Study of the different evaluation areas in the pesticide risk assessment process

Focus on pesticides based on microorganisms

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Abstract

Both chemical and microbial active substances can currently be approved as pesticides in the EU, the provisions of their approval being set under Regulation (EC) No 1107/2009. Although sharing the same legal framework, chemicals and microorganisms used as pesticides have different risk profiles especially because once released into the environment, microbial active substances may produce secondary metabolites, multiply, spread and possibly genetically adapt or transfer antimicrobial resistance genes to other microorganisms. Consequently, the risk assessment process must adjust to the specificities ensuing from the chemical or microbial nature of the active substance. This specific programme focused on the risk assessment of microorganisms used as pesticides, especially on the low-risk criteria linked to antimicrobial resistance and the risk assessment of secondary metabolites. The use of microorganisms in integrated pest management (IPM) programmes was also investigated. In 2020, the recently adopted Farm to Fork Strategy and the Biodiversity Strategy for 2030, two important action plans of the European Green Deal, called for a 50% reduction in the use of and risk from chemical and more hazardous pesticides. Many microorganisms are likely to be approved as low-risk active substances, thus representing important tools to achieve this goal. Given the central role that microbial active substances could play towards a more sustainable food system, a need for information regarding the actual production of secondary metabolites by the microorganisms of interest and projects investigating IPM programmes at national and EU levels was identified.

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Keywords: active substance, integrated pest management, low risk, microorganisms, pesticides, plant protection product, risk assessment

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Declarations of interest: The declarations of interest of all scientific experts active in EFSA's work are available at https://ess.efsa.europa.eu/doi/doiweb/doisearch.

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme. We thank all the members of the Unidad de Productos Fitosanitarios of INIA-CSIC for their kindness and availability as well as Jesús Jiménez-Ruiz and Elena Alonso-Prados for sharing their scientific knowledge and Mercedes Martínez for her administrative support during the fellowship.

Suggested citation: Mombert P, Guijarro Díaz-Otero B and Alonso-Prados JL, 2022. Study of the different evaluation areas in the pesticide risk assessment process. EFSA Journal 2022;20(S1): e200412, 14 pp. https://doi.org/10.2903/j.efsa.2022.e200412

ISSN: 1831-4732

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The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.
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1. Introduction

The main objective of the European Food Risk Assessment Fellowship Programme (EU-FORA) is to allow early to mid-career professionals from European Union (EU) and EFTA countries to widen their knowledge and gain hands-on experience in food safety risk assessment. The work was performed at the Spanish National Institute for Agricultural and Food Research and Technology (INIA – Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria), in the Plant Protection Products Unit (Unidad de Productos Fitosanitarios). This unit acts in Spain as the Independent Evaluation Institution authorised by the Spanish Ministry of Agriculture, Fisheries and Food (Ministerio de Agricultura, Pesca y Alimentación - MAPA) to conduct the risk assessment of pesticides in the following areas: identity and physico-chemical properties, analytical methods, efficacy, metabolism and residues, fate and behaviour in the environment and ecotoxicology; that is to say all aspects of the pesticide risk assessment except human toxicology, whose evaluation is carried out by the Spanish Ministry of Health, Consumer Affairs and Social Welfare.

Both chemical and microbial active substances can be authorised as pesticides in the EU. Regardless of the type of active substance, the placing of plant protection products (PPPs) on the market must comply with Regulation (EC) No 1107/2009 that defines microorganisms as ‘any microbiological entity, including lower fungi and viruses, cellular or non-cellular, capable of replication or of transferring genetic material’ (European Commission, 2009a). Although sharing the same legal framework, chemicals and microorganisms used as pesticides have different risk profiles mainly because once released into the environment, microorganisms can produce and release toxic metabolites, multiply, spread and possibly genetically adapt or transfer antimicrobial resistance genes to other microorganisms. Consequently, the risk assessment process must adjust to the specificities ensuing from the chemical or microbial nature of the active substance.

This specific programme focused on the risk assessment of microorganisms used as pesticides, especially on the low-risk criteria linked to antimicrobial resistance and the risk assessment of secondary metabolites. The fellow also investigated the use of microorganisms in integrated pest management (IPM) programmes.

1.1. Low-risk active substances

There is currently no simple regulatory definition of what a low-risk active substance is in the EU. Instead, the following exclusion criteria were set in Annex II, point 5 of Regulation (EC) No 1107/2009, regardless of the nature of the active substance considered:

‘An active substance shall not be considered of low risk where it is or has to be classified in accordance with Regulation (EC) No 1272/2008 as at least one of the following:

- carcinogenic,
- mutagenic,
- toxic to reproduction,
- sensitising chemicals,
- very toxic or toxic,
- explosive,
- corrosive.

It shall also not be considered as of low risk if:

- persistent (half-life in soil is more than 60 days),
- bioconcentration factor is higher than 100,
- it is deemed to be an endocrine disruptor, or
- it has neurotoxic or immunotoxic effects’.

However, the necessity to make a distinction between chemical active substances and microorganisms was identified thereafter and the low-risk criteria were amended by Regulation (EU) 2017/1432 to ‘reflect the current state of scientific and technical knowledge’. Two exclusion criteria were then established for microorganisms: demonstrated adverse effects on non-target insects for baculoviruses and demonstrated multiresistance to antimicrobials used in human or veterinary medicine for other microorganisms. Additionally, since microorganisms are evaluated at strain level, compliance with the low-risk criteria should be assessed at this same level, especially as the antimicrobial resistance mechanisms of different strains belonging to the same species can vary greatly.

Appendix A displays the current exclusion criteria of the different types of active substances.
1.2. Secondary metabolites

Microorganisms are known to produce metabolites. These metabolites are of two types: primary and secondary metabolites. Primary metabolites are directly involved in general metabolic processes such as the growth, development or reproduction of the microorganism and are not considered of potential concern. Secondary metabolites are biosynthesised from primary metabolites and show biological activities often relating to survival and ecological functions of the organism, such as competition against other micro- and macroorganisms, parasitism, symbiosis and transport of substances (OECD, 2018).

Although most of them are of non-concern, some secondary metabolites might produce undesirable adverse effects and threaten human and animal health or the environment. Consequently, the assessment of the potential risk caused by the production of these metabolites is part of the risk assessment performed in the framework of the approval of a microorganism as legally provided in EU data requirements (European Commission, 2013) and Uniform principles for the evaluation and authorisation of plant protection products (European Commission, 2011). However, various feedbacks revealed a need for guidance in interpreting the specific provisions on metabolites, materialising in a guidance document finalised by the Standing Committee on Plants, Animals, Food and Feed (ScoPAFF) at the end of 2020 (European Commission, 2020a). Applicable from 1 November 2021 onwards, this document proposed a practical stepwise approach to assess the risk related to the production of metabolites by microorganisms (intended to be) used as active substances that can be summed up as follows. After determining the type of assessment required, information on the microorganism and its metabolites is collected through literature searches and experimental data to establish a list of metabolites of potential concern. Then, based on data concerning the actual production of the metabolites by the strain under assessment, the possible routes of exposure and qualitative risk assessment, the list is refined to a list of metabolites of concern. Finally, a quantitative risk assessment is performed for each identified metabolite of concern.

1.3. Integrated Pest Management

According to the FAO, Integrated Pest Management is ‘an ecosystem approach to crop production and protection that combines different management strategies and practices to grow healthy crops and minimize the use of pesticides’. According to the European Commission, it implies the considerations of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of PPPs and other forms of interventions to levels that are economically and ecologically justified, while reducing or minimising risks to human health and the environment.

In May 2020, the European Commission presented the Farm to Fork Strategy, a road map towards a fair, healthy and environmental-friendly EU food system. This action plan includes two pesticide-related goals that should be achieved by 2030: the reduction by 50% of the overall use and risk of chemical pesticides and the reduction by 50% of the use of more hazardous pesticides. By encouraging the use of alternative control techniques such as appropriate cultivation techniques and crop rotation, IPM is one of the main tools in reaching these targets.

The general principles of IPM are set in Annex III to Directive 2009/128/EC and can be summed up as follows:

1) Prevention and/or suppression of harmful organisms by adequate cultivation techniques.
2) Monitoring of harmful organisms with adequate methods and tools.
3) Application of protection measures based on the monitoring of harmful organisms and threshold levels.
4) Preference to biological, physical and non-chemical methods over chemical methods if they provide satisfactory pest control.
5) Preference to specific pesticides with the least side effects on human health, non-target organisms and the environment.
6) Keeping the use of pesticides and other forms of interventions to levels that are necessary.
7) Prevention of resistance development.
8) Checking the success of the applied plant protection measures.

By fitting with several of these principles, microbial pest control products (MPCPs, that is, PPPs using microbial active substances) are an important component of IPM. Firstly, as indicated in principle
4, when providing a sufficient level of pest control, they should be preferred over chemical pesticides. Additionally, MPCPs are usually specific to a particular pest and, as microorganisms are organic matter, they tend to break down more easily and be less persistent than chemical pesticides (Principle 5). Furthermore, microorganisms’ modes of action differ from those of chemical pesticides. Consequently, if used with chemical pesticides or other means of protection, they can be a valuable tool in the resistance prevention strategy (Principle 7). All these principles rely on reducing pesticide applications or using lower doses of chemicals, in other words, reducing dependence on chemical control. Thus, microorganisms used as pesticides play a crucial role in reducing resort to chemical active substances (De Cal y Cortina et al., 2020).

2. Description of the work programme

2.1. Aims

As defined in the final work programme, the main aim of this work programme was to share expertise and develop training activities regarding risk assessment of active substances and PPPs. As agreed with the fellow upon arrival at the hosting sites, the work programme was adapted to focus on microorganisms used as active substances. This slight amendment of the work programme seemed particularly appropriate since two new SANCO guidance documents had been published shortly before the beginning of the fellowship. One deals with the approval and low-risk criteria linked to ‘antimicrobial resistance’ (European Commission, 2020a), while the other addresses the risk assessment of metabolites produced by microorganisms used as plant protection active substances (European Commission, 2020b). Additionally, as a former scientific risk assessor in the Residues and Consumer Safety Unit of Anses (French National Agency for Food, Environmental and Occupational Health Safety), the fellow already had good knowledge of the general concepts regarding the risk assessment of active substances and PPPs at European Level as well as a 6-year experience in the evaluation of the Residues and Metabolism section. Thus, the training sessions provided by INIA-CSIC related mainly to the efficacy section and the environmental fate and behaviours aspects assessed in a dossier.

As previously mentioned, although both chemicals and microorganisms used as active substances fall under the same regulations, the risk assessment process varies depending on the nature of the active substances. During her experience as an assessor of the Residues and Metabolism section, the fellow only evaluated chemical active substances. Thus, to get a comprehensive view of the specificities of the risk assessment of microbial active substances, the fellow had the opportunity to follow a 1-week training course on the risk assessment of microorganisms used as pesticides or biocides organised by the BTSF Academy. The course was very complete and addressed the regulatory framework and all the different scientific aspects (efficacy, toxicology, environmental fate and behaviour, ecotoxicology, sampling techniques) of the risk assessment of microorganisms used as active substances.

In parallel to the training activities, the fellow had to draw up a picture of the situation regarding the approval of microorganisms as active substances in the European Union, with particular attention to the low-risk status and the available information regarding the production of secondary metabolites. This task was then followed by a preliminary assessment of the applicability of the new SANCO Guidance on the risk assessment of metabolites produced by microorganisms used as plant protection active substances (European Commission, 2020b). As for every new guidance document, experience and feedback based on concrete case studies are key elements to assess its suitability. The objective of this assessment was to identify recurrent data gaps and critical points that would require further attention once the guidance document will be applicable for the approval of microorganisms as active substances.

Finally, following the training session on efficacy, the use of microorganisms in IPM programmes was investigated.

2.2. Activities

As a first activity, an Excel database compiling general information on all microbial active substances registered in the EU Pesticide database was created to provide an overview of the use of microorganisms as pesticides in the European Union. The type, approval and low-risk status, and, when available, the reference of the related EFSA conclusion, compliance to low-risk criteria, production of metabolites in the active substance, the product or in situ, known microbial resistances, data gaps related to secondary metabolites and high concerns were made available for 112 microbial active substances. The scientific data available in the database came from the EU Pesticides database
and the consultation of the EFSA Conclusions published at the time of the review. Appendix B lists the EFSA Conclusions consulted to populate the database.

Since numerous data gaps related to the possible production of secondary metabolites, a second database compiling all the metabolites mentioned in the EFSA conclusions of microorganisms used as active substances together with information regarding the production of these metabolites, when available. One of the aims of this task was also to capture the amount of data on secondary metabolites currently available in the monographs. Consequently, EFSA conclusions were also systematically reviewed for data on analytical methods, toxicity or environmental fate. One hundred and twenty secondary metabolites were registered in the database but specific analytical, toxicology and fate data could be reported for only 6, 20 and 5 of them, respectively.

Based on the experience of the UPF members, *Bacillus amyloliquefaciens* and *Beauveria bassiana* were chosen as case studies to assess the applicability of the new guidance document (European Commission, 2020b). As some of the oldest species approved as pesticide active substances in the European Union, sufficient data were presumed to be available in the literature to carry out the case studies. However, since neither the fellow nor members of the UPF team had experience in toxicology, only the first steps of the guidance document were investigated. Indeed, after a few steps, an assessment of the available toxicological data is required to refine the list of metabolites of potential concern that should be further assessed. Consequently, the task mainly consisted in systematic literature reviews on the two microorganism species to identify the secondary metabolites of potential concern. As recommended in the guidance document, the systematic literature reviews were conducted according to EFSA guidance on the submission of scientific peer-reviewed open literature (EFSA, 2011). Taking advantage of INIA’s subscriptions, the literature reviews were performed using Scopus, PubMed and Web of Science databases.

In order to acquire knowledge and deepen her understanding of the efficacy section, the fellow was provided with a lot of documentation and received personal training. This training helped the fellow understand the structure and the different aspects studied under the efficacy section that in reality goes way beyond assessing the sole effectiveness of the product since it also covers aspects like adverse effects and phytotoxicity on target plants, development of resistance, effects on yield and quality of plants or transformation process and other undesirable and unintended side effects.

Stand-alone MPCPs tend to have lower efficacy than chemical PPPs. Nonetheless, since these products tend to be generally less persistent, less harmful to the environment and the non-target organisms (NTOs) and intended as components of IPM programmes, reduced data packages and demonstrated efficacy are usually accepted. However, is the inclusion of MPCPs in IPM programmes used by the applicants to defend the authorisation of their product? To answer this question, the fellow reviewed the efficacy section of various registration reports available on the collaborative platform of the European Commission, CIRCABC. This review was retrained to the MPCPs using a strain of *Bacillus* as an active substance, assuming that this sample of products would permit an overview of the current situation. To complete the picture, the fellow also looked into the national guides for IPM, the ‘*Guías de Gestión Integrada de Plagas*’, developed by the Spanish Ministry of Agriculture, Fisheries and Food (Ministerio de Agricultura, Pesca y Alimentación) to analyse the proposed recommendations regarding the use of MPCPs.

Finally, the fellow received training in environmental fate and behaviour, especially in the different steps and software programmes of FOCUS used to determine the predicted environmental concentrations (PECs) of active substances and degradation products in surface and groundwater. As an application of this training, calculations of PEC$_{sw}$ and PEC$_{sed}$ using software programme STEPS1-2 were performed for crystal proteins, some secondary metabolites produced by Bacillus thuringiensis and the only metabolites for which some basic fate data (DT$_{50}$, $K_{OC}$) were available. However, due to the lack of specific fate data, the assessment could not be further refined using STEP 3 calculations. A summary of the calculations is available in Appendix C.

### 3. Conclusions

#### 3.1. Low-risk active substances

Since low-risk active substances have less-negative effects on human and animal health and the environment, their use should be promoted, according to Directive 2009/128/EC of the European Parliament and the Council on the sustainable use of pesticides (European Commission, 2009b). In particular, as provided in Article 12 of this directive, Member States shall consider in the first place
the use of low-risk pesticides, that is to say, pesticides containing only low-risk substances and for which no specific mitigation measures are required. However, some active substances were authorised in the European Union under Council Directive 91/414/EEC (European Commission, 1991), thus before the legal provisions for the approval of active substances as low-risk. Consequently, although some of these active substances would probably be approved nowadays as low risk, it is not until the renewal of their approval that they will be considered as such. Meanwhile, to help the EU Member States comply with Article 12 of the Commission Directive, the European Commission drew up a list of potentially low-risk active substances approved for use in plant protection (European Commission, 2018).

Of the 65 microorganisms authorised to date as active substances in the European Union, 20 are currently considered low risk. Although representing only 15% of the active substances approved in the European Union (65 of 448), microorganisms represent more than 60% of the low-risk active substances. However, when considering the list of potentially low-risk active substances established by the European Commission, an additional 35 microorganisms are expected to be of low risk, the number of potential low-risk microorganisms adding up to 55 of 65, that is, 85% of the microbial active substances. These numbers outline the central role that microorganisms play as low-risk active substances, thus their key importance in achieving the challenges of the Farm to Fork strategy.

3.2. Applicability of the new SANCO Guidance

Systematic literature reviews were performed to identify the secondary metabolites and toxins produced by the authorised strains of *Bacillus amyloliquefaciens* and *Beauvaria bassiana*. Thanks to the technological advances and important cost reductions of the last decade in whole-genome sequencing (WGS), it is nowadays relatively simple to obtain the genomic profile of a microorganism. Consequently, information was available regarding genes encoding for metabolites that could be produced by these microorganisms. However, little information was found regarding the conditions in which such genes would be expressed, leading to the synthesis of the metabolite. When such information was found, it mainly concerned metabolites already known to be produced by the strain in certain conditions.

The relevant articles captured in the search can be divided into two groups:

- Articles in which a complete genotyping of a specific strain is performed and whose outcome is a very long list of secondary metabolites that could be produced by the microorganism since encoded in its genome.
- Articles investigating the conditions of production of secondary metabolites known for a long time to be produced by a specific species of microorganisms.

The outcomes of the literature searches were thus very exhaustive lists of metabolites encoded in the genome of the strains with no specific information regarding their synthesis in the conditions of production of the Microbial Pest Control Agent (MPCA) or *in situ*, except for already well-known metabolites such as beaunvericin, a toxin produced by *Beauvaria bassiana*. In other words, too much information at the genomic level and too little at the proteomic one. Yet, when conducting a risk assessment of the secondary metabolites produced by a given strain, information regarding the production of the metabolites is essential to estimate the exposure component of the risk.

According to the guidance document, once the list of metabolites of potential concern is established, an additional literature search should be performed for each identified metabolite to determine if there is an indication for antimicrobial activity or hazardousness. When ending up with an exhaustive list of all the possible metabolites encoded in the genome of the microorganism, this represents a tremendous amount of work, especially since it implies searching for hazardous effects on human and non-target organisms (NTOs).

Following the SANCO guidance document, a few steps after establishing a list of metabolites of potential concern comes the question of the actual production of the metabolite. If it cannot be excluded, a risk assessment should be performed requiring the determination of toxicity reference values and ecotoxicity testing. It can therefore be foreseen that without information regarding the production of secondary metabolites, a significant amount of (eco-)toxicity data will have to be generated to avoid numerous data gaps.
3.3. Integrated Pest Management

As previously mentioned (see Section 2.2), data provided in the efficacy section for the assessment of MPCPs can be scarce. In particular, although MPCPs are an important component of IPM programmes and permit to reduce the use of chemical active substances, this aspect, which compensates for their sometimes lower effectiveness as stand-alone products, is not always mentioned by the applicants. Thus, out of the 31 reviewed Registration Reports of MPCPs using a strain of Bacillus as an active substance, only 71% of the documents included specific IPM data or repeated mentions of the possibility to use the product in IPM strategies. Additionally, despite the dedicated IPM section of the Registration Reports template, IPM was not mentioned at all in 10% of the cases.

Mentioned under Article 14 of Directive 2009/128/EC, integrated pest management is a key tool to ensure sustainable use of pesticides, which is one of the objectives of the European Green Deal. According to the regulation, Member States ‘shall ensure that professional users have at their disposal information and tools for pest monitoring and decision making, as well as advisory services on integrated pest management’ and ‘establish appropriate incentives to encourage professional users to implement crop or sector-specific guidelines for integrated pest management on a voluntary basis’ (European Commission, 2009b). To comply with these provisions, Spain developed and implemented 39 crop-type-specific guides for IPM. However, although several of the guides recommend using products containing microorganisms as a general measure, specific species of microorganisms are rarely mentioned and no recommendation about specific strains to be used to control specific pests was found. It could be argued that the Member States are required to respect the principle of free competition and thus cannot recommend a specific species or strain over another when several microbial active substances are authorised to control a given pest. However, this is probably more likely due to the lack of knowledge and scientific projects investigating crop-specific IPM programmes.

An interesting example of scientific projects to promote is the Operational Group FITOSCEREZO project conducted by INIA-CSIC from 2019 to 2021. The project aimed at designing and developing an IPM programme for cherry trees in two regions of Spain, Aragón and Extremadura. The outcome of the project was a new IPM programme allowing the control of six plant diseases1 and six plant pests2 (De Cal y Cortina et al., 2021).

In conclusion, to overcome the need for concrete IPM programmes, more projects should be conducted at national and EU levels to provide suitable sector-specific guidelines to professional users.

3.4. Fellowship experience

Apart from the personal enrichment that always brings an experience in a foreign country, the working programme has been the opportunity to gain experience in different areas of pesticide risk assessment, especially regarding efficacy and environmental fate and behaviour. The fellow also acquired a lot of knowledge about microorganisms, especially in the context of pesticide risk assessment. Finally, having learned to perform systematic literature reviews according to EFSA standards will serve the fellow’s future professional experiences for sure.

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1 Brown rot, caused by Monilinia spp., shot hole, caused by Stigmina carpophila, cherry leaf spot (CLS), caused by Blumeriella jaapii, cherry leaf scorch caused by Apiognomonia erythrostoma, bacterial canker, caused by Pseudomonas syringae, and crown gall, caused by Agrobacterium tumefaciens.

2 Myzus cerasi, Rhagoletis cerasi, Drosophila suzukii, Tetranychus urticae, Frankniella occidentalis and Thrips tabaci.
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European Commission, 2020a. Guidance on the approval and low-risk criteria linked to "antimicrobial resistance" applicable to microorganisms used for plant protection in accordance with Regulation (EC) No 1107/2009, SANTE/2020/12260, 23 October 2020.

European Commission, 2020b. Guidance on the risk assessment of metabolites produced by microorganisms used as plant protection active substances in accordance with Article 77 of Regulation (EC) No 1107/2009, SANTE/2020/12258, 23 October 2020.

OECD (Organisation for Economic Co-operation and Development), 2018. Series on Pesticides No. 98, Working document on the risk assessment of secondary metabolites of microbial biocontrol agents. ENV/JM/MONO\(2018\)33.

**Abbreviations**

Anses | Agence Nationale de la Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail - French Agency for Food, Environmental and Occupational Health & Safety

EU-FORA | EUropean FOod Risk Assessment

INIA | Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria – Spanish National Institute for Agricultural and Food Research and Technology

IPM | Integrated Pest Management

MPCA | Microbial Pest Control Agent

MPCP | Microbial Pest Control Product

NTO | Non-Target Organism

PEC | Predicted Environmental Concentration

PPP | Plant Protection Product

RR | Registration Report

SCoPAFF | Standing Committee on Plants, Animals, Food and Feed

WGS | Whole Genome Sequencing
Appendix A – Exclusion criteria applying for the approval of low-risk active substances in the European Union (consolidated version of Regulation (EC) No 1107/2009)

| Exclusion/Cut-off criteria applying for the approval as low-risk active substance | Chemical active substances | Microorganisms including viruses |
| --- | --- | --- |
|  | Synthetical | Naturally occurring | Emitted and used by plants, animals and other organisms for communication | Baculoviruses | Other microorganisms and viruses |
| (a) classified in accordance with Regulation (EC) No 1272/2008 as any of the following: | | | | |
| — carcinogenic category 1A, 1B or 2; | X | X | X |
| — mutagenic category 1A, 1B or 2; | | | |
| — toxic to reproduction category 1A, 1B or 2; | | | |
| — skin sensitiser category 1; | | | |
| — serious damage to eye category 1; | | | |
| — respiratory sensitiser category 1; | | | |
| — acute toxicity category 1, 2 or 3; | | | |
| — specific target Organ Toxicant, category 1 or 2; | | | |
| — toxic to aquatic life of acute and chronic category 1 on the basis of appropriate standard tests; | | | |
| — explosive, | | | |
| — skin corrosive, category 1A, 1B or 1C; | | | |
| (b) identified as priority substance under Directive 2000/60/EC; | X | X | X |
| (c) deemed to be an endocrine disruptor; | X | X | X |
| (d) neurotoxic or immunotoxic effects | X | X | X |
| persistent (half-life in soil is more than 60 days) or its bio-concentration factor is higher than 100. | | | |
| at strain level, demonstrated multiple resistance to antimicrobials used in human or veterinary medicine | X |
| at strain level, demonstrated adverse effects on non-target insects | | X |

N.B.: Information highlighted in green corresponds to the precisions and additional criteria listed under the Annex of Reg. (EU) 2017/1432, compared to Annex II, Point 5 of Reg. (EC) No 1107/2009.
### Appendix B – References of the EFSA Conclusions used to populate the databases

| EFSA Question Number | Microorganism                                                                 | Reference                                      |
|----------------------|------------------------------------------------------------------------------|------------------------------------------------|
| EFSA-Q-2009-00324    | Adoxophyes orana GV strain BV-0001                                            | EFSA Journal 2012;10(4):2654                   |
| EFSA-Q-2017-00055    | Akanthomyces muscarius Ve6 (formerly Lecanicillium muscarium strain Ve6)      | EFSA Journal 2020;18(6):6121                   |
| EFSA-Q-2015-00021    | Ampelomyces quisqualis strain AQ10                                             | EFSA Journal 2017;15(12):5078                  |
| EFSA-Q-2011-01200    | Aureobasidium pullulans (strains DSM 14940 and DSM 14941)                     | EFSA Journal 2013;11(4):3183                   |
| EFSA-Q-2016-00172    | Bacillus amyloliquefaciens (former subtilis) str. QST 713                    | EFSA Journal 2021;19(1):6381                   |
| EFSA-Q-2015-00614    | Bacillus amyloliquefaciens AH2                                                | EFSA Journal 2020;18(7):6156                   |
| EFSA-Q-2014-00323    | Bacillus amyloliquefaciens MBI 600                                            | EFSA Journal 2016;14(1):4359                   |
| EFSA-Q-2014-00322    | Bacillus amyloliquefaciens strain FZB24                                       | EFSA Journal 2016;14(6):4494                   |
| EFSA-Q-2013-00038    | Bacillus amyloliquefaciens subsp. plantarum D747                             | EFSA Journal 2014;12(4):3624                   |
| EFSA-Q-2011-00999    | Bacillus firmus I-1582                                                        | EFSA Journal 2012;10(10):2868                  |
| EFSA-Q-2012-00776    | Bacillus pumilus QST 2808                                                     | EFSA Journal 2013;11(8):3346                   |
| EFSA-Q-2015-00389    | Bacillus subtilis strain IAB/BS03                                             | EFSA Journal 2018;16(6):5261                   |
| EFSA-Q-2016-00696    | Bacillus thuringiensis subsp. Aizawai strain ABTS-1857                       | EFSA Journal 2020;18(10):6294                  |
| EFSA-Q-2016-00698    | Bacillus thuringiensis subsp. Aizawai strain GC-91                           | EFSA Journal 2020;18(11):6293                  |
| EFSA-Q-2016-00699    | Bacillus thuringiensis subsp. Israeliiens (serotype H-14) strain AM65-52     | EFSA Journal 2020;18(12):6317                  |
| EFSA-Q-2016-00697    | Bacillus thuringiensis subsp. Kurstaki strain ABTS 351                       | EFSA Journal 2021;19(10):6879                  |
| EFSA-Q-2017-00131    | Bacillus thuringiensis subsp. Kurstaki strain EG 2348                        | EFSA Journal 2021;19(4):6495                   |
| EFSA-Q-2017-00133    | Bacillus thuringiensis subsp. Kurstaki strain PB 54                          | EFSA Journal 2021;19(4):6496                   |
| EFSA-Q-2017-00132    | Bacillus thuringiensis subsp. Kurstaki strain SA-11                          | EFSA Journal 2020;18(10):6261                  |
| EFSA-Q-2016-00700    | Bacillus thuringiensis subsp. Kurstaki strain SA-12                          | EFSA Journal 2020;18(10):6262                  |
| EFSA-Q-2009-00250    | Bacillus thuringiensis subsp. Tenebrionis strain NB 176 (TM 14 1)            | EFSA Journal 2013;11(1):3024                   |
| EFSA-Q-2017-00593    | Beauveria bassiana 203                                                        | EFSA Journal 2020;18(11):6295                  |
| EFSA-Q-2015-00362    | Beauveria bassiana IMI389521                                                   | EFSA Journal 2017;15(9):4831                   |
| EFSA-Q-2015-00361    | Beauveria bassiana PPR1 5339                                                   | EFSA Journal 2018;16(4):5230                   |
| EFSA-Q-2014-00324    | Beauveria bassiana strain 147                                                 | EFSA Journal 2015;13(10):4261                  |
| EFSA-Q-2009-00251    | Beauveria bassiana strain ATCC 74040                                          | EFSA Journal 2013;11(1):3031                   |
| EFSA-Q-2009-00252    | Beauveria bassiana strain GHA                                                  | EFSA Journal 2013;11(1):3031                   |
| EFSA-Q-2014-00327    | Beauveria bassiana strain NPP111B005                                          | EFSA Journal 2015;13(10):4264                  |
| EFSA-Q-2009-00338    | Candida oleophila strain O                                                     | EFSA Journal 2012;10(11):2944                  |
| EFSA-Q-2013-00548    | Cerevisiae                                                                    | EFSA Journal 2014;12(6):3583                   |
| EFSA-Q-2015-00582    | Clonostachys rosea strain J1446 (Gliocladium catenulatum strain J1446)        | EFSA Journal 2017;15(7):4905                   |
| EFSA-Q-2014-00656    | Coniothyrium minitans Strain CON/M/91-08 (DSM 9660)                          | EFSA Journal 2016;14(7):4517                   |
| EFSA-Q-2009-00254    | Cydia pomonella Granulovirus (CpGV)                                           | EFSA Journal 2012;10(4):2655                   |
| EFSA-Q-2009-00341    | Helicoverpa armiger Granulovirus (HearNPV)                                    | EFSA Journal 2012;10(9):2865                   |
| EFSA-Q-2013-00833    | Isaria fumosorosea Aopka strain 97 (formerly Paecilomyces fumosoroseae)       | EFSA Journal 2014;12(5):3679                   |
| EFSA-Q-2017-00139    | Metarhizium anisopliae var. anisopliae strain BIPESCO 5/52                   | EFSA Journal 2020;18(10):6274                  |
| EFSA-Q-2015-00546    | Metschnikowia fructicola                                                      | EFSA Journal 2017;15(12):5084                  |
| EFSA-Q-2014-00474    | Mild Pepino Mosaic Virus isolate VC 1                                         | EFSA Journal 2017;15(1):4651                   |
| EFSA-Q-2014-00472    | Mild Pepino Mosaic Virus isolate VX 1                                         | EFSA Journal 2017;15(1):4650                   |
| EFSA-Q-2009-00323    | Paecilomyces fumosoroseus strain Fe9901                                      | EFSA Journal 2012;10(9):2869                   |
| EFSA-Q-2015-00405    | Pasteuria nishizawaiiae Pn1                                                   | EFSA Journal 2018;16(2):5159                   |
| EFSA Question Number | Microorganism | Reference |
|----------------------|--------------|-----------|
| EFSA-Q-2018-00110    | Pepino mosaic virus (PepMV) Chilean (CH2) strain, mild isolate Abp2 (PEPMVO) | EFSA Journal 2021;19(1):6388 |
| EFSA-Q-2018-00111    | Pepino mosaic virus (PepMV) European (EU) strain, mild isolate Abp1 (PEPMVO) | EFSA Journal 2021;19(1):6388 |
| EFSA-Q-2014-00054    | Pepino mosaic virus strain CH2 isolate 1906 | EFSA Journal 2015;13(1):3977 |
| EFSA-Q-2017-00140    | Phlebiopsis gigantea strain FOC PG 410.3 | EFSA Journal 2016;17(10):5820 |
| EFSA-Q-2017-00140    | Phlebiopsis gigantea strain VRA 1835 | EFSA Journal 2016;17(10):5820 |
| EFSA-Q-2017-00140    | Phlebiopsis gigantea strain VRA 1984 | EFSA Journal 2016;17(10):5820 |
| EFSA-Q-2015-00814    | Pseudomonas chlorophirps strain MA342 | EFSA Journal 2017;15(1):4668 |
| EFSA-Q-2011-01198    | Pseudomonas sp. Strain DSMZ 13134 | EFSA Journal 2012;10(12):2954 |
| EFSA-Q-2009-00315    | Pseudomyza flocculosa | EFSA Journal 2015;13(9):4250 |
| EFSA-Q-2015-00520    | Purpureocillium lilacinum strain 251 (former Paecilomyces lilacinus strain 251) | EFSA Journal 2020;18(9):6238 |
| EFSA-Q-2017-00141    | Pythium oligandrum M1 | EFSA Journal 2020;18(11):6296 |
| EFSA-Q-2014-00333    | Saccharomyces cerevisiae strain LAS02 | EFSA Journal 2015;13(12):4322 |
| EFSA-Q-2009-00507    | Spodoptera littoralis nucleopolyhedrovirus (SpliNPV) | EFSA Journal 2012;10(9):2864 |
| EFSA-Q-2017-00142    | Streptomyces K61 (formerly S. griseoviridis) | EFSA Journal 2020;18(7):6182 |
| EFSA-Q-2012-00775    | Streptomycyes lydics WYEC 108 | EFSA Journal 2013;11(11):3425 |
| EFSA-Q-2009-00300    | Trichoderma asperellum (formerly T. harzianum) strain ICC012 | EFSA Journal 2013;11(1):3036 |
| EFSA-Q-2009-00300    | Trichoderma asperellum (formerly T. harzianum) strain TV5 | EFSA Journal 2013;11(1):3036 |
| EFSA-Q-2009-00300    | Trichoderma asperellum (formerly T. harzianum) strain T11 | EFSA Journal 2013;11(1):3036 |
| EFSA-Q-2011-00899    | Trichoderma asperellum strain T34 | EFSA Journal 2012;10(5):2666 |
| EFSA-Q-2009-00297    | Trichoderma atroviride (formerly T. harzianum) strain IMI 206040 | EFSA Journal 2015;13(5):3056 |
| EFSA-Q-2009-00297    | Trichoderma atroviride (formerly T. harzianum) strain T11 | EFSA Journal 2015;13(5):3056 |
| cf. each strain     | Trichoderma atroviride (formerly T. harzianum) strain T11 and IMI 206040 | EFSA Journal 2015;13(5):3056 |
| EFSA-Q-2011-00900    | Trichoderma atroviride strain I-1237 | EFSA Journal 2012;10(10):2706 |
| EFSA-Q-2014-00334    | Trichoderma atroviride strain SC1 | EFSA Journal 2015;13(4):4092 |
| EFSA-Q-2012-00424    | Trichoderma gamssii (formerly T. viride) strain ICC080 | EFSA Journal 2013;11(1):3062 |
| EFSA-Q-2009-00298    | Trichoderma harzianum strain ITEM 908 | EFSA Journal 2013;11(10):3055 |
| EFSA-Q-2009-00298    | Trichoderma harzianum strain T-22 | EFSA Journal 2013;11(10):3055 |
| EFSA-Q-2009-00299    | Trichoderma polysporum strain IMI 206039 | EFSA Journal 2013;11(1):3035 |
| EFSA-Q-2017-00296    | Verticillium albo-atrum (formerly Verticillium dahliae) strain WCS850 | EFSA Journal 2019;17(1):5575 |
| EFSA-Q-2009-00346    | Zucchini yellow mosaic virus – weak strain | EFSA Journal 2012;10(6):2754 |
### Appendix C – Summary of PECSW and PECsed calculations using software programme STEPS1-2

Parameters considered for the calculations – based on data available for different crystal proteins (protoxin, Cry1Aa, Cry1Ab, Cry1Ac & Cry3Bb1)

| Water solubility (mg.L\(^{-1}\)) | 1 |
| DT\(_{50}\) in soil (days) | 41.3 |
| DT\(_{50}\) in sediment/water system (days) | 28 |
| DT\(_{50}\) in water (days) | 110.7 |
| DT\(_{50}\) in sediment (days) | 96 |
| K\(_{dOC}\) (L.kg\(^{-1}\)) | 1,000 |

| Microorganism | Most critical scenario | STEP 1 | STEP 2 |
|---------------|------------------------|--------|--------|
|               | PECSW (µg/L) | PECsed (µg/kg) | PECSW (µg/L) | PECsed (µg/kg) |
| ABTS-1857     | Pepper (vegetables, fruiting); 8 × 182* g/ha; 6-day interval No interception; Southern Europe, March-May | 221.39 | 2,090 | 59.46 | 584.04 |
| GC-91         | Pome fruits, early application; 6 × 80* g/ha; 7-day interval Minimal crop interception; Southern Europe, March-May | 115.29 | 864.26 | 32.36 | 289.51 |
| AM65-52       | Not performed - only indoor uses | | |
| ABTS-351      | Cabbage (Brassica (vegetables, root)); 8 × 64.8* g/ha; 7 day interval No interception; Southern Europe, March-May | 78.82 | 742.4 | 20.18 | 198.2 |
| EG-2348       | Pome fruits, early application; 10 × 234.6* g/ha; 7-day interval Minimal crop interception; Southern Europe, March-May | 563.46 | 4,220 | 135.8 | 1,210 |
| PB-54         | Ornamental trees (pome fruits, early application); 3 × 48* g/ha; 7-day interval Minimal crop interception; Southern Europe, March-May | 34.59 | 259.28 | 11.16 | 100.08 |
| SA-11         | Pome fruits, early application; 6 × 100.3* g/ha; 7-day interval Average crop interception; Southern Europe, March-May | 144.54 | 1,080 | 35.68 | 314.4 |
| SA-12         | Ornamental trees (pome fruits, early application); 6 × 411.4* g/ha; 7-day interval Minimal crop interception; Southern Europe, March-May | 592.86 | 4,440 | 166.42 | 1,490 |

*: The concentrations of applied crystal proteins used in the calculations are based on the average concentration of total Cry-proteins in the technical active substance and the content of technical grade active substance in each representative product.