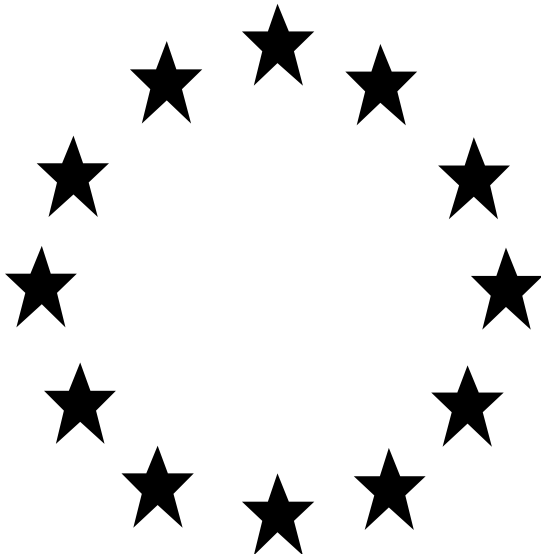

Draft Assessment Report (DAR)

- 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 1

August 2008

Draft
Assessment Report

22 November 2007

***Beauveria bassiana* GHA**

Volume 1

Report and
Proposed Decision

Rapporteur Member State: Germany

Contents

Level 1

Beauveria bassiana GHA

Statement of Subject Matter and Purpose of Draft Assessment Report

1 Statement of subject matter and purpose for which the DAR was prepared

1.1 Purpose for which the DAR was prepared (Dossier Document A)

1.2 Summary and assessment of information relating to collective provision of dossiers (Dossier Document B)

As Mycotech Europe Ltd. is the only notifier of the active ingredient *Beauveria bassiana* strain GHA, this point is not relevant.

1.3 Identity of the micro-organism (OECD IIM 1)

1.3.1 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 Registration Services Ltd.
PO Box 31
Robertsbridge TN 32 5ZL
England
Phone: +44 1580 882057
Fax: +44 1580 882057

1.3.2 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

1.3.3 Name and species description, strain characterisation (OECD IIM 1.3)

1.3.1.1 Accession number in culture collection

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

1.3.1.2 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)
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).

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.

1.3.4 Specification of the material used for manufacturing of formulated products (OECD IIM 1.4)

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).

It should be noted that the RMS has proposed a maximum limit for beauvericin of 5 mg/kg.

Confidential information, see Annex C.

1.4 Identity of the plant protection product (OECD IIM 3.1; OECD IIIM 1)

1.4.1 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

1.4.2 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

1.4.3 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

1.4.4 Type of the preparation and code (OECD IIIM 1.5)

Wettable powder (WP)

1.4.5 Function (OECD IIIM 1.6)

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

1.4.6 Composition of the preparation (OECD IIIM 1.7.1 – 1.7.3)

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

Regarding the formulants see Annex C/ Volume 4.

1.5 Uses of the plant protection product (OECD IIM 3; OECD IIIM 3)

1.5.1 Field of use (OECD IIM 3.3; OECD IIIM 1.6.1)

Beauveria bassiana is a fungal contact pathogen, acting as a microbiological insecticide, to control sucking insects on horticultural crops, such as ornamentals, tomatoes and cucumbers in greenhouses.

1.5.2 Effects on harmful organisms (OECD IIIM 3.1)

Beauveria bassiana is a fungal contact pathogen**,** acting as a microbiological insecticide. The conidia of *Beauveria bassiana* adhere to the insect cuticle by 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*.

1.5.3 Summary of intended uses (OECD IIM 3.4; OECD IIIM 3)

BotaniGard 22WP is intended for the control of insect pests such as whiteflies (Aleyrodidae), with some activity on thrips (Thysanoptera), and aphids (Aphididae) in vegetable and ornamental crops. Whiteflies, thrips, and aphids are major pests of greenhouse crops and cause mostly severe damage to plants if not controlled. Some of the pest species act also as virus vectors. BotaniGard 22WP is applied at rates of 0.0625 - 0.55 kg per ha by spraying corresponding to rates of 0.28 - 2.42 x 10^{13} CFU per ha.

1.5.4 Information on registrations in EU Member States

Plant protection products with the formulated product BotaniGard 22 WP are authorised in a number of countries in Northern and Southern Europe.

Level 2

Beauveria bassiana GHA

Overall Conclusions

2 Reasoned statement of the overall conclusions drawn by the regulatory authority

2.1 Identity, properties and further information

2.1.1 Identity of the micro-organism

2.1.1.1 Accession number in culture collection

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

2.1.1.2 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
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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.

2.1.2 Biological, physical and chemical properties

2.1.2.1 Biological properties of the micro-organism

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.

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.

Based on world-wide data, Li (1988*) listed 707 insect species as hosts of *B. bassiana*.

* cited in Zimmermann (2007)

Mode of action

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.

Host specificity range and effects on species other than the target harmful organism

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

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).
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, stemboring lepidoptera (including European and Southwestern corn borer).

B. bassiana can also be isolated from soil and plants in a wide range of habitats.

Potential of the micro-organism to produce metabolites of concern for human health and/or the environment

Certain strains of *Beauveria bassiana* have been shown to have the potential to produce metabolites such as beauvericin or bassianolide.
Contents of beauvericin, bassianolide and oosporein were determined for *Beauveria bassiana* strain GHA.

In addition, it is known from the literature that *B. bassiana* can produce also the following metabolites:

- bassiacridin
- beauveriolides and beauverolides
- bassianin and tenellin
- oxalic acid.

The RMS is of the opinion that only beauvericin should be regarded as relevant. The proposed specified maximum limit in the technical material of *Beauveria bassiana* strain GHA is 5 mg/kg. It is proposed to use the content of beauvericin in the technical material as an indicator for the presence of the other metabolites in order to exclude a risk to operators, workers and consumers.

Infectiveness, dispersal and colonisation ability

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)

*cited in Zimmermann (2007)

Relationships to known plant or animal or human pathogens

B. bassiana has no reported effects on plants. Infection mechanisms are highly evolved and specific only to insects and *Beauveria 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 and no reference to the occurrence of pathogenicity to plants, other animals than insects or humans in closely related species was found.

Genetic stability and factors affecting it

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.

2.1.2.2Physical, chemical and technical properties of the formulation

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.

2.1.3 Details of uses and further information

2.1.3.1 Details of uses

Field of use

Beauveria bassiana is a fungal contact pathogen, acting as a microbiological insecticide, to control sucking insects on horticultural crops, such as ornamentals, tomatoes and cucumbers in greenhouses.

Mode of action

Beauveria bassiana is a fungal contact pathogen**,** acting 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*.

Details of intended use

BotaniGard 22WP is intended for the control of insect pests such as whiteflies (Aleyrodidae), with some activity on thrips (Thysanoptera), and aphids (Aphididae) in 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.

Application rate

BotaniGard 22WP contains 4.4 x 10^{13} CFU/kg of the MPCA.
BotaniGard 22WP is applied at rates of 0.0625 - 0.55 Kg per ha corresponding to 0.28 - 2.42 x 10^{13} CFU per ha.

Method of application

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 per ha.

Necessary waiting periods or other precautions to avoid phytotoxic effects on succeeding crops

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.
For the list of uses evaluated for Annex I inclusion see point 2.7.4.1, Appendix III.

2.1.3.2 Further Information

Information on handling, storage, transport or fire, destruction or decontamination, and emergency measures for the active substance as manufactured and information on packaging,

cleaning procedures, handling, storage, transport or fire, emergency measures, and procedures for destruction or decontamination for the wettable powder have been supplied and are acceptable.

2.1.4 Classification and labelling

Active Substance

Health effects

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

Preparation

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

2.2 Analytical methods

2.2.1 Analytical methods for identification and determination of the micro-organism

Analytical methodology is available for the determination of the micro-organism in the technical material as manufactured.
To detect spontaneous changes in major characteristics of micro-organisms the identification of *B. bassiana* is determined by Restriction Fragment length polymorphism (RFLP) analysis, morphological examination and classification according to the taxonomic key.

Since 2003 a new Random Amplified Polymorphic DNA analysis (RAPD) is available to confirm the GHA strain.

2.2.2 Analytical methods for the preparation

Analytical procedures used to determine the spore count and the microbial contaminants in the product were provided.
The spore count of *B. bassiana* in the plant protection product is determined by using haemocytometer and enumeration of microbial contaminants and determining the presence/absence of enteric bacteria such as *Shigella*, *Salmonella* and *Vibrio spp.* by plating out on selective media.

2.2.3 Analytical methods for residue analysis

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 x 10^6 CFU/L.

Table 2.2-1: 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 x 10^6	CFU/L	Collins, M. K. (1993) MET2006-306

2.3 Effects on human and animal health

2.3.1 Effects having relevance to human and animal health arising from exposure to the micro-organism or to impurities contained in the organism, its residual traces and metabolites

The entomopathogenic fungus *Beauveria bassiana* is a rare human pathogen since, in very few cases, it was isolated from eye infections, from pulmonary disease or, in immunocompromised patients, from disseminated infections. Animal data suggest that both corneal and lung tissues provide suitable conditions for survival and even replication of this micro-organism that is otherwise considered not to grow at temperatures above 35 ^oC. It is not known whether these pathogenic properties can be attributed to certain strains since such an information is usually lacking in the case reports. Because eye infections were observed after mechanical damage to the cornea only and systemic disease was apparently confined to severely ill people under immunosuppression, the clinical evidence that infections in humans may occur, is of limited relevance for human health risk assessment of strain GHA with

regard to its intended use in plant protection. More important is the body of information suggesting allergenicity. Based on experience and tests in humans as well as on studies in laboratory animals, *Beauveria bassiana* in general should be considered a potential sensitiser by inhalation. Furthermore, skin sensitising properties of this micro-organism cannot be excluded.

A number of acute studies for infectivity, pathogenicity and toxicity of the strain GHA was submitted using the oral, intratracheal, intraperitoneal, and dermal routes. In addition, a Buehler test for skin sensitisation was provided. The available original studies in laboratory animals are summarised in Table B.2.3-1.

Table B.2.3-1: Summary of acute studies with *Beauveria bassiana* strain GHA

Annex II point	Study/Route/ Method	Species	Dose per animal	Results	Conclusion	Reference
IIM 5.3.1	Skin sensitisation (Buehler test 3 inductions)	Guinea pig	80 mg (*approx.* 8×10^9 CFU)	Negative (not sensitising)	No final conclusion can be drawn	Findlay (1998, TOX2006-870)
IIM 5.3.2	Oral gavage	Rat	*approx.* 1×10^8 CFU	No evidence of toxicity, pathogenicity or infectivity; rapid clearance (by day 3 post dosing)	$LD_{50} > 1 \times 10^8$ CFU; not to be classified, no concern	Barbera (1993, TOX2006-871)
IIM 5.3.3	Intratracheal	Rat	*approx.* 1×10^8 CFU	No evidence of infectivity but local effects on the lungs (inflammation/immune reaction, organ weight increase), transient reduction in body weight gain; clearance complete by day 7 post dosing	$LC_{50} > 1 \times 10^8$ CFU; evidence of some inhalation toxicity, sensitisation by inhalation cannot be excluded	Barbera (1993, TOX2006-872)
IIM 5.3.4	Intraperitoneal	Rat	*approx.* 1×10^7 CFU	No evidence of toxicity, pathogenicity or infectivity; rapid clearance (by day 3 post dosing)	$LD_{50} > 1 \times 10^7$ CFU; not to be classified, no concern	Barbera (1993, TOX2006-873)
IIM 5.5.1	Dermal	Rabbit	1.6×10^{11} CFU	No systemic effects but signs of slight	$LD_{50} > 1.6 \times 10^{11}$ CFU; not to be clas-	Johnson (1993, TOX2006-874)

Annex II point	Study/Route/ Method	Species	Dose per animal	Results	Conclusion	Reference
				but persisting local irritation	sified, no concern	

The results of these studies suggest that strain GHA was not infective by any route and not pathogenic in terms of inducing a systemic disease. Clearance from the body was rapid and virtually complete. In contrast, local pathological changes in the lungs, organ weight increase. and transient effects on body weight have been observed following intratracheal challenge. Thus, some toxicity may occur and seems to be route-dependent with the lung being the most sensitive target. This assumption is further substantiated by published data although apparently other strains were tested.

At least partly, these findings are in line with the assumption of an immunological response to the micro-organism. This is further supported by reports from the literature on allergic reactions following acute inhalative exposure of guinea pigs or chronic inhalative challenge of rats and mice. The negative outcome of a Buehler test with only three inductions is not sufficient to exclude sensitising properties of strain GHA. Furthermore, inhalation seems to be more critical than sensitisation by dermal contact and would not be elucidated by a Buehler test. Thus, classification and labelling (Xn, R42/43) of strain GHA is needed.

A genotoxic potential of strain GHA is not expected because there is no production of metabolites of concern at higher concentrations. This assumption was supported by the negative outcome of Ames tests with both the micro-organism itself and the metabolite beauvericin.

A short-term study to investigate infectivity, pathogenicity and toxicity of strain GHA under conditions of repeated exposure is lacking. However, taking into consideration that the inhalative route was the by far most critical (based on the outcome of the acute studies), a published long-term inhalation study with *Beauveria bassiana* on rats and mice (strain not specified) was regarded as sufficient proof that the effects were similar to those observed after acute challenge. Pulmonary toxicity and sensitisation by inhalation were confirmed. No further information is expected from a short-term study with strain GHA and, thus, this requirement may be waived.

In open literature, *Beauveria bassiana* is described to produce a range of different metabolites: beauvericin, bassianolide, bassiacridin, beauveriolides, beauverolides, bassianin, tenellin, oxalic acid and oosporein.
However, the three secondary metabolites bassianolide, beauvericin and oosporein, have usually not been detected in liquid cultures of strain GHA or in the plant protection product. As reported in the DAR the amount of beauvericin and bassianolide was in most batches below 5 ppm. In the batch (lot) 930210 that was used in the single dose studies for infectivity, pathogenicity and toxicity, in contrast, higher concentrations of both metabolites (48 ppm of beauvericin and 51 ppm of bassianolide) were detected.
There is only little information available on the toxicological properties of metabolites. As a precaution and due to the lack of reliable data, it is assumed that the metabolites act additively. The mycotoxin beauvericin is considered the most important metabolite produced by *B. bassiana.*

In the acute toxicity studies with *Beauveria bassiana* strain GHA with oral, dermal or i.p. administration, which were conducted with a technical material that contained approx. 50 ppm beauvericin and 50 ppm bassianolide, no adverse effects were noted.
The metabolite beauvericin was also not mutagenic in an Ames Test.

The available studies can not be used to derive safe levels for metabolite exposure. Therefore, it is proposed to use the concept of the threshold of toxicological concern (TTC). The TTC of 1.5 µg/d (i.e., 0.02 µg/kg bw/d, calculated with a bodyweight of 75 kg) is considered a safe exposure for the sum of all metabolites (due to the presumed additive action). If the daily exposure with the metabolites does not exceed this level, metabolites are not expected to be a safety concern.

For the specification, maximum levels for individual metabolites have to be given. Therefore, the concentration of 50 ppm used for risk assessment was divided by the number of metabolites (9), which are described in the literature. The result was rounded to 5 ppm. Due to the lack of individual analytical methods for all metabolites, it is proposed to specify only beauvericin with a maximum level in the technical material of 5 ppm. The beauvericin content can be used as an indicator for the presence of the other metabolites.

2.3.2 ADI

Beauveria bassiana strain GHA is a micro-organism which belongs to our natural environment and is considered to be a non-pathogen for humans. In the absence of any significant evidence for toxicity, pathogenicity or infectivity of the strain GHA of *Beauveria bassiana* in animal studies it is neither possible nor necessary to establish an ADI.

2.3.3 AOEL

For the above mentioned reasons (2.3.2) the establishment of an AOEL is not necessary.

2.3.4 ARfD

For the above mentioned reasons (2.3.2) the establishment of an ARfD is not necessary.

2.3.5 Drinking water limit

Not relevant.

2.3.6 Impact on human or animal health arising from exposure to the micro-organism or to impurities contained in the organism, its residual traces and metabolites

Operator
Due to the lack of a medium-term tolerable inhalation exposure level, a comparison with the estimated exposure is not possible. However, calculated inhalation and dermal exposure values are considerably below the observed acute LOAELs. It can be assumed that operators would not be at acute risk during mixing/loading and application of BotaniGard 22 WP. Al-

though situations of repeated exposure at subacute dose levels are not covered by the above considerations, it can be assumed that the estimated operator exposure is acceptable provided appropriate RPE is worn (necessary due to classification/labelling with Xn, R 42). In addition, precautionary the use of suitable gloves as well as wearing suitable protective clothing is considered necessary to minimise the risk of skin sensitisation because BotaniGard 22 WP is classified and labelled with R 43.
In addition, operators should be informed in a suitable way that eye infections were observed after mechanical injury of the cornea.
Taking into account a beauvericin concentration of 50 ppm, which is supposed to present the overall content of beauvericin and the other mycotoxins which might have occurred in the technical material, the operator exposure is unlikely to exceed the proposed TTC-value.

Bystander
Bystander exposure is not relevant for the intended uses (greenhouse).

Worker
After the spray solution has dried, the estimated exposure to Beauveria bassiana strain GHA during re-entry operations does not exceed acceptable values, even if no PPE is worn.
Taking into account a beauvericin concentration of 50 ppm, which is supposed to present the overall content of beauvericin and the other mycotoxins, the worker exposure is unlikely to exceed the proposed conservative threshold of toxicological concern (TTC).

In view of the recommended uses and application techniques, no harmful effects on the health of domestic or wild animals are assumed.

2.4 Residues

2.4.1 Non-viable residues

Various strains of *B. bassiana* have been shown to be capable of producing mycotoxins. Accumulation of metabolites may happen to occur during infestation. Since *B. bassiana* does not show any metabolic activity in the absence of a suitable host no formation of mycotoxins in food items is expected in this case.

The metabolites described in the literature might occur in the technical material. The beauvericin concentration of 50 ppm, used for consumer risk assessment, is supposed to present the overall content of beauvericin and the other mycotoxins. Based on a TTC approach, no acute and/or chronic adverse effects are expected from beauvericin concentrations up to 0.02 µg/kg bw/d.
This TTC value is used in the NESTI calculation. The amount of beauvericin in the tomatoes was calculated to be 0.00915 - 0.01105 µg corresponding to 0.0610 - 0.0737 µg/kg. The intake of beauvericin *via* tomatoes is 14 - 17 % of the TTC value thus not indicating an acute risk for consumers.
With the TTC value of 0.02 µg/kg bw/d for beauvericin and a mean consumption of 15.6 g tomatoes and 9.5 g cucumbers per day during a lifetime, the chronic risk for consumers resulting from the intended use in tomatoes and cucumbers is negligible.

Due to a lack of toxicological and analytical data, no separate quantitative risk assessment was possible for metabolites, described in the literature. It is assumed, that they were of comparable toxicity and concentration as beauvericin. To account for beauvericin and the other metabolites, it is therefore considered sufficiently conservative to base the consumer risk assessment on a concentration of 50 ppm while at the same time lowering the specification for beauvericin in the batches of the MPCA to levels as low as technically feasible (5 ppm). It is proposed to use the beauvericin content as an indicator for the presence of the other metabolites. The concentration of beauvericin in batches of the MPCA should be strictly controlled.

2.4.2 Viable residues

Residue trials have not been conducted on *B. bassiana* strain GHA on grounds of numerous strains of *Beauveria bassiana* occuring naturally in the environment, in soil and on plants as a result of natural infection of insects.
The fungus does not multiply outside of insect hosts. Under natural conditions the conidia germinate and die within few days in the absence of a suitable host in aqueous environments. *B. bassiana* conidia are thermo- and photo-labile; they are inactive above 33 ºC and are destroyed by UV sunlight.

2.5 Fate and behaviour in the environment

2.5.1 Persistence and multiplication

Soil
Beauveria bassiana can be present naturally in the environment, background levels of this organism vary from 0 (below detection limits) to 7.0 x 10^5 CFU/mL. Post-treatment levels vary from 0 to 4.4 x 10^8 CFU/mL. According to greenhouse and monitoring studies in the early and mid-season, there is more than 50 % decline of CFU *B. bassiana* after 48 hours. This speaks for a rapid decrease, whereby a complete decrease is not proven. In some cases *B. bassiana* cannot be detected after application. On the other hand there have been, where higher levels were determined after application than before treatment, but further measurements were lacking to confirm the results. This especially applies for tests with more than one application within a year.
It is difficult to consider persistence only with regard to soil, because multiplication does take place in the hosts. A low remaining residual level in soil can be sufficient for restarting of replication upon occurrence of a host, so that from its cadaver new spores can reach the soil again. On the other hand, in 5 of 9 monitoring studies no background levels of *B. bassiana* were detected, although *B. bassiana* is naturally present in the environment.
However, it should be kept in mind that the background level of *Beauveria bassiana* conidia may be strongly different in various parts of one field depending on the different appearance of potential host insects and also on possibly different microclimates.

Germination and multiplication of entomopathogenic fungi in natural, non-sterile soils can be excluded as long as no potential hosts are present, since microorganisms living in soil must have the ability to degrade heavily degradable and unsolvable substances like lignin and

humus. Furthermore the effect of fungistasis inhibits germination of conidia in non-sterile soils.
In this respect, there is no indication of accumulation.

Possible soil contamination with secondary metabolites

The only way of soil contamination is via a plentiful appearance of infected and dying insects. Concentrations of metabolites in natural environment and their stability are not known. However, most of them are peptides which are usually well degradable. Bassianin, tenellin and oosporein are non-peptide pigments, but due to the structure, biodegradability is assumed for all three substances. So many bacteria and fungi are able to cleave aromatic cycles in the manly presence of oxygen, but also without oxygen. Therefore the risk of soil contamination by secondary metabolites is considered to be negligible.

Surface water
On the basis of the information presented under this Annex point, it is only possible to notice a loss in conidial viability of *B. bassiana* in distilled water and aqueous suspensions at pH 5, 7 and 9 or in metal ion solutions in the presence of bacteria. The rapid growth of bacteria may be caused by the loss in conidial viability. There was no system with pond water. After 48 hours of incubation, there are 39 % viable conidia in distilled water, 59 % at pH 7, and 63 % at pH 9, which was the slowest loss. Conidia did not germinate in the different tested aqueous systems. In general, survival of spores in sterile water is well-known. However germination of conidia and therefore multiplication in water is not expected, since *B. bassiana* is no aquatic fungus and is therefore not adapted to the conditions of the aqueous environment (Campbell 1981). It may be concluded that conidia of *B. bassiana* in water will be degraded by bacteria and protozoa in natural non-sterile water bodies, rapidly.

Groundwater
Conidia of *B. bassiana* are not very mobile in soil and generally remain on the surface of the soil. Therefore a contamination of the groundwater can be excluded.

Air
It may be concluded that the degradation of *B. bassiana* on leaves under field condition is fast. This effect is assumedly not only caused by sunlight radiation, other unknown factors play a more important role. It is possible by adding a formulation to increase survival rate. Consequently, this should be considered by evaluating the final plant protection product. However, *B. bassiana* is not expected to persist in air, as the viability of conidia of *B. bassiana* are greatly reduced following exposure to sunlight for a period greater than 24 hours. Furthermore, in the absence of a specific host insect, conidia of *B. bassiana* will not persist in air for more than 2 days.

2.5.2 Mobility

Soil
Conidia of *B. bassiana* are not very mobile in soil and generally remain on the surface of the soil. The movement of conidia vertically, through the soil profile, is positively correlated with high infiltration rates in soil.

Water: the shape of conidia is not suitable for efficient movement in water.

Air
In the air, *B. bassiana* can be easily transported, as it is dry, of small size and it is produced in powdery clusters. Also, transmission of fungal spores through the air by insects is possible. However, all studies principally indicate that *B. bassiana* is not expected to persist in air, as the viability of conidia of *B. bassiana* is greatly reduced for a period greater than 24 hours. Furthermore, in the absence of a specific host insect, conidia of *B. bassiana* will not persist in air for more than 2 days.

2.6 Effects on non-target species

2.6.1 Effects on birds

One short-term dietary study with American kestrel (*Falco sparverius*) was submitted revealing a $LR_{50} > 2.5 \times 10^{10}$ CFU/kg bw. Further literature data reveal also that several feeding experiments with spores and conidia of *B. bassiana* and field experiments were performed. There is little indication that birds are susceptible to *B. bassiana*.

Birds may become exposed to entomopathogenic fungi directly by consuming spores deposited on their food, or indirectly by consuming fungus-infected insects. For greenhouse applications, exposure of birds is very limited (0.1 % of application rate based on generic drift from greenhouse). For the risk assessment it is considered that the acute scenario is covered by the short-term risk assessment due to the low toxicity, no external signs of pathogenicity and short direct exposure of birds to *B. bassiana*. The calculated Margin of safety (TER_{ST}) values for field exposure (leafy crops/cereals/grass) of insectivorous and small and medium herbivorous birds were between ≥ 6173 and > 34293. They exceed clearly the trigger value of 10 as described in Annex VI part I of Directive 91/414/EEC for all scenarios. Thus, no adverse effects on birds in short-term scenarios (and in acute scenarios) are expected following application of BotaniGard 22 WP at recommended use rates.
Literature data reveal that several feeding experiments with spores and conidia of *B. bassiana* and field experiments were performed. Studies show that *Beauveria bassiana* strain GHA growth is inhibited at 33 ºC and absent at 36 ºC, thus making growth untenable in living mammalian and avian tissues. There is also little indication that birds are susceptible to *B. bassiana*.

Secondary poisoning by infected insects:

However, studies with the metabolite oosporein indicated that there might be an intolerable risk for insectivorous birds consuming infected insects comprising various secondary metabolites. On the other hand no side-effects on birds, especially young ones, were noticed during a large field trail carried out in Germany with *B. brongniartii* blastospores against the forest chockchafer *Melolontha hippocastani* (1.5 – 2.8 x 10^{14} CFU/ha) (Havelka & Ruge 1988). Since the TER - calculation given in Vol.3 is based on information about *B. brongniartii*, this information indicates, that despite oosporein is the main secondary metabolite in *B. brongniartii* and the consumption of infected insects theoretically gives hints of concern according the calculation above, no detrimental effects can be observed after application in the field. Müller-Kögler (1967 cited in Zimmermann) mentioned that according to E. Devaux (in Giard 1892), chickens fed white grubs of *Melolontha* sp. infected with *B. brongniartii* (*B. tenella*) did not demonstrate any side-effects.
Johnson et al. (2002) demonstrated that young (4 and 9 day old) ring-necked pheasant chicks being fed two *Beauveria bassian*a strain GHA infected grasshoppers *Melanoplus sanguinipes* on each of 2 d separated by 4 d without treatment showed no external signs of pathogenicity or differences in behaviour, no changes in weight gain and no consistent changes in the histopathological examination of the tissues associated with the treatment.
Considering greenhouse applications the risk seems to be acceptable, since the target-organism (sucking insects) of *Beauveria bassiana* strain GHA tend to stay on the crops and not to fly around. Therefore it is very improbable, that they leave the greenhouse, e.g. via ventilation flaps and the exposure of bird is improbable.

2.6.2 Effects on terrestrial vertebrates

Mammals:
An acute oral toxicity study on rats conducted with *Beauveria bassiana* strain GHA (refer to the toxicology section) showed no adverse effects at a dose level of 1.05 x 10^{8} CFU/animal. Another study has been conducted with the product BotaniGard 22 WP. No mortalities or sublethal effects were observed at a dose level of 5000 mg MPCP/kg bw corresponding to 2.2 x 10^{14} CFU/kg bw.

On the basis that the product has very low mammalian toxicity, *Beauveria bassiana* strain GHA cannot grow at mammalian body temperatures (the *in vivo* growth temperature of the fungus is below 36 °C which prevents it from growing at the higher body temperature of mammals) and thus cannot infect mammalian hosts, and is not pathogenic to mammals, no short- or long-term effects are to be anticipated. Furthermore, sensitivity to low pH values encountered in the stomach of mammals renders survival and colonisation of the mammal's interior via ingestion unlikely.

BotaniGard 22WP is intended to be used in greenhouse. For greenhouse applications, exposure of terrestrial vertebrates is very limited based on the assumption that 0.1 % of the application rate enters the environment based on generic drift from greenhouse. Mammals dwelling in the field may be exposed to *Beauveria bassiana* after application of BotaniGard 22WP mainly by the consumption of contaminated feed. The scenarios "leafy crop/cereals/grass" was chosen as it best represents the type of habitat surrounding greenhouses. The LD_{50} value of $> 2.2 \times 10^{14}$ CFU /kg bw is chosen for an acute risk assessment. The Margin of safety (TER_A) values for small and medium herbivorous as well as

insectivorous mammals are > 9×10^6 and greater than the trigger of 10 as described in Annex VI part I of Directive 91/414/EEC indicating that there is no unacceptable risk for mammals from direct exposure to the spores following application of BotaniGard 22 WP according to Good Agricultural Practice. On the basis of a literature review, there is also little indication that terrestrial vertebrates are susceptible to *B. bassiana*. No unacceptable effects were detected for studies with rats, mice and rabbits.

Secondary poisoning by infected insects:

Concerning potential effects of secondary metabolites, only insectivorous mammals may be affected by consuming infected insects which had escaped from the greenhouse
Studies with the metabolite oosporein indicated that there might be an intolerable risk for insectivorous mammals consuming infected insects comprising various secondary metabolites. On the other hand in contrast to birds known to suffer from avian gout when exposed to oosporein, no lethal effects on mice were observed at the concentration used for this calculation.

Reptiles:
Fromtling et al. (1979, see B.6.2.4) reported an outbreak of pulmonary mycosis caused by *Beauveria bassiana* among captive American alligators (*Alligator mississipiensis*) in a zoo. Three of four animals died within the next 9 months. The authors also referred to a paper reporting pulmonary mycosis due to infection with *Beauveria bassiana* in giant tortoises (Georg et al., 1962, not available to the RMS).
In contrast to mammals and birds reptiles are poikilothermal animals with body temperatures that are often far lower as the maximum growth temperature of fungi like *B. bassiana*. However, the hazard of a possible pulmonary mycosis in wild reptiles is quite low, since conidia of *Beauveria bassiana* are not able to survive in air without their hosts for more than 2 days. The exposure of reptiles via air and therefore the risk of a pulmonary infection in wild reptiles is considered to be negligible.

2.6.3 Effects on aquatic species

Long-term studies with fish, daphnids and algae were submitted. *Beauveria bassiana* strain GHA is not expected to have any adverse effects on algae. Mild toxic effects were observed in studies on fish. There were no lethal or sublethal effects on daphnids, however, the impairment of reproduction could not be assessed due to outstanding data. However, as exposure of aquatic organisms to *Beauveria bassiana* from the intended indoor use of BotaniGard 22WP is expected to be minimal, it is anticipated that the potential risk posed to fish and *Daphnia* is low. To confirm this, a risk assessment was performed as it can be assumed that after greenhouse applications 0.1 % of the application rate enter the environment via generic drift from the greenhouse. Aquatic organisms may be exposed to *B. bassiana* entering surface waters *via* spray drift. The actual predicted environmental concentration ($PEC_{SW\ actual}$) of *Beauveria bassiana* resulting from input *via* this route was initially estimated. The calculation was based on five accumulated applications of BotaniGard 22WP (550 g *Beauveria bassiana GHA*/ha), assuming no degradation between applications and an entry resulting from spray drift at 1 m of 2.77 % of 0.1 % of the application rate according to Rautmann et al. (2001). The $PEC_{SW\ ini}$, is 0.0254 µg as/L. In terms of CFU, this is equivalent to 1118 CFU/L.

The calculated margin of safety (TER_{LT}) values are between 313059 and 8.6×10^9 exceeding clearly the limit value of 10. Thus, no adverse effects on aquatic invertebrates, fish and algae are expected after application of BotaniGard 22WP at recommended use levels. Though there was no definite NOEC in the fish and daohnia studies, it can be concluded that the risk to early life stages of fish and reproduction of daphnids is acceptable, due to the high margin of safety..

A literature research revealed that only small information is available. No toxicity or pathogenicity was observed in *Daphnia magna* and in the grass shrimp, *Palaemonetes pugio* after percutaneous and oral contamination. However, toxicity against fish cannot be excluded. However, according to the reported effect concentrations the effect seems to be less pronounced and the risk for aquatic organisms from the greenhouse application of BotaniGard 22 is assumed to be acceptable.

2.6.4 Effects on bees

Honeybees
In a study under practical conditions *Beauveria bassiana* strain GHA had no or negligible effects on honey bees. No treatment related increased mortality, latent infections or pathogenic effects on worker bees or bee brood could be observed .
However, bees are not exposed when *Beauveria bassiana* strain GHA is used as recommended for indoor or glasshouse application.

Bumblebees

Effects were observed on worker bees and drones via topical exposure. Due to the fact that the main entrance route is via topical exposure, it can be concluded that *Beauveria bassiana* (GHA) is harmful to bumble bees. Considering the mode of action of *Beauveria bassiana* (GHA) the infection process starts via contact with the cuticle of the susceptible insect. Due to the nature of *Beauveria bassiana* (GHA) as an entomopathogen microorganism, the observed effects are considered to be pathogenic. The concentration of the active ingredient *Beauveria bassiana* (GHA) bumble bees were exposed to (2.5×10^{10} CFU/L) in the study is approx. the half of the concentration of *Beauveria bassiana* (GHA) in the spray mixture used in field (4.84×10^{10} CFU/L). Conditions for hives in the ground are likely to offer even more conductive temperature and humidity conditions for these fungal pathogens making a spread of infection in the hives more probable. Therefore it can be concluded that *Beauveria bassiana* (GHA) poses an unacceptable risk to bumblebees at the intended application rate.
Unless higher tier studies may show that there are no adverse effects on bumblebees in the field, the application of products comprising *Beauveria bassiana* (GHA) should be restricted to greenhouse application. Further more it is proposed to prevent the exposure of bumblebees attracted from the outside by flowering plants in greenhouses by an adequate risk mitigation method, e.g. by the use of suitable networks covering the aperture of greenhouse ventilation flaps.

2.6.5 Effects on other arthropod species

Extended laboratory tests with treated soil or leaves were performed with the standard test organisms *Aphidius rhophalosiphi, Typhlodromus pyri, Orius laevigatus* and *Pardosa* spec..

For the test with *Pardosa*, there was no sufficient information concerning the tested dose. *B. bassiana* (GHA) was shown to be infective to the exposed arthropods under the specific laboratory conditions. All studies were conducted as limit tests. In the case of the studies with *T. pyri* and *O. laevigatus* significant adverse effects have been observed. These effects are considered to be caused by pathogenicity of *B. bassiana* (GHA).
According Annex VI part II of Directive 91/414/EEC pathogenic effects must not occur, consequently no effect concentrations have to be used for risk calculation. As it is not possible to determine no effect concentration from the submitted studies, the risk assessment on the basis of the HQ-approach is not applicable.
As *B. bassiana* (GHA) is intended to be used in greenhouses the exposure of the surrounding environment and hence the exposure of non-target arthropods is negligible. On the basis of a generic drift of 0.1 % and a maximum application number of five a maximum concentration outside, directly in front of the glasshouse of 2.75 g MPCP/ha can be calculated. Thus the concentration of *B. bassiana* (GHA) used in the laboratory studies were 344 and 346 fold higher than the worst case exposure directly in front of the glasshouse assuming no degradation between the five applications. In air the organism is not stable. The viability of conidia of *Beauveria bassiana* is greatly reduced during a period longer than 24 hours. Furthermore, in the absence of a specific host insect, conidia of *Beauveria bassiana* will not persist in air for more than 2 days (Reference IIM 7.1). Based on this information a calculation of exposure considering only one application rate appears to be feasible. The resulting exposure is more than 1700 fold lower than the test concentrations in the laboratory studies.
Based on the submitted field studies it can be concluded that there is a significant difference between the physiological host range (laboratory conditions) and the ecological host range (field conditions). Due to the fact that *Beauveria bassiana* (GHA) needs a microclimate of very high humidity in order to cause an infection, many arthropods being susceptible on the conditions of the laboratory test are not affected in the field. Furthermore the exposure of non-target-arthropods after greenhouse applications appears to be negligible. Thus the risk of *Beauveria bassiana* (GHA) to non-target arthropods is considered to be acceptable.

2.6.6 Effects on earthworms

An acute test with *Eisenia fetida* was performed. Up to the highest test concentration of 1000 mg *Beauveria bassiana* strain GHA/kg dry soil, corresponding to 8.6 x 10^{10} CFU, no negative impact on mortality was detected. With values of 538 - 896 the calculated Margin of safety (TERs) surpasses the required trigger value of 10 in Annex VI part I of the Directive 91/414/EC. This finding was supported by literature data on laboratory biotests. Therefore, it is assumed that the risk of possible adverse effects on earthworms is to be negligible.

2.6.7 Effects on other soil non-target macro-organisms

No further data submitted.

2.6.8 Effects on soil non-target micro-organisms

No further data submitted. A literature research has revealed that *B. bassiana* widely occurs in the soil as well as on insects in the aerial environment. Accordingly, there is a long-lasting evolutionary coexistence with other micro-organisms, which includes different forms of interactions. Artificial introduction of *B. bassiana* does not seem to interfere with the

microbial equilibrium of natural soils. Due to the natural occurrence, the presence of antagonists and lacking observation of uncontrollable growth of *B. bassiana* in soil resulting in non-tolerable effects with respect to microbial diversity in soil, the risk is assumed to be acceptable.

2.6.9 Effects on other non-target organisms (flora and fauna) believed to be a risk

No data on plant tests submitted.
In summarizing the past literature, it is assumed that side-effects or any phytopathogenic activity on plants are not expected.

2.6.10 Effects on biological methods of sewage treatment

No data submitted. Pathway does not seem to be relevant.

2.6.11 Effects of secondary metabolites

The literature research revealed that *B. bassiana* produces a wide variety of toxic compounds in artificial culture and, in a few cases, *in vivo*: beauvericin, bassianin and tenellin, bassianolide, bassiacridin, beauveriolides, beauverolides, oosporein, oxalic acid.
A majority of these insecticidal molecules are low molecular weight secondary metabolites, mainly cyclic peptides such as beauvericin and bassianolide.
Concentrations of metabolites in natural environment and their stability are not known. However, most of them are peptides which are usually well degradable. Also oxalic acid, a common product in metabolism, is biodegradable. Bassianin, tenellin and oosporin are non-peptide pigments. The chemical name of oosporin is [Bi-1,4-cyclohexadien-1-yl]-3,3',6,6'-tetrone. Tenellin and bassianin are the 3-[(*E*,*E*)-4,6-dimethylocta-2,4-dienoyl] and 3-[(*E*,*E*,*E*)-6,8-dimethyldeca-2,4,6-trienoyl] derivatives of 1,4-dihydroxy-5-(*p*-hydroxyphenyl)-2(1*H*)-pyridone. Due to the structure biodegradability is assumed for all three substances also.
Nevertheless, the metabolite oosporein might have intolerable effects on insectivorous birds and mammals. Since secondary metabolites are supposed to be concentrated in infected insects, a risk for insectivorous birds and by secondary poisoning via ingestion of infected insects cannot be completely excluded in the case of application in field, but seems to be negligible for greenhouse applications.

2.6.12 Overall conclusion

B. bassiana is common in the environment. The fungus occurs in soil and plants as well as on insects in the aerial environment. *B. bassiana* has been used for biocontrol of many leaf and root feeding pest insects in the past 100 years. Accordingly, there is a long-lasting evolutionary coexistence with other organisms which includes different forms of interactions.
Many laboratory and field experiments are reported in the literature covering many relevant exposure scenarios. Results concerning the adverse effects towards micro-organisms, soil organisms, insects, aquatic organisms and birds are available. With the exception of the pathogenic effects on bumblebees, the risk is assumed to be acceptable. In order to prevent the exposure of bumblebees the RMS proposes to dispose appropriate risk mitigation methods.

Furthermore, studies are available dealing on the persistence in soil and water as well as on the mode of action. The results show that stability in soil and water is influenced by biotic and abiotic factors.
Various strains of *B. bassiana* are known to produce a wide variety of potentially toxic compounds. Most of them are low molecular weight secondary metabolites, mainly cyclic peptides. No information is available concerning the stability of these substances. However, due to their chemical structure it has to be assumed that biodegradation will occur. Since secondary metabolites are supposed to be concentrated in infected insects, a risk for insectivorous birds and by secondary poisoning via ingestion of infected insects cannot be completely excluded in the case of application in field, but seems to be negligible for greenhouse applications.

There are some arguments implicating that the risk for the environment arising from the unintentional transport in the environment might be low:

- The fungus already occurs in the soil.
- There are natural antagonists (e.g. further micro-organisms, arthropods).
- In the past 100 years, *B. bassiana* has been used for biocontrol. Application occurred also in the field. Any detrimental effects of these fungi should have been noticed.
- Infectivity in the field seems to be lower than in laboratory experiments where stress situations for the organisms exist. This observation can be described by the difference between the physiological host range and the ecological host range.
- No negative impacts were observed in field experiments.

Appendix 1

Beauveria bassiana GHA

Standard Terms and Abbreviations

2.7 Appendices

2.7.1 Appendix I: Standard terms and abbreviations

Part 1 Technical Terms

A	ampere
ACH	acetylcholine
AChE	acetylcholinesterase
ADI	acceptable daily intake
ADP	adenosine diphosphate
AE	acid equivalent
AFID	alkali flame-ionisation detector or detection
A/G	albumin/globulin ratio
ai	active ingredient
ALD_{50}	approximate median lethal dose, 50 %
ALT	alanine amitrotransferase (SGPT)
AMD	automatic multiple development
ANOVA	analysis of variance
AOEL	acceptable operator exposure level
AP	alkaline phosphatase
approx	approximate
AR	applied radioactivity
ARC	anticipated residue contribution
ARfD	acute reference dose
as	active substance
AST	aspartate aminotransferase (SGOT)
ASV	air saturation value
ATP	adenosine triphosphate
BCF	bioconcentration factor
bfa	body fluid assay
BOD	biological oxygen demand
bp	boiling point
BSAF	biota-sediment accumulation factor
BSE	bovine spongiform encephalopathy
BSP	bromosulfophthalein
Bt	*Bacillus thuringiensis*
Bti	*Bacillus thuringiensis israelensis*
Btk	*Bacillus thuringiensis kurstaki*
Btt	*Bacillus thuringiensis tenebrionis*
BUN	blood urea nitrogen
bw	body weight
c	centi- (x 10^{-2})
°C	degree Celsius (centigrade)
CA	controlled atmosphere
CAD	computer aided design

CADDY	computer aided dossier and data supply (an electronic dossier interchange and archiving format)
cd	candela
CDA	controlled drop(let) application
cDNA	complementary DNA
CEC	cation exchange capacity
cf	confer, compare to
CFU	colony forming units
ChE	cholinesterase
CI	confidence interval
CL	confidence limits
cm	centimetre
CNS	central nervous system
COD	chemical oxygen demand
CPK	creatinine phosphatase
cv	coefficient of variation
Cv	ceiling value
CXL	Codex Maximum Residue Limit (Codex MRL)
d	day
DAT	days after treatment
DES	diethylstilboestrol
DFR	dislodgeable foliar residue
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
dna	designated national authority
DO	dissolved oxygen
DOC	dissolved organic carbon
dpi	days past inoculation
DRES	dietary risk evaluation system
DT_{50}	period required for 50 percent dissipation (define method of estimation)
DT_{90}	period required for 90 percent dissipation (define method of estimation)
dw	dry weight
DWQG	drinking water quality guidelines
ε	decadic molar extinction coefficient
E_bC_{50}	effective concentration on the biomass
EC_{50}	effective concentration
ECD	electron capture detector
ECU	European currency unit
ED_{50}	median effective dose
EDI	estimated daily intake
ELISA	enzyme linked immunosorbent assay
e-mail	electronic mail
EMDI	estimated maximum daily intake
EPMA	electron probe micro analysis
ERC	environmentally relevant concentration
E_rC_{50}	effective concentration on the growth rate
ERL	extraneous residue limit
F	field
F_0	parental generation

F_1	filial generation, first
F_2	filial generation, second
FIA	fluorescence immuno assay
FID	flame ionisation detector
FOB	functional observation battery
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
fp	freezing point
FPD	flame photometric detector
FPLC	fast protein liquid chromatography
g	gram
G	glasshouse
GAP	good agricultural practice
GC	gas chromatography
GC-EC	gas chromatography with electron capture detector
GC-FID	gas chromatography with flame ionisation detector
GC-MS	gas chromatography-mass spectrometry
GC-MSD	gas chromatography with mass-selective detection
GEP	good experimental practice
GFP	good field practice
GGT	gamma glutamyl transferase
GI	gastro-intestinal
GIT	gastro-intestinal tract
GL	guideline level
GLC	gas liquid chromatography
GLP	good laboratory practice
GM	geometric mean
GMO	genetically modified organism
GMM	genetically modified micro-organism
GPC	gel-permeation chromatography
GPPP	good plant protection practice
GPS	global positioning system
GSH	glutathion
GV	granulose virus
h	hour(s)
H	Henry's Law constant (calculated as a unitless value) (see also K)
ha	hectare
Hb	haemoglobin
HCG	human chorionic gonadotropin
Hct	haematocrit
HDT	highest dose tested
hL	hectolitre
HEED	high energy electron diffraction
HID	helium ionisation detector
HPAEC	high performance anion exchange chromatography
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	high pressure liquid chromatography – mass spectrometry
HPPLC	high pressure planar liquid chromatography
HPTLC	high performance thin layer chromatography

HRGC	high resolution gas chromatography
Hs	Shannon-Weaver index
Ht	haematocrit
I	indoor
I_{50}	inhibitory dose, 50 %
IC_{50}	median immobilisation concentration
ICM	integrated crop management
ID	ionisation detector
IEDI	international estimated daily intake
IGR	insect growth regulator
im	intramuscular
inh	inhalation
ip	intraperitoneal
IPM	integrated pest management
IR	infrared
ISBN	international standard book number
ISSN	international standard serial number
iv	intravenous
IVF	in vitro fertilisation
k	kilo
K	Kelvin or Henry's Law constant (in atmospheres per cubic meter per mole) (see also H)13
K_{ads}	adsorption constant
K_{des}	apparent desorption coefficient
K_{oc}	organic carbon adsorption coefficient
K_{om}	organic matter adsorption coefficient
kg	kilogram
L	litre
LAN	local area network
LASER	light amplification by stimulated emission
LBC	loosely bound capacity
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
LC_{50}	lethal concentration, median
LCA	life cycle analysis
LCLo	lethal concentration low
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, median; dosis letalis media
LDLo	lethal dose low
LDH	lactate dehydrogenase
LOAEC	lowest observable adverse effect concentration
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOEC	lowest observable effect concentration
LOEL	lowest observable effect level
LOQ	limit of quantification (determination)
LPLC	low pressure liquid chromatography
LSC	liquid scintillation counting or counter
LSD	least squared denominator multiple range test

LSS	liquid scintillation spectrometry
LT	lethal threshold
m	metre
M	molar
µm	micrometer (micron)
MC	moisture content
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MDL	method detection limit
MFO	mixed function oxidase
µg	microgram
mg	milligram
MHC	moisture holding capacity
min	minute(s)
mL	millilitre
MLT	median lethal time
MLD	minimum lethal dose
mm	millimetre
mo	month(s)
mol	Mol
MOS	margin of safety
mp	melting point
MRE	maximum residue expected
MRL	maximum residue limit or level
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
n	normal (defining isomeric configuration)
NAEL	no adverse effect level
nd	not detected
NEDI	no effect daily intake (mg/kg body wt/day)
NEL	no effect level
NERL	no effect residue level
ng	nanogram
nm	nanometer
NMR	nuclear magnetic resonance
no	number
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEAEC	no observed environmentally adverse effect concentration
NOEC	no observed effect concentration
NOED	no observed effect dose
NOEL	no observed effect level
NOIS	notice of intent to suspend
NPD	nitrogen-phosphorus detector or detection
NPV	nuclear polyhedrosis virus
NR	not reported

NTE	neurotoxic target esterase
OC	organic carbon content
OCR	optical character recognition
ODP	ozone-depleting potential
ODS	ozone-depleting substances
OM	organic matter content
op	organophosphorus pesticide
Pa	Pascal
PAD	pulsed amperometric detection
2-PAM	2-pralidoxime
pc	paper chromatography
PC	personal computer
PCV	haematocrit (packed corpuscular volume)
PEC	predicted environmental concentration
PEC_A	predicted environmental concentration in air
PEC_S	predicted environmental concentration in soil
PEC_{SW}	predicted environmental concentration in surface water
PEC_{GW}	predicted environmental concentration in ground water
PED	plasma-emissions-detector
pH	pH-value
PHED	pesticide handler's exposure data
PHI	pre-harvest interval
PIC	prior informed consent
pic	phage inhibition capacity
PIXE	proton induced X-ray emission
pK_a	negative logarithm (to the base 10) of the dissociation constant
PNEC	predicted no effect concentration
po	by mouth (per os)
P_{ow}	partition coefficient between n-octanol and water
POP	persistent organic pollutants
ppb	parts per billion (10^{-9})
PPE	personal protective equipment
ppm	parts per million (10^{-6})
ppp	plant protection product
ppq	parts per quadrillion (10^{-24})
ppt	parts per trillion (10^{-12})
PSP	phenolsulfophthalein
PrT	prothrombin time
PRL	practical residue limit
PT	prothrombin time
PTDI	provisional tolerable daily intake
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r	correlation coefficient
r^2	coefficient of determination
RBC	red blood cell
REI	restricted entry interval
R_f	ratio of fronts
RfD	reference dose

RH	relative humidity
RL_{50}	residual lifetime
RNA	ribonucleic acid
RP	reversed phase
rpm	reversed phase material
rRNA	ribosomal ribonucleic acid
RRT	relative retention time
RSD	relative standard deviation
s	second
SAC	strong adsorption capacity
SAP	serum alkaline phosphatase
SAR	structure/activity relationship
SBLC	shallow bed liquid chromatography
sc	subcutaneous
sce	sister chromatid exchange
SD	standard deviation
SE	standard error
SEM	standard error of the mean
SEP	standard evaluation procedure
SF	safety factor
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SIMS	secondary ion mass spectroscopy
SOP	standard operating procedure
sp	species (only after a generic name)
SPE	solid phase extraction
SPF	specific pathogen free
spp	subspecies
sq	square
SSD	sulphur specific detector
SSMS	spark source mass spectrometry
STEL	short term exposure limit
STMR	supervised trials median residue
t	tonne (metric ton)
$t_{1/2}$	half-life (define method of estimation)
T_3	tri-iodothyroxine
T_4	thyroxine
TADI	temporary acceptable daily intake
TAR	total applied radioactivity
TBC	tightly bound capacity
TCD	thermal conductivity detector
TCLo	toxic concentration low
TID	thermionic detector, alkali flame detector
TDLo	toxic dose low
TDR	time domain reflectrometry
TER	toxicity exposure ratio
TER_I	toxicity exposure ratio for initial exposure
TER_{ST}	toxicity exposure ratio following repeated exposure
TER_{LT}	toxicity exposure ratio following chronic exposure

tert	tertiary (in a chemical name)
TEP	typical end-use product
TGGE	temperature gradient gel electrophoresis
TIFF	tag image file format
TLC	thin layer chromatography
Tlm	median tolerance limit
TLV	threshold limit value
TMDI	theoretical maximum daily intake
TMRC	theoretical maximum residue contribution
TMRL	temporary maximum residue limit
TOC	total organic chlorine
Tremcard	Transport emergency card
tRNA	transfer ribonucleic acid
TSH	thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
UDS	unscheduled DNA synthesis
UF	uncertainty factor (safety factor)
ULV	ultra low volume
UV	ultraviolet
v/v	volume ratio (volume per volume)
WBC	white blood cell
wk	week
wt	weight
w/v	weight per volume
w/w	weight per weight
XRFA	X-ray fluorescence analysis
yr	year
<	less than
≤	less than or equal to
>	greater than
≥	greater than or equal to

Part 2 Organisations and Publications

ACPA	American Crop Protection Association
ASTM	American Society for Testing and Materials
BA	Biological Abstracts (Philadelphia)
BART	Beneficial Arthropod Registration Testing Group
CA	Chemical Abstracts
CAB	Centre for Agriculture and Biosciences International
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCFAC	Codex Committee on Food Additives and Contaminants
CCGP	Codex Committee on General Principles
CCPR	Codex Committee on Pesticide Residues
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Food
CE	Council of Europe

CIPAC	Collaborative International Pesticides Analytical Council Ltd
COREPER	Comité des Representants Permanents
EC	European Commission
ECB	European Chemical Bureau
ECCA	European Crop Care Association
ECDIN	Environmental Chemicals Data and Information of the European Communities
ECDIS	European Environmental Chemicals Data and Information System
ECE	Economic Commission for Europe
ECETOC	European Chemical Industry Ecology and Toxicology Centre
ECLO	Emergency Centre for Locust Operations
ECMWF	European Centre for Medium Range Weather Forecasting
ECPA	European Crop Protection Association
EDEXIM	European Database on Export an Import of Dangerous Chemicals
EHC (number)	Environment Health Criteria (number)
EHCD	Environmental Health Criteria Document
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMIC	Environmental Mutagens Information Centre
EPA	Environmental Protection Agency
EPO	European Patent Office
EPPO	European and Mediterranean Plant Protection Organisation
ESCORT	European Standard Characteristics of Beneficials Regulatory Testing
EU	European Union
EUPHIDS	European Pesticide Hazard Information and Decision Support System
EUROPOEM	European Predictive Operator Exposure Model
FAO	Food and Agriculture Organisation of the UN
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
FRAC	Fungicide Resistance Action Committee
GATT	General Agreement on Tariffs and Trade
GAW	Global Atmosphere Watch
GCOS	Global Climate Observing System
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GEDD	Global Environmental Data Directory
GEMS	Global Environmental Monitoring System
GIEWS	Global Information and Early Warning System for Food and Agriculture
GIFAP	Groupement International des Association Nationales de Fabricants de Produits Agrochimiques (now known as GCPF)
GRIN	Germplasm Resources Information Network
HRAC	Herbicide Resistance Action Committee
IARC	International Agency for Research on Cancer
IATS	International Academy of Toxicological Science
IBT	Industrial Bio-Test Laboratories
ICBB	International Commission of Bee Botany
ICBP	International Council for Bird Preservation
ICES	International Council for the Exploration of the Seas
ICPBR	International Commission for Plant-Bee Relationships
ILO	International Labour Organisation

IMO	International Maritime Organisation
IOBC	International Organisation for Biological Control of noxious Animals and Plants
IPCS	International Programme on Chemical Safety
IRAC	Insecticide Resistance Action Committee
IRC	International Rice Commission
ISCO	International Soil Conservation Organisation
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
JECFA	FAO/WHO Joint Expert Committee on Food Additives
JFCMP	Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme
JMP	Joint Meeting on Pesticides (WHO/FAO)
JMPR	Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
NATO	North Atlantic Treaty Organisation
NAFTA	North American Free Trade Agreement
NCI	National Cancer Institute (USA)
NCTR	National Centre for Toxicological Research (USA)
NGO	non-governmental organisation
NTP	National Toxicology Programme (USA)
OECD	Organisation for Economic Co-operation and Development
OLIS	On-line Information Service of OECD
PAN	Pesticides Action Network
RNN	Re-registration Notification Network
RTECS	Registry of Toxic Effects of Chemical Substances (USA)
SCPH	Standing Committee on Plant Health
SETAC	Society of Environmental Toxicology and Chemistry
SI	Systeme International d'Unites
SITC	Standard International Trade Classification
TOXLINE	Toxicology Information On-line
UN	United Nations
UNEP	United Nations Environment Programme
WCDP	World Climate Data Programme
WCP	World Climate Programme
WCRP	World Climate Research Programme
WFP	World Food Programme
WHO	World Health Organisation
WTO	World Trade Organisation
WWF	World Wide Fund for Nature

Appendix 2

Beauveria bassiana GHA

Specific Terms and Abbreviations

2.7.2 Appendix II: Specific terms and abbreviations

DNA	desoxyribonuleic acid
ITS	internal transcribed spacer
MPCA	Microbial pest control agent
MPCP	Microbial pest control product
RAPD	random amplified polymorphic DNA
rDNA	ribosomal DNA
RFLP	Restriction fragment length polymorphism
rRNA	ribosomal RNA
SSCP	Single strand conformation polymorphism
PAS	pure active substance
TAS	technical active substance

Mycological terms

Anamorph	An anamorph fungus only reproduces with asexual spores
Basionym	This name of the fungus is based on the first description of the fungus
Biotroph	The organism is only able to grow on living material and is unable to grow on artificial medium
Ectotrophic	The place of action of the fungus is on the surface of the leaf or root. Growth within the leaf or root is excluded by this term
Epiphyte	A plant that grows on another plant, which it uses as a mechacal support, but not as a sourse of food
Hyphomycete	Fungus with a mycelial form, which bears conidia on separate hyphae or aggregations of hyphae.
Hyperparasite	Parasite, which parasitises other parasites and grow on these
Mitosporic	Sexual reproduction only via mitosis (asexual). Sexual spores (via meiosis have never been found
Mycoparasite	Parasite of fungi
Necotroph	The organism is only able to grow on on death substrate
Phyllosphere	Area immediately surrounding the leaves of a plant
Rhizosphere	Area immediately surrounding the roots of a plant
Saprofyte	Organism which grows on dead organic material
Smut	Phytopathogenic fungus belonging to the Ustilaginales, Basidiomycetes causing smut (brandschimmel)
Yeast	Single celled fungi, which reproduce by budding
	Growth form exhibited in some cases by primary filamentous fungi as a part of the life-cycle or under particular environmental conditions

Appendix 4

Beauveria bassiana GHA

List of End Points

2.7.4 Appendix IV: Listing of endpoints

2.7.4.1 Appendix IV.1: Chapter 1 (identity, biological properties, details of uses, further information and proposed classification and labelling)

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

Active micro-organism:	*Beauveria bassiana* (Balsamo) Vuillemin
Function (e.g. control of fungi):	Control of insects

Identity of the Microbial Pest control Agent / Active substance (OECD data point IIM 1)

Name of the organism:	*Beauveria bassiana* (Balsamo) Vuillemin
Taxonomy:	Phylum: Deuteromycota, Class: Hyphomycetes, Order: Moniliales, Family: Moniliaceae, Genus: Beauveria
Species, subspecies, strain:	*B. bassiana* strain GHA
Identification / detection:	The organism has been identified according to the taxonomic descriptions of de Hoog 1972 (in particular conidiospore morphology) analysed by standard laboratory microscopy.
Culture collection:	*B. bassiana* strain GHA is maintained in the American Culture Collection under ATCC 74250.
Minimum and maximum concentration of the micro-organism used for manufacturing of the formulated product (CFU/g, CFU/L, etc.):	Nominal (mean) purity: 1.4×10^{11} viable CFU/g in the technical material. Acceptable range: 1.24×10^{11} to 1.47×10^{11} viable CFU/g
Identity and content of relevant impurities, in the technical grade micro-organism:	none
Is the MCPA genetically modified; if so provide type of modification	Not applicable

Biological properties of the micro-organism (OECD data point IIM 2)

Origin and natural occurrence, background level:	*Beauveria bassiana* strain GHA is an entomopathogenic fungus which is a common pathogen of many insect species. It is a ubiquitous and cosmopolitan fungus, found in a variety of soils from a broad range of climatic conditions in most countries.
Target organism(s):	Whiteflies (*Aleurodidae*), thrips (*Thysanoptera*) and aphids (*Aphididae*).
Mode of action:	The conidia of *B. bassiana* strain GHA adhere to the insect cuticle by means of hydrophobic interaction between the spore wall and epicuticle lipids. The conidia germinate, and the germ tube penetrates the cuticle, using a specific series of enzymes, which in turn degrade the lipids, protein and chitin in the insect cuticle. In the insect body, the fungus multiplies in the haemocœl as a blastospore,

	or yeast-like cell, and enzymes begin to destroy the internal structures of the host insect causing morbidity within 36 - 72 hours. Reduced feeding and immobility are rapidly evident, and the insect dies within between 4 to 10 days post-infection. The time to death will depend on the insect species, age and conidial dose. After death, the blastospores transform into mycelia, which emerge through the cuticle and form spores. These cover the cadaver as a characteristic white growth. Sporulation occurs only in conditions of high humidity.
Host specificity:	*B. bassiana* is specific to insects. Not pathogenic to humans or plants.
Life cycle:	The conidia adhere to the insect cuticle, germinate and penetrate in the insect body, where they replicate as yeast-like cells (blastospores) and destroy the internal structures, causing morbidity within 36 - 72 hours. After death of the insect, the blastospores transform into mycelia, which emerge through the cuticle and form spores.
Infectivity, dispersal and colonisation ability:	*B. bassiana* strain GHA has an optimal growth temperature range between 23 ºC and 28 ºC. The conidia are not able to germinate or grow at ≥36 ºC. *B. bassiana* strain GHA is stable in water at pH 5, 7 and 9 and has been shown to be unaffected when exposed to metallic ions (Na, Ca, Fe Mg). Detectable effects have only been noted when the fungus is exposed to copper, which is a known fungicide. Under natural conditions, the conidia of *B. bassiana* germinate and die in the absence of a suitable host insect within two days, and in aqueous environments. The conidia survive naturally in sheltered habitats and require specific environmental conditions of moderate temperature, high humidity and high insect population density for epizootic spread and dispersal.
Relationship to known pathogens:	*Beauveria bassiana* strain GHA is not related to any known human or plant pathogen.
Genetic stability:	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.
Production of relevant metabolites/toxins:	Beauvericin: max 5 mg/kg
Resistance/ sensitivity to antibiotics / antimicrobial agents used in human or veterinary medicine:	No specific information addressing 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.

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 applications (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

2.7.1.2 Appendix IV.2: Chapter 2 (methods of analysis)

Methods of analysis

Analytical methods for the micro-organism (OECD data point IIM 4.2, 4.3 and IIIM 5.1)

Manufactured micro-organism (principal of method):	Analytical methods for the identification of the technical *B. bassiana* strain GHA include the following: - Characterisation by microscopic examination of the visual morphology - Restriction Fragment Length Polymorphism (RFLP) profile analysis of chromosomal DNA (cDNA) and Random Amplified Polymorphic DNA analysis (RAPD) - Determination of fungal spore-count and spore viability by haemocytometer counts and *in vitro* germination assay.
Impurities and contaminating micro-organisms in manufactured material (principal of method):	Enumeration of microbial contaminants (bacteria and fungi), determining the presence/absence of enteric bacteria such as *Shigella, Salmonella* and *Vibrio spp.* by plating out on selective media. The method for beauvercin is not sufficiently validated. Moisture analyser balance.
Microbial plant protection product (principle of method):	Analytical methods for the formulation are as used for the active substance.

Analytical methods for residues (viable and non-viable) (OECD data point IIM 4.5)

of the active micro-organism (principle of method)	Validated methods are not required and not available. Nonetheless one method is validated for water with an LOQ of 1 x 10^6 CFU/L.
of relevant metabolites (principle of method):	None relevant

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

with regard to physical/chemical data:	none

2.7.4.3 Appendix IV.3: Chapter 3 (effects on human and animal health) + 4 (residues)

Impact on Human and Animal Health

Medical data: (including medical surveillance on manufacturing plant personnel) (OECD data point IIM 5.1, 5.2)	No adverse effects in employees who had been in occupational contact with strain GHA. For *Beauveria bassiana* in general, few cases of local (eye) and systemic infections were reported with the latter occurring only in immunocompromised or severely ill patients. Evidence of allergenicity by respiratory or skin contact.
Sensitisation: (OECD data point IIM 5.3.1 & IIIM 7.1.6)	Evidence of allergenicity by inhalation in laboratory animals and humans and indications of allergic skin reactions in humans. Buehler test in Guinea pigs negative for the micro-organism but positive for the formulation BotaniGard 22 WP.
Acute oral toxicity, pathogenicity and infectivity (OECD data point IIM 5.3.2 & IIIM 7.1.1)	LD_{50} > 1 x 10^8 CFU/animal in rats; no evidence of toxicity, pathogenicity or infectivity; rapid clearance (by day 3 post dosing)
Acute inhalation toxicity, pathogenicity and infectivity: (OECD data point IIM 5.3.3 & IIIM 7.1.3)	LC_{50} >1 x 10^8 CFU/animal in rats after intratracheal application; no evidence of infectivity but local effects on the lungs (inflammation/immune reaction, organ weight increase), transient reduction in body weight gain; clearance complete by day 7 post dosing
Intraperitoneal/Subcutaneous single dose: (OECD data point IIM 5.3.4 & IIIM 7.1.2)	LD_{50} > 1 x 10^7 CFU in rats; no evidence of toxicity, pathogenicity or infectivity; rapid clearance (by day 3 post dosing)
Genotoxicity: (OECD data point IIM 5.3.5)	No concern (negative in the Ames test)
Cell culture study: (OECD data point IIM 5.3.6)	Not available, not required
Information on short-term toxicity and pathogenicity: (OECD data point IIM 5.3.7)	Not available, not required)
Specific-toxicity, pathogenicity and infectivity: (OECD data point IIM 5.5.1)	Acute dermal study in rabbits: LD_{50} > 1.6x 10^{11} CFU; no systemic effects but signs of slight but persisting local irritation; Certain potential of the micro-organism (strain not specified) to damage the cornea confirmed in rabbits; Long-term inhalative exposure to *B. bassiana* (strain not specified) caused allergic lung reactions in rats and mice.
In vivo studies in somatic cells: (OECD data point IIM 5.5.2)	Not available, not required
Genotoxicity – *in vivo* studies in germ cells: (OECD data point IIM 5.5.3)	Not available, not required
Metabolites	Toxicological studies with strain GHA including a beauvericin and bassianolide contamination each with of approx. 50 mg/kg indicated no additional adverse effects after oral, dermal or ip administration. Effects seen after intratracheal administration are unlikely to be due to beauvericin and bassianolide. Beauvericin was not mutagenic in an Ames test. Exposure with (the sum of) the metabolites should stay below 1.5 µg/d (threshold of toxicological concern).

Exposure (operator, workers, bystander, consumer): (OECD data point IIM 6.1, IIIM 7.2, 7.3 and 8.0)	Operator Due to the lack of a medium-term tolerable inhalation exposure level, a comparison with the estimated exposure is not possible. However, calculated inhalation and dermal exposure values are considerably below the observed acute LOAELs. It can be assumed that operators would not be at acute risk during mixing/loading and application of BotaniGard 22 WP. Although situations of repeated exposure at subacute dose levels are not covered by the above considerations, it can be assumed that the estimated operator exposure is acceptable provided appropriate RPE is worn (necessary due to classification/labelling with Xn, R 42). In addition, precautionary the use of suitable gloves as well as wearing suitable protective clothing is considered necessary to minimise the risk of skin sensitisation because BotaniGard 22 WP is classified and labelled with R 43. In addition, operators should be informed in a suitable way that eye infections were observed after mechanical injury of the cornea. Taking into account the possible production of the metabolite beauvericin at a level of max. 50 mg/kg the operator exposure is unlikely to exceed the proposed conservative threshold of toxicological concern (TTC). In order to consider all metabolites sufficiently it is proposed to specify beauvericin at a maximum level of the technically feasible 5 ppm Bystander Bystander exposure is not relevant for the intended uses (greenhouse). Worker After the spray solution has dried, the estimated exposure to Beauveria bassiana strain GHA during re-entry operations does not exceed acceptable values, even if no PPE is worn. Nevertheless, precautionary the use of suitable gloves as well as wearing suitable protective clothing is considered necessary to minimise the risk of skin sensitisation because BotaniGard 22 WP is classified and labelled with R 43. In addition, it is concluded that the worker exposure to the metabolite beauvericin is unlikely to exceed the proposed conservative threshold of toxicological concern (TTC). Consumer Non viable residues: Acute Risk assessment: intake of beauvericin via tomatoes is 17 % of the TTC value. Chronic risk: negligible Viable rsidues: It is unlikely that viable residues of B. bassiana will occur in concentrations considerably higher than under natural conditions on harvested food and feed.

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

with regard to toxicological data:	Xn, R42/43

Residues (normally not required)

It is proposed to use the beauvericin content in the technical material as an indicator for the presence of the other metabolites. The maximum content of beauvericin in the technical material should be specified on 5 mg/kg.
Since a risk to consumers is not expected arising from the use of *Beauveria bassiana GHA* neither a residue definition nor an MRL are considered necessary.

2.7.1.4 Appendix IV.4: Chapter 5 (fate and behaviour in the environment)

Fate and Behaviour in the Environment (OECD IIM 7 & IIIM 9)

Persistence and multiplication

in soil:	It is difficult to regard the persistence only with regard to soil, because multiplication does take place in the hosts. A low remaining residual level in soil can be enough for restarting of replication on occurrence of a host so that from its cadaver new spores can reach the soil again. Germination and multiplication of entomopathogenic fungi in natural, non-sterile soils can be excluded as long as no potential hosts are present, since microorganisms living in soil must have the ability to degrade heavily degradable and unsolvable substances like lignin and humus. Furthermore the effect of fungistasis inhibits germination of conidia in non-sterile soils.
in water:	In an aquatic environment spores will be subject to sedimentation and can be expected to degrade rapidly in natural water bodies. The conidia did not germinate in the different tested aqueous systems.
in air:	In the absence of a specific host insect, conidia of *B. bassiana* will not persist in air for more than 2 days.
Mobility:	**In soil:** Conidia of *B. bassiana* are not very mobile in soil and generally remain on the surface of the soil. Therefore a contamination of the groundwater can be excluded. **In water:** The globose or ovoid shape of conidia is not suitable for efficient transport in water. **In air:** *Beauveria bassiana* is dry, of small size and it is produced in powdery clusters. Therefore, these types of conidia can be easily transported through the air. Also transmission of fungal spores through the air by insects is possible. However, all studies principally indicate that *Beauveria bassiana* is not expected to persist in air as the viability of conidia of *Beauveria bassiana* is greatly reduced for a period greater than 24 hours. Furthermore, in the absence of a specific host insect, conidia of *Beauveria bassiana* will not persist in air for more than 2 days..

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

with regard to fate and behaviour:	Not applicable

2.7.1.5 Appendix IV.5: Chapter 6 (effects on non-target organisms)

Effects on non-target organisms (OECD IIM 8 & IIIM 10)

Effects on terrestrial vertebrates

Risk assessment for mammals:	See effects on other terrestrial vertebrates
Effects on birds:	LR_{50}: > 2.5 x 10^7 CFU /g body weight, no effects on body weight, behaviour (*Falco sparverius*)
Risk assessment:	The calculated Margin of safety (TER_{ST}) values for field exposure (leafy crops/cereals/grass) of insectivorous and small and medium herbivorous birds were between > 6173 and > 34293. They exceed clearly the trigger value of 10 as described in Annex VI part I of Directive 91/414/EEC for all scenarios. Thus, no adverse effects on birds in short-term scenarios (and in acute scenarios) are expected following application of BotaniGard 22 WP at recommended use rates.
Effects on other terrestrial vertebrates (mammals):	LD_{50}: > 5000 mg BotaniGard 22WP/ kg bw corresponding to > 2.2 x 10^{14} CFU/ kg bw (rat)
Risk assessment:	On the basis that the product has very low mammalian toxicity, *Beauveria bassiana* strain GHA cannot grow at mammalian body temperatures (the *in vivo* growth temperature of the fungus is below 36 °C which prevents it from growing at the higher body temperature of mammals) and thus cannot infect mammalian hosts, and is not pathogenic to mammals, no short- or long-term effects are to be anticipated. Furthermore, sensitivity to low pH values encountered in the stomach of mammals renders survival and colonisation of the mammal's interior via ingestion unlikely. The LD_{50} value of > 2.2×10^{14} CFU /kg bw is chosen for an acute risk assessment. The margin of safety (TER_A) values for small and medium herbivorous as well as insectivorous mammals are > 9×10^6 and greater than the trigger of 10 as described in Annex VI part I of Directive 91/414/EEC indicating that there is no unacceptable risk for mammals from direct exposure to the spores following application of BotaniGard 22 WP according to Good Agricultural Practice. Since secondary metabolites are supposed to be concentrated in infected insects, a risk for insectivorous birds and by secondary poisoning via ingestion of infected insects cannot be completely excluded in the case of application in field, but seems to be negligible for greenhouse applications.

Effects on aquatic organisms

Effects on fish:	NOEC < 7.5 x 10^8 CFU/L initial (effects on growth < 25 %) no evidence of infectivity or pathogenicity (*Pimephales promelas)*
Risk assessment:	The actual predicted environmental concentration ($PEC_{SW\ actual}$) of *Beauveria bassiana* resulting from input *via* this route was initially estimated. The calculation was based on five accumulated applications of BotaniGard 22WP (550 g *Beauveria bassiana GHA*/ha), assuming no degradation between applications and an

	entry resulting from spray drift at 1 m of 2.77 % of 0.1 % of the application rate according to Rautmann et al. (2001). The $PEC_{SW\ ini}$, is 0.0254 µg as/L. In terms of CFU, this is equivalent to 1118 CFU/L. The calculated margin of safety (TER_{LT}) is 313059 exceeding clearly the limit value of 10. Thus, no adverse effects on fish are expected after application of BotaniGard 22WP at recommended use levels.
Effects on freshwater invertebrates:	NOEC = 4.7×10^8 CFU/L real (sublethal effects) EC_{50} > 9.3 x 108 CFU/L (*Daphnia magna*)
Risk assessment:	The actual predicted environmental concentration ($PEC_{SW\ actual}$) of *Beauveria bassiana* resulting from input *via* this route was initially estimated. The calculation was based on five accumulated applications of BotaniGard 22WP (550 g *Beauveria bassiana GHA*/ha), assuming no degradation between applications and an entry resulting from spray drift at 1 m of 2.77 % of 0.1 % of the application rate according to Rautmann et al. (2001). The $PEC_{SW\ ini}$, is 0.0254 µg as/L. In terms of CFU, this is equivalent to 1118 CFU/L. The calculated Margin of safety (TER_{LT}) is 4203934 exceeding clearly the limit value of 10. Thus, no adverse effects on fish are expected after application of BotaniGard 22WP at recommended use levels.
Effects on algae:	*Selenastrum capricornutum*, 3 days, static, nominal E_bC_{50} 114000 µg/L (0.98×10^{13} CFU/L) E_rC_{50} 237000 µg/L (2.04×10^{13} CFU/L) NOEC 75000 µg/L (2.64×10^{13} CFU/L) (all parameters)
Risk assessment:	The actual predicted environmental concentration ($PEC_{SW\ actual}$) of *Beauveria bassiana* resulting from input *via* this route was initially estimated. The calculation was based on five accumulated applications of BotaniGard 22WP (550 g *Beauveria bassiana GHA*/ha), assuming no degradation between applications and an entry resulting from spray drift at 1 m of 2.77 % of 0.1 % of the application rate according to Rautmann et al. (2001). The $PEC_{SW\ ini}$, is 0.0254 µg as/L. In terms of CFU, this is equivalent to 1118 CFU/L. The calculated Margin of safety (TER_{LT}) is $8{,}6 \times 10^9$ exceeding clearly the limit value of 10. Thus, no adverse effects on fish are expected after application of BotaniGard 22WP at recommended use levels.
Effects on aquatic plants:	Not performed, not required
Risk assessment:	no

Effects on arthropods

Effects on bees:	In a study under practical conditions *Beauveria bassiana* strain GHA had no or negligible effects on honey bees. No treatment related increased mortality, latent infections or pathogenic effects on worker bees or bee brood could be observed . However, bees are not exposed when *Beauveria bassiana* strain GHA is used as

	recommended for indoor or glasshouse application.
Effects on bumblebees:	**Bumblebees: LC_{50}** < 2.5 x 10^{10} CFU/L (pathogenic effects)
Risk assessment:	**Bumblebees:** The concentration of the active ingredient *Beauveria bassiana* (GHA) bumble bees were exposed to (2.5 x 10^{10} CFU/L) in the study is approx. the half of the concentration of *Beauveria bassiana* (GHA) in the spray mixture used in field (4.84 x 10^{10} CFU/L). Conditions for hives in the ground are likely to offer even more conductive temperature and humidity conditions for these fungal pathogens making a spread of infection in the hives more probable. Therefore it can be concluded that *Beauveria bassiana* (GHA) poses an unacceptable risk to bumblebees at the intended application rate.

Effects on terrestrial arthropods other than bees:

Species	Stage	Test Substance	Dose (kg MPCA/ha) (CFU/ha)	Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)
Aphidius rhopalosiphi	adult	BotaniGard 22 WP BotaniGard ES	0.947 (1.33 x 10^{14} CFU/ha) 1.695	Mortality: -5 % Reproduction: 20 % Mortality: -5 % Reproduction: 20 %
Typhlodromus pyri	protonymph	BotaniGard 22 WP BotaniGard ES	0.947 (1.33 x 10^{14} CFU/ha) 1.695	Mortality: 4 % Reproduction: 43 % Mortality: -4 % Reproduction: 34 %
Orius laevigatus	nymph	BotaniGard 22 WP BotaniGard ES	0.952 (1.33 x 10^{14} CFU/ha) 1.693	Mortality: 3 % Reproduction: 23 % Hatching succ: 24 % Mortality: 11 % Reproduction: -7 % Hatching succ: 29 %
Pardosa spec.	adult	BotaniGard 22 WP BotaniGard ES	No sufficient information No sufficient information	Mortality: 80 % Mortality: 45 %
Further information				
Melanoplus sanguinipes		Mycotrol ES	1.75 x 10^{13}/ha to rangeland 3.5 x 10^{13} conidia/ha to alfalfa	97.9 % lethal infections Logarithmically decrease of B. bassiana (less than 10 % after 2 days) Rangeland: minor and short-lived impact on non-target arthropods Alfalfa: tempory increases of B. bassiana in coccinelid beetles and harvestmen; small (< 20 %) or no

				increase in spiders and other insects; infection of a small number of bees under field conditions. No infection of leafcutting bee larvae, prepupae or adult emergence following overwintering diapause.
Eretmocerus sp.		Mycotrol WP	$5 * 10^{13}$ CFU/ha ($2 * 10^{13}$ CFU/acre)	No statistical significant effects
Xylocoris flavipes	nymphs	*Beauveria bassiana GHA*	Filter paper: 2.5×10^{13}, 2.5×10^{14}, 2.5×10^{15} CFU/ha	0, 16, 41 % infection after 10 days
Tenebrio molitor	adult	*Beauveria bassiana GHA*	$2 * 10^{4}$ CFU, $2.4 * 10^{8}$ CFU	No significant mortality (100 % mortality of target organism *Melanoplus sanguinipes* at $2 * 10^{4}$ conidia/ha)

Risk assessment:

B. bassiana (GHA) was shown to be infective to the exposed arthropods under the specific laboratory conditions. All studies were conducted as limit tests. In the case of the studies with *T. pyri* and *O. laevigatus* significant adverse effects have been observed. These effects are considered to be caused by pathogenicity of *B. bassiana* (GHA).

According Annex VI part II of Directive 91/414/EEC pathogenic effects must not occur, consequently no effect concentrations have to be used for risk calculation. As it is not possible to determine no effect concentration from the submitted studies, the risk assessment on the basis of the HQ-approach is not applicable.

Based on the submitted field studies it can be concluded that there is a significant difference between the physiological host range (laboratory conditions) and the ecological host range (field conditions). Due to the fact that *Beauveria bassiana* (GHA) needs a microclimate of very high humidity in order to cause an infection, many arthropods being susceptible on the conditions of the laboratory test are not affected in the field. Furthermore the exposure of non-target-arthropods after greenhouse applications appears to be negligible. Thus the risk of *Beauveria bassiana* (GHA) to non-target arthropods (with an exception of bumblebees) is considered to be acceptable.

Effects on soil organisms

Effects on other terrestrial invertebrates:	LC_{50} (Mortality) : > 1000 mg *Beauveria bassiana* strain GHA /kg artificial soil > 8.6×10^{10} CFU/ kg artificial soil (*Eisenia foetida*)
Risk assessment:	Margin of safety (TER) > 538 - > 896
Effects on soil micro-organisms:	No studies submitted.
Risk assessment:	Due to the natural occurrence, the presence of antagonists and lacking observation of uncontrollable growth of *B. bassiana* in soil resulting in intolerable effects considering microbial diversity in soil, the risk is assumed to be acceptable.

Additional studies

none

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

With regard to ecotoxicological data:	Not applicable

Level 3

Beauveria bassiana GHA

Proposal for the Decision

3 Proposed decision with respect to the application for inclusion of the MPCA in Annex I

3.1 Background to the proposed decision

Identity of the active substance

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.

Data on application

Beauveria bassiana strain GHA is used for biological control of sucking insects. The evaluation is based on the intended uses in tomatoes, cucumbers and ornamentals with an application rate of maximal 550 g as/ha containing 4.84 x 10^{10} *Beauveria bassiana* conidia/L spray solution. BotaniGard 22 WP should be applied when pest populations are manageable and before extensive damage occurs to crops. Number of applications: minimum 3 and maximum 5. Interval between applications 5 - 7 days.

Analytical methods for formulation analysis

Analytical methodology is available for the determination of the micro-organism in the technical material as manufactured. To detect spontaneous changes in major characteristics of micro-organisms the identification of *B. bassiana* is determined by Restriction Fragment length polymorphism (RFLP) analysis, morphological examination and classification according to the taxonomic key.
Since 2003 a new Random Amplified Polymorphic DNA analysis (RAPD) is available to confirm the GHA strain.

Analytical procedures used to determine the spore count and the microbial contaminants in the product were provided.
The spore count of *B. bassiana* in the plant protection product is determined by using haemocytometer and enumeration of microbial contaminants and determining the presence/absence of enteric bacteria such as *Shigella*, *Salmonella* and *Vibrio spp.* by plating out on selective media.

Analytical methods for residue analysis

Analytical methods for residue analysis are not required (no residue definition and no MRL's were set or proposed for food, feedstuff, soil, water or air).

Toxicology and metabolism

Beauveria bassiana strain GHA proved non-infective and non-pathogenic. Clearance of the micro-organism from the animal body was rapid and complete. There was clear evidence of local toxic effects on the lungs. In addition, there were transient systemic effects. At least partly, these effects might be due to an immunological reaction of the host. Based on experience in humans and in laboratory animals, the micro-organism must be considered a

potential inhalation and skin allergen. There is no concern about mutagenicity with relevance to humans.
There is only little information available on the toxicological properties of metabolites. It is known, that the metabolite beauvericin was not mutatgenic in an Ames Test.
The available studies can not be used to derive safe levels for metabolite exposure. Therefore, it is proposed to use the concept of the threshold of toxicological concern (TTC). The TTC of 1.5 µg/d is considered as a safe exposure for the sum of all metabolites.

No risk for operator and worker was indicated by the risk assessment.
Assessment of operator and worker exposure to beauvericin was performed with a concentration of 50 ppm in the MPCA. This is supposed to represent the overall content of beauvericin and the other toxins which might have occurred in the technical material. The beauvericin content could be used as an indicator for the presence of the other metabolites.

Residue data

The half-life of *Beauveria bassiana* in the field is very short. Under natural conditions, conidia perish within few days following germination in the absence of a suitable host insect.
Viable residues of *Beauveria bassiana* on crops are expected to be short-lived and concentrations dissipate to non-infective levels within few days.

Various strains of *Beauveria bassiana* have been shown to be capable of producing a range of mycotoxins, e.g. beauvericin. Since *Beauveria bassiana* does not show any metabolic activity in the absence of a suitable host no formation of mycotoxins in food items is expected in this case.

But the metabolites might occur in technical material. Based on a TTC approach, no acute and/or chronic adverse effects for consumers were indicated, if a beauvericin concentration of 50 ppm is used for consumer risk assessment. This is supposed to present the overall content of beauvericin and the other mycotoxins. To account for beauvericin and the other metabolites, it is therefore considered sufficiently conservative to base the consumer risk assessment on a concentration of 50 ppm while at the same time lowering the specification for beauvericin in the batches of the MPCA to levels as low as technically feasible (5 ppm).

Since a risk to consumers is not expected arising from the use of *Beauveria bassiana GHA* neither a residue definition nor an MRL are considered necessary.

Environmental fate and behaviour

Beauveria bassiana strain GHA can be present naturally in the environment, background levels of this organism vary from 0 (below detection limits) to 7.0 x 10^5 CFU/mL. Post-treatment levels vary from 0 to 4.4 x 10^8 CFU/mL. It is difficult to consider persistence only with regard to soil, because multiplication does take place in the hosts. Germination and multiplication of entomopathogenic fungi in natural, non-sterile soils can be excluded as long as no potential hosts are present. In this respect, there is no indication of accumulation. Conidia of *Beauveria bassiana* are not very mobile in soil and generally remain on the surface of the soil. The movement of conidia vertically, through the soil profile, is positively correlated with high infiltration rates in soil. Considering the fate data and the intended use a contamination of the groundwater can be excluded.

Conidia did not germinate in the different tested aqueous systems. Therefore multiplication in water is not expected, since *B. bassiana* is no aquatic fungus and is therefore not adapted to the conditions of the aqueous environment. It may be concluded that conidia of *B. bassiana* in water will be degraded by bacteria and protozoa in natural non-sterile water bodies, rapidly. *Beauveria bassiana* is not expected to persist in air as the viability of conidia of *Beauveria bassiana* are greatly reduced following exposure to sunlight for a period greater than 24 hours. Furthermore, in the absence of a specific host insect, conidia of *Beauveria bassiana* will not persist in air for more than 2 days.

Ecotoxicology

One short-term dietary study with American kestrel (*Falco sparverius*) was submitted. Together with further literature data little indication on infectivity, pathogenicity and toxicity to birds is given. The same can be stated for mammals, where also no mortalities or sublethal effects occurred in the submitted study on acute oral toxicity in rats. Since secondary metabolites are supposed to be concentrated in infected insects, a risk for insectivorous birds and mammals by secondary poisoning via ingestion of infected insects cannot be completely excluded in the case of application in field but seems to be negligible for greenhouse applications.

On the basis of the submitted studies *Beauveria bassiana* strain GHA is not expected to have any adverse effects on algae. Mild toxic effects were observed in studies on fish and presumably *Daphnia.* The risk assessment for outdoor and indoor uses leads to margins of safety (TER) clearly above the Trigger values set by Annex VI part I of Directive 91/414/EEC. No adverse effects on aquatic organisms are expected after application of BotaniGard 22WP at recommended use levels. Due to the high margin of safety derived from risk assessment no further data on fish or aquatic invertebrates seem to be necessary despite the observed effects. On the basis of the submitted studies *Beauveria bassiana* strain GHA is not expected to have any adverse effects on non-target arthropods, microorganisms and soil dwelling organisms or plants. Data from the literature however show that there are significant pathogenic effects on bumblebees exposed to *Beauveria bassiana* strain GHA at the recommended application rate. In order to prevent the exposure of bumblebees the RMS proposes to dispose appropriate risk mitigation methods.

3.2 Proposed decision

[redacted]

3.3 Rational for the postponement of the decision, or for the conditions and restrictions to be associated with a proposed inclusion in Annex I, as appropriate

[redacted]

The information in sections 3.2 and 3.3 has been removed upon request by the EU Commission as it relates to risk management recommendations or proposals.

Level 4

Beauveria bassiana GHA

Demand for Further Information

4 Further information to permit a decision to be made, or to support a review of the conditions and restrictions associated with the proposed inclusion in Annex I

4.1 Identity of the MPCA or MPCP

None

4.2 Biological properties of the MPCA and physical, chemical and technical properties of the MPCP

Annex IIIM, point 2.2
Information on the physical stability of the formulation is missing.
Justification:
Only information on the biological stability have been submitted.

Annex IIIM, point 2.3.1
Clarification about the explosive and oxidising properties of the formulation.
Justification:
Only a statement in document M-III was submitted yet. It is not clear, what properties are referred to in this statement.

Annex IIIM, point 2.4.4
A study on wet sieve test according to CIPAC MT 59.3 or MT 185 is missing.
Justification
This is a data requirement of 91/414/EG and according to FAO (2006).

4.3 Data on application and further information

None

4.4 Classification, packaging and labelling

None

4.5 Methods of analysis

Analytical methodes for formulation analysis
None

Analytical methods for formulation analysis
Annex IIM, point 4.3.5:
For the analysis of beauvercin and impurity 2 validation data in terms of linearity (either duplicate determinations at three or by single determinations at 5 or more concentrations) and repeatability (5 replicate sample determinations for each analyte) is missing. Furthermore, the

LOQ for beauvericin is too high taken the proposed specified maximum limit of 5 ppm into account.
Justification:
The linearity was just measured by single determinations at four concentrations.
The repeatability was just demonstrated for a fourfold sample determination. Additionally the obtained value for the repeatability is too high according to the Horwitz equation.

Analytical methods for residue analysis
None

4.6 Toxicology

None.

4.7 Residue data

None

4.8 Environmental fate and behaviour

Annex IIM, point 7.1
Survivability of *B. bassiana* in greenhouse cotton soil is reported. The results are presented as "CFU". It is not clear to which amount of soil the CFU refer to (per gram?)
The results have to be specified.

4.9 Ecotoxicology

Annex IIIM, point 10.4
Non-target arthropods
In the study Hoogendoorn, G (2000) (*BotaniGardTM: an extended laboratory test to determine effects of two formulations of Beauveria bassiana on spring generation spiders of the genus Pardosa; ANA 2006-210*) the applied amount of liquid is only reported for the first application. The further amounts are not reported. For the risk assessment the information of all applied amounts are necessary.
Information about all applied amounts are missing.