Peer review of the pesticide risk assessment of the active substance *Bacillus amyloliquefaciens* strain AH2

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Abstract

The conclusions of 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 amyloliquefaciens* strain AH2 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 use of *Bacillus amyloliquefaciens* AH2 as a fungicide on grapes. 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 amyloliquefaciens* AH2 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, the rapporteur Member State (RMS), the Netherlands, received an application from Probelte S.A. on 1 June 2015 for approval. 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 25 October 2015.

An initial evaluation of the dossier on *Bacillus amyloliquefaciens* AH2 was provided by the RMS in the draft assessment report (DAR) and subsequently, a peer review of the pesticide risk assessment on the RMS evaluation was conducted by EFSA in accordance with Article 12 of Regulation (EC) No 1107/2009. The following conclusions are derived.

According to the representative uses as a fungicide on grapes, the uses of *Bacillus amyloliquefaciens* strain AH2, as proposed at the European Union (EU) level, result in a sufficient fungicidal efficacy against the target grey mould.

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

For the mammalian toxicology, considering the lack of scientific evidence, the relationship between the presence of secondary metabolites and/or toxins and the risk of adverse effects in humans cannot be excluded leading to an issue that could not finalised.

*Bacillus amyloliquefaciens* AH2 may produce a range of metabolites, however not all of them were investigated as to whether these are produced on plants under good agricultural practice (GAP) directed use by the strain. Therefore, a consumer risk assessment cannot be finalised and a full characterisation of all potential metabolites relevant for this strain needs to be provided. In addition, it is recommended to establish the number of viable spores at the time of harvest for information and characterisation of the treated plant produce intended for human consumption.

The information and evidence provided was considered insufficient to conclude on the likely competitiveness, persistence and multiplication of *Bacillus amyloliquefaciens* AH2 in soil and surface water.

The risk assessment to birds, aquatic organisms, bees, other non-target arthropods, earthworms and soil microorganisms could not be finalised.
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Background

Regulation (EC) No 1107/2009 of the European Parliament and of the Council\(^1\) (hereinafter referred to as ‘the Regulation’) lays down, \textit{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).

\textit{Bacillus amyloliquefaciens} strain AH2 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 Probelte S.A. on 1 June 2015 for approval of the active substance \textit{Bacillus amyloliquefaciens} strain AH2. 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 25 October 2015.

The RMS provided its initial evaluation of the dossier on \textit{Bacillus amyloliquefaciens} strain AH2 in the DAR, which was received by EFSA on 21 December 2017 (Netherlands, 2017). The peer review was initiated on 2 March 2018 by dispatching the DAR for consultation of the Member States and the applicant, Probelte S.A., 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’s 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 23 July 2018. 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 area of ecotoxicology.

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 \textit{Bacillus amyloliquefaciens} strain AH2 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 April 2019 and April 2020.

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 use of \textit{Bacillus amyloliquefaciens} strain AH2 as a fungicide on grapes as proposed by the applicant. In accordance with Article 12(2) of Regulation (EC) No 1107/2009, risk mitigation options identified in the DAR and considered during the peer review are presented in the conclusion. 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 under Regulation (EC) No 1107/2009 without restrictions affecting the residue assessment. In the event of a non-approval of the active substance, no restrictions affecting the residue assessment will be approved under Regulation (EC) No 1107/2009 without restrictions affecting the residue assessment.

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\(^{1}\) 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.
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 and the formulation is provided in Appendix A.

In addition, a key supporting document to this conclusion is the peer review report (EFSA, 2020), 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 (26 July 2018);
- the evaluation table (8 May 2020);
- the report(s) of the scientific consultation with Member State experts (where relevant);
- the comments received on the assessment of the additional information (where relevant);
- the comments received on the draft EFSA conclusion.

Given the importance of the DAR including its revisions (Netherlands, 2019) 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 European Union (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 amyloliquefaciens* strain AH2 is a bacterium deposited at the Spanish Type Culture Collection (CECT), under the accession number CECT-7221. Initially, the strain AH2 was identified as *Bacillus velezensis*. *Bacillus amyloliquefaciens* strain AH2 is a naturally occurring, indigenous wild type bacterium, initially isolated from cultivated soils in the region of Murcia, Spain.

Due to availability of new data from fast sequencing tools, the taxonomy of the active substance was changed from the time of submission. It was proposed that the *Bacillus amyloliquefaciens* clade should be considered as a taxonomic unit above species level, designated as ‘operational group *Bacillus* amyloliquefaciens’, consisting of the soil-borne *Bacillus amyloliquefaciens*, and plant-associated *Bacillus siamensis* and *B. velezensis*, whose members are closely related.

The representative formulated product for the evaluation was ‘PB001’, a suspension concentrate (SC) containing 993 g/L (nominal $1 \times 10^{11}$ CFU/L, minimum content $7 \times 10^{10}$ CFU/L, maximum $7 \times 10^{11}$ CFU/L) *Bacillus amyloliquefaciens* strain AH2.

The representative uses evaluated comprise applications by spraying on grapes, as a fungicide against *Botrytis cinerea* (grey mould). Full details of the good agricultural practice (GAP) can be found in the list of end points in Appendix A.

Data were submitted to conclude that the uses of *Bacillus amyloliquefaciens* strain AH2 result in a sufficient fungicidal efficacy against grey mould, according to the representative uses proposed at EU level and following the guidance document SANCO/10054/2013 – rev. 3 (European Commission, 2013).

Conclusions of the evaluation

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

   The following guidance documents were considered in the production of this conclusion: European Commission, 2000, 2012 and EFSA, 2012.

   The technical grade of the microbial pest control agent (MPCA) is a hypothetical stage in a continuous production process of the end-use product.

   Identification of *Bacillus amyloliquefaciens* strain AH2 at the strain level can be performed with molecular methods for 16S rRNA and *gyrB* sequence amplification, with sequencing and phylogenetic analysis.

   The taxonomy of the *Bacillus subtilis* group is dynamically changing due to availability of new data (mostly due to fast sequencing tools). It is noted that until the final clarification on taxonomy, the originally proposed name of the active substance is used in the conclusion.
The analysis of contaminating microorganisms in commercially produced batches complies with the requirements of the Working Document on Microbial Contaminant Limits (European Commission, 2012). *Bacillus amyloliquefaciens* strain AH2 is able to produce iturin A, fengycin and surfactin C as well as subtilisin. *Bacillus amyloliquefaciens* strain AH2 does not code for genes necessary for bacilysin production. The production of amylosin by *Bacillus amyloliquefaciens* strain AH2 under optimum growth conditions was investigated by liquid chromatography–mass spectrometry (LC-MS) analysis and its presence was not detected. It should be mentioned, however, that the limit of detection (LOD) of the method was unknown. Comparative genome analysis between strains known for the production of amylosin and those where amylosin production could not be detected, revealed a cluster which is likely associated with amylosin production (the putative amylosin cluster). The production of amylosin associated with that gene cluster could not be identified in the *Bacillus amyloliquefaciens* strain AH2 genome. It is therefore acknowledged that currently all available evidence suggested that the possibility of amylosin production by strain AH2 is rather low.

Analysis of five batches of the microbial pest control product (MPCP) demonstrated that the formulated product contains very low amounts of surfactins, iturin A, fengycin and subtilisin. Their levels in the five batches were below 0.03 g/kg. Subtilisin was detected in the product below the quantification limit.

The growth temperature range of *Bacillus amyloliquefaciens* strain AH2 is between 15°C and 50°C whereas the optimum growth temperature is 30–40°C. It is able to grow at a large pH range and it is sensitive to ultra violet light. *Bacillus amyloliquefaciens* strain AH2 was sensitive to a wide range of antibiotics (24 antibiotics) from different classes: β-lactams such as penicillins and cephalosporins, aminoglycosides, fluoroquinolones and glycopeptides, covering a representative range of antibiotics commonly used in human and veterinary medicine. *Bacillus amyloliquefaciens* strain AH2 is resistant to colistin and metronidazole; however colistin is effective against most Gram-negative bacteria, while metronidazole is effective against Gram-negative and Gram-positive anaerobic bacteria.

The supported shelf-life of the product is 24 months at 5°C in COEX-HDPE and HDPE bottles. Acceptable methods are available for the determination of the microorganism in the technical material and for the determination of the content of contaminating microorganisms. Appropriate validated analytical methods were available for the determination of the content impurities surfactin C, iturin A, fengycin and subtilisin in the formulated product PB001.

A residue definition was not applicable for *Bacillus amyloliquefaciens* strain AH2, therefore post-registration monitoring methods are not needed.

2. **Mammalian toxicity**

**General data**

No indications of any toxicological or allergenic effects to the workers involved in the production or packaging of *Bacillus amyloliquefaciens* strain AH2 since 2008 have been observed, with use of protective clothing, gloves and eye protection where necessary.

From the literature review, some cases of allergenicity or sensitisation reactions caused by metabolites produced by strains of *Bacillus amyloliquefaciens* were reported but no publication on hypersensititivity or chronic sensitisation caused by *Bacillus amyloliquefaciens* strain AH2 was found. *Bacillus amyloliquefaciens* is recommended for the Qualified Presumption of Safety (QPS) list (EFSA BIOHAZ Panel, 2020) 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. Based on the available data, it cannot be concluded that these qualifications are met (see also Section 1).

**Toxicity studies**

Laboratory studies on mammalian toxicity of *Bacillus amyloliquefaciens* strain AH2 have been conducted in rats upon oral, intratracheal or intravenous applications of acute single doses. Adverse effects were not reported. Signs of infection or accumulation were not observed. Persistent low caecum concentrations after oral administration were not considered infectious, considering the overall clearance of the microorganism.

As the available methods for testing dermal sensitisation are not suitable for testing microorganisms and there are no validated test methods for sensitisation by inhalation, the following warning phrase is proposed: ‘Micro-organisms may have the potential to provoke sensitising reactions’.

From literature data, repeated oral administration of other strains of *Bacillus amyloliquefaciens* was also performed to assess potential probiotic effects of these microorganisms. The results were a
reduction of pathogenic clostridia in the faecal microbiota of dogs and a reduction of inflammatory bowel disease in a murine in vivo model.

Secondary metabolites/toxins

*Bacillus amyloliquefaciens* strain AH2 has the potential to produce the secondary metabolites iturin A, surfactin C, fengycin and subtilisin. Very low amounts were detected in the formulated product (see also Section 1). It is noted that iturins and surfactins belong to the lipopeptide family, and they are strong surfactants showing membrane-damaging properties (lytic activity) in vitro.

Toxicity studies with surfactin C, produced by strains of *Bacillus amyloliquefaciens* or *Bacillus subtilis*, were found in the literature. In a rat 28-day oral study with surfactin C (produced by *Bacillus subtilis*), the no observed adverse effect level (NOAEL) was 500 mg/kg body weight (bw) per day based on decreased body weight and liver toxicity. Surfactin C showed no genotoxic potential in vitro in the bacterial reverse mutation assay, or in vivo in the bone marrow micronucleus test. It is noted that the exposure of the bone marrow may not have been sufficiently demonstrated to conclude reliably on the results of this test and it should be considered supplementary. In a developmental study in mice, surfactin C did not show maternal toxicity or teratogenicity potential.

The toxin amylosin was shown to inhibit motility of boar sperm cells and to be cytotoxic to feline lung cells in vitro. Other in vitro tests with human cells indicated toxicity of amylosin produced by strains of *Bacillus amyloliquefaciens* isolated from moisture-damage buildings. Finally, amylosin was also identified in food poisoning outbreaks involving *Bacillus subtilis* and *Bacillus mojavensis*. Based on the available data (see Section 1), amylosin seems unlikely to be produced by *Bacillus amyloliquefaciens* AH2.

Considering the limited evidence available about their production and/or their toxicological profile, the relationship between the presence of secondary metabolites/toxins and the risk of illness in human cannot be excluded. The RMS is of the opinion that adequate information was provided to perform a risk assessment, considering that the microorganism is a natural component of the soil and that no adverse effects were observed in the human and in laboratory studies.

Reference values and exposure

Based on the lack of significant toxicity, infectivity of pathogenicity in the available toxicological studies, the setting of health-based reference values for the microorganism *Bacillus amyloliquefaciens* AH2 is not needed.

Pending on further investigations on toxins/secondary metabolites produced after application, further considerations will have to be given to their potential toxicity in order to conclude on the risk assessment for workers and residents (data gap and issue not finalised).

3. Residues

The strain *Bacillus amyloliquefaciens* strain AH2 is naturally occurring and was isolated from cultivated soils.

The representative use is a foliar treatment against grey mould (*Botrytis cinerea*) in grapes noting that this commodity belongs to the "high acid group" of plant commodities. Additional field uses on lettuce, tomatoes and courgettes are anticipated all of which are representing "high water commodities" (Netherlands, 2019). The high water commodities are less acidic compared to high acid commodities and can therefore be considered as a more favourable physiological environment with regard to survival, germination and growth of *Bacillus* spores.

*Bacillus amyloliquefaciens* strain AH2 spores are unlikely to multiply following application on edible parts and viable counts are declining to levels of around 1 % of the initially applied dose of around 10⁷ CFU per leaf. Nevertheless, exact viable spores at the time of harvest are not reported and do not appear to have been determined. While it can be concluded that germination of introduced spores and subsequently viable cell multiplication during wine production is unlikely, information on the fate of spores in products with treated high water commodities would be more important since in a low-acidic environments *Bacillus* spores could germinate and subsequently cell multiplication could occur.

*Bacillus amyloliquefaciens* strain AH2 may produce several metabolites; however, formation of all potential metabolites was not investigated following treatment considering the demonstrated decline characteristics of the strain. It seems unlikely that it produces amylosin (see Sections 1 and 2).

The RMS is of the opinion that adequate information was provided to perform a risk assessment, considering that the microorganism is a natural component of the soil and that no adverse effects were observed in the human and in laboratory studies. The RMS highlighted that antifungal
compounds are considered to be produced transiently during the direct interaction with the pathogens but will not accumulate thereafter.

The RMS outlined that the literature data provided and the fact that the microorganism has a global natural distribution in soil, is a natural component of the soil and food web and that there are no adverse effects found for the microorganism in humans and in the laboratory studies. They deemed it sufficient to perform a tentative risk assessment.

This assessment was supported by the fact that the species *Bacillus amyloliquefaciens* was included on the EFSA QPS list for more than a decade however with a qualification of absence of toxigenic activity. This absence of toxigenic activity remains an open issue for strain AH2 (see Section 2).

Overall, since a robust conclusion on all potentially relevant toxins/secondary metabolites formed by the strain cannot be drawn (see Sections 1 and 2), EFSA considers that the consumer risk assessment was not finalised and will need to be reconsidered when further information on metabolites becomes available. An inclusion in Annex IV of Regulation (EC) No 396/2005 cannot be recommended.

4. Environmental fate and behaviour

Satisfactory information has been provided in relation to potential interference of *Bacillus amyloliquefaciens* strain AH2 with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC2 (see specific Annex VI decision making criteria in Part II Commission Regulation (EU) No 546/20113). The provided information support that these methods utilise chromogenic agents to which *Bacillus amyloliquefaciens* strain AH2 does not give a response. Therefore it was considered unlikely that *Bacillus amyloliquefaciens* strain AH2 would interfere with the methodologies used for such determinations.

*Bacillus amyloliquefaciens* AH2 is a 'wild type' and 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 has not been ascertained, it would not be possible to distinguish any genetic drift from that in the wild population based on the information provided. Though it is acknowledged that the possibility and effects of transfer of genetic material is not different for *Bacillus amyloliquefaciens* AH2 than for other naturally occurring *Bacillus amyloliquefaciens* strains, transfer of genetic material by *Bacillus amyloliquefaciens* AH2 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 different strains of *Bacillus amyloliquefaciens* in relation to its persistence and multiplication in soil. There were no specific studies available on *Bacillus amyloliquefaciens* strain AH2. The studies on different strains of *Bacillus amyloliquefaciens* in soil were considered insufficient to conclude on the likely competitiveness, persistence and multiplication of *Bacillus amyloliquefaciens* strain AH2 in field soil. Consequently, EFSA concluded that the information is insufficient to address the uniform principles criterion of the strain not being expected to persist and multiply in soil in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years. This conclusion identifies a data gap (see Section 7) and an issue that could not be finalised (see Section 9.1).

With respect to the persistence and multiplication in surface water published studies were available providing information on the persistence of *Bacillus amyloliquefaciens* and *Bacillus subtilis* in water. There were no specific studies available for *Bacillus amyloliquefaciens* strain AH2. Consequently, EFSA concluded that the information is insufficient to address the uniform principles criterion of the strain not being expected to persist and multiply in surface water in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years. The information on the persistence/multiplication/germination of *Bacillus amyloliquefaciens* and *Bacillus subtilis* in natural surface water was considered insufficient to demonstrate that *Bacillus amyloliquefaciens* strain AH2 is likely to decline in surface water. This conclusion identifies a data gap

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2 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.
3 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.
Predicted environmental concentrations (PEC) in surface water for field use in grapes have 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 Bacillus amyloliquefaciens provided some information on occurrence and behaviour in air. Viable spores were determined to be stable in air of at least 24 h and could be detected in air up to 48 h at a maximum distance of 2.45 km from the point of release.

Regarding mobility in general, vertical distribution of the microbial organism through soil is unlikely to happen based on information in submitted published scientific paper on Bacillus subtilis and Bacillus subtilis cereus.

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

According to scientific papers from the literature search, the species Bacillus amyloliquefaciens is able to produce secondary metabolites such as iturins, fengycins, and surfactins. Production of iturins, fengycins and surfactin constitutes part of the mode of action of Bacillus amyloliquefaciens AH2.

It is not known to what extent Bacillus amyloliquefaciens strain AH2 will produce any metabolites following its application once the spores reach the soil, should they grow. Adequate information to address the potential concentrations of secondary metabolites/toxins to be produced by Bacillus amyloliquefaciens strain AH2 in all environmental compartments 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) 283/20134 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 amyloliquefaciens strain AH2 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 active substances) are triggered. Consequently, this resulted in a data gap (see Section 7) and assessment that could not be finalised, (see Section 9.1).

5. Ecotoxicology

Some aspects of the risk assessment for Bacillus amyloliquefaciens strain AH2 were discussed in the Pesticide Peer Review Meeting TC 200 on 22 January 2019.

Suitable studies were available to demonstrate that Bacillus amyloliquefaciens strain AH2 is not infectious or pathogenic to mammals. The risk to mammals was therefore considered to be low. No studies were conducted with the product or Bacillus amyloliquefaciens AH2 with birds. The assessment relies on literature on other Bacillus species or Bacillus amyloliquefaciens of unknown strain where no infectivity of pathogenicity was observed. Furthermore, it was referred to the use of some Bacillus species as bird feed additives. It was discussed in the experts’ meeting whether the available information (including information on secondary metabolites) is sufficient to conclude on a low risk to birds. Uncertainties were identified with regard to the comparability of the Bacillus amyloliquefaciens strain used in the plant protection product and the Bacillus species and Bacillus amyloliquefaciens strains for which information was retrieved from studies with birds.5 The majority of the experts agreed that it is possible in a weight of evidence approach to conclude on a low risk to birds considering all available information and provided that the concentrations of secondary metabolites in the environment are likely to be low. However, a data gap was identified in section 4 on the formation and concentration of metabolites in the environment. Therefore, the risk assessment for birds is considered as not finalised also taking into account the uncertainty related to the lack of specific

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4 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.

5 See discussion point 5.1 in the report from Pesticide Peer Review Meeting TC 200 from 22 January 2019.
information for potential effects for *Bacillus amyloliquefaciens* AH2 and secondary metabolites on birds (data gap and issue that could not be finalised).

Adequate studies were available with aquatic organisms showing no infectivity and pathogenicity to aquatic organisms. A high margin of safety was observed when comparing the endpoints with expected spore concentrations in the environment after spray drift entry into surface water. Therefore, a low risk to aquatic organisms was concluded. Information on the toxicity of the secondary metabolites iturin, fengycins, surfactin C and subtilisin to aquatic organisms is not available. It is unknown which amounts of secondary metabolites may be present in surface water (see data gap in Section 4). Therefore, no final conclusion can be drawn on the risk from secondary metabolites to aquatic organisms (data gap and issue that could not be finalised).

Adequate studies were available with aquatic organisms showing no infectivity and pathogenicity. Information was also available on the toxicity of some of the secondary metabolites iturin and surfactin C to bees. Exposure to iturin might affect bees (supplementary study, no information on the dose available). No effects on bees were observed from exposure to surfactin C (15.3 μg surfactin C/bee per day for 30 days). It is unknown which amounts of secondary metabolites are formed in the field (see data gap in Section 4) and to which amounts bees could be exposed. Therefore, no final conclusion can be drawn on the risk from secondary metabolites to bees (data gap and issue that could not be finalised).

Studies with the product and *Typhlodromus pyri* and *Aphidius rhopalosiphi* were available indicating a low risk to non-target arthropods. Effects on aphids were observed in a test with the secondary metabolite surfactin C. The product contains surfactin C in such low amounts that it is unlikely to cause effects in non-target arthropods which is confirmed by the available studies with the product and *Typhlodromus pyri* and *Aphidius rhopalosiphi*. However, it is unknown to which amounts surfactin C will be produced in the environment (see data gap in Section 4) and therefore the risk assessment for non-target arthropods other than bees cannot be finalised.

No study with earthworms and the product was available. Published literature was used in a weight of evidence approach. Information was available that *Bacillus subtilis* and probably all bacilli do not grow in the gut of earthworms and that the immune system of earthworms can cope with different *Bacillus* species. Based on all the information available, the majority of experts agreed that the risk can be regarded as likely to be low. A minority of the experts would require a new study for testing the effects on earthworms following the standard guidelines for microorganism testing. However, since no specific information regarding effects of *Bacillus amyloliquefaciens* AH2 on earthworms was available and a data gap was identified in Section 4, EFSA considers that it is it is not possible to finalise the risk assessment for earthworms (data gap and issue that could not be finalised).

The RMS expects no unwanted effects on soil microbial communities based on information from public literature on other *Bacillus amyloliquefaciens* strains and assuming low exposure of natural soils microbial communities. The RMS is of the opinion that further information is not relevant. However, no specific information was available to address the risk to soil microorganisms from *Bacillus amyloliquefaciens* AH2 and a data gap was identified in section 4 on fate and behaviour with regard to multiplication and persistence in soil. Hence, the risk assessment for soil microorganisms cannot be finalised.

The risk to non-target plants was assessed as low.

A further data gap was identified for a thorough evaluation of the available literature review for non-target organisms. An updated literature review was submitted by the applicant, but the information provided in the Renewal Assessment Report (RAR) in Volume 3, B9, was insufficient to conclude.

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6 See discussion point 5.2. in the report of Pesticide Peer Review Meeting TC200 from 22 January 2019.
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 |
|-----------------------------|-------------|---------------|
| *Bacillus amyloliquefaciens* AH2 | Data gap pending on their identification and quantification | Data gap |
| Toxins/secondary metabolites such as iturins, fengycins, subtilisin and surfactins | | No information was made available on the effects of secondary metabolites such as iturins, fengycins, subtilisin and surfactins on earthworms and other soil macro- and microorganisms |

Table 2: Groundwater

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

<sup>(a)</sup>: At least one FOCUS scenario or a relevant lysimeter.

Table 3: Surface water and sediment

| Compound (name and/or code) | Ecotoxicology |
|-----------------------------|---------------|
| *Bacillus amyloliquefaciens* AH2 | The risk from the product was assessed as low with a large margin of safety |
| Toxins/secondary metabolites such as iturins, fengycins, subtilisin and surfactins | The risk assessment from secondary metabolites to aquatic organisms is not finalised for the representative use of *Bacillus amyloliquefaciens* AH2 (data gap) |

Table 4: Air

| Compound (name and/or code) | Toxicology |
|-----------------------------|------------|
| *Bacillus amyloliquefaciens* AH2 | No mortality, no toxic effects and no pathogenicity were observed in rats following intratracheal instillation of *Bacillus amyloliquefaciens* AH2 at 9.1 × 10<sup>7</sup> CFU per animal |
| Toxins/secondary metabolites such as iturins, fengycins, subtilisin and surfactins | CFU: colony forming unit. |

CFU: colony forming unit.
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).

- Pending on further investigations on the production of toxins/secondary metabolites and levels present in the environment after application, further considerations will have to be given to their potential toxicity in order to conclude on the risk assessment for humans (workers and residents), consumers and non-target organisms (relevant for all representative uses evaluated; see Sections 2, 3, 4 and 5).
- Satisfactory information to demonstrate that *Bacillus amyloliquefaciens* AH2 cannot transfer genetic material, particularly the genes coding its resistance to antibiotics under the conditions of use (relevant for all representative uses evaluated; see Section 4).
- Adequate information to address the uniform principles criterion of the strain not being expected to persist and multiply in soil or plant growing media and in surface water in concentrations considerably higher than the natural background levels, provided that repeated applications over the years was not available (relevant for all representative uses evaluated; see Section 4).
- Information on potential effects (toxicity and infectivity) of *Bacillus amyloliquefaciens* AH2 to birds is missing (relevant for all representative uses evaluated; see Section 5).
- Information on effects of secondary metabolites to bees and other non-target arthropods is missing (relevant for all representative uses evaluated; see Section 5).
- Information on potential effects of *Bacillus amyloliquefaciens* AH2 to earthworms (relevant for all representative uses evaluated; see Section 5).
- Information on potential effects of *Bacillus amyloliquefaciens* AH2 to soil microorganisms (relevant for all representative uses evaluated; see Section 5).
- An evaluation of an updated literature review in the area of ecotoxicology taking into account the key words toxicity, infectivity and pathogenicity was not available. An updated literature review was submitted by the applicant but the information provided in the RAR in Volume 3, B9, was insufficient to conclude (relevant for all representative uses evaluated; 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 546/2011 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) The assessment of potential transfer of genetic material (e.g. responsible of antibioresistance) from *Bacillus amyloliquefaciens* strain AH2 to other organisms could not be finalised (see Sections 1 and 4).

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7 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.
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 workers, residents, consumers and the environment including the assessment of potential groundwater exposure (see Sections 2, 3, 4 and 5).

3) The information on the persistence/multiplication/germination of Bacillus amyloliquefaciens in soil was considered insufficient to demonstrate that Bacillus amyloliquefaciens strain AH2 is likely to decline in soil. These facts lead to an assessment not finalised (See Sections 4 and 5).

4) The risk assessment to birds is considered not finalised because information on toxicity and infectivity specific for Bacillus amyloliquefaciens AH2 is missing (See Section 5).

5) The risk assessment to aquatic organisms is considered not finalised because it is unknown to which extent secondary metabolites might be present in surface water and if present any effects that might occur to aquatic organisms (see Sections 4 and 5).

6) The risk assessment to bees cannot be finalised as information on the toxicity of secondary metabolites (other than surfactin C) is missing and the amounts up to which they can be produced in the environment is unknown (See Section 5).

7) The risk assessment for non-target arthropods other than bees could not be finalised as there is indication that the secondary metabolite surfactin C can be toxic to arthropods and the amounts up to which it can be produced in the environment is unknown. Also other secondary metabolites should be addressed in the assessment as they might also be toxic to non-target arthropods other than bees (see Section 5).

8) The risk assessment to soil microorganisms could not be finalised because no specific information was available for Bacillus amyloliquefaciens AH2 to address the risk to soil microorganisms (see Section 5).

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

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                        | Grapes Spray use |
|------------------------------------------|------------------|
| **Operator risk**                        |                  |
| Risk identified                          |                  |
| Assessment not finalised                 |                  |
| **Worker risk**                          |                  |
| Risk identified                          |                  |
| Assessment not finalised                 |                  |
| **Resident/bystander risk**              |                  |
| Risk identified                          |                  |
| Assessment not finalised                 |                  |
| **Consumer risk**                        |                  |
| Risk identified                          |                  |
| Assessment not finalised                 |                  |
| **Risk to wild non-target terrestrial vertebrates** |                  |
| Risk identified                          |                  |
| Assessment not finalised                 |                  |
| **Risk to wild non-target terrestrial organisms other than vertebrates** |                  |
| Risk identified                          |                  |
| Assessment not finalised                 |                  |
| **Risk to aquatic organisms**            |                  |
| Risk identified                          |                  |
| Assessment not finalised                 |                  |
| **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                 |                  |

The superscript numbers in this table relate to the numbered points indicated in Sections 9.1 and 9.2. Where there is no superscript number, see Sections 2–6 for further information.

(a): When the consideration for classification made in the context of this evaluation under Regulation (EC) No 1107/2009 is confirmed under Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008.

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

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**Abbreviations**

- **Bw**: body weight
- **CFU**: colony forming units
- **DAR**: draft assessment report
- **FOCUS**: Forum for the Co-ordination of Pesticide Fate Models and their Use
- **GAP**: Good Agricultural Practice
- **LC-MS**: liquid chromatography–mass spectrometry
- **LOD**: limit of detection
- **MPCA**: microbial pest control agent
- **MPCP**: microbial pest control product
- **NOAEL**: no observed adverse effect level
- **PEC**: predicted environmental concentration
- **QPS**: Qualified Presumption of Safety
- **RAR**: Renewal Assessment Report
- **RMS**: rapporteur Member State
- **SC**: suspension concentrate
- **SMILES**: simplified molecular-input line-entry system
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.2020.6156
## Appendix B – Used compound codes

| Code/trivial name(a) | Chemical name/SMILES notation(b) | Structural formula(c) |
|----------------------|----------------------------------|-----------------------|
| iturin A             | 3-[(3R,6S,13S,16R,19R,22S,27aS)-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-1H-pyrrole][2,1-i][1,4,7,10,13,16,19,22]octaazacyclopentacosin-22-yl] propanamide | ![Structural formula for iturin A](image) |
| surfactin C          | 3-[(3S,6R,9S,12S,15R,18S,21S)-9-(carboxymethyl)-3,6,15,18-tetrasobutyl-12-isopropyl-25-(10-methylundecyl)-2,5,8,11,14,17,20,23-octaox-1-oxa-4,7,10,13,16,19,22-heptaaazaclaycopentacosan-21-yl] propanoic acid | ![Structural formula for surfactin C](image) |
| fengycin             | 3-[(3S,6R,13R,16S,19S,22R,27aR)-3,6,13,19-tetrakis(2-amino-2-oxoethyl)-16-[(4-hydroxyphenyl)methyl]-9-(9-methylundecyl)-1,4,7,11,14,17,20,23-octaoxohexacosahydro-1H-pyrrolo][2,1-i][1,4,7,10,13,16,19,22]octaazacyclopentacosin-22-yl] propanamide | ![Structural formula for fengycin](image) |
| bacilysin            | L-alanyl-3-[(1R,2S,6R)-5-oxo-7-oxabicyclo[4.1.0]heptan-2-yl]-L-alanine | ![Structural formula for bacilysin](image) |

(a): The metabolite name in bold is the name used in the conclusion.
(b): ACD/Name 2017.2.1 ACD/Labs 2017 Release (File version N40E41, Build 96719, 6 September 2017).
(c): ACD/ChemSketch 2017.2.1 ACD/Labs 2017 Release (File version C40H41, Build 99535, 14 February 2018).