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## Peer review of the pesticide risk assessment of the active substance *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351

European Food Safety Authority (EFSA),  
Fernando Alvarez, Maria Arena, Domenica Auteri, Jorge Borroto, Alba Brancato,  
Luis Carrasco Cabrera, Anna Federica Castoldi, Arianna Chiusolo, Angelo Colagiorgi,  
Mathilde Colas, Federica Crivellente, Chloe De Lentdecker, Mark Egsmose, Gabriella Fait,  
Varvara Gouliarmou, Franco Ferilli, Luna Greco, Alessio Ippolito, Frederique Istace,  
Samira Jarrah, Dimitra Kardassi, Aude Kienzler, Renata Leuschner, Roberto Lava,  
Alberto Linguadoca, 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 the EFSA following the peer review of the initial risk assessments carried out by the competent authorities of the rapporteur Member State, Denmark, and co-rapporteur Member State, the Netherlands, for the pesticide active substance *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 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* subsp. *kurstaki* strain ABTS-351 as an insecticide on cabbage (field use) and tomato (permanent greenhouse and walk-in tunnel 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.

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**Correspondence:** pesticides.peerreview@efsa.europa.eu

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## 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 as amended by Commission Implementing Regulation (EU) No 2016/183. *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 is one of the active substances listed in that Regulation.

In accordance with Article 1 of Regulation (EU) No 844/2012, the rapporteur Member State (RMS), Denmark, and co-rapporteur Member State (co-RMS), the Netherlands, received an application from Sumitomo Chemical Agro Europe S.A.S for the renewal of approval of the active substance *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351.

An initial evaluation of the dossier on *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 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 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* subsp. *kurstaki* strain ABTS-351 according to the representative uses as an insecticide on cabbage (field uses) and tomato (permanent greenhouse and walk-in tunnel uses), as proposed at EU level result in a sufficient insecticidal efficacy against the target lepidopteran pests.

The assessment of the data package revealed no issues that could not be finalised or that need to be included as critical areas of concern with respect to identity, biological properties of the active substance and physical and technical properties of the representative formulation.

In the area of mammalian toxicology, two data gaps are identified, the first one concerning the potential adverse effects from repeated inhalation of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 and the second one related to the potential genotoxic effect of Cry proteins through non-dietary exposure. On this basis, the risk assessment for residents and bystanders cannot be concluded (issue not finalised).

In the area of residues, data gaps were identified related to the proposed threshold of  $1 \times 10^5$  colony forming units (CFU)/g for viable residues on edible plant commodities at harvest, where quantification of viable counts linked to specific preharvest intervals (PHIs) is requested for the representative uses on cabbage and tomatoes to ensure this threshold level is not exceeded and to allow finalisation of the consumer risk assessment. This information shall be supported by representative storage stability studies in high-water commodities and by a complete comprehensive search of the scientific peer reviewed open literature.

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 infectivity and pathogenicity to non-target arthropods for representative use in open field and walk-in tunnels. For the hazard characterisation and an assessment of the risk to non-target organisms from toxins/secondary metabolites such as crystal proteins present after the application of the product, satisfactory information was not provided leading to issues not being finalised for the non-target terrestrial organisms for the representative uses in open field and walk-in tunnels and for the aquatic organisms for all representative uses.

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## Background

Commission Implementing Regulation (EU) No 844/2012<sup>1</sup>, as amended by Commission Implementing Regulation (EU) No 2018/1659<sup>2</sup>, (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<sup>3</sup>. 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 an additional 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, Denmark, and co-RMS, the Netherlands, received an application from Sumitomo Chemical Agro Europe S.A.S for the renewal of approval of the active substance *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351. Complying with Article 8 of the Regulation, the RMS checked the completeness of the dossier and informed the applicant, the co-RMS, the European Commission and EFSA about the admissibility.

The RMS provided its initial evaluation of the dossier on *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 in the RAR, which was received by EFSA on 20 June 2019 (Denmark, 2019).

In accordance with Article 12 of the Regulation, EFSA distributed the RAR to the Member States and the applicant, Sumitomo Chemical Agro Europe S.A.S, for consultation and comments on 16 September 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 16 November 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 18 June 2020. 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 there was no need to conduct an expert consultation.

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 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 written consultation on the assessment of additional information, 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 June–July 2021.

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* subsp. *kurstaki* strain ABTS-351 as an insecticide on cabbage (field use) and

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> 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.

tomato (permanent greenhouse and walk-in tunnel uses), 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, if any, are presented in the conclusion.

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 RAR;
- the reporting table (24 June 2020);
- the evaluation table (13/9/2021);
- 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 RAR, including its revisions (Denmark, 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 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 thuringiensis* subsp. *kurstaki*<sup>4</sup> strain ABTS-351 is a bacterium deposited at the American Type Culture Collection, Rockville, Maryland, USA under the identification number ATCC-SD-1275. *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 is a naturally occurring wild-type bacterium, initially isolated from larvae of the insect *Pectinophora gossypiella* in Texas, USA.

The representative formulated product for the evaluation was 'DiPel® DF', a water-dispersible granule (WG) containing 540 g/kg of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 with a nominal biopotency of  $3.2 \times 10^7$  IU/g (min.  $1 \times 10^{13}$  CFU/kg, max.  $8 \times 10^{13}$  CFU/kg).

The representative uses evaluated were spray applications for the biological control of insect pests of the order of Lepidoptera on cabbage in the field and protected tomato (including permanent greenhouses and walk-in tunnels) in the EU. Full details of the good agriculture 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* subsp. *kurstaki* strain ABTS-351 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 (European Commission, 2012; EFSA FEEDAP Panel, 2018).

<sup>4</sup> A discussion has been ongoing for several years whether the phylogenetic evidence could need a re-assignment of *Bacillus cereus sensu lato* like strains to different species than their current assignments. These respective species might derive from classifications into different phylogenetic clusters, but for the time being the recent classification has been maintained. It cannot be excluded that in the future re-assigning selected strains of *Bacillus thuringiensis* as *Bacillus cereus* might occur (see reporting table comment 2(7) in the peer review report (EFSA, 2021) for further details and citations relating to this scientific discussion).

The specification of the technical grade microbial pest control agent (MPCA) has been expressed as dried technical slurry (identified as Dipel TP in Appendix A) and has been certified to have a minimum biopotency of  $9.5146 \times 10^7$  IU/g (Min.  $1 \times 10^{13}$  CFU/kg, max.  $9 \times 10^{13}$  CFU/kg).

*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 is characterised by flagella antigen serotyping, plasmid profiling, activity spectrum, fatty acid analysis, deoxyribonucleic acid (DNA) fingerprinting (amplified fragment length polymorphism; AFLP), and cry toxin analysis. A DNA and ribonucleic acid (RNA) microarray genotyping method is available to identify the strain ABTS-351. An annotated whole genome sequence (WGS) derived with the Illumina technology has been provided for the strain ABTS-351.

*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 contains genes encoding for potential production of cytotoxin type K<sub>2</sub> (CytK<sub>2</sub>), five crystal insecticidal proteins (Cry1Aa, Cry1Ab, Cry1Ac, Cry2Aa and Cry2Ab). Furthermore, *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 has the potential to form a non-haemolytic (Nhe) and haemolytic (Hbl) enterotoxin complex (which also includes CytK<sub>2</sub>) though the operon related to Hbl is incomplete (indicating haemolysin action is not functional). The genes for the highly cytotoxic Cyt K<sub>1</sub>, for cereulide and for  $\beta$ -exotoxins are missing in the strain. *Bacillus thuringiensis* subsp. *kurstaki* has been reported to produce Nhe enterotoxins, with its vegetative cells secreting vegetative insecticidal proteins (Vip) and secreted insecticidal proteins (Sip). The applicant has not provided information whether strain ABTS-351 produces this class of enterotoxins or these non-crystal insecticidal proteins. Therefore, this has been identified as a data gap.

The content of microbial contaminants of the microbial pest control product (MPCP) 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  $\delta$ -endotoxins are rapidly degradable and endospores are rapidly inactivated when exposed to UV radiation.

Optimum growth conditions for *Bacillus thuringiensis* subsp. *kurstaki* are 28°C to 30°C and pH 6.8–7.2.

As a member of the *Bacillus cereus* group, *Bacillus thuringiensis* subsp. *kurstaki* is closely related to *Bacillus anthracis* and *Bacillus cereus*. *Bacillus thuringiensis* strains are, however, distinguishable from *Bacillus cereus* and *Bacillus anthracis* using strain-specific quantitative polymerase chain reaction (qPCR) protocol based on the sequences of the whole genome and plasmids of strain ABTS-351.

*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 was shown to be sensitive to relevant antibiotics as provided in EFSA FEEDAP Panel Guidance (2018). It is sensitive to chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, trimethoprim/sulfamethoxazole and vancomycin. The strain ABTS-351 has intrinsic resistance to ampicillin, penicillin and cephalothin.

The main data regarding the identity of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 and its biological properties are given in Appendix A.

Acceptable methods for CFU counts of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 in the formulation for the determination of the microorganism in the MPCP 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 no maximum residues level (MRL) was set for any of the intended uses. The strain-specific DNA and RNA microarray genotyping can be used for monitoring of the strain upon field application.

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

Quantification of Cry1Ab in soil can be done with commercial enzyme-linked immunosorbent assay (ELISA) kit with a limit of quantification (LOQ) of 0.25  $\mu$ g/L. Determination of Cry1Ab in water can be done with ELISA with a detection limit of 2.1 ng/L.

## 2. Mammalian toxicity

*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 has not been the subject of a Pesticides Peer Review Meeting; however, reference is made to the Pesticides Peer Review Meeting Teleconference 25 in March 2020, where similar *Bacillus thuringiensis* subsp. were discussed.

### General data

As medical information, few data from the literature have reported clinical infections (e.g. ocular) and dermal irritation attributed to *Bacillus thuringiensis* species. The results of allergenicity observations indicate that increased IgE antibodies' levels can occur in greenhouses workers exposed to products containing *Bacillus thuringiensis*, but no effect on the occurrence of respiratory symptoms or lung function was observed. From direct observations, there are no major effects observed on

populations exposed to aerial spraying of bioinsecticides based on *Bacillus thuringiensis* subsp. *kurstaki*. Additionally, a new health surveillance report confirmed the absence of adverse reactions in or reported by operators resulting from exposure to *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 at the manufacturing facility.

*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 validated test methods for sensitisation by inhalation, it cannot be excluded that *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 may have the potential to provoke sensitising reactions.

No toxicity or infectivity was noted in experimental studies upon oral, dermal, respiratory or intraperitoneal exposure, even though an incomplete clearance has been observed in several studies (acute oral rat, acute intratracheal rat, acute intravenous rat) with technical material of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351.

In a repeated dose oral toxicity study on sheep with 'DiPel' (containing *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1<sup>5</sup>), no indication of pathogenicity or adverse clinical signs were observed during the study (5 months). Similarly, the oral administration of *Bacillus thuringiensis* subsp. *kurstaki* product 'Protecto' to rats for 12 weeks was not associated with any toxicological effects. In a repeated dose toxicity study with aerosol administration of a single dose of a biopesticide containing *Bacillus thuringiensis* subsp. *kurstaki* and *Bacillus thuringiensis* subsp. *israelensis*, an interstitial lung inflammation was observed in mice at day 70 after exposure. On the basis of the available data, a potential concern for serious health effects after repeated exposure by inhalation cannot be excluded as clearance was not sufficiently investigated after repeated exposure (data gap and issue not finalised for representative uses in field and walk-in tunnels).<sup>6</sup> The RMS disagreed, being of the opinion that the evidence is not sufficient to support a concern.

#### Secondary metabolites/toxins

*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 has the genetic determinants to express a non-haemolytic and haemolytic enterotoxin complex (Nhe and Hbl), and cytotoxin K2 (as most of the commercially used *Bacillus thuringiensis* subsp. *kurstaki* strains), though the operon related to Hbl is incomplete (see Section 1). It is noted that enterotoxins are inactivated at low pH, therefore, preformed enterotoxins are not relevant by oral exposure. 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). *In vitro* data (with human intestinal cells) showed lack of germination of *Bacillus thuringiensis* strains in DiPel. Considering the limitations of these data, this was not considered sufficient to dismiss any pathogenic potential of the strain ABTS-351. Considering the available evidence and uncertainties, the threshold of 10<sup>5</sup> CFU/g food as determined by the BIOHAZ Panel Opinion (EFSA BIOHAZ Panel, 2016) was concluded as applicable by a small majority of the experts to cover the risk of food-borne poisonings caused by the *Bacillus cereus* group of microorganisms.<sup>7</sup> The RMS, co-RMS and some MSs disagreed. Based on recent literature data,<sup>8</sup> it cannot be ruled out that the *Bacillus thuringiensis* subsp. used as pesticides may have a pathogenic potential.

Several studies investigating the toxic properties of the crystal proteins/endotoxins were identified in the literature. In a mouse micronucleus study with intraperitoneal administration, positive results were observed with the spore-crystal complex containing Cry1Aa and Cry1Ac. These results were considered equivocal especially regarding whether the Cry-proteins had been solubilised/activated prior to intraperitoneal administration or not (data gap).<sup>9</sup> It was concluded that genotoxicity is not a

<sup>5</sup> *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 is an isolate of HD-1.

<sup>6</sup> See experts' consultation 6.3 in the Report of the Pesticide Peer Review Teleconference 25 (March 2020) for *Bacillus thuringiensis* subsp. *aizawai* ABTS-1857 (EFSA, 2021).

<sup>7</sup> See experts' consultation 6.1 in the Report of Pesticide Peer Review Meeting Teleconference 25 (March 2020) for *Bacillus thuringiensis* subsp. *aizawai* strain ABTS-1857 (EFSA, 2021).

<sup>8</sup> Bonis M, Felten A, Pairaud S, Dijoux A, Maladen V, Mallet L, Radomski N, Dubois A, Arar C, Sarda X, Viral G, Mistou MY and Firmesse O, 2021 Comparative phenotypic, genotypic and genomic analyses of *Bacillus thuringiensis* associated with food-borne outbreaks in France. PLoS ONE, 16, e0246885.

<sup>9</sup> See experts' consultation 6.2 in the Report of Pesticide Peer Review Meeting Teleconference 25 (March 2020) for *Bacillus thuringiensis* subsp. *aizawai* strain ABTS-1857 (EFSA, 2021).



concern for dietary exposure (since Cry-proteins are not activated to their toxic form in the human digestive tract), but it was not possible to conclude for non-dietary exposure.

#### Reference values and exposure estimates

Regarding dietary exposure, considering the available evidence and uncertainties, the threshold of  $10^5$  CFU/g as determined by the BIOHAZ Panel Opinion is considered applicable for the consumers, in order to cover the risk of food-borne poisoning caused by the *Bacillus cereus* group of microorganisms.

With regard to non-dietary exposure, since toxicity/infectivity after repeated exposure by inhalation could not be concluded, 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 concluded, except for permanent greenhouses (issue not finalised). 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.

The RMS disagreed with this view, being of the opinion that *Bacillus thuringiensis* subsp. *kurstaki* preparations are not to be considered of health concern for operators, workers, bystanders and residents.

### 3. Residues

*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 has not been the subject of a Pesticides Peer Review Meeting; however, reference is made to the Pesticides Peer Review Meeting Teleconference 25 in March 2020, where similar *Bacillus thuringiensis* subsp. were discussed. The respective outcome derived for these strains is considered applicable for *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351.

Considering the available evidence and uncertainties, the threshold of  $10^5$  CFU/g plant commodity at the time of harvest 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 considered of concern for the dietary consumer exposure assessment (see Section 2).

Therefore, only information on viable residues, i.e. CFU per g or kg plant commodities at harvest in accordance with the representative uses, is needed to demonstrate that the threshold of  $10^5$  CFU/g edible plant commodity is respected.

Two representative uses are provided for *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 for which treatments are indicated to be started when infesting insecticidal larvae are hatching. The representative uses are an outdoor foliar use on cabbage and an indoor foliar use on tomatoes, both intended with a maximum of eight applications at a maximum application rate of  $1.17 \times 10^{13}$  CFU/ha (maximum seasonal application rate  $9.36 \times 10^{13}$  CFU/ha following eight applications) up to a BBCH of 92. A preharvest interval (PHI) is not indicated in the GAP table.

However, the setting of a PHI may be needed based on available experimental data e.g. in lettuces (up to  $3.07 \times 10^5$  CFU/g on the day of last application of three applications at  $9 \times 10^{12}$  CFU/g each). In the lettuce experiment 2 days after last treatment counts were reported to be  $1.78 \times 10^5$  CFU and on the third day a decline to values of  $4.47 \times 10^4$  CFU/g were reported. Data on greenhouse tomato indicated average counts of up to  $8.5 \times 10^4$  CFU/g on the day of the last of five treatments with an application rate of  $1.7 \times 10^{13}$  CFU/ha; however, a highest value of  $1.03 \times 10^5$  CFU/g in a tomato sample is also reported. Estimated counts on cabbage (up to  $5.29 \times 10^5$  CFU/g) for the representative use indicate that counts at harvest can exceed the threshold determined by the BIOHAZ Panel. It is further to be noted that in the first peer review assessment counts of  $2.25 \times 10^6$  CFU/g were estimated regarding the representative use on cabbage with a maximum application rate of  $6.3 \times 10^{12}$  CFU/ha and a PHI of 0 day (EFSA, 2012).

Lettuce and tomatoes samples were stored frozen at  $-18^\circ\text{C}$  and  $-20^\circ\text{C}$ , respectively, prior to analysis. In the study on tomato, frozen storage for one day did not indicate a significant effect on average viable counts. Beside this, storage stability data in crops representative of high-water content commodities were not available and the viable counts residues determined in lettuces should be considered as indicative only. Therefore, a storage stability study of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 in high-water plant commodities (tomatoes and cabbage) is still desirable.

Viable counts of commercial *Bacillus thuringiensis* strains including *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 were demonstrated in the scientific literature and by supporting experimental evidence to decline following application and to not persist or multiply on edible plant commodities (fruiting vegetable 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 in the RAR, a half-life of viable spores of around 1 day or 24 h can reasonably be assumed. The half-life of spores of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 on cabbage and tomatoes would nevertheless be preferably determined for conditions in line with the representative uses.

Some information including references, studies and their evaluation was identified as still missing with regard to the updated scientific peer-reviewed open literature review (**data gap**; see evaluation table data requirement points 7.4 and 7.9).

Therefore, sufficient residue data for the determination of the viable counts considering the representative uses on cabbages and tomatoes and linked to the specific PHIs is requested including quantification of the viable counts present on edible commodities on the day of harvest (**data gap**). The requested information should also be supported by acceptable storage stability data and the provision of requested additional information regarding the updated open peer-reviewed scientific literature search (**data gap**). The data gaps should be addressed before a consumer risk assessment can be finalised.

With regard to the five assessment criteria according to the Commission guidance SANCO/11188/2013 Rev. 2 (European Commission, 2015) for potential inclusion in Annex IV of Regulation (EC) No 396/2005<sup>10</sup>, none of the three criteria relevant for microorganisms (having no identified hazardous properties (criterion 3); natural exposure is higher than the one linked to the use as plant protection product (criterion IV) or consumer exposure is not expected (criterion V)) were considered to be met for of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 for the following reasons:

*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 can form spores and 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) (see Section 2);

It is unknown if *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 used as plant protection product would lead to significant increase of background levels of those *Bacillus thuringiensis* naturally occurring.

Consumer exposure to *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 can be expected for both representative uses an insecticide on cabbage and tomatoes (EU outdoor use on cabbage and EU indoor use on tomatoes:  $8 \times 1.17 \times 10^{13}$  CFU/ha up to a BBCH of 92).

Considering that the criteria laid down in the guidance are not fulfilled, **further risk management considerations are required** to decide whether the *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 **is qualified for being included** into Annex IV of Regulation (EC) No 396/2005.

#### 4. Environmental fate and behaviour

Satisfactory information was provided in relation to potential interference of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC<sup>11</sup> (see specific Annex VI decision-making criteria in Part II Commission Regulation (EU) No 546/2011<sup>12</sup>). It was concluded that *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 is unlikely to interfere with the methodologies routinely used for such determinations.

*Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 is a 'wild type' 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* subsp. *kurstaki* strain ABTS-351 than for other naturally occurring *Bacillus thuringiensis* strains, transfer of genetic material by *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 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

<sup>10</sup> 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.

<sup>11</sup> 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.

<sup>12</sup> 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.

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 (permanent and walk-in tunnel) uses were not provided. The applicant chose to address the representative use on tomatoes grown in greenhouses 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 ABTS-351 was not available. Information on subsp. *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 subsp. *kurstaki* strain ABTS-351 spores in the soil environment. Consequently, EFSA concluded that the information is sufficient to address the uniform principles criterion. 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. Predicted environmental concentrations (PEC) for 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 ABTS-351 was not available. Information on subsp. *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* subsp. *kurstaki* strain ABTS-351 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 and multiply in surface water in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years (**data gap**). The RMS disagreed. PEC surface water for the intended uses has been calculated (see Appendix A).

Information was provided on the occurrence and behaviour of *Bacillus thuringiensis* subsp. *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.

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

Fate and behaviour endpoints for  $\delta$ -endotoxins and their Cry and Cyt proteins were discussed at the Pesticides Peer Review Meeting Teleconference 25 in March 2020.

According to scientific papers from the literature search, the subspecies *Bacillus thuringiensis* *kurstaki* is able to produce secondary metabolites, which are crystal proteins e.g. contain the  $\delta$ -endotoxins, Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ia, Cry2Aa and Cry2Ab. Strain ABTS-351 has genes encoding these crystal proteins except Cry1Ia. The crystal proteins except Cry1Ia and Cry2Ab constitute components in the formulated product within and outside spores and are responsible for the insecticidal mode of action of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351. It may also be that vegetative cells can produce Vip and Sip proteins.

It is not known to what extent products containing *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 will produce crystal proteins or vegetative cells following its application. However, as the concentrations of the crystal proteins in the formulated product 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 endpoints between the different crystal proteins from the available data set that contains measured endpoints from only a subset of these different  $\delta$ -endotoxins and/or crystal proteins. Full details of the

available experimental endpoints and which  $\delta$ -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  $\delta$ -endotoxins present in *Bacillus thuringiensis* subsp. *kurstaki*, the experts agreed that the most conservative values available should be selected and used in the exposure calculations. These values were a DT<sub>50</sub> soil of 41.3 days, K<sub>doc</sub> estimated at 1,000 mL/g and DT<sub>50</sub> water system of 28 days. EFSA considers that this information is also likely to cover the Vip and Sip proteins if produced. Satisfactory calculations were provided for an environmental exposure assessment of the crystal proteins in soil, surface water, sediment and groundwater covering the representative uses. The FOCUS surface water Step 1 and 2 calculator (v3.2) was used for surface water and sediment calculations (FOCUS, 2001). For groundwater calculations PEARL 4.4.4 was used (European Commission, 2014a)<sup>13</sup> (All the PEC are included in Appendix A). It was concluded that the potential for leaching of the crystal and Vip and Sip 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

Two studies on the toxicity and pathogenicity of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 to the mallard duck and northern bobwhite quail were available and did not indicate any adverse effects. Investigation of infectivity was not performed in the studies. Based on the lack of toxicity and pathogenicity in the available studies, a low risk to **birds** from *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 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* subsp. *kurstaki* strain ABTS-351 in **mammals**. A low risk to wild mammals was concluded (relevant for all representative uses).

Adequate studies were available with **aquatic organisms** showing low toxicity and no pathogenicity to aquatic organisms from *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351. A margin of safety was observed when comparing the endpoints with expected spore concentrations in the environment after entry into surface water from the intended field uses. Based on the low toxicity and lack of infectivity and pathogenicity in the available studies, a low risk to aquatic organisms from *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 was concluded for all representative uses.

Adequate studies of sufficient duration were available with **honeybees** and honeybee larvae showing low toxicity and no pathogenicity from *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351. Infectivity was not investigated. A margin of safety was observed when comparing the endpoints with the maximum application rate for the intended field uses. Based on the low toxicity and lack of pathogenicity in the available studies, a low risk to honeybees and honeybee larvae from *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 was concluded for all representative uses.

Insufficient data were available to address infectivity and pathogenicity to **non-target arthropods** from *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351. In the two-glass plate limit test studies on *Aphidius rhopalosiphi* and *Typhlodromus pyri* with strain ABTS-351, no adverse effects were observed on reproduction. The study designs did not take the relevant oral route into account and no assessment of infectivity and pathogenicity was performed. In an extended laboratory test on *Metaseiulus occidentalis* with strain ABTS-351 slight significant mortality was observed in the two highest treatment doses. No effects were observed on the number of eggs laid; however, significant effects on the percentage of larvae hatched were observed at all treatment rates. Observation of infectivity and pathogenicity was not performed in the study. In an extended laboratory test on *Tetranychus urticae* with strain ABTS-351 significant mortality was found at the highest test substance dose. Observation of infectivity and pathogenicity was not performed in the study. Consequently, a **data gap** leading to an assessment not finalised was identified for the infectivity and pathogenicity of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 to non-target arthropods for the representative uses in open field and walk-in tunnels. For representative uses in permanent greenhouses, the risk is low as the exposure to non-target arthropods is expected to be negligible.

A study was available with **earthworms** showing no toxicity and pathogenicity from *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351. Infectivity was not investigated. Based on the lack of toxicity and pathogenicity in the available study, a low risk to earthworms and other soil macro-organisms was concluded (relevant for all representative uses). Adequate data showing no adverse effects were available on **soil microorganisms** exposed to *Bacillus thuringiensis* subsp. *kurstaki* strain

<sup>13</sup> Simulations utilised the agreed Q10 of 2.58 (following EFSA, 2008) and Walker equation coefficient of 0.7.

ABTS-351. Low risk from *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 was concluded to soil microorganisms for all representative uses.

The risk assessment of toxins/secondary metabolites such as crystal proteins could not be finalised for terrestrial non-target organisms for the representative field and walk-in tunnel uses and for aquatic organisms for all representative uses. Toxicity data were not available to perform a hazard characterisation (resulting in data gap and issue not finalised). The RMS disagreed. For aquatic organisms, exposure to surface water cannot be excluded for the representative use in permanent greenhouse (resulting in a data gap and issue not finalised). The RMS was in disagreement.

## 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
<i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> strain ABTS-351	Low risk to soil macro- and microorganisms from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> strain ABTS-351 for all representative uses
Toxins/secondary metabolites such as crystal proteins, Cry1Aa, Cry1Ab, Cry1Ac and Cry2Aa and Vip and Sip	A data gap and an assessment not finalised was identified for the non-target soil macro- and microorganisms for the representative field and walk-in tunnels uses.

**Table 2:** Groundwater<sup>(a)</sup>

Compound (name and/or code)	> 0.1 µg/L at 1 m depth for the representative uses <sup>(b)</sup> Step 2	Biological (pesticidal) activity/relevance Step 3a.	Hazard identified Steps 3b. and 3c.	Consumer RA triggered Steps 4 and 5	Human health relevance
Toxins/secondary metabolites such as crystal proteins, Cry1Aa, Cry1Ab, Cry1Ac and Cry2Aa and Vip and Sip	No	Yes	Assessment not triggered. Equivocal results in a micronucleus test (with intraperitoneal administration)	No	Assessment not triggered for the representative uses assessed.

(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
<i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> strain ABTS-351	Low risk to aquatic organisms from <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> strain ABTS-351 for all representative uses
Toxins/secondary metabolites such as crystal proteins, Cry1Aa, Cry1Ab, Cry1Ac and Cry2Aa and Vip and Sip	A data gap and an assessment not finalised for aquatic organisms was identified for all representative uses

**Table 4:** Air

Compound (name and/or code)	Toxicology
<i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> strain ABTS-351	Rat LC <sub>50</sub> > 1 × 10 <sup>8</sup> CFU <i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> strain ABTS-351/animal
Toxins/secondary metabolites such as crystal proteins, Cry1Aa, Cry1Ab, Cry1Ac and Cry2Aa	No data

## 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.

### 7.1. Particular conditions proposed for the representative uses evaluated

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

Representative use	Cabbage (field)	Tomato (permanent greenhouse)	Tomato (walk-in tunnel)
	Foliar spray	Foliar spray	Foliar spray
<b>Operator risk</b>	Use of PPE/RPE might be considered to reduce non-dietary exposure (dermal and inhalation).	Use of PPE/RPE might be considered to reduce non-dietary exposure (dermal and inhalation).	Use of PPE/RPE might be considered to reduce non-dietary exposure (dermal and inhalation).
<b>Worker exposure</b>	Use of PPE/RPE might be considered to reduce non-dietary exposure (dermal and inhalation).	Use of PPE/RPE might be considered to reduce non-dietary exposure (dermal and inhalation).	Use of PPE/RPE might be considered to reduce non-dietary exposure (dermal and inhalation).

## 8. Concerns and related data gaps

### 8.1. 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<sup>14</sup> 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.

**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 risk assessment by inhalation for residents and bystanders (see Section 2) could not be concluded for the outdoor (field and walk-in tunnel) use on vegetables considering the identified data gaps:
  - a) Lack of sufficient information on the pathogenicity/infectivity of *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 after repeated inhalation exposure (relevant for all representative uses except permanent greenhouses, see Section 2)

<sup>14</sup> 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.

- b) Lack of data on the genotoxic potential of the Cry proteins resulting from non-dietary exposure (relevant for all representative uses except permanent greenhouses, see Section 2)
- 2) The consumer dietary risk assessment was not finalised because it could not be concluded that the threshold level of  $1 \times 10^5$  CFU/g edible commodity was not exceeded considering the identified data gaps:
  - a) Quantification of viable count residues linked to specific PHIs is requested to demonstrate that the threshold of  $10^5$  CFU/g is not exceeded at harvest for the representative uses. This information should be supported by storage stability data on crops representative of the high-water content commodities (relevant all representative uses; see Section 3).
  - b) Provision of requested additional information regarding the open peer-reviewed scientific literature search (see Section 3);
- 3) Satisfactory information was not available for the potential infectivity and pathogenicity to non-target arthropods from *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 for the assessment of the representative uses in open field and walk-in tunnels 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 arthropods from *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 (relevant for the representative uses in open field and walk-in tunnels, see Section 5).
- 4) Satisfactory information was not available for a hazard characterisation and an assessment of the risk to non-target terrestrial organisms and aquatic organisms from toxins/secondary metabolites such as crystal proteins present in the environment after application of the product. For non-target terrestrial organisms, relevant for the representative use in open field and walk-in tunnels (see Sections 4 and 5); for aquatic organisms, relevant for all the representative uses (see Sections 4 and 5).
  - a) Further hazard characterisation and assessment of the risk to non-target terrestrial organisms and aquatic organisms from toxins/secondary metabolites such as crystal proteins present in the environment after application of the product. For non-target terrestrial organisms, relevant for the representative use in open field and walk-in tunnels (see Sections 4 and 5); for aquatic organisms, relevant for all the representative uses (see Sections 4 and 5).

## 8.2. 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.

### 8.3. Critical areas of concern, including associated data gaps, have not been identified

#### 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		Cabbage (field)	Tomato (permanent greenhouse)	Tomato (walk-in tunnel)
		Foliar spray	Foliar spray	Foliar spray
<b>Operator risk</b>	Risk identified			
	Assessment not finalised			
<b>Worker risk</b>	Risk identified			
	Assessment not finalised			
<b>Resident/bystander risk</b>	Risk identified			
	Assessment not finalised	X <sup>1</sup>		X <sup>1</sup>
<b>Consumer risk</b>	Risk identified			
	Assessment not finalised	X <sup>2</sup>	X <sup>2</sup>	X <sup>2</sup>
<b>Risk to wild non-target terrestrial vertebrates</b>	Risk identified			
	Assessment not finalised	X <sup>4</sup>		X <sup>4</sup>
<b>Risk to wild non-target terrestrial organisms other than vertebrates</b>	Risk identified			
	Assessment not finalised	X <sup>3,4</sup>		X <sup>3,4</sup>
<b>Risk to aquatic organisms</b>	Risk identified			
	Assessment not finalised	X <sup>4</sup>	X <sup>4</sup>	X <sup>4</sup>
<b>Groundwater exposure to active substance</b>	Legal parametric value breached			
	Assessment not finalised			
<b>Groundwater exposure to metabolites</b>	Legal parametric value breached			
	Parametric value of 10 µg/L <sup>(a)</sup> breached			
	Assessment not finalised			

The superscript numbers relate to the numbered points indicated in Sections 8.1 and 8.2. Where there is no superscript number, see Sections 2–7 for further information.

(a): 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:**

Information whether *Bacillus thuringiensis* subsp. *kurstaki* strain ABTS-351 produces the non-haemolytic enterotoxins (Nhe) or non-crystal: vegetative insecticidal proteins (Vip) and secreted insecticidal proteins (Sip) was not available (relevant for all representative uses evaluated; see Section 1).

Adequate information to address the uniform principles criterion of the strain not being expected to persist and multiply in surface water in concentrations considerably higher than the natural background



levels, taking into account repeated applications over the years was not available (relevant for all representative uses evaluated; see Section 4).

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## Abbreviations

$\lambda$	wavelength
$\epsilon$	decadic molar extinction coefficient
$\mu\text{g}$	microgram
$\mu\text{m}$	micrometer (micron)
ADE	actual dermal exposure
ADI	acceptable daily intake
AF	assessment factor
AFLP	amplified fragment length polymorphism

AP	alkaline phosphatase
AR	applied radioactivity
AR	androgen receptor
Bp	base pair
BUN	blood urea nitrogen
Bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming units
CHO	Chinese hamster ovary cells
CI	confidence interval
CL	confidence limits
Cm	centimetre
DAR	draft assessment report
DAS-ELISA	double-antibody sandwich enzyme linked immunosorbent assay
DAT	days after treatment
DM	dry matter
DNA	deoxyribonucleic acid
DT <sub>50</sub>	period required for 50% dissipation (define method of estimation)
EEC	European Economic Community
ELISA	Enzyme-linked immunosorbent assay
FID	flame ionisation detector
FIR	food intake rate
FOB	functional observation battery
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
G	gram
GAP	Good Agricultural Practice
GC	gas chromatography
GC-FID	gas chromatography with flame ionisation detector
GC-MS	gas chromatography – mass spectrometry
GC-TEA	gas chromatography with thermal energy analyser
GM	geometric mean
GS	growth stage
GPC	gel permeation chromatography
H	hour(s)
Ha	hectare
HR	hazard rate
ICP-AES	inductively coupled plasma atomic emission spectroscopy
IMI	International Mycological Institute [CABI Bioscience, Eggham, UK ( <i>formerly</i> International Mycological Institute; <i>same as</i> CMI).]
ISO	International Organization for Standardization
ITS	internal transcribed spacer
IUPAC	International Union of Pure and Applied Chemistry
Iv	intravenous
K <sub>doc</sub>	organic carbon linear adsorption coefficient
Kg	kilogram
L	litre
LC	liquid chromatography
LC <sub>50</sub>	lethal concentration, median
LC-MS	liquid chromatography–mass spectrometry
LC-MS/MS	liquid chromatography with tandem mass spectrometry
M	metre
M	mol
Mg	milligram
M/L	mixing and loading
mm	millimetre (also used for mean measured concentrations)
MOA	mode of action
MPCA	active agent of the microbial pest control product

MPCP	microbial pest control product
MRL	maximum residue level
MS	mass spectrometry
NOEL	no observed effect level
OECD	Organisation for Economic Co-operation and Development
OM	organic matter content
Pa	pascal
PD	proportion of different food types
PDA	Potato Dextrose Agar
PEC	predicted environmental concentration
pH	pH-value
PHI	preharvest interval
PIE	potential inhalation exposure
PPE	personal protective equipment
PT	proportion of diet obtained in the treated area
RAR	Renewal Assessment Report
RBC	red blood cells
REACH	Registration, Evaluation, Authorisation of Chemicals Regulation
RNA	ribonucleic acid
RPE	respiratory protective equipment
S	svedberg, S ( $10^{-13}$ s)
SAR	systemic acquired resistance
SCAR	sequence characterized amplified region
SD	standard deviation
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
WG	water dispersible granule
WGS	whole genome sequence
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.2021.6879>