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Peer review of the pesticide risk assessment of the active substance *Bacillus subtilis* strain IAB/BS03

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Abstract

The conclusions of the EFSA following the peer review of the initial risk assessments carried out by the competent authority of the rapporteur Member State the Netherlands, for the pesticide active substance *Bacillus subtilis* strain IAB/BS03 are reported. The context of the peer review was that required by Regulation (EC) No 1107/2009 of the European Parliament and of the Council. The conclusions were reached on the basis of the evaluation of the representative uses of *B. subtilis* strain IAB/BS03 as a fungicide on field lettuce, orchards and protected cucurbits. The reliable endpoints appropriate for use in regulatory risk assessment are presented. Missing information identified as being required by the regulatory framework is listed. Concerns are identified.

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Summary

Bacillus subtilis strain IAB/BS03 is a new active substance for which, in accordance with Article 7 of Regulation (EC) No 1107/2009 of the European Parliament and of the Council (hereinafter referred to as 'the Regulation'), the rapporteur Member State (RMS), the Netherlands, received an application from Investigaciones y Aplicaciones Biotecnológicas S.L. on 16 December 2014 for approval. In accordance with Article 8(1)(g) of the Regulation, Investigaciones y Aplicaciones Biotecnológicas S.L. submitted an application to include *Bacillus subtilis* strain IAB/BS03 in Annex IV of Regulation (EC) No 396/2005. Complying with Article 9 of the Regulation, the completeness of the dossier was checked by the RMS and the date of admissibility of the application was recognised as being 23 June 2015.

The RMS provided its initial evaluation of the dossier on *Bacillus subtilis* strain IAB/BS03 in the draft assessment report (DAR), which was received by the European Food Safety Authority (EFSA) on 24 February 2017. The peer review was initiated on 24 March 2017 by dispatching the DAR for consultation to the Member States and the applicant, Investigaciones y Aplicaciones Biotecnológicas S.L.

Following consideration of the comments received on the DAR, it was concluded that additional information should be requested from the applicant and that EFSA should conduct an expert consultation in the areas of mammalian toxicology and environmental fate and behaviour.

In accordance with Article 12 of the Regulation, EFSA should adopt a conclusion on whether *Bacillus subtilis* strain IAB/BS03 can be expected to meet the approval criteria provided for in Article 4 of the Regulation taking into consideration recital (10) of the Regulation. Furthermore, this conclusion also addresses the assessment required from EFSA under Article 12 of Regulation (EC) No 396/2005, provided that the active substance will be approved under Regulation (EC) No 1107/2009 without restrictions affecting the residue assessment.

The conclusions laid down in this report were reached on the basis of the evaluation of the representative uses of *Bacillus subtilis* strain IAB/BS03 as a fungicide on field lettuce, orchards and protected cucurbits, as proposed by the applicant. Full details of the representative uses can be found in Appendix A of this report.

According to the representative uses proposed at European Union (EU) level, the uses of *Bacillus subtilis* strain IAB/BS03 result in a sufficient fungicidal efficacy against the target organisms.

In the section identity, physical-chemical and technical properties and analytical methods, a data gap was identified for a good laboratory practice (GLP)-compliant growth temperature study, including human body temperature.

Because of outstanding issues regarding proper investigation of clearance and pending on information on metabolite production following application, the human health risk assessment to the microorganism and secondary metabolites cannot be finalised.

In view of the concerns flagged in the section on mammalian toxicology with regard to viable residues (lack of infectivity still to be supported) and non-viable residues (potential source of food poisoning), currently, it cannot be concluded that the use of *Bacillus subtilis* IAB/BS03 in apple, lettuce and cucurbits is unconditionally safe for consumers. Currently, the consumer risk assessment to the microorganism and its secondary metabolites cannot be finalised and inclusion in Annex IV of (EC) No 396/2005 cannot be recommended.

The information and evidence provided were considered insufficient to conclude on the likely competitiveness, persistence and multiplication of *Bacillus subtilis* strain IAB/BS03 in field soil. This conclusion is also applicable regarding soil and other growing media used in glasshouse production systems.

The available information was considered insufficient to conclude on the infectivity and pathogenicity of the microorganism *Bacillus subtilis* strain IAB/BS03 for non-target organisms; thus, the risk assessment for non-target organisms other than aquatic invertebrates and algae could not be finalised. Additionally, a data gap was identified with regard to the toxicological characterisation of secondary metabolites/toxins if they should be produced in the environment.

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Background

Regulation (EC) No 1107/2009 of the European Parliament and of the Council¹ (hereinafter referred to as 'the Regulation') lays down, inter alia, the detailed rules as regards the procedure and conditions for approval of active substances. This regulates, for the European Food Safety Authority (EFSA), the procedure for organising the consultation of Member States and the applicant(s) for comments on the initial evaluation in the draft assessment report (DAR), provided by the rapporteur Member State (RMS), and the organisation of an expert consultation, where appropriate.

In accordance with Article 12 of the Regulation, EFSA is required to adopt a conclusion on whether an active substance can be expected to meet the approval criteria provided for in Article 4 of the Regulation (also taking into consideration recital (10) of the Regulation) within 120 days from the end of the period provided for the submission of written comments, subject to an extension of 30 days where an expert consultation is necessary, and a further extension of up to 150 days where additional information is required to be submitted by the applicant(s) in accordance with Article 12(3).

Bacillus subtilis strain IAB/BS03 is a new active substance for which, in accordance with Article 7 of the Regulation, the RMS, the Netherlands (hereinafter referred to as the 'RMS'), received an application from Investigaciones y Aplicaciones Biotecnológicas S.L. on 16 December 2014 for approval of the active substance *Bacillus subtilis* strain IAB/BS03. In accordance with Article 8(1)(g) of the Regulation, Investigaciones y Aplicaciones Biotecnológicas S.L. submitted an application to include *Bacillus subtilis* strain IAB/BS03 in Annex IV of Regulation (EC) No 396/2005.² Complying with Article 9 of the Regulation, the completeness of the dossier was checked by the RMS and the date of admissibility of the application was recognised as being 23 June 2015.

The RMS provided its initial evaluation of the dossier on *Bacillus subtilis* strain IAB/BS03 in the DAR, which was received by EFSA on 24 February 2017 (Netherlands, 2017a). The peer review was initiated on 24 March 2017 by dispatching the DAR for consultation of the Member States and the applicant, Investigaciones y Aplicaciones Biotecnológicas S.L., for consultation and comments. EFSA also provided comments. In addition, EFSA conducted a public consultation on the DAR. The comments received were collated by EFSA and forwarded to the RMS for compilation and evaluation in the format of a reporting table. The applicant was invited to respond to the comments in column 3 of the reporting table. The comments and the applicant response were evaluated by the RMS in column 3.

The need for expert consultation and the necessity for additional information to be submitted by the applicant in accordance with Article 12(3) of the Regulation were considered in a telephone conference between EFSA and the RMS on 6 July 2017. On the basis of the comments received, the applicant's response to the comments and the RMS's evaluation thereof, it was concluded that additional information should be requested from the applicant and that EFSA should conduct an expert consultation in the areas of mammalian toxicology and environmental fate and behaviour.

The outcome of the telephone conference, together with EFSA's further consideration of the comments, is reflected in the conclusions set out in column 4 of the reporting table. All points that were identified as unresolved at the end of the comment evaluation phase and which required further consideration, including those issues to be considered in an expert consultation, were compiled by EFSA in the format of an evaluation table.

The conclusions arising from the consideration by EFSA, and as appropriate by the RMS, of the points identified in the evaluation table, together with the outcome of the expert consultation where this took place, were reported in the final column of the evaluation table.

In accordance with Article 12 of the Regulation, EFSA should adopt a conclusion on whether *Bacillus subtilis* strain IAB/BS03 can be expected to meet the approval criteria provided for in Article 4 of the Regulation, taking into consideration recital (10) of the Regulation. A final consultation on the conclusions arising from the peer review of the risk assessment took place with Member States via a written procedure in February 2018.

This conclusion report summarises the outcome of the peer review of the risk assessment on the active substance and the representative formulation evaluated on the basis of the representative uses of *Bacillus subtilis* strain IAB/BS03 as a fungicide on field lettuce, orchards and protected cucurbits as proposed by the applicant. Furthermore, this conclusion also addresses the assessment required from EFSA under Article 12 of Regulation (EC) No 396/2005, provided the active substance will be approved

¹ Regulation (EC) No 1107/2009 of 21 October 2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.

² Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, p. 1–16.

under Regulation (EC) No 1107/2009 without restrictions affecting the residue assessment. In the event of a non-approval of the active substance or an approval with restrictions that have an impact on the residue assessment, this conclusion might no longer be relevant and a new assessment under Article 12 of Regulation (EC) No 396/2005 will be required. A list of the relevant end points for the active substance is provided in Appendix A.

In addition, a key supporting document to this conclusion is the peer review report (EFSA, 2018), which is a compilation of the documentation developed to evaluate and address all issues raised in the peer review, from the initial commenting phase to the conclusion. The peer review report comprises the following documents, in which all views expressed during the course of the peer review, including minority views where applicable, can be found:

- the comments received on the DAR;
- the reporting table (6 July 2017);
- the evaluation table (26 March 2018);
- the report(s) of the scientific consultation with Member State experts (where relevant);
- the comments received on the assessment of the additional information;
- the comments received on the draft EFSA conclusion.

Given the importance of the DAR including its revisions (Netherlands, 2017b) and the peer review report, both documents are considered as background documents to this conclusion.

It is recommended that this conclusion report and its background documents would not be accepted to support any registration outside the EU for which the applicant has not demonstrated that it has regulatory access to the information on which this conclusion report is based.

The active substance and the formulated product

Bacillus subtilis subsp. *subtilis* strain IAB/BS03 is a bacterium deposited at the Spanish Type Culture Collection (CECT), Spain, under the accession number CECT 7254 and also at the German Type Culture Collection (DSMZ), Germany, under the accession number DSM 24682. *Bacillus subtilis* strain IAB/BS03 is a naturally occurring, indigenous wild-type bacterium, initially isolated in 2005 from agricultural soil in Valencia, Spain, from rhizosphere of cucurbits.

The representative formulated product for the evaluation was 'Bacillus subtilis BS8 IABBS03 WP', a wettable powder (WP) containing 10 g/kg (nominal 2×10^{11} CFU/kg, minimum and maximum content 1×10^{11} CFU/kg to 5×10^{11} CFU/kg) *Bacillus subtilis* strain IAB/BS03.

The representative uses evaluated comprise field applications by spraying on apple, lettuce and applications on protected cucurbits, as a fungicide against scab, downy and powdery mildew. Full details of the good agricultural practices (GAPs) can be found in the list of end points in Appendix A.

Data were submitted to conclude that the uses of *Bacillus subtilis* strain IAB/BS03 according to the representative uses proposed at EU level result in a sufficient fungicidal efficacy against plant pathogenic fungi, following the guidance document SANCO/10054/2013 – rev. 3 (European Commission, 2013).

Bacillus subtilis strain IAB/BS03 was discussed at the pesticides peer review microorganism teleconference 161 in December 2017 in the sections on mammalian toxicology and fate and behaviour.

Conclusions of the evaluation

1. Identity of the microorganism/biological properties/physical and technical properties and methods of analysis

The following guidance documents were followed in the production of this conclusion: Working Document on Microbial Contaminant Limits for Microbial Pest Control Products, SANCO/12116/2012 (European Commission, 2012), Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. (EFSA FEEDAP Panel, 2012).

The minimum and maximum content of *Bacillus subtilis* strain IAB/BS03 in the microbial pest control agent was in the range between 1 to 5×10^{13} CFU/kg. The specification was based on batch data from pilot scale production. New batch data will be required after the stabilisation of the industrial production.

The molecular identification of *Bacillus subtilis* strain IAB/BS03 was conducted by the determination of the complete sequence of the 16S rRNA gene. The combined use of specific primers either in two single PCR or in a multiplex PCR allowed to identify the strain IAB/BS03; however, the method was considered not powerful enough.

The content of microbial contaminants of the microbiological pest control agent (MPCA) and the microbiological pest control product (MPCP) were below the limits defined in the Working Document on Microbial Contaminants (European Commission, 2012). In the literature, *B. subtilis* has been associated with food poisoning and identified in infections of immunocompromised humans; however, there was no evidence of direct relationship of *B. subtilis* strain IAB/BS03 to known plant, animal or human pathogens. It can produce cyclolipopeptides such as surfactins, fengycins and iturin A. These metabolites were present in the growing medium and separated from the MPCA; their content in the MPCP was negligible. *Bacillus subtilis* strain IAB/BS03 is present in the product as spores.

The growth temperature range of *Bacillus subtilis* strain IAB/BS03 was between 4°C and 35°C. A data gap was identified for a GLP-compliant growth temperature study, including human body temperature, to support the lack of infectivity (see also Section 2). The growth of *Bacillus subtilis* strain IAB/BS03 in the growth media was favoured at pH 8 and was not possible at pH lower than 3. The strain was able to tolerate relatively high-saline conditions, as growth was still observed at 7% sodium chloride concentration.

From the 39 antibiotics tested, *Bacillus subtilis* strain IAB/BS03 showed susceptibility to 33 antibiotics and resistance was shown only to aztreonam, nyastin and streptomycin.

The assessment of the data package revealed no issues that need to be included as critical areas of concern with respect to the identity, biological properties of the active substance and physical and technical properties of the representative formulation.

Acceptable methods are available for the determination of the microorganism in the MPCA and the MPCP and for the determination of the content of contaminating microorganisms. As a maximum residue level (MRL) for *Bacillus subtilis* strain IAB/BS03 was not proposed, a monitoring method for detection of *Bacillus subtilis* strain IAB/BS03 is not needed. Post registration monitoring methods are not needed since residue definition for *Bacillus subtilis* strain IAB/BS03 in food or in the environment was not proposed.

2. Mammalian toxicity

General data

In the literature, some *B. subtilis* species have been associated with food poisoning and identified in infections of immunocompromised humans.

B. subtilis is recommended for the Qualified Presumption of Safety list (EFSA BIOHAZ Panel, 2018) if it is qualified for the absence of toxigenic activity and if the strain does not harbour any acquired antimicrobial resistance genes to clinically relevant antibiotics. *Bacillus subtilis* strain IAB/BS03 has been shown to be resistant to only to aztreonam, nyastin and streptomycin out of 39 antibiotics tested (see Section 1). It is unknown whether this resistance is acquired and due to transfer of genetic material. In view of the fact that the microorganism showed susceptibility to 33 antibiotics, a data gap is not proposed.

Toxicity studies

The applicant submitted a basic set of tier I toxicity studies to evaluate the risk of the microorganism *Bacillus subtilis* strain IAB/BS03. In these toxicity studies, performed with *Bacillus subtilis* strain IAB/BS03, there was no indication for acute toxicity of *Bacillus subtilis* strain IAB/BS03 following oral, intratracheal or intraperitoneal administration to rats. After a single intravenous injection, slow clearance of *Bacillus subtilis* strain IAB/BS03 was observed in the liver and spleen. It was also present in the caecum contents. Since clearance was not investigated in other studies, the experts of the peer review teleconference agreed that a growth temperature study (GLP-compliant and including human body temperature) should be provided to support the lack of infectivity of the microorganism. Pending on the results, an additional acute inhalation study including investigations of the clearance could be initially required. This data gap leads to an issue that could not be finalised. No study on the sensitisation potential of *Bacillus subtilis* strain IAB/BS03 was submitted. However, microorganisms in general are considered sensitising unless there is sufficient experimental evidence that there is no concern. In an Ames test with the supernatant of the fermentation broth, negative results were obtained. The product is not acutely toxic by the dermal route and it is neither a skin nor eye irritant.

Secondary metabolites/toxins

In the available studies, *Bacillus subtilis* strain IAB/BS03 has been shown to produce cyclic lipopeptides including surfactin, fengycin and iturin A. Lipopeptides might be associated to food-borne diseases in humans, even though their role is not clear (no further information has been found in the

literature). Iturins and surfactins belong to the lipopeptide family; they are strong surfactants showing membrane damaging properties (lytic activity) *in vitro*. Data from a subacute oral toxicity study with surfactin C in rats are reported. After intragastric gavage for 28 days, the no observed adverse effect level (NOAEL) was 500 mg/kg body weight (bw) per day, based on changes in clinical chemistry parameters, increased liver weight and zonal hepatocyte necrosis. Negative results were observed in an Ames test *in vitro* and in a micronucleus assay *in vivo*. No maternal or developmental toxicity was observed in mice, treated up to 500 mg/kg bw per day. Pending on information on metabolite production following application, further data might need to be required to characterise the toxicity profile of the secondary metabolites except surfactin C.

Reference values and exposure

Because of outstanding issues regarding the proper investigation of clearance and pending on information on metabolite production following application, the human health risk assessment for the microorganism and secondary metabolites cannot be finalised including the setting of reference values.

3. Residues

A single efficacy field trial in grape vines, determining the colony-forming unit (CFU) in grapes treated with *Bacillus subtilis* IAB/BS03 in comparison with untreated controls, showed an initial increase of CFU while within 7 days after treatment, CFU had declined to numbers comparable to natural background counts. The plate count method, which was used, was not specific to *Bacillus subtilis* IAB/BS03. Thus, the study observations cannot be correlated with growth and decline behaviour of the strain under assessment but merely demonstrated that, in this trial, *Bacillus subtilis* IAB/BS03 did not grow to significant numbers on the treated grapes within a 7 days period after treatment.

With regard to secondary metabolites/toxins, information is not available whether and to which extent *Bacillus subtilis* IAB/BS03 does produce its cyclic lipopeptides such as surfactin, fengycin and iturin A following application on crops.

In view of the concerns flagged in the section on mammalian toxicology (see Section 2) with regard to viable residues (lack of infectivity still to be supported) and non-viable residues (potential source of food poisoning), it can currently not be concluded that the use of *Bacillus subtilis* IAB/BS03 in apple, lettuce and on cucurbits is unconditionally safe for consumers, also considering that the GAP does not preclude consumer exposure very soon after treatment of the crops (possible preharvest interval (PHI) of 0 days).

A low exposure potential for consumers to non-viable residues may be assumed only for the use on cucurbits with inedible peel; however, lack of infectivity has still to be supported by data for *Bacillus subtilis* IAB/BS03 as cross-contamination of the edible crop portion with the microorganism during food preparation is possible.

Currently, the consumer risk assessment to the microorganism and secondary metabolites cannot be finalised and inclusion in Annex IV of (EC) No 396/2005 cannot be recommended.

4. Environmental fate and behaviour

Satisfactory information has been provided in relation to potential interference of *Bacillus subtilis* strain IAB/BS03 with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC³ (see specific Annex VI decision-making criteria in Part II Commission Regulation (EU) No 546/2011⁴). As these methods utilise chromogenic agents to which *B. subtilis* does not give a response, it was considered unlikely that *Bacillus subtilis* IAB/BS03 would interfere with the methodologies used for such determinations.

As *Bacillus subtilis* strain IAB/BS03 is a 'wild type', there are no marker genes in the strain which would permit analysis of a frequency of genetic exchange. As the genetic diversity and drift in the wild-type population have not been ascertained, it would not be possible to distinguish any genetic drift from that in the wild population. Although it is acknowledged that the possibility and effects of transfer of genetic material are no different for *Bacillus subtilis* strain IAB/BS03 than for other naturally

³ Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.98, p. 32-54.

⁴ Commission Regulation (EU) 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127-175.

occurring *B. subtilis* strains and that this has generally only been observed to occur in high density cell cultures, transfer of genetic material by *Bacillus subtilis* strain IAB/BS03 after application is possible and could not be excluded based on the information in the dossier.

4.1. Fate and behaviour in the environment of the microorganism

Information was derived from published literature on the species of *B. subtilis* in relation to its **persistence and multiplication in soil**. The information and evidence provided were considered insufficient by EFSA experts and some Member States' experts to conclude on the likely competitiveness, persistence and multiplication of *Bacillus subtilis* strain IAB/BS03 in field soil by considering this more general species level information. This conclusion is also applicable regarding soil and other growing media used in glasshouse production systems. Consequently, EFSA concluded that it is unclear if the strain will respect the uniform principles criterion of not being expected to persist and multiply in soil or plant-growing media in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years. Experts from two member states including the RMS had the contrary view and considered that the available information on the species was sufficient to conclude that *Bacillus subtilis* strain IAB/BS03 is likely to respect the uniform principles criterion. This conclusion, however, identifies a data gap (see Section 7) and an assessment not finalised (see Section 9). Predicted environmental concentrations (PEC) in soil have been calculated (see Appendix A).

With respect to the **persistence and multiplication in water**, published peer-reviewed literature was available for *B. subtilis* and *Bacillus thuringiensis*. The scientific papers provided information on the persistence of *B. subtilis* in water. The information on the persistence/multiplication/germination of *B. subtilis* in natural surface water was considered sufficient to demonstrate that the microorganism is likely to decline in surface water. PEC surface water has been calculated considering the spray drift and runoff routes of exposure (see Appendix A).

The literature search according to the EFSA guidance (EFSA, 2011) on *B. subtilis* did not provide any information on occurrence or behaviour in **air**.

Regarding **mobility** generally, vertical distribution of the microbial organism through soil is unlikely to happen based on information in submitted published scientific papers on *B. subtilis*. Local dispersal through aerosol particles formed at the time of spraying is possible.

4.2. Fate and behaviour in the environment of any relevant metabolite formed by the microorganism under relevant environmental conditions

The species *B. subtilis* is able to produce secondary metabolites such as fengycin, iturin and surfactin. Some of these are inhibitory to fungi or bacteria, including human pathogens.

It is not known to what extent *Bacillus subtilis* strain IAB/BS03 will produce any metabolites following its application once the spores reach the soil, should they grow. Scientific papers on *B. subtilis* and the closely related *Bacillus amyloliquefaciens* show the production of surfactin and iturin A in the rhizosphere; all at amounts in the nano- or microgram range per gram of root/rhizosphere. Adequate information to address the potential for *Bacillus subtilis* strain IAB/BS03 to produce secondary metabolites/toxins was not available. Therefore, a data gap was identified. Consequently, it is not clear if such metabolites might fulfil the criteria according to Part B Section 7 (iv) of Commission Regulation (EU) 283/2013⁵ namely:

- the relevant metabolite is stable outside the microorganism;
- a toxic effect of the relevant metabolite is independent of the presence of the microorganism;
- the relevant metabolite is expected to occur in the environment in concentrations considerably higher than under natural conditions.

Therefore, data on the potential for *Bacillus subtilis* strain IAB/BS03 to produce metabolites in relation to these criteria are necessary to assess if the further data requirements and the corresponding risk assessment according to Commission Regulation (EU) No 283/2013, part A, Section 7 (standard data requirements and assessment mandatory for chemical plant protections

⁵ Commission Regulation (EU) 283/2013 of 1 March 2013 setting out the data requirements for active substances in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93, 3.4.2013, p. 1-84.

active substances) are triggered. Consequently, this resulted in a data gap (see Section 7) and assessments being not finalised, (see Section 9).

5. Ecotoxicology

Surfactins, fengycin and iturins were identified as the main metabolites which are known to be produced by *Bacillus subtilis* strain IAB/BS03 and that may occur in the environment. Mycosubtilin has also been identified from open literature to be produced as well by *B. subtilis* (not necessarily specific to the strain under assessment). Furthermore, a data gap for identification of further secondary metabolites in the environment was identified (see Section 4). Sufficient information for the toxicological characterisation of secondary metabolites/toxins that may be produced in the environment is missing (data gap; see also Sections 2 and 4).

Ecotoxicity tests were carried out following the guidelines for the testing of chemicals. The test duration in most of the cases was not suitable for investigating infectivity and pathogenicity; thus, a data gap has been identified to demonstrate the absence of pathogenicity and infectiveness of *Bacillus subtilis* strain IAB/BS03 to birds, mammals, fish, aquatic plants, honeybees, non-target arthropods, earthworms and other soil organisms. Based on the available information, low risk was concluded for aquatic invertebrates and algae. The RMS does not agree with the data gaps identified for non-target organisms as the RMS considers that the available studies were sufficient to exclude the potential for infectivity and pathogenicity.

Information from open literature was insufficiently well reported to consider its relevance for the strain under assessment.

6. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments (Tables 1–4)

Table 1: Soil

| Compound (name and/or code) | Persistence | Ecotoxicology |
|--|---|-----------------------------|
| <i>Bacillus subtilis</i> strain IAB/BS03 | Data gap | No data available; data gap |
| Toxins/secondary metabolites such as fengycins, iturins and surfactins | Open, data gap pending on their identification and quantification | No data available; data gap |

Table 2: Groundwater

| Compound (name and/or code) | Mobility in soil | > 0.1 µg/L at 1 m depth for the representative uses ^(a) | Pesticidal activity | Toxicological relevance |
|--|--|--|---------------------|-------------------------|
| Toxins/secondary metabolites such as fengycins, iturins and surfactins | Open, possible data gap pending on their identification and quantification | Open | Yes | Open |

(a): At least one FOCUS scenario or relevant lysimeter.

Table 3: Surface water and sediment

| Compound (name and/or code) | Ecotoxicology |
|--|--|
| <i>Bacillus subtilis</i> strain IAB/BS03 | Low risk to aquatic invertebrates. Data gap for other non-target aquatic organisms |
| Toxins/secondary metabolites such as fengycins, iturins and surfactins | No data available; data gap |

Table 4: Air

| Compound (name and/or code) | Toxicology |
|--|---|
| <i>Bacillus subtilis</i> strain IAB/BS03 | Low acute inhalation toxicity to rats. Clearance not investigated by the inhalation route |
| Toxins/secondary metabolites such as fengycins, iturins and surfactins | Open |

7. Data gaps

This is a list of data gaps identified during the peer review process, including those areas in which a study may have been made available during the peer review process but not considered for procedural reasons (without prejudice to the provisions of Article 56 of the Regulation concerning information on potentially harmful effects).

- Growth temperature study including human body temperature (GLP compliant, or done in an officially recognised testing facility but only when not supporting human health risk assessment) was not available to support the lack of infectivity of the microorganism. Pending on the results, an additional acute inhalation study including investigations of the clearance could be required (relevant for all representative uses evaluated; submission date proposed by the applicant unknown; see Sections 1 and 2).
- Adequate information to address the uniform principles criterion of the strain not being expected to persist and multiply in soil or plant-growing media in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years was not available (relevant for all representative uses evaluated; submission date proposed by the applicant unknown; see Section 4).
- Information on the toxicity of secondary metabolites/toxins is missing and would be required should they be produced, with evidence for non-production currently lacking (relevant for all representative uses evaluated; submission date proposed by the applicant: unknown; see Sections 2, 3, 4 and 5)
- Information is needed to demonstrate that the representative uses of *Bacillus subtilis* strain IAB/BS03 will not pose pathogenicity and infectiveness to birds, mammals, fish, honeybees, non-target arthropods, earthworms and other soil organisms (relevant for all representative uses evaluated; submission date proposed by the applicant unknown; see Section 5).

8. Particular conditions proposed to be taken into account to manage the risk(s) identified

No particular conditions are proposed for the representative uses evaluated.

9. Concerns

9.1. Issues that could not be finalised

An issue is listed as 'could not be finalised' if there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the uniform principles in accordance with Article 29(6) of the Regulation and as set out in Commission Regulation (EU) No 546/2011⁶ and if the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

An issue is also listed as 'could not be finalised' if the available information is considered insufficient to conclude on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of the Regulation.

- 1) Because of outstanding issues regarding proper investigation of clearance of the microorganism, the human health risk assessment for the microorganism cannot be finalised (see Sections 2 and 3).
- 2) The production of relevant toxins/secondary metabolites known to be of concern for humans and the environment cannot be excluded. Therefore, the risk assessment cannot be finalised for operators, workers, residents, bystanders, consumers and the environment including the assessment of potential groundwater exposure. (see Sections 2, 3, 4 and 5)
- 3) The assessment of all non-target organisms other than aquatic invertebrates and algae could not be finalised with the available information (see Section 5).

⁶ Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127–175.

9.2. Critical areas of concern

An issue is listed as a critical area of concern if there is enough information available to perform an assessment for the representative uses in line with the uniform principles in accordance with Article 29(6) of the Regulation and as set out in Commission Regulation (EU) No 546/2011, and if this assessment does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if the assessment at a higher tier level could not be finalised due to lack of information, and if the assessment performed at the lower tier level does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if, in the light of current scientific and technical knowledge using guidance documents available at the time of application, the active substance is not expected to meet the approval criteria provided for in Article 4 of the Regulation.

- None identified.

9.3. Overview of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in Section 8, has been evaluated as being effective, then 'risk identified' is not indicated in Table 5.)

Table 5: Overview of concerns

| Representative use | | Lettuce field | Cucurbits protected | Orchards |
|---|---|------------------|---------------------|------------------|
| Operator risk | Risk identified | | | |
| | Assessment not finalised | X ^{1,2} | X ^{1,2} | X ^{1,2} |
| Worker risk | Risk identified | | | |
| | Assessment not finalised | X ^{1,2} | X ^{1,2} | X ^{1,2} |
| Resident/bystander risk | Risk identified | | | |
| | Assessment not finalised | X ^{1,2} | X ^{1,2} | X ^{1,2} |
| Consumer risk | Risk identified | | | |
| | Assessment not finalised | X ^{1,2} | X ^{1,2} | X ^{1,2} |
| Risk to wild non-target terrestrial vertebrates | Risk identified | | | |
| | Assessment not finalised | X ^{2,3} | X ^{2,3} | X ^{2,3} |
| Risk to wild non-target terrestrial organisms other than vertebrates | Risk identified | | | |
| | Assessment not finalised | X ^{2,3} | X ^{2,3} | X ^{2,3} |
| Risk to aquatic organisms | Risk identified | | | |
| | Assessment not finalised | X ^{2,3} | X ^{2,3} | X ^{2,3} |
| Groundwater exposure to active substance | Legal parametric value breached | | | |
| | Assessment not finalised | | | |
| Groundwater exposure to metabolites | Legal parametric value breached ^(a) | | | |
| | Parametric value of 10 µg/L ^(b) breached | | | |
| | Assessment not finalised | X ² | X ² | X ² |

Columns are grey if no safe use can be identified. The superscript numbers relate to the numbered points indicated in Sections 9.1. Where there is no superscript number, see Sections 2–6 for further information.

(a): Based on classification made in the context of this evaluation procedure under Regulation (EC) No 1107/2009. It should be noted that harmonised classification and labelling are formally proposed and decided in accordance with Regulation (EC) No 1272/2008 or it should be noted that the classification proposed in the context of this evaluation procedure under Regulation (EC) No 1107/2009 concurs with the harmonised classification and labelling in accordance with Regulation (EC) No 1272/2008.

(b): Value for non-relevant metabolites prescribed in SANCO/221/2000 – rev. 10 final, European Commission, 2003.

References

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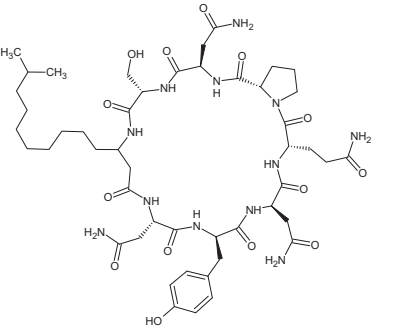
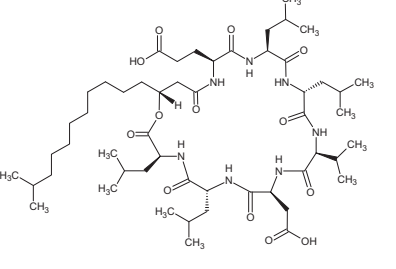
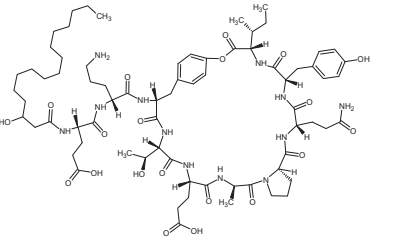
Abbreviations

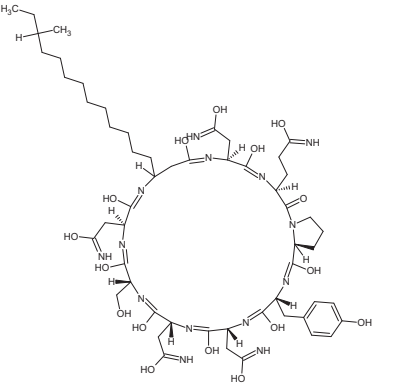
| | |
|--------|--|
| bw | body weight |
| CECT | Spanish Type Culture Collection |
| CFU | colony-forming units |
| DSMZ | German Type Culture Collection |
| DAR | draft assessment report |
| FOCUS | Forum for the Co-ordination of Pesticide Fate Models and their Use |
| GAP | Good Agricultural Practice |
| GLP | Good laboratory practice |
| IUPAC | International Union of Pure and Applied Chemistry |
| MPCA | microbiological pest control agent |
| MPCP | microbiological pest control product |
| MRL | maximum residue level |
| NOAEL | no observed adverse effect level |
| PEC | predicted environmental concentration |
| PHI | preharvest interval |
| RMS | rapporteur Member State |
| SMILES | simplified molecular-input line-entry system |
| WP | Wettable powder |

Appendix A – List of end points for the active substance and the representative formulation

Appendix A can be found in the online version of this output ('Supporting information' section):
<https://doi.org/10.2903/j.efsa.2018.5261>

Appendix B – Used compound codes

| Code/trivial name ^(a) | IUPAC name/SMILES notation/InChiKey ^(b) | Structural formula ^(c) |
|----------------------------------|--|--|
| iturin A | <p>3-[(3<i>R</i>,6<i>S</i>,13<i>S</i>,16<i>R</i>,19<i>R</i>,22<i>S</i>,27<i>aS</i>)-3,13,19-tris(2-amino-2-oxoethyl)-6-(hydroxymethyl)-16-[(4-hydroxyphenyl)methyl]-9-(9-methyldecyl)-1,4,7,11,14,17,20,23-octaoxohexacosahydro-1<i>H</i>-pyrrolo[2,1-<i>f</i>][1,4,7,10,13,16,19,22]octaazacyclopentacosin-22-yl]propanamide</p> <p><chem>NC(=O)CC[C@@H]3NC(=O)[C@@H](CC(N)=O)NC(=O)[C@@H](Cc1ccc(O)cc1)NC(=O)[C@H](CC(N)=O)NC(=O)CC(CCCCCCCC(C)C)NC(=O)[C@H](CO)NC(=O)[C@H](NC(=O)[C@@H]2CCCN2C3=O)CC(N)=O</chem></p> <p>RDUGMXONDQDIRN-QZBZMMCASA-N</p> |  |
| Surfactin C | <p>3-[(3<i>S</i>,6<i>R</i>,9<i>S</i>,12<i>S</i>,15<i>R</i>,18<i>S</i>,21<i>S</i>,25<i>R</i>)-9-(carboxymethyl)-3,6,15,18-tetrakis(2-methylpropyl)-25-(10-methylundecyl)-2,5,8,11,14,17,20,23-octaoxo-12-(propan-2-yl)-1-oxa-4,7,10,13,16,19,22-heptaazacyclopentacosan-21-yl]propanoic acid</p> <p><chem>CC(C)C[C@@H]1NC(=O)[C@@H](CC(C)C)NC(=O)[C@H](CC(=O)O)NC(=O)[C@@H](NC(=O)[C@@H](CC(C)C)NC(=O)[C@@H](NC(=O)[C@@H](NC(=O)C[C@@H](CCCCCCCC(C)C)OC1=O)CCC(=O)O)CC(C)C(C)C</chem></p> <p>NJGWOFRZMQRKHT-WGVNQQGSSA-N</p> |  |
| fengycin | <p><i>N</i>-(3-hydroxyhexadecanoyl)-<i>L</i>-α-glutamyl-<i>N</i>-{(3<i>S</i>,6<i>S</i>,9<i>S</i>,17<i>R</i>,20<i>S</i>,23<i>S</i>,26<i>R</i>,31<i>aS</i>)-3-(3-amino-3-oxopropyl)-9-[(2<i>R</i>)-butan-2-yl]-23-(2-carboxyethyl)-20-[(1<i>S</i>)-1-hydroxyethyl]-6-[(4-hydroxyphenyl)methyl]-26-methyl-1,4,7,10,18,21,24,27-octaoxo-1,2,3,4,5,6,7,8,9,10,16,17,18,19,20,21,22,23,24,25,26,27,29,30,31,31<i>a</i>-hexacosahydro-12,15-ethenopyrrolo[2,1-<i>f</i>][1,4,7,10,13,16,19,22]oxaheptaacyclononacosin-17-yl}-<i>D</i>-ornithinamide</p> <p><chem>O=C(O)CC[C@H](NC(=O)CC(O)CCCCCCCCCCCCC)C(=O)N[C@H](CCCN)C(=O)N[C@@H]2Cc1ccc(cc1)OC(=O)[C@@H](NC(=O)[C@@H](NC(=O)[C@H](CCCC(N)=O)NC(=O)[C@@H]3CCCN3C(=O)[C@@H](C)NC(=O)[C@H](CCC(=O)O)NC(=O)[C@@H](NC2=O)[C@H](C)O)Cc4ccc(O)cc4)[C@H](C)CC</chem></p> <p>CUOJDWBMJMRDHN-RLLVTFBRSA-N</p> |  |

| Code/trivial name ^(a) | IUPAC name/SMILES notation/InChiKey ^(b) | Structural formula ^(c) |
|----------------------------------|---|--|
| mycosubtilin | <p>3-[(3<i>R</i>,6<i>R</i>,9<i>R</i>,12<i>R</i>,15<i>S</i>,22<i>S</i>,25<i>S</i>,30<i>aS</i>)-6,9,15,22-tetrakis (2-amino-2-oxoethyl)-12-(hydroxymethyl)-3-[(4-hydroxyphenyl)methyl]-18-(11-methyltridecyl)-1,4,7,10,13,16,20,23,26-nonaoxotriacontahydropyrrolo [1,2-<i>g</i>][1,4,7,10,13,16,19,22,25]nonaazacyclooctacosin-25-yl]propanamide</p> <p><chem>N=C(O)CC[C@@H]1N=C(O)[C@H](CC(=N)O)N=C(O)CC(CCCCCCCCCC(C)CC)N=C(O)[C@H](CC(=N)O)N=C(O)[C@H](CO)N=C(O)[C@@H](CC(=N)O)N=C(O)[C@@H](CC(=N)O)N=C(O)[C@H](N=C(O)[C@@H]2CCCC2C1=O)Cc3ccc(O)cc3</chem></p> <p>RCIPRGNHNAEGHR-ZLHAWHIKSA-N</p> |  |

(a): The metabolite name in bold is the name used in the conclusion.

(b): ACD/Name 2015 ACD/Labs 2015 Release (File version N20E41, Build 75170, 19 December 2014).

(c): ACD/ChemSketch 2015 ACD/Labs 2015 Release (File version C10H41, Build 75059, 17 December 2014).