatment of Sf-9 cells (from Spodoptera frugiperds) for 72 h with concentrations of 60 µg/mL (and above) inhibited reduction of MTT dye to formazane, which is an indicator for cytotoxicity. Lower concentrations showed no cytotoxic properties.

Beauveriolides

Beauveriolides are a group of compounds having 13-membered cyclic skeleton composed of L-amino acids, one D-amino acid and one 3-hydroxy fatty acid. Several members have been reported (as reviewed by Namatame, 2004 and the references cited there, but not available to RMS). Beauveriolides I and III were able to inhibit lipid droplet accumulation in mouse macrophages (which were incubated with liposomes) (Namatame, 1999 & 2004). Formation of cholesteryloleat was inhibited by treatment of macrophages with beauveriolides I and III but formation of trigrlycerides was not inhibited. In contrast, beauvericin inhibited both reactions and showed cytotoxic properties. Beauveriolides I and III inhibited acyl-CoA-cholesterol acyl transferase (ACAT) in microsomes of mouse peritoneal macrophages, mouse livers and human CaCo-2 cells. Beauvericin inhibited ACAT with greater potency (i.e., lower IC_{50} values) (Namatame, 2004).

Nine male mice (apoE-knockout) per group were fed a 0.15 % cholesterol-supplemented diet. Animals were treated daily with beauveriolid III (25 mg/kg bw) or the vehicle CM-cellulose (0.05 %) for 2 months. Atherosclerotic lesions areas in aortas were reduced by approx. 50 %; no significant differences occurred in bodyweight or clinical chemistry values (data were not shown). In LDL-R-knockout mice treated with 50 mg/kg bw, atherosclerotic lesions in aorta and heart were also reduced by approx. 50 % (neither details on experimental set-up nor resulting data were presented) (Namatame, 2004).

No antimicrobial activity of beauveriolides I and III were observed against a range of selected micro-organisms (i.e., inhibition of growth when cultures covered with paper disks soaked up with solutions of concentrations up to 1 mg/mL). The compounds showed no insecticidal or nematocidal activity against *Artemia salina* or *C. elegans* at concentrations of up to 200 µM (Namatame, 1999).

In further studies (Ohshiro et al., 2006 & 2007), Beauveriolides I and III inhibited cholesterol ester synthesis mediated by the enzyme ACAT1 but not by ACAT2 in a cell based assay using CHO cells expressing African monkey ATAC1 or ATAT2, respectively. In a cell-free assay with microsomes of these cells, beauveriolides I and III inhibited cholesterol ester synthesis by the two enzymes by a similar extent. Beauvericin inhibited both enzymes in the cellular and the cell-free assays in a similar extent. The authors proposed to use beauveriolides as molecular leads for the development of selective ATAC inhibitors that might be used as antiatherosclerotic agents.

Beauverolides

Beauverolides are peptides with a similar structure to beauvericin and bassianolide (Namatame et al. 1999*, 2004*).

No studies on the toxicological properties of beauverolides were submitted by the notifier, nor could RMS identify any relevant articles in open literature.

Bassianin and tenellin

Bassianin and tenellin are yellow non-peptide pigments found in extracts of *B. bassiana* and *B. tenella* (Jeffs & Khachatourians, 1997).

Jeffs & Khachatourians (1997) tested the influence of bassianin, tenellin and oosporein on equine erythrocyte Na/K-, Ca- and Mg-ATPase. Membrane-bound ATPases were prepared from lysed erythrocytes. The compounds inhibited total ATPase activity at concentrations of approx. 75 µg/mL and above. A concentration of 200 µg/mL inhibited total ATPase activity by 40 to 50 % (all three compounds). Bassianin and oosporein inhibited Ca-ATPase to a lower extent than tenellin. Na/K-ATPase was inhibited in lower extent than Ca-ATPase by these compounds. Inhibition of Mg-ATPase by bassianin and oosporein was higher than inhibition by tenellin.
Intact erythrocytes lysed after incubation with a concentration of 200 µg/mL of tenellin (almost complete haemolysis within 1 h) and bassianin (partial haemolysis after 1 h, complete after 2 h) as observed by phase contrast microscopy.
Similar results (i.e., inhibition of Na/K- and Ca-ATPase, shrinking of erythrocytes) were also obtained with beauvericin.

Oxalic acid

Oxalic acid is secreted by *B. bassiana* and *B. brongniartii* (Müller-Kögler 1965*, Roberts 1981*) and is considered to be an important pathogenicity determinant (Vey et al. 2001*).
Oxalic acid was recently evaluated as veterinary medicinal product. The following information was extracted from the summary report (EMEA, 2003):
Oxalic acid is an organic dicarboxylic acid occurring in human food stuff (up to 200 g/kg dry wt). Mean daily dietary intake is 50 mg (depending on type of food there is a large interindividual variation, range: 5 - 500 mg). It is the product of metabolism of some amino acids, glycolates and ascorbic acid. Endogenous sources constitute approx. 30 - 70 % of the oxalic acid excreted via urine (20-30 mg). Oral absorption rate was low in rodents (< 30 %) and humans (3 - 20 %); free calcium and magnesium ions and solubility of oxalic acid under the different pH levels in GI influenced the degree of absorption.
Oxalic acid is moderately toxic to rats (oral LD_{50}: 475 or 375 mg/kg bw for males or females, respectively). Main target organs after oral administration was the kidney with formation of calcium oxalate crystals, associated with focal necrosis, mineralisation and impairment of kidney function. Oxalic acid was irritating to skin, eye and the respiratory tract. After intravenous administration (40 mg/kg bw) to dogs, all animals died shortly after injection. Oxalate binds to blood calcium and induces neurotoxicity and cardiac arrest.
In studies with repeated oral (70-d, 2000 and 5000 mg/kg bw/d) or subcutaneous administration (1-wk, 75 mg/kg bw/d; 3-wk, 50 mg/kg bw/d; 2-wk, 25 mg/kg bw/d), nephrotoxicity was observed (macroscopic and microscopic findings, urine parameters) [due to limitations in the study designs, no NOELs were derived].
In a 2-generation study in mice, number of litters, litter pup weight and total number of live pups was decreased (F1 generation, 275 mg/kg bw/d via drinking water) and in F2 males the incidence of abnormal sperm was increased. Furthermore, reduced water intake, low prostate gland weights and increased relative kidney weights were observed.
In teratogenicity studies in rats, marked vacuolisation in cells of proximal tubules and tubular nephrosis in the pups were observed in the preliminary study, while this effect was not seen in the main study (despite using similar dosages). Renal oxalosis was observed in lambs of sheep treated during the second half of or during the whole gestation. Gross malformations were seen neither in rats, nor in sheep. EMEA concluded, that it could draw no final conclusion on teratogenic or embryotoxic effects.
Oxalic acid was negative in Ames test and chromosomal aberration assay in Chinese hamster lung fibroblast cells. In vivo studies were not available.

It was not carcinogenic in a 2-yr rats study with dietary administration up to 600 mg/kg bw/d (12000 ppm). Slight periportal hypertrophy of hepatic cells was noted during pathology examination.
Intravenous doses of approx. 25 mg/kg bw of oxalic acid inadvertently administered to humans led to kidney failures, cardiac arrest and death in spit of intensive care measures. High oral intake via diet rich in oxalic acid has also occasionally led to severe poisoning and death. Oral fatal doses of oxalic acid were reported to range from 3 to 30 g/person. The susceptibility of individuals varies greatly, depending on prior kidney damage, certain intestinal disease states or genetic abnormalities such as primary hyperoxaluria.

Oosporein
Oosporein (isoloated from cultures of *Chaetomium trilaterale* Chivers ATCC 24912) was found to be a red crystalline compound (Cole *et al.*, 1974). In day-old DeKalb cockerels (60 animals per dose level: 200, 300, 400 µg/animal, average weight: 38 g; dosing solution was prepared by dissolving the compound in ethyl acetate, adding the desired amount of corn oil and removing ethyl acetate under vacuum at 70° [RMS: unit not reported, probably Celsius]; administration by crop intubation of 1 mL dosing solution) it had a LD_{50} of 6.12 mg/kg bw (245 µg/animal). Mortality began to appear approx. 2 d post-dosing, with higher doses, death appeared earlier. Approximately 10 mg/kg bw killed all dosed animals. Maximum observation period was not reported.
The mycotoxin oosporein (isolated from cultures of *Chaetomium trilaterale* Chivers ATCC 24912) was added to broiler starter ration in dose levels of up to 600 µg/g feed (Pegram & Wyatt, 1979, 1981). These rations were fed to male broiler chicks (Cobb) from hatching for 3 weeks with feed and water *ad libitum*. Each experimental diet was fed to three (experiment 1: 0, 2.5, 5.0, 10, 20, 40 µg/g; experiment 2: 0, 12.5, 25, 50, 100, 200 µg/g; experiment 4: 0, 200, 250, 300, 350, 400 µg/g) or four (experiment 3: 0, 200, 400, 600 µg/g) replicate groups of 10 birds each. Bodyweight and feed intake were recorded weekly, mortality and clinical signs of toxicity were recorded daily. On the final day of experiments, cloacal temperatures were taken (only experiment 4), blood (only experiments 2 and 3, PCV) and plasma samples were taken. Animals were killed and gross pathological observations recorded, weights of selected organs (heart, liver, spleen, bursa; additionally in experiments 3 and 4: left kidney, proventriculus, gizzard).
No advers effects were observed in animals fed dose levels up to 100 µg/g. Seven percent of animals receiving 200 µg/g died. Animals treated with higher dietary dose levels showed high mortality and various lesions including visceral and articular gout. At 400 and 600 µg/g dose groups, most of the deaths occurred in first week. Total mortality in the 3-wk treatment period was 2.5 %, 10.0 %, 62.5 %, 95.0 % for 0, 200, 400, 600 µg/g dose groups. In a further experiment with narrower dose levels, high mortality in the first week was observed in animals fed dose levels of 300 µg/g and above, leading to a cumulated mortality with in the three-week treatment period of 0 %, 13.3 %, 20.0 %, 30.0 %, 46.7 %, 56.7 % for 0, 200, 250, 300, 350, and 400 µg/g dose groups.
Animals that died within the first week, appeared normal 24 h prior to death, but approx. 12 h before death a rapid progression of symptoms began: morbid birds abstained from eating, began to consume large quantities of water, became lethargic and eventually collapsed. Fluid droppings were observed in pens containing morbid birds and dehydration was evident in the feet and legs prior to death.
In animals that died in the first week, dominant lesion was extensive visceral gout with urate deposition in internal organs, mesentery, air sacs, muscles, within the gall bladder and especially intense concentration of urates on the pericardium. In some birds, articular tophi were also present in the hock joints and interphalangeal toe joints. The second prominent

finding was severe swelling and paleness of the kidneys. Additional gross pathological observations included dehydration, focal necrosis and mottling of liver, a dark green discolouration of the gizzard lining and proventriculitis; at 400 µg/g and above, mucosal necrosis was visible, especially in the isthmus. The gall bladder was often enlarged and filled with bile varying in colour from translucent green to yellow.

Dosed animals that survived the first week had higher water intake (400 and 600 µg/g groups) and had lower bodyweight (250 - 400 µg/g groups, 600 µg/g group not shown as only 2 animals survived), feed intake was reduced in 300 µg/g group and above. Animals that died after the first week showed articular and muscular urate deposition with principle involvement of the legs and feet. Oosporein-induced gout was of sufficient severity to produce visible urate deposits in the hock joints and feet of these birds 1 to 3 days before death. Necropsy findings were similar to findings in animals that died earlier and occurred in all dosed animals. Liver, kidney (300 µg/g and above) and proventriculus (200 µg/g and above) weights were significantly increased. Plasma uric acid was increased (+ 48 %) in 400 µg/g dose group animals.

The NOAEL in this study was a dietary dose level of 100 µg/g (equivalent to 12.5 mg/kg bw/d) with increased mortality, decreased feed intake and toxicity in liver, kidney and digestive tract in the higher dose levels. Furthermore, there was urate deposition in various organs.

Day-old large white male turkey poults (received from a commercial ghatchery) were housed in electrically heated batteries and illuminated continuously (Pegram et al., 1982). Feed and water were provided *ad libitum*. Oosporein (extracted and purified from cultures of *Chaetomium trilaterale* Chivers ATCC 24912) was incorporated into turkey started ration at dietary dose levels of 0, 500, 1000 and 1500 µg/g feed. Poults were treated for tree weeks with oosporein-containing feed. Five replicates of 5 animals each were employed per treatment.

After three weeks of treatment, bodyweight was significantly lower (compared to control) in all treatment groups (-10 %, -21 % or -43 % for dose levels of 500, 1000 or 1500 µg/g). Feed consumption during the whole treatment period was significantly reduced in 1000 and 1500 µg/g-dose groups. Water consumption was significantly increased in all treatment groups. First mortalities were observed on day 6 in 1000 and 1500 µg/g groups. Cumulative mortality in this experiment was 0, 0, 24, 54 % for control group, or 500, 1000 and 1500 µg/g groups.

Prior to death, susceptible poults showed retarded growth, fluid-distended crops, approximately 12 to 18 h before death they were lethargic and had impaired mobility. Subcutaneous urate deposits surrounding the hock joint and interphalangeal toe joints were visible in approximately half of the dying animals. At later time-points during the experiment, articular gout was visible for 3 to 5 days before death. Both, visceral and articular gout with massive urate deposition in many organs or joints were observed. Urate deposition was more severe in 1500 µg/g group than in 1000 µg/g group. As in chickens, kidneys were swollen and pale, ureters were distended with semifluid, white urine. Proventricular enlargement with small haemorrhages on the serous surface were observed. A pseudo-membranous exudates lined the mucosa of proventriculous and a peculiar lesion consisting of necrotic tissue and clotted blood was found in the isthmus. In some cases, the necrosis of the isthmus extended into the gizzard, creating eroded chasms and perforations in the lining. An olive-green discolouration of the gizzard lining and of the content of the large intestines and caeca were often observed. In surviving animals, urate deposition was not observed, but the further findings on kidney and proventriculus. Changes in organs accompanied by changes in relative organ weights (increase in all treated groups: kidney, proventriculus; increase in medium and high dose grous: liver, pancreas, gizzard).

Glutamic-oxalacetic transaminase activity was increased in all treated groups, whereas LDH activity was increased only in the high dose group. Urea nitrogen (all treated groups) and uric acid (medium and high dose group) levels were significantly increased.

To determine oral LD_{50} (Manning and Wyatt, 1984) within a 10 d post-dosing observation period, oosporein was dissolved in corn oil or aqueous vehicle and administered to day-old male broiler chicks (Hubbard x Hubbard). For each dose level, 25 animals were used. To evaluate the influence of chemical form (i.e., free organic acid, Na salt and K salt), oosporein was dissolved either in 0.1 M $NaHCO_3$ or 0.1 M K_2HCO_3 (sic! Probably $KHCO_3$ is meant, as this would allow to evaluate the influence of Na or K salt on the toxic properties) and evaporated to dryness.

LD_{50} values (mg/kg bw) were as follows:

	Oosporein (organic acid)	Na salt	K salt
Corn oil	5.77	5.59	5.57
Aqueous vehicle	Not done	5.00	4.56

Birds began to die approximately 30 h post-dosing and majority of deaths had occurred by 72 h post-dosing

For further evaluation day-old male broiler chicks were treated for three weeks with the different preparations of oosporein. The compounds were incorporated into the feed. Animals were housed in electrically heated batteries under continuous illumination. Feed and water were provided *ad libitum*. Each dose group consisted of three (K salt, organic acid) or four (Na salt, organic acid) replicates of 10 birds each.
Bodyweight was not influenced by treatment with any of the chemical forms of oosporein. Mortality within the three-week feeding period (300 µg/g), was 65 % for the Na salt, 43 % for the K salt and 23 or 48 % in two experiments with the organic acid. The findings were similar to the earlier study (Pegram & Wyatt, 1981): green discolouration of the gizzard lining and articular gout; the findings were more severe in animals treated with the Na or K salts than in animals treated with the organic acid. Animals that died during the treatment period, exhibited large nodules of urate deposits on the feet (some formed nodular sacs approx. 1 cm in diameter). Relative weights from kidneys (approx. + 10 - 15 %) and proventriculus (approx. + 20 %, in one experiment this increase was significant but not in the other experiment) were significantly increased, whereas gizzard and liver weights were not altered. Serum uric acid was slightly but dose-dependently increased (9 or 60 % for 150 or 300 µg/g K salt in feed; increases with organic acid were slightly lower). Serum protein and serum glucose were not altered.

Brown et al. (1987) evaluated kidneys of broiler chicks treated with oosporein K salt by microscopic and transmission electron microscopic techniques. Two replicate groups of 8 one-day-old male Arbor Acres chicks (obtained from a commercial hatchery) received feed containing oosporein (300 µg/g) from day 0 to day 21; an additional replicate received untreated feed. Chicks that died on day 3 had nephrosis of initial proximal tubular segments, necrosis and necrotic debris was observed in proximal tubular lumens (gross lesions were enlarged pale kidney, white granular urate deposits on pericardial and peritoneal surfaces). Basement membrane was exposed and in some areas it had gaps. In subsequent proximal tubular segments, microvilli were absent or damaged and necrosis was present in scattered

foci. In chicks that survived the 21-d treatment period, kidneys were enlarged; tubules were enlarged and raised above the adjacent renal surface. Some glomeruli were enlarged and hypercellular and had cuboidal metaplasia of parietal epithelium. Others had periglomerular fibrosis, a dilated urinary space and an atrophied sclerotic tuft. Centrilobular distal tubules were dilated and filled with hyaline basophilic casts. Interstitial fibrosis was prominent in cortical and medullary zones.

Toxicity studies with oosporein in mice and hamsters indicated a LD_{50} value of 0.5 mg/kg bw after intraperitoneal injection. Whereas, daily oral doses of 7 mg/kg bw over 47 d in mice were not lethal (cited from Strasser et al., 2000, original reference (Wainwright et al., 1986, Trans Br Mycol Soc (86) 168-170) not available to RMS).

LDH release from isolated rat renal proximal tubules was increased after exposure to oosporein in concentrations of 1 mM (1 h and longer incubation) or 0.5 mM (4 h incubation) (Aleo *et al.*, 1991). Basal and nystatin-induced oxygen consumption was not reduced by treatment of these cells with oosporein in concentrations of 1 mM (0.5 h) or 0.5 mM (1 h). Oosporin (1 mM, 0.5 h) did not induce lipid peroxidation (measured by malondialdehyde formation) in the suspended rat renal proximal tubules.
Favilla et al. (2006) treated newly hatched brine shrimps *Artemia salina* and water flea *Daphnia magna* with oosporein. They observed no influence on viability (i.e., forward motion) of brine shrimps when treated with up to 200 µM oosporein (dissolved in methanol, final concentration in medium: 1 % (vol/vol)) for 24 and 36 h. Water flea treated with oosporein showed increased mortality (i.e., dead or immobilised neonates) with an LC_{50} of 224 or 19 µM after 24 or 36 h of treatment, respectively. At the later observation time, mortality showed a very steep dose-response curve.

Incubation of isolated rat liver mitochondria with oosporein reduced the oxygen consumption in state 3 (0.41 mM oosporein and above) and 4 (1.63 mM oosporein) of oxidative phosphorylation (Taniguchi *et al.*, 1984). Respiratory control ratio (i.e., tightness of coupling between respiration and phosphorylation) and phosphorylation rate (i.e., measure for ATP synthesis) were also reduced. ADP/oxygen ratio was not altered. The authors concluded that oosporein did neither act as an uncoupler nor as a phosphorylation inhibitor; therefore, oosporein had an action on some respiratory chain enzyme.

*cited in Zimmermann (2007)

B.2.1.9 Information on resistance / sensitivity to antibiotics / anti-microbial agents used in human or veterinary medicine (OECD IIM 2.12)

No specific information adressing this issue has been submitted by the notifier or could be found in the open literature. However, because spread of resistance (genes) to antimycotic agents between fungal species is not of that concern as for bacteria with regard to antibiotics, and since *Beauveria bassiana* itself is only an opportunistic pathogen that has very rarely caused infections in humans and is not related to any known human pathogen, this lack of information is not considered a critical data gap.

B.2.2 Physical, chemical and technical properties of the plant protection product (OECD IIIM 2, OECD IIIM 6.4)

Product name: **BotaniGard** (containing 4.4 x 10^{13} CFU/kg, equivalent to 22 % (w/w), *Beauveria bassiana* strain GHA, WP)

Table B.2.2-1: Summary of the physical, chemical and technical properties of the plant protection product

Section (OECD Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
B.2.2.1.1 (IIIM 2.1)	Appearance: colour	Visual	grey (near Pantone #12-1006)	acceptable	Jaronski, S.T. (1997) PHY2006-355
B.2.2.1.2 (IIIM 2.1)	Appearance: odour	Olfactory	slight petroleum odour	acceptable	Jaronski, S.T. (1997) PHY2006-355
B.2.2.1.3 (IIIM 2.1)	Appearance: physical state	Visual	non-dusty, slightly cohesive powder	acceptable	Jaronski, S.T. (1997) PHY2006-355
B.2.2.2 (IIIM 2.2)	Storage stability and shelf life		Product will remain stable if stored for 14 d at 40 °C. Spore viability decreased by 15 % after 29 d at 40 °C. The product may be safely used at 85 % of its original activity without loss of efficacy. A shelf life of 8 month is proposed.	Only biological stability is examined, data on the physical stability of the formulation further information are missing.	Anonymous (1996) PHY2006-480

Section (OECD Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
			Eight commercial batches of BotaniGard 22Wp have been analysed after storage for 12 month at 5 °C and 25 °C, respectively. The viabilities were: Month 5 °C 25 °C 0 90.2% 82.3% 12 87.6% to 96.5 % 88.7% to 88.7%	Data on the physical stability are missing.	Chatriand (2002) BVL no 1677991
B.2.2.3.1.1 (IIIM 2.3.1)	Explosive properties	statement	The properties of BotaniGard 22 WP indicate that the product has little or no explosive properties.	It is not clear, which properties are referred to here. A clarification is missing. The applicant refers also to a MSDS. However, there is only stated that the ppp has no "unusual explosion hazard". This does not address the Annex point.	

Section (OECD Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
B.2.2.3.1.2 (IIIM 2.3.1)	Oxidising properties	statement	The properties of BotaniGard 22 WP indicate that there is little or no potential for this material to act as an oxidising or reducing agent.	It is not clear, which properties are referred to here. A clarification is missing.	
B.2.2.3.2.1 (IIIM 2.3.2)	Flash point	EEC A 9	not applicable (no liquid)		
B.2.2.3.2.2 (IIIM 2.3.2)	Flammability	statement	Caolin clay is used as a carrier in the product. This material is not flammable and often used as an extinguishing material.	acceptable	
B.2.2.3.2.3 (IIIM 2.3.2)	Spontaneous ignition		no information submitted		
B.2.2.3.3.1 (IIIM 2.3.3)	Acidity/alkalinity		not applicable (due to pH)		
B.2.2.3.3.2 (IIIM 2.3.3)	pH	SOP 09-06-02	6.3 (1 g of test substance in 100 mL of distilled water)	acceptable	Jaronski, S.T. (1997) PHY2006-355
B.2.2.3.4.1 (IIIM 2.3.4)	Viscosity		not applicable		
B.2.2.3.4.3 (IIIM 2.3.4)	Surface tension		not applicable		

Section (OECD Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
B.2.2.4.1 (IIIM 2.4.1)	Wettability	CIPAC MT 53.3.1	63 min 10 s 36 min (repetition)	not acceptable values clearly above general limit of 2 min (FAO, 2006, p.213)	Kang, J. (2005) PHY2006-357
B.2.2.4.2 (IIIM 2.4.2)	Persistent foaming	CIPAC MT 47.2	after 10 s: 6 mL foam after 1 min: 4 ml foam after 3 min: 2 mL foam after 12 min: 0 mL foam	acceptable no concentration stated	Foster, B. (2006) PHY2006-758
B.2.2.4.3 (IIIM 2.4.3)	Suspensibility	CIPAC MT 15	54.2 % (0.0625 %) 58.7 % (0.125 %) (gravimetric determination)	values slightly below general limit of 60 %.	Kang, J. (2005) PHY2006-357
B.2.2.4.4 (IIIM 2.4.4)	Dry sieve test and wet sieve test		no information submitted	data on a wet sieve test are missing	

Section (OECD Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
B.2.2.4.5.1 (IIIM 2.4.5)	Particle size distribution	CIPAC MT 58	**sieve [µm] result 1 [%] result 2 [%]** > 850 0.9 0.51 710 - 850 0.6 0.19 500 - 710 1.6 0.75 425 - 500 0.6 0.32 355 - 425 1.1 0.58 250 - 355 4.8 6.15 150 - 250 47.5 21.09 (< 150 42.9) 75 - 150 38.98 45 - 75 23.40 < 45 8.03	acceptable	Kang, J. (2005) PHY2006-357
B.2.2.4.5.2 (IIIM 2.4.5)	Dust content		not applicable		
B.2.2.4.5.3 (IIIM 2.4.5)	Friability and attrition		not applicable		
B.2.2.4.6 (IIIM 2.4.6)	Emulsifiability, re-emulsifiability and emulsion stability		not applicable (WP formulation)		
B.2.2.4.7.1 (IIIM 2.4.7)	Flowability		not applicable		
B.2.2.4.7.2 (IIIM 2.4.7)	Pourability (rinsability)		not applicable		
B.2.2.4.7.3 (IIIM 2.4.7)	Dustability		not applicable		

Section (OECD Annex point)	Study	Method	Results	Comment/ Conclusion	Reference
B.2.2.5 (IIIM 2.5)	Density	ASTM D4512	0.512 g/cm^3	acceptable	Jaronski, S.T. (1997) PHY2006-355
B.2.2.6 (IIIM 2.6)	Adherence and distribution to seeds, for seed treatment products	statement	not applicable (not intended for seed treatment)		
B.2.2.7.1 (IIIM 6.4.1)	Physical compatibility	statement	not applicable (no tank mixes recommended)		
B.2.2.7.2 (IIIM 6.4.2)	Chemical compatibility	statement	not applicable (no tank mixes recommended)		
B.2.2.7.3 (IIIM 6.4.3)	Biological compatibility	statement	not applicable (no tank mixes recommended)		

B.2.2.1 Summary and evaluation of data on properties of the MPCP presented under points B.2.2.1.1 to B.2.2.6 (IIIM 2.7)

BotaniGard is a grey, non-dusty and slightly cohesive powder with a faint petroleum odour. It is not explosive or flammable and shows no oxidising properties.
The shelf life of 8 month is only based on biological stability. For the physical stability of the formulation no information was submitted.
On the basis of the values for wettability and suspensibility it seems questionable whether the product can be applied without problems.

B.2.3 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 2.1 KIIM 2.2 KIIM 2.3 KIIM 2.5 (OECD)	Bradley, C.	1991	Product identity and disclosure of ingredients N/A GLP: N, published: N 1300940 / BWS2006-63	Y	LAM
KIIM 2.1 (OECD)	Humber, R.A.; Hansen, K.S.	2003	Catalog of isolates including all indices USDA-ARS Plant Protection Research Unit , GLP: N, published: Y 1677989 /	Y	LIT
KIIM 2.12 (OECD)	Laverlam International Corp	1999	BotaniGard 22 WP: Fungicide compability GLP: N, published: N 1678063 /	Y	MEU
KIIM 2.2 KIIM 2.4 KIIM 2.6 KIIM 2.7.2 KIIM 2.8 (OECD)	Zimmermann, G.	2007	Review on safety of the entomopathogenic fungi Beauveria bassiana ans Beauveria brongiartii Biocontrol Science and Technology 17 (5/6), 553-596 GLP: N, published: Y 1673970 /	Y	LIT
KIIM 2.4 KIIM 2.8 (OECD)	Fargues, J. et al.	1997	Effect of temperature on vegetative growth of Beauveria bassiana isolates from different origins 3, 383-392 Mycologia, 89(8) GLP: N, published: Y 1300943 / BWS2006-65	N	LAM

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 2.4 (OECD)	Anonymous	2000	EPA: Beauveria bassiana strain ATCC 74040 (128818) Technical Document Envirnmental Protection Agency United States GLP: N, published: Y 1673974 /	N	LIT
KIIM 2.5 (OECD)	Fernandez, S.	2001	Bassiana (bals.) vuill. in colorado potato beetle (leptinotarsadecemlineata) GLP: N, published: Y 1677994 /	N	LIT
KIIM 2.6 KIIM 2.7.2 (OECD)	Bradley, C.	1993	Discussion of formation of unintentional ingredients GLP: N, published: N 1300937 / BWS2006-72	Y	LAM
KIIM 2.6	Aleo MD, Wyatt RD, Schnellmann RG	1991	Mitochondrial dysfunction is an early event in ochratoxin A but not oosporein toxicity to rat renal proximal tubules. Toxicol Appl Pharmacol (107) 73-80 GLP: N, published: Y BVL No. 1690628	N	LIT
KIIM 2.6	Benz R	1978	Alkali ion transport through lipid bilayer membranes mediated by enniatin A and B and beauvericin. J Membr Biol (43) 367-394 GLP: N, published: Y BVL No. 1690635	N	LIT
KIIM 2.6	Brown TP, Fletcher OJ, Osuna O, Wyatt RD	1987	Microscopic and ultrastructural renal pathology of oosporein-induced toxicosis in broiler chicks. Avian Dis (31) 868-877 GLP: N, published: Y BVL No. 1690636	N	LIT
KIIM 2.6	Calo L, Fornelli F, Ramires R, Nenna S, Tursi A, Caiaffa MF, Macchia L	2004	Cytotoxic effects of the mycotoxin beauvericin to human cell lines of myeloid origin. Pharmacol Res (49) 73-77 GLP: N, published: Y BVL No. 1690637	N	LIT
KIIM 2.6	Calo L, Fornelli F, Ramires R, Nenna S, Tursi A, Caiaffa MF, Macchia L	2004	Erratum to "Cytotoxic effects of the mycotoxin beauvericin to human cell lines of myeloid origin" [Pharm. Res. 49 (1) 73-77]. Pharmacol Res (49) 299 BVL No. 1690642	N	LIT
KIIM 2.6	Chen BF, Tsai MC, Jow GM	2006	Induction of calcium influx from extracellular fluid by beauvericin in human leukemia cells. Biochem Biophys Res Commun (340) 134-139 GLP: N, published: Y BVL No. 1690640	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 2.6	Cole RJ, Kirksey JW, Cutler HG, Davis EE	1974	Toxic effects of oosporein from Chaetomium trilaterale. J Agric Food Chem (22) 517-520 GLP: N, published: Y BVL No. 1690644	N	LIT
KIIM 2.6	Cook JL, Routes BA, Walker TA, Colvin KL, Routes JM	1999	E1A oncogene induction of cellular susceptibility to killing by cytolytic lymphocytes through target cell sensitization to apoptotic injury. Exp Cell Res (251) 414-423 GLP: N, published: Y BVL No. 1690645	N	LIT
KIIM 2.6	Cook JL, Routes BA, Leu CY, Walker TA, Colvin KL	1999	E1A oncogene-induced cellular sensitization to immune-mediated apoptosis is independent of p53 and resistant to blockade by E1B 19 kDa protein. Exp Cell Res (252) 199-210 GLP: N, published: Y BVL No. 1690648	N	LIT
KIIM 2.6	Dombrink-Kurtzman MA	2003	Fumonisin and beauvericin induce apoptosis in turkey peripheral blood lymphocytes. Mycopathologia (156) 357-364 GLP: N, published: Y BVL No. 1690913	N	LIT
KIIM 2.6	EMEA (Commitee for veterinary medicinal products)	2003	Summary report: Oxalic acid. EMEA/MRL/891/03-FINAL GLP: N, published: Y BVL No. 1690914	N	LIT
KIIM 2.6	Favilla M, Macchia L, Gallo A, Altomare C	2006	Toxicity assessment of metabolites of fungal biocontrol agents using two different (Artemia salina and Daphnia magna) invertebrate bioassays. Food Chem Toxicol (44) 1922-1931 GLP: N, published: Y BVL No. 1690915	N	LIT
KIIM 2.6	Fotso J, Smith JS	2003	Evaluation of beauvericin toxicity with the bacterial bioluminescence assay and the Ames mutagenicity bioassay. Journal of Food Science (68) 1938-1941 GLP: N, published: Y BVL No. 1690916	N	LIT
KIIM 2.6	Harnois DM, Que FG, Celli A, LaRusso NF, Gores GJ	1997	Bcl-2 is overexpressed and alters the threshold for apoptosis in a cholangiocarcinoma cell line. Hepatology (26) 884-890 GLP: N, published: Y BVL No. 1690917	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 2.6	Ivanova L, Skjerve E, Eriksen GS, Uhlig S	2006	Cytotoxicity of enniatins A, A1, B, B1, B2 and B3 from Fusarium avenaceum. Toxicon (47) 868-876 GLP: N, published: Y BVL No. 1690918	N	LIT
KIIM 2.6	Jeffs LB, Khachatourians GG	1997	Toxic properties of Beauveria pigments on erythrocyte membranes. Toxicon (35) 1351-1356 GLP: N, published: Y BVL No. 1690919	N	LIT
KIIM 2.6	Jow GM, Chou CJ, Chen BF, Tsai JH	2004	Beauvericin induces cytotoxic effects in human acute lymphoblastic leukemia cells through cytochrome c release, caspase 3 activation: the causative role of calcium. Cancer Lett (216) 165-173 GLP: N, published: Y BVL No. 1690921	N	LIT
KIIM 2.6	Kamyar MR, Kouri K, Rawnduzi P, Studenik C, Lemmens-Gruber R	2006	Effects of moniliformin in presence of cyclo-hexadepsipeptides on isolated mammalian tissue and cells. Toxicol In Vitro (20) 1284-1291 GLP: N, published: Y BVL No. 1690629	N	LIT
KIIM 2.6	Klaric MS, Pepeljnjak S, Domijan AM, Petrik J	2007	Lipid peroxidation and glutathione levels in porcine kidney PK15 cells after individual and combined treatment with fumonisin B(1), beauvericin and ochratoxin A. Basic Clin Pharmacol Toxicol (100) 157-164 GLP: N, published: Y BVL No. 1690632	N	LIT
KIIM 2.6	Kouri K, Lemmens M, Lemmens-Gruber R	2003	Beauvericin-induced channels in ventricular myocytes and liposomes. Biochim Biophys Acta (1609) 203-210 GLP: N, published: Y BVL No. 1690634	N	LIT
KIIM 2.6	Kouri K, Duchen MR, Lemmens-Gruber R	2005	Effects of beauvericin on the metabolic state and ionic homeostasis of ventricular myocytes of the guinea pig. Chem Res Toxicol (18) 1661-1668 GLP: N, published: Y BVL No. 1690638	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 2.6	Krska R, Schuhmacher R, Grasserbauer M, Lemmens M, Lemmens-Gruber R, Adler A, Lew H	1997	Effects of beauvericin to mammalian tissue and its production by Austrian isolates of Fusarium proliferatum and Fusarium subglutinans. Mycotoxin Research (13) 11-16 GLP: N, published: Y BVL No. 1690639	N	LIT
KIIM 2.6	Lemmens-Gruber R, Rachoy B, Steininger E, Kouri K, Saleh P, Krska R, Josephs R, Lemmens M	2000	The effect of the Fusarium metabolite beauvericin on electromechanical and -physiological properties in isolated smooth and heart muscle preparations of guinea pigs. Mycopathologia (149) 5-12 GLP: N, published: Y BVL No. 1690643	N	LIT
KIIM 2.6	Lin HI, Lee YJ, Chen BF, Tsai MC, Lu JL, Chou CJ, Jow GM	2005	Involvement of Bcl-2 family, cytochrome c and caspase 3 in induction of apoptosis by beauvericin in human non-small cell lung cancer cells. Cancer Lett (230) 248-259 GLP: N, published: Y BVL No. 1690647	N	LIT
KIIM 2.6	Manning RO, Wyatt RD	1984	Comparative toxicity of Chaetomium contaminated corn and various chemical forms of oosporein in broiler chicks. Poult.Sci (63) 251-259 GLP: N, published: Y BVL No. 1690649	N	LIT
KIIM 2.6	Massini P, Naf U	1980	Ca2+ ionophores and the activation of human blood platelets. The effects of ionomycin, beauvericin, lysocellin, virginiamycin S, lasalocid-derivatives and McN 4308. Biochim Biophys Acta (598) 575-587 GLP: N, published: Y BVL No. 1690651	N	LIT
KIIM 2.6	Nakajyo S, Kometani A, Shimizu K, Urakawa N	1981	Effects of Bassianolide on Drug Induced Contractions of Isolated Guinea-Pig Aorta. Bulletin of the Nippon Veterinary and Animal Science University (30) 71-76 GLP: N, published: Y BVL No. 1690652	N	LIT

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KIIM 2.6	Nakajyo S, Shimizu K, Kometani A, Kato K, Kamizaki J, Isogai A, Urakawa N	1982	Inhibitory effect of bassianolide, a cyclodepsipeptide, on drug-induced contractions of isolated smooth muscle preparations. Jpn J Pharmacol (32) 55-64 GLP: N, published: Y BVL No. 1690653	N	LIT
KIIM 2.6	Nakajyo S, Shimizu K, Kometani A, Isogai A, Urakawa N	1982	The Effect of Bassianolide on A Nicotine Induced Contraction in Isolated Smooth and Skeletal Muscle Preparations. Bulletin of the Nippon Veterinary and Animal Science University (31) 33-39 GLP: N, published: Y BVL No. 1690654	N	LIT
KIIM 2.6	Nakajyo S, Shimizu K, Kometani A, Yuyama A, Kobayashi H, Suzuki A, Urakawa N	1982	Effects of Bassianolide on Muscarinic and Nicotinic Responses to Acetyl Choline in Various Tissue Preparations. Bulletin of the Nippon Veterinary and Animal Science University (31) 40-50 GLP: N, published: Y BVL No. 1690659	N	LIT
KIIM 2.6	Nakajyo S, Shimizu K, Kometani A, Suzuki A, Ozaki H, Urakawa N	1983	On the inhibitory mechanism of bassianolide, a cyclodepsipeptide, in acetylcholine-induced contraction in guinea-pig taenia coli. Jpn J Pharmacol (33) 573-582 GLP: N, published: Y BVL No. 1690661	N	LIT
KIIM 2.6	Nakajyo S, Shimizu K, Urakawa N	1984	Effects of Bassianolide on the Muscle Contraction Induced by Electrical Stimulation in the Guinea-Pig Hypogastric Nerve-Vas Deferens Preparation. Bulletin of the Nippon Veterinary and Animal Science University (33) 63-70 GLP: N, published: Y BVL No. 1690662	N	LIT
KIIM 2.6	Nakajyo S, Matsuoka K, Kitayama T, Yamamura Y, Shimizu K, Kimura M, Urakawa N	1987	Inhibitory effect of beauvericin on a high K+-induced tonic contraction in Guinea-Pig taenia coli. Jpn J Pharmacol (45) 317-325 GLP: N, published: Y BVL No. 1690671	N	LIT

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KIIM 2.6	Namatame I, Tomoda H, Si S, Yamaguchi Y, Masuma R, Omura S	1999	Beauveriolides, specific inhibitors of lipid droplet formation in mouse macrophages, produced by Beauveria sp. FO-6979. J Antibiot.(Tokyo) (52) 1-6 GLP: N, published: Y BVL No. 1690672	N	LIT
KIIM 2.6	Namatame I, Tomoda H, Ishibashi S, Omura S	2004	Antiatherogenic activity of fungal beauveriolides, inhibitors of lipid droplet accumulation in macrophages. Proc Natl Acad Sci U.S A (101) 737-742 GLP: N, published: Y BVL No. 1690673	N	LIT
KIIM 2.6	Norred WP, Plattner RD, Dombrink-Kurtzman MA, Meredith FI, Riley RT	1997	Mycotoxin-induced elevation of free sphingoid bases in precision-cut rat liver slices: specificity of the response and structure-activity relationships. Toxicol Appl Pharmacol (147) 63-70 GLP: N, published: Y BVL No. 1690674	N	LIT
KIIM 2.6	Ohshiro T, Namatame I, Nagai K, Sekiguchi T, Doi T, Takahashi T, Akasaka K, Rudel LL, Tomoda H, Omura, S	2006	Absolute stereochemistry of fungal beauveriolide III and ACAT inhibitory activity of four stereoisomers. J Org Chem (71) 7643-7649 GLP: N, published: Y BVL No. 1690676	N	LIT
KIIM 2.6	Ohshiro T, Namatame I, Nagai K, Sekiguchi T, Doi T, Takahashi T, Akasaka K, Rudel LL, Tomoda H, Omura, S	2006	Erratum to "Absolute stereochemistry of fungal beauveriolide III and ACAT inhibitory activity of four stereoisomers". J Org Chem (71) 9252 GLP: N, published: Y BVL No. 1690677	N	LIT
KIIM 2.6	Ohshiro T, Rudel LL, Omura S, Tomoda H	2007	Selectivity of microbial acyl-CoA: cholesterol acyltransferase inhibitors toward isozymes. J Antibiot.(Tokyo) (60) 43-51 GLP: N, published: Y BVL No. 1690681	N	LIT
KIIM 2.6	Ojcius DM, Zychlinsky A, Zheng LM, Young JD	1991	Ionophore-induced apoptosis: role of DNA fragmentation and calcium fluxes. Exp Cell Res (197) 43-49 GLP: N, published: Y BVL No. 1690685	N	LIT

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KIIM 2.6	Pegram RA, Wyatt RD	1979	Effect of dietary oosporein on broiler chicks. Poult.Sci (58) 1092 BVL No. 1690688	N	LIT
KIIM 2.6	Pegram RA, Wyatt RD	1981	Avian gout caused by oosporein, a mycotoxin produced by Caetomium trilaterale. Poult.Sci (60) 2429-2440 GLP: N, published: Y BVL No. 1690689	N	LIT
KIIM 2.6	Pegram RA, Wyatt RD, Smith TL	1982	Oosporein-toxicosis in the turkey poult. Avian Dis (26) 47-59 GLP: N, published: Y BVL No. 1690691	N	LIT
KIIM 2.6	Prince RC, Crofts AR, Steinrauf LK	1974	A comparison of beauvericin, enniatin and valinomycin as calcium transporting agents in liposomes and chromatophores. Biochem Biophys Res Commun (59) 697-703 GLP: N, published: Y BVL No. 1690693	N	LIT
KIIM 2.6	Que FG, Gores GJ, LaRusso NF	1997	Development and initial application of an in vitro model of apoptosis in rodent cholangio-cytes. Am J Physiol (272) G106-G115 GLP: N, published: Y BVL No. 1690694	N	LIT
KIIM 2.6	Roeske RW, Isaac S, King TE, Steinrauf LK	1974	The binding of barium and calcium ions by the antibiotic beauvericin. Biochem Biophys Res Commun (57) 554-561 GLP: N, published: Y BVL No. 1690696	N	LIT
KIIM 2.6	Strasser H, Vey A, Butt TM	2000	Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of Metarhizium, Tolypocladium and Beauveria species? Biocontrol Science and Technology (10) 717-735 GLP: N, published: Y BVL No. 1690697	N	LIT
KIIM 2.6	Suzuki A, Ka-naoka M, Isogai A, Murakoshi S, Ichinoe M, Tamura S	1977	Bassianolide - New Insecticidal Cyclodepsi-peptide from Beauveria-Bassiana and Verticil-lium-Lecanii. Tetrahedron Letters (25) 2167-2170 GLP: N, published: Y BVL No. 1690698	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 2.6	Tang CY, Chen YW, Jow GM, Chou CJ, Jeng CJ	2005	Beauvericin activates Ca2+-activated Cl- currents and induces cell deaths in Xenopus oocytes via influx of extracellular Ca2+. Chem Res Toxicol (18) 825-833 GLP: N, published: Y BVL No. 1690699	N	LIT
KIIM 2.6	Taniguchi M, Kawaguchi T, Tanaka T, Oi S	1984	Antimicrobial and Respiration-Inhibitory Activities of Oosporein. Agricultural and Biological Chemistry (48) 1065-1067 GLP: N, published: Y BVL No. 1690701	N	LIT
KIIM 2.6	Tomoda H, Huang XH, Cao J, Nishida H, Nagao R, Okuda S, Tanaka H, Omura S, Arai H, Inoue K	1992	Inhibition of acyl-CoA: cholesterol acyltransferase activity by cyclodepsipeptide antibiotics. J Antibiot.(Tokyo) (45) 1626-1632 GLP: N, published: Y BVL No. 1690702	N	LIT
KIIM 2.6	Wu SN, Chen H, Liu YC, Chiang HT	2002	Block of L-type Ca2+ current by beauvericin, a toxic cyclopeptide, in the NG108-15 neuronal cell line. Chem Res Toxicol (15) 854-860 GLP: N, published: Y BVL No. 1690703	N	LIT
KIIM 2.6	Zhan J, Burns AM, Liu MX, Faeth SH, Gunatilaka AA	2007	Search for cell motility and angiogenesis inhibitors with potential anticancer activity: beauvericin and other constituents of two endophytic strains of Fusarium oxysporum. J Nat Prod (70) 227-232 GLP: N, published: Y BVL No. 1690706	N	LIT
KIIM 2.6 (OECD)	Strasser, H; Vey, A. and Butt, T.M.	2000	Are there any Risks in using Entomopathogenic Fungi for pest Control, with Particular Reference to the Bioactive Metabolites of Metarhizium, Tolypocladium and Beauveria species? Biocontrol Science and Technology (10) , 717-735 N/a GLP: N, published: J 1300945 / BWS2006-66	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 2.7.2 (OECD)	Isaka, M.; Kittakoop, P.; Kirtikara, K.; Hywel-Jones, N.; Thebtaranonth, Y.	2005	Bioactive substances from insect pathogenic fungi National Center for Genetic Engineering and Biotechnology, Thailand , GLP: N, published: J 1677998 /	N	LIT
KIIM 2.7.2 (OECD)	Seger, C.; Erlebach, D.; Stuppner, H.; Griesser,, U.; Strasser, H.	2005	Physicochemical properties of oosporein, the major secreted metabolite of the entomopathogenic fungus beauveria brongniartii Helvetica Chimica Acta 88, 802-810 GLP: N, published: Y 1678001 /	N	LIT
KIIM 2.7.2 (OECD)	Längle, T.	2007	A biocontrol agent with more than 100 years of history of safe use Agriculture and Agri_Food Canada , GLP: N, published: Y 1678002 /	N	LIT
KIIM 2.7.2 (OECD)	Quesada-Moraga, E.; Vey, A.	2004	Bassiacridin, a protein toxic for locusts secreted by the entomopathogenic fungus beauveria bassiana Mycol.Res. 108 (4) , 441-452 GLP: N, published: Y 1678003 /	N	LIT
KIIM 2.7.2 (OECD)	Quesada-Moraga, E.; Vey, A.	2003	Intra-specific variation in virulence and in vitro production of macromolecular toxins active against locust among beauveria bassiana strains and effects of in vivo and in vitro passage on these factors Biocontrol Science and Technology 13, 323-340 GLP: N, published: Y 1678004 /	N	LIT
KIIM 2.7.2 (OECD)	Wat, C.; McInnes, A.G.; Smith, D.	1977	The yellow pigments of beauveria species. Structures of tenellin and bassianin J. Chem 55, 4090-4098 GLP: N, published: Y 1678006 /	N	LIT
KIIM 2.7.2 (OECD)	Kikuchi, H.; Takashani, N.; Oshima, Y.	2004	Novel aromatics bearing 4-O-methylglucose unit isolated from the oriental crude drug bombyx batryticatus Tetrahedron Letters 45, 367-370 GLP: N, published: Y 1678051 /	N	LIT

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KIIM 2.7.2 (OECD)	Champlin, F.R.; Grula, E.A.	1979	Noninvolvement of beauvericin in the entomopathogenicity of beauveria bassiana Applied and Environmental Microbiology , 1122-1125 GLP: N, published: J 1678057 /	N	LIT
KIIM 2.8 (OECD)	Jaronski,S.T. and Britton, J.A.	1993	Effect of pH and metal ions on Beauveria bassiana strain GHA in aqueous suspension , 93-025 GLP: J, published: N 1300947 / BWS2006-68	Y	LAM
KIIM 2.8 (OECD)	Jaronski S.T.	1993	TGAI Physical stability study of Beauveria bassiana strain GHA: sunlight effects. 93-001 GLP: J, published: N 1300948 / BWS2006-67	Y	LAM
KIIM 2.8 (OECD)	Jaronski, S.T.	1993	TGAI Physican stability study of Beauveria bassiana strain GHA: temperature extremes 93-002 GLP: N, published: N 1300950 / BWS2006-62	Y	LAM
KIIIM1 2.1 (OECD) KIIIM1 2.3.3	Jaronski, S. T. and Jaronski, S.	1997	Physical and Chemical Properties of Mycotrol WP/BotaniGard WP9616B (Wettable Powder) (Beauveria bassaiana Strain GHA): Color, Odor, Physical State, Bulk Density, and pH 96-05a GLP: J, published: N 1301007 / PHY2006-355	Y	LAM
KIIIM1 2.2 (OECD)	Anonymous	2000	Effect of temperature on the Shelf - life of BotaniGard emulsionsfiable suspension (code ES9601) and Botani Gard 22 WP (Code WP9616B) N/A GLP: J, published: N 1301008 / PHY2006-480	Y	LAM
KIIIM1 2.2 (OECD)	Chatriand, G.	2006	Eight-lot analysis of BotaniGard 22WPshelf life stability GLP: N, published: N 1677991 /	J	MEU
KIIIM1 2.2 (OECD)	EPA	2004	40 CFR Ch. I (7-1-04 Edition) Part 180 - Tolerances and exemptions for pesticide chemicals in food EPA , 300-318 GLP: N, published: J 1678070 /	N	LIT

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KIIIM1 2.2 (OECD)	EPA	2004	U.S. Environmental protection agency - Office of pesticide programs - List of inert pesticide ingredients - List 4A - Minimal risk inert ingredients EPA , GLP: N, published: J 1678074 /	N	LIT
KIIIM1 2.2 (OECD)	EPA	2004	U.S. Environmental protection agency - Office of pesticide programs - List of inert pesticide ingredients List 4B - Minimal risk inert ingredients EPA , GLP: N, published: J 1678077 /	N	LIT
KIIIM1 2.2 (OECD)	EPA	2006	Inerts reassesment: five exemptions from the requirement of a tolerance for petroleum hydrcarbons EPA , GLP: N, published: J 1678081 /	N	LIT
KIIIM1 2.3.1 (OECD)	Anonymous	2006	MSDS for BotaniGard 22 WP Laverlahm International Corp. , GLP: N, published: J 1678085 /	N	LIT
KIIIM1 2.4.2 (OECD)	Foster, B.	2006	Analysis of Botaniguard 22WP for Persistent foam CEMR-3034 GLP: J, published: N 1301011 / PHY2006-758	Y	LAM
KIIIM1 2.4.3 KIIIM1 2.4.4 KIIIM1 2.4.5 (OECD)	Kang, J.	2005	Analysis of Botaniguard 22WP CEMR-2173 GLP: J, published: N 1301012 / PHY2006-357	Y	LAM

Codes of owner

LAM Laverlam International Corporation
LIT Literature
MEU Mycotech Europe Ltd.

Annex B

Beauveria bassiana GHA

B-3: Data on application and further data

B.3 Data on application and further information

B.3.1 Further information on the micro-organism (OECD IIM 3)

B.3.1.1 Function (OECD IIM 3.1)

Fungal contact pathogen**,** functioning as a microbiological insecticide. The formulated products can be an important part of an IPM programme.

B.3.1.2 Field of use envisaged (OECD IIM 3.3)

Horticulture, protected crops, greenhouse use.

B.3.1.3 Information on target crop and target organism(s) (OECD IIM 3.4)

Different horticultural greenhouse crops, where whiteflies (Aleyrodidae), thrips (Thysanoptera), and aphids (Aphididae) occur as pests.

B.3.1.4 Information on the occurrence or possible occurrence of the development of resistance of the target organism(s) (OECD IIM 3.6)

So far no hints on the occurrence of resistance to *Beauveria bassiana* in the target pests exist.

B.3.1.5 Method of production and quality control (OECD IIM 1.4.3)

Confidential information, see Volume 4/Annex C.

B.3.1.6 Methods to prevent loss of virulence of seed stock of the micro-organism (OECD IIM 4.1)

The seed culture is replenished once per year. The purity/viability is checked by bioassay and/or DNA fingerprinting. The strain is also deposited at ATCC and could be retrieved from there.

Confidential information, see Volume 4/Annex C.

B.3.1.7 Recommended methods and precautions concerning handling, storage, transport or fire (OECD IIM 3.7)

Reference:
Anonymous (2005), Material safety data sheet *Beauveria bassiana* GHA, MEU, (CHE2006-583)

B.3.1.7.1 Handling

Information on safe handling:	Keep out of reach of children and away from food for human and animal consume.
Advice on protection against fire and explosion	Not applicable, but maintain from fire materials.

Personal Precautions:

Personal protective equipment:	Wear protective equipment including waterproof gloves, coveralls, over long-sleeved shirt, long pants and an approved dust musk.
General protective measures:	Avoid contact with eyes and skin.
Hand protection:	Waterproof gloves
Eye protection:	Wear goggles, glasses with side shields, or face shield to protect eyes.
Applicable exposure limits:	There are no known exposure limits applicable to this product.

Information on stability and reactivity:

Stability:	This product is stable at ambient temperature. It is not self-reactive.
Thermal decomposition:	The only compound thermally unstable is the fungus *Beauveria bassiana* which dies or inhibits his growth at temperatures higher than 35 °C.
Incompatibility:	Acids, bases, oxidising chemicals, disinfectants, and biocides may inactivate the product.
Polymerisation:	Hazardous polymerisation will not occur.

B.3.1.7.2 Storage

General information:	Do not store this material with chemical pesticides. Do not store this material near food, feed or drinking water.
Storage conditions	Do not store above 15 °C (Freezing is acceptable). Protect from sunlight. Store in a cool, dry location away from food.

B.3.1.7.3 Transport

Land transport ADR/RID:	Not classified.
Maritime transport IMDG:	Not classified.
Air transport IATA:	Not classified.

B.3.1.7.4 Fire fighting measures

Flammable limits:	Upper explosion / lower explosion limit: not known.
Suitable extinguishing media:	Dry chemical, foam and/or water fog, or carbon dioxide extinguishing agents.
Personal protective equipment:	Full protective equipment and self-contained breathing apparatus are recommended.
Unusual fire and explosion hazards:	None.
Special fire fighting procedures:	None.

B.3.1.8 Procedures for destruction or decontamination (OECD IIM 3.8)

There is no readily available method for decontamination of water for this microbial product. Antimicrobial disinfectants would be marginally effective, even at full strength, and the water would dilute them. Based on the submitted ecological effects studies, minimal adverse ecological effects would be anticipated from contamination of water by *Beauveria bassiana* strain GHA. Furthermore, it has been shown that *B. bassiana* degrades quite rapidly under natural conditions, in the absence of optimal environmental conditions and a viable host.

B.3.1.9 Measures in case of an accident (OECD IIM 3.9 and OECD IIM 3.12)

First-aid measures:

General information:	In case of an accident or if you feel unwell, seek medical advice immediately.
After eye contact:	Immediately flush eyes with water for at least five minutes. Get medical attention if irritation persists.
After skin contact:	Wash off immediately with soap and water.
After inhalation:	No known problems for inhalation.
After ingestion:	If swallowed, give several large glasses of water to dilute. Do not induce vomiting. Seek medical advice immediately.

Accidental release measure:

General information:	If accidental spilled, isolate the spill area and restrict entry to the area by non-essential personnel. Wearing appropriate protective equipment, sweep the spilled material into a properly labelled container. Wash the contaminated surface with water and flush residual material into sewer system. Do not wash residue into surface water system (i. e. lake, river, stream). Dispose of the container according to directions on product label.

B.3.2 Data on application (OECD IIIM 3)

B.3.2.1 Field of use envisaged (OECD IIIM 1.6.1)

Control of insects on horticultural greenhouse crops.

B.3.2.2 Mode of action (OECD IIIM 3.1)

Fungal contact pathogen**,** functioning as a microbiological insecticide. The conidia of *Beauveria bassiana* adhere to the insect cuticle by means of hydrophobic interaction between the spore wall and epicuticle lipids. The conidia germinate, and the germ tube penetrates the cuticle, using a specific series of enzymes, which in turn degrade the lipids, protein and chitin in the insect cuticle. No toxins or toxic metabolites are produced; it is purely a physical attack. In the insect body, the fungus replicates in the haemocœl as a blastospore, or yeast-like cell, and enzymes begin to destroy the internal structures of the host insect causing morbidity within 36 - 72 hours. Reduced feeding and immobility are rapidly evident, and the insect dies within between 4 to 10 days post-infection. The time to death will depend on the conidial dose and the physical condition of the insect. After death, the blastospores transform into mycelia, which emerge through the cuticle and form spores. These cover the cadaver as a characteristic white growth. Sporulation occurs only in conditions of high humidity. At low humidity the fungal mycelia die within the insect cadaver. This completes the infection process and life-cycle of *Beauveria bassiana*.

B.3.2.3 Details of intended uses (OECD IIIM 3.1)

BotaniGard 22WP is intended for the control of insect pests such as whiteflies (Aleyrodidae), with some activity on thrips (Thysanoptera), and aphids (Aphididae) in protected vegetable and ornamental crops. Whiteflies, thrips, and aphids are major pest of greenhouse crops and cause mostly severe damage to plants if not controlled. Some of the pest species act also as virus vectors.

B.3.2.4 Application rate (OECD IIIM 3.3 - 3.4)

Minimum application rate: 0.014 kg as/ha (0.063 kg product/ha).
Maximum application rate: 0.121 kg as/ha (0.550 kg product/ha).

BotaniGard 22WP contains 4.4 x 10^{13} CFU/kg.

B.3.2.5 Content of the micro-organism in material used (e.g. in the diluted spray, baits or treated seed) (OECD IIIM 3.3)

0.14 - 4.84 x 10^{10} *Beauveria bassiana* CFU/L spray solution.

B.3.2.6 Method of application (OECD IIIM 3.5)

The product is applied by spraying, typically by an air-assisted knapsack sprayer at low pressure and using a Tee-Jet cone or fan nozzle. The product is mixed with 500 – 2000 litres of water/ha.

B.3.2.7 Number and timing of applications (OECD IIIM 3.6)

Number of applications: 3 to 5.
Interval between applications: 5 to 7 days.
Conditions of applications: BotaniGard 22WP should be applied when pest populations are manageable and before extensive damage occurs to crops. It should not be tank mixed with fungicides.

B.3.2.8 Necessary waiting periods or other precautions to avoid phytopathogenic effects on succeeding crops (OECD IIIM 3.7)

Beauveria bassiana strain GHA is not a plant pathogen nor is it related to any known plant pathogens. Consequently, no specific precautions are recommended for use on protected or succeeding crops.

B.3.2.9 Proposed instructions for use (OECD IIIM 3.8)

Copies of all available labels are provided in Document C.

B.3.3 Summary of data on application

Summary of uses supported by available data (*Beauveria bassiana* strain GHA)

Crop and/or situation (a)	Member State or Country	Product name	F, G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (l)	Remarks (m)
					Type (d-f)	Conc. of MPCA (i)	Method Kind (f-h)	Growth stage & season (j)	Number min max (k)	Interval between applica-tions (min)	Kg MPCA/hL	water L/ha min max	Kg MPCA/ha Viable granules min max		
Ornamentals	Northern and Southern Europe	BotaniGard 22 WP	I	*Sucking insects*	WP	220 g/kg 4.4 x 10^{13} CFU/kg	High volume Spraying	Maturity (BBCH89)	3 - 5	5-7 days	0.14 - 4.84 x 10^{12} CFU	500 - 2000	0.014 – 0.121 // 0.28 - 2.42 x 10^{13} CFU	0	MPCP 0.0625-0.55 Kg/ha
Tomatoes, Cucumbers	Northern and Southern Europe	BotaniGard 22 WP	I	*Sucking insects*	WP	220 g/kg 4.4 x 10^{13} CFU/kg	High volume Spraying	Ripeness (BBCH89)	3 - 5	5-7 days	0.14 - 4.84 x 10^{12} CFU	500 - 2000	0.014 – 0.121 // 0.28 - 2.42 x 10^{13} CFU	0	MPCP 0.0625-0.55 Kg/ha

(a) For crops, the EU and codex classifications (both) should be used; where relevant, the situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) e.g. biting and sucking insects, soil born insects, foliar fungi, weeds
(d) e.g. wettable powder (WP), emusifiable concentrate (EC), granule (GR)
(e) GCPF codes - Crop Life Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, spreading, dusting, drench
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plant - type of equipment used must be indicated
(i) viable granules = colony forming units and g/kg or g/L
(j) Growth stage at last treatment (BBCH Monograph, growth stages of Plants, 1997, Blackwell ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) Indicate the minimum and maximum number of application possible under practical conditions of use
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

B.3.4 Further information on the plant protection product (OECD IIIM 4)

B.3.1.1 Packaging and compatibility of the preparation with proposed packaging materials (OECD IIIM 4.1 - 4.2)

Packaging description:
BotaniGard 22WP is packaged in 1 litre HDPE bottles fitted with a PE screw cap. The label is affixed directly to the packaging:

Bottle capacity:	64 oz
Bottle dimensions:	5 x 5 x 6.5 inch
Opening size:	3 ⅛ inch
Materials:	HDPE
Closure:	White screw on
Seal type:	Induction
Box:	20 ⅞ x 15 ⅞ x 7 ⅛ inch single wall 44 lb test

Specifications of packaging and measures of its suitability:
BotaniGard 22WP will be packaged in polyethylene packs, precluding the possibility of corrosion.

B.3.4.2 Procedures to clean equipment and protective clothing; measures of their effectiveness (OECD IIIM 4.3 - 4.4)

Label instructions: cleaning equipment and protective clothing
Remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing. Remove PPE immediately after handling the product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

Procedures to clean equipment and protective clothing; measures of their effectiveness
The application equipment (tank, filters, boom and nozzles) must be thoroughly washed with water immediately after use following the sprayer manufacturer's procedure. Following this standard rinsing procedure, the anticipated low levels of BotaniGard 22WP in the tank do not present any particular risk for crops to be treated with the next product.

B.3.4.3 Re-entry periods, necessary waiting periods or other precautions to protect man, livestock and the environment (OECD IIIM 4.5)

No recommandations are possible at this moment because further data and information are necessary (Annex IIM, point 5.3: repeated dose (subacute) study with inhalative exposure; Annex IIM, point 1.4.4: evaluation of the five batch analysis from the last 10 years in consideration to the beauvericin levels in the active substance)

B.3.4.4 Recommended methods and precautions concerning: handling, storage, transport or fire (OECD IIIM 4.6 - 4.7)

Reference:
Document MIII

B.3.4.4.1 Storage and Handling

General:	The product should be stored in a cool, dry place away from direct sunlight or excessive heat. Optimum storage temperatures are 4 °C to 27 °C. The spray solution should not be allowed to stand for more than 4 hours before use. Contamination and loss of potency of opened containers should be avoided by closing containers tightly after use. It is suggested that opened containers be used in their entirety when possible.
Advice on safe handling:	Use in the open air or well ventilated areas. Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove clothing immediately if pesticide gets inside. Clean clothing thoroughly. Do not reuse the empty container.
Disposal procedures:	Triple rinse (or equivalent), puncture and dispose of in a sanitary landfill, or incinerate if allowed by authorities. Alternatively, the empty container may be returned to the manufacturer at the address listed on the product label. Wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility.

B.3.4.4.2 Transport

There are no specific precautions relating to transport.

B.3.4.4.3 Fire

Suitable extinguishing media:	water, carbon dioxide, dry chemical, foam
Extinguishing media that must be not used for safety reasons:	not known
Special protective equipment for firefighting:	Full protection by suitable clothing and positive pressure, self contained breathing apparatus.

B.3.4.5 Measures in case of an accident (OECD IIIM 4.8 - 4.9)

Environment protection measures:	Do not contaminate water by cleaning of equipment or disposal of equipment wash waters. Do not discharge into lakes, streams, ponds or public waterways.

B.3.4.5.1 Cleanup of spillage

Remove persons not wearing protective clothing from spill area. Contain and ventilate spill area. Avoid contamination of streams and sewers. Absorb onto clay or other absorbent material.

B.3.4.5.2 Protection of emergency workers and bystanders

Impervious clothing to minimise contact, long-sleeved shirt and long trousers. Shoes and socks must be worn. Dust/mist NIOSH approved filtering respirator R95, rubber/chemical resistant gloves, goggles/glasses with side shields.

B.3.4.5.3 First aid measures

After eye contact:	Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after 5 minutes, then continue rinsing. Call a poison control centre or doctor for further treatment.
After skin or clothing contact:	Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control centre or doctor for treatment advice.
After swallowing:	Immediately call a poison control centre or doctor. Do not induce vomiting unless told to do so by a poison control centre or doctor. Do not consume any liquid. Do not orally administer anything to an unconscious person.
After inhalation:	Potential sensitising agent. If the person is not breathing, call emergency services, then give artificial respiration (preferably mouth-to-mouth if possible).

B.3.4.6 Procedures for destruction or decontamination of the plant protection product and its packaging (OECD IIIM 4.10)

There is no readily available method for decontamination of water for this microbial product. Antimicrobial disinfectants would be marginally effective, even at full strength, and the water would dilute them. Based on the submitted ecological effects studies, minimal adverse ecological effects would be anticipated from contamination of water by *Beauveria bassiana* Strain GHA. Furthermore, it has been shown that *B. bassiana* degrades quite rapidly under natural conditions, in the absence of optimal environmental conditions and a viable host.

Controlled incineration
Disposal by controlled incineration is recommended. Unused material may be returned to the manufacturer at the address listed on the product label.

Methods other than controlled incineration
Unused material may be returned to the manufacturer at the address listed on the product label.

B.3.5 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 3.7 (OECD)	Anonymous	2005	MSDS: Beauveria bassiana Strain GHA GLP: N, published: N 1300951 / CHE2006-583	Y	MEU

Codes of owner
MEU Mycotech Europe Ltd.

[1] Only notifier listed

Annex B

Beauveria bassiana GHA

B-4: Proposals for the classification and labelling

B.4 Proposals for classification and labelling

B.4.1 Proposals for the classification and labelling of the micro-organism

Based on the observations in humans and on studies in laboratory animals when using the intratracheal/inhalatory route, a sensitising potential of *Beauveria bassiana* strain GHA at least by inhalation must be assumed. Furthermore, the only available study for skin sensitisation (*i.e.*, a Buehler test with three topical inductions), in spite of its negative outcome, is not considered sufficient to exclude skin sensitising properties. Accordingly, the following classification and labelling for the micro-organism is proposed:

Hazard symbol(s)	Xn	
Indications of danger	Harmful	
Risk phrase(s)	R 42	May cause sensitisation by inhalation
	R 43	May cause sensitisation by skin contact

B.4.2 Proposals for the classification and labelling of the plant protection product

Based on the observations in humans and on studies in laboratory animals when using the intratracheal/inhalatory route, a sensitising potential of *Beauveria bassiana* strain GHA at least by inhalation must be assumed. Furthermore, the only available study for skin sensitisation of *Beauveria bassiana* strain GHA is not considered sufficient to exclude skin sensitising properties. Accordingly, classification and labelling of the micro-organism with Xn, R42/43 is proposed (see B.4.1).

In accordance with Directives 67/548/EEC and 1999/45/EC and in the absence of a dermal sensitisation test with the plant protection product, the following classification/labelling requirements are derived for BotaniGard 22 WP:

Hazard symbol(s)	Xn	
Indications of danger	Harmful	
Risk phrase(s)	R 42	May cause sensitisation by inhalation
	R 43	May cause sensitisation by skin contact

B.4.3 References relied on

No references submitted.

Annex B

Beauveria bassiana GHA

B-5: Methods of analysis

B.5 Analytical methods

B.5.1 Methods for the analysis of the micro-organism as manufactured (OECD IIM 4.3 - 4.4)

B.5.1.1 Methods for the identification of the micro-organism

Identification of *B. bassiana* in technical product is determined by Restriction Fragment Length Polymorphism (RFLP) techniques, morphological examination and classification according to a taxonomic key. Since 2003 newer updated techniques have been developed using random amplified polymorphic DNA (RAPD) analysis.

Reference:
Jaronski, S.T., Butler, E. and Kahler, A. (1993): *Beauveria bassiana* strain GHA cDNA library construction and RFLP analysis, (BWS 2006-54)

Method:

Preparation of the samples:

All *B. bassiana* isolates (i.e. ARSEF201 (parent strain), GHA (Master used in production), 3 cultures from single spore isolations of GHA Master (GHA1f1, GHA3f1, GHA4f1), GHA-GH (passed through Orthoptera), GMB6A (deliberately mislabelled to serve as a blind control) and GMB6B (another Mycotech isolate of *B. bassiana*)) were cultured in CSYE medium. Inoculation was from agar slant cultures. Cultures were incubated at 24 - 28 °C for 3 days. Checks for contamination were performed at this stage. Secondary cultures were provided with a sterile air supply and incubated for 10 days at 25 °C. Each culture was harvested by *in vacuo* filtration of the mycelium and blastospores or by centrifugation for GHA master and GHA-GH. Culture filtrate were transferred into 50 mL sterile centrifuge tubes and frozen. These samples were then prepared for RFLP analyses. Cells were lyophilised.

cDNA library construction:

The cDNA library was constructed with poly(A+)-RNA that was purified from the *B. bassiana* GHA Master culture. The *B. bassiana* GHA total cellular RNA was extracted following a number of centrifugation and precipitation cycles in the appropriate medium. Incubation of this GHA total RNA was conducted in the presence of biotinylated oligo (dT) which allows poly(A+)-RNA to be captured on paramagnetic beads. Poly(A+)-RNA was then purified using a series of washes. Clones of GHA cDNA were then prepared from poly(A+)-RNA.

Hybridisation probes were also prepared using the polymerase chain reaction technique (PCR) to obtain GHA cDNA inserts and amplify the GHA cDNAs from crude lysates of colonies. Aliquots of PCR reaction mixtures were analysed *via* electrophoresis (1.5 % agarose gel). 20 GHA cDNA sequences were chosen and purified to be probes.

Determination of the length of fragment

Total cellular DNA was purified from each of the eight *B. bassiana* cultures provided. Lyophilised cells were ground with glass beads and dispersed in cationic detergent solution. Cells were then incubated at 65 °C for 60 minutes. The suspensions were extracted and centrifuged. Crude RNAs were obtained by isopropanol precipitation of the clarified lysates. Each DNA sample, dissolved in a buffer was sedimented and then recovered to be purified. Yields of DNA in each sample were determined by fluorometry.

Aliquots of each DNA samples were digested with enzymes (Cla I, EcoRI, Eco Rv, Hind III, Pvu II, and Xhu I) and eluted using a gel electrophoresis. Each set was flanked by molecular weight marker fragments. Hybridisations were conducted at 63 - 65 °C in the solution specified by Budwole and Baechtel. Radiography was then performed with Kodak XAR or XRP.

Findings:

It was concluded that GHA strains were consistent within single spore isolations of the parent strain and after passage through an orthopteran host. The method differentiates strain GHA from at least one other *B. bassiana* strain.

Reference:
Castrillo *et al.* (2003), Strain –specific detection of introduced *Beauveria bassiana* in agricultural fields by use of sequence-characterized amplified region markers

RAPD
Since 2003, newer updated techniques have been developed to detect *B. bassiana* strain GHA. Using random amplified polymorphic DNA (RAPD) analysis, unique fragments that distinguished GHA from other strains of *B. bassiana* have been obtained. Three amplicons, OPA-$14_{0.44}$, OPA-$15_{0.44}$, and OPB-$9_{0.67}$, generated with RAPD primers were cloned and sequenced and used as bases for designing SCAR primers OPA14 F/R_{445}, OPA15 F/R_{441}, and OPB9 F/R_{677}, respectively. All three SCAR primers were highly sensitive, capable of detecting 100 pg *B. bassiana* strain GHA genomic DNA.

Reference:
Jaronski, S.T. (1993): Characterization of *Beauveria bassiana* (SOP #3.5; Edition #01) and including sample characterizations, (CHE 2006-750)

Taxonomic key:

Colony characteristics:
On agar medium, colonies are white to off-white, beige or very slightly pinkish and powdery in appearance.

Morphological characteristics:
Strains are virtually indistinguishable. Conidiogenous cells are elongated. Conidia are round and conidiophores round to flask-shaped in dense clusters. Conidiophores may also be broad, arising scattered or in irregular clusters on swollen stalk cells.

B.5.1.2 Methods for providing information on possible variability of seed stock/active micro-organism

Identity of the reisolate can be confirmed by RFLP/RAPD and by taxonomic identification. A set of replicate master cultures are deposited at the ATCC Patent Deposit and could be retrieved from there.

Further details on the methodology used are described under point B.5.1.1 and in Volume 4/Annex C under point C.1.1.1.

B.5.1.3 Methods to differentiate a mutant of the micro-organism from the parent wild strain

B. bassiana strain GHA is not a mutant strain.

B.5.1.4 Methods for the establishment of purity of seed stock from which batches are produced and methods to control that purity

B. bassiana strain GHA can be identified by morphological examination and RFLP/RAPD techniques and also the microbial contaminants are determined.

Further details on the methodology used are described under point B.5.1.1 and in Volume 4/Annex C under point C.1.1.1.

B.5.1.5 Methods to determine the content of the micro-organism in the manufactured material used for the production of formulated products and methods to show that contaminating micro-organisms are controlled to an acceptable level

Reference:
Jaronski, S.T. (1993): Fungal spore counts (SOP #03-02;Edition #02) (BWS 2006-55)

The number of fungal spores/g of *Beauveria bassiana* strain GHA was determined by enumeration from serial dilution. The number of spores in each sample was then determined using a haemacytometer.

Method to enumerate the micro-organism

The number of fungal spores/g of *B. bassiana* GHA as a powder was determined by dissolving 0.1 g of sample into 9.9 mL of Tween 80 % (0.1 %) or 0.04 % aqueous Silwet L-77. A ten thousand-fold serial dilution (1:10000 in 10 mL volumes) in water was made. The theoretical spore concentration was expected to be 1×10^{11} spores/g. The number of spores in each sample was then determined using a hemacytometer under 400 x magnification. The total organism count per gram of samples is calculated by the average number per plate times the reciprocal of the dilution factor.

Methods to show that contaminating micro-organisms are controlled to an acceptable level are described in Volume 4/Annex C point C.1.1.2 and C.1.2.1.

B.5.1.6 Methods for the determination of relevant impurities in the manufactured material

beauvericin and bassianolide

A specified maximum content of 5 mg/kg is proposed by the RMS. The submitted method is not sufficiently validated for this low level.

Reference:

Jaronski, S.T.; Becarra M. and Sears, J. (1995), Analysis of Mycotrol GH TGAI (Beauveria bassiana strain GHA Conditional Powders) for the metabolites Beauvericin and Bassianolide, 94-009, LAM, (CHE2006-752)

Becerra, M.A. (1995), Extraction procedure, SOP # 3.8, LAM, (CHE2006-767)

Principle of the method:

Spore extraction was carried out by sonicating in methanol followed by a clean up using solid phase extraction (C-18 SPE tubes) using methanol as elution phase. After the extracts were blown to dryness and redissolved in 200 µL acetonitrile:water:acetic acid (50:50:1) containing 1 mM each of sodium iodide and potassium bromide the analyses were performed by electrospray mass spectrometry (accuracy: better than 2 amu) using external standard calibration.

Findings:

Table B.5.1-1 Validation of the HPLC method for determination of beauvericin :

Impurity		Specificity/ interferences	Linearity (R^2) (conc. range)	Accuracy (n = 4)		Repeatability (% RSD) (n = 4)
				fortification level (mg/kg)	mean recovery (%)	
1	beauvericin	demonstrated; no interferences	0.983 (250-2500 ppb)	30	75	14 (too high according to Horwitz equation)

The specificity of the method was demonstrated by electrospray mass spectrometry. The identity of the impurities has been confirmed by electrospray mass spectrometry.

Conclusion:

The method is not fully validated. The LOQ is too high. In addition, the linearity must be shown by either duplicate determinations at three or by single determinations at 5 or more concentrations.

The repeatability must be determined for 5 replicate sample determinations for each analyte. Moreover, the value for the repeatability is too high according to the Horwitz equation.

B.5.1.7 Methods to control the absence and to quantify (with appropriate limits of determination) the possible presence of any human and mammalian pathogen

Reference:
Jaronski, S.T. (1993): Enumeration of Microbial Contaminants in Beauveria bassiana TGAI (03-06; Edition 01) (CHE 2006-784)

Crase, C. (2006): Enumeration of microbial contaminants in whole culture, TGAI, and formulated product

B. bassiana strain GHA (whole culture or technical grade material) is analysed to determine if the following enteric pathogens are present: total presumptive Enteriobacteriaceae, *Vibrio sp.*, *Salmonella sp.*, and *Shigella sp.*.
B. bassiana strain GHA cultures were prepared onto SDAY + RB (Sabouraud Dextrose plus Yeast Extract Agar (SDAY) with Rose Bengal) for fungi enumeration, TSA (Trypticase Soy Agar for total bacteria) and XLD (Xylose-Lysin-Desoxycholat) agar plates for enumeration of enterics. Control isolates of pathogens were plated onto selective media for quality control. *Escherichia coli* and *Proteus mirabilis* positive controls were plated onto XLD agar. Salmonella-Shigella (SS) agar is used to confirm the presence of *Salmonella* and *Shigella* which form colourless colonies. TCBS (Thiosulfate-Citrate-Bile-salts-Sucrose Agar) plates are used for the detection of *Vibrio*.

All plates are incubated at 37 °C in the dark. Plates are read after 18 - 24 hours Any colonies appearing to be *Shigella, Salmonella* or other enterics were subcultured on TSA plates for pure colony isolation and in the API 20E test strip to verify the identity. Following incubation, appearance of colonies was examined. The presence of *Vibrio*, in very small amounts, was confirmed by preparing gram-stained microscope slides.

B.5.1.8 Methods to determine storage stability, shelf-life of the micro-organism, if appropriate

Additional data not required according to Directive 91/414/EEC.

Storage stability of *Beauveria bassiana* strain GHA technical grade (Mycotrol TGAI) was determined using the following tests:

- 56-day stability over temperature extremes (5 - 50 °C)
- Stability to pH and metal ions (1 hour at 23 °C)
- Stability to sunlight (24 hour artificial light)

56-day stability over temperature extremes (5 - 50 °C)

Reference:
Jaronski, S.T. (1993), TGAI physical stability study of *Beauveria bassiana* Strain GHA: temperature extremes, (CHE2006-755)

Principle of the method:
For each temperature regime and sampling, 0.5 g of *B. bassiana* strain GHA was placed into a sterile, 20 mL, glass, screw cap vial, for a total of 21 vials. Another 0.5 g of *Beauveria*

bassiana strain GHA were reserved for an initial viability test. Seven vials were placed into an incubator set at 25 °C. Another seven vials were placed at 50 °C and the remaining seven vials of the test substance were placed in a refrigerator operating at 5 °C. One vial each was removed from the 5 °C, the 25 °C and the 50 °C stations 7, 14, 21, 28, 42 and 56 days after study initiation. Viability determinations were made by plating dilute aqueous suspensions of *B. bassiana* strain GHA on Sabouraud Dextrose Yeast Agar and incubating the plates overnight before microscopic examination at 400x and phase contrast illumination.

Findings:
B. bassiana strain GHA did not suffer any loss in viability during 56 days of incubation at 5 and 25 °C. At 50 °C, *Beauveria* conidia rapidly lost viability. After 28 days viability was less than 1 % at 50 °C. *B. bassiana* strain GHA was found to be unstable at 50 ± 2 °C. Results are summarised in Table B.5.1-2.

Table B.5.1-2: Storage stability testing of *B. bassiana* strain GHA: over 56 days

Temperature	Percent conidial viability/germination (after n days of incubation) ± SD						
	0	**7**	**14**	**21**	**28**	**42**	**56**
5 °C	97.4 ± 0.01	99.1 ± 0.00	98.6 ± 0.07	98.6 ± 0.07	99.7 ± 0.5	99.7 ± 0.5	97.6 ± 0.05
25 °C		97.2 ± 0.17	**99.8 ± 0.45**	98.2 ± 0.25	97.6 ± 0.04	98.6 ± 0.07	97.9 ± 0.05
50 °C		65.9 ± 0.03	6.8 ± 0.13	1.4 ± 0.09	0.2 ± 0.47	0.0	0.0

Stability to pH and metal ions (1 hour at 23 °C)

Reference:
Jaronski, S.T. and Britton, J.A. (1993), Effect of pH and metal ions on *Beauveria bassiana* Strain GHA in aqueous suspension, (CHE2006-766)

Principle of the method:
0.15 g of *B. bassiana* strain GHA conidia was suspended in 25 ml of 0.01 M aqueous buffers at pH 5, 7 or 9), in 0.01 M solutions of Na^+, Cu^{2+}, Fe^{2+}, $Mg^{2+,}$ Ca^{2+} ions and in glass-distilled water. Initial samples were removed and placed on Sabouraud dextrose agar with yeast plus antibiotic and incubated overnight to determine initial spore viability. All flasks were incubated at 22 - 26 °C. Three replicate sub-samples of each buffer and metal ion solution were removed at 24 and 48 h. Observation of conidial germination and relative number of bacteria in each sample were made using a 400x magnification and phase contrast illumination.

Findings:
At no point during the observation period more than 1 % of the conidia germinated in the test system. Results of percent of germination are summarised in Table B.5.1-3.

Table B.5.1-3: Conidial germination of *B. bassiana* strain GHA in the test systems

Test system	% germination after 24 hours	% germination after 48 hours
Water control [1]	0.3	0.0
pH 5	0.5	0.5
pH 7	0.7	0.3
pH 9	0.5	0.0
0.01M NaCl	0.2	0.0
0.01M $CuSO_4$	0.2	0.2
0.01M $CaCl_2$	0.5	0.3
0.01M $FeSO_4$	0.0	0.0
0.01M $MgCl_2$	0.7	0.3

[1]unbuffered glass-distilled water

Bacterial levels

Bacterial levels in all test systems except 0.01M $CuSO_4$ increased greatly during the incubation period. The rapid growth of bacteria in all test systems with the exception of $CuSO_4$ may have caused the viability loss. According to Mycotech, bacteria were at levels of 6000 CFU/g and there were enough nutrients in the inerts of the TGAI to cause these micro-organisms to proliferate.

Stability to sunlight

Reference:
Jaronski, S.T. (1993), TGAI physical stability study of *Beauveria bassiana* Strain GHA: sunlight effects, (CHE2006-765)

Principle of the method:
0.1 mL of a *B. bassiana* strain GHA suspension containing approximately 2.6 x 10^9 viable conidia/mL was sprayed on cover slips. These cover slips were dried for about 0.5 hour indoors at room temperature (19 °C) and in the dark. Following the drying period, samples were placed into a closed insulated box and exposed to sunlight for two days. Samples were collected at study initiation and at 1, 2, 4, 6 and 8 hours after the start of exposure. Each sample was plated on Sabouraud Dextrose agar prior to incubation at 22 - 26 °C overnight. Results are summarised in Table B.5.1-4.

Findings:
Conidia of *B. bassiana* strain GHA were rapidly inactivated by natural sunlight. The half-life of *B. bassiana* strain GHA conidia was 2.58 hours.

Table B.5.1-4: Stability of *B. bassiana* strain GHA to sunlight

Time (h)	Irradiance MJ/m^2	Mean percent viability ± SD
0	0	97.7 ± 0.22
1	3.197	98.9 ± 0.01
2	6.795	86.9 ± 0.41
4	14.343	0.22 ± 0.17
6	20.748	0.0
8	27.108	0.0

- sampling was discarded as conidial viability was < 5 %.
SD = Standard Deviation

B.5.2 Methods for the analysis of the preparation (OECD IIIM 5.1 - 5.2)

B.5.2.1 Methods for the identification and the determination of the content of the micro-organism(s) in the preparation

In order to proceed with the analysis of BotaniGard 22WP, the micro-organism *Beauveria bassiana* strain GHA in the preparation must be cultured. Colonies of *B. bassiana* strain GHA are enumerated using a hemacytometer (see point B.5.1.5).

B.5.2.2 Methods to establish regular control of the preparation to show that it does not contain other organisms than the indicated ones and to establish uniformity

The method for determination of impurities in the technical product is applicable to the preparation.
The method to show presence of any human and mammalian pathogens in the technical product is applicable to the preparation.

B.5.2.3 Methods to identify any contaminating micro-organisms of the preparation

The methods provided for the technical product are also used for the preparation.

B.5.2.4 Methods used to determine the storage stability and shelf life of the preparation

Methods for the determination of storage stability are described by Anonymous (2000) and Jaronski, S.T. (1993).

References:
Anonymous (2000), Effect of temperature on the Shelf - life of BotaniGard emulsionsfiable suspension (code ES9601) and Botani Gard 22 WP (Code WP9616B) (CHE2006-770)

Jaronski, S.T. (1993), Fungal spore viability test (SOP #03-03-02;Edition#02) (CHE 2007-389)

Test procedure

Multiple lots of BotaniGard 22WP were held in HDPE containers at constant temperatures of 25, 35 and 40 °C for lengths of time sufficient for conidial viability to degrade to below 50 % of orginal values. Viability were generally determined monthly (Mycotech SOP 03-03). Therefore each sample was serially diluted and two drops of the final dilution were placed on the surface of an SDAY agar plate to evaluate the germination. The rate of viability loss was determined at 25, 35 and 40 °C. The number of days needed for spores viabilities to drop to a given value, either 85 % (LT15) or 50 % (LT50) of original viability was calculated.

Further details concerning the determination of viability are described in Volume 4 under point C.1.1.

See also point B.2.2.2

B.5.3 Methods to determine and quantify residues (viable or non-viable) (OECD IIM 4.5, IIIM 5.4)

B.5.3.1 Analytical methods (residue) for plants, plant products, foodstuffs of plant and animal origin, feedingstuffs

B.5.3.1.1 Plant material

Analytical methods which meet requirements of Guidance document on residue analytical methods (SANCO/825/00) are not supplied.

However, residue analytical methods for *B. bassiana* strain GHA are not considered necessary, because maximum residue levels are not set.

B.5.3.1.1.1 Methods which do not meet the requirements

Reference: OECD IIM 4.5.1

Report: Lord, J. C. (1995) Quantification of spores per unit area on leaf surfaces; Report No. SOP # 01-08, Edition # 01
MET2006-303

Guidelines: Not reported

Deviations: Not relevant

GLP: No

Acceptability: The study is considered not to be acceptable.

Materials and Methods:

Test material: Green leaves

Fortified analyte(s): none

Analyte(s) determined as: Beauveria bassiana

Principle of the method: A 2 cm diameter disk was cut from each leaf and was placed in 3 mL of sterile 0.1% Tween 80 in a screw cap tube. Each tube was then shaken and vortexed at high speed. The leaf solution was analysed using an hemacytometer. The number of spores was determined with phase contrast microscopy at 400x magnification. Microbial counts are based on the number of spores per mm^2 of leaf surface.

Findings:
none provided

Conclusion:
Only the standard operation procedure is submitted but neither validation data nor details on the detection limits etc. Therefore this method cannot be accepted for the determination of residues of *Beauveria bassiana* strain GHA in food.

B.5.3.1.2 Animal material

Analytical methods which meet requirements of Guidance document on residue analytical methods (SANCO/825/00) are not supplied.

However, residue analytical methods for *B. bassiana* strain GHA are not considered necessary, because maximum residue levels are not set.

B.5.3.2 Analytical methods (residue) soil, water, air

B.5.3.2.1 Soil

Analytical methods which meet requirements of Guidance document on residue analytical methods (SANCO/825/00) are not supplied.

There is no need for a monitoring method since no residue definition for enforcement purposes is proposed.

B.5.3.2.1.1 Methods which do not meet the requirements (if appropriate)

Reference: OECD IIM 4.5.4

Report: Grace, J. (1997) Standard operating procedure for the analysis of *Beauveria bassiana* in soil
Report No. SOP # 13.1
MET2006-305

Guidelines: Not reported

Deviations: Not relevant

GLP: No

Acceptability: The study is considered not to be acceptable.

Materials and Methods:

Test material: soil

Fortified analyte(s): none

Analyte(s) determined as: Beauveria bassiana strain GHA

Principle of the method: A 10g soil sample was suspended in 90 mL of sterile 0.1% Tween 80 in a screw cap dilution bottle. The sample was shaken and sonicated for 15 minutes. Ten fold serial dilutions in sterile water were made. Each diluted sample was plated in duplicate on plates of Dodine, Sabouraud-dextrose agar or other selective media. To isolate *Beauveria bassiana* strain GHA, 2 mL of gentamicin/L agar medium were added. Plates were then incubated at 25°C for 4-7 days. Following incubation, the colonies formed were enumerated. Enumeration was made by counting the number of compact bright white colonies of characteristic spore clusters.

Findings:
none provided

Conclusion:
Only the standard operation procedure is submitted but neither validation data nor details on the detection limits etc. Therefore this method cannot be accepted for the determination of residues of *Beauveria bassiana* strain GHA in soil.

B.5.3.2.2 Water

There is no need for a monitoring method since no residue definition for enforcement purposes is proposed.

However, a method has been submitted and described below.

Reference:	OECD IIM 4.5.5
Report:	Collins, M. K. (1993) Beauveria bassiana (Bb GHA 1991) 21-day toxicity to Daphnids (Daphnia magna) under static renewal conditions SLI Report #: 93-7-4883, SLI Study #: 13072.0493.6103.110, Document No. 24 MET2006-306
Guidelines:	Not reported
Deviations:	Not relevant
GLP:	No
Acceptability:	The study is considered acceptable.

Materials and Methods:

Test material: well water used for toxicity testing with Daphnids, passed through XAD polymer material to remove contaminants. Final concentration of dissovled organic carbon (DOC) 1 – 1.5 mg/L.

Fortified analyte(s): *Beauveria bassiana* strain GHA was added in concentrations of 6×10^7 to 1×10^9 CFU/L

Analyte(s) determined as: viable spores of *Beauveria bassiana* strain GHA

Principle of the method: Fungal spore levels in fresh test solutions were. Test solution was droped onto agar plates and incubated for 20 hours at 25°C. Viable fugal spores were quantitatively determined by counting germinated spores, charactericed by swollen size of the spores or by emergent hypha detected with a phase contrast microscope with 4000X magnification.

Findings:
Reproducibility: 70 samples from the toxicity test were analyzed in duplicate with a mean RSD of 17%.
Limit of quantification: Lowest reported viable spore concentration was 8×10^5 CFU/L and this is considerd as the limit of quantification.
Recovery: Concentration of viable fungal spores from freshly prepared solutions was 95 % on average in a concentration range of 6×10^7 to 1×10^9 CFU/L (Σ n = 12) (see Table 5.3-1).

Conclusion:
This method is considered suitable and sufficiently validated for the determination of spores of *Beauveria bassiana* strain GHA from groundwater at the concentration level of 1 x 10^6 to 1 x 10^9 CFU/L.

Table B.5.3-1: Validation data for the analytical methods for the determination of residues of *Beauveria bassiana* strain GHA in groundwater

References	Matrix	Determined analyte	Detection method	LOQ [CFU/L]	Fortification level [CFU/L]	Average recovery [%]	RSD [%]	n
Collins,) 1993 (MET2006-306)	groundwater	Beauveria bassiana strain GHA	spore counting	1×10^6	6.6×10^7	87	10	2
					1.3×10^8	108	0	2
					2.6×10^8	100	4	2
					5.0×10^8	102	8	2
					1.0×10^9	89	2	2

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B.5.3.2.3 Air

Despite of documented sensitising effects no residue definition is proposed for *Beauveria bassiana* GHA in air, as data that would have allowed to derive an AOELs are lacking. Therefore, no residue analytical methods for air are required.

B.5.3.3 Analytical methods (residue) for body fluids and tissues

The submission of an analytical method for the determination of residues in body fluids and tissues is not necessary, because the active substance is not classified as toxic or highly toxic.

B.5.3.3.1 Methods which do not meet the requirements (if appropriate)

The following method was provided by the notifier.

Reference:	OECD IIM4.5.3
Report:	Barbera, P. W.. (1993) Sensitivity of Detection of *Beauveria bassiana* strain GHA for toxicity/ pathologenicity testing in rats, IITRI Project No. L08433, Study No.1 MET2006-304
Guidelines:	Not reported
Deviations:	Not relevant
GLP:	No
Acceptability:	The study is considered not to be acceptable.

Materials and Methods:

Test material: lung and ceacum tissue of rats

Fortified analyte(s): two concentrations of *Beauveria bassiana* strain GHA (1 x 10^4, 1 x 10^6 CFU/L) to homogenised samples of lung and ceacum tissue in peptone.

Analyte(s) determined as: colony forming units of *Beauveria bassiana* strain GHA

Principle of the method: an aliquot of the amended tissue homogenate was spread on duplicate plates (three different media) for enumeration. Plates were incubated for 4 days at 25 °C. Colony forming units of *Beauveria bassiana* strain GHA were identified and counted by nacked eye.

Findings:
The suitability of the different agar media for growing of *Beauveria bassiana* strain GHA depends upon the kind of tissue matrix.
Recovery and RSD is provided (see Table B.5.3-2:) but no original data. Only two levels in duplicate were tested.

Conclusion:
Method is not sufficiently validated to be suited for the determination of residues of *Beauveria bassiana* strain GHA in body tissues.

Table B.5.3-2: Sensitivity of detection of *Beauveria bassiana* strain GHA

Tissue	Target dose	Percent recovery ± SD					
		Pre[c] ODA	Pre[c] PDA	Pre[c] SDAY	Post[d] ODA	Post[d] PDA	Post[d] SDAY
None[a]	9.9 x 10^1	108.59 ± 3.57	123.74 ± 3.57	126.26 ± 14.28	27.78 ± 3.57	73.23 ± 10.71	60.61 ± 14.28
Lung[b]		51.77 ± 8.63	121.21 ± 14.28	117.42 ± 7.58	103.54 ± 2.92	108.59 ± 6.52	104.80 ± 2.53
Caecum[b]		0.00 ± 0.00	61.87 ± 28.08	49.24 ± 21.18	82.07 ± 13.28	92.17 ± 9.56	80.81 ± 9.22
None[a]	9.9 x 10^3	97.47 ± 1.43	108.33 ± 1.79	104.80 ± 8.93	47.73 ± 3.21	78.54 ± 4.64	66.67 ± 2.86
Lung[b]		89.39 ± 7.11	111.62 ± 2.97	112.37 ± 3.97	63.01 ± 3.33	83.71 ± 3.79	77.78 ± 6.91
Caecum[b]		58.84 ± 4.15	97.85 ± 7.64	98.11 ± 8.52	45.45 ± 5.17	75.25 ± 4.34	56.06 ± 5.13

[a] Two samples tested
[b] Four samples tested
[c] Pre: samples mixed by vortexing and plated
[d] Post: sample mixed by vortexing , homogenised and plated

[e]) ODA: Dodine oatmeal agar
[f]) PDA: Potato extrose agar
[g]) SDAY: Sabouraud dextrose agar with yeast extract

B.5.4 Evaluation and assessment

B.5.4.1 Analysis of the micro-organism

Adequate methodology exists for identification of *Beauveria bassiana*, strain GHA, in the technical product. Techniques used to ensure a uniform product and assay methods for its standardisation as well as information on quality control measures during production process were provided.

B.5.4.2 Formulation analysis

Analytical procedures used to determine the spore count and the microbial contaminants in the product were provided.

B.5.4.3 Residue analysis

Currently, no MRL and no residue definition for monitoring is proposed. Therefore, no analytical methods for the determination of residues in plants, in plant products, foodstuffs of plant and animal origin, or in feedingstuffs are required. The same is true for soil, water and air.

Nevertheless the notifier has submitted several methods that were evaluated. Only for one of the methods sufficient data were provided to allow comparison with the following validation criteria:

- Mean recovery rates at each fortification level in the range of 70 to 110% with a relative standard deviation of ≤ 20%
- No interfering blanks (< 30% of the LOQ)

With respect to these criteria the method was sufficient for the determination of residues in groundwater at a level above 1×10^6 CFU/L.

Table 5.4-1: Methods for the determination of residues

Matrix-type	Matrix	Residue component	Method	Limit of quantification		Reference
water	groundwater	*B. bassiana* Strain GHA	counting after incubation on agar plates	1×10^6	CFU/L	Collins, M. K. (1993) MET2006-306

B.5.5 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 4.1 (OECD)	Castrillo, L.A. Vandenberg, J.D., Wraight, S.P	2003	Strain-specific detection of introduced *Beauveria bassiana* in agricultural fields by use of sequence-characterized amplified region markers Journal of Invertebrate Pathology 82, 75-83 GLP: N, published: N 1675896	Y	LIT
KIIM 4.2 (OECD)	Jaronski, S.T.; Butler,E.; Kahler, A.	1993	*Beauveria bassiana* strain GHA cDNA libaray construction and RFLP analysis R145-93/003 GLP: N, published: N 1300920 / BWS2006-54	Y	LAM
KIIM 4.3.1 (OECD)	Jaronski, S.T.	1993	Fungal spore counts (SOP #03-02;Edition #02) N/A GLP: N, published: N 1300960 / CHE2006-757	J	LAM
KIIM 4.3.2 (OECD)	Jaronski, S.T.	1993	Characterisation of Beauveria bassiana (SOP #3.5; Edition #01) and Including Sample Characterisations N/A GLP: N, published: N 1300961 / CHE2006-750	J	LAM
KIIM 4.3.5 (OECD)	Becerra, M.A.	1995	Extraction procedure SOP # 3.8 GLP: N, published: N 1300963 / CHE2006-767	J	LAM
KIIM 4.3.5 (OECD)	Jaronski, S.T.; Becarra M.; Sears, J.	1995	Analysis of Mycotrol GH TGAI (Beauveria bassiana strain GHA Conditional Powders) for the metabolites Beauvericin and Bassianolide 94-009 GLP: N, published: N 1300964 / CHE2006-752	J	LAM
KIIM 4.3.5 (OECD)	Jaronski, S.T.	1993	Analysis of GH TGAI (Beauveria bassiana strain) for the metabolite Oosporein 93-032 GLP: N, published: N 1300965 / CHE2006-753	J	LAM
KIIM 4.3.6 (OECD)	Jaronski, S.T.	1993	Enumeration of Microbial Contaminants in Beauveria bassiana TGAI (03-06; Edition 01) 5 GLP: O, published: N 1300966 / CHE2006-784	J	MEU

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 4.4 (OECD)	Jaronski,S.T. and Britton, J.A.	1993	Effect of pH and metal ions on Beauveria bassiana strain GHA in aqueous suspension 93-025 GLP: J, published: N 1300967 / CHE2006-766	J	LAM
KIIM 4.4 (OECD)	Jaronski, S.T.	1993	TGAI Physican stability study of Beauveria bassiana strain GHA: temperature extremes 93-002 GLP: N, published: N 1300968 / CHE2006-755	J	LAM
KIIM 4.4 (OECD)	Jaronski S.T.	1993	TGAI Physical stability study of Beauveria bassiana strain GHA: sunlight effects. 93-001 GLP: J, published: N 1300969 / CHE2006-765	J	LAM
KIIM 4.5.5 (OECD)	Collins, M.K.	1993	Beauveria bassiana (Bb GHA 1991) 21-day toxicity to Daphnids (Daphnia magna) under static renewal conditions 93-7-4883 GLP: J, published: N 1300973 / MET2006-306	J	LAM
KIIIM1 5.2 (OECD)	Jaronski, S.T.	1993	Fungal spore viability test (SOP #03-03-02;Edition#02) N/A GLP: N, published: N 1301015 / CHE2007-389	J	LAM
KIIIM1 5.2 (OECD)	Anonymous	2000	Effect of temperature on the Shelf - life of BotaniGard emulsionsfiable suspension (code ES9601) and Botani Gard 22 WP (Code WP9616B) N/A GLP: J, published: N 1301016 / CHE2006-770	J	LAM
KIIIM1 5.3 (OECD)	Bradley, C.	1998	Minjor Modifications to Manufacturing Process for Mycotrol TGAI and End Product Formulation N/A GLP: N, published: N 1301017 / CHE2006-769	J	LAM

Codes of owner

LAM Laverlam International Corporation
MEU Mycotech Europe Ltd.
LIT Literature