Peer review of the pesticide risk assessment of the active substance Bacillus amyloliquefaciens strain IT-45

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Abstract

The conclusions of the European Food Safety Authority (EFSA) following the peer review of the initial risk assessments carried out by the competent authority of the rapporteur Member State, France, for the pesticide active substance Bacillus amyloliquefaciens strain IT-45 and the considerations as regards the inclusion of the substance in Annex IV of Regulation (EC) No 396/2005 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 strain IT-45 as a fungicide on citrus (field use, application to soil via drip irrigation systems). 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* strain IT-45 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), France, received an application from Danstar Ferment AG and Comercial Quimica Masso on 26 June 2017 for approval. In addition, the applicants submitted an application for inclusion of the substance 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 1 December 2017.

An initial evaluation of the dossier on *Bacillus amyloliquefaciens* strain IT-45 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.

Due to availability of new data, 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 *Bacillus velezensis*, whose members are closely related.

*Bacillus amyloliquefaciens* strain IT-45 was reclassified as *Bacillus velezensis* strain IT-45; however, for consistency reasons, the name *Bacillus amyloliquefaciens* strain IT-45 was used in this conclusion.

The use of *Bacillus amyloliquefaciens* strain IT-45 by drip irrigation as a fungicide on citrus according to the representative use as proposed at EU level results in some fungicidal efficacy against the target *Phytophthora citrophthora* and *Phytophthora nicotianae* var *parasitica*. Considering the limited data provided, more detailed consideration will be fully assessed in the context of subsequent applications for products authorisation.

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.

With regard to mammalian toxicity, no critical areas of concern were identified.

The consumer risk assessment cannot be finalised with regard to the toxins/secondary metabolites that might be produced in soil and further considerations will have to be given to their residue behaviour in relation to the representative use and if relevant the toxicity of these compounds should also be addressed to finalise the consumer exposure assessment.

The information and evidence provided was considered insufficient to conclude on the likely competitiveness, persistence and multiplication of *Bacillus amyloliquefaciens* strain IT-45 in soil for the representative use. An assessment not finalised was concluded in relation to the potential for the production of secondary metabolites in soil following application.

The risk assessment to non-target organisms, birds, wild mammals, aquatic organisms, non-target soil arthropods, earthworms, other soil macro- and microorganisms could not be finalised.
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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 amyloliquefaciens* strain IT-45 is a new active substance for which, in accordance with Article 7 of the Regulation, the RMS, France (hereinafter referred to as the ‘RMS’), received an application from GAB Consulting on behalf of the Task Force formed by Danstar Ferment AG and Comercial Quimica Masso on 27 June 2017 for approval of the active substance *Bacillus amyloliquefaciens* strain IT-45. In addition, the applicant submitted an application for inclusion of the substance 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 1 December 2017.

The RMS provided its initial evaluation of the dossier on *Bacillus amyloliquefaciens* strain IT-45 in the DAR, which was received by EFSA on 15 May 2019 (France, 2019). The peer review was initiated on 4 July 2019 by dispatching the DAR to the Member States and the applicants, GAB Consulting on behalf of the Task Force formed by Danstar Ferment AG and Comercial Quimica Masso, 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 applicants were 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 13 December 2019. 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 applicants, and that EFSA should conduct an expert consultation in the area of mammalian toxicology.

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 and the written consultation on the assessment of additional information, where these 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 amyloliquefaciens* strain IT-45 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 and on the proposal for inclusion of the substance in Annex IV of Regulation (EC) No 396/2005 took place with Member States via a written procedure in February–March 2021.

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 *Bacillus amyloliquefaciens* strain IT-45 as a fungicide on citrus (field use, application to soil via drip

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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.
irrigation systems) 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, if any, are presented in the conclusion.

Furthermore, this conclusion also addresses the requirement for an assessment by 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.

A list of the relevant end points for the active substance and the formulation is provided in Appendix A.

A key supporting document to this conclusion is the peer review report (EFSA, 2021), 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 (13 December 2019);
- the evaluation table (6 April 2021);
- 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 (France, 2021), and the peer review report, both documents are considered as background documents to this conclusion and thus are made publicly available.

It is recommended that this conclusion 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 identity of the microorganism and the properties of the formulated product

*Bacillus amyloliquefaciens* strain IT-45 is a bacterium deposited at the National Collection of Pasteur Institute (CNRM), Paris, France, under the accession number CNCM I-3800. The strain *Bacillus amyloliquefaciens* IT-45 is a naturally occurring, indigenous wild-type bacterium, initially isolated in France from soil on which corn has been cultivated.

The representative formulated product for the evaluation was ‘CILUS PLUS’, a wettable powder (WP) containing 100 g/kg (nominal content $1 \times 10^{13}$ CFU/kg, minimum $2 \times 10^{12}$ CFU/kg, maximum $5 \times 10^{13}$ CFU/kg) *Bacillus amyloliquefaciens* strain IT-45.

The representative uses evaluated comprise field applications by drip irrigation as a fungicide on citrus against root rot (*Phytophthora citrophthora* and *Phytophthora nicotianae var parasitica*) in the EU. Full details of the Good Agricultural Practices (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 IT-45 according to the representative uses proposed at EU level result in a sufficient fungicidal efficacy against root rot, following the guidance document SANCO/10054/2013-rev. 3 (European Commission, 2013).

A data gap has been identified for a search of the scientific peer-reviewed open literature on the active substance for the biological properties to be conducted and reported in accordance with EFSA guidance on the submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA, 2011).

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 (European Commission, 2000, 2012; EFSA FEEDAP Panel, 2012).

Due to newer information on genetic data, *Bacillus subtilis* strain IT-45 was reclassified as *B. amyloliquefaciens* strain IT-45 and subsequently as *Bacillus amyloliquefaciens* ssp. *plantarum* IT-45.
Based on more recent phylogenetic analysis from additional literature identified, a further reclassification was considered necessary as *Bacillus velezensis* strain IT-45.

The specification of the technical grade microbial pest control agent (MPCA) is based on five-batch data and on quality control data. The specification for the MPCA is min. $2 \times 10^{13}$ CFU/kg and max. $6 \times 10^{14}$ CFU/kg of *Bacillus amyloliquefaciens* strain IT-45. The analysis of contaminating microorganisms in commercially produced batches complies with the requirements (European Commission, 2012).

Sequence characterised amplified region (SCAR) markers can distinguish *Bacillus amyloliquefaciens* strain IT-45 from the other strains. Two SCAR markers have been identified to be specific of the strain IT-45.

*Bacillus amyloliquefaciens* strain IT-45 has the genetic capacity to produce the cyclic lipopeptides: iturin A, bacillomycin, fengycin and surfactin; the polyketides: macrolactin, bacilaene, oxydiflacidin, diflacidin and a putative triketide pyrone; the iron siderophore bacillibactin; the antimicrobial dipeptide bacilysin, the lantibiotics amylocyclicin, amylolysin, the volatile compound 2,3-butanediol. The strain does not have the genetic capacity to produce the following secondary metabolites: amylosin, subtilosin, plantazolicin, mersacidin. Iturin A, surfactin and fengycin were investigated in the product (levels higher than 1 g/kg for surfactin and iturin A were found) and amylosin in the fermentation broth (not detected). There were not analytical determinations for the presence of the metabolites macrolactin, bacilaene, diflacidin, bacillibactin, bacilysin, amylocyclicin and amylolysin.

The growth temperature range of *Bacillus amyloliquefaciens* strain IT-45 is between 22°C and 50°C. The optimal temperatures for the growth are between 30°C and 45°C. However, information on the optimum pH for growth, sensitivity to UV light of the strain was not available.

*Bacillus amyloliquefaciens* strain IT-45 showed to be sensitive to all relevant antibiotics as provided in the EFSA FEEDAP Panel (2012) guidance document (chloramphenicol, tetracycline, streptomycin, clindamycin, erythromycin, kanamycin, gentamicin and vancomycin).

The supported shelf-life of the product is 2 years at 20°C in the original packaging. As the wettability increased significantly after storage, it should be mentioned on the label that mixing during the application is needed.

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 are available for the determination of the content of secondary metabolites surfactin, iturin A and fengycin in the formulated product.

Residue definitions were not applicable for *Bacillus amyloliquefaciens* strain IT-45; therefore, post-registration monitoring methods are not needed.

2. Mammalian toxicity

*Bacillus amyloliquefaciens* strain IT-45 was discussed at the Pesticide Peer Review Meeting Teleconference 35 in November 2020.

General data

From the literature review, medical data reported few clinical cases (e.g. by opportunistic mode of action), and isolation of *Bacillus* species from the gut of humans and animals was reported without any adverse effects. No indications of any toxicological or allergenic effects to the workers involved in the production, formulation and handling of *Bacillus amyloliquefaciens* strain IT-45 for more than 10 years have been observed, with use of protective clothing and gloves where necessary.

Some cases of allergenicity or sensitisation reactions caused by metabolites produced by strains of *Bacillus amyloliquefaciens* were reported, but no publication on hypersensitivity caused by *Bacillus amyloliquefaciens* strain IT-45 was found.

*Bacillus amyloliquefaciens* is recommended for the Qualified Presumption of Safety 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, the absence of toxigenic activity cannot be concluded (see also Section 1).

Toxicity/Infectivity/Pathogenicity studies

The available methods for testing dermal sensitisation are not suitable for testing microorganisms and there are no validated test methods for sensitisation by inhalation. Based on their characteristics,
microorganisms such as *Bacillus amyloliquefaciens* strain IT-45 may have the potential to provoke sensitising reactions.

Laboratory studies on mammalian toxicity of *Bacillus amyloliquefaciens* strain IT-45 have been conducted in rats upon single oral and intratracheal administration. No mortality or pathogenicity was observed. Some transitory clinical signs due to intratracheal administration were observed. Clearance was not investigated after oral administration and was incomplete in one out of 10 animals 35 days after intratracheal administration. Considering this incomplete clearance, the lack of investigations of the clearance in the acute oral study and the absence of an intraperitoneal study, the experts agreed that the potential infectivity of *Bacillus amyloliquefaciens* strain IT-45 was not sufficiently addressed (data gap).

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 IT-45 has the potential to produce compounds including cyclic lipopeptides (e.g. iturin, fengycin, surfactin), polyketides and bacteriocins/lantibiotics but cannot produce amylosin (see Section 1).

It is noted that compounds of the lipopeptide family are strong surfactants showing membrane damaging properties (lytic activity) *in vitro*. 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 cannot be produced by *Bacillus amyloliquefaciens* strain IT-45.

Intragastric administration of *iturin* to mice (7 and 28 days) did not produce mortality, clinical signs, organ damage or abnormal blood indices.

With regard to the other metabolites/toxins potentially produced by *Bacillus amyloliquefaciens* strain IT-45 (see Section 1), no information/data on their possible toxicity has been provided.

**Reference values and exposure**

Based on the lack of significant toxicity and pathogenicity in the available toxicological studies, together with the absence of adverse finding for the species *Bacillus amyloliquefaciens* in the literature review, the setting of health-based reference values for the microorganism *Bacillus amyloliquefaciens* strain IT-45 is not considered necessary. In the absence of a quantitative risk assessment, the use of personal/respiratory protective equipment for operators and workers might be considered to reduce the non-dietary exposure.

For the metabolites surfactin C and iturin, in combination with a low toxicity profile from the available literature, the expected exposure levels due to their presence in the plant protection product do not raise a concern for operators and bystanders. Considering the method of application (automatic drip irrigation), the exposure of operators, workers, residents and bystanders to toxins/secondary metabolites produced after application is not expected to be significant or trigger a concern even though some uncertainties are still present (e.g. presence of toxins/secondary metabolites other than surfactin and iturin in the plant protection product, produced amounts of toxins/secondary metabolites after application).

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2 Pesticide Peer Review Meeting Teleconference 35 in November 2020: Experts’ consultation 6.1.
3. Residues

The representative use is a soil treatment via drip irrigation against root rot (*Phytophthora citrophthora* and *Phytophthora nicotianae var. parasitica*) in citrus fruits (lemon, orange, clementine) which is performed once during spring. Viable spores of *Bacillus amyloliquefaciens* strain IT-45 are unlikely to be transported in the plant by uptake via the roots to edible plant parts considering their size (~1 μm) and the representative use. However, an enumeration of viable spores of edible plant parts at harvest is not reported to confirm this assumption. It was demonstrated that following application to soil viable spore counts reached a steady plateau concentration of around $1 \times 10^7$ CFU/g soil one month after treatment.

*Bacillus amyloliquefaciens* strain IT-45 has the potential to produce several metabolites (see Section 1); notably, a formation of all potentially formed metabolites was not investigated in soil following treatment according to the representative use.

This information was provided for two main metabolites, namely surfactin and iturin. It was demonstrated that their formation in soil would be significantly (by a factor of 100 and 1,000, respectively) below natural background levels considering the representative use of *Bacillus amyloliquefaciens* strain IT-45. Furthermore, it was shown and concluded that lipoprotein metabolites are highly unlikely to reach edible plant parts following uptake by root because they are large molecules (Molecular Weight ~1 kDa).

It is to be noted that since additional metabolites were reported to be potentially formed by this strain in the soil (see Section 1), their residue behaviour and if relevant their magnitude in the edible parts of the plants should be characterised (data gap). If it were to be concluded that quantifiable levels of metabolites and/or their degradation products are present in edible plant parts, their toxicological properties should be addressed to conclude the consumer exposure assessment. The RMS expressed disagreement and is of the opinion that, considering the intended GAP (application by drip irrigation during spring), no further investigation is required to finalise the consumer risk assessment because consumer exposure to metabolites of *Bacillus amyloliquefaciens* strain IT-45 can be considered as negligible. The RMS supports the finalisation of the consumer dietary risk assessment.

Overall, a robust conclusion on all potentially relevant toxins/secondary metabolites formed by the strain in the soils, on their potential uptake by the plants and on their presence in edible plant commodities cannot be drawn considering remaining uncertainties and lack of information (see Section 1 and identified data gaps in Sections 3 and 4). EFSA therefore concludes that the consumer risk assessment cannot be finalised and will need to be reconsidered when this information on the metabolites becomes available. An inclusion in Annex IV of Regulation (EC) No 396/2005 cannot be recommended at this stage.

4. Environmental fate and behaviour

Satisfactory information has been provided in relation to potential interference of *Bacillus amyloliquefaciens* strain IT-45 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). The provided information support that these methods utilise specific media and chromogenic agents to which *Bacillus amyloliquefaciens* strain IT-45 does not give a response. Therefore, it was considered unlikely that *Bacillus amyloliquefaciens* strain IT-45 would interfere with the methodologies used for such determinations.

*Bacillus amyloliquefaciens* strain IT-45 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 are not different for *Bacillus amyloliquefaciens* strain IT-45 than for other naturally occurring *Bacillus amyloliquefaciens* strains, transfer of genetic material by *Bacillus amyloliquefaciens* strain IT-45 after application is possible and could not be excluded based on the information in the dossier.

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3 Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.1998, 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.
4.1. Fate and behaviour in the environment of the microorganism

Information was derived from published literature on Bacillus amyloliquefaciens and other Bacillus species in relation to its persistence and multiplication in soil. Species of Bacillus amyloliquefaciens produce spores that persist in soil. An unpublished study investigating the population dynamics of Bacillus spp. and Bacillus amyloliquefaciens strain IT-45 in two different natural soils was available. The density of total bacteria and Bacillus spp. spores was stable over the 30-day study duration, but due to the non-specific enumeration method, it could not be related to the growth/ decrease of Bacillus amyloliquefaciens strain IT-45 in soil. It was confirmed that the study was not performed by an officially recognised testing facility. The studies on Bacillus amyloliquefaciens and other Bacillus species in soil were considered insufficient to conclude on the likely competitiveness, persistence and multiplication of Bacillus amyloliquefaciens strain IT-45 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 and an issue that could not be finalised. PEC soil for the representative use was calculated (see Appendix A).

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. These studies indicate that Bacillus spp. may survive in particular nutrient-rich water. There were no specific studies available for Bacillus amyloliquefaciens strain IT-45. 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 IT-45 is likely to decline in surface water. However, for the representative use by drip irrigation exposure of Bacillus amyloliquefaciens strain IT-45 to surface water can be considered negligible due to the low mobility of the microorganism in soil.

An increase of the natural level of Bacillus amyloliquefaciens strain IT-45 in air is not expected from the representative use by drip irrigation.

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 bacillomycins, iturins, fengycins and surfactins which contribute to the mode of action. Strain IT-45 has the genetic capacity to synthesise certain bacillomycins, surfactins, fengycins, polyketides, antimicrobial dipeptides, iturin A and other metabolites (see Section 1). The active substance as manufactured contains measurable levels of iturin A, surfactin and fengycin. The concentrations of iturin A and surfactin were greater than 1g/kg in the formulated product. Information on PECs in soil, PECs in surface water (FOCUS STEP 1-2 v3.2) and PEC groundwater (FOCUS PELMO 5.5.3 and FOCUS PEARL 4.4.4) for the secondary metabolites iturin A, surfactin and fengycin. The concentrations of iturin A and surfactin were greater than 1g/kg in the formulated product. Information on PECs in soil, PECs in surface water (FOCUS STEP 1-2 v3.2) and PEC groundwater (FOCUS PELMO 5.5.3 and FOCUS PEARL 4.4.4) for the secondary metabolites iturin A and surfactin based on the concentrations in the formulated product were provided (see Appendix A). It was concluded that the potential for leaching of iturin A and surfactin to groundwater above the parametric drinking water limit of 0.1 µg/L is low for the representative uses assessed in geoclimatic situations represented by the FOCUS groundwater scenarios.

It is not known to what extent Bacillus amyloliquefaciens strain IT-45 will produce any metabolites following its application when the spores reach the soil and the rhizosphere. Adequate information to address the potential concentrations of secondary metabolites/toxins to be produced by Bacillus amyloliquefaciens strain IT-45 in all environmental compartments was not available.6 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) 283/20136 namely:

- the relevant metabolite is stable outside the microorganism;

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5 Information was provided for the secondary metabolites surfactin and iturin indicating that their formation in soil would be below natural background levels considering the representative use of Bacillus amyloliquefaciens strain IT-45 (see Section 3).

6 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.
• 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 IT-45 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 and assessment that could not be finalised.

### 5. Ecotoxicology

Strain-specific data were not available to address potential effects, infectivity and pathogenicity to birds from *Bacillus amyloliquefaciens* strain IT-45. The assessment relied on literature on other *Bacillus* species or *Bacillus amyloliquefaciens* of unknown strain where no infectivity or pathogenicity was observed. Furthermore, it was referred to the use of some *Bacillus* species as bird feed additives. Insufficient information was available to support that the other *Bacillus amyloliquefaciens* strains would have similar traits as for *Bacillus amyloliquefaciens* strain IT-45 to allow to draw a conclusion on whether the infectivity and pathogenicity to birds is sufficiently addressed with the available information. Exposure to birds cannot be excluded when birds feed on soil organisms in the irrigated topsoil. Consequently, a data gap leading to an assessment that could not finalised was identified for the potential infectivity and pathogenicity of *Bacillus amyloliquefaciens* strain IT-45 to birds for the representative use.

No mortality or pathogenicity was observed in mammalian oral and intratracheal toxicity studies with *Bacillus amyloliquefaciens* strain IT-45 (see Section 2). A low risk to wild mammals was concluded for the representative use.

Adequate data were available for algae and aquatic plants from *Bacillus amyloliquefaciens* strain IT-45 resulting in a low risk from the representative use. Acute toxicity studies were available for fish and freshwater invertebrate from *Bacillus amyloliquefaciens* strain IT-45 and low toxicity was observed; however, such studies were not considered of sufficient duration to address potential infectivity and pathogenicity to fish and freshwater invertebrates. Overall low risk was concluded for aquatic organisms (fish, freshwater invertebrates, algae and aquatic plants) from *Bacillus amyloliquefaciens* IT-45 as the exposure from the representative use by drip irrigation was assessed to be negligible.

Acute oral and contact studies for honeybees with *Bacillus amyloliquefaciens* strain IT-45 were available. Laboratory studies with foliar species of non-target arthropods (*Typhlodromus pyri* and *Aphidius rhopalosiphi*) exposed to *Bacillus amyloliquefaciens* strain IT-45 were available. These studies on honeybees and non-target arthropods indicated low effects. The honeybee studies were not of sufficient duration to address potential infectivity and pathogenicity. For the studies on non-target arthropods potential infectivity and pathogenicity was not reported. Due to the biology and behaviour of honeybees and non-target foliar arthropods, the exposure to these organisms from the intended use is expected to be low. Therefore, low risk is concluded for honeybees and non-target foliar arthropods from the representative use by drip irrigation as exposure is assessed to be low. A data gap leading to an assessment that could not be finalised was identified for the potential infectivity and pathogenicity of *Bacillus amyloliquefaciens* strain IT-45 to soil-dwelling non-target arthropods for the representative use.

Strain-specific data were available from an acute toxicity study on earthworms indicating low toxicity from *Bacillus amyloliquefaciens* strain IT-45. Infectivity and pathogenicity was not reported in the study, but the study was not considered of sufficient duration to address potential infectivity and pathogenicity to earthworms. Sufficient strain-specific data were available to address potential adverse effects to soil microorganisms exposed to a representative formulation containing *Bacillus amyloliquefaciens* strain IT-45. A low risk to soil microorganisms was concluded from these studies for the representative use. A data gap leading to an assessment that could not be finalised was identified for the potential infectivity and pathogenicity to earthworms and other soil macro-organisms from *Bacillus amyloliquefaciens* strain IT-45 for the representative use.

Toxicity data on mammals were available for the secondary metabolites surfactin C and iturin (see Section 2). However, an environmental risk assessment for wild mammals was not provided for these metabolites. From the published scientific literature, short-term toxicity studies were available on freshwater invertebrates (Daphnia magna), algae (Selenastrum capricornutum) and the terrestrial...
arthropod (Aphids) exposed to the secondary metabolite surfactin. Details on which surfactin was included in the study was not provided (see Appendix A). A margin of safety as demonstrated and a low risk was concluded for freshwater invertebrates, algae and non-target foliar arthropods from surfactin for the representative use. For honeybees and non-target foliar arthropods, exposure from toxins/secondary metabolites is assessed to be low and low risk is concluded for the representative use. Toxicity data were not available to perform a hazard characterisation of toxins/secondary metabolites such as iturins, surfactins and fengycins for terrestrial non-target organisms (birds, wild mammals, non-target soil arthropods, earthworms, other soil macro- and microorganisms) and aquatic non-target organisms (other than freshwater invertebrates and algae for surfactin). The risk assessment of toxins/secondary metabolites such as bacillomycins, iturins, surfactins and fengycins could not be finalised for terrestrial non-target organisms (birds, wild mammals, non-target soil arthropods, earthworms, other soil macro- and microorganisms) and aquatic non-target organisms (other than freshwater invertebrates and algae for surfactin) for the representative use (resulting in data gap and issue that could not be finalised).

6. Overview of the risk assessment of the organism or metabolite 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) | Ecotoxicology |
|---------------|-----------------------------|---------------|
| Bacillus amyloliquefaciens strain IT-45 | A data gap and an assessment not finalised for the potential infectivity and pathogenicity to earthworms and other soil macro-organisms from Bacillus amyloliquefaciens strain IT-45 was identified for the representative use |
| Toxins/secondary metabolites such as bacillomycins, iturins, surfactins and fengycins | A data gap and an assessment not finalised was identified for the non-target organisms; non-target soil arthropods, earthworms, other soil macro- and microorganisms for the representative use |

| Table 2: Groundwater(a) | Compound (name and/or code) | Biological activity/ relevance | Hazard identified Steps 3b and c | Consumer RA triggered Steps 4 and 5 | Human health relevance |
|-------------------------|-----------------------------|-------------------------------|-------------------------------|-------------------------------|-----------------------|
| Toxins/secondary metabolites such as bacillomycins, iturins, surfactins and fengycins | Unlikely | Yes | No | Unlikely for the representative use | Unlikely for the representative use |
| | > 0.1 µg/L at 1 m depth for the representative uses(b) Step 2 | | | | |

(a): Assessment according to European Commission guidance of the relevance of groundwater metabolites (2003).
(b): FOCUS scenarios or relevant lysimeter.

| Table 3: Surface water and sediment | Compound (name and/or code) | Ecotoxicology |
|-------------------------------------|-----------------------------|---------------|
| Bacillus amyloliquefaciens strain IT-45 | Low risk was concluded for aquatic organisms (fish, freshwater invertebrates, algae and aquatic plants) from Bacillus amyloliquefaciens strain IT-45 as the exposure from the representative use by drip irrigation is considered negligible |
| Toxins/secondary metabolites such as surfactins | A data gap and an assessment not finalised for aquatic organisms (other than freshwater invertebrates and algae) was identified for the representative use |
| Toxins/secondary metabolites such as bacillomycins, iturins and fengycins | A data gap and an assessment not finalised was identified for the representative use |
7. Particular conditions proposed to be taken into account by risk managers

Risk mitigation measures (RMMs) identified following consideration of Member State (MS) and/or applicant's proposal(s) during the peer review, if any, are presented in this section. These measures applicable for human health and/or the environment leading to a reduction of exposure levels of operators, workers, bystanders/residents, environmental compartments and/or non-target organisms for the representative uses are listed below. The list may also cover any RMMs as appropriate, leading to an acceptable level of risks for the respective non-target organisms.

It is noted that final decisions on the need of RMMs to ensure the safe use of the plant protection product containing the concerned active substance will be taken by risk managers during the decision-making phase. Consideration of the validity and appropriateness of the RMMs remains the responsibility of MSs at product authorisation, taking into account their specific agricultural, plant health and environmental conditions at national level.

As the wettability increased significantly after storage, it should be mentioned on the label that mixing during the application is needed.

7.1. Particular conditions proposed for the representative uses evaluated (Table 5)

Table 5: Risk mitigation measures proposed for the representative uses assessed

| Representative use | Citrus (field use) |
|--------------------|--------------------|
| Drip irrigation    | Use of PPE/RPE might be considered to reduce non-dietary exposure (dermal and inhalation). |
| Operator risk      | Use of PPE/RPE might be considered to reduce non-dietary exposure (dermal and inhalation). |

8. Concerns and related data gaps

8.1. Concerns and related data gaps for the representative uses evaluated

8.1.1. 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 one or more of the representative uses in line with the uniform principles in accordance with Article 29(6) of Regulation (EC) No 1107/2009 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 Regulation (EC) No 1107/2009.

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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.
The following issues or assessments that could not be finalised have been identified, together with the reasons including the associated data gaps where relevant, which are reported directly under the specific issue to which they are related:

1) The consumer dietary risk assessment could not be concluded (see Section 3).
   a) Pending on further investigations on the production of toxins/secondary metabolites and levels present in the soil after application, further considerations will have to be given to their residue behaviour in relation to the representative use and if relevant for the consumer the potential toxicity of these compounds should also be addressed to conclude on the risk assessment for consumers (see Sections 3).

2) Satisfactory information was not available on the persistence and multiplication of the strain in soil and the information was not sufficient to demonstrate that *Bacillus amyloliquefaciens* strain IT-45 will not occur in concentrations considerably higher than the natural background levels from repeated applications over the years leading to an assessment not finalised (see Section 4).
   a) Adequate information 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, provided that repeated applications be made over the years, was not available (relevant for all representative uses, see Section 4)

3) Satisfactory information was not available for the potential infectivity and pathogenicity to non-target terrestrial organisms (birds, non-target soil arthropods and soil macro-organisms) from *Bacillus amyloliquefaciens* strain IT-45 for the assessment of the representative use leading to an assessment not finalised (see Section 5).
   a) Data and information for the assessment of the potential infectivity and pathogenicity to non-target terrestrial organisms (birds, non-target soil arthropods and soil macro-organisms) from *Bacillus amyloliquefaciens* strain IT-45 (relevant for the representative use, see Section 5).

4) Satisfactory information was not available on the production of toxins/secondary metabolites such as bacillomycins, iturins, surfactins and fengycins and levels present in the environment after application and their potential toxicity in order to conclude on the risk assessment for non-target terrestrial organisms (birds, wild mammals, non-target soil arthropods, earthworms, other soil macro- and microorganisms) and aquatic non-target organisms (other than freshwater invertebrates and algae for surfactin) leading to an assessment not finalised (relevant for the representative use; see Sections 4 and 5).
   a) Pending on further investigations on the production of toxins/secondary metabolites such as bacillomycins, iturins, surfactins and fengycins 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 terrestrial non-target organisms (birds, wild mammals, non-target soil arthropods, earthworms, other soil macro- and microorganisms) and aquatic non-target organisms (other than freshwater invertebrates and algae for surfactin) (relevant for the representative use; see Sections 4 and 5).

8.1.2. 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 Regulation (EC) No 1107/2009 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.

8 Satisfactory PEC calculations for the metabolites (iturin A and surfactin) present above 1 g/kg in the formulated product.
9 Low risk was concluded for freshwater invertebrates and algae for the representative use. For honeybees and non-target foliar arthropods, exposure from toxins/secondary metabolites is assessed to be low and low risk is concluded for the representative use.
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 Regulation (EC) No 1107/2009.

**The following critical areas of concern are identified, together with any associated data gaps, where relevant, which are reported directly under the specific critical area of concern to which they are related:**

No critical areas of concern have been identified.

### 8.1.3. Overview of the concerns identified for each representative use considered (Table 6)

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in Section 7, has been evaluated as being effective, then ‘risk identified’ is not indicated in Table 6).

**Table 6:** Overview of concerns reflecting the issues not finalised, critical areas of concerns and the risks identified that may be applicable for some but not for all uses or risk assessment scenarios

| Representative use                             | Citrus (field use)                                      | Drip irrigation |
|-----------------------------------------------|--------------------------------------------------------|-----------------|
| 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)                    | Assessment not finalised |
|                                              | Parametric value of 10 l g/L (b) breached              |                  |

The superscript numbers relate to the numbered points indicated in Sections 8.1.1 and 8.1.2. Where there is no superscript number, see Sections 2–7 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).

### 9. List of other outstanding issues

Remaining data gaps not leading to critical areas of concern or issues not finalised but considered necessary to comply with the data requirements, and which are relevant for some or all of the representative uses assessed at EU level. Although not critical, these data gaps may lead to uncertainties in the assessment and are considered relevant.
These data gaps refer only to the representative uses assessed and are listed in the order of the sections:

- A search of the scientific peer-reviewed open literature on the active substance for the biological properties, to be conducted and reported in accordance with EFSA guidance on the submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA, 2011) has not been provided. (Relevant for the representative use evaluated).
- The potential infectivity of *Bacillus amyloliquefaciens* strain IT-45 was not sufficiently addressed by a robust argumentation and weight of evidence assessment in the absence of an acute intraperitoneal study (relevant for the representative use evaluated; see Section 2).

References

EFSA (European Food Safety Authority), 2011. Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092, 49 pp. https://doi.org/10.2903/j.efsa.2011.2092

EFSA (European Food Safety Authority), 2021. Peer review report to the conclusion regarding the peer review of the pesticide risk assessment of the active substance *Bacillus amyloliquefaciens* strain IT-45. Available online: www.efsa.europa.eu

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2020. Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017 –2019). EFSA Journal 2020;18(2):5966, 56 pp. https://doi.org/10.2903/j.efsa.2020.5966

EFSA FEEDAP Panel (EFSA Panel on Additives or Substances used in Animal Feed), 2012. Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA Journal 2012;10(6):2740, 10 pp. https://doi.org/10.2903/j.efsa.2012.2740. Available online: www.efsa.europa.eu/efsajournal

European Commission, 2000. Technical material and preparations: guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (Part A, Section 4) and Annex III (Part A, Section 5) of Directive 91/414. SANCO/3030/99-rev. 4, 11 July 2000.

European Commission, 2003. Guidance Document on Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC. SANCO/221/2000-rev. 10 final, 25 February 2003.

European Commission, 2012. Working Document on Microbial Contaminant Limits for Microbial Pest Control Products. SANCO/12116/2012 -rev. 0, September 2012.

European Commission, 2013. Guidance document on data requirements on efficacy for the dossier to be submitted for the approval of new active substances contained in plant protection products. SANCO/10054/2013 -rev. 3 July 2013.

France, 2019. Draft Assessment Report (DAR) on the active substance *Bacillus amyloliquefaciens* strain IT-45 prepared by the rapporteur Member State France, in the framework of Regulation (EC) No 1107/2009 of the European Parliament and the Council, May 2019. Available online: www.efsa.europa.eu

France, 2021. Revised Renewal Assessment Report (RAR) on *Bacillus amyloliquefaciens* strain IT-45 prepared by the rapporteur Member State France in the framework of Regulation (EC) No 1107/2009 of the European Parliament and the Council, January 2021. Available online: www.efsa.europa.eu

Abbreviations

| Symbol | Term |
|--------|------|
| λ      | wavelength |
| ε      | decadic molar extinction coefficient |
| µg     | microgram |
| µm     | micrometer (micron) |
| ADE    | actual dermal exposure |
| AF     | assessment factor |
| AP     | alkaline phosphatase |
| AST    | aspartate aminotransferase (SGOT) |
| AV     | avoidance factor |
| BLAST  | Basic Local Alignment Search Tool |
| BUN    | blood urea nitrogen |
| bw     | body weight |
| CAS    | Chemical Abstracts Service |
| CFU    | colony forming units |
| CHO    | Chinese hamster ovary cells |
| Abbreviation | Full Form |
|--------------|-----------|
| PPE          | personal protective equipment |
| PT           | proportion of diet obtained in the treated area |
| PTT          | partial thromboplastin time |
| RAR          | Renewal Assessment Report |
| RBC          | red blood cells |
| REACH        | Registration, Evaluation, Authorisation of Chemicals Regulation |
| RPE          | respiratory protective equipment |
| SAR          | systemic acquired resistance |
| SCAR         | sequence characterised amplified region |
| SMILES       | simplified molecular-input line-entry system |
| TK           | technical concentrate |
| TWA          | time-weighted average |
| UV           | ultraviolet |
| W/S          | water/sediment |
| w/v          | weight per unit volume |
| w/w          | weight per unit weight |
| WBC          | white blood cell |
| WHO          | World Health Organization |
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.2021.6594
### Appendix B – Used compound codes

| Code/trivial name<sup>(a)</sup> | Chemical name/SMILES notation/InChiKey<sup>(b)</sup> | Structural formula<sup>(c)</sup> |
|---------------------------------|-----------------------------------------------|---------------------------------|
| **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-methyldeca-1,4,7,11,14,17,20,23-octaoxohexacosahydro-1H-pyrrolo[2,1-β]octaazacyclopentacosin-22-yl]propanamide | ![Structural formula](image1) |
|                                | | ![Structural formula](image2) |
|                                | RDUGMXONDQDIRN-QZBZMMCASA-N | |
| **surfactin C**                 | 3-[(3S,6R,9S,12S,15R,18S,21S)-9-(carboxymethyl)-3,6,15,18-tetraisobutyl-12-isopropyl-25-(10-methylundecyl)-2,5,8,11,14,17,20,23-octaoxo-1-oxa-4,7,10,13,16,19,22-heptaazacyclopentacosan-21-yl]propanoic acid | ![Structural formula](image3) |
|                                | | ![Structural formula](image4) |
|                                | NJGWOFRZMQRKHT-VKBYPPDESA-N | |
| **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-β]octaazacyclopentacosin-22-yl]propanamide | ![Structural formula](image5) |
|                                | | ![Structural formula](image6) |
|                                | CMYBONFRMPHHAP-IFGWIXSHSA-N | |

<sup>(a)</sup> The metabolite name in bold is the name used in the conclusion.

<sup>(b)</sup> ACD/Name 2019.1.1 ACD/Labs 2019 Release (File version N05E41, Build 110555, 18 July 2019).

<sup>(c)</sup> ACD/ChemSketch 2019.1.1 ACD/Labs 2019 Release (File version C05H41, Build 110712, 24 July 2019).