Biocidal Products Committee (BPC)

Opinion on the application for approval of the active substance:

***Bacillus amyloliquefaciens* strain ISB06**

Product type: 3

ECHA/BPC/085/2015

Adopted

10 December 2015

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Opinion of the Biocidal Products Committee

on the application for approval of the active substance *Bacillus amyloliquefaciens* strain ISB06 for product type 3

In accordance with Article 89(1) of Regulation (EU) No 528/2012 of the European Parliament and of the Council 22 May 2012 concerning the making available on the market and use of biocidal products (BPR), the Biocidal Products Committee (BPC) has adopted this opinion on the approval in product type 3 of the following active substance:

Common name:	***Bacillus amyloliquefaciens* strain ISB06**
Chemical name(s):	**not applicable**
EC No.:	**not applicable**
CAS No.:	**not applicable**
Existing active substance	

This document presents the opinion adopted by the BPC, having regard to the conclusions of the evaluating Competent Authority. The assessment report, as a supporting document to the opinion, contains the detailed grounds for the opinion.

Process for the adoption of BPC opinions

During the early stage of identification in 2000, the microorganism has been allocated to the species *B. subtilis* based on initial gene sequence analysis. According to this finding, the microorganism has been notified as *Bacillus subtilis* according to biocides directive 98/8/EC. Subsequent gene sequence analysis in 2007 indicated that the strain belonged to the species *Bacillus amyloliquefaciens*. The redefinition of the microorganism was published in October 2015.

Following the submission of an application by COBIOTEX SNE on 30th July 2007, the evaluating Competent Authority Germany submitted an assessment report and the conclusions of its evaluation to the Agency on 22nd September 2014. In order to review the assessment report and the conclusions of the evaluating Competent Authority, the Agency organised consultations via the BPC and its Working Groups. Revisions agreed upon were presented and the assessment report and the conclusions were amended accordingly.

Adoption of the BPC opinion

Rapporteur: BPC member for Germany

The BPC opinion on the approval of the active substance *Bacillus amyloliquefaciens* strain ISB06 in product type 3 was adopted on 10 December 2015.

The BPC opinion was adopted by consensus.

Detailed BPC opinion and background

1. Overall conclusion

The overall conclusion of the BPC is that the active substance *Bacillus amyloliquefaciens* strain ISB06 in product type 3 may be approved. The detailed grounds for the overall conclusion are described in the assessment report.

2. BPC Opinion

2.1. BPC Conclusions of the evaluation

a) Presentation of the active substance including the classification and labelling of the active substance

This evaluation covers the use of *Bacillus amyloliquefaciens* strain ISB06 in product type 3, veterinary hygiene biocidal products.

The mode of action of *B. amyloliquefaciens* strain ISB06 (hereafter ISB06) is still unclear. The microbiocidal effect may arise from several factors including competition with target microorganisms by nutritive competition and by competitive exclusion. The competitive exclusion may be triggered by the synthesis of antibacterial compound(s) like biosurfactants. Strains of the *Bacillus subtilis* group have been reported to produce lipopeptides like surfactins. Lipopeptides bear the capability of plasma membrane disruption via formation of small vesicles and aggregation of intramembranous particles in microorganisms (yeast cells). These surface-active amphiphilic molecules act on the target cell by formation of membrane pores. Consequently, cytoplasmic components are released from the cell in an uncontrolled fashion resulting in cell death. Also in the case of *B. amyloliquefaciens* there are indications on the synthesis of antibacterial compounds which are not regarded as being of (veterinary) medicinal relevance.

ISB06 has been isolated from an agricultural environment and is a wild-type; hence it has not been modified genetically or in any other way.

Cells are gram-positive, mobile medium rods with rounded edges and subterminal spores.

ISB06 has been identified by means of physiological and molecular methodologies as a strain of the species *Bacillus amyloliquefaciens.* Furthermore, it could be excluded that it belongs to closely related species, e.g. *Bacillus subtilis*, as well as to facultative and obligate pathogens of the genus *Bacillus*.

Independent production batches have been analysed for absence of toxins and contaminants with pathogenic potential, *i.e. Salmonella*, *Staphylococcus aureus*, coliforms, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Vibrio parahaemolytiocus*, *Shigella*, *Listeria monocytogenes*, anaerobic spore-forming microorganisms, and moulds. All batches were negative or within specified limits concerning the investigated contaminants. The analysed batches represent the technical grade active ingredient.

The biological, physico-chemical properties of the active substance and biocidal product have been evaluated and are deemed acceptable for the appropriate use, storage and transportation of the active substance and biocidal product.

Adequate analytical methods for the identification at the strain level are available for the active substance.

No Classification and Labelling is necessary with regard to Regulation (EC) No 1272/2008. However, based on the precautionary principle all microorganisms should be considered to have the potential to provoke sensitising reactions.

b) Intended use, target species and effectiveness

The biocidal product is designed to control potentially harmful bacteria in livestock buildings and equipment of animal rearing facilities, e.g. for poultry and pig.

Target organisms of *B. amyloliquefaciens* ISB06 are bacteria including potential pathogens, e.g. *Enterococcus*, *Listeria*, *Staphylococcus*, *Escherichia*, *Pasteurella*, *Salmonella* and *Yersinia*. The product is intended to complement but not to substitute chemical disinfection measures as a prophylactic treatment. The biocidal product is applied by spraying on abiotic surfaces.

The claimed application rate is 0.01g of the product/mL corresponding to about 10^4 cfu/mL of the active substance ISB06. The proposed application rate to cover $1m^2$ is 1g of the product (containing 10^6 cfu of the active substance) dissolved in 0.1 L water.

The biocidal activity of ISB06 has been investigated by studies performed with both the active substance and the representative biocidal product, the latter containing approximately 10^6 cfu/g of the active substance.

The studies revealed reliable results for basic efficacy assessment.

Knowledge on resistance mechanisms against antimicrobial activity mediated by *Bacillus* strains including *B. amyloliquefaciens* is scarce. Specific mechanisms which confer resistance to *Bacillus*-mediated antimicrobial activity have been described for bacteria, *i.e.* for *Bacillus subtilis*. Likewise, the target organisms could bear the potential to develop resistances.

c) Overall conclusion of the evaluation including need for risk management measures

Human health

Bacillus amyloliquefaciens strain ISB06 has been shown to exert no pathogenic or toxic impacts on humans or animals. This is supported by data on other *B. amyloliquefaciens* strains and data on the related *Bacillus subtilis*. *Bacillus amyloliquefaciens* is a ubiquitously occurring bacterium that neither possesses genes encoding *Bacillus* enterotoxins nor the key gene implicated in the synthesis of emetic toxins, nor otherwise demonstrates phenotypic characteristics of any toxin production of human health relevance. Qualified presumption of safety (QPS) status has been granted by the EFSA (2008) for non-toxin-producing strains of *B. amyloliquefaciens* used in feed/food production. In addition, cytotoxicity testing did not reveal production of significant quantities of extracellular enzymes or toxins by *B. amyloliquefaciens* ISB06.

The table below summarises the exposure scenarios assessed.

Summary table: human health scenarios			
Scenario	**Primary or secondary exposure and description of scenario**	**Exposed group**	**Conclusion**
Spray application of livestock buildings and breeding equipment	Primary and inhalation exposure during manual dilution of the powdery biocidal product. Primary dermal and inhalation exposure (aerosol) during spraying.	Professional user	Acceptable

Misting of buildings and breeding equipment	Primary and inhalation exposure during manual dilution of the powdery biocidal product. Primary dermal and inhalation exposure (aerosol) during misting.	Professional user	Acceptable

Due to the potential sensitizing effects of the microorganisms the use of protective gloves, coverall and respiratory protective equipment is recommended.

Professional spraying and misting application in livestock buildings is considered to be of no concern since signs of toxicity, pathogenicity or infection in the acute studies is missing. According to German and Swiss guidelines on biological agents (Technical Rules for Biological Agents (TRBA) 466: "Classification of Prokaryotes (Bacteria and Archaea) into Risk Groups") *B. amyloliquefaciens* is a member of biological agents of the Risk group 1, which means that this microorganism is unlikely to cause human, plant or animal disease. For work with Risk group 1 biological agents the principles of good occupational safety and hygiene should be observed. Due to the potential sensitizing effect and expected dermal and inhalation exposure the use of protective gloves for all phases, in addition protective coverall during spraying/misting and RPE for mixing and loading and spraying/misting is recommended.

Non-professional use was not assessed, since the representative product is intended for professional use only. Secondary exposure of the general public is not expected since animal production facilities, where the product is used, are not open to the general public.

Relevant residues in food or feed are also not expected from the intended use.

Environment

The representative product is intended for indoor use only, for the treatment of livestock buildings and breeding equipment by professionals. Therefore direct exposure of the environment is not expected. However, application of manure and/or sewage sludge (after releases to sewage treatment plants; STP) in agriculture will result in a potential indirect exposure of soil, groundwater and, via run-off, also of surface water. Emissions to surface water (recipient) may also occur after sewage treatment through the STP effluent.
Emissions to manure and waste water following cleaning of the treated surface areas seem to be the most relevant release pathways into environment after indoor use of the reference product. Emission to air is considered negligible, as the reference product would only be used indoors by spray application of an aqueous solution.

The risk for the environment is usually characterized by comparing the toxicity of the substance (PNEC) with the exposure estimates (PEC). However, the reference product is a microbial product with a very specific mode of action based on the endospores of *Bacillus amyloliequefaciens* strain ISB06 which does not produce any toxins of environmental concern. A quantitative risk assessment is therefore not considered appropriate.

However, a semi-quantitative environmental exposure assessment for the soil compartment was performed according to the approach described in Emission Scenario Documents (ESD) for Product Type 3: Veterinary hygiene biocidal products and applied only for veal calves as worst case animal (sub-) category based on the amount of active ingredient released to soil via manure/slurry in dependence of the nitrogen immission standard. The assessment indicated that the number of cells and spores introduced into soil following product application in animal housings and subsequent manure/slurry deposition on agricultural land (grassland, arable land) can be considered negligible. A highest PECsoil of 102.59 cfu*kg^{-1} was calculated for grassland and compared with natural abundances reported for *Bacillus sp.* and *Bacillus subtilis*, being in the range of 10^2-10^5 cfu per g of soil. This background level has been considered as realistic also for *B. amyloliquefaciens*, which belongs to the *B.*

subtilis group and is well known as plant- and soil-associated bacteria. Based on this it can be assumed that application of the product and subsequent environmental exposure is unlikely to cause increased abundance of ISB06 in the environment.

The table below summarises the exposure scenarios assessed.

Summary table: environment scenarios		
Scenario	**Description of scenario including environmental compartments**	**Conclusion**
Disinfection of animal housings (in complement to chemical disinfection as a prophylactic indoor treatment in livestock buildings and breeding equipment in animal production sites, e.g., poultry and pig)	Emissions to manure/slurry system as result of spray application. The application of manure/slurry on agricultural land (arable land or grassland) may lead to indirect exposure of soil. Emission to air is considered negligible.	The number of cells and spores of *B. amyloliquefaciens* introduced into soil due to product application in animal housings and subsequent manure/slurry application on agricultural land can be considered negligible compared to the natural abundance of this microorganism in soil.

In addition, the available ecotoxicological studies implicate that no adverse effects will occur after application of the product in the prescribed concentrations because effects on exposed organisms occurred only in concentrations that were considerably higher than levels of *Bacillus spp*. found after anthropogenic release.

From there, it can be concluded, that the potential indirect exposure of the environment (soil, groundwater, surface water) resulting from the recommended indoor use of the product, does not present any adverse impact on the environment when used as intended.

Overall conclusion

Professional spraying and misting application in livestock buildings is considered to be acceptable for human health. Due to the potential sensitizing effect and expected dermal and inhalation exposure the use of PPE is recommended.

Compared to the natural abundance of 10^2-10^5 cfu per g of soil the number of cells and spores introduced into soil following product application can be considered negligible. It is therefore assumed that application of the product and subsequent environmental exposure is unlikely to cause increased abundance of ISB06 in the environment.

The environmental risk assessment indicates that for the scenario investigated, the application of *Bacillus amyloliquefaciens* strain ISB 06 would not result in unacceptable risks for environment. However, this assessment only covers the indoor use of the biocidal product. This includes the assumption that mixing and application of the product is only done indoors by professionals and on impermeable ground to avoid unintentional direct release into the environment.

2.2. Exclusion, substitution and POP criteria

2.2.1. Exclusion and substitution criteria

The table below summarises the relevant information with respect to the assessment of exclusion and substitution criteria:

Property		Conclusions
CMR properties	Carcinogenicity (C)	Not applicable (n.a.)
	Mutagenicity (M)	n.a.
	Toxic for reproduction (R)	n.a.
PBT and vPvB properties	Persistent (P) or very Persistent (vP)	n.a.
	Bioaccumulative (B) or very Bioaccumulative (vB)	n.a.
	Toxic (T)	n.a.
Endocrine disrupting properties	Not applicable	
Respiratory sensitisation properties	No classification required, but all microorganisms are considered as potential sensitisers.	
Concerns linked to critical effects	*B. amyloliquefaciens* ISB06 does not fulfil criterion (e) of Article 10(1).	
Proportion of non-active isomers or impurities	*B. amyloliquefaciens* ISB06 does not fulfil criterion (f) of Article 10(1).	

For microorganisms the assessment of exclusion criteria is not relevant.

The assessment of substitution criteria is relevant for micro-organisms. It has been agreed that substitution criteria are assessed in line with the "Note on the principles for taking decisions on the approval of active substances under the BPR" agreed at the 54th meeting of the representatives of Member States Competent Authorities for the implementation of Regulation 528/2012 concerning the making available on the market and use of biocidal products (CA-March14-Doc.4.1 - Final - Principles for the approval of AS.doc). This implies that the assessment of the substitution criteria is based on Article 10(1)(a, b and d). Of these Article 10(1)(b) may be relevant although micro-organisms are not covered by CLP. All microorganisms are considered as potential sensitizers. In the absence of data indicating respiratory sensitization *B. amyloliquefaciens* strain ISB06 does not meet Article 10 (1)(b). The other criteria (Article 10(1)(a and c-f) are not applicable for microorganisms.

Therefore, *B. amyloliquefaciens* strain ISB06 does not meet the conditions laid down in Article 10 of Regulation (EU) No 528/2012, and is therefore not considered as a candidate for substitution.

2.2.2. POP criteria

For microorganisms the assessment of POP criteria is not relevant.

2.3. BPC opinion on the application for approval of the active substance *B. amyloliquefaciens* strain ISB06 in product-type 3

In view of the conclusions of the evaluation, it is proposed that *B. amyloliquefaciens* strain ISB06 shall be approved and be included in the Union list of approved active substances, subject to the following specific conditions:

1. Specification: *Bacillus amyloliquefaciens* strain ISB06 and "no relevant impurities".
2. The product assessment shall pay particular attention to the exposures, the risks and the efficacy linked to any uses covered by an application for authorisation, but not addressed in the Union level risk assessment of the active substance.
3. The authorisations of biocidal products are subject to the following condition(s):
    a. In view of the risks identified for the uses assessed, the product assessment shall pay particular attention to professional users.

As all microorganisms are considered as potential sensitisers, based on the precautionary principle, the active substance may not fulfil the criteria according to Article 28(2) to enable inclusion in Annex I of Regulation (EU) 528/2012.

2.4. Elements to be taken into account when authorising products

1. The following recommendations and risk mitigation measures have been identified for the uses assessed. Authorities should consider these risk mitigation measures when authorising products, together with possible other risk mitigation measures, and decide whether these measures are applicable for the concerned product:

    a. If an unacceptable risk for professional users is identified for the concerned product, safe operational procedures and appropriate organisational measures shall be established. Where exposure cannot be reduced to an acceptable level by other means, products should be used with appropriate personal protective equipment.

    b. Considering that all microbials should be regarded as potential sensitisers, the product label should include the agreed warning phrase "Microorganisms may have the potential to provoke sensitising reactions".

2.5. Requirement for further information

Sufficient data have been provided to verify the conclusions on the active substance, permitting the proposal for the approval of *Bacillus amyloliquefaciens* strain ISB 06.

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