Peer review of the pesticide risk assessment of the active substance Bacillus thuringiensis ssp. aizawai strain GC-91

European Food Safety Authority (EFSA), Maria Anastassiadou, Maria Arena, Domenica Auteri, Alba Brancato, Laszlo Bura, Luis Carrasco Cabrera, Eugenia Chaideftou, Arianna Chiusolo, Federica Crivellente, Chloe De Lentdecker, Mark Egsmose, Gabriella Fait, Luna Greco, Alessio Ippolito, Frederique Istace, Samira Jarrah, Dimitra Kardassi, Renata Leuschner, Alfonso Lostia, Christopher Lythgo, Oriol Magrans, Iris Mangas, Ileana Miron, Tunde Molnar, Laura Padovani, Juan Manuel Parra Morte, Ragnor Pedersen, Hermine Reich, Miguel Santos, Rachel Sharp, Csaba Szentes, Andrea Terron, Manuela Tiramani, Benedicte Vagenende and Laura Villamar-Bouza

Abstract

The conclusions of EFSA following the peer review of the initial risk assessments carried out by the competent authorities of the rapporteur Member State The Netherlands and co-rapporteur Member State Germany for the pesticide active substance Bacillus thuringiensis ssp. aizawai strain GC-91 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 Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659. The conclusions were reached on the basis of the evaluation of the representative uses of Bacillus thuringiensis ssp. aizawai strain GC-91 as an insecticide on pome fruits (apple, pear), grapes and sports turf (field uses) and tomato (greenhouse uses). The reliable end points, appropriate for use in regulatory risk assessment, are presented. Missing information identified as being required by the regulatory framework is listed. Concerns are identified.

© 2020 European Food Safety Authority. EFSA Journal published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

Keywords: Bacillus thuringiensis ssp. aizawai strain GC-91, peer review, risk assessment, pesticide, insecticide

Requestor: European Commission

Question number: EFSA-Q-2016-00698

Correspondence: pesticides.peerreview@efsa.europa.eu
Acknowledgments: EFSA wishes to thank the rapporteur Member State The Netherlands for the preparatory work on this scientific output.

Suggested citation: EFSA (European Food Safety Authority), Anastassiadou M, Arena M, Auteri D, Brancato A, Bura L, Carrasco Cabrera L, Chaideftou E, Chiusolo A, Crivellente F, De Lentdecker C, Egsmose M, Fait G, Greco L, Ippolito A, Istace F, Jarrah S, Kardassi D, Leuschner R, Lostia A, Lythgo C, Magrans O, Mangas I, Miron I, Molnar T, Padovani L, Parra Morte JM, Pedersen R, Reich H, Santos M, Sharp R, Szentes C, Terron A, Tiramani M, Vagenende B and Villamar-Bouza L, 2020. Conclusion on the peer review of the pesticide risk assessment of the active substance Bacillus thuringiensis ssp. aizawai strain GC-91. EFSA Journal 2020;18(11):6293, 18 pp. https://doi.org/10.2903/j.efsa.2020.6293

ISSN: 1831-4732

© 2020 European Food Safety Authority. EFSA Journal published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the Creative Commons Attribution-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.
Summary

Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659, lays down the procedure for the renewal of the approval of active substances submitted under Article 14 of Regulation (EC) No 1107/2009. The list of those substances is established in Commission Implementing Regulation (EU) No 686/2012. *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 is one of the active substances listed in Regulation (EU) No 686/2012.

In accordance with Article 1 of Regulation (EU) No 844/2012, the rapporteur Member State (RMS), The Netherlands, and co-rapporteur Member State (co-RMS), Germany, received an application from Certis USA LLC for the renewal of approval of the active substance *Bacillus thuringiensis* ssp. *aizawai* strain GC-91.

An initial evaluation of the dossier on *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 was provided by the RMS in the renewal assessment report (RAR) and subsequently, a peer review of the pesticide risk assessment on the RMS evaluation was conducted by the European Food Safety Authority (EFSA) in accordance with Article 13 of Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659. The following conclusions are derived.

The uses of *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 according to the representative uses as an insecticide on pome fruits against *Cydia pomonella*, on grapes against *Lobesia botrana* and *Eupoecilia ambiguella*, on turf against *Spodoptera* spp. and on protected tomato (greenhouse, walk-in tunnels) against *Tuta absoluta*, as proposed at the European Union (EU) level, result in a sufficient insecticidal efficacy against the target insect pests.

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

In the area of mammalian toxicology, potential adverse effects after repeated exposure by inhalation to *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 and genotoxic potential of the insecticidal proteins by non-dietary exposure could not be excluded. On this basis, the risk assessment for residents and bystanders cannot be concluded (issue not finalised).

In the area of residues a data gap was identified related to the proposed threshold of $1 \times 10^5$ colony forming units (CFU)/g for viable residues on edible plant commodities at the time of harvest for the representative uses on pome fruits grapes and tomatoes, where quantification of viable counts of *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 linked to specific preharvest intervals (PHIs) is requested to finalise the consumer dietary risk assessment.

*Bacillus thuringiensis* ssp. *aizawai* strain GC-91 is not proposed to be included into Annex IV of Regulation (EC) No 396/2005 because limited available residue data do not allow to conclude on a general basis that residues will always be below the threshold of $10^5$ CFU/g at harvest.

In the area of environmental fate and behaviour, the available information was considered sufficient to complete the necessary environmental exposure assessments.

Satisfactory information was not provided leading to issues not being finalised for the potential for toxicity, infectivity and pathogenicity to bees and non-target arthropods for representative field and walk-in tunnel uses; the potential for infectivity and pathogenicity to earthworms for all representative uses and for a hazard characterisation and an assessment of the risk to non-target organisms from toxins/secondary metabolites such as crystal proteins.

---

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

Abstract ................................................................................................................................................... 1
The active substance and the formulated product .................................................................................. 3
Conclusions of the evaluation .................................................................................................................. 6
1. Identity of the microorganism/biological properties/physical and technical properties and methods of analysis ............................................................................................................ 6
2. Mammalian toxicity ............................................................................................................................ 7
3. Residues ........................................................................................................................................... 9
4. Environmental fate and behaviour ...................................................................................................... 9
4.1. Fate and behaviour in the environment of the microorganism ............................................................... 10
4.2. Fate and behaviour in the environment of any relevant metabolite formed by the microorganism under relevant environmental conditions ....................................................................................................... 11
5. Ecotoxicology .................................................................................................................................... 11
6. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments (Tables 1-4) ................................................................................................. 13
7. Data gaps ......................................................................................................................................... 14
8. Particular conditions proposed to be taken into account to manage the risk(s) identified ....................... 14
9. Concerns ............................................................................................................................................. 14
9.1. Issues that could not be finalised ..................................................................................................... 14
9.2. Critical areas of concern .................................................................................................................. 15
9.3. Overview of the concerns identified for each representative use considered .................................. 15
References ............................................................................................................................................... 16
Abbreviations ........................................................................................................................................... 17
Appendix A – List of end points for the active substance and the representative formulation ................. 18
Background

Commission Implementing Regulation (EU) No 844/2012\(^2\), as amended by Commission Implementing Regulation (EU) No 2018/1659\(^3\), (hereinafter referred to as 'the Regulation'), lays down the provisions for the procedure of the renewal of the approval of active substances, submitted under Article 14 of Regulation (EC) No 1107/2009\(^4\). This regulates for the European Food Safety Authority (EFSA) the procedure for organising the consultation of Member States, the applicant(s) and the public on the initial evaluation provided by the rapporteur Member State (RMS) and/or co-rapporteur Member State (co-RMS) in the renewal assessment report (RAR), and the organisation of an expert consultation where appropriate.

In accordance with Article 13 of the Regulation, unless formally informed by the European Commission that a conclusion is not necessary, EFSA is required to adopt a conclusion on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 within 5 months from the end of the period provided for the submission of written comments, subject to an extension of up to 3 months where additional information is required to be submitted by the applicant(s) in accordance with Article 13(3).

In accordance with Article 1 of the Regulation, the RMS The Netherlands and co-RMS Germany received an application from Certis USA LLC for the renewal of approval of the active substance Bacillus thuringiensis ssp. aizawai strain GC-91. Complying with Article 8 of the Regulation, the RMS checked the completeness of the dossier and informed the applicant, the co-RMS (Germany), the European Commission and EFSA about the admissibility.

The RMS provided its initial evaluation of the dossier on Bacillus thuringiensis ssp. aizawai strain GC-91 in the RAR, which was received by EFSA on 31 July 2018 (The Netherlands, 2018).

In accordance with Article 12 of the Regulation, EFSA distributed the RAR to the Member States and the applicant, Certis USA LLC, for consultation and comments on 2 April 2019. EFSA also provided comments. In addition, EFSA conducted a public consultation on the RAR. EFSA collated and forwarded all comments received to the European Commission on 2 June 2019. At the same time, the collated comments were 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 13(3) of the Regulation were considered in a telephone conference between EFSA and the RMS on 27 August 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 applicant, and that EFSA should conduct an expert consultation in the areas of effects on human health of the microorganism and of the plant protection product and environmental fate and behaviour.

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

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

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 August-September 2020.

\(^2\) Commission Implementing Regulation (EU) No 844/2012 of 18 September 2012 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in 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 252, 19.9.2012, p. 26-32.

\(^3\) Commission Implementing Regulation (EU) No 2018/1659 of 7 November 2018 amending Implementing Regulation (EU) No 844/2012 in view of the scientific criteria for the determination of endocrine disrupting properties introduced by Regulation (EU) 2018/605.

\(^4\) 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.
This conclusion report summarises the outcome of the peer review of the risk assessment of the active substance and the representative formulation, evaluated on the basis of the representative uses of Bacillus thuringiensis ssp. aizawai strain GC-91 as an insecticide on pome fruits (apple, pear), grapes, sports turf and tomato, as proposed by the applicant. In accordance with Article 12(2) of Regulation (EC) No 1107/2009, risk mitigation options identified in the RAR and considered during the peer review are presented in the conclusion. 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 RAR;
- the reporting table (28 August 2019);
- the evaluation table (29 September 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 RAR, including its revisions (The Netherlands, 2020), 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 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 thuringiensis ssp. aizawai strain GC-91 is a bacterium deposited at the National Collection of Type Cultures (NCTC), at the Health Protection Agency, Centre for Emergency Preparedness and Response, Porton Down, Salisbury, UK under the reference number NCTC 11821. Bacillus thuringiensis ssp. aizawai strain GC-91 is a transconjugant strain, not genetically modified.

The representative formulated product for the evaluation was ‘Agree 50 WG’, a water-dispersible granule (WG) containing 500 g/kg of Bacillus thuringiensis ssp. aizawai strain GC-91 (min. 3.1 × 10^{13} colony forming units (CFU)/kg, max. 4 × 10^{13} CFU/kg) with a biopotency of min. 25,000 IU/mg.

The representative uses evaluated were field spray applications on pome fruits against Cydia pomonella, on grapes against Lobesia botrana and Eupoecilia ambiguella, on turf against Spodoptera spp. and on protected tomato (greenhouse, walk-in tunnels) against Tuta absoluta in the EU. Full details of the Good Agricultural Practices (GAPs) can be found in the list of end points in Appendix A.

Data were submitted to conclude that the use of Bacillus thuringiensis ssp. aizawai strain GC-91 according to the representative uses proposed at EU level results in a sufficient insecticidal efficacy against the target organisms, following the guidance document SANCO/2012/11251-rev. 4 (European Commission, 2014b).

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: SANCO/12116/2012-rev. 0 (European Commission, 2012) and Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance (EFSA FEEDAP Panel, 2018).

Based on the scientific progress on the taxonomy of the Bacillus cereus group in general and that of Bacillus thuringiensis in particular, it was proposed that Bacillus cereus sensu stricto and Bacillus thuringiensis are in fact one and the same species which differ only in some phenotypic traits. Nevertheless, a proposal has been put forward which maintains the current species assignment. Accordingly, both the phenotypic assignment ‘Bacillus thuringiensis serovar aizawai’ and the
phylogenetic assignment ‘Bacillus cereus sensu stricto serovar aizawai biovar Thuringiensis’ would be valid for strain GC-91. For taxonomic conservation, ‘ssp.’ is used throughout this conclusion.

GC-91 is not a mutant but a transconjugant strain, incorporating genes from two different Bacillus thuringiensis strains. This strain has flagella serotype aizawai and δ-endotoxin genes mixture kurstaki-aizawai. Morphological and biochemical characterisation, serotyping, plasmid profiling, activity spectrum, fatty acid analysis, DNA fingerprinting (amplified fragment length polymorphism; AFLP), crystal insecticidal (Cry) proteins toxin analysis, strain specific markers can be used to clearly identify strain GC-91.

The technical grade microbial pest control agent (MPCA) used for manufacturing of the formulated product (microbial pest control product; MPCR) contains minimum $6.2 \times 10^{13}$ CFU/kg and maximum $7.9 \times 10^{13}$ CFU/kg Bacillus thuringiensis ssp. aizawai strain GC-91, with a minimum biopotency of 50,000 IU/mg. The δ-endotoxin content of the MPCA was 80 g/kg; however, a data gap was identified for recent 5-batch data which includes the δ-endotoxin content. The δ-endotoxin is stated to be constituted by the Cry1Ac, Cry1C, Cry1D and Cry2A proteins, which are those reported to be expressed by the strain.

Whole genome sequence (chromosomal and plasmids) was screened for the genes coding for exotoxins and enterotoxins. Bacillus thuringiensis ssp. aizawai strain GC-91 lacks the majority of genes required for exotoxin production and lacks haemolysin II (HlyII) encoding genes. Bacillus thuringiensis ssp. aizawai strain GC-91 contains genes for one cytotoxin K (type Cyt K2), three genes related to non-haemolytic enterotoxins and eight genes related to haemolytic enterotoxins, of which three are located on plasmid 2. Genes for cereulide biosynthesis are missing in the Bacillus thuringiensis ssp. aizawai strain GC-91 genome. The genetic determinants for the typical Bacillus subtilis/amyloliquefaciens metabolites in the genome of Bacillus thuringiensis ssp. aizawai strain GC-91 showed that none of the substances iturin A, bacillomycin D, mycosubtilisin, surfactin, fengycin, entomycin 110, zwittemycin can be produced by the strain, except for zwittemycin A.

The content of microbial contaminants of the MPCR was below the limits defined in the SANCO/12116/2012 working document (European commission, 2012). Bacillus thuringiensis spores can remain viable for years in soil, but applied as a spray, the δ-endotoxins are rapidly degradable and endospores are rapidly inactivated when exposed to UV radiation.

As a member of the Bacillus cereus group, Bacillus thuringiensis ssp. aizawai is closely related to Bacillus anthracis and Bacillus cereus. Bacillus thuringiensis strains are however distinguishable from Bacillus cereus and Bacillus anthracis. Bacillus cytotoxicus is known to produce the highly cytotoxic variant of CytK, the CytK-1, which is not produced by any of the other members of this group.

Bacillus thuringiensis ssp. aizawai strain GC-91 was sensitive to chloramphenicol, tetracycline, streptomycin, clindamycin, erythromycin, kanamycin, gentamycin and vancomycin but not sensitive to penicillin, ampicillin. Resistance to β-lactam antibiotics is intrinsic.

Strains of thuringiensis are capable of plasmid and gene transfer. However, during manufacture, the Bacillus thuringiensis ssp. aizawai strain GC-91 was proven to be stable by regular quality control checks.

The main data regarding the identity of Bacillus thuringiensis ssp. aizawai strain GC-91 and its biological properties are given in Appendix A.

Acceptable methods for CFU counts of Bacillus thuringiensis ssp. aizawai strain GC-91 in the formulation for the determination of the microorganism in the MPCR and for the determination of the content of contaminating microorganisms are available.

Methods for the determination and quantification of residues are currently not required as no residue definition applies to the microorganism and maximum residue level (MRL) have not been set for any of the intended uses. However, it is noted that a validated enumeration method in high water commodities (lettuce) is available with a limit of quantification (LOQ) of $1.3 \times 10^3$ CFU/g and unambiguous identification of colonies of Bacillus thuringiensis ssp. aizawai strain GC-91 can be achieved using DNA fingerprinting AFLP markers. The methods can be used for monitoring of the strain upon field application.

Methods of analysis for viable residues in the environment are not required.

2. Mammalian toxicity

Bacillus thuringiensis ssp. aizawai strain GC-91 was discussed at the Pesticides Peer Review Meeting Teleconference 25 in March 2020.
General data

From the medical data, no adverse reactions or sensitisation reactions due to Bacillus thuringiensis ssp. aizawai strain GC-91 exposure were reported during production, formulation, handling of microbial products, filling and packaging. The results of allergenicity observations indicate that increased IgE antibodies levels can occur in greenhouses workers exposed to products containing Bacillus thuringiensis ssp. kurstaki and aizawai but no effect on the occurrence of respiratory symptoms or lung function was observed. Bacillus thuringiensis is not recommended for the Qualified Presumption of Safety list (EFSA BIOHAZ Panel, 2020).

Toxicity/infectivity/pathogenicity studies

As the available methods for testing dermal sensitisation are not suitable for testing microorganisms and there are no validate test methods for sensitisation by inhalation, the following warning phrase is proposed: ‘Contains Bacillus thuringiensis ssp. aizawai strain GC-91. Micro-organisms may have the potential to provoke sensitising reactions’.

On the basis of the acute studies submitted for Bacillus thuringiensis ssp. aizawai strain GC-91, there is no evidence that the microorganism may cause acute oral, intraperitoneal or intravenous toxicity, pathogenicity or infectivity in mammals. Regarding the acute inhalation, the incomplete lung clearance in one study did not allow to exclude a potential for infectivity. Bacillus thuringiensis ssp. aizawai strain GC-91 was not skin or eye irritant. Studies on acute oral, inhalation, dermal toxicity were also submitted for the formulation ‘Agree 50 WP’.

No genotoxicity studies were reported given that no validated methods are currently available for microorganisms.

With regard to repeated dose toxicity by oral exposure, no adverse effects were observed in a 90-day rat study with Bacillus thuringiensis ssp. aizawai strain GC-91. As regards short-term toxicity by inhalation, in a literature study a lung interstitial inflammation was observed in mice after repeated exposure by inhalation (2 × 5 days) to products containing Bacillus thuringiensis israelensis and Bacillus thuringiensis kurstaki, and was still present 70 days after the exposure.3 The adversity and possible treatment relationship of the finding of the interstitial lung inflammation were discussed during the experts’ meeting considered also applicable to Bacillus thuringiensis ssp. aizawai strains. The potential concern for adverse health effects after repeated exposure by inhalation could not be excluded for the intended field uses on the basis of the available data (data gap).5 This was agreed by the majority of experts.

Secondary metabolites/toxins

Bacillus thuringiensis ssp. aizawai strain GC-91 can produce certain enterotoxin and δ-endotoxin (Cry1Ac, Cry1C, Cry1D and Cry2A insecticidal proteins) (see Section 1). However, humans and other mammals will not be directly exposed to enterotoxins as they are inactivated at low pH in the stomach.

Based on the available evidence, the peer review concluded that only the spores are able to survive the stomach passage and to germinate and produce enterotoxins in the intestinal tract (potentially leading to diarrhoeal-associated food-borne disease in humans). RMS and co-RMS did not agree.6

In a mouse micronucleus study with intraperitoneal administration, positive results were observed with the spore-crystal complex containing Cry1Aa, Cry1Ab, Cry1Ac and Cry2Aa. These results were considered equivocal especially regarding whether the Cry proteins had been solubilised/activated prior to administration or not (data gap). It was concluded that genotoxicity of the insecticidal proteins is not a concern for dietary exposure but a possible concern for non-dietary exposure could not be excluded.7 This was agreed by the majority of experts.

Reference values and exposure estimates

For the representative uses on turf sports, pome fruits, grapes, and solanaceous fruits by professional and non-professional users, the threshold of 10⁵ CFU/g food as determined by the BIOHAZ Panel Opinion (EFSA BIOHAZ Panel, 2016) was concluded as applicable to all Bacillus thuringiensis to cover the risk of food-borne poisonings caused by the Bacillus cereus group of microorganisms (EFSA BIOHAZ Panel, 2016), has to be taken into account. RMS and co-RMS did not agree.6

5 Refer to experts’ consultation 6.3 in the Report of Pesticides Peer Review Meeting Teleconference 25 (March 2020) of Bacillus thuringiensis ssp. aizawai ABTS-1857.
6 Refer to experts’ consultation 6.1 in the Report of Pesticides Peer Review Meeting Teleconference 25 (March 2020).
7 Refer to experts’ consultation 6.2 in the Report of Pesticides Peer Review Meeting Teleconference 25 (March 2020) of Bacillus thuringiensis ssp. aizawai ABTS-1857.
With regard to non-dietary exposure, toxicity/infectivity after repeated exposure to *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 by inhalation could not be concluded, and a genotoxic potential of the Cry proteins could not be excluded. Therefore, the risk assessment by inhalation for residents and bystanders for field uses cannot be concluded for the microorganism and the Cry proteins (issue not finalised except for permanent greenhouses). In the absence of a quantitative risk assessment, the use of respiratory protective equipment for the operators and workers might be considered to reduce the exposure via inhalation (for field and greenhouse uses).

### 3. Residues

*Bacillus thuringiensis* strains were discussed at the Pesticides Peer Review Meeting Teleconference 25 in March 2020. Considering the available evidence and uncertainties, the threshold of $10^5$ CFU/g plant commodity at the time of harvest as determined by the BIOHAZ Panel Opinion (EFSA BIOHAZ Panel, 2016) is considered applicable to all *Bacillus thuringiensis* strains to cover the risk of food-borne poisonings (caused by the *Bacillus cereus* group of microorganisms; see Section 2). Non-viable residues are not of concern for the dietary consumption (see Section 2). The RMS wished to inform that work was ongoing on the development of a new diagnostic tool to discriminate *Bacillus thuringiensis* biocontrol strains from *Bacillus cereus sensu lato* species and strains. The expectation is that a tool would become available to distinguish between *Bacillus cereus sensu lato* strains and commercial *Bacillus thuringiensis* strains and to ensure correct conclusions and decisions can be taken with regard to the origin of food borne outbreaks.

Therefore, only information on viable residues, i.e. CFU per g or kg of *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 on plant commodities at harvest is needed to demonstrate that the threshold of $10^5$ CFU/g edible plant commodity is not exceeded.

To ensure this, the setting of a preharvest interval (PHI) may be needed. For greenhouse tomatoes, considering a slightly higher application rate of the representative use on tomatoes, mean viable counts of around $6.5 \times 10^4$ CFU/g can be estimated.

Residue data provided for greenhouse lettuce indicate CFU following treatment of up to $5.4 \times 10^4$ CFU/g. However, when proportionality was applied from the dose rate applied to lettuce compared to the applied dose rates on pome fruits and grapes of the representative uses which is around two times higher, the threshold of $10^5$ CFU/g maybe exceeded. Lettuce samples were stored frozen at $-18^\circ C$ prior to analysis. The storage time is not indicated. Therefore, the residue trials on lettuce are considered as supporting information only.

A calculated estimate of viable spore counts on grapes following treatment with a slightly lower application rate than the representative use was performed and counts between 3.7 and $7.4 \times 10^6$ CFU/g were estimated.

Viable counts of commercial *Bacillus thuringiensis* strains were demonstrated in the scientific literature and by supporting experimental evidence to decline following application and not to persist or multiply on edible plant commodities (fruiting vegetables and leafy crops). Furthermore, in the literature a body of evidence supports inactivation and decline of viable spores by environmental factors such as solar radiation, rainfall, plant growth and temperature. Based on the available data, a half-life of viable spores of up to 1 day or 24 h can reasonably be assumed.

Since measured residue levels are below however close to the threshold of $1 \times 10^5$ CFU/g for tomatoes, indicatively above the threshold for lettuce and based on a calculated estimate on grape wine leaves (maximally around $10^7$ CFU/g when considering a ca. 1.4 times higher application rate of the representative use), a data gap on viable counts of *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 linked to specific PHIs for the representative uses on pome fruits, grapes and tomatoes considering uncertainties related to variability of viable counts (see also data gap in Section 1) needs to be addressed before a consumer risk assessment can be finalised (issue that could not be finalised).

*Bacillus thuringiensis* ssp. *aizawai* strain GC-91 is not proposed to be included into Annex IV of Regulation (EC) No 396/2005 because the few available residue data do not allow to conclude on a general basis that residues are always below the threshold of $10^5$ CFU/g at harvest.

### 4. Environmental fate and behaviour

*Bacillus thuringiensis* ssp. *aizawai* strain GC-91 was discussed at the Pesticides Peer Review Meeting Teleconference 25 in March 2020.
Satisfactory information was provided in relation to potential interference of *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC8 (see specific Annex VI decision making criteria in Part II Commission Regulation (EU) No 546/20119). It was concluded that *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 is unlikely to interfere with the methodologies routinely used for such determinations.

*Bacillus thuringiensis* ssp. *aizawai* strain GC-91 is a transconjugant of two ‘wild-type’ strains and there are no marker genes in the strain which would permit analysis of a frequency of genetic exchange. Though it is acknowledged that the possibility and effects of transfer of genetic material is not different for *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 than for other naturally occurring *Bacillus thuringiensis* strains, transfer of genetic material by *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 after application is possible (the strain has plasmids), so could not be excluded based on the information in the dossier. Information in the dossier confirms that plasmid exchange between vegetative cells of different strains of the species can be measured when applications were made to leaf surfaces. Note the applied material in the product is spores and not vegetative cells.

Specific environmental exposure estimates for greenhouse uses were not provided. The applicant chose to address the representative use on protected tomato by stating that greenhouse uses are covered by the exposure assessments provided for the field uses.

### 4.1. Fate and behaviour in the environment of the microorganism

Information was derived from published literature on different strains of *Bacillus thuringiensis* in relation to its persistence and multiplication in soil. Information specific to strain GC-91 was not available. Information on ssp. *kurstaki* demonstrated that spores remain viable for many years (more than 7). The species has been reported to have spores that can germinate in the rhizosphere of some plants. Based on a weight of evidence, it appears that germination of spores does not occur in the bulk soil where nutrient levels are generally more limited than in the rhizosphere. Overall, it is considered that repeated use over the years would result in the accumulation of ssp. *aizawai* strain GC-91 spores in the soil environment. The RMS disagreed. The spores of the strain are expected to persist and be present above natural background levels in soil, taking into account repeated applications over the years, but multiplication in bulk soil will not occur. Consequently, EFSA concluded that the information is sufficient to address the uniform principles criterion associated with persistence and accumulation in the environment regarding soil. Predicted environmental concentration (PEC) in soil covering the intended uses have been calculated (see Appendix A).

With respect to the persistence and multiplication in surface water information specific to strain GC-91 was not available. Information on ssp. *kurstaki* demonstrated that in a flowing water catchment levels of CFU declined after applications were made, but the authors attributed this to the dilution and removal effect of the flowing water. The available literature indicates the species *Bacillus thuringiensis* is present in surface water and that it is likely that the species is capable of growing in freshwater environments under nutrient/oxygen-rich conditions. Overall, it is concluded that the information available on the persistence/multiplication/germination of the strain in natural surface water was insufficient to demonstrate that *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 is likely to decline in surface water. Consequently, EFSA concluded that the information is insufficient to address the uniform principles criterion of the strain not being expected to persist in surface water environment in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years. This conclusion identifies a data gap in this respect. The RMS disagreed. PEC surface water for the intended use on pome fruit (use pattern with greatest potential for spray drift exposure) have been calculated (see Appendix A).

Information was provided on the occurrence and behaviour of *Bacillus thuringiensis* ssp. *kurstaki* spores in air. Re-aerolisation of applied spores occurred but spore transport distances were limited being up to 30 m. Spores rapidly lost viability following release to air. It was considered that this information might be read across to spores of *Bacillus thuringiensis* ssp. *aizawai* strain GC-91.

---

8 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.

9 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.
4.2. 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 subspecies Bacillus thuringiensis aizawai is able to produce secondary metabolites, which are crystal proteins, e.g. contain the δ-endotoxins, Cry1Ac, Cry1C, Cry1D and Cry2A. These crystal proteins constitute components in the formulated product within and outside spores and are responsible for the insecticidal mode of action of Bacillus thuringiensis ssp. aizawai strain GC-91. Genes encoding zwittermycin A are also present in strain GC-91.

It is not known to what extent Bacillus thuringiensis ssp. aizawai strain GC-91 will produce crystal proteins following its application. However, as the concentrations of the crystal proteins in the formulated product from historical production batches is known it was considered appropriate to complete an exposure assessment for them for surface water and groundwater based on their content in the product (Pesticides Peer Review Meeting Teleconference 25). For the crystal proteins, the experts agreed it would be appropriate to read across degradation and adsorption end points between the different crystal proteins from the available data set that contains measured endpoints from only a subset of these different δ-endotoxins and/or crystal proteins. Full details of the available experimental endpoints and which δ-endotoxins or crystal protein test material they were derived from can be found in Appendix A. As these endpoints were not available for all the δ-endotoxins present in Bacillus thuringiensis ssp. aizawai, the experts agreed that the most conservative values available should be selected and used in the exposure calculations. These values were a DT$_{50}$ soil of 41.3 days, $K_{doc}$ estimated at 1,000 mL/g and DT$_{50}$ water system of 28 days. Satisfactory calculations were provided for an environmental exposure assessment of the crystal proteins in soil, surface water, sediment and groundwater covering the representative uses. Soil exposure was calculated covering all representative uses. The FOCUS surface water Step 1 and 2 calculator (v3.2) for the crop pome/stone fruit was used (use pattern with greatest potential for spray drift exposure) for surface water and sediment calculations (FOCUS, 2001). For groundwater calculations, PEARL 4.4.4 was used for the crop grass (European Commission, 2014a)\(^\text{10}\) (see Appendix A). It was concluded that the potential for leaching of the crystal proteins 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.

5. Ecotoxicology

Some toxicity, infectiveness and pathogenicity studies on birds for Bacillus thuringiensis ssp. aizawai strain GC-91 were available and did not indicate any adverse effects. Based on the lack of toxicity or pathogenicity in the available studies, a low risk was concluded (relevant for all representative uses).

As concluded in Section 2, sufficient information is available to finalise the assessment of infectivity and pathogenicity of Bacillus thuringiensis ssp. aizawai strain GC-91 in mammals. A low risk to wild mammals was concluded (relevant for all representative uses).

Adequate studies were available with aquatic organisms showing no infectivity and pathogenicity to aquatic organisms from Bacillus thuringiensis ssp. aizawai strain GC-91. Based on the lack of toxicity, infectivity and pathogenicity in the available studies, a low risk to aquatic organisms was concluded for all representative uses.

Insufficient data were available to address toxicity, infectivity and pathogenicity to bees from Bacillus thuringiensis ssp. aizawai strain GC-91. Consequently, a data gap leading to an assessment not finalised was identified for the representative uses in open field and in walk-in tunnels. The RMS disagreed. Low risk identified for representative uses in permanent greenhouses as the exposure to bees is expected to be negligible.

Insufficient data were available to address toxicity, infectivity and pathogenicity to non-target arthropods from Bacillus thuringiensis ssp. aizawai strain GC-91. Consequently, a data gap leading to an assessment not finalised was identified to non-target arthropods for the representative uses in open field and walk-in tunnels. The RMS disagreed. For representative uses in permanent greenhouses, the risk is low as the exposure to non-target arthropods is expected to be negligible.

\(^{10}\) Simulations utilised the agreed Q10 of 2.58 (following EFSA, 2008) and Walker equation coefficient of 0.7.
For representative uses in permanent greenhouses, a risk assessment to soil organisms is required as spores of the strain are expected to persist and be present above natural background levels in soil (see Section 4.1). Insufficient information was available to address infectivity or pathogenicity to earthworms from exposure to *Bacillus thuringiensis* ssp. *aizawai* strain GC-91. Consequently, a data gap leading to an assessment not finalised was identified for the all representative uses. The RMS disagreed. Adequate data were available demonstrating the effects of *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 on soil microorganism and therefore a low risk is concluded for all representative uses. Adequate data were available and indicated that *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 is unlikely to be toxic to non-target plants and therefore a low risk to non-target plants is concluded for all representative uses.

The risk assessment of toxins/secondary metabolites such as crystal proteins could not be finalised for non-target terrestrial organisms, due to the lack of toxicity data resulting in data gap and issue not finalised (relevant for representative field- and walk-in tunnel uses). The RMS disagreed.

The risk assessment of toxins/secondary metabolites such as crystal proteins could not be finalised for non-target aquatic organisms, due to the lack of toxicity data resulting in data gap and issue not finalised (relevant for all representative uses). The RMS disagreed.
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 thuringiensis* ssp. *aizawai* strain GC-91 | Spores remain viable for many years (more than 7) multiplication in bulk soil will not occur | Data gap for potential effects on earthworms for all representative uses |
| Toxins/secondary metabolites such as crystal proteins, Cry1Ac, Cry1C, Cry1D, and Cry2A | Very low to moderate persistence | Data gap for potential effects on soil organisms for all representative uses in open field and walk-in tunnels |
| DT$_{50}$: period required for 50% dissipation. |

**Table 2: Groundwater**

| Compound (name and/or code) | Mobility in soil | > 0.1 μg/L at 1 m depth for the representative uses$^{(a)}$ | Pesticidal activity | Toxicological relevance |
|-----------------------------|------------------|-------------------------------------------------|--------------------|------------------------|
| Toxins/secondary metabolites such as crystal proteins, Cry1Ac, Cry1C, Cry1D, and Cry2A | The mobility of the crystal proteins in soil is low | No | Yes | Not triggered (for dietary exposure) Data gap (for non-dietary exposure) |

(a): FOCUS scenarios or a relevant lysimeter.

**Table 3: Surface water and sediment**

| Compound (name and/or code) | Ecotoxicology |
|-----------------------------|---------------|
| *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 | Low risk for the strain for all representative uses |
| Toxins/secondary metabolites such as crystal proteins, Cry1Ac, Cry1C, Cry1D, and Cry2A | Data gap for non-target aquatic organisms for all representative uses |

**Table 4: Air**

| Compound (name and/or code) | Toxicology |
|-----------------------------|------------|
| *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 | Rat LC$_{50}$ > 3.16 mg/L (corresponding to 3.77 $\times$ 10$^7$ CFU/kg bw) |
| Toxins/secondary metabolites such as crystal proteins, Cry1Ac, Cry1C, Cry1D and Cry2A | No data |

LC$_{50}$: lethal concentration, median; CFU: colony forming unit; bw: body weight.
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 Regulation (EC) No 1107/2009 concerning information on potentially harmful effects).

Five-batch data which includes the δ-endotoxin content (relevant for all representative uses evaluated; see Section 1).

Further assessment of potential health effects after repeated exposure by inhalation to *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 should be provided (relevant for field uses; see Section 2).

Further assessment of the genotoxic potential of the Cry insecticidal proteins by non-dietary exposure should be provided (relevant for field uses; see Section 2).

Viable counts of *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 linked to specific PHIs (relevant for the representative uses on pome fruits, grapes and tomatoes; see Section 3).

Adequate information to address the uniform principles criterion of the strain not being expected to persist in surface water in concentrations considerably higher than the natural background levels, resulting from repeated applications over the years was not available (relevant for all representative uses evaluated; see Section 4).

Further data to address the toxicity, infectivity and pathogenicity to bees and non-target arthropods (relevant for representative field and walk-in tunnel uses; see Section 5).

Further data to address the infectivity and pathogenicity to earthworms (relevant for all representative uses; see Section 5).

Further hazard characterisation and assessment of the risk to non-target terrestrial organisms from toxins/secondary metabolites such as crystal proteins (relevant for the representative field and walk-in tunnel uses; see Section 5).

Further hazard characterisation and assessment of the risk to non-target aquatic organisms from the toxins/secondary metabolites such as crystal proteins (relevant for all representative uses; see Section 5).

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

- In the absence of a quantitative risk assessment, the use of respiratory protective equipment for the operators and workers might be considered to reduce the exposure via inhalation (see Section 2).

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 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.

1) Since adverse effects after repeated exposure by inhalation could not be excluded, and a genotoxic potential of the Cry proteins could not be excluded by non-dietary exposure, the risk assessment by inhalation for residents and bystanders cannot be finalised (relevant for all representative uses except permanent greenhouses, see Section 2).

---

11 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) Since it is not demonstrated that the threshold of $10^5$ CFU/g is respected at harvest for the representative uses on pome fruits, grapes and tomatoes, the consumer risk assessment cannot be finalised (see Section 3).

3) The risk assessment to bees and non-target arthropods cannot be finalised as satisfactory information for the potential toxicity, infectivity and pathogenicity to bees and non-target arthropods was not provided (relevant for representative field and walk-in tunnel uses; see Section 5).

4) The risk assessment to earthworms cannot be finalised as satisfactory information for the potential infectivity and pathogenicity to earthworms was not provided (relevant for all representative uses; see Section 5).

5) Satisfactory information was not provided for a hazard characterisation and an assessment of the risk to non-target terrestrial organisms from toxins/secondary metabolites such as crystal proteins present after the application of the product (relevant for the representative field and walk-in tunnel uses; see Section 5).

6) Satisfactory information was not provided for a hazard characterisation and an assessment of the risk to non-target aquatic organisms from the toxins/secondary metabolites such as crystal proteins present after the application of the product (relevant for all representative uses; 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 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.

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.

- No critical areas of concern were identified.

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

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

| Representative use                                      | Pomefruit | Grapes | Tomato permanent greenhouse | Tomato walk-in tunnel | Sports turf |
|---------------------------------------------------------|-----------|--------|-----------------------------|-----------------------|------------|
| 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 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).

References

EFSA (European Food Safety Authority), 2008. Opinion on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil. EFSA Journal 2008;6(1):622, 32 pp. https://doi.org/10.2903/j.efsa.2008.622

EFSA (European Food Safety Authority), 2020. Peer review report to the conclusion regarding the peer review of the pesticide risk assessment of the active substance Bacillus thuringiensis ssp. aizawai strain GC-91. Available online: www.efsa.europa.eu

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2016. Scientific opinion on the risks for public health related to the presence of Bacillus cereus and other Bacillus spp. including Bacillus thuringiensis in foodstuffs. EFSA Journal 2016;14(7):4524, 93 pp. https://doi.org/10.2903/j.efsa.2016.4524

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordenez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cocconcelli PS, Fernandez Escamez PS, Maradona MP, Querol A, Suarez JE, Sundh I, Vlak J, Barizzone F, Correia S and Herman L, 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 and Products or Substances used in Animal Feed), 2018. Guidance on the characterisation of micro-organisms used as feed additives or as production organisms. EFSA Journal 2018;16(3):5206, 24 pp. https://doi.org/10.2903/j.efsa.2018.5206

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, 2014a. Assessing potential for movement of active substances and their metabolites to ground water in the EU. Report of the FOCUS Workgroup. EC Document Reference SANCO/13144/2010-v. 3, 613 pp., as outlined in Generic guidance for tier 1 FOCUS groundwater assessment, v. 2.2, May 2014.
European Commission, 2014b. Guidance document on the renewal of approval of active substances to be assessed in compliance with Regulation (EU) No 844/2012. SANCO/2012/11251-rev. 4, 12 December 2014.
FOCUS (Forum for the Co-ordination of Pesticide Fate Models and their Use), 2001. FOCUS surface water scenarios in the EU evaluation process under 91/414/EEC. Report of the FOCUS Working Group on Surface Water Scenarios. EC Document Reference SANCO/4802/2001-rev. 2, 245 pp., as updated by Generic guidance for FOCUS surface water scenarios, v. 1.4, May 2015.
The Netherlands, 2018. Renewal Assessment Report (RAR) on the active substance *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 prepared by the rapporteur Member State The Netherlands, in the framework of Commission Implementing Regulation (EU) No 844/2012, July 2018. Available online: [www.efsa.europa.eu](http://www.efsa.europa.eu)
The Netherlands, 2020. Revised Renewal Assessment Report (RAR) on *Bacillus thuringiensis* ssp. *aizawai* strain GC-91 prepared by the rapporteur Member State The Netherlands, in the framework of Commission Implementing Regulation (EU) No 844/2012, May 2020. Available online: [www.efsa.europa.eu](http://www.efsa.europa.eu)

**Abbreviations**

- **AFLP** amplified fragment length polymorphism
- **bw** body weight
- **CFU** colony-forming units
- **Cry** crystal insecticidal protein
- **DT₅₀** period required for 50% dissipation (define method of estimation)
- **EEC** European Economic Community
- **FOCUS** Forum for the Co-ordination of Pesticide Fate Models and their Use
- **GAP** Good Agricultural Practice
- **HlyII** haemolysin II
- **K-doc** organic carbon linear adsorption coefficient
- **LOQ** limit of quantification
- **MPCA** microbial pest control agent
- **MPCP** microbial pest control product
- **MRL** maximum residue level
- **PEC** predicted environmental concentration
- **PHI** preharvest interval
- **RAR** renewal assessment report
- **RMS** rapporteur Member State
- **WG** water-dispersible granule
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.2020.6293