Peer review of the pesticide risk assessment of the active substance *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348

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Abstract

The conclusions of the European Food Safety Authority (EFSA) following the peer review of the initial risk assessments carried out by the competent authorities of the rapporteur Member State, Denmark, and co-rapporteur Member State, the Netherlands, for the pesticide active substance *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 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* *kurstaki* strain EG2348 as an insecticide on pome fruits (field use), solanaceous fruiting vegetables (uses in permanent greenhouse and walk-in tunnel) and ornamentals (field, 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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**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 EG2348 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 CBC (Europe) S.r.l. and Certis USA LLC for the renewal of approval of the active substance *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348.

An initial evaluation of the dossier on *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 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 EG2348 according to the representative uses as an insecticide on pome fruits (apple, pear; field uses), solanaceous fruiting vegetables (tomato, aubergine, sweet pepper; uses in permanent greenhouses and walk-in tunnels) and ornamentals (field, permanent greenhouse and walk-in tunnel uses), as proposed at EU level result in a sufficient insecticidal efficacy against the target pests (Lepidopteran larvae).

The uses of *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 according to the representative uses as an insecticide on pome fruit and ornamentals (field uses) and solanaceous fruiting vegetables and ornamentals (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 EG2348 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, two data gaps were identified. A storage stability study of *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 at −18°C in high-water plant commodities is required (data gap). Related to the upper proposed threshold of $1 \times 10^5$ colony forming units (CFU)/g for viable residues on edible plant commodities at harvest, quantification of viable counts linked to specific preharvest intervals (PHIs) (data gap) is requested to finalise the consumer risk assessment. *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 is not proposed to be included into Annex IV of Regulation (EC) No 396/2005 as the consumer dietary risk assessment cannot currently be finalised.

In the area of environmental fate and behaviour, the available information was considered sufficient to complete the necessary environmental exposure assessments with the exception that adequate information to address the uniform principles criterion of the strain EG2348 not being expected to persist and multiply in surface water in concentrations considerably higher than the natural background levels, provided that repeated applications be made over the years, was not available. This has led to the identification of an assessment not finalised.

Satisfactory information was not provided leading to issues not being finalised for the potential effects, infectivity and pathogenicity to non-target terrestrial organisms (bees and non-target arthropods) for representative uses in open field and walk-in tunnels and for the potential effects, infectivity and pathogenicity to soil organisms and to non-target aquatic organisms (algae and aquatic plants) for all representative uses. Satisfactory information was not provided leading to issues not being finalised for the potential effects of toxins/secondary metabolites such as crystal proteins to non-target terrestrial organisms (birds, wild mammals, bees, non-target arthropods and soil organisms) for representative uses in open field and walk-in tunnels and to non-target aquatic organisms (fish, freshwater invertebrates, algae and aquatic plants) for all representative uses.
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Background

Commission Implementing Regulation (EU) No 844/2012\(^1\), as amended by Commission Implementing Regulation (EU) No 2018/1659\(^2\), (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\(^3\). 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 CBC (Europe) S.r.l. and Certis USA LLC for the renewal of approval of the active substance Bacillus thuringiensis subsp. kurstaki strain EG2348. Complying with Article 8 of the Regulation, the RMS checked the completeness of the dossier and informed the applicants, the co-RMS (the Netherlands), the European Commission and EFSA about the admissibility.

The RMS provided its initial evaluation of the dossier on Bacillus thuringiensis subsp. kurstaki strain EG2348 in the RAR, which was received by EFSA on 28 June 2019 (Denmark, 2019). In accordance with Article 12 of the Regulation, EFSA distributed the RAR to the Member States and the applicants, CBC (Europe) S.r.l. and Certis USA LLC, for consultation and comments on 5 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 5 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 applicants were invited to respond to the comments in column 3 of the reporting table. The comments and the applicants’ 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 applicants in accordance with Article 13(3) of the Regulation were considered in a telephone conference between EFSA and the RMS on 20 May 2020. On the basis of the comments received, the applicants’ response to the comments and the RMS’s evaluation thereof, it was concluded that no additional information should be requested from the applicants, 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, 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 January 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 EG2348 as an insecticide on pome fruits (apple, pear; field uses), solanaceous fruiting vegetables (tomato, aubergine, sweet pepper; uses in permanent

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\(^1\) 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.

\(^2\) 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.

\(^3\) 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.
greenhouses and walk-in tunnels) and ornamentals (field, permanent greenhouse and walk-in tunnel uses), as proposed by the applicants. 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 (26 May 2020);
- the evaluation table (24 February 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 applicants have 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* strain EG2348 is a bacterium deposited at the ARS Culture Collection (Northern Regional Research Laboratory (NRRL)), at the Microbial Properties Research Unit, U.S. Department of Agriculture Peoria, Illinois, USA, under the reference number NRRL B-18208.

*Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 is a transconjugant strain derived from a *Bacillus thuringiensis* subsp. *kurstaki* strain host into which plasmids from a *Bacillus thuringiensis* subsp. *aizawai* strain were transferred via conjugation. *Bacillus thuringiensis* subsp. *kurstaki* as a bacterial species is indigenous in the intended areas of application.

The representative formulated product for the evaluation was 'Lepinox Plus', a wettable powder (WP) containing 375 g/kg of *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 with a nominal biopotency of $32 \times 10^9$ IU/g (min. $8.5 \times 10^{12}$ CFU/kg, max. $1.1 \times 10^{13}$ CFU/kg).

The representative uses evaluated were field spray applications for the biological control of insect pests of the order Lepidoptera on pome fruits and ornamentals and spray applications on protected solanaceous fruiting vegetables and ornamentals (in both 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 EG2348 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) and EFSA FEEDAP Panel (2012).

Minimum and maximum specification of the technical grade microbial pest control agent (MPCA) used for manufacturing the microbial pest control product (MPCP) is $8.53 \times 10^7$ to $1.35 \times 10^9$ IU/g, respectively. (Min. $1 \times 10^{13}$ CFU/kg, max. $2.9 \times 10^{13}$ CFU/kg).
**Bacillus thuringiensis** subsp. **kurstaki** strain EG2348 is characterised by serotyping, plasmid profiling, activity spectrum, fatty acid analysis, deoxyribonucleic acid (DNA)fingerprinting (amplified fragment length polymorphism; AFLP) and cry toxin analysis. To allow an unequivocal identification of strain EG2348, a set of four highly specific primers based on the sequences of the whole genome and plasmids of strain EG2348 were developed allowing an unequivocal identification of the strain except for the parental strain a **Bacillus thuringiensis** subsp. **aizawai** strain. Identification can be done by whole genome sequence analysis of the strain EG2348.

**Bacillus thuringiensis** subsp. **kurstaki** strain EG2348 contains genes encoding for potential production of cytotoxin type K2 (CytK2), five crystal insecticidal proteins (Cry1Aa, Cry1Ac, Cry1Ia, Cry2Aa and Cry2Ab), two Cry-like proteins and one vegetative insecticidal protein (VIP 3Aa10). Furthermore, **Bacillus thuringiensis** subsp. **kurstaki** strain EG2348 has the potential to form a non-haemolytic (Nhe) and haemolytic (Hbl) enterotoxin complex (which also includes CytK2). The genes for the highly cytotoxic Cyt K1, for the cytotoxic variant of haemolysin II (HlyII), for cereulide and for β-exotoxins are missing in the strain.

The content of microbial contaminants of the MPCP were 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 β-endotoxins are rapidly degradable and endospores are rapidly inactivated when exposed to UV radiation.

Optimum growth conditions for **Bacillus thuringiensis** subsp. **kurstaki** strain EG2348 are 28–30°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 EG2348.

**Bacillus thuringiensis** subsp. **kurstaki** strain EG2348 was shown to be sensitive to all relevant antibiotics as provided in EFSA FEEDAP Panel Guidance (2012). It is sensitive to chloramphenicol, tetracycline, streptomycin, clindamycin, erythromycin, kanamycin, gentamicin and vancomycin. The strain EG2348 has intrinsic resistance to ampicillin and penicillin G.

The main data regarding the identity of **Bacillus thuringiensis** subsp. **kurstaki** strain EG2348 and its biological properties are given in Appendix A.

Acceptable methods for CFU counts of **Bacillus thuringiensis** subsp. **kurstaki** strain EG2348 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 MRL was set for any of the intended uses. The strain-specific molecular markers 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 μg/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 EG2348 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**

From medical data, no adverse health effects were observed in manufacturing personnel working with **Bacillus thuringiensis** subsp. **kurstaki** strain EG2348 during production and packaging of the microbial formulations, when wearing standard personal protective equipment. Few data from the literature have reported clinical infections (e.g. ocular) and dermal irritation caused by **B. 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.

**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 EG2348 may have the potential to provoke sensitising reactions.

For acute oral, pulmonary and intravenous toxicity studies, the studies performed for *Bacillus thuringiensis* subsp. *kurstaki* strain SA-12 have been reported, since *Bacillus thuringiensis* subsp. *kurstaki* strains SA-11 and SA-12 were considered applicable to *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 during the first peer review (EFSA, 2012). For *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348, only acute studies with the formulation were available and were considered sufficient to fulfil the data requirements for the microorganism.

After oral administration of *Bacillus thuringiensis* subsp. *kurstaki* strains SA-11 or SA-12, no mortality, pathogenicity or significant toxic effect was observed but infectivity/persistence and clearance from the gastro-intestinal tract and other organ/tissue were not investigated. In a repeated dose oral toxicity study on sheep with Dipel (containing *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1), no indication of pathogenicity or adverse clinical signs were observed during the study (5 months).

After acute intratracheal administration of the formulation Condor OF (containing *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348), no signs of toxicity or pathogenicity were observed, indications of clearance were noted, but full clearance from the lungs was not reached at the end of the study. 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, the potential concern for serious health effects after repeated exposure by inhalation cannot be excluded (data gap).\(^4\) The RMS disagreed, being of the opinion that the evidence is not sufficient to support a concern. One MS disagreed with this view during the written consultation on the draft EFSA conclusion.

After acute intravenous administration of Condor OF (containing *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348), no signs of toxicity or pathogenicity were detected, and incomplete clearance at day 21 was observed in spleen, liver and lungs.

No acute dermal toxicity study with Lepinox Plus was provided, a study with the closely related strain *Bacillus thuringiensis* subsp. *kurstaki* strain SA-12 (in Costar) revealed no mortality, toxicity or pathogenicity.

No skin irritation and slight eye irritation were observed in studies with Condor OF.

Secondary metabolites/toxins

*Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 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). 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). Considering the available evidence and uncertainties, the threshold of \(10^5\) 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.\(^5\) The RMS, co-RMS and some MSs disagreed. It is noted that the adoption of this threshold is also supported by recent literature data.\(^6\)

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

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\(^4\) 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).

\(^5\) 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).

\(^6\) Bonis M, Felten A, Pairaud S, Dijoux A, Maladen V, Mallet L, 2021. Comparative phenotypic, genotypic and genomic analyses of *Bacillus thuringiensis* associated with foodborne outbreaks in France. PLoS ONE, 16, e0246885.
to intraperitoneal administration or not (data gap). It was concluded that genotoxicity is not a 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. The RMS and one MS disagreed during the written consultation on the draft EFSA conclusion, being of the opinion that these studies were not relevant.

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 *B. 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 (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. Another MS also disagreed during the written consultation on the draft EFSA conclusion.

3. Residues

*Bacillus thuringiensis* subsp. *Kurstaki* strain EG2348 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* strainEG2348.

Considering the available evidence and uncertainties discussed at the expert meeting (see Section 2), 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).

Therefore, only information on viable residues i.e., CFU per g or kg plant commodities at harvest is needed to demonstrate that the threshold of $1 \times 10^5$ CFU/g edible plant commodity is not exceeded.

To ensure this, the setting of a PHI may be needed because available experimental data from residue trials in greenhouse lettuce and peppers (up to $1.78 \times 10^5$ CFU/g 2 days after treatment following three applications of $9.82 \times 10^{15}$ CFU/ha and $1.2 \times 10^5$ CFU/g 5 days after treatment following three application of $1.6 \times 10^{14}$ CFU/ha, respectively), and calculated estimated counts in tomato (up to $9.9 \times 10^5$ CFU/g after the first application) for *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 demonstrate that counts at harvest may be very close to or even exceed this threshold.

A study on greenhouse lettuce conducted with *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 under GAP relevant conditions; however, with three instead of 10 applications, reported levels of up to $9.03 \times 10^4$ CFU one day after treatment. An accumulation of viable counts after each of the three treatments was not observed. Since samples in the lettuce study were stored at –18°C prior to analysis; however, the time period of storage prior to analyses was not reported this study is not considered fully reliable. In addition, storage stability studies of *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 in pome fruits and fruiting vegetables at –18°C are not available and are according to the current international OECD guideline still required (data gap).

Viable counts of commercial *Bacillus thuringiensis* strains including *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 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 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. In the respective experiment in greenhouse lettuce with *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348, a decline by an order of magnitude within 3 days after last treatment was reported.

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7 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).
Considering the above-mentioned threshold of $1 \times 10^5$ CFU/g edible commodity, it is to be noted that while mostly the threshold is respected, several samples were very close to or even slightly exceeding the threshold level. Considering measurement uncertainties and the variability in quantifying viable counts, it can be concluded that there is a considerable risk that the consumer may be exposed to concentrations above the threshold level. This assumption is supported by reported concentration at retail in lettuce samples where *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348, a PCR method with specific primer pairs is available and considered as appropriate. It is, however, noted that whole genome sequencing is available to unambiguously identify the strain (see also Section 1).

Furthermore, it is to be noted that the representative uses on pome fruits and on solanaceous fruiting vegetables (tomatoes, aubergines and sweet peppers) are indicating a BBCH of up to 89 for final treatments which corresponds to the ripe fruits stage and can therefore readily be consumed since a PHI was not specified. Therefore, to ensure that consumers are not exposed to levels of *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 above the agreed threshold level, a quantification of viable counts for the representative uses linked to specific PHIs is requested (data gap).

This information would still need to be addressed before a consumer risk assessment can be finalised.

Because of the above-mentioned data gaps and the consumer dietary risk assessment not being finalised, *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 is not proposed to be 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 EG2348 with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC (see specific Annex VI decision-making criteria in Part II Commission Regulation (EU) No 546/2011). It was concluded that *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 is unlikely to interfere with the methodologies routinely used for such determinations.

*Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 is a laboratory conjugated strain for which marker genes in the strain have not been identified 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 EG2348 than for other naturally occurring *Bacillus thuringiensis* strains, transfer of genetic material by *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 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.

#### 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 EG2348 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 *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 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

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8 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.
9 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.
10 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.

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present above natural background levels in soil, taking into account repeated applications over the years, but multiplication in bulk soil will not occur. PEC 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 EG2348 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 EG2348 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. This conclusion identifies a data gap and assessment not finalised in this respect (see Section 8.1). PEC surface water for the intended use on pome fruit (use pattern with greatest potential for spray drift exposure) and on ornamentals have been calculated. PEC surface water covering the intended protected uses on solanaceous fruiting vegetables were also 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 end points for δ-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 δ-endotoxins, Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ia, Cry2Aa and Cry2Ab. Strain EG2348 has genes encoding these crystal proteins except Cry1Ab. The crystal proteins except Cry1Ab and Cry1Ia 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 EG2348. Genes encoding two Cry-like proteins and VIP 3Aa10 protein are also present in strain EG2348.

It is not known to what extent *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 will produce crystal proteins 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 end points between the different crystal proteins from the available data set that contains measured end points from only a subset of these different δ-endotoxins and/or crystal proteins. Full details of the available experimental end points and which δ-endotoxins or crystal protein test material they were derived from can be found in Appendix A. As these end points were not available for all the δ-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 DT50 soil of 41.3 days, Kdoc estimated at 1,000 mL/g and DT50 water system of 28 days. It can also be considered that these properties might be read across to the two Cry-like proteins and the VIP3 Aa10 protein should they be produced by strain EG2348. 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 for the use on pome fruit. The FOCUS surface water Step 1 and 2 calculator (V3.2) for the crop pome/stome 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 apples (European Commission, 2014a)11 (see Appendix A). It was concluded that the potential for

11 Simulations utilised the agreed Q10 of 2.58 (following EFSA, 2008) and Walker equation coefficient of 0.7.
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

A study investigating potential effects including pathogenicity from *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 to birds was available. Pathogenicity was not observed. Data on infectivity were not reported. Sufficient information was available to address toxicity and pathogenicity to birds from *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348. As concluded in Section 2, sufficient information was available to finalise the assessment of infectivity and pathogenicity of *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 in mammals. A low risk from *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 to birds and wild mammals is concluded for all representative uses.

Sufficient information to assess potential effects including infectivity and pathogenicity from *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 to fish were available and no effects were observed. No toxicity was observed in a 21-day limit test investigating effect of *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 on the reproduction of *Daphnia magna*. Although infectivity and pathogenicity were not reported, such effects would likely have been observed considering the duration of the study. Therefore, sufficient information is available to conclude on low risk to freshwater invertebrates for the representative uses. Insufficient data were available to address potential effects to aquatic organisms (algae and aquatic plants) from *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348. Consequently, a data gap leading to an assessment not finalised was identified for potential effects from *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 to aquatic organisms (algae and aquatic plants) for all representative uses.

Two acute oral studies on honeybee that were terminated after 5 and 11 days were available with mortality in the control groups reaching 22% and 18%, respectively. Although treatment-related effects were not observed the studies were considered too short to address infectivity and pathogenicity from *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 to honeybees. A neutral pH medium is reported to be the optimal condition for production of the microorganism and the neutral pH may occur in some life stages of honeybee larvae according to a published paper. Honeybee larvae may therefore be more sensitive to *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348. Insufficient information was available to address potential effects to honeybee larvae and infectivity and pathogenicity to honeybee from *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348. Consequently, a data gap leading to an assessment not finalised was identified for the potential effects to honeybee larvae and for the infectivity and pathogenicity of *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 to honeybee for the representative uses in open field and in walk-in tunnels. Low risk identified for representative uses in permanent greenhouses as the exposure to honeybees is expected to be negligible. No information was available on potential effects to bumblebees intentionally introduced in permanent greenhouses.

Insufficient data were available to address potential toxicity, infectivity and pathogenicity to non-target arthropods from *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348. In short-term lab studies on two foliar species of non-target arthropods exposed to the formulated product containing the strain EG2348 at a rate more than three times higher than the maximum application rate, no treatment-related effects on mortality and reproduction were observed. Infectivity and pathogenicity was not reported. It is to be noted that the exposure route in the studies was through dermal exposure (glass-plate) which is less relevant than the oral exposure route considering the mode of action of *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 (see Section 4.2). Consequently, a data gap leading to an assessment not finalised was identified for the toxicity, infectivity and pathogenicity of *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 to non-target arthropods for the representative uses in open field and in walk-in tunnels. Low risk was identified for representative uses in permanent greenhouses as the exposure to non-target arthropods is expected to be negligible.

No strain specific information was available on earthworms and other soil macro-organisms to indicate if *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 would be toxic, infectious or pathogenic to earthworms or other soil macro-organisms. Insufficient data were available to address potential adverse effects to soil micro-organisms exposed to *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348. The spores of strain EG2348 are expected to persist and be present above natural background levels in soil (see Section 4.1). A data gap leading to an assessment not finalised was identified for the potential adverse effects, infectivity and pathogenicity to soil organisms from *Bacillus*
thuringiensis subsp. kurstaki strain EG2348 for representative uses in walk-in tunnels and open field. A data gap leading to an assessment not finalised was identified for the potential adverse effects, infectivity and pathogenicity to soil organisms from Bacillus thuringiensis subsp. kurstaki strain EG2348 for permanent greenhouses to account for possible change of destination of the soil within the structure in the longer term (e.g. if the soil is removed and used outside and/or the structure is removed).

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

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

Table 1: Soil

| Compound (name and/or code) | Ecotoxicology |
|-----------------------------|--------------|
| Bacillus thuringiensis subsp. kurstaki strain EG2348 | A data gap and an assessment not finalised were identified for all representative uses |
| Toxins/secondary metabolites such as crystal proteins, Cry1Aa, Cry1Ac, Cry1Ia, Cry2Aa, Cry2Ab, 2 Cry-like proteins and Vip 3Aa10 protein | A data gap and an assessment not finalised were identified for representative field and walk-in tunnel uses |

Table 2: Groundwater (a)

| Compound (name and/or code) | Biological (pesticidal) activity/relevance Step 2 | Hazard identified Steps 3b and 3c | Consumer RA triggered Steps 4 and 5 | Human health relevance |
|-----------------------------|-----------------------------------------------|----------------------------------|---------------------------------|-----------------------|
| Toxins/secondary metabolites such as crystal proteins, Cry1Aa, Cry1Ac, Cry1Ia, Cry2Aa, Cry2Ab, 2 Cry-like proteins and Vip 3Aa10 protein | No | Yes | Assessment not triggered. Equivocal results in a micronucleus test (with intraperitoneal administration) | No |

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

Table 3: Surface water and sediment

| Compound (name and/or code) | Ecotoxicology |
|-----------------------------|--------------|
| Bacillus thuringiensis subsp. kurstaki strain EG2348 | A data gap and an assessment not finalised were identified for all representative uses |
| Toxins/secondary metabolites such as crystal proteins, Cry1Aa, Cry1Ac, Cry1Ia, Cry2Aa, Cry2Ab, 2 Cry-like proteins and Vip 3Aa10 protein | A data gap and an assessment not finalised were identified for all representative uses |
Particular conditions proposed to be taken into account by risk managers

Risk mitigation measures (RMMs) identified following consideration of Member State (MS) and/or applicants’ 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 | Ornamentals (field) | Ornamentals (permanent greenhouse) | Ornamentals (walk-in tunnels) | Pome fruit (field) | Solanaceous fruiting vegetables (permanent greenhouse) | Solanaceous fruiting vegetables (walk-in tunnels) |
|--------------------|---------------------|-----------------------------------|-------------------------------|-------------------|--------------------------------------------------------|--------------------------------------------------|
| Operator risk      | Foliar spray        | Foliar spray                       | Foliar spray                  | Foliar spray      | Foliar spray                                           | Foliar spray                                     |
| Worker exposure    | 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) | 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\(^{12}\) 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 considering the identified data gaps:

a) Lack of sufficient information on the toxicity/infectivity of Bacillus thuringiensis subsp. kurstaki strain EG2348 after repeated inhalation exposure (relevant for all representative uses except permanent greenhouses, see Section 2)

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 could not be concluded in view of the identified data gaps (see Section 3).

a) A storage stability study with Bacillus thuringiensis subsp. kurstaki strain EG2348 in high-water commodities at \(-18^\circ\text{C}\) (relevant for the uses in pome fruits and solanaceous fruiting vegetables, see Section 3).

b) To ensure that consumers are not exposed to levels of Bacillus thuringiensis subsp. kurstaki strain EG2348 above the agreed threshold level, a quantification of viable counts for the representative uses linked to specific PHIs is requested (relevant for the uses in pome fruits and solanaceous fruiting vegetables, see Section 3).

3) Satisfactory information was not available for the potential effects, infectivity and pathogenicity to non-target terrestrial organisms (bees and non-target arthropods) from Bacillus thuringiensis subsp. kurstaki strain EG2348 for the assessment of representative uses in open field and walk-in tunnels and for soil organisms all representative uses (see Section 5).

a) Data and information for the potential effects, infectivity and pathogenicity to non-target terrestrial organisms (bees and non-target arthropods) from Bacillus thuringiensis subsp. kurstaki strain EG2348 (relevant for the representative uses in open field and walk-in tunnels and for soil organisms all representative uses, see Section 5).

4) Satisfactory information was not available on the potential for Bacillus thuringiensis subsp. kurstaki strain EG2348 to persist and multiply in surface water nor for its potential effects, infectivity and pathogenicity to aquatic organisms (algae, and aquatic plants) for the assessment of all representative uses (see Sections 4 and 5).

a) Adequate information to address the uniform principles criterion of the strain EG2348 not being expected to persist and multiply in surface water in concentrations considerably higher than the natural background levels, provided that repeated applications be made over the years, was not available (relevant for all representative uses evaluated; see Section 4).

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\(^{12}\) 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.

\(^{13}\) A data gap leading to an assessment not finalised was identified for the potential adverse effects, infectivity and pathogenicity to soil-organisms from Bacillus thuringiensis subsp. kurstaki strain EG2348 for permanent greenhouses to account for possible change of destination of the soil within the structure in the longer term (e.g. if the soil is removed and used outside and/or the structure is removed).
b) Data and information for the potential effects, infectivity and pathogenicity to non-target aquatic organisms (algae and aquatic plants) from *Bacillus thuringiensis* subsp. *kurstaki* strain EG2348 (relevant for all representative uses, see Section 5).

5) Satisfactory information was not available for the potential effects of toxins/secondary metabolites such as crystal proteins to non-target terrestrial organisms (birds, wild mammals, bees, non-target arthropods and soil organisms) for the assessment of representative uses in open field and walk-in tunnels (see Section 5).

   a) Data and information for the potential effects of toxins/secondary metabolites such as crystal proteins to non-target terrestrial organisms (birds, wild mammals, bees, non-target arthropods and soil organisms) (relevant for the assessment of the representative uses in open field and walk-in tunnels, see Section 5).

6) Satisfactory information was not available for the potential effects of toxins/secondary metabolites such as crystal proteins to non-target aquatic organisms (fish, freshwater invertebrates, algae and aquatic plants) for the assessment of all representative uses (see Section 5).

   a) Data and information for the potential effects of toxins/secondary metabolites such as crystal proteins to non-target aquatic organisms (fish, freshwater invertebrates, algae, and aquatic plants) (relevant for the assessment of all representative uses, see Section 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.

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

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

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in Section 7, has been evaluated as being effective, then 'risk identified' is not indicated in Table 6.)
Table 6: Overview of concerns reflecting the issues not finalised, critical areas of concerns and the risks identified that may be applicable for some but not for all uses or risk assessment scenarios

| Representative use | Ornamen-tuals (field) | Ornamen-tuals (permanent greenhouse) | Ornamen-tuals (walk-in tunnels) | Pome fruit (field) | Solanaceous fruiting vegetables (permanent greenhouse) | Solanaceous fruiting vegetables (walk-in tunnels) |
|--------------------|-----------------------|--------------------------------------|--------------------------------|-------------------|------------------------------------------------------|------------------------------------------------------|
| Operator risk      | Foliar spray          | Foliar spray                          | Foliar spray                   | Foliar spray      | Foliar spray                                         | Foliar spray                                         |
| Worker risk        | Risk identified       | Assessment not finalised              |                                 |                   |                                                      |                                                      |
| Resident/ bystander risk | Risk identified | Assessment not finalised             | $\chi^2$                        | $\chi^2$          | $\chi^2$                                             | $\chi^2$                                             |
| Consumer risk      | Risk identified       | Assessment not finalised              | $\chi^2$                        | $\chi^2$          |                                                      |                                                      |
| Risk to wild non-target terrestrial vertebrates | Risk identified | Assessment not finalised | $\chi^{1.5}$ | $\chi^{3.5}$ | $\chi^{3.5}$ | $\chi^{3.5}$ | $\chi^{3.5}$ |
| Risk to wild non-target terrestrial organisms other than vertebrates | Risk identified | Assessment not finalised | $\chi^{1.5}$ | $\chi^{3.5}$ | $\chi^{3.5}$ | $\chi^{3.5}$ | $\chi^{3.5}$ |
| Risk to aquatic organisms | Risk identified | Assessment not finalised | $\chi^{4.6}$ | $\chi^{4.6}$ | $\chi^{4.6}$ | $\chi^{4.6}$ | $\chi^{4.6}$ |
| 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 8.1 and 8.2. Where there is no superscript number, see Sections 2–7 for further information.

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

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

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

Remaining data gaps not leading to critical areas of concern or issues not finalised have not been identified.

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Abbreviations

AFLP amplified fragment length polymorphism
CFU colony forming units
DT₅₀ period required for 50% dissipation (define method of estimation)
ELISA enzyme-linked immunosorbent assay
FOCUS Forum for the Co-ordination of Pesticide Fate Models and their Use
GAP Good Agricultural Practice
ISO International Organization for Standardization
iv intravenous
K_{doc} organic carbon linear adsorption coefficient
LC_{50} lethal concentration, median
LOQ limit of quantification
mm millimetre (also used for mean measured concentrations)
MPCA microbial pest control agent
MPCP microbial pest control product
MRL maximum residue level
OECD Organisation for Economic Co-operation and Development
PD proportion of different food types
PEC predicted environmental concentration
PEC_{soil} predicted environmental concentration in soil
pF2 pF value of 2 (suction pressure that defines field capacity soil moisture)
PHI preharvest interval
PPE personal protective equipment
ppm parts per million (10^{-6})
qPCR quantitative Polymerase chain reaction
RAR Renewal Assessment Report
RPE respiratory protective equipment
SC suspension concentrate
t_{1/2} half-life (define method of estimation)
UV Ultraviolet
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.2021.6495