Draft Assessment Report (DAR)

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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

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Annex B

***Beauveria bassiana* GHA**

B-7: Residue data

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

B.7.1 Persistence and likelihood of multiplication in or on crops, feedingstuffs or foodstuffs (OECD IIM 6.3 and OECD IIIM 8)

Beauveria bassiana is a naturally occurring entomopathogenic fungus found in soils throughout the world. Its infection mechanisms are highly evolved and specific to insects. After contact with such a host, *B. bassiana* will multiply and take over the body of the insect, but usually dies when the insect dies. Unless the environment is exceptionally humid, there is no further reproductive activity, since continued multiplication and reproduction are entirely dependent upon another specific insect host becoming infested. Under natural conditions, *B. bassiana* conidia germinate and die within few days in the absence of a suitable host insect in aqueous environments.

B. bassiana requires an insect host in order to multiply and reproduce. The conidia of *B. bassiana* are hyaline and rapidly killed by sunlight (Jaronski, 1993, BWS2006-67) (Inglis et al., 1995, 1690316). Furthermore they are highly sensitive to higher temperatures, i.e. they become inactive above 33 °C (Fargues et al, 1997, 1690318).

B.7.2 Exposure to consumers (OECD IIM 6.2 and OECD IIIM 8)

B.7.2.1 Non-viable residues

Beside the MPCA residual materials remaining from the fermentation process are contained in the technical grade material. These substances, besides insoluble starch mainly nutrients, hyphae fragments and extracellular proteins are not considered part of the residue.

Entomogenous fungi of the various genera such as entomo- and phytopathogenic *Fusarium, Beauveria, Verticillium*, etc. are known to secrete secondary metabolites at levels which potentially may be of concern. In open literature, *Beauveria bassiana* is described to produce a range of different metabolites such as beauvericin, bassianolide, bassiacridin, beauveriolides, beauverolides, bassianin, tenellin, oxalic acid and oosporein. Production of these metabolites depends on the culture conditions during production of the inoculum and possibly on the strain. Toxin quantities produced *in vivo* are usually by far lower than those produced in nutrient rich liquid media. Oosporine was found in related species (*Beauveria brogniartii*) but was absent in cultures of *B. bassiana* (Strasser et al., 2000, 1690320). Apart from beauvericin (< LOQ of 50 ppm), oosporein (not detected, no LOQ given), bassianolide (< LOQ of 50 ppm) and bassianin/tenellin (cultures of GHA do not show the yellow colour of these metabolites) there is no information available if the above mentioned metabolites produced by *B. bassiana* can also be formed in the particular *B. bassiana* strain GHA.
The mycotoxin beauvericin is considered the most important metabolite produced by *B. bassiana* (see B.6.2.4). Besides a moderate insecticidal activity beauvericin has been reported to exhibit antibiotic properties but can also induce apoptosis in murine and human cell lines. However time course of pathogenesis and mortality is consistent with a general invasive mode of infection, i.e. decomposition of internal structures of the insect host. Some authors presume that production of endotoxins like beauvericin might serve for protection of the “prey” from competing micro-organisms rather than killing the host.

B. bassiana strain GHA was found to contain detectable concentrations of beauvericin in one batch with 50 mg/kg. The analysis of 21 other batches gave no detectable residues above the LOD of 5 mg/kg or the LOQ of 50 mg/kg, respectively (Jaronski, 1995 et al., CHE2006-752). Assessment of consumer exposure was performed with a beauvericin concentration in the MPCA of 50 ppm. This is supposed to represent the overall content of beauvericin and the other toxins which might have occurred in the technical material (see above). Due to a lack of toxicological and analytical data, no separate quantitative risk assessment was possible for those compounds. It is assumed, that they were of comparable toxicity and concentration as beauvericin. To account for beauvericin and the other metabolites, it is therefore considered sufficiently conservative to base the consumer risk assessment on a concentration of 50 ppm while at the same time lowering the specification for beauvericin in the batches of the MPCA to levels as low as technically feasible (5 ppm). Due to the lack of individual analytical methods for all metabolites, it is proposed to specify only beauvericin. The concentration of beauvericin in batches of the MPCA should be strictly controlled.

Highest beauvericin concentrations were assumed to be present at the time of application. The critical GAP involves 5 applications in tomatoes or cucumbers with a single application rate of 0.121 kg MPCA/ha. Only one single application was taken into account for the assessment because an accumulation was not considered likely given that *B. bassiana* does not show any metabolic activity in the absence of a suitable host. Assuming a beauvericin concentration of 50 ppm in the technical product, the application rate corresponds to 0.61 µg beauvericin/m^2.

Acute Risk Assessment

The short-term dietary risk assessment is based on the 97.5th percentile consumption estimate on a commodity basis using the German consumption data (VELS). Based on a TTC (threshold of toxicological concern) approach, no acute and/or chronic adverse effects are expected from beauvericin concentrations up to 0.02 µg/kg bw/d (see B.6.2.4). This value is used in the NESTI calculations.

No supervised residue trials are available for tomatoes. Therefore a very rough worst-case exposure calculation was performed which was based on the following assumptions:

- a large portion of 150 g tomatoes or of 150 g cucumbers is consumed (consumption data for children aged 2-<5 years, bw 16,15 kg, German VELS study, 2005), the large portion consists of more than one single unit for tomatoes and of fewer than one single unit for cucumbers
- the tomatoes are spherical and have an average diameter of 5-6 cm (resulting in a surface area of 0.00785-0.0113 m^2 and a volume of 65-113 ml, which corresponds well with the unit weight of 99 g from the VELS study)
- the cucumbers are cylindrical and have an average length of 35 cm and an average diameter of 4 cm (resulting in a surface area of 0.0465 m^2 and a volume of 440 ml, which corresponds well with the unit weight of 458 g from the VELS study)
- the relative density of tomatoes and cucumbers is considered to be δ = 1 kg/L (due to the high water content)
- tomatoes as well as cucumbers are fully covered by the spraying solution during application (no interception by leaves etc.)

With these assumptions, the amount of beauvericin in the “large portion” of tomatoes was calculated to be 0.00915-0.01105 µg corresponding to 0.0610-0.0737 µg/kg. This was used as the HR in the NESTI calculations. The intake of beauvericin *via* tomatoes is 14-17 % of the TTC value thus not indicating an acute risk for consumers.

Chronic Risk Assessment

With the TTC value of 0.02 µg/kg bw/d for beauvericin and a mean consumption of 15.6 g tomatoes and 9.5 g cucumbers per day during a lifetime, the chronic risk for consumers resulting from the intended use in tomatoes and cucumbers is negligible. However it should be kept in mind that according to open literature naturally occurring background concentrations of beauvericin in cereal grains and maize kernels might be more than three orders of magnitude higher than the concentrations expected in tomatoes or cucumbers from the intended use.

B.7.2.2 Viable residues

Residue studies have not been conducted on *Beauveria bassiana* Strain GHA as *B. bassiana* occurs naturally in the environment, in soil and on plants as a result of natural infection of insects. Hence there is every possibility that the fungus would be found in a wide range of crops and raw foodstuffs. Moreover, it is difficult to distinguish between the conidia of sprayed organisms and those which occur naturally. Only advanced DNA-based techniques have sufficient resolution to differentiate strains of *B. bassiana*.

B. bassiana requires an insect host in order to multiply and reproduce; unless humidity is exceptionally high, the fungus dies with the insect host. The half-life of *B. bassiana* in the field is very short. Under natural conditions, conidia perish within few days following germination in the absence of a suitable host insect.

Thermo- and particularly photolability of *B. bassiana* conidia have been demonstrated in various studies:. A half-life of 2.6 hours was measured for conidial viability under simulated sunlight conditions in the laboratory (Jaronski, 1993, BWS2006-67). In line with this result rapid reduction of *B. bassiana* germination from 88 % to 41 % was observed in a further study, following two hours exposure to simulated sunlight (Ignoffo et al, 1992,). Almost complete inactivation for unprotected *B. bassiana* spores was found after an exposure to direct sunlight after 60 min or 20 s of UV light of 302 nm wavelength (Edgington et al., 2000)
A maximum thermal threshold of 37 °C was determined for growth of 65 isolates of *B. bassiana* from different geoclimatic and host origins (Fargues et al., 1997, 1690318) while under storage conditions of 50 °C a half-life of 4.6 days was determined for viable residues of the MPCA (Jaronski, 1993, BWS2006-62).

It is concluded that any residue of *B. bassiana* on crops is expected to be short-lived and concentrations dissipate to non-infective levels within few days.

B.7.3 Summary and evaluation of residue behaviour (OECD IIM 6.5 and OECD IIIM 8)

Studies on residue levels in food crops have not been conducted for *Beauveria bassiana* strain GHA as *B. bassiana* occurs naturally in the environment in soil and on plants as a result of natural infection of insects. Hence there is every possibility that the fungus would be found in a wide range of crops and raw foodstuffs. Moreover, it is difficult to distinguish between the conidia of sprayed organisms and those which occur naturally. Only advanced DNA-based techniques have sufficient resolution to differentiate strains of *B. bassiana*.

B. bassiana requires an insect host in order to multiply and reproduce. Unless humidity is exceptionally high, the fungus dies with the insect host. Under natural conditions, conidia perish within few days following germination in the absence of a suitable host insect. *B. bassiana* conidia are thermo- and photo-labile; they are inactive above 33 ºC and are destroyed by UV-sunlight. Thus any residues on crops would be short-lived and there is no possibility of *B. bassiana* having a harmful effect on the food chain, or from multiplying within foods and feedstock.

B. bassiana strain GHA was found to contain detectable concentrations of beauvericin in one batch with 50 mg/kg. The analysis of 21 other batches gave no detectable residues above the LOD of 5 mg/kg or the LOQ of 50 mg/kg. The amount of beauvericin or other metabolites which might be expected from the intended use is not likely to pose an acute or chronic risk for consumers.

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

Neither a residue definition nor an MRL are considered necessary.

B.7.4 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]
OECD KIIM2.8	Jaronski, S.T.	1993	TGAI Physical stability study of Beauveria bassiana strain GHA: sunlight effects. Report-No. 93-001 GLP: no Published: no BWS2006-67	Y	LAM
KIIA1 2.4. (OECD)	Fargues	1997	Effect of temperature on vegetative growth of Beauveria bassiana isolates from different origins, Mycologia 89 (3), 1997, 389-392 Report-No. Not applicable GLP: no Ublished: Yes BWS2006-65	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 6.1 (OECD)	Jaronski, S.T., Becerra, M., Sears, J.	1999	Analysis of Mycotrol/BotaniGard TGAI (beauveria bassiana strain GHA Conidial Powers) for the metabolites Beauvericin and bassianolide. 98-06 GLP: Y, published: N 1679387 / CHE2006-752	Y	MEU
KIIM 6.1 (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: O, published: N 1690320 / BWS2006-66	N	MEU
KIIM 6.3 (OECD)	Jaronski, S.T.	1993	TGAI Physical stability study of Beauveria bassiana strain GHA: sunlight effects. 93-001 GLP: Y, published: N 1690316 / BWS2006-62	Y	LAM
OECD KIIM7.1 and KIIIM9	Inglis, G. D., Goettel, M. S. and Johnson, D. L.	1995	Influence of ultraviolet light protectants on persistence of the entomopathogenic fungus, Beauveria bassiana Biological Control 5, (1995), 581-590 Report-No. not applicable GLP: no Published: yes BOD2006-319	N	LIT
OECD KIIM... (B.7.2.2)	Edgington, S., Segura, H.; De la Rosa, W., Trevor, W.	2000	Photoprotection of Beauveria bassiana: testing simple formulations for control of the coffee berry borer International Journal of Pest Management 46 (3), (2000), 169-176 BNWS...	N	LIT
OECD KIIM. (B.7.2.2)	Ignoffo, C.M., Garcia, C.	1992	Influence of conidial color on inactivation of several entomogenous fungi (Hyphomycetes) by simulated sunlight. Env. Ent. 21 : 913-917 Report-No. not applicable GLP: no Published: yes BWS...	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]
KIIIM1 8 (OECD)	Anonymous	2000	Beauveria bassiana strain GHA (128924) technical document 128924 GLP: Y, published: N 1679406 /	Y	MEU

Codes of owner

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