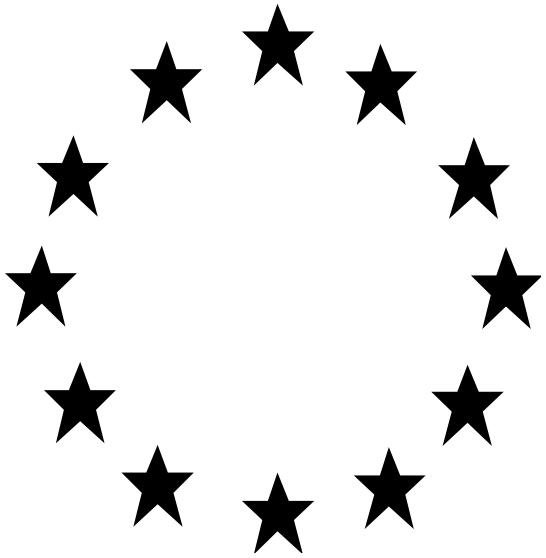

Draft Assessment Report (DAR)

- public version -

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

BEAUVERIA BASSIANA GHA

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

Volume 3, Annex B, part 5, B.9

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Annex B

Beauveria bassiana GHA

B-9: Ecotoxicology

B.9 Ecotoxicology data and assessments of risks for non-target species (OECD IIM 8, IIIM 10)

General information

Mode of action

The infection pathway consists of the following steps: 1. Attachment of the spore to the cuticle, 2. Germination, 3. Penetration through the cuticle, 4. Overcoming the host response and immune defence reactions, 5. Proliferation within the host by formation of hyphal bodies or blastospores, i.e. yeast like cells and 6. Saprophytic outgrowth from the dead host and production of new conidia.
The incubation period depends on the host, the host stage, temperature and virulence of the fungus strain. In aphids, it may take 3 - 4 days, while in white grubs of scarabs, 2 - 4 weeks are needed. After the death of the host and under humid conditions, the fungus starts its saprophytic growth emerging out of the host body. Conidia are produced outside the cadaver. Under very dry condions, the fungus may also persist in the hyphal stage inside the cadaver or, e.g. in locusts in Africa, and produce its conidia inside the body.
During the incubation period, the fungus may already affect its host insect by behavioural and feeding changes, the reduction of body weight or fecundity, by malformations or by the increase of temperature (Müller-Kögler 1965, Ekesi 2001, Ouedraogo et al. 2003 cited in Zimmermann 2007).

Production and exposure of secondary metabolites (toxins)

Beauveria bassiana is known to produce a variety of toxins. In order to assess the risk coming from these toxins, the time of their production in relation to the life cycle of fungi and therefore the exposure should be assessed.
It is important to be aware that almost all toxins produced by fungi belong to the group of secondary metabolites. Secondary metabolites are produced during the changeover from the logarithmic growth stage (log stage) of a micro-organism to the steady-state stage, when micro-organisms stop growing because of several reasons, e.g. the lack of nutrients and oxygen, abiotic stress, limited space and so on. With beginning growth (corresponding with the lag stage) and the log stage, micro-organisms mainly produce primary metabolites that are non-toxic and that are produced by every living organism. Secondary metabolites are synthesized on the basis of primary metabolites declining growth speed and reduction of primary metabolism. (Madigan 2001, page 430-432).
Environmental conditions inhibiting the growth of conidia and the building of mycelium, therefore, prevent the synthesis of secondary metabolites, since during the dormancy of conidia, metabolism is inhibited in general.
Dormancy continues as long as the environmental conditions remain unchanged.
In the case of the entomopathogenic fungus *B. bassiana* the germination of conidia starts with the contact between conidia and a suitable host, provided that the temperature range is between 0 °C and 38 °C (Müller-Kögler 1965; Roberts & Campbell 1977 cited in Zimmermann 2007) and especially the microhumidity at the surface of the host is sufficiently high (100 – 92 %) (Prior et al. 1988; Bateman et al. 1993; Vidal et al. 2003 cited in Zimmermann 2007).
The effect of fungistasis in non-sterile soils inhibits the germination of conidia. This has been shown by Watson & Ford, 1972 (cited in Zimmermann 2007). A widespread fungistasis in

soils was found and postulated about 50 years ago (Dobbs & Hinson 1953 cited in Zimmermann 2007). According to Clerk, 1969 (cited in Zimmermann 2007), several authors have reported that conidia of *B. bassiana* are subject to fungistatic effects in natural soils. However, the nature of the inhibitor(s) responsible for soil fungistasis is still unknown, although several authors consider that inhibitory substances released by soil micro-organisms play a major role.
From this, it can be concluded that secondary metabolites including fungal toxins cannot be produced as long as the germination of conidia is inhibited, e.g. in natural soils.
Conidia of *B. bassiana* start germinating when in contact with the cuticle of a suitable host insect. They penetrate the cuticle and proliferate within the host by formation of hyphal bodies or blastospores. These steps correspond with the lag-stage in the beginning and the log-stage, when the fungus grows through the body of the insect. The fungal growth enters the steady-state stage, when all nutrients have been consumed and the fungus has been grown out of the dead insect and produces new conidia. At this point of time, secondary metabolites (fungal toxins) are mainly produced.
In the case of *B. bassiana* and possibly also other entomopathogenic fungi, the highest concentrations of toxins that can occur in nature are expected to be found in the host insects.

Secondary metabolites produced by *B. bassiana*

The following secondary metabolites are known to be produced by different strains of *B. bassiana*:

Beauvericin is the most important metabolite; it is also a common metabolite of many phytopathogenic *Fusarium* species. It is a toxic cyclic hexadepsipeptide comprising a cyclic repeating sequence of three molecules of N-meethyl phenylalanine alternating with three molecules of 2-hydroxyisovaleric acid. Investigations on beauvericin have demonstrated that this metabolite has insecticidal, antibiotic, cytotoxic, and ionophoric properties.
Bassianin and Tenellin are two yellow-coloured non-peptide secondary metabolites which inhibit the erythrocyte membrane ATPases (Jeffs & Khatchatourians (1997) cited in Zimmermann 2007).
Bassianolide is a cyclo-octadepsipeptide produced by *B. bassiana* with ionophoric and anti-biotic activity similar to beauvericin (Strasser et al. (2000), Vey et al. (2001) cited in Zimmermann 2007).
Bassiacridin is a toxic protein which was purified from a strain of *B. bassiana* infecting locusts (Queseda-Moraga & Vey (2004) cited in Zimmermann 2007). Injection of fourth instar nymphs of *Locusta migratoria* with the pure protein at relatively low dosage (3.3 µg toxin/g body weight) caused approx. 50 % mortality. This insecticidal protein showed specific activity against locusts and has a limited similarity to a chitin binding protein from yeasts.
Beauveriolides and **Beauverolides** are peptides with a similar structure to beauvericin and bassianolide (Namatame et al., (1999 and 2004) cited in Zimmermann 2007).
Oosporein is the major secondary metabolite produced by *B. brongniartii,* but is also known to be produced by *B. bassiana*. A comprehensive overview on oosporein is presented by Strasser et al. (2000) and Vey et al. (2001) cited in Zimmermann 2007). This red-coloured pigment is a dibenzoquinone, which is also produced by many soil fungi. It is an antiviral compound and has antibiotic activity against gram-positive bacteria, but little effect on gram-negative bacteria. Obviously, oosporein has no antifungal and phytotoxic effects. It has been reported to cause avian gout in broiler chicks and turkeys and has been found to be toxic to 1-day-old chicks (LD_{50} = 6 mg/kg). Studies on its toxicity in mice and hamsters indicated an

LD_{50} value of 0.5 mg/kg body weight after intraperitoneal injection and a NOEC value of 7 mg/kg*d in a long term oral study (42 d) (see Strasser et al. 2000; Vey et al. 2001 and Wainwright et al. 1986 cited in Strasser et al. 2000). In vivo and in vitro studies on the distribution of oosporein in the environment revealed negligible amounts. The maximum amount of oosporein produced in a culture medium was 300 mg/L, in the commercial product 'Melocont®-Pilzgerste' 3.2 mg/kg, in a mycosed larva 200 µg, in soil enriched with the commercial product 0.02 mg/m^2, and in soil enriched by mycosed larvae 6.4 mg/m^2 (Strasser et al. 2000). The results demonstrate, that the quantities of secondary metabolites produced in vivo by these fungi are usually much smaller than those secreted in nutrient-rich liquid media.
Oxalic acid is secreted by *B. bassiana* and *B. brongniartii* (Müller-Kögler 1965; Roberts 1981 cited in Zimmermann 2007) and is considered to be an important pathogenicity determinant (Vey et al. 2001 cited in Zimmermann 2007).

Toxic metabolites as beauvericin, bassianolide and oosporein are produced during the infection process, in order to overcome the insect defence response and lead to immunosuppression or titanic paralysis. Further more those metabolites may be involved in the competitive exclusion of competing microorganisms from the cadaver (Duperchy 2003).

B.9.1 Effects on birds (Annex IIM 8.1; Annex IIIM 10.1)

B.9.1.1 Active substance

Reference:	**IIM 8.1**
Author:	McEwen, L.C.
Title:	Response of young American kestrels (*Falco sparverius*) to *Beauveria bassiana* strain GHA.
Date:	1993
Doc ID:	Report No. 93-012, BVL No. AVS2006-168
Guideline:	US EPA Guideline 154A-16
GLP:	Not documented
Validity:	Acceptable (not valid according to guideline), results plausible, some raw data missing

Material and methods:

Micro-organism:	*Beauveria bassiana* (strain GHA)
Test species:	*Falco sparverius*
Number of test animals:	4
Treatments:	5 x 10^{10} conidia/mL (sun oil); 5 x 10^6 or 2.5 x 10^7 conidia/g body weight
Duration:	5 d dosing / 18 d observation

Test conditions: A study was conducted with young American kestrels (*Falco sparverius*), which is representative in behaviour as both an insectivorous bird and a raptor. In this study, 3 birds (8-16 days old) were randomly selected from each of 13 nest boxes (55 birds in total), and dosed with a single dose each of one of the following: *Beauveria bassiana* strain GHA in carrier (paraffin) oil with 5 x 10^{10} conidia/mL, carrier oil alone, and corn-oil (control groups). The birds were dosed orally with 1 or 5 µL of solution containing 5 x 10^{6} and 2.5 x 10^{7} conidia/g body weight respectively by capsule at the higher dose for the challenge group and by syringe for the remainder. Behavioural patterns and growth rates were observed over a period of 5 - 18 days post dosing. These included observation of a righting reflex and visual awareness. When the birds had reached 26 - 31 days of age, transmitters were attached and the birds' behaviour and travelling distances from the boxes observed at random times daily or on alternate days.

Deviations from guideline: None of the requirements of the guideline were met. Examinations of infectivity and pathogenicity were not conducted. Results of necropsy were not reported.

Endpoint: Mortality, body weight gain

Observations: Behavioural patterns, growth rates, righting reflexes, visual awareness, gross pathology.

Results:

There were no mortalities related to *Beauveria bassiana* strain GHA in any groups. There were no differences between treated groups in terms of body weight gain and behavioural patterns or movements, 7 birds, aged 31 - 42 days were collected for examination. Two additional birds were found dead. Necropsy showed that no visible gross pathology was evident. A summary of endpoints is given in the table below.

Table B.9.1-1: Adverse effects of *Beauveria bassiana* strain GHA spores to American kestrels

Test species	**American kestrels (*Falco sparverius)***
Toxicity	LR_{50}: > 2.5 x 10^{7} conidia/g body weight
Pathogenicity	No external symptoms of pathogenicity. The results of the necropsy were not submitted.

Conclusion:

The young kestrels showed no harmful effects from doses of 5 x 10^{6} or 2.5 x 10^{7} conidia/g body weight of *Beauveria bassiana* strain GHA.

The chosen test design did not reflect the recommendations of the cited guideline 154A-16. None of the requirements was met. Examinations of infectivity and pathogenicity were not conducted. The results of necropsy were not included in the test report and the notifier is asked to provide the information. Thus, the results can only be used as supplemental information. Based on the submitted data, the hazard to birds could not be assessed completely.

B.9.1.1.1 Toxicity

No toxic effects have been observed from doses of 5 x 10^6 or 2.5 x 10^7 conidia/g body weight of *Beauveria bassiana* strain GHA.

B.9.1.1.2 Infectiveness/Pathogenicity

No external symptoms of pathogenicity have been observed. The results of the necropsy were not submitted. Histopathological examinations have not been conducted. Results from other studies with birds and another strain of *Beauveria bassiana* confirm the finding that there is no evidence of pathogenicity from the exposure to *Beauveria bassiana* (please refer to W41-6155 Draft Assessment Report on *Beauveria bassiana* strain ATTC 74040; Reference number KIIM 8.1(OECD), Foster, J.W., Campbell, S.M. and Beavers J.B., 1994, *Beauveria bassiana* ATCC 74040 - An avian oral pathogenicity and toxicity study in the Northern Bobwhite(AVS2006-136).

B.9.1.1.3 Toxin/metabolite from active substance

No data submitted. Toxic effects coming from secondary metabolites within the technical substance are covered by the oral pathogenicity and toxicity study described in B.9.1.1. Possible effects of toxins produced after application in nature are discussed in chapter B.9.1.5.

B.9.1.2 Plant protection product

No data submitted. The effects of the plant protection product on birds can be predicted on the basis of the data available for the active substance *Beauveria bassiana* strain GHA.

B.9.1.3 Summary of the studies on birds on toxicity, infectiveness and pathogenicity

Table B.9.1-2: Summary of the studies on effects on birds treated with *Beauveria bassiana* strain GHA spores

Species	Test duration	Dose range	Results/ Endpoint	Observations	Reference
Toxicity					
Falco sparverius	5 d	single dose of 5 x 10^6 or 2.5 x 10^7 conidia/g body weight	LR_{50}: > 2.5 x 10^{10} conidia/kg bw/day, no effects on other parameters observed	No mortality, no differences in body weight, behavioural patterns or movements	McEwen 1993, IIM 8.1/02 (Report No. 93-012)
Infectiveness	no data				
Pathogenicity	5 d	single dose of 5 x 10^6 or 2.5 x 10^7 conidia/g body weight	No data.	No external symptoms of pathogenicity. The results of the necropsy were not submitted.	

B.9.1.4 Risk assessment for birds

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. *Beauveria bassiana* strain GHA is not related to any known human / plant / animal pathogens. This is confirmed following exposure of young kestrels (see Table B.9.1-2).

Exposure and relevant indicator birds

BotaniGard 22 WP is intended to be used in greenhouse. For greenhouse applications, exposure of birds outside the glasshouse is very limited and assumed to be 0.1 % of the application rate based on generic drift from greenhouse. However, a risk assessment was performed to address potential hazard to birds.

Birds may be exposed directly and indirectly via the ingestion of sprayed plant parts and via infected arthropods, respectively. Indirect exposure cannot be excluded when infected targets may become available for ingestion, e.g. infected insects after outdoor application.

The expected direct exposure via the diet is calculated according to SANCO/4145/2000 (2002) and based on 0.1 % of the application rate and the model scenario leafy crop/cereals. This scenario was chosen as it best represents the type of habitat outside a greenhouse.

According to the scenario, the indicator bird species supposed to be at risk are insectivorous and herbivorous birds.

Table B.9.1-3: Relevant indicator bird species for locations and crops intended to be sprayed with *B. bassiana* strain GHA

Crop	Indicator species	Example
Leafy crops	Small insectivorous bird	10 g Wren, tit
	Medium herbivorous bird	300 g Partridge, pigeon
Cereals/grass	Small insectivorous bird	10 g Wren, tit
	Large herbivorous bird	3000 g Goose

Risk assessment

In order to judge the environmental concentration of the technical material exposed to birds the short-term ETE according to SANCO/4145/2000 (2002) is calculated, since no alternative approach is available. The RUD value generated for chemical pesticides is used provisionally, since there are no specific data about residues of microbial pesticides.

The short-term ETE and the resulting TER for insectivorous and medium herbivorous birds following the application of BotaniGard 22WP are presented in Table B.9.1-4.

Calculations on the short-term exposure for herbivorous and insectivorous birds conform to the following equation:

$$ETE = FIR/bw \times C \times AV \times PT \times PD \times MAF \times RUD$$

ETE [Estimated Theoretical Exposure; herbivorous and insectivorous birds are assumed to have covered their daily caloric demand fully with treated plants and infected arthropods, respectively, obviously worst-case assumptions; dimension mg/kg bw/day]
FIR [Feed Intake Rate; dimension, g fresh weight/day]
bw [body weight; g]
C [Concentration of the active substance in fresh feed; dimension, mg as/kg feed fresh weight]
AV [Avoidance factor: 1 is no avoidance, 0 is complete avoidance; for worst-case purposes, the AV for treated plant parts is 1]

PT [The fraction of diet obtained in the treated area: number between 0 and 1; for worst-case purposes, the PT is 1]
PD [Fraction of feed type in diet: number between 0 and 1; one type or more types; for worst-case purposes, the PD is 1: in this way the whole diet is assumed to consist of sprayed plant parts or infected arthropods]
MAF Multiple application factor
RUD Residue per unit dose (mg/kg fresh weight)

Based on the estimated theoretical exposure (ETE), the margin of safety (MoS) [corresponding to the short-term toxicological exposure ratio (TER)] for birds is derived from the higest test concentration (corresponding to the NOEC and LD_{50} value) according to the formula:

$$TER_{ST} = \frac{LD_{50}(NOEC)\ [mg/kg\ bw\ per\ day]}{ETE\ [mg/kg\ bw\ per\ day]}$$

These indicative calculations on the short-term exposure are based on the ingestion of contaminated grass, leafy crop or contaminated insects, assuming that diets of herbivorous birds consist of grass or leafy crop only. Insectivorous small birds are assumed to eat infected insects only and foraging is entirely in the active substance treated area. These are obviously worst-case assumptions.

Table B.9.1-4: Short-term ETE and resulting MoS (TER_{ST}) for birds exposed to *B. bassiana* according to SANCO/4145/2000 (2002) after use of BotaniGard WP 22 in tomato (field)

Indicator species	Food category	LD_{50} [mg as/kg bw per day]	Application rate[1)] [kg as/ha]	FIR/ bw	MAF	RUD	ETE [mg/kg bw*d]	TER_{ST} (10)
Insectivorous bird	Small insects	> 2.5×10^{10} CFU/kg bw/day	0.00055 (2.42×10^{13} CFU/ha)	1.04	1	29	0.0166 (7.29×10^{5} CFU/kg bw*d)	34293
Medium herbivorous bird	Leafy crops			0.76	5	40	0.0836 (3.7×10^{6} CFU/kg bw*d)	6757
Large herbivorous bird	Short grass			0.44	5	76	0.092 (4.05×10^{6} CFU/kg bw*d)	6173

[1)] single application rate
Please note that the MAF for leafy crop scenario is set to 5, assuming, as a worst case, no breakdown of the product between applications

In terms of potentially toxic effects of the technical substance the calculated MoS (TER_{ST}) clearly exceeds the trigger value of 10 as described in Annex VI part I of Directive 91/414/EEC for all scenarios. The potential risk of pathogenicity and infectiveness of MPCAs can also be excluded considering these margins of safety. 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.

Because birds are exposed to this micro-organism as part of their natural environment, no long-term effects are to be anticipated. Sensitivity to low pH values encountered in the stomach of birds renders survival and colonisation of the birds' interior via ingestion unlikely. Moreover, the *in vivo* growth temperature of the fungus is below 35 °C which prevents it from growing at the higher body temperature of birds.

Conclusion:
There seems evidence, also in view of the type of exposure, experimental values and the microbiology of *B. bassiana* strain GHA to support the assumption that the filamentous fungus has neither acute nor short term effects on birds, if exposed. Long-term effects cannot be evaluated strictly speaking as there are no test results on reproduction. However, in view of the acute bird toxicity data, the mode of action, the 'natural' host or target range, *in vitro* temperature preferences, and a lack of reported field cases, long-term risks following short-term exposure are considered unlikely.
However, indirect exposure by infected insects may occur via ingestion of infected target organisms (larvae) escaped from treated greenhouse. Infected larvae may contain substantial amounts of toxins produced in the expanding hyphens, whereas there may be days before the targets actually die. Such exposure has not been tested. There is information from literature that metabolites of *B. bassiana* can be toxic to birds.

B.9.1.5 Further information from literature:

Submitted by the notifier:

Reference:	IIM 8.1
Author:	Hartmann, G.C. and Wasti, S.S.
Title:	Avian safety of three species of entomogenous fungi
Date:	1980
Doc ID:	Literature: Comp. Physiol. Ecol. (1980), 5 (4), pp. 242-245 BVL No. AVS2006-167
Guideline:	US EPA Guideline 154A-16
GLP:	Not documented
Validity:	Not applicable, publication

Material and methods:

Micro-organism:	*Beauveria bassiana* (strain ATCC 18514)
Test species:	*Coturnix coturnix japonica*
Number of test animals:	4
Treatments:	29.45 x 10^9 viable spores/bird
Duration:	14 d

Test conditions:	Japanese quail (*Coturnix coturnix japonica)* were tested for infectivity and toxicity of three species of entomogenous fungi as part of this study four birds were individually housed and fed with suspensions of *Beauveria bassiana* (strain ATCC 18514) in water (at the rate of 29.45 x 10^9 viable spores/bird), and four birds served as a control (water only). All birds were then fed their daily rations of standard poultry feed, followed by sterile distilled water *ad libitum*. Birds were monitored daily over a 14-day period for indicators such as mortality, abnormal behaviour, and changes in appearance or physiology, body weights, feed consumption *etc*. The test suspensions were prepared in sterile distilled water and tested daily for viability on sterile nutrient plates. Faecal samples (10 mg aliquots) were collected from each bird, mixed in sterile distilled water and plated on sterile media. A second faecal suspension was centrifuged with Tween-80, then washed and centrifuged to remove debris and collect only the spores, which were then plated out as before and observed.
Deviations from guideline	4 birds instead of 30 birds (testing of one level); Observation period 14 days instead of 30 days; Age of birds at test start: 56 days instead of 14 - 24 days
Endpoint:	Mortality, behaviour, body weight
Observations:	Mortality, abnormal behaviour, and changes in appearance or physiology, body weight, feed consumption

Results:

Table B.9.1-5: Toxic effects of *Beauveria bassiana* spores to Japanese quail

Test species	Japanese quail
Toxicity	No mortality at 29.45 x 10^9 viable spores/bird.

Conclusion:
No mortality was recorded, and the birds were healthy and increased in body weight. No spores were found in the bodies, as all had been excreted. Spores were probably inactivated in the gut, since they appeared to remain viable in the droppings. Since the information is presented as a publication, raw data are not available and the results could not be validated. Even though this *Beauveria bassiana* strain was different to the present one in use and being supported in this dossier, the presented data provide additional information confirming the low toxicity of *Beauveria bassiana* to birds, as observed in the following study of McEwen, 1993 (Rep-No 93-012). Furthermore, it can be seen that the spores are still viable in the excreta of the birds and thus can be distributed in the environment by the birds.

Further information:

Several feeding experiments with spores and conidia of *B. bassiana* and field experiments are reported. There is little indication that birds are susceptible to *B. bassiana*.
In field experiments, young *Falco sparvensis* were fed with 5 x 10^6 spores of *B. bassiana* per kg body weight (Althouse et al., 1997 cited in Zimmermann 2007). No differences were found among any treatments and the control in growth, body mass or survival. Male and female ring-

necked pheasants (*Phasianus colchicus*) were challenged per os with conidia of *B. bassiana* strain GHA and with B. bassiana strain GHA infected grasshoppers (Johnson et al. 2002). In both sexes, the weight gain at 17 and 25 days was not significantly different between challenged and control groups. Histopathological changes were generally undetectable. In 1987, a large field trial was carried out in Germany with *B. brongniartii* blastospores against the forest cockchafer *Melolontha hippocastani* (1.5 – 2.8 x 10^{14} blastospores/ha). During this experiment, no side-effects on birds, especially young ones, were noticed (Havelka & Ruge 1988). According to Copping (2004 cited in Zimmermann 2007), the non-target bird toxicity for *B. bassiana* is: oral LD_{50} (5 days) for quail > 2000 mg/kg daily (by gavage); for *B. brongniartii*: dietary LD_{50} (5 days) for quail and mallard ducks > 4000 mg/kg.

The highest concentrations of toxins that can occur in nature are expected to be found in the host insects. The only information about the amount of toxins synthesized in arthropods is described by Strasser et al. (2000) for oosporein produced in *Melolontha melolontha* larvae by the related fungus *Beauveria brongniartii*.
Therefore, calculation based on these parameters is used to assess the range of potential risks in this case:

Parameters:
Concentration of oosporein in one larva = 200 µg
LD_{50} (chicken) = 6 mg/kg

Risk assessment for insectivorous birds:

Assuming an insectivorous bird having a body weight of 0.01 kg would eat **only one** larva:

$$\text{TER} = \frac{LD_{50}}{\text{amount of oosporein in one larva}}$$

$$\text{TER} = \frac{6*10^{-5}\text{ g oosporein}}{0.2*10^{-3}\text{ g oosporein}}$$

TER = 0.30

According to these calculations a risk to insectivorous birds cannot be excluded.
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 calculation above 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.
This generally speaks for low risk for birds consuming *B. bassiana* or *B. brongniartii* infected insects.

Exposure- and risk assessment regrading the application in greenhouses:
Products comprising *B. bassiana* GHA are intended to be used against sucking insects. Sucking insects 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. The exposure of insectivorous birds or mammals by infected, possibly toxin comprising sucking insects seems to be negligible. Moreover a transfer of infections from infected arthropods within the greenhouse to arthropods outside the greenhouse appears to be very improbable.
Overall it may be concluded, that the exposure by infected arthropods is minimal and therefore the risk to insectivorous birds and mammals acceptable.

B.9.2 Effects on aquatic organisms (Annex IIM 8.2; Annex IIIM 10.2)

B.9.2.1 Effects on fish (Annex IIM 8.2; Annex IIIM 10.2)

B.9.2.1.1 Active substance

Reference:	IIM 8.2
Author:	Collins, M. K.
Title:	*Beauveria bassiana* (Bb GHA 1991) – Evaluation of potential embryo larval toxicity and pathogenicity to fathead minnow (*Pimephales promelas*) under static renewal conditions.
Date:	1993
Doc ID:	Report No. 93-8-4910, BVL No. WAT2006-386
Guideline:	Following US EPA Subdivision M, Guideline Section Series 154A-19 (OPPTS 885.4200).
GLP:	Yes
Validity:	Acceptable

Material and methods:

Micro-organism:	*Beauveria bassiana* (Bb GHA 1991)
Test species:	*Pimephales promelas*
Number of test animals:	40 fathead minnow eggs (≤ 24 hours old) per incubation cup, 240 eggs per treatment level and control, each
Treatments:	Single aqueous concentration equal to 7.5 x 10^8 CFU/L, semi-static
Duration:	31 d

Test conditions:	The effect of *Beauveria bassiana* spores on different phases of the life-cycle of the Fathead minnow (*Pimephales promelas*) was studied over a period of 31 days. Eggs, embryos and larvae were exposed to a single aqueous concentration equal to 7.5 x 10^8 CFU/L, being the theoretical maximum concentration attainable for this material. Controls with killed *Beauveria bassiana* (heat inactivated spores) and water were also set up.
Deviations from guideline	The guideline is designed to study effects on fish weighing between 0.5 and 5 g. The aim of the presented study was to study effects on early life stages of fish starting with eggs. *P. promelas* was used as test species instead of rainbow trout as recommended in the guideline. The study was performed as a limit test. Route of exposure: the MPCA was not additionally administered through the oral route of exposure.
Endpoint:	Egg hatchability, larval survival, pathogenicity
Observations:	During, and at the end of the study, fish were examined for infectivity and/or pathogenicity in the form of lesions, necroses or tumours formed on the external surfaces of the fish

Results:
Results of variability determinations demonstrated that the quantities of remaining *Beauveria bassiana* spores remained viable throughout the study. External examination of the surviving fish at test termination established that no infectivity or pathogenicity in the form of lesions, necroses or tumours were observed on the skin, scales, fins or gills of the examined fish. Histopathological examination showed no evidence of infection or pathogenicity associated with the *Beauveria bassiana* (Bb GHA 1991) under the conditions of this study.
It was found that larval growth was significantly inhibited in the test group in comparison to that in the control groups. The test concentration of 7.5 x 10^8 CFU/L (initial) did not cause effects on growth higher than 25 percent. There was no significant difference between egg hatchability or larval survival in the treatment and control group.
A summary of endpoints is given in the table below.

Table B.9.2-1: Toxic effects of the active substance *Beauveria bassiana* (strain GHA) to early life stages of *Pimephales promelas*

Test species	***Pimephales promelas***
Toxicity	NOEC < 7.5 x 10^8 CFU/L, initial concentration
Evidence of pathogenicity or infectivity	none

Conclusion:
Exposure to 7.5 x 10^8 CFU/L of *Beauveria bassiana* strain GHA significantly affected fish larval growth in comparison to growth in the dilution water and attenuated control larvae. Based on the absence of signs of infectivity or pathogenicity among the exposed fathead minnow embryos and larvae, the observed decrease in larval growth was determined to be related to toxic effects of the technical material of *Beauveria bassiana* strain GHA.
The recommendations of the guideline FIFRA 154A-19 to additionally evaluate the effects of *Beauveria bassiana* (GHA) to fish after oral intake were not followed. However, the applied study design enables the determination of possible effects of *Beauveria bassiana* (GHA) to

the early life stages of *Pimephales promelas* including pathogenicity and infectivity after exposure via the aqueous phase. To address possible effects on free feeding fish, further test with young fish exposed via oral intake according to FIFRA 154A-19 is necessary.
The results on effects on survival and hatching success could not be checked due to missing raw data.
However, as the product is used only in greenhouse and the risk assessment based on drift entry of 0.1 % from the application rate resulted in very high TER values, further data are not required.

B.9.2.1.1.1 Toxicity

Exposure to 7.5 x 10^8 CFU/L of *Beauveria bassiana* strain GHA significantly affected fish larval growth in comparison to growth in the dilution water and attenuated control larvae. Based on the absence of signs of infectivity or pathogenicity among the exposed fathead minnow embryos and larvae, the observed decrease in larval growth was determined to be related to toxic effects of the technical material of *Beauveria bassiana.*

B.9.2.1.1.2 Infectiveness/Pathogenicity

Results from the study with early-life stages of *Pimephales promelas* indicate, that there is no evidence of infectivity and pathogenicity from the exposure to *Beauveria bassiana* strain GHA (Collins, M.K., 1993; IIM 8.2/02, WAT2006-386).

B.9.2.1.1.3 Toxin/metabolite from active substance

No data submitted. Toxic effects coming from secondary metabolites within the technical substance are covered by the study with the active ingredient (technical material) described in B.9.2.1.1.
Since conidia of *Beauveria bassiana* strain GHA are in a stage of dormancy, and no germination is expected in natural water bodies a proceeding synthesis of fungal metabolites in aquatic ecosystems can be excluded.

B.9.2.1.2 Plant protection product

Results from the study with early-life stages of *Pimephales promelas* indicate, that there is no evidence of pathogenicity from the exposure to *Beauveria bassiana* strain GHA (Collins, M.K., 1993; IIM 8.2/02, WAT2006-386).
No data submitted. The effects of the plant protection product on fish can be predicted on the basis of the data available for the active substance *Beauveria bassiana* strain GHA.

B.9.2.2 Effects on freshwater invertebrates (Annex IIM 8.3; Annex IIIM 10.2)

B.9.2.2.1 Active substance

Reference:	IIM 8.3
Author:	Collins, M. K.
Title:	*Beauveria bassiana* (Bb GHA 1991) – 21-day toxicity to Daphnids (*Daphnia magna*) under static renewal conditions.
Date:	1993
Doc ID:	Report No. 93-7-4883, BVL No. WAT2006-387
Guideline:	Following US EPA Subdivision M, Guideline Section Series 154A-20 (OPPTS 885.4240).
GLP:	Yes
Validity:	results plausible (not valid according to guideline), for the assessment of reproduction raw data on offspring required

Material and methods:

Micro-organism:	*Beauveria bassiana* (Bb GHA 1991)
Test species:	*Daphnia magna* (≤ 24 h old)
Number of test animals:	10 daphnids per replicate, 2 replicates per concentration
Treatments:	Five nominal test concentrations, semi-static: Nominal 6.6 x 10^7, 1.3 x 10^8, 2.6 x 10^8, 5.0 x 10^8 and 1.0 x 10^9 CFU/L, Mean measured 6.4 x 10^7, 1.3 x 10^8, 2.5 x 10^8, 4.7 x 10^8 , 9.3 x 10^8 CFU/L
Duration:	21 d
Test conditions:	The potential toxicity and pathogenicity of *Beauveria bassiana* was studied in *Daphnia magna* over a 21-day exposure period. An attenuated control of heat inactivated *Beauveria bassiana* spores was set up to see if there were any effects from culture metabolites or non-infective components which could influence toxicity. The number of immobilised daphnids was recorded daily. Fungal spore viability determinations of the test sample were also performed.
Deviations from guideline	The guideline requires a benthic invertebrate for MCPAs having terrestrial use patterns. Where direct aquatic exposure is anticipated, two aquatic species shall be used. In the presented study *Daphnia magna* was used to assess effects on aqueous spores on aquatic invertebrates.
Endpoint:	Survival and growth of adults, pathogenicity
Observations:	Immobilisation, daphnid survival and organism growth at test termination were recorded to determine the effects of *Beauveria bassiana*. Daily observation of offspring.

Results:

There were no effects observed on any animals at any dose-rate tested. Survival was not affected, but there did seem to be a very slight dose-related effect on overall growth. Although a decrease in spore concentration was observed in the aged solutions, the results of spore viability determinations established that the quantities of *Beauveria bassiana* spores were

viable. After 21 days exposure, 95 % and 90 % survival was observed among the control and attenuated control organisms, respectively. Daily observations for offspring established that daphnids at all treatment levels reproduced during the 21-day study. The time for appearance of the first brood was 7 days. However, there are no data presented in the study report and effects on fecundity can not be assessed. A slight difference in organism length was noted, however, the reduction of only 4 % compared to controls is not considered to be ecologically relevant (Table B.9.2-2). The parameters determined from this study are summarised in Table B.9.2-3.

Table B.9.2-2: Effects of *Beauveria bassiana* strain GHA to body weight and body length of *Daphnia magna* after a 21-day exposure period

Mean measured concentration	Mean organism body length ± standard deviation (mm)	Percent of mean body length compared to control (%)	Mean organism body weight ± standard deviation (mg)	Percent of mean body weight compared to control (%)
Control	5.0 ± 0.4		1.6 ± 0.3	
Attenuated control	5.1 ± 0.1		1.4 ± 0.2	
Pooled control	5.1	100	1.5	100
6.4×10^7	5.0 ± 0.2	98	1.7 ± 0.3	113
1.3×10^8	5.1 ± 0.2	100	1.6 ± 0.3	107
2.5×10^8	5.1 ± 0.2	100	1.7 ± 0.3	113
4.7×10^8	5.1 ± 0.2	100	1.8 ± 0.2	113
9.3×10^8	4.9 ± 0.2 [a]	96	1.6 ± 0.3	107

[a] significantly different as compared to the pooled controls

Table B.9.2-3: Endpoints of toxic effects of *Beauveria bassiana* strain GHA to *Daphnia magna* over a 21-day exposure period

Test species	***Daphnia magna***
21-day EC_{50}	> 9.3×10^8 CFU/L
LOEC:	9.3×10^8 CFU/L
NOEC for sublethal effects on adults	4.7×10^8 CFU/L

Conclusion:

The test with *Daphnia magna* did not fully meet the requirements of the EPA-Guideline OPPTS 885.4240. However, toxic effects of *Beauveria bassiana* (GHA) on mortality and growth of adult *Daphnia magna* could be assessed. A slight difference in organism length was noted, however, the reduction of only 4 % compared to controls is not considered to be ecologically relevant. An assessment of effects on number of offspring/female and survival of offspring was not included in the study report. Therefore, the risk on reproduction of *Daphnia magna* cannot be assessed.

However, as the product is used only in greenhouse and the risk assessment based on drift entry of 0.1 % from the application rate resulted in very high TER values, further data are not required.

For an assessment of risk to daphnia after field application further data on reproduction of the daphnids would be required. However, as the product is used only in greenhouse and the risk assessment based on drift entry of 0.1 % from the application rate resulted in very high TER values, further data are not required.

B.9.2.2.1.1 Toxicity

A slight difference in organism length was noted, however, the reduction of only 4 % compared to controls is not considered to be ecologically relevant.

B.9.2.2.1.2 Infectiveness/Pathogenicity

Results from the 21-day toxicity study with *Daphnia magna* indicate, that there is no evidence of pathogenicity from the exposure to *Beauveria bassiana* strain GHA (Collins, M.K., 1993; IIM 8.3/01, WAT2006-387).

B.9.2.2.2 Toxin/metabolite from active substance

No data submitted. Toxic effects coming from secondary metabolites within the technical substance are covered by the study with the active ingredient (technical material) described in B.9.2.2.1.
Since conidia of *Beauveria bassiana* strain GHA are in a stage of dormancy, and no germination is expected in natural water bodies a proceeding synthesis of fungal metabolites in aquatic ecosystems can be excluded.

B.9.2.2.3 Plant protection product

No data submitted. The effects of the plant protection product on daphnids can be predicted on the basis of the data available for the active substance *Beauveria bassiana* strain GHA.

B.9.2.3 Effects on algae growth (Annex IIM 8.4; Annex IIIM 10.2)

B.9.2.3.1 Active substance

Reference:	IIM 8.4
Author:	Palmer, S. J. and Krueger, H. O.
Title:	*Beauveria bassiana* strain GHA – A 96-hour toxicity test with the freshwater alga (*Selenastrum capricornutum*)
Date:	1998
Doc ID:	Report No. 488A-101, BVL No. WAT2006-388
Guideline:	US EPA Subdivision M, Guideline Section Series 154A-22 (EPA OPPTS 850.5400), OECD 201 (1984).
GLP:	Yes
Validity:	**Acceptable**

Material and methods:

Micro-organism:	*Beauveria bassiana* (Bb GHA 1991), 8.59 x 10^{10} CFU/g
Test species:	*Pseudokirchneriella capricornutum* (formerly *Selenastrum capricornutum*)
Number of test organisms:	10 000 cells/mL at test start

Treatments:	0 (negative control), 0 (attenuated control), five concentrations of 19, 38, 75, 150 and 300 mg/mL
Duration:	96 h, endpoints for 72 h exposure were used
Test conditions:	A 96-hour toxicity study was conducted on the fresh water green alga *Selenastrum capricornutum.* The cultures were exposed to nominal test concentrations of *Beauveria bassiana* strain GHA under normal pH and lighting conditions for such organisms. Test substance viability was checked at 0 and 72 hour and at study termination.
Deviations from guideline	None
Endpoint:	Effects on algal growth and growth rate (EC_{50} values)
Observations:	Mean cell densities, mean areas under the growth curve, mean growth rates and percentage inhibition were determined

Results:
Changes in mean cell density in the negative control indicated that exponential growth occurred, but cell density, area under the growth curve and growth rate were significantly reduced *($p < 0.05$)* in the attenuated control at 72 and 96 hours when compared to the negative control. Reductions in cell density, area under the growth curve, and growth rate observed in the two highest treatment rates (150 and 300 mg/L) at 72 and 96 hours were significantly reduced ($p = 0.05$) in comparison to the negative control. Due to lacking uniform exponential growth of control cultures between 72 and 96 hours, the endpoints were calculated for the 72 hour exposure period (the coefficient of variation of the sectional growth rates in the controls was 16.8 % for the 72 h exposure period).
There was no evidence of cellular flocculation or aggregation in neither negative or attenuated control groups nor the three lower dose-rates (19, 38 and 75 mg/L). In the cultures treated at 150 and 300 g/L *Beauveria bassiana* there was evidence of cell enlargement, flocculation and aggregation. Although growth was inhibited in these two groups, there was no maximal inhibition, thus a recovery phase study to differentiate algicidal and algistatic effects was not conducted. Measured concentrations of treatment samples collected at test initiation ranged from 80 to 113 % of nominal, while those collected at 72 hours ranged from 69 to 118 % of nominal. A summary of endpoints is given in the table below.

Table B.9.2-4: Effects of the active substance *Beauveria bassiana* strain GHA to the green alga *Pseudokirchneriella subcapitata*

Test species:	***Pseudokirchneriella capricornutum***	
		Confidence limits (not available)
Biomass (area under growth curve)	72-hour $E_BC_{50:}$	114 mg/L (0.98 x 10^{13} CFU/L)
	72-hour NOEC:	75 mg/L (2.64 x 10^{13} CFU/L)
Growth rate	72-hour E_r $C_{50:}$	237 mg/L (2.04 x 10^{13} CFU/L)
	72-hour NOEC:	75 mg/L (2.64 x 10^{13} CFU/L)

Conclusion:
Due to lacking uniform exponential growth of cells in the control between 72 h and 96 h, the test could only be analysed at 72 h. The effects can only be compared to the growth of cells in the blank control, since there was a significant effect on algal growth between the attenuated control and the blank control.

The 72 hour EC_{50} values for *Pseudokirchneriella subcapitata* exposed to *Beauveria bassiana* strain GHA was calculated in terms of biomass integral and growth rate based on nominal concentrations and was determined to be 114 mg/L (0.98 x 10^{13} conidia/L) and 237 mg/L (2.04 x 10^{13} conidia/L), respectively. The 72 h NOEC was 75 mg/L (2.64 x 10^{13} conidia/L) relative to the untreated controls for biomass and growth rate.

B.9.2.3.2 Toxin/metabolite from active substance

No data submitted. Toxic effects coming from secondary metabolites within the technical substance are covered by the study with the active ingredient (technical material) described in B.9.2.3.1.
Since conidia of *Beauveria bassiana* strain GHA are in a stage of dormancy, and no germination is expected in natural water bodies a proceeding synthesis of fungal metabolites in aquatic ecosystems can be excluded.

B.9.2.3.3 Plant protection product

No data submitted. The effects of the plant protection product on algae can be predicted on the basis of the data available for the active substance *Beauveria bassiana* strain GHA.

B.9.2.4 Effects on plants other than algae (Annex IIM 8.5; Annex IIIM 10.2)

It is assumed that a study addressing the effects on aquatic plants is not required, as the plant protection product BotaniGard 22WP is an insecticide intended for use on protected ornamental plants and vegetables indoor. Its use pattern would not result in significant exposure to aquatic plants. *Beauveria bassiana* strain GHA itself is selective to insects according to its mode of action and no harmful effects to plants are known.

B.9.2.5 Summary of the studies on aquatic organisms toxicity, infectiveness and pathogenicity

Beauveria bassiana strain GHA is not expected to have any adverse effect on algae. Mild toxic effects were observed in studies on fish. There were no lethal or sublethal effects on daphnids. 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. For a risk assessment please refer to the following chapter.

Table B.9.2-5: Summary of the studies on effects on aquatic organisms treated with the active substance *Beauveria bassiana* strain GHA

Species	Test duration	Concentration range	Results/ Endpoint	Observations	Reference
Toxicity					
Pimephales promelas	31 d	7.5×10^8 CFU/L	NOEC < 7.5×10^8 CFU/L / (< 1179 µg as/L)	Effects on larval growth	Collins 1993 IIM 8.2 WAT2006-386
Daphnia magna	21 d	6.6×10^7 - 1.0×10^9 CFU/L	EC_{50} > 9.3×10^8 CFU/L / NOEC 4.7×10^8 CFU/L / (739 µg CFU/L)	Mortality Sublethal effects on adults	Collins 1993 IIM 8.3 WAT2006-387
Physical effects					
Pseudokirchneriella capricornutum	72 h	19 - 300 mg/L (1.6×10^{12} - 2.6×10^{13} conidia/L)	E_BC_{50} 114 mg/L $E_r C_{50}$ 237 mg/L NOEC 75 mg/L	Biomass Growth rate Biomass and Growth rate	Palmer and Krueger 1998 IIM 8.4 WAT2006-388
Infectiveness/Pathogenicity					
Pimephales promelas	31 d	7.5×10^8 CFU/L	no evidence of infectivity and pathogenicity	Lesions, necroses or tumours on the skin, scales, fins or gills	Collins 1993 IIM 8.2 WAT2006-386
Daphnia magna	21 d	6.6×10^7 - 1.0×10^9 CFU/L	no evidence of pathogenicity	Sublethal effects on adults	Collins 1993 IIM 8.3 WAT2006-387

B.9.2.6 Risk assessment for aquatic organisms

Based on the intended indoor use of BotaniGard 22WP, it is expected that the exposure of aquatic organisms to *Beauveria bassiana* strain GHA is minimal, and it is anticipated that the potential risk posed to algae, fish and *Daphnia* is low. After greenhouse applications it can be assumed that 0.1 % of the application rate enter the environment via generic drift from the greenhouse.

Aquatic organisms may be exposed to *B. bassiana* strain GHA entering surface waters *via* spray drift. The actual predicted environmental concentrations ($PEC_{SW\ actual}$) of *Beauveria bassiana* strain GHA resulting from input *via* this route were 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.

Margin of safety (TER) calculation for the active substance

The risk of *Beauveria bassiana* strain GHA to aquatic organisms was assessed from margin of safety (TER) values according to the following equation.

$$\text{Margin of safety (Chronic TER)} = \frac{\text{NOEC}\ (EC_{50\ \text{algae}})}{\text{PEC}}$$

Margin of safety (TER) values were calculated using the lowest toxicity endpoint for standard representative freshwater species. The NOEC obtained in the 21 day test with *Daphnia magna* was the most sensitive endpoint. The resulting TER values following spray drift exposure after application of BotaniGard 22WP in greenhouse are summarised below.

The calculated margin of safety (TER_{LT}) value exceeds clearly the limit value of 10 as described in Annex VI part I of Directive 91/414/EEC. Thus, no adverse effects on aquatic invertebrates, fish and algae are expected after application of BotaniGard 22WP at recommended use levels. Due to the high margin of safety it can be concluded that the risk to early life stages of fish and reproduction of daphnids is acceptable and a new study with daphnids is not required.

Table B.9.2-6: Margin of safety (TER) for aquatic organisms exposed to *B. bassiana* strain GHA after use of BotaniGard 22WP in glasshouse (N/S Europe)

Organism	Test substance	PEC_{SW} [1)]	NOEC / EC_{50} [2)]	Margin of safety (TER_{LT})
Fish	*B. bassiana* GHA	0.0254 µg as/L (1118 CFU/L)	< 1179 µg as/L (< 3.5 x 10^8 CFU/L)	313059
Daphnia	*B. bassiana* GHA		739 µg as/L (4.7 x 10^8 CFU/L)	4203934
Algae	*B. bassiana* GHA		114 000 µg as/L (9.6 x 10^{12} CFU/L)	8.6×10^9

1) based on spray-drift from five accumulated applications in glasshouse, assuming no degradation between applications
2) EC_{50} refers to endpoint "algal biomass and growth rate"

Conclusion:
Based on the submitted data on aquatic ecotoxicity and the intended use of BotaniGard 22WP in glasshouses the calculated Margin of safety (TER_{LT}) values are above the trigger of 10 as described in Annex VI part I of Directive 91/414/EEC and it is anticipated that the potential risk posed from *Beauveria bassiana* to algae, fish and *Daphnia* is low and acceptable.
In view of the nature of this living organism and its lack of toxicity, pathogenicity and infectivity, it is appropriate to conclude that *B. bassiana* GHA poses no environmental risk to aquatic organisms.

B.9.2.7 Further information from literature

Only small information is available. Beauvericin has been found to be highly toxic towards *Artemia salina* larvae and murine cell lines and can induce apoptosis (Pascale et al. 2002 cited in Zimmermann 2007).
No toxicity or pathogenicity was observed in *Daphnia magna* (Goettel & Jaronski 1997 cited in Zimmermann 2007) and in the grass shrimp, *Palaemonetes pugio* after percutaneous and oral contamination (Genthner et al. 1994 cited in Zimmermann).
Depending on the species, toxicity for fish can occur. No adverse effects of strain ATCC were observed in embryos and larvae of the fish *Pimephales promelas*, when exposed for 31 days to 1 x 10^9 CFU per litre (Goettel & Jaronski, 1997 cited in Zimmermann 2007) Naturalis-L® did

not affect fish embryos, larvae or adults; the LC_{50} (31 days) for rainbow trout was 7300 mg/L (Copping, 2004 cited in Zimmermann 2007). In contrast, Genthner & Middaugh (1992 cited in Zimmermann 2007) found that developing embryos of the inland silverside fish, *Menidia beryllina*, showed rupture and death when exposed to conidia of *B. bassiana*. Embryo rupture did not always result in death, nor was death always associated with embryo rupture. Conidia treated with a detergent showed significantly less binding to embryos than did untreated spores.

Conclusion:
Adverse effects towards fish cannot be excluded. However, according to the reported effect concentrations, the effect seems to be less pronounced and the risk is assumed to be acceptable.

B.9.3 Effects on terrestrial vertebrates others than birds

Acute oral toxicity studies on rats were conducted with *Beauveria bassiana* strain GHA 74040 (refer to chapter B.6). No mortalities occurred and no sublethal effects were observed at a dose level of 5000 mg BotaniGard 22WP/ kg bw corresponding to > 2.2 x 10^{14} CFU/ kg bw. Another study has been conducted with the active ingredient *Beauveria bassiana* strain GHA 74040. No mortalities or sublethal effects were observed at a dose level of 1.05 x 10^{8} CFU/animal. The LD_{50} value of > 2.2 x 10^{14} CFU /kg bw is chosen for risk assessment.

Table B.9.3-1: Effects on terrestrial vertebrates other than birds:

Application rate (kg MPCA/ha)	Crop	Category (*e.g.* insectivorous bird)	Time-scale	Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)
5 x 0.550, interval 5-7 days	ornamentals, tomatoes and cucumbers, indoor	rat	acute	LD_{50}: > 5000 mg BotaniGard 22WP/ kg bw corresponding to > 2.2 x 10^{14} CFU/ kg bw (product containing 4.4 x 10^{13} CFU/kg)
		rat	acute	*Beauveria bassiana* strain GHA was not toxic, pathogenic, or infective to male and female rats at 1.05 x 10^{8} CFU and 1 x 10^{8} CFU respectively.

Short-term adverse effects
On the basis that the product has very low mammalian toxicity, 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 mammals' interior via ingestion unlikely.

Exposure

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* strain GHA after application of BotaniGard 22WP mainly by the consumption of contaminated feed. The expected exposure via the diet is calculated according to SANCO/4145/2000 (2002) and based on the intended uses and the model scenario leafy crop/cereals. This scenario was chosen as it best represents the type of habitat surrounding greenhouses.

According to the scenario, the model organisms supposed to be at risk are small and medium herbivorous mammals as well as insectivorous mammal.

Table B.9.3-2 Relevant indicator species for locations and crops: intended to be: sprayed with *B. bassiana* strain GHA

Crop	Indicator species	Example
Cereals/short grass	Small herbivorous mammal	25 g vole
Cereals/short grass	Insectivorous mammals	10 g shrew
Leafy crops (incl. tomatoes)	Medium herbivorous mammal	3000 g hare

In order to judge the environmental concentration of the technical material exposed to birds the acute ETE according to SANCO/4145/2000 (2002) is calculated, since no alternative approach is available. The RUD value generated for chemical pesticides is used provisionally, since there are no specific data about residues of microbial pesticides.

The acute estimated theoretical exposure (ETE) for the exposed mammals following the application of BotaniGard 22WP is presented in Table B. 9.3-3.

Based on the estimated theoretical exposure (ETE), the margin of safety (MoS) [corresponding to the acute toxicological exposure ratio (TER_{acute}) for mammals is derived from the LD_{50} value according to the formula:

$$ETE = FIR/bw \times C \times AV \times PT \times PD \times MAF \times RUD$$

ETE [Estimated Theoretical Exposure; herbivorous and insectivorous birds are assumed to have covered their daily caloric demand fully with treated plants and infected arthropods, respectively, obviously worst-case assumptions; dimension mg/kg bw/day]
FIR [Feed Intake Rate; dimension, g fresh weight/day]
bw [body weight; g]
C [Concentration of the active substance in fresh feed; dimension, mg as/kg feed fresh weight]
AV [Avoidance factor: 1 is no avoidance, 0 is complete avoidance; for worst-case purposes, the AV for treated plant parts is 1]
PT [The fraction of diet obtained in the treated area: number between 0 and 1; for worst-case purposes, the PT is 1]
PD [Fraction of feed type in diet: number between 0 and 1; one type or more types; for worst-case purposes, the PD is 1: in this way the whole diet is assumed to consist of sprayed plant parts or infected arthropods]
MAF Multiple application factor
RUD Residue per unit dose (mg/kg fresh weight)

The indicative calculations on the acute exposure are based on the ingestion of contaminated grass or leafy crop, assuming that diets of small and medium herbivorous mammals consist of

grass or leafy crop only. The factors AV, PT and PD were set to the default value of 1. The other values are presented in the table below. For an initial worst case assessment, the MAF for both crop scenarios is set to 5, assuming no breakdown of the product between applications.

Table B. 9.3-3: Acute ETE for mammals exposed to *B. bassiana* strain GHA according to SANCO/4145/2000 (2002) after use of BotaniGard 22WP in greenhouse

Crop scenario	Indicator species	Application rate [kg as/ha]	FIR/bw	RUD	ETE [mg as/kg bw/day]	ETE [CFU/kg bw/day] [2)]
Leafy crops / cereals/grass	Small herbivorous mammal	0.00055 x 5	1.39	142	0.543	2.39×10^7
	Medium herbivorous mammal		0.28	87	0.0067	2.95×10^5
	Insectivorous mammal	0.00055	0.63	14	0.0049	2.16×10^5

[2)] CFU/kg bw/day were calculated based on the information on the intended uses of BotaniGard 1 mg = 4.4×10^7 CFU. Please note that MAF for herbivorous crop scenarios is set to 5, assuming, as a worst case, no breakdown of the product between applications

Margin of safety (TER_{acute})

Based on the estimated theoretical exposure (ETE), the acute toxicological exposure ratio (TER) for mammals is derived from the LD_{50} value according to the formula:

$$\text{Margin of safety} = \frac{LD_{50}\ [\text{CFU/kg bw per day}]}{\text{ETE}\ [\text{CFU/kg bw per day}]}$$

As discussed above, short-term and long-term exposure of terrestrial vertebrates following the intended use of BotaniGard 22WP are considered not relevant. The margin of safety values are shown in the following table.

Table B. 9.3-4: Acute TER values for mammals exposed to *B. bassiana* according to SANCO/4145/2000 (2002) after use of BotaniGard 22WP in greenhouse

Crop scenario	Indicator species	Application rate [kg as/ha]	LD_{50} [CFU/kg bw/day]	ETE [CFU/kg bw/day] [1)]	TER_A (10)
	Small herbivorous mammal	0.00055 x 5	LD_{50}: > 2.2×10^{14} CFU/ kg bw	2.39×10^{7}	> 9.2×10^{6}
Leafy crops /cereals / grass	Medium herbivorous mammal			2.95×10^{5}	> 7.5×10^{8}
	Insectivorous mammal			2.16×10^{5}	> 1.0×10^{9}

[1]CFU/kg bw/day were calculated based on the information on the intended uses of BotaniGard 1 mg 4.4×10^{7} CFU. Please note that MAF for herbivorous crop scenarios is set to 5, assuming, as a worst case, no breakdown of the product between applications

The calculated margin of safety values are determined to be greater than the trigger of 10 as described in Annex VI part I of Directive 91/414/EEC.
This value is based on the highest tested dose from an acute study where no adverse effects were observed, thus leading to an over-exaggeration of any theoretical risk. Moreover, exposure to *B. bassiana* is also overestimated, as the calculation does not take into account the rapid inactivation of *B. bassiana* in the absence of host insect. Furthermore, in the environment, small mammals are constantly exposed to *B. bassiana* spores, as this is a naturally occurring soil fungus and application only represents a transient shift in population density. No short- or long-term effects are to be anticipated. Sensitivity to low pH values encountered in the stomach of mammals renders survival and colonization of the mammals' interior via ingestion unlikely. Moreover, the *in vivo* growth temperature of the fungus is below 36 °C which prevents it from growing at the higher body temperature of mammals. Regarding direct exposure of mammals in the field to *B. bassiana* from the use of BotaniGard WP 22 in greenhouse, it is concluded that no unacceptable risk is expected to exist for mammals following application of BotaniGard WP 22 according to Good Agricultural Practice.

However, indirect exposure by infected insects may occur via ingestion of infected target organisms (larvae) escaped from treated greenhouse. Infected larvae may contain substantial amounts of toxins produced in the expanding hyphens, whereas there may be days before the targets actually die. Such exposure has not been tested. For a discussion of information taken from literature and a respective discussion of potential risk see the following chapter.

B.9.3.1 Further information from literature

Several experiments were performed with rats, mice, rabbits to get information on the toxicity of *B. bassiana* with respect to mammals (e.g. Scharffenberg, 1968, Mel'nikova & Murza, 1980; Semalulu et al., 1992; Copping, 2004 cited in Zimmermann 2007). They include injection, inhalation and feeding tests. LD_{50} oral and inhalation was > 10^{8} CFU/kg. It was demonstrated that the organism may be moderately irritant to eyes, skin and respiratory system, but no signs of infection were observed.

Conclusion:
There is little indication that terrestrial vertebrates are susceptible to *B. bassiana*. However, studies on the metabolite oosporein show that intolerable effects may occur (see below).

Secondary metabolites (toxins):
The highest concentrations of toxins that can occur in nature are expected to be found in the host insects. The only information about the amount of toxins synthesised in arthropods is described by Strasser et al. (2000) for oosporein produced in *Melolontha melolontha* larvae by the related fungus *Beauveria brongniartii*.
Therefore, calculation based on these parameters is used to assess the range of potential risks in this case:

Parameters:
Concentration of oosporein in one larva = 200 µg
Toxicity studies of oosporein in mice and hamsters indicated a LD_{50} value of 0.5 mg/kg bw, when injected intraperitoneally (Wainwright et al. 1986 cited in Strasser et al. 2000). A daily oral administration of 7 mg/kg oosporein to mice over 47 days was non-lethal.

NOEC (mice, 47 days) = 7 mg/kg*day

Risk assessment for insectivorous mammals:

Assuming an insectivorous mammal having a body weight of 0.01 kg would eat **only one** larva:

$$\text{TER} = \frac{\text{NOEC}}{\text{amount of oosporein in one larva}}$$

$$\text{TER} = \frac{7*10^{-5}\text{ g oosporein}}{0.2*10^{-3}\text{ g oosporein}}$$

TER = 0.35

According to this calculation a risk to insectivorous birds and mammals cannot be excluded.

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.
Although studies with birds exposed to oosporein in the lab show adverse effects, 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* ($1.5 – 2.8 \times 10^{14}$ CFU/ha) (Havelka & Ruge 1988). Since the calculation above 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.
This generally speaks for low risk for birds as well as for mammals consuming *B. bassiana* or *B. brongniartii* infected insects.
However, there is no information about the impact of insects infected with *B. bassiana* strain GHA on birds or mammals. Therefore a risk seems to be improbable, but cannot be fully excluded.

Exposure- and risk assessment regarding the application in greenhouses:
Products comprising *B. bassiana* GHA are intended to be used against sucking insects. Sucking insects 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. The exposure of insectivorous birds or mammals by infected, possibly toxin comprising sucking insects seems to be negligible. Moreover a transfer of infections from infected arthropods within the greenhouse to arthropods outside the greenhouse appears to be very improbable.
Overall it may be concluded, that the exposure by infected arthropods is minimal and therefore the risk to insectivorous birds and mammals acceptable.

Reptiles (see also B.6.2.4):

Fromtling et al. (1979), (TOX2007-472) see B.6.2.4 reported an outbreak of pulmonary mycosis caused by *Beauveria bassiana* among captive American alligators (*Alligator mississipiensis*) in a zoo. Following a drop in temperature because of a heating system failure in a hibernation grotto (decrease by 8^{o}C for 12 hours) three of four animals died within the next 9 months. Pathological and microbiological examinations revealed a severe systemic mycosis that affected the lungs and the pleura but was strictly confined to the thoracic cavity. 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).

Conclusion:
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 are considered to be negligible.

B.9.4 Effects on bees (Annex IIM 8.7; Annex IIIM 10.3)

B.9.4.1 Honeybees

B.9.4.1.1.1 Toxicity

No data submitted.

B.9.4.1.2 Active ingredient

No data submitted.

B.9.4.1.3 Toxin/Metabolite from active ingredient

No data submitted.

B.9.4.1.4 Plant protection product

No data submitted.

B.9.4.2 Infectiveness

See under 'pathogenicity'.

B.9.4.3 Pathogenicity

Report: Bromenshenk, J.J. *et al* (1996): Multiple Endpoint, Holistic Assessment of the Effects of Mycotrol WP (*Beauveria bassiana* strain GHA) on Outdoor *Apis mellifera* L. Colonies. Study Number: Mycotech 95-05. Division of Biological Sciences, University of Montana, Missoula.
BVL-Reg.-No.: BIE2006-88
Testguideline: FIFRA 154-24
GLP compliance: no
valid: yes
Test design:
test species: *Apis mellifera* L.
test substance: Mycotrol WP (*Beauveria bassiana* strain GHA; 4.4 x 10^{13} spores/kg; EPA Reg. No. 65626-7)
reference substance: *Ascophera apis* (ascospores from larval bee cadavers)
control variants: Attenuated control with heat-killed *Beauveria bassiana* and untreated

Test procedure:
Outdoor nucleus honeybee hives were used as test units. The colonies were examined for absence of Varroa mite and chalk brood and characterized for hygenic behaviour. 3 colonies

each were used for the treatments with Mycotrol WP, *Ascophera apis* and heat-killed *Beauveria bassiana* spores as well as for the untreated control. Each treatment was replicated 3 times in approximately 7 day intervals. About 1/3 of the worker bees from each colony were exposed by aqueous spray containing the corresponding treatment at equal dose rates. Each bee received a mean dose of 364 000 spores/bee representing a field rate of 1.12 kg/ha Mycotrol WP. Hive temperatures averaged 35 ºC. In each colony brood areas containing eggs and young larvae were marked including cell-by-cell census. Samples of live and dead worker bees were taken during the test. Observation period and brood assessment ended 27 days after the last treatment. 1 colony treated with heat-killed *Beauveria bassiana* treated and 2 untreated were substituted due to loss of the queen.

Findings:
No significant differences in mortality were observed between *Beauveria bassiana* treated colonies, treatments with the reference substance and the control variants. No latent infections were observed among live workers in the *Beauveria bassiana* treated colonies. Prevalence of *Beauveria bassiana* in cadavers of worker bees related to the number of bees actually exposed to the treatment was 1.2 % in the average of the 3 colonies. No *Beauveria bassiana* was detected in the bee brood. Brood survival was below 50 % with high variation between all colonies and treatments but there were no significant differences between *Beauveria bassiana* treated colonies, treatments with the reference substance and the control variants. Low brood survival may be caused by the late test period (fall) and the repeated disturbance of the colonies by treatments and brood observations. In the reference substance treatments *Ascophera apis* was present only in a few cadavers.

B.9.4.4 Summary and risk assessment for 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.

B.9.4.5 Bumblebees

B.9.4.5.1 Active substance

No data submitted.

B.9.4.5.2 Toxin/Metabolite from active ingredient

No studies were submitted with toxins or metabolites. Toxic effects coming from secondary metabolites within the technical substance are covered by the studies described with the and the plant protection product (B.9.4.5.3)

B.9.4.5.3 Plant protection product

Reference:

Author:	Mommaerts, V.; Boulet, J.; Sterk, G.; Smagghe, G.
Title:	Effects of biological control agents (BCAs) on the pollinator, *Bombus terrestris*
Date:	Data not submitted
Doc ID:	
Guideline:	none
GLP:	none
Validity:	

Material and methods:

Test substance:	Botanigard (Beauveria bassiana strain GHA)
Test species:	*Bombus terrestris*
Number of test animals:	20
Treatments:	1,25 mL/L (2 x 10^{10} spores/mL)

The test was conducted to investigate the compatibility of four biofungicides and three biological insecticides including Botanigard (*Beauveria bassiana* GHA) with the pollinator *Bombus terrestris*. Bumblebee workers were treated under laboratory conditions with the biological control agents at their respective concentration as recommended to be used in the field. The exposure follows the routes of dermal contact and orally via the drinking of sugar and via the pollen. For the experiments artificial nests were used, each containing 5 worker bees. During a period of about 10 weeks the nests were observed. To evaluate acute adverse effects, dead worker bees were counted on a weekly interval. In addition, the number of drones was weekly counted per nest as biological endpoints of effects on insect growth, development, brood care and reproduction.

Results:

Table B.9.4-1: Effects of Botanigard on pollen to *Bombus terrestris*

Number of living worker Bees				Drones		
	Reference	Water	Botanigard	Reference	Water	Botanigard
25. Oct	20	20	20	0	0	0
02. Nov	20	18	19	0	0	0
08. Nov	4	16	18	0	0	0
15. Nov	3	16	18	0	0	0
24. Nov	3	16	18	0	0	0
01. Dec	2	16	18	0	3	11
08. Dec	0	16	17	0	9	22
14. Dec	0	16	17	0	22	26
22. Dec	0	16	17	0	29	31
10. Jan	0	15	15	0	49	44
24. Jan	0	14	14	0	65	65
14. Feb	0	12	8	0	65	68

Table B.9.4-2 Effects of Botanigard in sugarwater to *Bombus terrestris*

Number of living worker Bees				Drones		
	Reference	Control	Botanigard	Reference	Control	Botanigard
06. Oct	20	20	20	0	0	0
13. Oct	0	19	18	0	0	0
18. Oct	0	19	18	0	0	0
25. Oct	0	19	18	0	0	0
02. Nov	0	19	18	0	0	0
08. Nov	0	19	18	0	0	1
15. Nov	0	19	18	0	23	18
24. Nov	0	19	18	0	54	37
01. Dec	0	19	15	0	85	62
08. Dec	0	18	14	0	92	72
14. Dec	0	17	13	0	95	77
22. Dec	0	17	12	0	102	79
09. Jan	0	16	11	0	113	84
21. Jan	0	12	10	0	113	87

Table B.9.4-3: Effects of Botanigard to *Bombus terrestris* topical exposure

Number of living worker Bees			Drones	
	Control	Botanigard	Control	Botanigard
06. Oct	20	20	0	0
13. Oct	20	12	0	0
18. Oct	20	5	0	0
25. Oct	20	3	0	0
02. Nov	20	3	0	0
08. Nov	20	3	6	0
15. Nov	20	3	29	0
24. Nov	20	3	63	4
01. Dec	20	3	79	6
08. Dec	20	2	88	9
14. Dec	20	2	98	9
22. Dec	20	2	104	9
10. Jan	18	2	114	9
24. Jan	14	2	115	9
02. Feb	8	2	118	9

Comments:

The study is not published yet.
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.
Since the study is not yet published, and the design follows no standard guideline the results could be used as additional information to address the risk to bumble bees.

B.9.4.5.4 Toxicity/Infectiveness/Pathogenicity

Considering the mode of action of *Beauveria bassiana* (GHA) the infection process starts via contact with the cuticle of the susceptible insect. Since significant effects on bumblebees being topically exposed have been found, bumblebees are considered to be affected by *Beauveria bassiana* (GHA). Due to the nature of *Beauveria bassiana* (GHA) as an entomopathogen microorganism, those effects are considered to be pathogenic.

B.9.4.5.5 Risk assessment for bumble bees

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 nets covering the aperture of greenhouse ventilation flaps.

B.9.4.6 Further information from literature

Laboratory experiments with bumblebees carried out under conditions that correspond to those prevailing in exposed bumblebee hives revealed that infection of bumblebees cannot be excluded. Conditions for hives in the ground are likely to offer even more conductive temperature and humidity conditions for these fungal pathogens. However, the degree and severity of risk by *B. bassiana* to bumblebees or their colonies cannot be reliably estimated at present, and requires further field experiments (Hokkanen et al., 2003).
The laboratory study considered in B.9.4.5.3 assures this concern with regard to the strain GHA.

B.9.5 Effects on arthropods other than bees (Annex IIM 8.8; Annex IIIM 10.4)

B.9.5.1 Active substance

Reference:	IIM 8.8
Author:	Hildreth, M. and Jaronski, S. T.
Title:	Acute toxicity/pathogenicity of *Beauveria bassiana* strain GHA to *Tenebrio molitor* [Coleoptera: Tenebrionidae].
Date:	October 19, 1993
Doc ID:	Unpublished report No. 93-015; BVL No. ANA2006-204
Guideline:	US EPA Guideline 154A-23
GLP:	Not documented
Validity:	Plausible, additional information

Material and methods:

Micro-organism:	*Beauveria bassiana* strain GHA
Test species:	*Tenebrio molitor; Melanoplus sanguinipes*
Number of test animals:	30 beetles per treatment group

Treatments:	30 beetles per treatment group were sprayed with air (control), 0.09 or 0.2 mL oil (oil control) or a 0.2 mL oil-conidia suspension containing 1 x 10^5 conidia/mL (or 2 x 10^4 conidia per spray). 30 4th instar *Melanoplus sanguinipes* were also exposed to each of the three treatments. The grasshoppers were included in the test to determine the relative efficacy of the fungus toward a target insect species under the conditions of the test. In a separate series of tests, 0.09 mL of oil-*Beauveria* solution at 2.64 x 10^9 conidia/mL (or 2.4 x 10^7 conidia per spray) was sprayed onto beetles. A control group and an oil control group were also included in this test series. Grasshoppers were not included in these tests.
Duration:	10 days
Test conditions:	Temperature: 26.7°C - 29.4 °C Humidity: 40 - 50 %
Deviations from guideline	none
Endpoint:	Mortality
Observations:	Dead animals

Results:
Mortality was 3.3 % for the untreated control group after 10 days, 10 % for the group treated with 0.2 mL oil and 10 % for the group treated with 0.2 mL oil-conidia suspension containing 1 x 10^5 CFU/mL. In contrast, mortalities in grasshoppers were 6.7 %, 6.7 % and 100 % respectively. Grasshopper mortalities in the *Beauveria bassiana* treatment group occurred mostly on Day 6, typical of *Beauveria bassiana*-induced kill. The mortality data for tests 2 to 4 are presented in Table B. 9.5-1.

Table B. 9.5-1: *Tenebrio molitor* mortalities ten days after treatment with *Beauveria bassiana* strain GHA in tests 2 to 4

Treatment	No. of insects	Percent mortality (%)		
		Test 2	Test 3	Test 4
Control	30	16.7	20	16.7
0.09 mL Oil	30	16.7	23.2	6.7
0.09 mL *B. bassiana* + Oil	30	26.7	26.7	13.3

Conclusion:
It is clear from the data provided that adult *Tenebrio molitor* beetles did not suffer any significant mortality when exposed to *B. bassiana* strain GHA at the rates used in this test. In comparison however, the fourth instar nymphs of the grasshopper *Melanoplus sanguinipes* subjected to the same treatment suffered up to 100 % mortality. This demonstrates the selective nature of *Beauveria bassiana* strain GHA and that *Tenebrio molitor* is not susceptible to the fungus.

Reference:	IIM 8.8
Author:	Jaronski, S. T. and Dunkel, F.V.
Title:	Acute toxicity/pathogenicity of *Beauveria bassiana* strain GHA to Predators / Parasites: *Xylocoris flavipes* (Hemiptera: Anthocoridae)
Date:	October 8, 1993
Doc ID:	Unpublished report No. 93-015; BVL No. ANA2006-205
Guideline:	US EPA Guideline 154A-23
GLP:	Yes
Validity:	Acceptable

Material and methods:

Micro-organism:	*Beauveria bassiana* strain GHA
Test species:	*Xylocoris flavipes*
Number of test animals:	5 nymphs per vial; 30 vials per treatment
Treatments:	*Xylocoris flavipes*, nymphs were continuously exposed to conidia on filter paper at concentrations of 2.7×10^7, 2.6×10^6 and 2.6×10^5 conidia per cm^2 of filter which is equivalent to a field application rate of 1×10^{15}, 1×10^{14} and 1×10^{13} conidia per acre (2.5×10^{13}, 2.5×10^{14}, 2.5×10^{15} CFU/ha). As well as the test substance suspensions, three concentrations of an attenuated control suspension were prepared.
Duration:	10 days
Test conditions:	20 µL of test concentrations and control were applied to each of 30 filter paper discs in vials per treatment. A set of 30 vials for untreated controls were prepared with deionised water. Fifth instar nymphs were introduced to the test vials until vials were infested. All dead insects were placed in petri dishes containing water soaked cotton (to maintain high humidity) and incubated for 5 days.
Deviations from guideline:	None
Endpoint:	Mortality Parasitation
Observations:	The numbers of live, moribund and dead nymphs were recorded daily for 10 days (*Xylocoris* nymphs were fed daily during the observation period with larval *Tribolium castaneum*). The frequency of cadavers developing the white sporulating mycelium typical of *B. bassiana* was recorded.

Results:

Mortality in excess of control mortality was observed with the active test substance at concentrations of 1×10^{14} and 1×10^{15} CFU/acre respectively, but not at 1×10^{13} CFU/acre. There was minimal but statistically insignificant mortality at the 1×10^{15} CFU/acre rate of attenuated control. Maximum control mortality was 10 %, on the last day of the observation period. *Beauveria bassiana* was observed to grow from and sporulate on 100 % of the cadavers from the active test substance treatments. Cadavers from the of 1×10^{14} CFU/acre and 1×10^{15} CFU/acre rates of the attenuated control had 100 % prevalence of *B. bassiana*. *Beauveria bassiana* did grow and sporulate on two of the three cadavers from the untreated control

treatment, but neither of the two cadavers for the attenuated control treatment had *B. bassiana* under identical environmental condition. The mortality data for each of the test treatments are presented in Table B. 9.5-2. Probit analysis of the 10-day mortality data indicated an ED_{50} of 1.55 x 10^{15} CFU/acre (155 x recommended use rate) and an ED_{90} of 3.3 x 10^{16} CFU/acre (3300 x recommended use rate). These rates are much higher than those proposed by current EU practices.
Contamination of the cadavers from the untreated control and attenuated control groups was observed. However, this deviation was not considered to have affected the scientific integrity of the study.

Table B. 9.5-2: Mortalities over ten days following treatment with *Beauveria bassiana* strain GHA, untreated and attenuated controls at 1 x 10^{13}, 1 x 10^{14} and 1 x 10^{15} CFU/acre*

Day	Mortality (%) in each treatment						
	Untreated control	Attenuated control (CFU/acre)			Active *Beauveria bassiana* (CFU/acre)		
		1 x 10^{13}	1 x 10^{14}	1 x 10^{15}	1 x 10^{13}	1 x 10^{14}	1 x 10^{15}
1	3	0	0	0	0	0	0
2	3	0	0	4	0	0	4
3	3	0	0	7	0	0	7
4	3	0	0	7	0	11	21
5	3	0	0	7	0	14	24
6	7	0	0	4	0	15	25
7	7	0	0	7	0	15	28
8	7	0	0	11	0	19	28
9	7	0	0	11	0	19	39
10	10	0	0	7	0	16	41

* In cases where the control mortality exceeded experimental mortality, the latter value became zero; when control mortality increased but experimental mortality did not, the latter decreased, e.g., day 10 for attenuated control treatment at 2.7 x 10^{7} conidia per cm^2.

Conclusion:
The potential impact on *X. flavipes* by *B. bassiana* GHA is probably much lower in the field than observed in this study. The data presented resulted from continuous exposure of insects to the test substance rather than a short-term or acute exposure, as would occur in the field. As *B. bassiana* is known to be degraded by sunlight and dispersed from leaf surfaces by rainfall, it can be assumed that field exposure of hemipterans would be much shorter than exposure which occurred in the study presented here. The indicated ED_{50} value would occur only at a significantly higher application rate than those proposed by current EU practice.

B.9.5.1.1 Toxin/metabolite from active substance

No studies were submitted with toxins or metabolites. Toxic effects coming from secondary metabolites within the technical substance are covered by the studies described with the active ingredient (B.9.5.1) and the plant protection product (B.9.5.2).

B.9.5.2 Plant protection product

B.9.5.2.1 Laboratory experiments

Reference:	IIIM 10.4
Author:	Aldershof, S.A.
Title:	BotaniGardTM: an extended laboratory test to determine effects of two formulations of *Beauveria bassiana* on the predatory mite *Typhlodromus pyri* (DeStefani-Perez)
Date:	10 July 2000
Doc ID:	Unpublished Report No. MT001TPE; BVL No. ANA2006-207
Guideline:	No specific guideline was used. A combination of the following was followed throughout the course of the study: EPPO guideline 151, Bakker et al (1992), Baier et al, ring tested guideline in prep (3rd draft, 1999), Overmeer (1988), SETAC/ESCORT (Barrett et al., 1994).
GLP:	Yes
Acceptability:	The study is considered as acceptable**.**

Material and methods:

Test substance:	BotaniGard 22WP BotaniGard ES 9601
Test species:	*Typhlodromus pyri* Scheuten
Number of test animals:	10 groups of 10 individuals per treatment (except in the toxic standard: 5 groups of 10 animals).
Treatments:	BotaniGard were applied to potted grapevines 7 times with a 7 or 8-day spray interval at rates of 125 g product/100 L (BotaniGard 22WP) and 250 mL product/100 L (BotaniGard ES 9601) to the point of incipient run-off. The control was treated with demineralised water. Dimethoate was used as toxic standard and applied at the first and at the last 2 application dates.
Duration:	14 days

Test conditions:

	Exposure period	Oviposition period
Temperature (°C):	25.0 - 25.5	24.5 - 25.0
Relative humidity (%):	60 - 68	63 - 67
Light intensity (lux):	210 - 295	823 - 1648

Typhlodromus pyri Scheuten was confined to the test substance residues on detached leaves immediately after drying of residues of the last application in 10 groups of 10 individuals per treatment (except in the toxic standard: 5 groups of 10 animals). Mortality was assessed after a 7-day exposure period. Surviving individuals of the water control treatment and both BotaniGard treatments were transferred to open untreated glass arenas and reproduction success was determined during the following 7 days.

Deviations from guideline:	No specific guideline was used.

Endpoint:	Juvenile mortality after a 7-day exposure period to treated grapevine leaf. Reproduction of surviving females during a successive 7-day oviposition period. Proportion of cadavers displaying *Beauveria bassiana* sporulation (following min. 3 days incubation)
Observations:	Dead and escaped mites; Number of young mites

Results:
The results are presented in Table B. 9.5-3.

Table B. 9.5-3: Results of the extended laboratory test with *Typhlodromus pyri*

Test substance	**BotaniGard**	
Test object	***Typhlodromus pyri***	
Exposure	**Grapevine leaf disks**	
	Mortality after 7 days	Fecundity (eggs/female/7 days)
Control	19 %	7.4
BotaniGard Treatments	Corrected mortality after 7 days	Reduction of Reproduction relative to the control
BotaniGard 22WP	4 % (ns)	43 %
BotaniGard ES 9601	4 % (ns)	34 %
Additional observations	Adult mortality during the 7-day oviposition period was statistically higher in both BotaniGard treatments than in the water control. *T. pyri* exposed to residues of BotaniGard can be infected with spores of *B. bassiana* under the specific conditions tested in this study.	

ns = difference not statistically significant

Conclusion:
Exposure to residues of the test product applied at 125 g product/100 L for BotaniGard 22WP (947 g product/ha) and of 250 mL/100 L (1695 mL product/ha) of BotaniGard ES 9601 has no adverse effect on *T. pyri* survival or fecundity after a 7-day exposure period. Exposure resulted in a statistically significant adverse effect on female mortality. *Typhlodromus pyri* exposed to residues of BotaniGard can be infected with spores of *Beauveria bassiana* under the specific conditions tested in this study. Although exposure to BotaniGard 22WP has no statistically significant adverse effect on juvenile mortality and reproductive success of surviving females, reproductive success of the original batch of test animals exposed to both BotaniGard treatments is considerably reduced due to high female mortality.

Reference:	**IIIM 10.4**
Author:	Aldershof, S.A.
Title:	BotaniGardTM: an extended laboratory test to determine effects of two formulations of *Beauveria bassiana* on the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez)
Date:	14 June 2000
Doc ID:	Unpublished report MT002ARE; BVL No. ANA206-208

Guideline: No specific guideline was used. A combination of the following was followed throughout the course of the study: Mead-Briggs (1992), Polgar (1988), SETAC/ESCORT (Barrett et al., 1994)
GLP: Yes
Acceptability: The study is considered as acceptable.

Material and methods:

Test substance: BotaniGard 22WP
BotaniGard ES 9601

Test species: *Aphidius rhopalosiphi* Scheuten

Number of test animals: 5 groups of 20 (21 in one case) individuals per treatment (except in the toxic standard: 3 groups of 20 animals)

Treatments: Two formulations of the entomopathogene BotaniGard were applied to potted grapevines 7 times with a 7 or 8-day spray interval at rates of 125 g product/100 L (BotaniGard 22WP) and 250 mL product/100 L (BotaniGard ES 9601) to the point of incipient run-off. The control was treated with demineralised water. Dimethoate was used as toxic standard and applied at the first and at the last 2 application dates.

Duration: 26/04 - 09/06: application
09/06 - 25/06: test performance

Test conditions:

	Exposure period	Reproduction period
Temperature (°C):	19 - 20	19 - 20
Relative humidity (%):	70 - 72	70 - 73
Light intensity (lux):	2500 - 3000	1550 - 2200*

* measured one day after the end of the reproduction period

Aphidius rhopalosiphi Scheuten was confined to the test substance residues on detached leaves immediately after drying of residues of the last application in 5 groups of 20 (21 in one case) individuals per treatment (except in the toxic standard: 3 groups of 20 animals). Mortality was assessed after a 2-day and a 4-day exposure period. The toxic standard was stopped after the last mortality assessment. Dead specimens were transferred to Petri-dishes with 5 % agar and incubated for 5 days at 25 °C to check for fungal growth of *Beauveria*. 15 females per remaining treatment were transferred and kept on untreated aphid infested cereal. After 1 day the females were removed. The number of mummies (parasitised aphids) produced during this 1-day period was assessed 11 days after removal of the females. Surviving individuals of the water control treatment and both BotaniGard treatments were transferred to open untreated glass arenas and reproduction success was determined during the following 7 days.

Deviations from guideline: No major deviations

Endpoint: Adult mortality
Reproduction of surviving females
Overall effect

Observations: Mortality was assessed after a 2-day and a 4-day exposure period
The number of mummies (parasitised aphids) produced during a 1-day period was assessed 11 days after removal of the females.

Results:
The results are presented in Table B. 9.5-4.

Table B. 9.5-4: Results of the test with *Aphidius rhopalosiphi*

Test substance	**BotaniGard**	
Test object	***A. rhopalosiphi***	
Exposure	**Grapevine leaf disks**	
	Mortality after 4 days	Reproduction (mummies/female/day)
Control	30 %	7.4
BotaniGard Treatments	Corrected mortality after 4 days	Reduction of Reproduction relative to the control
BotaniGard 22WP	-5 % (ns)	20 % (ns)
BotaniGard ES 9601	-5 % (ns)	20 % (ns)
Additional observations	*A. rhopalosiphi* exposed to residues of BotaniGard can be infected with spores of *B. bassiana* under the specific conditions tested in this study. Control mortality after 4 days of exposure was above the 20% validity criterion. This was probably too strict as it is known that natural mortality rapidly increases over time, but no data is available for long term mortality. Mortality after 2 days of exposure was below 15 % which is the current accepted criterion for extended laboratory tests and semi-field test.	

ns = difference not statistically significant

Mortality in the toxic standard, dimethoate, was 100 % at 100 mg/as/L, showing that test animals were sufficiently sensitive.

Conclusion:
Exposure to residues of the test product applied at 125 g product/100 L (947 g product/ha) for BotaniGard 22WP and 250 mL/100 L (1695 mL product/ha) BotaniGard ES9601, resp., has no adverse effect on *Aphidius rhopalosiphi* survival or fecundity after a 4-day exposure period. *Aphidius rhopalosiphi* exposed to residues of BotaniGard can be infected with spores of *Beauveria bassiana* (GHA) under the specific conditions tested in this study.

Reference: **IIIM 10.4**
Author: Aldershof, S.A.
Title: BotaniGardTM: an extended laboratory test to determine effects of two formulations of *Beauveria bassiana* on the minute pirate bug *Orius laevigatus*
Date: 10 July 2000
Doc ID: Unpublished report MT003OLE; BVL No. ANA206-209
Guideline: No specific guideline was used. A combination of the following was followed throughout the course of the study: Austin et al. (1997), SETAC/ESCORT (Barrett et al., 1994).

GLP: Yes

Acceptability: The study is considered as acceptable**.**

Material and methods:

Test substance: BotaniGard 22WP
BotaniGard ES 9601

Test species: *Orius laevigatus* (Fieber)

Number of test animals: 10 groups of 6 individuals per treatment.

Treatments: Two formulations of the entomopathogene BotaniGard were applied to potted grapevines 6 times with a 7 or 8-day spray interval at rates of 125 g product/100 L (BotaniGard 22WP) and 250 mL product/100 L (BotaniGard ES 9601) to the point of incipient run-off. The control was treated with demineralised water. Dimethoate was used as toxic standard and applied at the first and at the last application dates.

Duration: 26/04 - 02/06: Application
02/06 - 26/06: test period

Test conditions:

	Exposure period	Reproduction period
Temperature (°C):	25.0 - 25.5	not recorded
Relative humidity (%):	60 - 65	not recorded
Light intensity (lux):	1400 - 1900	733 - 1100

Nymphs of *Orius laevigatus* (Fieber) were confined to the test substance residues on detached leaves approximately 8 - 9 hours after drying of residues in 10 groups of 6 individuals per treatment. Mortality was assessed after a 9-day exposure period, after which time the toxic standard was stopped. Dead specimens were transferred to Petri-dishes with 5 % agar and incubated for 5 days at 25 °C to check for fungal growth of *Beauveria bassiana*. Surviving animals in remaining treatments were transferred and kept together for 5 days to ensure mating. Reproductive success was determined on a sub-batch of 12 - 15 females per treatment which were kept individually on untreated cowpea bean leaves during 2 consecutive 2 or 3-day periods. The number of hatched and unhatched eggs was counted 5 days after females were removed from the oviposition substrates.

Deviations from guideline: No major deviations

Endpoint: Mortality
Reproduction

Observations: Juvenile mortality after a 9-day exposure period to treated grapevine leaf.
Oviposition of surviving females during 2 successive periods.
Egg hatch success of the eggs laid during the oviposition periods.
Proportion of cadavers displaying *Beauveria bassiana* sporulation (following min. 3 days incubation).

Results:
The results are presented in Table B. 9.5-5.

Table B. 9.5-5: Results of the test with *Orius laevigatus*

Test substance	**BotaniGard**		
Test object	***O. laevigatus***		
Exposure	**Grapevine leaf disks**		
	Mortality after 9 days	Per capita daily oviposition	Egg hatch success
Control	19 %	5.7	82 %
BotaniGard Treatments	Corrected mortality after 4 days	Reduction of ovipostion relative to the control	Reduction of egg hatch success relative to the control
BotaniGard 22WP	3 % (ns)	23 % (ns)	24 % *
BotaniGard ES 9601	11 % (ns)	-7 % (ns)	29 %*
Additional observations	*O. laevigatus* exposed to residues of BotaniGard can be infected with spores of *B. bassiana* under the specific conditions tested in this study.		

ns = difference not statistically significant,
* difference statistically significant (mortality data: Fisher's Exact test, ovipositon data: ANOVA followed by Fischer's LSD test, egg hatch success data: Mann-Whitney U test

Conclusion:
Exposure to residues of the test product applied at 125 g product/100 L (952 g product/ha) for BotaniGard 22WP and 250 mL/100 L (1693 mL product/ha) BotaniGard ES9601, resp. has no adverse effect on *Orius laevigatus* juvenile survival after a 9-day exposure period or on oviposition after a 5-day exposure period. Exposure resulted in a statistically significant adverse effect on egg hatch success. *O. laevigatus* exposed to residues of BotaniGard, can be infected with spores of *Beauveria bassiana* under the specific conditions tested in this study.

Reference: IIIM 10.4
Author: Hoogendoom, G.
Title: BotaniGardTM: an extended laboratory test to determine effects of two formulations of Beauveria bassiana on spring generation spiders of the genus Pardosa
Date: 15 May 2000
Doc ID: Unpublished report MT004PSE; BVL No. ANA2006-210
Guideline: No specific guideline was used. A combination of the following was followed throughout the course of the study: Wehling et al. (1998), SETAC/ESCORT (Barrett et al., 1994).
GLP: Yes
Acceptability: The study is considered as acceptable.

Material and methods:
Test substance: BotaniGard 22WP
BotaniGard ES
Test species: Pardosa spp.

Number of test animals:	Each treatment consisted of 20 replicate test units, containing 1 female spider each at the last application.
Treatments:	Two formulations of the entomopathogene BotaniGard were applied to soil 6 times with a 6 to 8-day spray interval at concentrations of 125 g product/100 L water (BotaniGard 22WP) and 250 mL product/100 L water (BotaniGard ES). The control was treated with demineralised water. Karate (lambda-cyhalothrin) was tested as toxic standard and applied at the first and at the last application dates.
Duration:	21 days
Test conditions:	Temperature (°C): 19.2 - 20.4, except for 10 days after exposure, when a temperature of 24.8 °C was measured. Relative Humidity (%): 63.5 - 69.3 Illumination: 16 h light/8 h dark; 736 - 840 lux during light
Deviations from guideline:	No major deviations
Endpoint:	Mortality Food consumption
Observations:	Assessments of spider condition took place 2 to 4 hours after starting exposure and 1, 4, 6, 8, 11 and 13 days after starting exposure. The number of eaten and uneaten flies was recorded. Mortality, behavioural changes, the occurrence of moulting skins and the occurrence of fungal growth on dead spiders of the BotaniGard treatments was monitored. Dead spiders were incubated at approx. 100 % relative humidity to determine if they were indeed infected by *Beauveria bassiana*. 13 days after starting exposure, it was decided to prolong the bioassay for a week as mortality in the BotaniGard treatments in the second week was high. Additional assessments of spider condition and food consumption were carried out 15, 18 and 21 days after starting exposure.

Results:
The results are summarised in Table B. 9.5-6.

Table B. 9.5-6: Results of the test with *Pardosa* ssp.

Treatment	No spiders tested	Mortality [%]	Mean cumulative no. flies eaten per spider
Water	19 **	0	6.88
Karate	20	65 (nt)	4.90 (nt)
BotaniGard ES	20	45 *	5.40 (ns)
BotaniGard WP22	20	80 *	5.19 (ns)

** spider went missing; nt = not tested; * Fisher's exact test $P \leq 0.001$; ns overall treatment effect not significant (Kruskal-Wallis, $P = 0.074$)

Conclusion:
Under the specific conditions of this test, exposure to BotaniGard when applied at 125 g product/100 L water as a WP formulation or at 250 mL/100 L as ES formulation can cause an infection with spores of *Beauveria bassiana* and have a lethal effect to *Pardosa* spp..

Exposure does not cause statistically significant (Fisher's exact test , P < 0.001, Kruskal-Wallis P = 0.074) adverse effects on food consumption.

B.9.5.2.2 Field test

Reference:	IIM 8.8
Author:	Jaronski, S. T., Lord, J., Simmons, G. and Hoelmer, K.
Title:	Field evaluation of the effects of Mycotrol WP® (*Beauvaria bassiana* strain GHA) on the Whitefly parasite, *Eretmocerus* sp. (Hymenoptera: Aphelinidae) in commercial melons.
Date:	April 24, 1996
Doc ID:	Unpublished report No. 95-006A; BVL No. ANA2006-206
Guideline:	US EPA Guideline 154A-23
GLP:	No
Acceptability	not acceptable (The test design is suitable to determine efficiency and not to determine effects. The establishment of an artificial population in the field is questionable, as migration at the borders can occur. Therefore, effects may be not obvious. The results after the first application show only a low rate of paratisation.)

Material and methods:

Micro-organism / Test substance	BotaniGard 22WP
Test species:	*Eretmocerus* sp.
Number of test animals:	Field test with an artificial population
Treatments:	Artificially augmented field populations of *Eretmocerus* sp. were exposed to Mycotrol WP (which contains the fungus *Beauveria bassiana* Mycotech strain GHA). The test was conducted in two commercial cantaloupe fields in Imperial County, California, during the spring of 1995. The experiment involved several weeks of wasp releases onto 0.75 acre plots, followed by three applications of Mycotrol WP, at 0.454 kg per acre (equal to 2 x 10^{13} viable *B. bassiana* conidia per acre), at approximately five-day intervals. Applications were made by a Solo® motorised backpack airblast sprayer. No comparisons with chemical insecticide treatments were made. An estimated 40,000 pupae were released in the first field with an adult emergence rate of ca. 50 %. In the second field an estimated total of 120,000 parasite pupae were released with an emergence rate of ca. 50 %. Prior to the first application 60 leaves were randomly taken from the entire experimental area within each field site to determine initial whitefly and parasite population levels.
Duration:	Parasite release: 21 March - 15 May Applications: 19 May - 31 May Sampling: 19 May - 16 June
Test conditions:	Field test

Deviations from guideline	none
Endpoint:	Parasite population level
Observations:	12 and 28 days after the first application samples of 30 leaves were taken randomly from both experimental sites, to determine the percentage parasitism. Additional samples of leaves were taken 7 (field 1) or 9 (field 2) days after the third and last fungus application. These samples were taken to measure the overall efficacy of the sprays.

Results:
In one site (field 1) differences in the number of *Eretmocerus* sp. in untreated, carrier-treated and Mycotrol WP treated plots, were not statistically significant on each of two sample dates. The percent parasitism was numerically higher in Mycotrol WP plots than in control plots, while peak whitefly population reduction by the fungus was 71 - 72 %. In the second site (field 2) numerical differences among treatments in both parasite numbers and percent parasitism were not statistically significant. Whitefly control from the Mycotrol applications here was only 43 - 44 %. The fungus overtly infected a low, variable percentage of parasitised nymphs.

Conclusion:
Even though parasites can be infected by the fungus, large percentages of whitefly parasites (*Eretmocerus* sp.) survived field use of Mycotrol WP which contains *Beauveria bassiana* in cantaloupe melon. Overall levels of parasitism were unaffected, which may have been at least partly due to an increase in the parasite to host ratio.

Reference:	IIM 8.9.2
Author:	Goettel, M., Douglas Inglis, G., Duke, G. M. and Lord, J. C.
Title:	Effect of Beauveria bassiana (Mycotech strain GHA) on invertebrates in Rangeland and Alfalfa Agroecosystems.
Date:	March 14, 1997
Doc ID:	Unpublished report No. 95-04; BVL No. ARW2006-100
Guideline:	US EPA Guideline 154A-23
GLP:	No
Acceptability:	The study is considered as acceptable**.**

Material and methods:

Test substance:	Mycotrol ES formulation
Test species:	Field test with natural population of non-target arthropods
Number of test animals:	Field test

Treatments:	In order to determine the extent to which non-target arthropods would be impacted by field applications of *B. bassiana* for grasshopper control, the most abundant non-target arthropods in two agroecosystems, rangeland and alfalfa were collected and processed to determine the prevalence rates of the fungus before and at intervals after application of the product at the rates of 1.75×10^{13} *B. bassiana* conidia/ha (rangeland) or 3.5×10^{13} conidia/ha (alfalfa). These application rates are higher than those proposed for the EU. For both rangeland and alfalfa, treatments consisted of Mycotrol ES (*Beauveria bassiana* conidia in emulsifiable oil) and a carrier control. For the rangeland experiment, an untreated control was also included in the experimental design.
Duration:	In life initiation date: July 10, 1995 In life termination date: May 1, 1996
Test conditions:	The infectivity of the *B. bassiana* conidia applied to alfalfa leaves was tested by feeding nymphs of a laboratory strain of *Melanoplus sanguinipes* (Fabricus) leaves collected immediately and 12 h after conidial application. Conidial persistence was assayed by arbitrarily collecting ten rangeland grasses or alfalfa leaves from each subplot. Sampling times were as follows: 0, 2, 5 or 7, 10 and 15 days post-application.
Deviations from guideline:	None
Endpoint:	Infectivity of conidia
Observations:	The prevalence rates of *B. bassiana* in non-target arthropods was measured by two methods, surface disinfection followed either by homogenisation and plating on selective medium, or incubation of cadavers in a high humidity chamber. Grasshopper population densities were monitored by counting living grasshoppers in sample areas delimited by permanent sampling frames one day prior to application, 0, 4, 11 and 15 days post-application of *B. bassiana* conidia.

Results:

The deposition of spray droplets on water-sensitive paper was similar in the rangeland and alfalfa experiments. Grasshopper nymphs exposed to alfalfa leaves sprayed with *B. bassiana* displayed disease 5 days after ingestion of the leaves. After 10 days, 97.9 % of nymphs had died of mycosis compared to 1.2 % of nymphs that had ingested alfalfa leaves collected from the control treatment. Conidial survival decline logarithmically over time and no conidia were recovered on leaves from control plots. Arthropods sampled before conidial application or from control plots yielded no or very small populations of *B. bassiana*. The greatest number of *B. bassiana* CFU recovered from any arthropod from rangeland was 2 x 103 CFU. Mean populations of *B. bassiana* on spiders, carabid and tenebrionid beetles collected from sprayed plots were less than 25 CFU per arthropod and populations of *B. bassiana* appeared to decrease at later sampling times. The following arthropod species were collected from treated plots but showed absence of mycosis when assayed: araneida spiders, carabid beetles, tenebrionid beetles, scarab beetles, tiger beetles, burying beetles, rove beetles, click beetles, blister beetles, skin beetles, weevils, grasshoppers, robber flies, ants, velvet ants, plant bugs, ladybird beetles, sunscorpions, bees, flies and lepidopterans. Ladybird beetles collected in alfalfa plots

by sweep netting 2 days after application showed colonisation by *B. bassiana*. Similarly, alfalfa weevils and lygus bugs collected 5 days after application and placed on filter paper displayed colonisation by *B. bassiana*. Overwintering leafcutting bee survival was shown to be minimally affected by *B. bassiana* treatment. Conidial survival on or in grasshopper nymphs declined over time. A low number of *B. bassiana* CFU were recovered from control plot nymphs at all sampling times. Application of *B. bassiana* did not affect field population numbers of grasshoppers. Nymphs collected from control treatment plots and maintained in cages displayed less than 7 % mycosis after 12 days.

Conclusion:
Infection of grasshoppers by assay of treated alfalfa demonstrated that there were sufficient spores delivered to kill target arthropods. No significant effects were recorded in non-target arthropods.

Reference:	IIM 8.8.4
Author:	Brinkmann, M.A., Fuller,B.W.
Title:	Influence of *Beauveria bassiana* strain GHA on Nontarget Rangeland Arthropod
Date:	1999
Doc ID:	Publication; BVL No. 1679474
Guideline:	OECD Guideline 207
GLP:	Yes, US EPA GLP Standards, 40 CFR 160
Acceptability:	The study is considered as acceptable.

Material and methods:

Test substance:	*Beauveria bassiana* (Balsamo) Vuillemin strain GHA at 1 x 10^{13} conidia in 1.89 L oil per 0.405 ha, carbaryl at 559.88 g (as) in 0.59 L total volume per hectare, or no insecticide (untreated controls)
Test species:	Field test with natural population of non-target arthropods
Number of test animals:	Field test
Treatments:	Infectivity of *B. bassiana* was determined by capturing grasshoppers from treatment plots 7 d after application and monitoring them for external growth of the fungus. Major predators, parasitoids, and pollinators were captured with pitfall and malaise traps weekly throughout the summer season. Abundance of individuals belonging to Formicidae, Araneae, *Pasimachus elongates, Amara obesa, Platynus decentis,* Carabidae, Ichneumonidae, Mutillidae, Sphecidae, Chalcididae, Noctuidae, Coccinellidae, Meloidae, 2 Dipteran families (Nemestrinidae, Asilidae), 10 other Hymenopteran families (Vespidae, Cephidae, Braconidae, Proctotrupidae, Figitidae, Halictidae, Tiphiidae, Apidae, Andrenidae, Megachilidae), and 3 Neuropteran families (Chrysopidae, Myrmeleontidae, Hemerobiidae) was evaluated.
Duration:	No information
Endpoint:	Infectivity of conidia

Results:
About one-half of the grasshoppers collected from *B. bassiana* treatment plots exhibited external growth of the fungus. Formicidae, Araneae, and Carabidae decreased in all plots during periods of heavy precipitation after the treatment date, but these declines were not caused by mortality as a result of *B. bassiana* or carbaryl spray treatments. Ground-dwelling arthropod abundance rebounded to pretreatment levels 2 weeks after treatments. No statistical differences in the abundance of aerial insects were detected with respect to treatment effects; however, natural increases in abundance in all plots were observed as the season progressed.

Conclusions:
Results from this large-scale field study suggest that the grasshopper-derived strain of *B. bassiana* can be used for grasshopper suppression on rangeland without major impacts on non-target arthropods.

B.9.5.3 Toxicity/Infectiveness/Pathogenicity

Studies submitted show, that *Beauveria bassiana* strain GHA can infect all tested arthropods under conditions of laboratory tests. Observed cases of mortality and/or effects on reproduction are considered to be pathogenic effects, due to the nature of *Beauveria bassiana* (GHA) as an entomopathogen microorganism.
Therefore all tested arthropods belong to the physiological host range of *Beauveria bassiana* (GHA). On the other hand the two submitted field tests indicate no harmful effects to beneficial insect populations.
So it can be concluded that there is a significant difference between the physiological host range (laboratory conditions) and the ecological host range (field conditions).

B.9.5.4 Summary and risk assessment for non-target arthropod species other than bees

Extended laboratory tests with applied soil or leaves were performed with the standard test organisms *Aphidius rhophalosiphi, Typhlodromus pyri, Orius laevigatus* and *Pardosa* spec. The results are summarised in Table B. 9.5-7.

Table B. 9.5-7: Summary of effects of BotaniGard 22WP and BotaniGard ES on non-target arthropods - extended laboratory tests (part A)

Test organism	Exposure	LR_{50} (kg MPCP/ha) BotaniGard 22WP	LR_{50} (L MPCP/ha) BotaniGard ES 9601	Reference
A. rhophalosiphi	Grape vine leaf disks	> 0.947	> 1.695	IIIM 10.4 Aldershof S.A.., 2000 (ANA2006-208)
T. pyri	Grape vine leaf disks	> 0.947	> 1.695	IIIM 10.4 Aldershof S.A.., 2000 (ANA2006-207)
O. laevigatus	Grape vine leaf disks	> 0.952	> 1.693	IIIM 10.4 Aldershof S.A.., 2000 (ANA2006-209)
Pardosa spec.	Soil	No sufficient information	No sufficient information	IIIM 10.4 Hogendoom G., 2000 (ANA2006-210)

Table B.9.5-8: Summary of effects of BotaniGard 22WP and BotaniGard ES on non-target arthropods - extended laboratory tests (part B)

Test organism	Exposure	50 % effect (conidia/ha)	Reference
Eretmocerus sp.	Field test	> 5 x 10^{13} condidia/ha (parasitation) (test regarded as not valid)	IIM8.8 Jaronski et al., 1996 (ANA2006-206)
Xylocoris flavipes	Filter paper	> 10^{15} (effect: infection)	IIM 8.8 Jaronski & Dunkel, 1993 (ANA2006-205)
Tenbrio molitor	No information	> 10^{8} conidia/test (effect: mortality)	IIM 8.8 Hildreth & Jaronski, 1993 (ANA2006-204)

For the test with *Pardosa spec.* the test concentrations are not properly reported. Only the first concentration of the multiple applications is mentioned. If this concentration (510 g/ha) is assumed to be representative for the further applications it has to be concluded that the multiple application of BotaniGard 22WP results in negative impacts for Pardosa spec. (80 % mortality). As no information is available concerning the total number of spores the results cannot be used for risk assessment.
In a filter paper test with *Xylocoris flavipes* a maximum application rate of 2.5 x 10^{15} conidia/ha was applied resulting in 41 % effect. A single application rate of the product equals to about 10^{13} conidia/ha. This concentration results in no effect in the filter paper test.
The test results of *T. molitor* cannot be used for risk assessment as no information concerning the application rate/ha is given in the report.

Field studies with alfalfa and rangeland revealed no unacceptable negative impacts.
1.75 x 10^{13} conidia/ha were applied to rangeland and 3.5 x 10^{13} conidia/ha to alfalfa (IIM 8.9.2/01; Goettel et al. (1997), ANA2006-100). There was a logarithmically decrease of *B. bassiana* (less than 10 % after 2 days). In the rangeland minor and short-lived impact on non-target arthropods were observed. In Alfalfa there were tempory increases of *B. bassiana*

in coccinelid beetles and harvestmen as well as small (< 20 %) or no increase in spiders and other insects. Furthermore a small number of bees were infected under field conditions. However, there was no infection of leafcutting bee larvae, prepupae or adult emergence following overwintering diapause.
The field study described in Brinkman and Fuller (1999, BVL-1679474) showed that *B. bassiana* strain GHA can be used for grasshopper suppression on rangeland without major impacts on non-target arthropods.

For BotaniGard 22WP following application is intended:

Crop:	tomatoes, cucumbers, ornamentals indoor
Concentration of as:	220 g/kg, 4.4 x 10^{13} CFU/kg
Number of application:	3 - 5
Interval between applications	5 d
Application rate per treatment:	max. 550 g MPCP/ha

BotaniGard is applied indoor. After greenhouse applications it can be assumed that not more than 0.1 % of the application rate enters the environment via generic drift from the greenhouse.

HQ-approach:
From the RMS point of view appropriate no effect concentrations (NOECs) are necessary in order to accomplish a risk assessment on the basis of the HQ-approach, since in all conducted extended laboratory tests *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.

Exposure:
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.

Risk assessment:
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, see in B.9.4.5) is considered to be acceptable.

B.9.5.5 Further information from literature

B. bassiana has a wide host range, occurring on several hundred arthropod species. However, host specificity depends on specific strains.
Generally, there is a difference between the physiological host range and the ecological host range (Hajek & Butler 2000 cited in Zimmermann 2007). The physiological host range demonstrates the range of insect species that can be infected in the laboratory, while the ecological host range demonstrates which insects can be infected in nature or under field conditions. Non-target insects which are infected under laboratory conditions may not necessarily be infected in nature. There are numerous papers on the effect of *B. bassiana* on beneficial and other non-target organisms. Most of the studies were done in the laboratory and only a few in the field. Laboratory bioassays demonstrated that it was possible that *B. bassiana* and *B. brongniartii*, resp., infect collembolans, cicindellid and carabid beetles as well as honey bees under stress conditions (e.g. Ludwig & Oetting, 2001, Vandenberg, 1990 cited in Zimmermann 2007). Data from field investigations did not indicate any indication of possible adverse effects on vertebrates, honeybees, beneficial insects, earthworms and plants (Vestergaard et al. 2003 cited in Zimmermann 2007).

B.9.6 Effects on earthworms (Annex IIM 8.9.1; Annex IIIM 10.5)

B.9.6.1 Active substance

Reference:	**IIM 8.9.1**
Author:	Hoxter, K. A., Palmer, S. J. and Krueger, H. O.
Title:	*Beauveria bassiana* strain GHA: An acute toxicity study with the earthworm in an artificial soil substrate.
Date:	1998
Doc ID:	Unpublished report No. 488-101A; BVL No. ARW2006-99
Guideline:	OECD Guideline 207
GLP:	Yes
Validity:	**Acceptable**

Material and methods:

Micro-organism:	*Beauveria bassiana* (8.59 x 10^{10} conidia/g)
Test species:	*Eisenia foetida*
Number of test animals:	280
Treatments:	0 (negative control), 0 (attenuated control), 130, 216, 360, 600, and 1000 mg/kg
Duration:	14 days

Test conditions:	20 ± 2 °C; 24 h photoperiod (400 to 800 lx)
Deviations from guideline:	None
Endpoint:	Mortality, Biomass
Observations:	Observations were made of the number of surviving worms and behavioural abnormalities on day 7 and 14. Body weights were recorded on day 0 and 14.

Results:
At study termination, there were no treatment related mortalities in the 130, 360, 600 and 1000 mg/kg treatment groups. All worms were normal in appearance and behaviour through-out the test period. None of the relevant groups showed any aversion to the treated soil. There were no treatment related effects in any group, although 2/39 worms died in the 216 g/kg treatment group, and 1/40 worm died in the attenuated control groups at test termination, these are not thought to be compound-related. There were no apparent treatment related effects upon bodyweight in the attenuated control or any of the treatment groups compared to the negative control group.

A summary of endpoints is given in the table below.

Table B.9.6-1: Toxic effects of active substance to *Eisenia foetida*

Test species	**EC_{50}**
Toxicity of active substance	LC_{50}: > 1000 mg/kg soil (8.6 x 10^{10} CFU/kg soil)
Body weight change	EC_{50} (weight change): > 1000 mg/kg soil (8.6 x 10^{10} CFU/kg soil)

Conclusions:
The 14-day LC_{50} to *Eisenia foetida* exposed to *Beauveria bassiana* strain GHA was found to be > 1000 mg/kg (8.6 x 10^{10} CFU/kg soil), with a NOEC = 1000 mg/kg dry soil (8.6 x 10^{10} CFU/kg soil).

B.9.6.1.1 Toxicity

No mortality was observed at the tested concentration. The biomass development was not statistically different compared to the control at the tested concentration.

B.9.6.1.2 Infectiveness/Pathogenicity

No histopathological examination has been carried out, but there were no external signs of illness. The fungus is not known to be pathogenic to earthworms.

B.9.6.2 Toxin/metabolite from active substance

No studies were submitted with toxins or metabolites. Toxic effects coming from secondary metabolites within the technical substance are covered by the studies with the active ingredient described (technical material) in B.9.6.1.

B.9.6.3 Plant protection product

Beauveria bassiana is a naturally-occurring soil borne fungus that has not been modified in any respect. It is likely that the fungus (and its relatives) constitutes food for earthworms and is expected not to cause harm to earthworms at the expected exposures levels for the use patterns of BotaniGard 22WP. An acute toxicity study on earthworms was carried out using the MPCA *Beauveria bassiana* strain GHA. The results of this study demonstrated that *B. bassiana* has no adverse effects on earthworms (Please refer to point 9.6.1). It is therefore proposed that further toxicity tests on earthworms are not required.

B.9.6.4 Summary and risk assessment for earthworms

The LC_{50} (14-day) and the LOEC were determined to be greater than 1000 mg *Beauveria bassiana* strain GHA per kg soil dry weight. The NOEC was determined to be greater or equal to 1000 mg *Beauveria bassiana* strain GHA per kg soil dry weight, the highest concentration tested, corresponding to 8.6 x 10^{10} CFU/kg soil (Hoxter, Palmer and Krueger, 1998; ANA2006-99).

For BotaniGard 22WP following application is intended:

Crop:	Tomatoes, cucumbers, ornamentals indoor
Concentration of as:	220 g/kg = 4.4 x 10^{13} CFU/kg
Number of application:	3 - 5
Intervall between applications	5 d
Application rate per treatment:	550 g MPCA/ha corresponding to 0.733 mg MPCA/kg 3.5 x 10^{14} CFU/ha corresponding to 4.7 x 10^{8} CFU/kg

Organism	LC_{50} [CFU/kg]	Application rate [CFU/kg]	MAF	PEC [CFU/kg]	Margin of safety (TER)	Trigger
Laboratory test						
E. foetida	≥ 8.6 x 10^{10}	4.7 x 10^{8}	3 - 5	9.6 x 10^{7} – 1.6 x 10^{8}	> 538 - > 896	10

Based on the predicted environmental concentration (PEC_{soil}) calculated previously as 9.6 x 10^{7} – 1.6 x 10^{8} CFU/kg soil for multiple applications in tomatoes, cucumbers, or ornamentals (indoor), assuming as a worst case that no degradation occurs between applications, the margin of safety (TER) for earthworms is derived from the LC_{50} value according to the formula:

$$\text{Margin of safety} = \frac{LC_{50}\ [\text{mg as/kg soil}]}{PEC_{soil}\ [\text{mg as/kg soil}]}$$

With values of > 538 - > 896, the calculated margin of safety exceeds the limit value of 10. Thus, no adverse effects on earthworms are expected after application of BotaniGard 22WP at recommended use levels even under the worst case assumption of no degradation in the soil.

Generally, it has to be taken into consideration that *B. bassiana* is a naturally occurring fungus. Earthworms are thus expected to be frequently exposed to this fungal species in nature. The earthworms' digestive tract does not contain chitin, in contrast to arthropods. The chitinolytic enzymes produced by *B. bassiana* will therefore not have a negative effect on earthworms. Production of toxins at significant levels has not been demonstrated and a long-term effect of the fungus on earthworms by application of BotaniGard 22WP according to Good Agricultural Practice is not expected.
Furthermore, it should be noted that *Beauveria bassiana* is mainly for use in glasshouses and hydroponic systems where earthworms do not occur. Therefore, the risk to earthworms from the use of BotaniGard 22WP is assumed to be negligible.

B.9.6.5 Further information from literature

The findings show that there are no or very low detrimental effects on the tested soil-dwelling collembolans and mites. In contrast, collembolans act as vectors of *Beauveria* spp. and thus play an important role for the dispersal and transmission of these fungi in soil (e.g. Broza et al., 2001; Visser et al., 1987, Dromph, 2001; Samšiňáková & Samšiňák, 1970 cited in Zimmermann 2007).
Laboratory bioassays demonstrated that earthworms were not affected (Hozzank et al., 2003; Arregger-Zavadil, 1992 cited in Zimmermann 2007).

Conclusion:
The risk of possible adverse effects on collembola, mites and earthworms is assumed to be negligible. However, organisms such as collembola might be used as vectors for dispersal and transmission of the fungi in soil.

B.9.7 Effects on non-target soil micro-organisms (Annex IIM 8.6; Annex IIIM 10.6)

Beauveria bassiana is a naturally occurring soil borne fungus. It is not likely to cause harm at the expected exposures according to the use patterns of BotaniGard 22WP. Therefore, a study to investigate the effects on soil micro-organisms is not required.

B.9.7.1 Information from literature

- Investigations on the natural prevalence of *B. bassiana* have shown that this fungus widely occurs in the soil as well as on insects in the aerial environment. Accordingly, there is a long-lasting evolutionary coexistence with other microorganisms which includes different forms of interactions.

In Canada, for example, the most abundant species in 266 soil samples from 86 locations were *B. bassiana* (187 isolates) and *M. anisopliae* (357 isolates) (Biodochka et al., 1988 cited in Zimmermann 2007). Furthermore, the natural occurance of *B. bassiana* is documented for the Czech Republic (Landa, 2002 cited in Zimmermann 2007), Finland (Vänninen, 1996 cited in Zimmermann 2007), Germany (Kleespies et al. 1989 cited in Zimmermann 2007), Italy (Southern part; Tarasco et al., 1997 cited in Zimmermann 2007), Japan (Shimazu et al., 2002 cited in Zimmermann 2007), Macquarie Islands (Roddam & Rath, 1997 cited in Zimmermann 2007), Nepal (Dhoj & Keller, 2003 cited in Zimmermann 2007), New Zealand (Barker & Barker, 1998 cited in Zimmermann 2007), Norway (Northern parts; Klingen et al., 2002 cited in Zimmermann 2007), Panama (Tropical forest; Hughes et al., 2004 cited in Zimmermann 2007), Poland (Sapieha-Waszkiewicz et al., 2003; Mietkiewski et al., 1996; 1994; Tkaczuk & Mietkiewski, 1996 cited in Zimmermann 2007), Spain (Asensio et al., 2003 cited in Zimmermann 2007), Switzerland (Keller et al., 2003 cited in Zimmermann 2007), USA (Shapiro-Ilan et al., 2003 cited in Zimmermann 2007).

- Artificial introduction of *B. bassiana* does not seem to interfere with the microbial equilibrium of natural soils.
  Wang et al. (2004 cited in Zimmermann 2007) monitored the fate of inundatively applied strains of *B. bassiana* against *Dendrolimus punctatus* in Southwest China. During one year, the indigenous and exotic strains were reisolated. However, the indigenous strains were predominant in the local environment, indicating that they were not displaced by the exotic ones. Comparable results were obtained for *B. brongniartii* in Switzerland (Enkerli et al., 2004 cited in Zimmermann 2007).
- There are several reports on interactions of *Beauveria* spp. with hyperparasitic, antagonistic and, phytopathogenic fungi.
  Inhibition of growth of *B. bassiana* by other micro-organisms: A hyperparasitic fungus attacking *B. bassiana* is the ascomycete *Syspastospora parasitica*, formerly known as *Melanospora parasitica* (Müller-Kögler 1961, Markova 1991, Posada et al. 2004 cited in Zimmermann 2007). Lingg & Donaldson (1981 cited in Zimmermann 2007) reported that the survival of conidia of *B. bassiana* in amended nonsterile soil was possibly related to *Penicillium urticae*, which produced a water-soluble inhibitor of *B. bassiana*. There are also various interactions with *Clonostachys* spp. and *Trichoderma* spp. which may suppress or overgrow *B. bassiana* in Petri dishes (Krauss et al. 2004 cited in Zimmermann 2007).
  Inhibition of phytopathogens by *B. bassiana*: The mycelial growth of phytopathogens of genera such as *Fusarium*, *Armillaria* und *Rosellinia* is reduced by filtrates of *B. bassiana* (Reisenzein & Tiefenbrunner 1997 cited in Zimmermann 2007).

Conclusion:
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.

B.9.8 Additional studies (Annex IIM 8.10; Annex IIIM 10.7)

Information from scientific literature:

Effects on plants

In summarizing the past literature, Müller-Kögler (1965 cited in Zimmermann 2007) concluded, that side-effects or any phytopathogenic activity on plants are not known. Due to the long history of application - in the past 100 years, *B. bassiana* has been used for biocontrol of many leaf- and root-feeding pest insects - the literature review, also performed in 1965, still seems to be relevant.
B. bassiana has been reported to be an endophyte of certain plants, especially in corn (Bing & Lewis 1992 cited in Zimmermann 2007), in coffee (Posada & Vega 2005 cited in Zimmermann 2007) and in banana tissue culture plants (Dubois et al. 2005 cited in Zimmermann 2007).
Beauvericin did not cause any symptoms on roots of melon, tomato, wheat and barley (Moretti et al. 2002 cited in Zimmermann 2007). The observed toxicity towards the protoplasts of these plants does not seem to be relevant with respect to the intended use, where protoplasts do not play a role.

Conclusion:
The risk of *B. bassiana* towards plants seems to be tolerable.

B.9.9 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 8 (OECD)	Strasser, H.; Vey, A. and Butt, T.M.	2000	Are there any Risks in using Entomopathogenic Fungi for pest Control, with Particular Reference to the Bioactive Metabolites of Metarhizium, Tolypocladium and Beauveria species? N/a GLP: N, published: N 1689733 / BWS2006-66	N	MEU
KIIM 8 (OECD)	Campbell, R.	1981	Mikrobielle Ökologie Verlag Chemie GmbH, Weinheim page 190 GLP: N, published: Y	N	LIT

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 8 (OECD)	Zimmermann, G.	2007	Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Science and Technology , Volume 17, Issue 6: 553-598 GLP: N, published. Y	N	LIT
KIIM 8.1 (OECD)	McEwen, L. C.	1993	Response of young American kestrals (*Falco sparverius*) to *Beauveria bassiana* strain GHA 93-012 GLP: N, published: N 1300995 / AVS2006-168	Y	MEU
KIIM 8.1 (OECD)	Hartmann, G. C. and Wasti, S. S.	1980	Avian safety of three species of entomogenous fungi on Japanese quail Comp. Physiol. Ecol. 5, 242-245 154A-16 GLP: N, published: Y 1300996 / AVS2006-167	N	MEU
KIIM 8.1 (OECD)	Johnson, D.L. et al.	2002	Assessment of health and growth of ring-necked pheasants following consumption of infected insects or conidia of entomopathogenic funge Metarhizium anisopliae var. acridum and *Beauveria bassiana* from Madagascar and North America J. of Toxicology and Environmental Health, Part A 65, 2145-2162 GLP: N, published: Y 1679420 /	N	LIT
KIIM 8.11 (OECD)	Fromtling, R.A., Kosanke, S.D., jensen, J.M., Bulmer, G.S.	1979	Fatal *Beauveria bassiana* infection in a captive American alligator. J Am Vet Med Assoc 175, 934-936 GLP: N, published: Y 1690115 / TOX2007-472	N	MEU
KIIM 8.2 (OECD)	Collins, M.K.	1993	*Beauveria bassiana* - evaluation of potential embryo larval toxicity and pathogenicity to fathead minnow (*Pimephales promelas*) under static renewal conditions 93-8-4910 GLP: Y, published: N 1300997 / WAT2006-386	Y	MEU
KIIM 8.4 (OECD)	Palmer, S. J. and Krueger, H. O.	1998	*Beauveria bassiana* strain GHA: a 96-hour toxicity test with the freshwater alga *Selenastrum capricornutum* 488A-101 GLP: Y, published: N 1300999 / WAT2006-388	Y	MEU

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 8.7 (OECD)	Bromenshenk, J.J. et al.	1996	Multiple endpoint, holistic assessment of the effects of Mycotrol WP (*Beauveria bassiana* strain GHA) on outdoor *Apis mellifera* L. colonies Study No. 95-05 GLP: N, published: N 1301000 / BIE2006-88	Y	MEU
KIIM 8.7 (OECD)	Hokkanen, H.M.T., Zeng, Q-Q. and Menzler-Hokkanen, I..	2003	Assessing the impacts of *Metarhizium* and *Beauveria* on bumblebees. In: Hokkanen HMT & Hajek AE (eds.) Environmental impacts of microbial insecticides, 63-72. Kluwer Academic Publishers. Published: Y	N	LIT
KIIM 8.7 (OECD)	Mommaerts, V., Boulet, J., Sterk, G. and Smagghe, G.	2007	Effects of bilogical control agents (BCAs) on pollinator, *Bombus terrestris* Not yet published.	—	—
KIIM 8.8 (OECD)	Jaronski, S.T. Dunkel, F.V.	1993	Acute toxicity/pathofenicity of *Beauveria bassiana* strain GHA to predators/parasites: *Xylocoris flavipes* [Hemiptera: Anthrocoridae] Lab.Rep. 93-019 GLP: Y, published: N 1301001 / ANA2006-205	Y	MEU
KIIM 8.8 (OECD)	Duperchy, E.	2003	Identification of up-regulated genes of the hyphomycete, *Beauveria bassiana*, during infection of *Leptinotarsa decemlineata*. Dissertation, Ruberto-Carola University of Heidelberg, Germany	N	LIT
KIIM 8.8 (OECD)	Hildreth, M. Jaronski, S.T.	1993	Acute toxicity/pathofenicity of *Beauveria bassiana* strain GHA to *Tenebrio molitor* [Coleoptera: Tenebrionidae] Lab.Rep. 93-015 GLP: N, published: N 1535005 / ANA2006-204	N	MEU
KIIM 8.8 (OECD)	Havelka, P. and Ruge, K.	1988	Auswirkungen der Bekämpfung des Waldmaikäfers (*Melolontha hippocastani* F.) im Forstbezirk Karlsruhe-Hardt auf die Avi-Fauna. Mitteilungen der Forstlichen Versuchs- und Forschungsanstalt Baden-Württemberg, Freiburg/B. 132: 117-139. (In German).	N	LIT
KIIM 8.8.4 (OECD) KIIM1 10.4 (OECD)	Brinkman, M.A. Fuller, B.W.	1999	Influence of *Beauveria bassiana* strain GHA on nontarget rangeland arthropod populations Environmental Entomology 28, 863-867 GLP: Y, published: Y 1679474 /	N	LIT

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIM 8.9.1 (OECD)	Hoxter, K.A. et al.	1998	An acute toxicity study with the earthworm in an artificial soil substrate Proj.No. 488-101A GLP: Y, published: N 1301003 / ARW2006-99	Y	MEU
KIIM 8.9.2 (OECD)	Goettel, M. et al.	1997	Effect of *Beauveria bassiana* (Mycotech strain GHA) on invertebrates in rangeland and alfalfa agroecosystems Proj.No. 95-04 GLP: N, published: N 1301004 / ARW2006-100	Y	MEU
KIIM 8.11 (OECD)	Madigan, M.T., Martinko, J.M., Parker, J.	2001	Brock Mikrobiologie, Spektrum Akad. Verlag, Heidelberg: 430-432 GLP: N, published: Y	N	LIT
KIIIM1 9 (OECD)	Rautmann, D. et al.	2001	New spray drift values in the authorization procedure for plant protection products In: Forster et al.: Workshop on risk assessment and risk mitigation measures in the context of the authorisation of plant protection products; Mitt. Biol. Bundesanst. (Berlin-Dahlem) , GLP: N, published: Y 1639813 / WAS2006-329	N	LIT
KIIIM1 10.4 (OECD)	Hoogendoorn, G.	2000	BotaniGard: An extended laboratory test to determine effects of two formulations of *Beauveria bassiana* on spring generation spiders of the genus Pardosa MT004PSE GLP: Y, published: N 1301021 / ANA2006-210	Y	MEU
KIIIM1 10.4 (OECD)	Aldershof, S.	2000	BotaniGard: An extended laboratory test to determine effects of two formulations of *Beauveria bassiana* on the minute pirate bug *Orius laevigatus* MT003OLE GLP: Y, published: N 1301022 / ANA2006-209	Y	MEU
KIIIM1 10.4 (OECD)	Aldershof, S.	2000	BotaniGard: An extended laboratory test to determine effects of two formulations of *Beauveria bassiana* on the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez) MT002ARE GLP: Y, published: N 1301023 / ANA2006-208	Y	MEU

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner[1]
KIIIM1 10.4 (OECD)	Aldershof, S.	2000	BotaniGard: An extended laboratory test to determine effects of two formulations of *Beauveria bassiana* on the preadatory mite *Typhlodromus pyri* (DeStefani-Perez) MT001TPE GLP: Y, published: N 1301024 / ANA2006-207	Y	MEU

Codes of owner

LAM	Laverlam International Corporation
LIT	Literature
MEU	Mycotech Europe Ltd.