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Explanatory notes

for the implementation of the data requirements on micro-organisms and plant protection products containing them in the framework of Reg. (EC) No 1107/2009

These Explanatory Notes compile the main elements for the implementation on the data requirements related to micro-organisms laid down under Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013, as amended in 2022. Any views expressed may not in any circumstances be regarded as stating an official position.

The document is not intended to create any legally binding effect and does not substitute the legal requirements laid down in the relevant applicable EU laws. It cannot be relied upon for any legal purposes and it does not establish any binding interpretation of EU laws.

Although detailed information on a number of issues is provided, the content of the document is not exhaustive. Additional data might be required.

Test methods and guidance documents cited, in light of the current scientific and technical knowledge, are to be considered updated at the time of the publication of these Explanatory Notes.

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Version history

October 2023	First version of the explanatory notes

Regulatory framework and purpose of this document

The regulatory framework for Plant Protection Product (PPP) containing an active substance that is a micro-organism is set by Regulation (EC) No 1107/2009. This regulation states that a plant protection product can only be authorised when the active substance has been approved, the product is sufficiently effective and the use of the product does not have immediate or delayed harmful effects on human or animal health and has no unacceptable effects on the environment. These conditions must be met for all PPP independent of the type of active substance (microbial or chemical).

The rules to determine if these conditions are met, are set by the evaluation criteria and the data requirements, i.e. Article 4(3) and Annex II to Regulation (EC) No 1107/2009, Regulation (EU) No 283/2013 (data requirements for active substances), Regulation (EU) No 284/2013 (data requirements for plant protection products), Regulation (EU) No 546/2011 (uniform principles). The relevant provisions in these acts were amended in 2022 as regards active substances which are micro-organisms, and apply from 21 November 2022 onwards[1,2,3,4]. These explanatory notes (hereinafter also referred to as "EN") relate to the updated acts.

To include references in cases where a differentiation is needed between the versions of the regulations before and after the amendment, the correct wording is (taking Regulation (EU) No 283/2013 as an example):

- *To refer to the "old" regulation: "…Regulation (EU) No 283/2013 as it stood before being amended by Regulation (EU) 2022/1439…"*
- *To refer to the "new" regulation: "…Regulation (EU) No 283/2013 as amended by Regulation (EU) 2022/1439…"*

In these EN, citations concerning test methods and guidance documents are made with the sole purpose of supporting a better reading and implementation of the data requirements. However, please note that test methods and guidance documents recommended to fulfil the data requirements on micro-organisms are those listed in the Commission Communications in the framework of the implementation of Part B of the Annexes of Commission Regulation (EU) No 283/2013[5] and Commission Regulation (EU) No 284/2013[6]; which will be updated regularly.

For the assessment of the representative use of an active substance and for PPP (including microbial PPP), the first step of dossier preparation and evaluation is therefore to use all available information to identify what needs to be assessed (problem formulation).

[1] Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market (OJ L 93, 3.4.2013, p. 1).
[2] Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market (OJ L 93, 3.4.2013, p. 85).
[3] 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).
[4] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 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).
[5] Communication from the Commission concerning Part B of the Annex to Commission Regulation (EU) No 283/2013 setting out the data requirements for active substances in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market C/2023/3552 (OJ C 202, 9.6.2023, p. 14).
[6] Communication from the Commission concerning Part B of the Annex to Commission Regulation (EU) No 284/2013 setting out the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market C/2023/3548 (OJ C 202, 9.6.2023, p. 2).

Furthermore, information becoming available during dossier preparation from literature searches or experimental data may trigger (or exclude) the need for further information for other areas of the assessment – the initial strategy for the risk assessment needs to be adapted during this process. For those areas where a hazard has been identified, the assessment should conclude on whether this hazard leads to a foreseeable risk. This assessment for microbial PPP will in most cases be a qualitative assessment, often using a weight-of-evidence approach.

These EN aim to provide relevant information for all stages of dossier preparation and assessment. However, due to the complexity and diversity of micro-organisms, what these EN do not provide is a one-size-fits-all tick-the-box approach for microbial PPP. For each microbial PPP, the appropriate specific approach for the risk assessment should be determined and discussed with the respective Rapporteur Member State prior to submission of a dossier, as provided for in Article 32a of Regulation (EC) No 178/2002[7].

For each area of the risk assessment, the EN provide technical information on how the data requirements can be addressed, or which guidance document or guidelines may apply, and also on the purpose of the section for the risk assessment. By describing why the information is needed and how the information can be used in the risk assessment, the relevance of a section and the best approach to address the section for a particular micro-organism can be better determined. This should be supportive to compile thorough and coherent dossiers.

A draft of these EN has been prepared in a joint effort of the Danish Environmental Protection Agency and the Dutch board for the authorisation of plant protection products and biocides (Ctgb). Upon completion of the first draft, the document has been made available to the European Commission and further elaborated by the EU Biopesticide Working Group experts. On 12 October, these EN have been endorsed by the Standing Committee on Plants, Animals, Food and Feed.

[7] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (OJ L 031 1.2.2002, p. 1).

Glossary of abbreviations and acronyms

5-BA	Five-Batch Analysis
ADI	Acceptable Daily Intake
AMR	Anti-Microbial Resistance
AOAC	Association of Official Analytical Collaboration
ARfD	Acute Reference Dose
CA	Competent Authority
CC-MPCA	Communication from the Commission concerning Part B of the Annex to Commission Regulation (EU) No 283/2013[8]
CC-PPP	Communication from the Commission concerning Part B of the Annex to Commission Regulation (EU) No 284/2013[9]
CFU	Colony-Forming Unit
CoA	Certificate of Analysis
dRR	draft Registration Report
EFSA	European Food Safety Authority
EN	Explanatory Notes
EPPO	European and Mediterranean Plant Protection Organisation
FAO	Food and Agriculture Organization of the United Nations
GAP	Good Agricultural Practice (colloquially refers to GAP-table)
GD	Guidance Document
GEP	Good Experimental Practice
GLP	Good Laboratory Practices
GMO	Genetically Modified Organism
IPM	Integrated Pest Management
IU	International Unit
LOQ	Limit Of Quantification
LWA	Leaf Wall Area
MED	Minimum Effective Dose
MPCA-AM	Microbial Pest Control Agent As Manufactured
MHC	Maximum Hazard Concentration
MHD	Maximum Hazard Dose
MoA	Mode of Action
MoC	Metabolite of Concern
MoPC	Metabolite of Potential Concern
NTO	Non-Target Organism
OB	Occlusion Body
OECD	Organisation for Economic Co-operation and Development
PAE	Pesticide Application Equipment
PEC	Predicted Environmental Concentration
PED	Predicted Environmental Density
PHI	Pre-Harvest Interval
PPP	Plant Protection Product

[8] Communication from the Commission concerning Part B of the Annex to Commission Regulation (EU) No 283/2013 setting out the data requirements for active substances in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market C/2023/3552 (OJ C 202, 9.6.2023, p. 14).

[9] Communication from the Commission concerning Part B of the Annex to Commission Regulation (EU) No 284/2013 setting out the data requirements for plant protection products in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market C/2023/3548 (OJ C 202, 9.6.2023, p. 2).

QPS	Qualified Presumption of Safety
RMS	Rapporteur Member State
RRF	Relative Response Factor
SM/RF	Spent Medium / Rest Fraction
TTC	Threshold of Toxicological Concern
UVCB	Unknown or Variable composition, Complex reaction products or Biological materials

Definitions used in this document

The definitions below, in addition to those provided in the Regulations, are included for the purpose of clarifying the terms used in the context of these EN.

'Claimed active metabolite' means a secondary metabolite present in the MPCA-AM that is claimed to be part of the plant protection action and whose quantitative presence in the final product is considered indispensable to the effect (see A.1.4.1 for further explanation). Claimed active metabolites are included in the specification.

'Consortium' means a qualitatively defined combinations of strains as they occur naturally or by manufacture.

'Consort' means an individual strain that is part of a consortium of strains or isolates.

'Formulation process' means the part of the production process that starts with combining the MPCA-AM (hypothetical or not) with co-formulants, other active substances and/or safeners/synergists, and ends with a finished PPP. This part of the process is absent in process flows where the MPCA-AM is the PPP.

'Formulation process plant' means the industrial plant employed to carry out the formulation process.

'Host range' means the range of different biological host-species that can be infected by a microbial species or strain.

'Infection' means the non-opportunistic introduction or entry of a micro-organism into a susceptible host, where the micro-organism is able to reproduce to form new infective units and persist in the host, whether or not the micro-organism causes pathological effects or disease;

'Infectivity' means the ability of a micro-organism to cause an infection.

'Manufacturing (process)' means the part of the production process that starts with the first operation performed with the seed stock and/or starting materials and ends with a finished a MPCA-AM.

'Manufacturing (process) plant' means the industrial plant employed to carry out the manufacturing process.

'Microbial Pest Control Agent as manufactured' ('MPCA-AM) means the outcome of the manufacturing process of the micro-organism(s) intended to be used as active substance in plant protection products, consisting of the micro-organism(s) and any additives, metabolites (including metabolites of concern), chemical impurities (including relevant impurities), contaminating micro-organisms (including relevant contaminating micro-organisms) and the spent medium/rest fraction resulting from the manufacturing process or, in case of a continuous manufacturing processes where a strict separation between the manufacturing of the micro-organism(s) and the production process of the plant protection product is not possible, a non-isolated intermediate.

'**Minimum Effective Dose**' ('**MED**') means the minimal dose rate that is necessary to achieve sufficient control for the intended use(s).

'Metabolite of concern' means a metabolite produced by the micro-organism under assessment, with known toxicity or known relevant antimicrobial activity, which is present in the MPCA as

manufactured at levels that may present a risk to human health, animal health or the environment, and/or for which it cannot be adequately justified that in-situ production of the metabolite is not relevant for the risk assessment.

'Non-viable micro-organism' means a microbiological entity that is no longer capable of replication or transfer of genetic material.

'Part A active substance' means a substance for which a dossier must be submitted in accordance with Part A (of Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013). This group concerns chemical substances, extracts from biological material, semiochemicals, and secondary metabolites produced by a micro-organism (either purified or as part of a fermentate in which the micro-organism has been deactivated).

'Part B active substance' means a substance for which a dossier must be submitted in accordance with Part B (of Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013). This group concerns (a consortium of) micro-organisms, either with or without secondary metabolites that are claimed to contribute to the substance's overall plant protection action.

'Pathogenicity' means the non-opportunistic ability of a micro-organism to inflict injury and damage to the host upon infection.

'Production process' means the total of the manufacturing process and the ensuing formulation process (if any). In line with the definitions of the two sub-processes, the production process starts with the first operation performed with the seed stock and/or starting materials and ends with a finished PPP.

'Regulatory framework' means the totality of regulatory texts (e.g., Regulations, Directives, guidance documents, working documents, and technical reports) that apply in the context of active substance approval under (EC) No 1107/2009.

'Specification element' means a component, either an active (component), additive, contaminating micro-organism, relevant impurity, or MoC, that has been included in the specification.

'Viability' means the potential of microbial cells (e.g., spores, vegetative cells) to multiply under favourable conditions (e.g., to develop into colonies). Quantitatively, this parameter is approximated as ratio with respect to a defined mass or volume unit (CFUs / spores / cells per g (or mL)). In certain cases, this may be expressed as a % if the mass/volume can be defined. In the context of storage stability, 'viability' refers to the fraction of the content of the micro-organism which is viable.

'Virulence' means the degree of pathogenicity that a pathogenic micro-organism is able to exert in the host.

Reading of the framework in specific cases

The three terms "micro-organism", "active substance", and "MPCA-AM" are already defined in the regulatory texts. However, it is useful to further clarify their use for the purposes of in this document.

- "*micro-organism*"

Defined in Article 3(15) of the Regulation (EC) No 1107/2009, this term is used in this document to refer to the micro-organism which is the active substance, or part of the active substance if secondary metabolites (the "claimed active metabolite") are part of the active substance. If the active substance is a consortium, the term "micro-organism" as used in these EN applies to the consortium itself,

unless otherwise specified. Where the EN refers to micro-organisms other than the active substance (e.g. contaminating micro-organism), this is specified in the text.

- "*active substance*"

Defined in Article 2(2) of Regulation (EC) No 1107/2009, this term is used in this document taking into consideration that there might be cases where the active substance can be a micro-organism alone, a consortium, or one of the two with "claimed active metabolite" as part of the active substance.

- "*MPCA-AM*"

Defined in the Introduction to Part B of the Annexes to Regulations (EU) No 283/2013, 284/2013, and 546/2011, this term is used in these EN when referring to the active substance (i.e., the micro-organism alone, a consortium, or one of the two, with "claimed active metabolite") together with possible additives, secondary metabolites, chemical impurities, contaminating micro-organisms and the spent medium/rest fraction.

Introduction to general concepts and principles of the risk assessment of microbial PPP

SELECTION OF THE APPROPRIATE ASSESSMENT TYPE

(Regulation (EU) No 283/2013, Annex, Introduction)

Before starting to prepare a dossier for active substance approval (or renewal of approval), it is important to determine if Part A (for chemical active substances) and/or Part B (for micro-organisms) of Regulations (EU) No 283/2013 and 284/2013 apply.

Part A covers:

- Chemical substances (including semiochemicals and extracts from biological material);
- secondary metabolites[10] that are purified from a micro-organism by physical means (e.g., filtration, solvent-extraction, crystallisation, (co-)precipitation);
- secondary metabolites (not separated from a micro-organism which produced them and is no longer viable).

Part B covers:

- Micro-organisms (i.e. viable), either as single strain or as multi-strain consortium;
- Micro-organisms (i.e. viable), either as single strain or as multi-strain consortium, and one or more secondary metabolites that are claimed to contribute to the overall efficacy. In this situation, the *direct or indirect* contribution of the micro-organisms themselves is always significant.

  NB: it is pragmatically assumed that, as long as a micro-organism is capable of replication or transfer of genetic material, it will always provide a significant contribution to the overall efficacy, even when the secondary metabolites are solely responsible for the actual pest control activity in terms of the MoA. In those cases, the micro-organisms will at least act as secondary metabolite vector or 'sustained releaser' of the metabolites that exert the pest control.

For active substances not falling under the definition of "micro-organism" (i.e. Article 3(15) of Regulation (EC) No 1107/2009), Part A applies even if these substances are of biological origin (e.g., non-viable microbiological entities). However, upon discussion with the RMS during pre-submission meetings, some information required may be not provided if well justified, as provided for by point 1.5 of the Introduction to the Annex to Regulation (EU) No 283/2013. These EN, even though referred to Part B of Regulations (EU) No 283/2013 and 284/2013, may support applicants and RMS during pre-submission meetings for decision on which information required according to Part A may be not submitted in the dossier, according to the specific case.

QUALITATIVELY DEFINED COMBINATIONS OF STRAINS; CONSORTIA

(Regulation (EU) No 283/2013, Annex, Introduction)

The new versions of the data requirements and uniform principles explicitly include the possibility of having active substances which are a qualitatively defined combination of strains (a microbial consortium). While in plant protection most uses of micro-organisms currently rely on the use of a single strain, in many other areas where micro-organisms are used in the food

[10] Primary metabolites are wholly excluded from consideration, as they are by definition not employed by the micro-organism for any purpose beside maintenance. Even if these substances may add to the plant protection action (as is likely the case for non-viable micro-organisms), identification is not required due to (i) their reasonably assumed trivial nature, (ii) their short half-lives outside the microbial cell, or (iii) their unidentifiable contribution to the MoA.

chain, the use of consortia is more common. This holds not only for brewing, fermenting and bread making, but also for probiotics for humans and animals and for biostimulants (covered by Regulation (EU) No 1009/2019[11]).
From an ecological point of view, a consortium can for example be more efficient as regards plant protection when the separate strains function optimally under different environmental conditions or have different host ranges. Also, the efficacy of the consortium can be increased if the members have different modes of actions against the target, or when certain strains act as helper strains to the micro-organisms responsible for the control activity. It should be noted that also in the case of microbial consortia more is not always better; strains can also negatively affect each other and thereby lower overall efficacy of the active substance.
To note is that active substances which are viruses so far have been approved at species level, i.e. as mixture of several isolates (e.g., isolates of baculoviruses).
While regulatory frameworks regulating microbial consortia intentionally added in the food chain already exist, currently no guidance is available on the risk assessment for microbial consortia for plant protection purpose. It is advised to start a dialogue between applicant and competent authority (and EFSA if this concerns application for approval/renewal of active substances) at an early stage of dossier preparation, i.e. during the pre-submission meetings.
The relevance of data requirements for specific consorts or for the full consortium (active substance) will depend on the characteristics of the consortium and on the proposed use. While certain data requirements will apply unequivocally to all members of a consortium (e.g., the absence of relevant AMR genes for bacterial strains), for other data requirements it may be justified not to provide certain data for all strains in the consortium. A minimum requirement is the qualitative definition of the consortium: all strains must be identified and deposited in a culture collection. It is also always required to define the minimum and maximum content of the micro-organisms in the MPCA-AM (see point 1.4.1 of the Annex Part B to Regulation (EU) No 283/2013), including when the active substance is a consortium of micro-organisms. As for all microbial active substances, which data requirements are relevant should be determined by for example the identity and ecology of the strains, whether the micro-organisms are sufficiently well-known, and from the proposed use (e.g., seed treatment versus post-harvest treatment of fruits). Where new data need to be generated through studies, and where possible, all the consorts may be tested together.

FORESEEABLE RISK
(Regulation (EU) No 283/2013, Annex, Introduction, point 1.1)

The introduction of the data requirements state that a submitted dossier should contain information which is sufficient to evaluate the *foreseeable* risks which the active substance or PPP may entail (point 1.1 of Regulation (EU) No 283/2013 and No 284/2013, respectively). Furthermore, the introduction states that at least the information and results which are referred to in the data requirements themselves should be submitted, but not when this information is not needed due to the nature of the PPP or the proposed use, or when it is technically not possible to supply (see point 1.5 of the Introduction to the Annex of Regulation (EU) No 283/2013).
In effect, this means that all the information needed to conclude that the use of a PPP is sufficiently effective and does not have harmful effects on human and animal health and has no unacceptable effects on the environment must be submitted. It also means that information should not be required if – due to the properties of the substance or its use – the information is not necessary to evaluate foreseeable risks. Taken together, this leads to the question: what are these foreseeable risks for which information is required?

[11] Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003 (OJ L 170, 25.6.2019, p. 1).

In general, a risk is the likelihood of a hazard causing harm. In turn, a hazard is something that has the potential to harm humans, animals or non-target organisms (EFSA, 2016[12]). Risk therefore depends on hazard and the exposure to this hazard. In turn, a foreseeable risk is a risk which is anticipated. For living micro-organisms, including their MoC, a quantitative risk assessment based on thresholds is often not applicable (e.g. due to a low concentration of microbial secondary metabolites that cannot be quantitatively determined with reasonable effort, or because living micro-organisms are capable of multiplication), and qualitative-based approach and weight of evidence are more appropriate.
For instance, based on the guidance SANCO/2020/12258, a qualitative approach is used for starting the assessment to identify which secondary metabolites produced by the micro-organism may be of concern.
Information on what is considered to be a foreseeable risk has been included in the amended Regulations. For example, while prior to the amendment information on genetic stability was required for all micro-organisms 'where appropriate', the amended Regulation indicates that this information is only needed for non-virulent variants of plant pathogenic viruses. Indeed, it is known that the risk of a micro-organism which is not closely related to human pathogens to suddenly mutate into a human pathogen is negligible. Therefore, such risks are excluded as foreseeable risk based on the text of the data requirements.
For those sections of the risk assessment where such information on what is considered to be a foreseeable risk is not included in the requirements or principles, expert judgment is needed to determine what should be and what should not be considered to be a foreseeable risk. For this expert judgment, knowledge on normal microbial ecology (without the use of the microbial PPP) and the body of knowledge on the particular microbial species is highly relevant.
If the applicant has submitted a dossier to another regulatory agency or an authorisation was granted in a country outside the EU, this information should be submitted to the EU CA. Additionally, if the organism was assessed and approved under other regulations (e.g., micro-organism used as probiotic in feed) this information should also be submitted (n.b. information on criteria to dismiss data-provision is included in each section, if applicable).

CASES WHERE INFORMATION IS NOT REQUIRED
(Regulation (EU) No 283/2013, Annex, point 1.5)

Microbial diversity is vast; it includes viruses, bacteria, archaea, protozoa, and fungi (including yeasts). Furthermore, the hazards which may apply from the use of a micro-organism are diverse: for instance possible toxicity of secondary metabolites they can produce, possible pathogenicity, or possible transfer of genetic information to human pathogens that may render these resistant to antibiotics. As a result, the risk assessment of a microbial PPP should be always case by case and able to deal with this diversity of micro-organisms and their potential hazards.

The EU regulatory framework aims to address this diversity by setting data requirements to cover for all potential hazards of all micro-organisms. At the same time, however, because data requirements are set to cover this full range of diversity, not all data requirements will be relevant for the micro-organism under assessment. This is acknowledged by the Regulations (EU) No 283/2013 and (EU) No 284/2013. Three different elaborations of this principle are used in the data requirements:

- Conditional data requirement for which the text of the data requirement clearly indicates for which kind of micro-organisms the data requirement is relevant. An example is data requirement 5.1.1 of Regulation (EU) No 283/2013: '*For micro-organisms excluding viruses*, …'. In this case, it is clear that no information is required

[12] https://www.efsa.europa.eu/en/discover/infographics/hazard-vs-risk

in case the micro-organism is a virus – a statement to this extent suffices.

- Conditional data requirements for which the text of the data requirements, despite acknowledging that this data is needed only in certain cases, does not indicate the specific criteria to dismiss data-provision. An example of this type of conditionality is data requirement 4.2 of Regulation (EU) No 283/2013: '*Where relevant, methods for post-approval monitoring shall be described*.' In this case, a more elaborate statement should be included in the dossier, for example to justify that methods for post-approval are not relevant for the micro-organism as no metabolites of concern have been identified (including references to the sections of the dossier where metabolites of concern are excluded).
- All data requirements where the requested information is not necessary '*owing to the nature of the PPP or its proposed uses, or it is not scientifically necessary, or it is technically not possible to supply*' (point 1.5 of the introduction to the Annex of Regulation (EU) No 283/2013). Also in this case, a justification should be provided to demonstrate the fact that information is not needed or not possible to supply. An example of the latter is when the micro-organism cannot be assigned to a described species (as it is not sufficiently closely related to a described species) – in this case it is not possible to provide the information requested under point 1.3 (ii) of the Annex, Part B of Regulation (EU) No 283/2013.

Please note that in all cases a sound and robust justification is needed as to why certain information or studies are not included in the dossier. It is strongly recommended to discuss the appropriateness of such justifications with the competent authorities (and EFSA, if this concerns application for approval/renewal of active substances) in pre-submission meetings.

NATIONAL REQUIREMENTS

At authorisation level, applicants are also invited to take into consideration possible national requirements which may apply in addition to the EU legal framework.

LOW-RISK STATUS

(Regulation (EU) No 283/2013, Annex, point 1.11(z))

The conditions under which an active substance that is a micro-organism may not be considered a low-risk active substance are given in point 5.2 of the Annex II to Regulation (EC) No 1107/2009 (see Article 22 of Regulation (EC) No 1107/2009).

A PPP must be authorized as a low-risk PPP when all the active substances contained in the PPP are low-risk active substances and no specific risk mitigation measures are needed following a risk assessment (see Article 47 of Regulation (EC) No 1107/2009). Please note the specification that personal protective equipment (e.g., masks) must be worn. Because micro-organisms *per se* are always regarded as potential sensitisers due to the unavailability of validated test methods, the recommendation to wear personal protective equipment is considered to be a non-specific risk mitigation measure (see Point 2.5.1.4 of Part B of the Annex of Regulation (EU) No 546/2011).

GOOD LABORATORY PRACTICE

(Regulation (EU) No 283/2013, Annex, point 3)

Studies within the scope of Directive 2004/10/EC must in principle be performed under Good Laboratory Practice (GLP). However, as stated in point 3.2 of the Introduction of Annex to Regulation (EU) No 283/2013, a derogation is in place for active substances that are micro-organisms. For these substances, tests and analyses performed to obtain data for other aspects than human health may be conducted by official or officially recognised facilities which satisfy at least the requirements set out in points 3.2 and 3.3 of the Introduction of the Annex to Commission Regulation (EU) No 284/2013.

This implies that all studies used for the assessment of the potential effects on human health

should be GLP-compliant, irrespective of the section of the dossier for which the studies are submitted. For example, phenotypic testing concerning the assessment of sensitivity to antimicrobial agents (in the context of the assessment of transferability of relevant antimicrobial resistance genes - see A.2.9 - and the assessment of availability of treatment options – see A.5.1) must be GLP-compliant. However, generation and analysis of WGS data which can be used e.g., to identify AMR or MoC genes, are considered part of the characterisation process of the micro-organisms; consequently, generation and analysis of WGS data can be subject to the above-mentioned derogation from GLP compliance.

SPECIFICATION DATA ON TEST BATCHES
(Regulation (EU) No 283/2013, Annex, Introduction, point 4, and Introduction to Part B, point (vi))

According to Regulation (EU) No 283/2013 and 284/2013, Annex, Introduction, point 4, the test material used in any study included in a dossier must be fully characterised in analogy with the corresponding Reference specification, i.e., it must include data on all defined constituents (see A.1.4). The information also needs to cover batch number, the weight and/or volume of the batch, the manufacturing date, the site where the batch has been manufactured, and the scale of the process (i.e., commercial or pilot).
As most tests are being conducted prior to the assessment of the active substance, batches may lack full compliance with the reference specification as it is ultimately established. In these cases, evidence needs to be submitted that the deviation is not critical to the purposes of the test in which the batch has been used.

GENETIC MODIFICATION
(Regulation (EU) No 283/2013, Annex, point (vii))

As highlighted in (EC) No 1107/2009, Article 48, any genetically modified biological entity must comply with EU Directive 2001/18/EC[13]. Organisms modified through mutagenesis are exempt from compliance to this Directive, as well as organisms that are incapable of replication or transfer of genetic material. Furthermore, GMO that do not end up in the PPP are not considered within the 2001/18/EC-framework.

WHOLE GENOME SEQUENCE DATA

Whole genome sequence (WGS) data can be highly effective to inform the risk assessment and for identification purposes. However, this effectiveness stands or falls by the availability of correctly annotated sequence information. Although annotation may be performed using publicly available databases, the information in these databases is not always properly curated and may be erroneous. The use of WGS data as a screening step in hazard identification should therefore consider such challenge.

In contrast, targeted inquiries of the WGS data in case the sequence data of a specific property of the micro-organism is known are appropriate. This is the case of bacteria for the assessment of anti-microbial resistance (AMR), where the hazard is due to the presence of a known AMR gene in the genome (see A.2.9). Other cases where WGS data may be useful is for the exclusion of the presence of certain virulence factors including the production of secondary metabolites. When using WGS data in the risk assessment, the relevant guidance documents listed under the section “General test methods and guidance documents” of the

[13] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (OJ L 106, 17.4.2001, p. 1).

CC-MPCA should be taken into consideration. If WGS data is included in the dossier, confidentiality of this data may be requested (see Article 63 of Regulation (EC) No 1107/2009).

Please note that, as mentioned under the section “good laboratory practice”, WGS does not need to be GLP compliant (see point 3.2 of the Introduction of Annex to Regulation (EU) No 283/2013), because DNA sequencing and bioinformatic analyses can still be considered as belonging to the characterisation process of the micro-organism.

IUCLID

Active substance dossiers should be submitted using the software application IUCLID (International Uniform ChemicaL Information Database). To aid this process, a crosswalks document[14] is available for the table of contents in the IUCLID versions for dossiers based on the Regulations before and after the amendment (i.e., the Regulations as they stood before being amended by Regulation (EU) 2022/1439 and Regulation (EU) 2022/1440 versus the Regulations as amended by Regulation (EU) 2022/1439 and Regulation (EU) 2022/1440).

OTHER SOURCES OF INFORMATION

- *CC-MPCA and CC-PPP*

  These are reference documents listing guidance documents and test methods recommended to be used to fulfil the requirements set out in the Part B of the Annexes to Regulation (EU) No 283/2013 and to Regulation (EU) No 284/2013.

  Please note that the reference made in these EN to useful guidance documents and test methods are made, as much as possible, through reference to these two Commission Communications. This is due to the fact that the Commission Communication will be regularly updated and will be capturing the evolution of available guidance documents and test methods more than what these EN could do.

  Please note that where provisions of Part B of the Annex to Regulation (EU) No 283/2013 (or No 284/2013) require generation of data based on requirements laid down in Part A of the Annex to Regulation (EU) No 283/2013 (or No 284/2013), the relevant test methods and guidance are listed in the Commission Communication relevant to the implementation of Part A of the Annex to Regulation (EU) No 283/2013[15] (or No 284/2013[16], i.e. regarding chemical active substances or chemical PPP).

- *Commission website*

  This includes information of general regulatory developments and useful links[17].

[14] https://zenodo.org/record/7427313#.Y-p8fHbMI2w

[15] Communication from the Commission in the framework of the implementation of Part A of the Annex of the Commission Regulation (EU) No 283/2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market C/2023/6245 (OJ C 344, 29.9.2023, p. 37)

[16] Communication from the Commission in the framework of the implementation of Part A of the Annex of the Commission Regulation (EU) No 284/2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market C/2023/6285 (OJ C 344, 29.9.2023, p. 1)

[17] https://food.ec.europa.eu/plants/pesticides/micro-organisms_en

FUTURE DEVELOPMENTS

The requirements and criteria for the risk assessment acknowledge the importance of the body of knowledge on the species or higher taxon of a micro-organism for the assessment of individual strains within this taxon. As a result, the same body of knowledge on a taxon should be included and assessed for each strain within a taxon. The risk assessment for a micro-organism could be performed more efficiently while maintaining the same level of safety if the body of knowledge would not need to be assessed as part of the assessment of each strain within the assessment. However, at the time of writing the first version of these EN (December 2022), no procedure is in place to circumvent the re-assessment of the body of knowledge for each new dossier within a taxon. The EU Commission acknowledges such a procedure as a way to make the assessment procedure more efficient and harmonised. Currently (October 2023), work is ongoing at OECD on consensus documents on microbial species used in plant protection. In case a consensus document is available for the micro-organism under assessment, the way the consensus document can be used for the risk assessment will depend on the section of the risk assessment (e.g., human health, biological properties, MoC) and the body of knowledge of the taxonomical group.

For some sections a reference to the consensus document can fully address the data requirements or risk assessment. For these sections of the dossier, the risk assessment of specific strains belonging to a micro-organism taxonomic group can be concluded based on the body of knowledge for the taxonomical group. For example, based on the body of knowledge for *B. amyloliquefaciens*, it can be concluded that *B. amyloliquefaciens* strains are not pathogenic to humans (as it is reflected by the inclusion of this taxonomical unit in the most updated QPS list[18]). The data requirements and the assessment of the pathogenicity to humans can therefore be addressed by referring to the conclusion in the consensus document. Other areas of dossier which may be addressed by the consensus document are for example the history of use, relationship to known pathogens and effects on certain non-target organisms.

For other sections, focused, strain-level information may be needed as indicated by the consensus document and the data requirements. For these sections of the dossier the risk assessment of strains cannot be concluded based on the body of knowledge for the taxonomical group, but the body of knowledge can be used to focus on which data are needed for individual strains within this taxonomical group. An example would be the identity of the micro-organism (i.e., the unequivocal identification of a certain strain as belonging to a certain species): while information at strain level is always needed for this section of the dossier, the body of knowledge as presented in the consensus document can provide information on which analyses are appropriate to determine if a strain belongs to the taxonomical group. For example, for *B. amyloliquefaciens* the relevant genes needed for identification at species level can be indicated, including references to studies describing the methods and results (e.g., which primers, which criteria). In the dossier, strain-specific experimental data can be generated based on the methods selected from the consensus document information.

Other areas where the information from a consensus document can be used to focus the information needed in the dossier are for example the production of secondary metabolites (e.g., by indicating for which secondary metabolites information at strain level is needed – thereby circumventing the need to perform a full secondary metabolite assessment for each strain within the taxonomical group), the production of virulence factors or the delineation of the host range of pathogenic micro-organisms.

Other sections of the risk assessment cannot be addressed by the body of knowledge: for these areas strain-level information is needed. This applies to obvious datapoints such as the

[18] https://www.efsa.europa.eu/en/topics/topic/qualified-presumption-safety-qps

deposition number of the strain, but also for antimicrobial resistance genes transferred horizontally among different bacterial strains. To which sections of the dossier this applies will vary between taxonomical groups. For example, while strain-level information on the effects on non-target organisms may be needed for a dossier of a *Metarhizium* strain, for a bacteriophage dossier no information on non-target organisms may be needed.

Explanatory notes on the data requirements concerning the active substance

The Explanatory Notes provided under this section refers to the data requirements laid down in Part B of the Annex to the Regulation (EU) No 283/2013, relevant to dossiers submitted in the context of applications for approval of active substances that are micro-organisms.

A.1 IDENTITY OF THE APPLICANT, IDENTITY OF THE ACTIVE SUBSTANCE AND MANUFACTURING INFORMATION

A.1.1 Applicant

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 1.1
Relevant evaluation criterion:	-
Relevant decision making criterion:	-
GLP-compliance:	Not relevant
Purpose of this point:	
The applicant must, as such, be identified as entity addressing all issues relating to the active substance, either directly or through a notified representative.	

Conditionality
Not relevant.

Confidentiality
No confidentiality can be claimed for the identity of the applicant.

A.1.2 Producer

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 1.2
Relevant evaluation criterion:	-
Relevant decision making criterion:	-
GLP-compliance:	Not relevant
Purpose of this point	
The producer acts as contact point with regard to manufacturing. Furthermore, producer and corresponding plant locations are fundamental identifiers for the manufacturing process.	

Conditionality
Not relevant.

Confidentiality
Confidentiality can be claimed for the identity of the producer and the manufacturing location, as this information complies with the criteria in (EC) No 1107/2009, Article 63.

A.1.3 Identity, taxonomy and phylogeny of the micro-organism

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 1.3
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.1.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.1.4
Relevant approval criterion	Reg (EC) No 1107/2009, ANNEX II, point 3.4.3

Confidentiality

Confidentiality can be claimed for WGS-data relating to the micro-organism.

(i) Deposition in culture collection

Purpose of this point: Through the deposition of the micro-organism before the time of dossier submission, a sample of the micro-organism is preserved for future reference. Furthermore, the unique deposition number can be useful for the evaluation of scientific literature.

Assessment principle:
To be able to verify the status of the culture collection and the deposition of the strain, the official documents relevant for the deposition of the micro-organism should be included in the dossier.

(ii) Species to which the micro-organism belongs to

Purpose of this point: The micro-organism needs to be identified as unambiguously belonging to a certain species, based on up-to-date methodologies and current knowledge. The identification of the correct species is of crucial importance, as the assessment of a micro-organism may be largely based on the body of knowledge on the species to which the micro-organism is assigned. Please note that the methods used to unequivocally classify the micro-organism to a certain microbial species (this data point) are not necessarily the same methods which are needed to determine if a microbial sample contains the micro-organism under assessment (the latter are the methods included in the dossier to be able to identify the micro-organism at strain level, see A.4.1(a)).

Assessment principle:
To determine whether the micro-organism is correctly identified at species level, both the relevance of the methods used for this classification and the results are evaluated. Which methods are appropriate for a certain micro-organism is determined based on scientific literature describing the most appropriate method(s) for the specific species. Therefore, this information and a justification for the methods used to identify the micro-organism at species level should be included in the dossier. Note that the method itself should also be included under analytical methods (see A.4.1).

Nowadays DNA sequencing (and the comparison with sequences of reference strains) is in principle considered the most appropriate method. For example, sequence analysis can be performed on (several) genes that are conserved within the genus to which the micro-organism belongs. Data from Whole Genome Sequencing (WGS) of the micro-organism can be used.

In the EFSA “Guidance on the characterisation of micro-organisms used as feed additives or as production organisms”[19] it is stated:

- Bacteria: whole genome sequence (WGS) analysis can be used for the characterisation of bacteria (Section 2.1.1 of the same EFSA guidance). Therefore, data from WGS should be used for identification of the micro-organism. This can be achieved by computational approach for taxonomic assignments (e.g. phylogenomics or average nucleotide identity (ANI)), or by comparing the sequences commonly used

[19] EFSA Guidance on the characterisation of micro-organisms used as feed additives or as production organisms (EFSA Journal 2018 ; 16(3) : 5206).

for taxonomic identification (e.g. 16S rRNA gene), or other characteristic genes (e.g. housekeeping genes) to relevant databases.

- Yeasts: As for bacteria, WGS can also be used for the characterisation of yeasts (point 2.1.1). Therefore, data from WGS analysis should be used for identification of the micro-organism. This should be done by phylogenomic analysis (e.g. using a concatenation of several conserved genes to produce a phylogeny against available related genomes).
- Filamentous fungi: When WGS is available, identification should be made by a phylogenomic analysis comparing the genome against available related genomes. If no WGS is available, identification should be made by comparing the 18S rRNA gene and/or ITS regions and other characteristic genes (e.g. tubulin) with sequences deposited in databases.

These methods are also recommended for micro-organisms used as active substances under Regulation (EC) No 1107/2009. However, WGS is in principle not a legally-binding requirement, but it is more or less indispensable for bacteria to investigate potential of transfer of genes conferring resistance to antimicrobials of clinical relevance, and may be useful to exclude potential production of known secondary metabolites.

For Viruses, WGS can also be used, even though they can also be classified based on morphology, chemical composition, and mode of replication. Even though viruses differ in classification, all viruses are similar in structure and contain a nucleic acid (genome made up of DNA or RNA) enclosed in a protein coat (capsid).

In case WGS data of the micro-organism are provided, the ”EFSA statement on the requirements for whole genome sequence analysis of micro-organisms intentionally used in the food chain” should be taken into consideration[20]. This document provides recommendations to applicants on how to describe the analysis and results of WGS data, including quality criteria/thresholds that should be provided/reached (e.g. sequence depth, number of contigs). In addition, several examples are provided of how WGS-based data can be used for the identification of the micro-organism. For bacteria for instance digital DNA-DNA hybridisation (dDDH), average nucleotide identity (ANI), or phylogenomic methods are proposed (e.g. Multi Locus Sequence Typing, MLST). While the first two methods compare sequences genome-to-genome, the latter focusses on sequence similarities of conserved sequences within a species/genus. For fungi, phylogenomic analysis or alignment to a complete reference genome from the same species is proposed.

Reading of the framework in specific cases

Micro-organism belongs to undescribed taxon

In case a micro-organism does not belong to a formally described and named species, it is not possible to identify the micro-organism at species level. How this situation may be dealt with in a regulatory context is described in the ‘Guidance on the characterisation of micro-organisms used as feed additives or as production organisms’. This guidance provides the following information regarding this situation: “In the case that the data do not allow the assignment of the strain under assessment to a known microbial species, its phylogenetic position with respect to the closest relatives should be provided”.

The fact that the micro-organism belongs to an undescribed species will have implications for the dossier, as by definition the body of knowledge on this undescribed species is non-existent. Although the body of knowledge on related described species can (and should) be used for the dossier, more

[20] EFSA statement on the requirements for whole genome sequence analysis of micro-organisms intentionally used in the food chain (EFSA Journal 2021; 19(7):6506).

information at strain level may be required (see for example the approach described for less well-known species in the "Guidance on the risk assessment of metabolites produced by micro-organisms used as plant protection active substances"[21].

(iii) Synonymous, alternative and superseded names

Purpose of this point:
All synonymous, alternative and superseded names are needed as these names may be used in scientific literature or other reports. To facilitate the interpretation of literature and reports in case a different name is used for the micro-organism, information on the relevance of these names for the micro-organism should be provided. This means for example that in addition to listing the superseded names, it should be explained why the name of the species has changed (e.g., reclassification of specific micro-organism or revision of microbial classification), when the name was changed and how the superseded names of the micro-organism and closely related micro-organisms should be interpreted in the context of the risk assessment.

Assessment principle:
The synonymous, alternative and superseded names should be correctly listed, and this information should be incorporated accordingly in the remainder of the dossier. Superseded names must be included in literature searches. According to the "Further guidance on performing and presenting the literature search", an appendix to the EFSA Guidance on the submission on scientific peer-reviewed open literature[22], if in the previous 10 years the strain had been ascribed to a different species, the name of that other species also needs to have been included in the search terms to ensure a comprehensive search is carried out. However, this does not prevent applicants from providing peer-reviewed literature information older than 10 years, if relevant.

(iv) Phylogenetic tree

Purpose of this point:
Information on the relationship of the micro-organism to closely related strains, species or higher taxonomical units is needed to support the risk assessment in several ways. The phylogenetic tree provides information on the possible relationship to human pathogens and to pathogens to non-target organisms (see also A.2.6). In addition, the phylogenetic tree can be used to support the justification for read across between the micro-organism and closely related micro-organisms. However, please note that a close relationship in itself is not sufficient to justify the use of read across, as information on the nature of the trait under study (e.g., if it is a trait encoded by genes harboured in the core genome) should always be provided on the applicability of read across for the property or trait for which information is needed.

Also, a phylogenetic tree will provide supporting evidence in case of a (future) change of taxonomy, as the phylogenetic tree will provide information on the relevance of the change in taxonomy for the risk assessment of the micro-organism (e.g., are the search terms used in

[21] EU Guidance document on the risk assessment of metabolites produced by microorganisms used as plant protection active substances (SANCO/2020/12258).

[22] EFSA Guidance on submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA Journal 2011;9(2):2092 - including appendix https://efsa.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.2903/j.efsa.2011.2092&file=efs22092-sup-0001-Appendix.pdf).

the literature searches still appropriate considering the changes in taxonomy?).

Assessment principle:
The choice for the micro-organisms included in the phylogenetic tree and the methods to build the tree should be adequately justified. The method itself should be submitted under analytical methods (see A.4.1.)

(v) Wild type, mutant or genetically modified micro-organism

Purpose of this point: Whether a micro-organism is a wild type or differs from the wild type is relevant for the interpretation of natural exposure of humans and the environment to related micro-organisms. In addition, for genetically modified organisms, additional regulation applies (see Directive 2001/18/EC on the deliberate release into the environment of GMOs).

Assessment principle:
If the micro-organism is a mutant or genetically modified the differences between the parental strain and the mutant should be explained. This does not only refer to (epi)genetic differences, but also to the effect the (epi)genetic differences may have on the biological properties of the micro-organism, such as persistence, phenotypic difference in host range of a pathogenic micro-organism or the levels of secondary metabolite production under certain conditions. In addition, methods to differentiate the mutant strain from the parental wild type strain should be provided, in accordance to A.4.1(d).

A.1.4 Specification of the microbial pest control agent as manufactured

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 1.4
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.1.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.1.2
GLP-compliance:	5-BA data on relevant contaminating micro-organisms, metabolites of concern, and relevant impurities must be produced under GLP

Purpose of this point:

The specification established for the MPCA-AM provides an acceptable range for the micro-organism itself and possible claimed active metabolites, and furthermore for any additives, metabolites of concern, relevant impurities and relevant contaminating micro-organisms. Its main purpose is to ensure consistency in the manufacturing output in terms of safety and efficacy.

Conditionality

In some cases, the MPCA-AM specification would not even be as meaningful as its PPP-level counterpart, e.g. (i) when micro-organism-viability is significantly modified during the formulation process causing the micro-organism-limits established for the MPCA-AM to be less representative, or (ii) when the micro-organism-content is derived from a bioassay that needs to be performed with the PPP anyway.
Moreover, in cases where the MPCA-AM represents a non-isolated intermediate (or 'hypothetical phase'), the PPP-level specification is simply the only one available.
Regardless of the nature of the case, requests for dismissing data-provision must be adequately substantiated.

N.B. A major disadvantage of lacking an MPCA-AM specification is that any change to the PPP process would be interpreted as a change to the manufacturing process, which triggers evaluation of technical equivalence according to the relevant guidance document listed under Section 1 of the CC-MPCA, albeit in a somewhat stripped-down format; all changes must be described in detail, upon which the Competent Authority decides whether an updated PPP-

specification can be simply derived from the existing one (often the case when the change only affects the co-formulants), or whether a wholly new 5-BA may be required (that corresponds with the existing specification in terms of elements).

Confidentiality
Confidentiality can be claimed for the distinct data relating to production batches; i.e., results of the 5-BA, as well as for the identity and specification of additives. Specifications of the micro-organism including claimed active metabolites, if present, as well as of relevant impurities, relevant contaminating micro-organisms and MoC(s) are not confidential.
Data relating to the 5-BA may be placed in the confidential part of the DAR / RAR, but the specified results (e.g., the practical abstract of the 5-BA in terms of the content of the micro-organism, claimed active metabolites, relevant impurities, contaminating micro-organisms, MoCs) must appear in the respective non-confidential sections.

A.1.4.1 Content of the active substance
Background information
Primarily, a specification must provide clear information on the representative range of the content of the micro-organism and that of claimed active metabolites (see 'Defining a specification', directly below on representativeness). For Part B active substances, the present framework does not consider any active substance categories beyond 'micro-organism' and 'claimed active metabolite'. Fundamental characteristics of the micro-organism and claimed active metabolites are that they (i) are present in the MPCA-AM (and in the PPP[23]), (ii) are sufficiently stable throughout a practical shelf-life (*N.B. Stability is addressed at the product-level*), and (iii) are quantifiable by conventional microbiological, molecular, or analytical methods.

For the purpose of defining a specification, the conceptualisation of what is actually causing the plant protection action is necessarily simplified. After all, in reality a micro-organism's mode of action is the resultant of a complex orchestration of effects against a target organism, many of which rely on untraceable, short-lived chemicals that are produced under specific circumstances *in situ*.
For regulatory purposes, characterisation of the micro-organism itself is in most cases considered to cover this complexity.
The micro-organism must always be included in the specification because for as long as it is capable of replication and – depending on its type – gene transfer, the micro-organism is assumed to either directly or indirectly contribute to the overall plant protection action (even in cases where its direct plant protection action appears to be marginal compared with that of any claimed active metabolites).

Reading of the framework in specific cases

Claimed active metabolites
In case secondary metabolites are, based on appropriate justifications, claimed to contribute significantly to the MoA (i.e., in case they are "claimed active metabolites"), specifications must be included on their regard. To actually distinguish between the secondary metabolites that "significantly" contribute to the MoA and others for which a claim might be meaningless (e.g. due to a minimal or undefined contribution), the applicant may take into consideration whether it is expected that claimed active metabolites can either be predominantly produced during manufacturing, or can

[23] To maintain consistency between the substance -and product-level risk assessments, the MPCA-AM -and PPP-specification must always be equivalent in terms of defined elements and how they are expressed. *N.B. This only applies when both specification levels are relevant to the dossier* (see also A.1.5.1, 'Suggestions on dealing with non-standard manufacturing in the regulatory context', Principle 2).

accumulated in the MPCA-AM to a degree that cannot be achieved by *in situ* production upon proposed use. These considerations may take into account also the stability of such claimed active metabolite, or technical limitations such as the lack of analytical standards which must not, however, limit the risk assessment if the claimed active metabolite is also a MoC (please see SANCO/2020/12258).

Such specification must also include quantitative information concerning the presence of claimed active metabolites in the manufacturing batch to support evaluation on quality of the micro-organism (A.1.5.1) and efficacy of the PPP (P.6).

In case the claimed active metabolite is also a MoC, please note that the specification on its content in the manufacturing batch:

- would also support the risk assessment for possible effects on humans and animals, and on the environment;
- must be provided under this point, rather than under point 1.4.2.3 where identity and quantification of MoC must be provided only if they are unintendedly present in the manufacturing batch (i.e. as impurities).

A.1.4.2 *Identity and quantification of additives, relevant contaminating micro-organisms and relevant impurities*

A.1.4.2.1 Identity and quantification of additives

Additives (e.g., silicon oil, sorbic acid, kaolin) are specifically added to increase the micro-organism's stability and/or to facilitate handling. Restrictions on such additives are described in P.6.1. The identity of additives is considered confidential by default.

The choice of the additive and of its content must be proportionally related to its intended function. Additives may neither significantly enhance nor mitigate the overall efficacy of the micro-organism. In case an additive may reasonably be suspected of such behaviour, dedicated field trials can be requested for verification. As additives are intentionally added under controlled circumstances, their content is not affected by spontaneous variation, other than weighing error. Additives are defined in the specification in terms of chemical identity, content range (min. and max., whenever the content requires batch-specific adaptation to ensure functionality), and function. Moreover dedicated analytical methods are required for the determination of additives in the MPCA-AM (see A.4.1, 'Quantitative methods'). Changes that affect the identity and/or content of additives would trigger an equivalence assessment according to the relevant guidance document listed under Section 1 of the CC-MPCA.

A.1.4.2.2 Identity and content of relevant contaminating micro-organisms

It must be shown that the level and nature of relevant contaminating micro-organisms are within the acceptable limits as stated in the relevant guidance document listed under point.

1.4.2.2 of the CC-MPCA.

In case of indications for the presence of a relevant contaminating micro-organism that is not covered by the set proposed by the relevant guidance document, it must nevertheless be included in the routine screening. Certified screening methodology must be used (see A.4.1, 'Quantitative methods' for additional details), and it is advisable to consult with the RMS and EFSA on a consensus limit prior to data generation.

A.1.4.2.3 Identity and quantification of relevant impurities

Relevant impurities are chemicals that are of concern to humans, animals or the environment, and that may unintentionally end up in production batches during manufacturing. In A.1.5.1,

'The essential process checkup; Potential sources of relevant impurities', likely sources of such impurities are discussed, in order to provide a starting point for effective identification. Note that one often used method of Regulation (EU) No 283/2013, Part A, 1.11 (i.e., making an inventory of all components present in quantities of 1 g/kg or more and typically up to analytical coverage of at least 980 g/kg), is considered an inappropriate method to identify relevant impurities in the micro-organism-context.
Information on the identity and quantification of MoC which are not claimed to be part of the plant protection action must also be provided under this datapoint (see also A.1.4.1 and A.2.8).

A.1.4.3 *Analytical profile of batches*

REPRESENTATIVENESS
An analytical profile of batches is established based on the 5-BA. Stating the batch sizes or submitting a statement whether the batches are from either industrial or pilot scale is part of the key information to be submitted. Representativeness is key in this analysis; the five batches[24] that are tested are pragmatically considered to indicate the variation in the output of the relevant manufacturing process[25], thus ensuring that the assessment relates to the actually produced material. To this end, the examined batches:

- are produced within five years before dossier submission (as evidenced by manufacturing dates on the respective CoAs),
- are produced within a time window that is sufficiently representative of the manufacturing calendar (again, as evidenced by manufacturing dates on the respective CoAs – if needed, amended by a confirmation on the yearly window of operation of the relevant manufacturing plant), and
- are produced according to the relevant process (as evidenced by statement).

EXPRESSION OF CONTENTS
The 5-BA data must present the contents of the specification elements in a meaningful way; the content of the micro-organism must be expressed in a way that most accurately reflects plant protection action.
Although the micro-organism is frequently presented in terms of CFUs, this is not always a good estimator of the numbers or biomass of the micro-organism. For example, for filamentous fungus viable spores is a more appropriate unit to express the content of the micro-organism.
When the content of the micro-organism does not directly relate to efficacy, a less apparent, indirect association is assumed, and inclusion of the micro-organism in the specification is still required – although the accuracy of expression may be less critical.
When plant protection action is expressed in terms of biopotency, which is essentially a parameter that is defined by the conditions set in the appending bioassay, further speciation may be warranted (e.g. correspondence between Biopotency and CFU). An especially relevant condition that directly relates to the GAP is the choice of test species. In these cases, the specified biopotency range must therefore be clearly linked to the target species that has been investigated. The choice of species needs to be justified, mostly with respect to sensitivity. Biopotency should only be established for the product.
Claimed active metabolites (either if there are also MoC or not) and relevant impurities are generally expressed in gravimetric terms, although other terms appropriate to their nature may be considered (e.g., mol per g or mL).

[24] For micro-organisms, five batches may not suffice in a statistical sense to allow derivation of a truly representative range. On the other hand, the number holds a middle ground that allows obtaining a meaningful indication while maintaining a reasonable amount of regulatory burdening.
[25] Here, 'relevant manufacturing process' is defined as the actual process employed in the manufacture of the MPCA that will eventually end up in products to be marketed in the EU (see also A.1.5.1, 'The essential process checkup; Relevance for EU-context and fundamental process characteristics').

For relevant contaminating micro-organisms, it must simply be demonstrated that their content remains below the corresponding threshold indicated by the relevant guidance document listed under point 1.4.2.2 of the CC-MPCA (or, in case of relevant contaminating micro-organisms not mentioned in the guideline, a relevant threshold – see point A.1.4.2.2).

ESTABLISHING RANGES

The specification range for the micro-organism and any claimed active metabolites serves to establish a reference quality for production of the MPCA-AM; it must not be too broad, in order to ensure that batches at either extreme of the range will perform equally with regard to efficacy. At the same time, the range must not be too narrow so that it allows for the variation inherent in the manufacturing process and post-manufacturing productivity of the micro-organism – for this reason, the micro-organism - content range is preferably not directly derived from the 5-BA, as batches often tend to have very similar contents.

For the micro-organism, the minimum of the specified range is therefore primarily proposed by the applicant based on knowledge of the MED, supported by specification data of the batches used in the field trials in which minimal effectivity has been observed. The range maximum is established by simply multiplying the minimum content with a factor of ten (i.e., by 'adding' one log unit). In case a risk assessment is necessary the maximum content must be determined.

Whether the proposal is appropriate in terms of representativeness is subsequently verified with results from the 5-BA, from which a minimum is derived by subtracting three standard deviations from the 5-result average, and a maximum by adding three standard deviations to the average[26]. When the 5-BA range reasonably coincides with the 'one log unit range' based on minimal effectiveness, the latter is considered to be sufficiently appropriate to serve as specification range for the micro-organism.

Establishing a range that exceeds the one log unit broadness is not desirable, as this would at some point result in non-trivial performance differences between minimally and maximally specified batches. Still, when necessitated by unavoidable variation, a broader range will be accepted once adequately justified.

When activity is expressed in terms of biopotency, the above-mentioned approach may be less appropriate and another way to define a range may be warranted.

To establish a range for claimed active metabolites which are not MoC, provisional guidance is given at this point: in principle, a minimum is derived by subtracting three standard deviations from the 5-BA result average, and a maximum by adding three standard deviations. For established relevant impurities and MoCs, only a specified maximum content-threshold is relevant. It is also calculated by adding three standard deviations to the 5-batch result average.

In some cases, the maximum content in the specification for one MoC (or a relevant impurity) could be set based on a level which is a toxicological concern (and not based on the levels in the batches). MoCs which are considered to be of concern only due to *in situ* production are logically not included in the specification.

A.1.5 Information on manufacturing process and control measures for the active substance

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 1.5
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.1.2
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.1.3 and 2.1.5
GLP-compliance:	Not relevant

[26] Although the sample set may be too small, and the contents not purely normally distributed, this practice should reasonably approximate the situation in which about 99 % of all produced batches fall within the established range.

Purpose of this point:

The manufacturing process must (i) be well-controlled, efficient, logical in terms of design and process flows, (ii) adhere to good manufacturing practices, (iii) be conducted under adequate hygienic conditions, and (iv) include a tight, sufficiently sensitive, and fail-safe monitoring system – all to ensure consistent quality in MPCA-AM (and thus PPP) output that complies with the established specification in terms of identity and content.

Conditionality

Describing the manufacturing process is not conditional, and information must be provided.

Confidentiality

Confidentiality can be claimed for data relating to the manufacturing process, as this information complies with the criteria in Regulation (EC) No 1107/2009, Art. 63.

Background information

A.1.5.1 Production and quality control

THE ESSENTIAL PROCESS CHECK-UP

This point provides a practical checklist of things that need to be covered in the manufacturing process description to allow drawing a conclusion on process control, good manufacturing practice, hygiene, and monitoring.

Relevance for EU-context and fundamental process characteristics

For any Part B active substance, the applicant needs to refer to at least one reference process[27] involved in the approval of that substance. All other processes involved in serving the EU are considered additional. Typically, the manufacturing process assessed within the context of substance approval (or Renewal) is designated as reference process. Furthermore, the specification assigned to the material that is being produced by the reference process is considered the reference specification. The reference process retains its special status for the whole substance approval duration, irrespective of any changes made to it afterwards.

Additional manufacturing processes relevant for the EU market must be assessed against the reference limits so that quality and safety of the material used for manufacturing of formulated products is guaranteed regardless of the manufacturing origin.

For any given manufacturing process, reference included, the key identifiers, i.e., technical details, applicant, manufacturer, plant location address and scale – pilot or industrial – must be indicated. Changes in any of these three characteristics (manufacture, plant location or scale of production), or notification of a wholly new process, trigger assessment according to the relevant guidance document listed under Section 1 of the CC-MPCA[28].

Functionality of critical conditions

The manufacturing process may have implications for the assessment. For instance, fermentation conditions may either propagate or inhibit the production of secondary metabolites. In rarer cases the manufactured material may be sufficiently diluted to allow filtration, so that undesirable components may be physically excluded. Given their potential relevance, critical conditions of the manufacturing process and their intended function must be clearly defined, and their functionality evaluated in terms of their effect on the test material. The type of data needed depends on the nature of the respective process design.

[27] Here, the process is considered, rather than the location. Theoretically, multiple, non-equivalent manufacturing processes of the same substance can be performed at a single site.

[28] A technical equivalence assessment triggered by changes of an existing process in terms of scale, location and/or technical details, or by the notification of an additional process can be performed within the course of the substance assessment, or at any given moment after approval of the active substance (but before commissioning of the changed/new process for the EU).

Potential sources of relevant impurities
In rare cases, relevant impurities may be introduced to the MPCA-AM. Of the limited number of conceivable contamination sources, starting materials and additives are the most likely. Adequate descriptions of these ingredients are required, stating e.g., identity, origin, supplier, and purity (whenever relevant). Along with *a priori* knowledge on likely contaminants associated with a given material (e.g., mycotoxins in cereal grains), these data should provide sufficient leads for any further investigation.
Next, contaminated equipment may be considered as source, but the nature of its contribution is considered to be accidental, and therefore unlikely to be picked up within the (long-term) context of approval dossier evaluation. The description of sanitation measures and how equipment is prepared for the process (e.g., removal of residual cleaning agents) should in general suffice to identify systematic issues.

Quality control
As provided for by point 1.5.1 of Part B to the Annex to Regulation (No) 283/2013, quality control steps need to ensure that all MPCA-AM (and thus PPP-) batches produced by the process concerned comply with the established specification with regard to micro-organism-identity and content, and the content of claimed active metabolites, additives, relevant contaminating micro-organisms, relevant impurities, and MoCs.
Commonly, the contents of the specification elements have been established based on results produced by one or more contracted labs that will rarely, if ever, be hired to analyse all batches that will henceforth be produced by the respective manufacturer. Rather, in-house analyses must be capable of accurately identifying incompliant batches.
Contrary to the analytical methods employed by the contracted lab, the in-house counterparts do not need to be validated; pragmatically, the 5-BA is assumed to represent the expected variation among batches. Checking and guaranteeing compliance of production batches with the specification is the responsibility of the applicant.
The robustness of the in-house analytical methods – or assay methods for standardisation, maintenance, and purity of the product – may be examined on a case-by-case basis[29]. First, the analytical methods must be described and specified under Regulation (EU) No 283/2013, Part B, point 4.1, and the quality assurance criteria and results of the analytical investigation must be submitted under Regulation (EU) No 283/2013, Part B, point 1.5.1. To ensure fitness for purpose, the description should at least allow comparison with a typical analytical method (most conveniently, the one employed by the contracted lab) in terms of equipment, materials, conditions, and terms in which the measured contents are expressed.
Secondly, equivalence of performance may be assessed by comparing in-house analytical method results (that are expected to be available anyway) with those generated by the contracted lab for the same batches.
Third, for all monitored parameters the test criteria maintained by the manufacturer must be made explicit so that they can be related with corresponding international thresholds (such as for contaminating micro-organisms) or thresholds that are established in the course of the assessment. The method's LOQ needs to be stated to check whether the method is sufficiently sensitive.

Ideally, quality control batches are drawn at strategic instances during the manufacturing process to allow early detection of unintended changes to the material being manufactured. Of course, this is mostly in the interest of process efficiency, and therefore particularly relevant to the manufacturer. Broadly, regulators are primarily concerned with the quality control steps performed with the starting cultures, the MPCA-AM and PPP, and will at least demand that

[29] This is considered most relevant for the methods used to check for microbial contaminants, relevant impurities, and MoCs.

these materials are under routine monitoring.
The assay results for the starting cultures must evidence preservation of purity and activity, whereas those for the MPCA-AM and PPP should be relatable to the respective specification. Often, for the purpose of routine control, the micro-organism is characterized in terms of spores (per g or mL), as their quantification does not require a laborious incubation step. In many cases however, the micro-organism is specified in terms of CFUs. In these cases, it should be substantiated that checking for viability measures provides a good surrogate for quantifying CFUs (e.g. viable spores for fungi, plaques for bacteriophages, infectivity unit for viruses).

Storage and repurposing
In some cases, the MPCA-AM is stored for a prolonged duration prior to be used for the formulation process. No data are required to show that the specification elements remain within acceptable ranges throughout this period, as (long as) these parameters will be routinely checked further downstream – which is always assumed to be the case, as is discussed above in the context of strategic sampling.

Reading of the framework in specific cases

Suggestions on dealing with non-standard manufacturing in the regular context

The manufacturing process is at the basis of the development of new concepts of microbial active substances and of the design of more efficient process flows. To avoid that novel concepts are abandoned due to some seeming mismatch with the framework, a set of fundamental principles are defined that provide a clear understanding of the aspects that require compliance in any case, for both regulatory and practical reasons. This small set of principles is presented below.

Note that the current information does not pretend to encompass everything that is imaginable today, let alone that which lies beyond. Cases that appear to confuse the principles should not immediately be abandoned. Rather, they should be discussed with the Competent Authority early on in the process to explore possibilities for alignment.

Principle 1 – Defining the MPCA-AM(s) and PPP(s) in the process flow
It is good to remember that the designation 'MPCA-AM' is mainly a regulatory label that clearly identifies the material for which a specification has been established, for reasons that are discussed in detail under A.1.4. Only one practical insight serves to identify the actual MPCA-AM in any process: the MPCA-AM is the material at the boundary of manufacturing and formulation process that directly and without further modification[30] enters the formulation process. Figure A.1.5.1-01 below visualizes the separation between the 'manufacturing-part' of the complete process (depicted in blue), and the 'formulation process-part' (red). As the figure implies, multiple MPCA-AMs may exist within one process flow; it could be the material resulting from (i) post-processing (e.g., after drying of the fermentate), (ii) post-post-processing (e.g., after harvesting spores from a solid phase that has been inoculated after the main fermentation process. In this case, inoculation is the post-processing step), (iii) parallel manufacturing process, or (iv) blending of the materials resulting from (i), (ii), and/or (iii).
Deciding which of these should be considered MPCA-AM is a strategic choice to be taken by the applicant. A pivotal argument in this decision involves planned future amendments to micro-organism manufacturing that would trigger equivalence assessment. Any change to the MPCA-AM described in A.1.5.1, 'The essential process check-up; Relevance for EU-context and fundamental process characteristics' necessitates re-evaluation of the equivalence status, whereas simple blending of

[30] There can be no 'pre-step' within the formulation process. Any modification to the material that carries the micro-organism preceding formulation is automatically considered a manufacturing step. This ensures that consistency between MPCA-AM and PPP is maintained (see Principle 2).

(unmodified) MPCA-AMs does not.

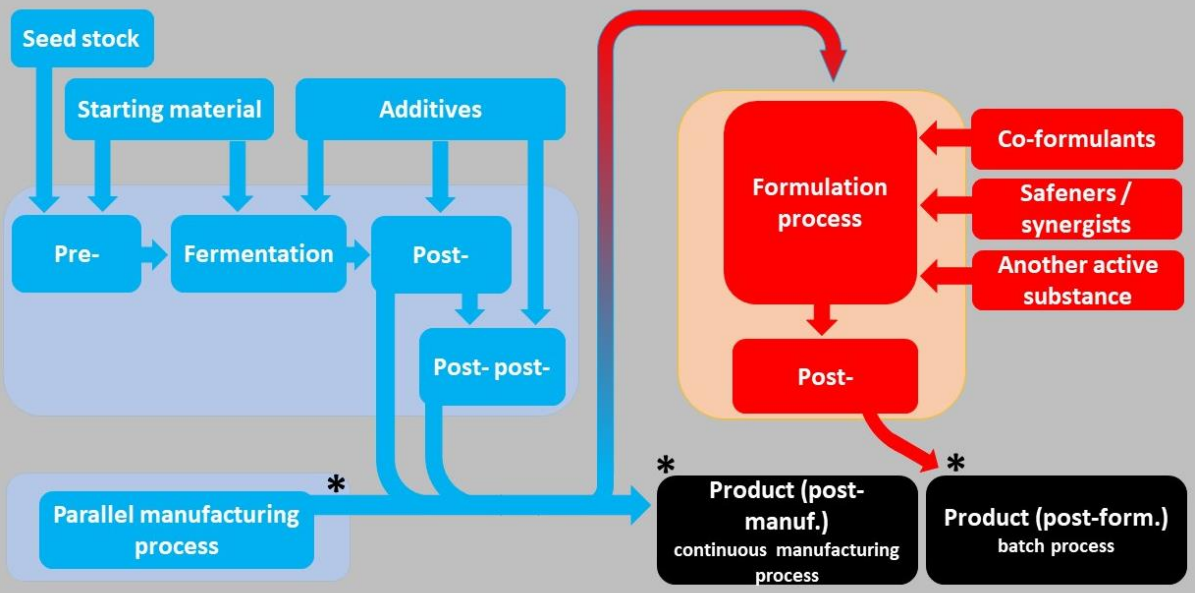

Figure A.1.5.1-01: Generic graphical representation of a typical manufacturing (blue) / formulation (red) process flow. The asterisks indicate the likely instances where material may be sampled for which a specification may be established. Note that the steps 'pre-', 'post-', and 'post-post-' are designated as such just to save space. They should be considered relative to the main process within their box, i.e., as pre-fermentation, post-preparation, etc.

Principle 2 – Consistency between MPCA-AM and PPP

In principle, the PPP-specification should be derivable from the MPCA-AM-specification, by simple multiplication of the concentration of the specification elements in the MPCA-AM, and the concentration of MPCA-AM in the PPP (see also P.1.4). This relationship between both specification levels requires that the element in the MPCA-AM and its corresponding counterpart in the PPP cannot be fundamentally different. For example, from a regulatory perspective it is not possible that the MPCA in the MPCA-AM is viable, while it has been deactivated in the PPP. Any step that involves such a fundamental change to the material is therefore considered part of the 'manufacturing-part' of the process. Further, such steps are excluded during the post-preparation step.

Of course, modifications that are perfectly normal during preparation and post-preparation (e.g., addition of co-formulants, drying) may affect micro-organism viability. In extreme cases where loss of viability would cause a significantly different efficacy, additional actions may be required (e.g., improvement of the formulation process, submission of additional efficacy data and an accompanying 5-BA for the PPP).

Principle 3 – Consortia

A major feature of the new Data Requirements is the consortium concept (see General introduction to Reg (EU) No 283/2013 for the essential details). Aside from the fact that consorts should relate to each other in a meaningful way, they must on an individual level adhere to the criteria of a Part B active substance.

With regard to combining consorts, blending of single-strain/isolate MPCA-AMs, and multi-strain/isolate fermentation are acceptable, although both have their own disadvantages.

Blending of single-strain/isolate MPCA-AMs would require separate 5-BAs for each of the materials involved. For fermentation of multiple consorts in one vessel, the process requires sufficient control to ensure reasonably constant quantities of each participating strain/isolate.

A.1.5.2 *Recommended methods and precautions concerning handling, storage, transport, or fire*

No specific reading necessary for this point.

A.1.5.3 *Procedures for destruction or decontamination*

No specific reading necessary for this point.

A.2 BIOLOGICAL PROPERTIES OF THE MICRO-ORGANISM

General introduction

According to the uniform principles the biological properties and the mode of action of a micro-organism are the first and crucial step in the evaluation process, because they define which are the aspects and elements on which the evaluation should focus, and also which aspects are not relevant for this specific micro-organism. The information provided in this Section can be used as (part of) a justification, by following a weight of evidence approach[31], to address certain points in other sections of the evaluation.

In this section, explanations are included for each data requirement on how the information can support the risk assessments conducted in the other sections, e.g. on human health, residue, environmental occurrence and ecotoxicology (see A.5, A.6, A.7 and A.8).

A.2.1 Origin, occurrence and history of use

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 2.1
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.1.1
Relevant decision making criterion:	-
Criteria for approval	-

Purpose of this point:

In contrast to many conventional chemical active substances, the majority of micro-organisms used as active substances in PPP occurs naturally in EU. Due to this natural occurrence, humans and the environment may already be exposed to micro-organisms which are closely related to the micro-organism under assessment. As a result, the body of knowledge on a microbial species can include information on the (absence of) adverse effects due to this natural exposure. Information on the location from where the micro-organism was isolated (geography and habitat) and the natural occurrence of the species provide information on the extent of the natural exposure of humans and the environment. In addition to this natural exposure, exposure to the micro-organism or closely related micro-organisms may result from (other) uses of these micro-organisms. Therefore, information on the uses of the micro-organism and closely related micro-organisms can provide indication on the extent of the exposure of humans and the environment. These data can be used in the risk assessment to better interpret the information on (the absence of) adverse effects.

Besides providing information on the exposure of humans and the environment, the information on the origin, occurrence and history of use provide information on the biological properties of the micro-organism which is relevant for the risk assessment. These properties include for example the habitat in which the micro-organism is expected to occur and its growing conditions.

<u>Required information</u>

2.1.1. Origin and isolation source

[31] Please refer to the relevant guidance document listed under the section “General test methods and guidance documents” of the CC-MPCA.

In this point, among others, the geographical location and environmental compartment from which the micro-organism was isolated must be given, including the method of isolation and the selection procedure. The description of the method of isolation can provide information on the substrate onto which the strain can grow on, possible host specificity and natural occurrence in an environmental compartment.

Information on the geographical location is especially relevant for micro-organisms with pathogenic MoA against the target pest. As described in A.7.1.2 and the introduction of A.8, the natural occurrence of closely related micro-organisms - and thereby the geographical location from which the micro-organism was isolated - is an important factor in the risk assessment of micro-organisms with pathogenic MoA.

2.1.2. Occurrence

The geographical distribution of the micro-organism and environmental compartment in which the micro-organism occurs should be described at a relevant taxonomical level. A special attention should be given to the occurrence of the micro-organisms in EU environments. As the populations of micro-organisms can be highly dynamic in the environmental matrices (e.g. due to seasonal effect, agricultural practices, dynamic occurrence of hosts), information on absence or presence of the micro-organism may be as informative as information on actual population densities.

Which taxonomical level is relevant for this data point may differ per micro-organism as well as per section of the risk assessment. For example, while species-level information will in general be relevant for micro-organisms of which the MoA is competition, for micro-organisms with pathogenic MoA the natural occurrence of a specific virulence factor of the species may be even more relevant. Similarly, when hazards are identified for a certain toxin produced by the micro-organism which is the active substance, information on the natural occurrence of micro-organisms producing this toxin (i.e., on other strains/genetic variants belonging to the same species of the active substance, or even on different species) may be more relevant than a detailed description of the natural occurrence of the microbial species to which the active substance belongs to (n.b., as other strains/genetic variants belonging to the same species might be not able to produce the same toxin). Therefore, the selected taxonomical level to provide information on the occurrence should be explained.

The origin of the strain under evaluation itself is already described under Regulation (EU) No 283/2013 point 2.1.1. Please note that by definition, a microbial strain does not occur naturally – and the strain can occur in the environment only upon application (see Part B of the Reg (EU) No 283/2013 for definitions relevant for micro-organisms).

2.1.3. History of use

Information on all previous and current uses of the micro-organism (and closely related micro-organsims if relevant) can be used in the risk assessment as it may provide information e.g. on the extent of exposure of human and/or the environment. This information may include research, other commercial uses (biostimulant, probiotics, bioremediation, etc) and uses applied to micro-organisms granted with the QPS status[32]. These uses should therefore not be limited to plant protection or agricultural uses. Information provided in assessment under other relevant regulatory frameworks can be useful to better interpret the information on (the absence of) adverse effects. The relevance for the risk assessment of the information on the history of use of closely related micro-organisms such as microbial strains from the same species or closely related species should be explained.

[32] https://www.efsa.europa.eu/en/topics/topic/qualified-presumption-safety-qps

A.2.2 Ecology and life cycle of the micro-organism

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 2.2
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.1.2
Relevant decision making criterion:	-
Criteria for approval :	-

Purpose of this point:

Information on the ecology of the micro-organism is basic information for any risk assessment of a micro-organism. The ecology of a micro-organism describes how the micro-organisms interacts with its environment including other organisms. Information on the life cycle of the micro-organism also provides general information, such as whether the micro-organism can produce resistant resting stages.

Required information:

The information presented in this point should provide a clear overview of the available information on the ecology and life cycle of the micro-organism. Specific topics which should be addressed are for example:

- Whether the micro-organism is known to be a parasite (e.g., a mycoparasite), a saprophyte, symbiont, endophyte or pathogen. Regarding endophytes, a distinction can be made between obligate and facultative (passenger) endophytes. In case the micro-organism is known to be able to live endophytically, information on the plant parts in which the micro-organism occurs may be relevant for the exposure of humans and non-target organisms.
- What is the life cycle of the organism? For example, can the micro-organism form resting structures and if so, which types and under which conditions?

Fungi and bacteria

All forms in which the micro-organism can occur need to be described. For instance, for fungi and bacteria an overview of available information on resting stages, resistance of spores against environmental conditions (e.g. UV light, heat or possible chemicals present in the environment), survival time of the spores and conditions for germination of spores needs to be provided. In general, the information presented under this point can be based on publicly available scientific information and information obtained for example in preliminary trials.

Likewise, for fungi and bacteria information should be presented on whether the micro-organism is capable of biofilm formation. Micro-organisms in a biofilm are typically embedded by an extracellular matrix which makes them less vulnerable for adverse environmental conditions (as opposite to single planktonic cells), like for instance desiccation. Biofilm formation can also play an important role in pathogenicity (if relevant) as the micro-organism will be more protected against compounds produced by the immune systems of insects or defence system of plants. Moreover, micro-organisms in a biofilm are less susceptible for therapeutic antimicrobials in cases of opportunistic infections.

Bacteriophages

For bacteriophages – viruses which infect bacteria – information should be provided on their lytic and lysogenic properties. Because of their strictly lytic life cycle, virulent bacteriophages have the ability to kill their pathogen hosts and are good candidates for plant protection. Strictly lytic phages do not pose a concern for public health. This is in contrast to temperate bacteriophages, due to the potential transfer of AMR genes. The lysogenic life cycle of temperate bacteriophages does not decrease the population density of their plant pathogenic hosts and can potentially lead to the transfer of genes of concern (including AMR genes) to their host bacteria. Therefore, temperate bacteriophages are of less interest for plant

protection in terms of efficacy and safety (higher probability of horizontal gene transfer).

A.2.3 Mode of action on the target organism and host range

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 2.3
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.1.2 Reg (EU) No 546/2011, Annex, Part B, 1.2.1.3 Reg (EU) No 546/2011, Annex, Part B, 1.2.1.4
Relevant decision making criterion:	-
Criteria for approval :	-

Purpose of this point:

Information on MoA on the target organisms is needed, as this not only explains the function of the active substance that is a micro-organism, but is also extremely helpful for the identification of hazards (e.g. possible production of MoC which are also claimed active metabolites). Hence, special attention should be paid to possible infectivity, pathogenicity, toxicity, and relevant antimicrobial activity in the mode of action against the target organism.

Information regarding the host range of the micro-organism should be given (if applicable), including information on possible population density of host organisms.

Required information:
All available information on mode(s) of action against the target organism(s) need to be provided. Although it is not needed to identify a single mode of action as most important, available information on the relative contributions of the different MoA to the effectiveness should be provided. The MoA supports the understanding of the intended use and function. In other words, it explains why the product(s) based on the micro-organism(s) will be effective against the intended target pest species, when applied at the proposed method, timing, and proposed application rate. As such, the level of detail that is required should be such that it will provide useful information regarding the mechanism of the MoA(s) on the target organism to facilitate the assessment (e.g. to explain how the product will be effective against the target and what could be the hazards). According to point 2.3 all available information on the MoA must be provided. Thus, a concise summary of the available information may suffice and generating additional data would not be required in most cases.

The MoA against the target pest can be direct and/or indirect, based on pathogenicity, parasitism, toxigenicity, production of antimicrobial agents, competition for nutrients or space, plant defences induction, and other mechanisms (n.b. either as a single MoA, or as a combination of different ones).
The efficacy evaluation (addressed in more detail under P.6, Efficacy data) distinguishes direct and indirect MoAs; this influences extrapolation possibilities and the way the risk of possible development of resistance in the target organism(s) is evaluated. When the MoA is direct, the micro-organism will have a direct effect on the target organism(s), e.g. by pathogenicity, infectivity or parasitism or by the production of toxins or antimicrobial compounds. In contrast, during competition for nutrients or space, or the induction of plant defences, the effect of the micro-organism on the target organism is of an indirect nature. During the induction of plant defences, the micro-organism will trigger a systemic resistance in the plant that is (typically) active against a broad range of pathogens. Hence it is not the micro-organism itself that acts against the target organism but host defences of the plant that are induced by the micro-organism.

With a direct MoA, the claimed crops are considered of less relevance and extrapolation of data between crops may be possible (taking into account crop morphology, cropping system,

application technique, feeding are on the plant etc.). With an indirect MoA, the claimed pest is considered as less relevant and extrapolation to other pests may be possible (taking into account life cycle of the pest, feeding behaviour etc.). This is well explained in EPPO standard PP1/296[33] on "The principles of efficacy evaluation for low-risk plant protection products". In addition, specific guidance is available for certain MoAs. There is a general EPPO standard for plant protection products with a predominant mode of action as plant defence inducers (elicitors), EPPO standard PP1/319[34]. For more information regarding the efficacy evaluation is further referred to P.6 (Efficacy data).

Special attention should be paid to possible infectivity, pathogenicity, toxicity, and relevant antimicrobial activity in the MoA against the target organism (note, these are all direct MoAs) to better understand the risks that should be assessed in other Sections.

Infectivity, pathogenicity, parasitism
When the MoA on the target pest is based on infectivity, pathogenicity or parasitism, it is needed to provide information on the site of infection and mode of entry into the target organism(s), infective dose and susceptible stages of the target organism(s). In addition is referred to point A.2.5 on infectivity to the target organism.

Host range
All known organisms must be listed, regardless of the nature of the interaction (being either beneficial, neutral or detrimental to the host). Available information on possible density of these host organisms must be provided, as this will support the indication on natural occurrence of the micro-organism and is relevant for the environmental occurrence of the micro-organism upon application. In case of infectivity and pathogenicity the indicated host range may provide information on the capacity of the micro-organism to infect hosts other than the target or vector (possible risk for NTO).

Toxicity/antimicrobial activity
The production of compounds that have a toxic or antimicrobial effect on the target organisms can be part of the MoA. Here, only the toxic or antimicrobial effect on the target organisms will be discussed. In this case, information should be provided on the mode of action of the secondary metabolite and the exposure route (e.g. way of uptake) of the secondary metabolite to the target organism. See also the relevant information on secondary metabolites and MoA in the general introduction (under selection of the appropriate assessment type).

Note that the assessment of secondary metabolites (including those that can be part of the MoA) regarding potential harmful effects on human and animal health and non-target organisms is discussed in point A.2.8.

A.2.4 Growth requirements

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 2.4
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.1.5
Relevant decision making criterion:	-
Criteria for approval :	-

Purpose of this point:

The information provided in this point should allow defining limiting factors (e.g. UV light, humidity, pH, temperature, and other relevant agro-environmental conditions) influencing the growth of the micro-

[33] https://pp1.eppo.int/standards/PP1-296-1

[34] https://pp1.eppo.int/standards/PP1-319-1

organism. The growth requirements of the micro-organism may give an understanding of its physiological needs and thus to its potential occurrence (e.g. distribution, viability, persistence) in the environment. This information is also relevant for test protocols, for example for non-target testing. Moreover, the provided information may give insights on preferential conditions of use to ensure maximal effectiveness (e.g. the product should be protected from light or preferably applied under conditions of certain humidity).

Required information:
The conditions required for growth and proliferation of the micro-organism needs to be described. It should for instance be stated which nutrients are required. Or in case when a host organism is required for production (e.g. for viruses), which host organism. Growth limiting factors (e.g. UV light, humidity, pH, temperature, osmotic potential) should also be described. Often information on growth conditions is known from scientific literature on closely related strains. However, if the information is insufficient, small scale in vitro laboratory tests may be performed to determine the growth conditions of the micro-organism.

The minimum, optimum and maximum temperature required for growth and proliferation must be reported. This may for instance support the exclusion of an infectivity/pathogenicity potential for human and certain terrestrial vertebrates (e.g. mammals and birds), in case growth at body temperature can be ruled out, as may be the case for psychrophilic (cold-loving) micro-organisms. If the growth temperature data is used as justification (along with other relevant information provided for e.g. point A.2.1., A.2.3 and A.2.6 and data provided for point A.5.1) for non-submission of studies to assess the potential infectivity and pathogenicity of the micro-organism to humans (point A.5.2), the growth temperature study should be carried out under GLP or in a GLP compliant laboratory.

The generation time under favourable growth conditions must be reported. This information is for example relevant for the design and interpretation of tests (e.g., regarding infectivity and pathogenicity).

A.2.5 Infectivity to the target organism

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 2.5
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.1.3
Relevant decision making criterion:	-
Criteria for approval :	-

Purpose of this point:

In case of a pathogenic mode of action (MoA) to the target organism, the factors that enhance the pathogenicity/virulence of a micro-organism and environmental factors affecting them need to be described. This information will justify the conditions of use (e.g. explain how the product should be applied to ensure maximal efficacy).

Required information:
In case of a pathogenic mode of action on the target organism (see A.2.3), information on known virulence factors and (if applicable) environmental factors affecting them need to be provided. Virulence factors (e.g. toxins, surfaces receptors) are factors that enhance the pathogenicity/virulence of a micro-organism. Information may be obtained from experimental studies and/or information from existing literature at the relevant taxonomic level. Please note that information on the infectivity to other organisms than the target organism should be included in either the Section on human health (see A.5.3) or the Section on ecotoxicology (see A.8).

A.2.6 Relationship to known human pathogens and to pathogens to non-target

organisms

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 2.6
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.1.4
Relevant decision making criterion:	-
Criteria for approval:	-

Purpose of this point:

Information on the relationship of the micro-organism to known pathogens to humans, animals and non-target organisms is fundamental information for the hazard identification in Sections A.5 (humans health) and A.8 (ecotoxicological studies). Any available information on the relationship of the micro-organism to known pathogens to humans, animals, plants, and other non-target species should be described at this point, at the most appropriate taxonomic level.

Required information:

In case the micro-organism is related to any known pathogens to humans, animals, crops or other non-target species, these pathogens and type of disease they caused needs to be listed. Known virulence factors of the listed pathogens should be described and (if relevant) compared to known virulence factors belonging to the micro-organism proposed as active substance. The phylogenetic relationship between the micro-organism and the related pathogens needs to be described. Consequently, this data requirement is strongly related to the datapoint described under A.1.3, as the phylogenetic tree provided there should include all relevant known pathogens described for the current datapoint. The chosen taxonomic level to address this point should be explained (e.g. is information provided on genus level or any other (mono)phyletic clade). Lastly, the way or means to distinguish the active micro-organism from pathogenic strains and species needs to be clearly described.

A.2.7 Genetic stability and factors affecting it

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 2.7
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.1.6
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.2.1
Relevant approval criterion (low-risk):	Related to non-target plants: Reg (EC) No 1107/2009 Annex II, point 5.2.2.
Eligible for substantiated dismissal of data-provision:	Yes (see text).

Purpose of this point:

If the micro-organism is a non-virulent variation of a plant pathogen virus, the likelihood of regaining virulence through mutation after application under the proposed conditions of use needs to be discussed. This is needed to assess the hazard of regaining virulence.

Conditionality:

This data requirement is only applicable for non-virulent isolates of plant pathogenic viruses. Hence, data can be dismissed for all other types of micro-organisms.

Required information:

This data requirement is only applicable for non-virulent variants of plant pathogenic viruses (a.k.a mild virus isolates). This specific category of micro-organisms may trigger gene silencing in plants. Gene silencing is a plant defence mechanism. This mechanism will also be effective against more aggressive (virulent) isolates (the target organisms). As gene silencing is based on sequence similarities between the two virus isolates, the non-virulent and virulent virus isolates will by default be very closely related. Consequently, the possibility may exist that the non-virulent virus isolate will regain virulence through mutation. To assess this hazard,

information should be provided for non-virulent virus isolates on the likelihood of regaining virulence through mutation. This can be done for instance by describing the underlying genetic basis that differentiates the non-virulent virus isolate from the virulent virus isolates. If only a single point mutation is underlying the difference between being virulent or non-virulent, the likelihood of (re)gaining virulence is higher than those cases where the difference is based on frameshifts or complete absence of essential virulence genes in the non-virulent isolate. Information regarding possible risk mitigation measures to reduce the likelihood of this to occur should also be provided.

According to Regulation (EU) No 546/2011, Annex, Part B, 2.2.1, no authorisation can be granted for non-virulent virus isolates when the likelihood of (re)gaining virulence and causing adverse effects in target and non-target plants is not negligible (even with possible risk mitigation measures in place).

Regarding low risk criteria, Regulation (EC) No 1107/2009. Annex II, point 5.2.2 indicates that non-virulent isolates of plant pathogenic virus can be considered as low-risk substances, unless they have demonstrated adverse effects on non-target plants.

For micro-organisms other than non-virulent isolates of plant pathogenic viruses, information on genetic stability upon application is in principle not required for this data requirement. For bacteria, information on the presence of transferable AMR genes should be provided as discussed under point A.2.9. Genetic stability of micro-organisms before application is considered as part of the quality assurance process during manufacturing (see A.4.1)

A.2.8 Information on metabolite(s) of concern

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 2.8
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.1.7 (evaluation of metabolites of concern) 1.5 (relevant antimicrobial activity)
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.5.1 (effects on human health[35])
Criteria for approval (low-risk):	-

Purpose of this point

For all micro-organisms except for viruses, information is needed on secondary metabolites which can be produced by the micro-organism. The phrasing of data requirement 2.8 is focused on MoC; however, it should be noted that in order to be able to identify and list the MoC, it is required also to provide all the information on the secondary metabolites produced by the micro-organisms which was collected (e.g., from the published literature or any other reliable source) or generated by the applicant and used to assess whether the secondary metabolites are of concern or not.

A summary and conclusion of the assessment performed by the applicant on the secondary metabolites must be included under the current point (for example in the overview table for the secondary metabolite assessment – see text below). As a result, the information submitted for this datapoint functions as a reading guide for the dossier on the subject of secondary metabolites – without this reading guide it may not be clear why certain information on secondary metabolites is included in other sections of the dossier.

The full form of the information which the above-mentioned summary refers to must be

[35] Please note that “effects on human health” refers to the possible antimicrobial activity of the MoC, and not its possible toxicity (which is dealth with the relevant sections on Effects on Human Health and Ecotoxicological Studies).

submitted under the respective sections of the dossier (i.e., Sections 5, 6, 7 and 8). In contrast, the underlying studies on relevant antimicrobial properties of secondary metabolites which may be present in the plant protection product (as referred to in Regulation (EU) No 546/2011, Annex, Part B, 1.5) should be included under the current point 2.8 along with their summary and conclusion (see Figure A.2.8-01).

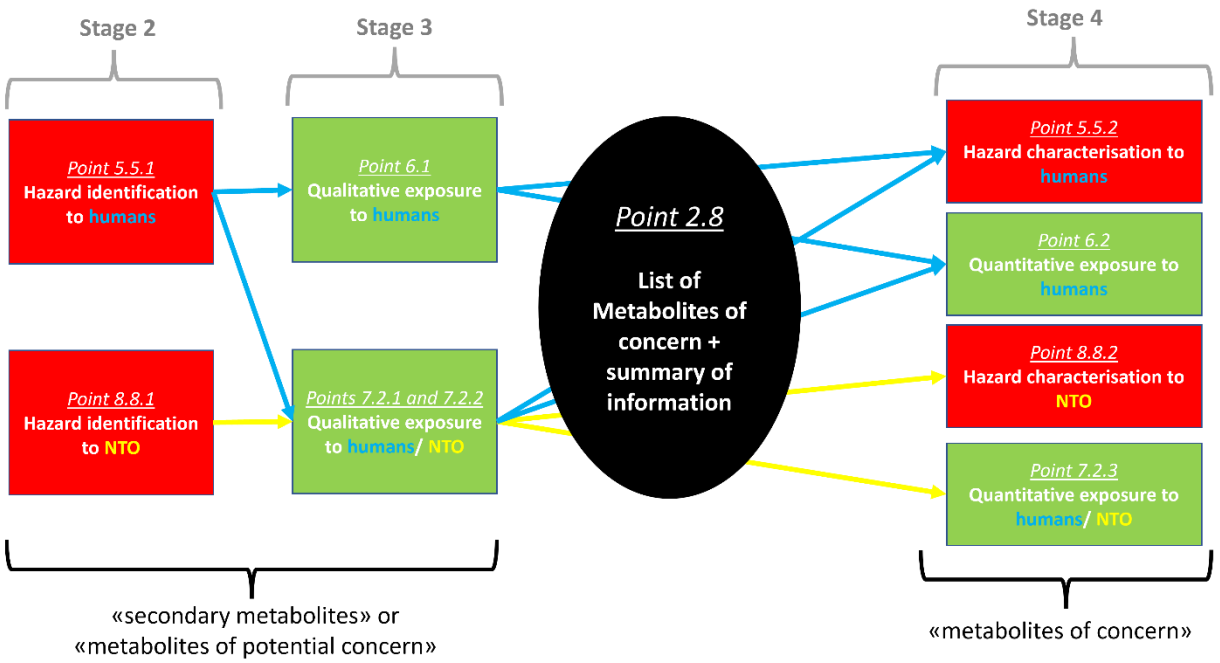

Figure A.2.8-01: Graphical representation of the distribution of information concerning secondary metabolites across the different data requirements of Regulation (EU) No 283/2013. Red boxes represent information concerning **hazards**. Green boxes represent information concerning **exposure**. Light blue text and arrows represent information concerning hazard or exposure of **humans**. Yellow text and arrows represent information concerning hazard or exposure of **non-target organisms**. Black curly brackets represent the right **nomenclature** of the metabolites across the different stages. Grey curly brackets represent the corresponding **stages of the guidance SANCO/2020/12258**. Please note that SANCO/2020/12258 and the Regulation (EU) No 283/2013 describe the same data provision on secondary metabolites, but they follow a different order:

- SANCO/2020/12258 describes the data provision following a time-based "decision tree", to facilitate a step-wise strategy of the applicant when deciding which data generating or collecting (and, analogously, to facilitate risk assessors' evaluation);
- Regulation (EU) No 283/2013 describes the data provision following the dossiers structures, hence indicating where the data generated/collected following SANCO/2020/12258 should be filed in the dossier.

Required information and assessment principle: Guidance for the assessment of secondary metabolites is given in the 'Guidance on the risk assessment of metabolites produced by micro-organisms used as plant protection active substances' (SANCO/2020/12258). The aims of this guidance are twofold: to describe how to exclude or identify metabolites of concern produced by the micro-organism and how to perform a risk assessment for secondary metabolites which are of concern.

Two hazards may apply to secondary metabolites produced by micro-organisms: toxicity and relevant antimicrobial activity (for the latter, please see the definition as provided in the introduction of Part B in the Annex of Regulation (EU) No 283/2013). The outcome of the secondary metabolite assessment as presented for this point in the dossier should include an outcome for each secondary metabolite which either excludes the secondary metabolite as

being of concern or which identifies the secondary metabolite as being of concern, and describing the concern.

To be able to exclude the production of MoC by the micro-organism or to perform a risk assessment in case the micro-organism does produce a MoC, the appropriate information should be included in the dossier. The guidance document SANCO/2020/12258 therefore provides a step-by-step approach which describes which information is needed for the assessment and how this assessment can be performed. Please note that the approach described in the guidance document should not be seen as a fixed route that should be followed for each secondary metabolite of each micro-organism: depending on the specific situation another approach may be more appropriate.

It is expected that several of the secondary metabolites investigated though already available information or newly generated data would be identified as of no-concern. For those secondary metabolites identified as MoC, as a general rule it is expected that they are present in the MPCA-AM due to production during the fermentation process, even though *in situ* production can still be possible.

For the sake of efficiency and harmonisation of the assessment of secondary metabolites, it is highly recommended to use the template for the overview table for secondary metabolites as provided in Appendix I to this document, and to include this overview table in the dossier/assessment report.

Relevant antimicrobial activity
The definition of a MoC as provided in Regulation (EU) No 283/2013 includes ”known relevant antimicrobial activity”. In turn, relevant antimicrobial activity is defined as being caused by relevant antimicrobial agents which are included either in the WHO list of medically important antimicrobials[36] or in an EU list of antimicrobials reserved for the treatment of certain infections in humans[37]. Furthermore, in SANCO/2020/12258 information is given on when the production of relevant antimicrobial agents by micro-organisms used in plant protection products are considered to be a foreseeable risk: relevant antimicrobial agents are only considered to be a foreseeable risk when they are present in detectable amounts in the formulated product (see page 6 of the introduction of the guidance and Step 14), hence *in situ* production is excluded from being of concern. It is important to underline that this data requirement A.2.8 concerns information on antimicrobial activity (or absence of it) which may interfere with the effectiveness of antimicrobials used in human and veterinary medicine. Information on antimicrobial activity which the micro-organism may exert against the target pest as part of its MoA must be provided under point A.2.3.

When analyses are performed to exclude the presence of detectable amounts of relevant antimicrobial agents in the product, the relevance of these analyses should be justified based on available information on which antimicrobials may be produced by the micro-organism.

A.2.9 Presence of transferable antimicrobial resistance genes

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 2.9
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.1.8

[36] https://www.who.int/publications/i/item/9789241515528

[37] Commission Delegated Regulation (EU) 2021/1760 of 26 May 2021 supplementing Regulation (EU) 2019/6 of the European Parliament and of the Council by establishing the criteria for the designation of antimicrobials to be reserved for the treatment of certain infections in humans (OJ L 353, 6.10.2021, p. 1).

Relevant approval criterion:	Reg (EC) No 1107/2009, Annex II, Point 3.6.6(b)
Eligible for substantiated dismissal of data-provision:	Yes (see text).

Purpose of this point:

Bacteria may have the potential to transfer anti-microbial resistance genes to bacteria which are pathogenic to humans, potentially affecting the effectiveness of antimicrobials used in human or veterinary medicine. Due to this hazard, bacteria can only be approved if it is concluded that they do not carry in their genome any known, functional and transferable genes coding for resistance to relevant antimicrobial agents.

Conditionality: This data requirement is only applicable for bacteria.

Assessment principle:

The assessment of antimicrobial resistance is described in three main steps:
- Whole Genome Sequencing (WGS) data screening
- Phenotypic testing
- Decision making

Whole genome sequencing (WGS) data screening

In accordance to the 'Guidance on the approval and low-risk criteria linked to "antimicrobial resistance" applicable to micro-organisms used for plant protection in accordance with Regulation (EC) No 1107/2009' (SANTE/2020/12260), WGS data should be screened for the presence of genetic material known to encode for, or contributing to, resistance to antimicrobials (AMR genes) relevant for use in humans and animals. Regarding WGS data generation, the 'EFSA statement on the requirements for whole genome sequence analysis of micro-organisms intentionally used in the food chain' should be taken into consideration (EFSA Journal 2021; 19(7):6506, 14 pp.). This document also provides information regarding the percentage of sequence identity and sequence length that can be used as threshold (also see the general introduction of these EN concerning for further information regarding the use of WGS data).

According to the guidance document on AMR (SANTE/2020/12260), screening for AMR genes should be done against at least two up-to-date and curated international databases (see for examples the guidance document itself). If the WGS screening identifies a hit for an AMR gene, this should be phenotypically investigated. In addition, it should be investigated whether this AMR gene is located on a mobile genetic element (MGE), and thus is transferable. This latter may be done by looking at the neighbouring sequences. For instance, if the neighbouring sequence is derived from plasmid DNA, the AMR can be considered transferable (note that this underlines the importance of including plasmid DNA during WGS assembly). Other MGE are described in a review by Partide *et al.*, 2018 (as indicated in SANTE/2020/12260). Information regarding the functionality of AMR genes may be gained from frameshifts, deletions or preliminary stop codons, in combination with phenotypic testing.

Phenotypic testing

Information on the micro-organism's resistance or sensitivity to antibiotics or other antimicrobial agents must be provided by performing phenotypic testing based on determination of a minimum inhibitory concentration (MIC) for a selected group of antimicrobials. European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) defined MIC breakpoint values for different micro-organism species based on published data. In case no MIC breakpoint values are available for micro-organisms, EUCAST proposes different approaches to determine which breakpoint value can be used for these micro-

organisms in the guidance document: Antimicrobial susceptibility tests on groups of organisms or agents for which there are no EUCAST breakpoints (2021)[38].

The same guidance document highlights that phenotypic susceptibility for at least two antimicrobial agents with different modes of action has to be demonstrated for bacteria and fungus to ensure treatment options in any case of opportunistic infection. It has to be noted that this data is not required under this point, but under the Section on effects on human health (see A.5.1).

Please note that all experimental data for the assessment for human and animal health should be GLP-compliant as laid down in the Introduction to the Annex of Regulation (EU) No 283/2013; this includes experimental data on phenotypic susceptibility to antimicrobials, but not the analytical phase of WGS analyses as it can be considered as a part of the characterisation process of the micro-organism.

Reading of the framework in specific cases

Bacteriophages
Although the data requirements on the presence of AMR genes only explicitly mention bacteria, information may be needed also in other cases where bacteria are involved in the production of the active substance. This is for example the case for bacteriophages, as the production of bacteriophages depends on using bacterial hosts. Therefore, to rule out the spread of AMR genes by horizontal gene transfer (HGT) by bacteriophages, the genome of the bacterial hosts used in production for AMR genes can be screened. This is also indicated in a recent published OECD guidance document for bacteriophages "Guidance Document for the Regulatory Framework for the Micro-organism Group: Bacteriophages[39]".

A.3 FURTHER INFORMATION

The information required under this Section mainly concerns efficacy information for the active substance. In addition, the information on the literature search(es) performed for the micro-organism and its secondary metabolites should be included in this Section.

A.3.1 Function and target organism

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 3.1
Relevant evaluation criterion:	See for relevant evaluation and decision making Criteria P.3.3 where information regarding the target organism(s) is discussed in more detail.
Relevant decision making criterion:	
Criteria for approval	
Purpose of this point:	
To provide information on the disease or target organisms against which protection is afforded.	

Assessment principle:
Regulation (EU) No 283/2013 , Annex, Part B, 3.1 lists the following biological functions:
- control of bacteria,

[38] See EUCAST Guidance "Antimicrobial susceptibility tests on groups of organisms or agents for which there are no EUCAST breakpoints" - https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Guidance_documents/When_there_are_no_breakpoints_Guidance_1_Dec_2021.pdf

[39] OECD Guidance Document for the Regulatory Framework for the Microorganism Group: Bacteriophages Series on Pesticides No. 108 https://one.oecd.org/document/env/cbc/mono(2022)40/en/pdf

- control of fungi,
- control of viruses,
- control of insects,
- control of mites,
- control of molluscs,
- control of nematodes,
- control of plants,
- other (needs to be specified)

Under “other” for example micro-organisms that act as plant defence inducer (a.k.a. elicitors) can be listed. For more information regarding micro-organisms that act as plant defence inducers, please see A.2.3.

Although the title encompasses both function and target organism, information on the specific target organisms for proposed uses should be included at product level (see Reg (EU) No 283/2013, Annex, Part B, 3.3 (Function, target organisms and plants or plants products to be protected and possible risk mitigation measures).

A.3.2 Field of use envisaged

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 3.2
Relevant evaluation criterion:	-
Relevant decision making criterion:	-
Criteria for approval	-

Purpose of this point:

The existing and proposed field(s) of use of the PPP containing the micro-organism must be specified here (n.b., the field of use concerning the representative PPP should be listed also under P3.1).

Assessment principle:
The field(s) of use, existing (if relevant) and proposed, for the micro-organism can be specified from among the following:

- agriculture, horticulture, forestry, or viticulture,
- protected crops (e.g. in greenhouses)
- non-cultivated areas,
- home gardening,
- houseplants,
- stored food/feed items,
- seed treatment,
- other (needs to be specified).

If amateur/non-professional use is intended (whether or not in addition to professional use), this should be clearly indicated.

Reading of the framework in specific cases

Protected crops
For protected crops the type of protection should be indicated (e.g. greenhouse, walk-in tunnel, shade house). Different types of protected structures are described in the “EFSA Guidance Document on clustering and ranking of emissions of active substances of plant protection products

and transformation products of these active substances from protected crops (greenhouses and crops grown under cover) to relevant environmental compartments"[40]. In addition to the type of protected structure (e.g. permanent or non-permanent), the growing system should be indicated (soil-bound versus soil-less). This information is used to determine the exposure scenarios of humans and the environment for the risk assessment, then supporting the identification of what needs to be assessed (problem formulation).

A.3.3 Crops or products protected or treated

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 3.3
Relevant evaluation criterion:	-
Relevant decision making criterion:	-
Criteria for approval	-

Purpose of this point:

The details of existing or intended use(s) in terms of crops, groups of crops, plants or plant products protected is not only used for the efficacy assessment, but also for the risk assessment as it provides information on the exposure of humans and the environment to the micro-organism. N.b., the same information specifically concerning the representative PPP should be listed also under P.3.3.

Assessment principle:

This information is needed to know the extent of use of the substance. To avoid misinterpretation of ambiguous terms (e.g. ornamentals can encompass different plant groups in different member states) it is advisable to also include the relevant EPPO codes and scientific names.

A.3.4 Information on possible development of resistance in the target organism(s)

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 3.4
Relevant evaluation criterion:	
Relevant decision making criterion:	
Criteria for approval	

Purpose of this point:

Information on the possible development of resistance in the target organism(s) is needed to assess whether a lasting efficacy of the micro-organism used in the plant protection product(s) is ensured. Please note that for the current data requirement, only the inherent properties of the micro-organism to trigger the development of resistance in the target organism(s) is discussed; the resistance risk assessment which is performed at product level is described in P.6.4.

If there is a risk on development of resistance in the target organism(s), it is essential that the likelihood of resistance developing in target species is, if applicable, minimised by relevant resistance management strategies which must be described under P.6.4.

Assessment principle:

PPPs based on micro-organisms often have MoA that do not trigger cross-resistance with existing products. This can be due to indirect MoA (e.g., by competition for nutrients or space), or due to the fact that MOs often have more than one direct MoA (e.g., antibiosis and parasitism). As such they can offer advantages to resistance management. However, pests or pathogens may develop resistance to certain micro-organisms. In these cases, resistance

[40] EFSA Guidance document on clustering and ranking of emissions of active substances of plant protection products and transformation products of these active substances from protected crops (greenhouses and crops grown under cover) to relevant environmental compartments, Section 2 (EFSA Journal 2014;12(3):3615).

management needs to be addressed. In some cases, target organisms may have developed resistance to some strains of a micro-organism, but not to other strains of the same species (e.g. resistance to baculoviruses is isolate specific). This differs from conventional PPPs, where often cross-resistance exists between many active substances. If there are indications (e.g. from scientific peer-reviewed literature or other reliable sources) of intrinsic ability of the target pest to develop resistance against the micro-organism under evaluation, the applicant must take them into consideration in order to define, if applicable, resistance management strategies. Such strategies must be described under point P.6.4, taking also into consideration the information provided under that point.

Resistance risk depends for a large part on the MoA (see also the information provided under A.2.3), and therefore, it is important to clearly describe the MoA[41].

A.3.5 Literature data

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 3.5
Relevant evaluation criterion:	-
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, General introduction, 3.10
Criteria for approval	-

Purpose of this point:

Together with the correct identification of the micro-organism at species level, literature data form the basis of the dossier. The information on the literature searches performed to retrieve the relevant literature data should be included at the current point.

Assessment principle:

The literature search should be carried out in accordance with the relevant guidance on the submission of scientific peer-reviewed open literature listed under the “General test methods and guidance documents” section of the CC-MPCA. The summary of the search (e.g., used search terms for each section, search strategy, overview tables of the retrieved publication) must be submitted under the current data point 3.5 of Reg (EU) No 283/2013, Annex, Part B. Literature information retrieved from this search must be reported in the relevant sections of the dossier (e.g., A.5 Effects on Human Health and A.8 Ecotoxicological Studies).

In addition to the species name of the micro-organism, the search terms should include any previous names given in the 10 years prior to dossier submission. Likewise, in case of alternative names for the micro-organisms these should be included (see A.1.3).

As indicated in SANCO/2020/12258, additional literature searches are needed for each metabolite of potential concern to determine if these secondary metabolites have known toxic effects on humans and/or non-target organisms or are known relevant antimicrobials. Please note that these searches should not include the name of micro-organism in the search terms, as information on a specific secondary metabolite produced by a different species should also be retrieved. Please note that the literature search information submitted under this point must contain the search terms relevant to the secondary metabolites investigated, in combination with search terms relevant for known adverse effects (toxicity or relevant antimicrobial activity). Please refer to point A.2.8 for more detailed information regarding the literature search for the risk assessment of secondary metabolites produced by the micro-organism.

Should the dossier be making use of read across between different species because of similar

[41] Please see EPPO Standards PP1/213 (Resistance risk analysis) and PP1/276 (Principles of efficacy evaluation for microbial plant protection products).

biology or other traits / factors, then a systematic search for that other species should in principle be included at least in relation to the property for which read across is proposed. This is needed to ensure all relevant information on this property is included.

Applicants should also make use of the systematic literature reviews that EFSA procured and published (Mudgal *et al*., 2013[42], Hackl *et al*., 2015[43]) ensuring publications identified there have been considered in the dossier. The search strategies reported in these two references may be helpful to determine the appropriate search strategy (including search terms) for the micro-organism.

Instructions on how to report a literature search and literature reference studies (e.g. in case they are used to address a specific endpoint/ data requirement) can be found in the IUCLID Microbial active substances manual[44]. The IUCLID report generator function will present individual study summaries based on the information included in the dossier.
The applicant must include also a justification or reasoning on why the specific literature information is relevant to address the cited data point.

A.4 ANALYTICAL METHODS

A.4.1 Methods for the analysis of the MPCA as manufactured

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 4.1
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.4.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.4.1
GLP-compliance:	Not required for method validation
Purpose of this point:	
Methods for analysis of the MPCA-AM, used (i) to verify the identity of the micro-organism as unequivocally belonging to a certain species, (ii) to distinguish the micro-organism from other strains / isolates, (iii) to check any genetic variability of the micro-organism and its seed stock, (iv) to determine the content of the micro-organism, claimed active metabolites, and any MoCs and relevant impurities established for the MPCA-AM, and (v) to detect possible contaminating micro-organisms, must be evidenced to be sufficiently specific, linear, accurate, and precise – whichever criterion is relevant for the respective method – to serve their purpose.	

Conditionality
Not relevant.

Confidentiality
Confidentiality can be claimed for WGS-data relating to the micro-organism, for methods implemented in the context of quality control – as they directly relate to the manufacturing process, and for methods for the determination of additives.

Background information on methods currently available

MICRO-ORGANISM IDENTIFICATION METHODS

(a) Methods for unequivocal identification of the micro-organism
used for identification at strain level, based on unique genotypic or phenotypic markers or a

[42] Mudgal, S; De Toni, A; Tostivint, C; Hokkanen, H; Chandler, D. EFSA Supporting Publications 2013:EN-518.
[43] Hackl, E; Pacher-Zavisin, M; Sedman L.; Arthaber, S; Bernkopf, U.; Brader G; Gorfer, M; Mitter, B; Mitropoulou, A; Schmoll, M; Van Hoesel, W; Wischnitzky, E; Sessitsch, A. EFSA Supporting Publication 2015:EN-801.
[44] https://doi.org/10.5281/zenodo.4773526

combination thereof to distinguish the strain from other strains belonging to the same species.

(b) Methods for the characterisation of the micro-organism
used for characterisation of the micro-organism at species level (see A.1.3 (ii)). This method is not necessarily the same method used to determine if a microbial sample contains the micro-organism under assessment. Additionally, the analytical methods used for building the phylogenetic tree (see A.1.3 (iv)) must be described.
The provided methods must be evidenced to be capable of verifying the results obtained for the identification of the micro-organism at species level and for establishing the position in the submitted phylogenetic tree.

(c) Methods for providing information on possible variability of seed stock / micro-organism and its storability
The data detailing the manufacturing process must include a full description of quality assurance measures, regarding e.g., validation, maintenance and storage conditions of the seed stock, drawing from the seed stock to initiate manufacturing, viability, and contamination checks during manufacturing. Taken together, the precautionary steps must reasonably suffice to maintain purity of the micro-organism.

To limit variability, for micro-organisms it is often essential to generate sufficient aliquots of the master seed stock, stored in such a way that, while remaining viable, the micro-organism will not multiply. Frequent sub-culturing may result in genetic or epigenetic changes which may lead to loss of activity (e.g. due a reduced production of virulence factors).

Here, storability is interpreted as the ability of the seed stock of a micro-organism to maintain its viability over a longer period of time. The required analytical methodology is in most cases the same as the ‘Methods to determine the content of the micro-organism which is the active substance’ (see point f below).

(d) Methods to differentiate a spontaneous or induced mutant from the parent wild strain
In case the micro-organism is a mutant (either spontaneous or induced) it is essential that the mutant strain can be distinguished from its original parental wild-type strain. A method should be provided for this purpose.

This point is only relevant when the micro-organism is a mutant. This can either be a spontaneous mutant (e.g. picked up in the laboratory during subculturing) or an artificially induced mutation (e.g. by exposure to radiation or a chemical mutagen). Lastly, the mutant may be genetically modified (in which case Directive 2001/18/EC should be considered, as discussed previously under point A.1.3).

This point is not related to the one above that considers possible variability of seed stock. Whereas possible variability of seed stock is considered unintentionally, the difference between the mutant micro-organism and the parental wild type strain is purposefully provoked. The mutant may for instance have slightly different biological properties compared to the wild type strain, making the mutant more suitable for the use as PPP. According to A.1.3, both the genetic and biological differences between the mutant and the parental strain should be explained. The method provided here should be able to differentiate the mutant strain from the parental wild type strain. While a molecular method based on (any of the) genetic difference may be the most obvious method, alternative methods may be acceptable. But note that under A.1.3, it will be still required to list all known genetic differences between the mutant and the wild type strain.

(e) Methods for the establishment of purity of seed stock
Please refer to point *(c)*.

QUANTITATIVE METHODS

(f) Methods to determine the content of the micro-organism which is the active substance, and methods to detect relevant contaminating micro-organisms
The choice of method to quantify the micro-organism depends on how its activity is best expressed. Below, the main approaches to quantify the content of the micro-organism which is the active substance and/or its activity, and of relevant contaminating micro-organisms, are presented. Other methodologies for quantification of the micro-organism which is the active substance exist (e.g., qPCR, Most Probable Number), but have, to our knowledge, not yet been employed within the context of active substance approval. Therefore, no framework-dedicated validation criteria have yet been established for such methods. This, however, should not prevent applicants from using them e.g. upon discussion with competent authority during pre-submission meetings.

Enumeration methods – Bacterial and fungal spores, and virus particles[45] are generally counted in a counting chamber (hemocytometer).
Colony-forming micro-organisms whose activity relies on their viability can be enumerated by plating on an appropriate type of nutrient agar, and subsequent incubation and colony enumeration. Conversely, bacteriophages are counted by the purged areas, or plaques, that they leave when incubated on a plate colonized by their target bacterium.
Currently, there is no formal guidance on validation criteria for these methods. Regulation (EU) No 283/2013, Part B, Section 4 states the universal validation parameters, i.e., specificity, linearity, accuracy, and precision, but leaves the actual acceptance thresholds undefined.
Methods that have been validated (e.g., according to the relevant guidance document listed under point 4.1of the CC-MPCA), at least for linearity and precision, are commonly encountered. The document is however intended for evaluation of analytical chemistry methods, and is less suitable for the microbiological methods discussed here. Nevertheless, compliance automatically means acceptability for enumeration methods, again, at least with regard to linearity and precision.
To provide a degree of systematisation, the following pragmatic rules may be considered until a micro-organism-dedicated guideline has been adopted:

- *Specificity:* the morphological characteristics based on which the colonies of the micro-organism are identified during counting must be described. These characteristics must be sufficiently distinctive to recognise the micro-organism among any consorts, whenever relevant.
  For the plaque-forming bacteriophages, that are approved as e mixture of isolates, isolate-by-isolate differentiation may not be possible. These micro-organisms are preferably enumerated in single-isolate suspension (or preparations, as alternative to suspensions);
- *Linearity:* the hemocytometer -and plate count range of a single sample roughly covers a factor of 10 (typically 30 – 300 per (plate) area). Given this limited broadness, triplicate counts at three dilution levels (typically a factor of three apart) are advised. A linearity plot and regression equation must be presented, along with the coefficient of determination r^2. Acceptability is assessed based on fitness for the purpose;
- *Accuracy:* a possibly critical feature affecting the method's accuracy is the dilution

[45] Often, expressing the micro-organism in terms of spores or virus particles per g or L of matrix may not be the most accurate way in relation to activity. Still the spore content may be a useful metric in the context of quality control (see A.1.5.1, "The essential process checkup" for an explanatory note on this). Moreover, especially for viruses, the virus particle content is commonly required for a meaningful expression of dosing levels (e.g. in virus particles per g of diet). See "Bioassays" in this subsection for more details.

chain. As enumeration procedures often include five to seven subsequent dilution steps, the cumulative error introduced by inhomogeneous distribution of the micro-organism in the increasingly diluted matrix could be substantial. Methods should be in place to prevent an inhomogeneous distribution during serial dilutions.

- *Precision:* Precision data must include at least 5 independent determinations (replicates) performed at the same dilution. The mean, %RSD, and number of determinations must be reported. Precision criteria may be adopted from ISO -or EN-standards that are appropriate with regard to species and matrix. If none are available, acceptability is assessed based on fitness for purpose.

Biopotency assays – Bioassays provide a middle ground between the oversimplification inherent in the expression of virus particles or colony formers per matrix quantity and the complexity surrounding their actual activity in the field.
Similar to dose-effect testing for toxins, a bioassay includes exposure of test organisms to a range of levels of micro-organism, plotting of response against dose, and subsequent derivation of a median lethal dose (LD_{50}). Currently, tests are far from being standardised, which limits the evaluation efficiency. Furthermore, test outcomes are reported in various ways, which may complicate a harmonised interpretation of results. To promote standardisation of the evaluation, and the derivation of better-referenced and more communicable metrics, the following points, taken as an example from the case of an insecticidal virus, should be considered:

- First, it is important to have the test batch characterized in a way that is meaningfully related to the observed effect. Based on this information, subsequent exposure concentrations can be expressed in terms of the actual component causing lethality in the test organism per unit of feeding medium (e.g., as ‘mg of δ-endotoxin’ or ‘virus particles’, instead of the unnecessarily inaccurate ‘mL of product’, per gram of exposure medium).
- The amount of dosing matrix (e.g., diet material, water) to which the organism is actually exposed must be a non-negligible fraction of the total of prepared matrix, in order to minimize bias due to the invariably inhomogeneous distribution of the micro-organism in the matrix.
- Next, the test species should be justified. Ideally, it is the species for which a biopotency minimum has been established in the specification. The tests must be performed with healthy individuals.
- The test must at least include five separate dosing groups, with a concentration difference of about 0.5 log units between neighbouring groups. A sixth group will be the control and receives unspiked exposure medium. The number of individuals per group should account for the overall variability in test performance, and needs to be justifiable from a statistical point of view.
  Mortality in the lowest dose group should be about 15%, mortality in the highest dose group about 90% and mortality in the control group should be about 10%. The LD_{50} ideally coincides with a group in the middle between highest and lowest dose. Preliminary range finding experiments should help optimizing the test design.
- The test report should present the raw data, and sufficient details of the data analysis. Probit analysis could be regarded as default, but other statistical operations may be warranted. The median LD_{50} and the 99% confidence interval limits must be reported. By rule of thumb, a lower limit of 0.5 x LD_{50}, and an upper limit of twice the LD_{50} is amply acceptable, whereas a factor of >9 difference between the upper and lower limit suggests poor data quality.
- As an internal performance check, the test item is preferably compared with a reference item – often a benchmark batch of the microbial active substance itself – that undergoes synchronous testing. In these cases, resulting biopotency, reflected by the

LD_{50}, is commonly presented as *relative* biopotency, i.e., LD_{50} (reference item) / LD_{50} (test item). An important criterion for a reference item is that, under well-controlled circumstances, it presents as little variation in performance as possible. To evidence this, supporting data should be made available that show a workable degree of consistency in the reference item's LD_{50} over multiple standard test runs, if possible over a timespan of several years.

Contaminating micro-organism screening methods – Beside safe limits and context-dependent information on relevant contaminating species, the relevant guidance document listed under point 1.4.2.2 of the CC-MPCA provides guidance on recommended methodology. Having drawn its inspiration from food/feed-legislation, the document typically advises use of internationally standardized reference methods (e.g. FDA BAM, USDA MLG, MFLP, AOAC, and ISO), commonly employed in screening of food and feed. Whereas the recommended methods still only include the more traditional plating methods that were the norm at the time of drafting, the EU food/feed-framework has evolved in the meantime to allow the use of more innovative, alternative methods (e.g. qPCR-based) – mainly through translational standard ISO 16140-2, that validates alternative methods against reference methods.
To be able to benefit from more advanced methodology within the PPP-context as well, alternative methods that are ISO 16140-2 -or AOAC-certified[46] are acceptable. Of course, if a modified/different method than the international standards is used, validation data should be addressed (specificity of the medium to the target pathogen, if medium is changed, positive (if possible), negative control, accuracy, precision, LOD/LOQ).
Relatively recently, the Competent Authority has seen initiatives for setting up screening methods that are distinctly PPP-dedicated and therefore explicitly depart from the existing food law-related certification context. Though there is a solid rationale to encourage such developments, safe implementation of such 'non-certified alternative methods' requires PPP-specific reinterpretation of ISO 16140-2 criteria to ensure fitness for purpose in terms of e.g., test design, matrix preparation, inclusivity, and sensitivity[47].
By definition, validation of such methods is extensive and typically takes place outside of the dossier context. As such, validation of contaminating micro-organism screening methods is not covered by the assessment systematic described in this EM.

(g) Methods for the determination of relevant impurities, metabolites of concern, and additives

Below, the approaches to quantify specification elements with a chemical nature are presented.

Chromatographic methods – The Regulation does not provide any specific criteria for analytical methods that may be used during the assessment of the relevance of impurities. Beside focused routine analyses of the material for components expected in a given context, CoAs issued by adequately certified screening labs may also be accepted. Although this 'Tier I'-type of data needs to be sufficiently reliable, GLP-compliance is not per se required at this stage.
An issue that is especially problematic for MoCs, is the potential unavailability of analytical standards. Since purification or synthesis of the MoCs are not deemed realistic due to the disproportionate investments involved, and/or technical feasibility to obtain the isolate metabolite (e.g. volatiles, quick degradation of large peptides), alternative approaches are considered by default. Often, the use of structurally similar chemicals is advised in these cases

[46] Within the EU food framework, non-standard screening techniques are validated against translational criteria (such as those in ISO 16140-2) and, in case of successful validation, certified by designated normalisation bodies (e.g., Afnor, AOAC, or NordVal).
[47] Definition of PPP-dedicated criteria is currently in progress.

– if suitable candidates may be found in the first place given the complex molecular structure of most secondary metabolites. This approach is however debatable, as the representativeness of a substitute standard does not depend on its structural similarity, but rather on the agreement between its RRF[48] and that of the actual secondary metabolite. The availability of reliable RRFs for substances for which no analytical standards are obtainable depends heavily on how intensively the substances have been studied. Custom *in silico* methods have shown promising results in specific contexts, but their success in dealing with complex molecular structures and their applicability for the purpose of dossier drafting may (yet) be limited.
In summary, finding a substitute that may perform well enough is likely to present a challenge due to the highly specialized nature of the investigation involved.
To conclude on a pragmatic note, the secondary metabolite assessment process (see Appendix 1) requires quantitative data at a stage in which it has already been largely established whether a given secondary metabolite will be of concern or not. Therefore, quantification is not required for those secondary metabolites which are not of concern. For the extreme cases where a MoC has been established for which no analytical-quality grade standards are available, a substitute standard must simply have an RRF that is lower than that of the MoC (for monitoring purposes) or higher (for (eco)tox-testing).

Other methods – Chromatographic methods may not be compatible with any conceivable specification element with a chemical nature, like e.g., relatively large proteins that rather necessitate the use of other methods. As no dedicated guidelines are yet in place, the method evaluation will focus on the universal quality criteria, i.e., specificity, linearity, accuracy, and repeatability, stated in Regulation (EU) No 283/2013, Part B, Section 4.

A.4.2 Methods to determine the density of the micro-organism and quantify residues

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 4.2
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.4.2
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.4.2
GLP-compliance:	Not required for method validation

Purpose of this point:

The method used to determine and quantify the density of the micro-organism is required for human toxicology and ecotoxicology studies. Methods for analysis of residues may be required for risk assessment of the micro-organism and/or MoCs in relevant crops, foodstuffs, feeding stuffs, animal and human tissues and fluids, and environmental matrices. Any monitoring methods that may be required for MoCs must be sufficiently simple, robust, and sensitive.

Conditionality
Not relevant.

Confidentiality
Not relevant.

Background information
METHODS TO QUANTIFY THE DENSITY OF THE MICRO-ORGANISM IN RELEVANT ENVIRONMENTAL COMPARTMENTS

[48] A RF (response factor) of a substance is the ratio between the abundance of this substance (i.e., its peak area or height) and its concentration in a respective sample. The factor is mainly a resultant of ionisation efficiency, and to a lesser degree of matrix effects and ion transport. The RRF (relative response factor) of a substance is the ratio between the RF of a substance and the RF of a reference substance also contained in (i.e. often explicitly added to) the same sample (internal standard). Therefore, to compare the RRF of the MoC with the RRF of the substitute" standard an internal standard is needed, in order to calculate the RRFs in the first place.

In case experimental data is required for the risk assessment under Point 7.1.4 of the Annex Part B of Regulation (EU) No 283/2013 or to provide information on the density of the micro-organism to support the estimation of exposure to residues (see Point 6.1 of the Annex Part B of Regulation (EU) No 283/2013) a method to quantify the density of the micro-organism in the relevant environmental compartments (e.g., edible part of crop, soil, plant surfaces) is needed. A description of these methods (including for example sampling strategy, extraction of nucleic acids, PCR-protocols) should be provided.
Methods for post-approval monitoring of the density of the micro-organism (viable residues) are in principle not needed. Indeed, for none of the currently approved microbial active substances monitoring definition has been set (nor for viable residues, nor for metabolites of concern). If methods to quantify viable residues would be required, no guidance is available. Until such guidance is available, methods will be evaluated on a case-by-case basis.

METHODS TO QUANTIFY RESIDUES OF METABOLITES OF CONCERN
When pre- or post-approval methods are required to quantify metabolites of concern, criteria for method validation are available (see SANTE/2020/12830).

A.5 EFFECTS ON HUMAN HEALTH

Scope
As mentioned in Regulation (EU) No 283/2013, the information provided by the applicant must be sufficient to:

'— Permit a decision to be made as to whether or not the micro-organism is to be approved,
— specify appropriate conditions or restrictions to be associated with the approval,
— specify risk and safety phrases for the protection of human and animal health and the environment to be included on packaging (containers),
— identify relevant first aid measures as well as appropriate diagnostic and therapeutic measures to be followed in the event of infection or another adverse effect in humans.'

The hazards related to the use of micro-organisms in plant protection products are different to those of chemicals. In addition to the potential hazard related to the toxicity of secondary metabolites produced by micro-organisms, micro-organisms may have the potential to cause infection or pathogenicity in humans, which must be carefully assessed and excluded. Although if unlikely, infections may also be caused opportunistically by micro-organisms which are not known for being infective to humans; hence the information provided must suffice to support the identification of aid measure and diagnostic strategies. They may also have the potential to cause sensitising reactions and non-specific effects such as an inflammatory response (up to anaphylactic shock) after exposure via inhalation. To assess these hazards of a micro-organism the scientific knowledge on the biology of micro-organisms should be taken into account.

According to the amended uniform principles (Regulation (EU) No 546/2011, Annex Part B, 1.5) the most important aspects that must be assessed are:

'— infectivity and pathogenicity;

— toxicity of metabolites of concern, safeners, synergists, and relevant impurities;

— relevant antimicrobial activity of metabolites present in the plant protection product;

— susceptibility to relevant antimicrobial agents to ensure the availability of sufficient treatment options in case of an opportunistic infection.

These aspects comprise a complex set of interactions between micro-organisms and the hosts, and need to be assessed in an integrated way and applying a weight of evidence approach.

An assessment of infectivity and pathogenicity is always necessary.'

This assessment of infectivity and pathogenicity is described in the current Section (A.5). Information on the other effects on human health is either included in the current Section, or in other Sections as indicated below. The susceptibility to relevant antimicrobial agents to ensure the availability of sufficient treatment options in case of an opportunistic infection is described in A.5.1 and A.2.9. The assessment of infectivity and pathogenicity is described in A.5.2 to A.5.4 and toxicity of secondary metabolites and whether they are of (potential) concern is described in A.5.5. The quantitative exposure assessment for metabolites of concern is described in Sections A.6 and P.7. Identification of metabolites of potential concern produced by the micro-organism and the assessment of relevant antimicrobial activity of secondary metabolites present in the plant protection product is described in A.2.8. The information on toxicity of co-formulants as e.g. safeners, synergists and impurities is described in point P.7.6.

Literature search

According to the Introduction to the Annex to Regulation (EU) No 283/2013, all available relevant data from the scientific peer reviewed and open literature on the micro-organism should be provided. Please refer to A.3.5 for further information on the literature search.
The literature search on secondary metabolites relevant for human health is discussed in A.5.5 and should be based on the 'Guidance on the risk assessment of secondary metabolites produced by micro-organisms used as plant protection active substances, SANCO/2020/12258'.

Hazard testing

Although it is stated that an assessment of infectivity and pathogenicity is always necessary, it is important to realise that a weight of evidence approach can be sufficient to address infectivity and pathogenicity as explained further in the text under point 1.5.1.2 of Part B of the Annex to Regulation (EU) No 546/2011 and data requirement 5.2 of Regulation (EU) No 283/2013. The explicit mentioning of the weight of evidence approach is an important update in the revised data requirements and supports the 3-R principle for replacement, reduction and refinement of animal use. Moreover, due to the host range of the micro-organism and differences in the immune system of humans and test animals, the relevance of animal tests to assess the pathogenicity of micro-organisms to humans is not a priori clear. Based on the body of knowledge on the micro-organism, further specific studies may be required, as indicated in points 5.3.1, 5.3.2 and 5.4 of Part B of the Annex to Regulation (EU) No 283/2013 and explained further in the text under point 1.5.1.2 of Regulation (EU) No 546/2011. These tests may for example involve inquiring WGS data for virulence factors or non-animal methods (a.k.a. "New Approach Methodologies") such as *in vitro* testing with cell lines. As the use of these methods may be considered on a case-by-case bases, it is strongly recommended to discuss the testing strategy with RMS and EFSA during pre-submission meetings.

The typical OECD test guidelines are not tailored towards micro-organisms. This is acknowledged by the ongoing activities initiated at the 2022 OECD Conference on Innovating Microbial Pesticide Testing. In case experimental data is necessary for the assessment of infectivity and pathogenicity to humans, and pending the acceptance of specific guidelines at international level, it is recommended to reach agreement with the competent authority on the test guidelines that may be used for the specific micro-organism (e.g. US EPA's microbial

pesticide test guidelines[49], please also refer to the CC-MPCA, Section 5). As regards testing toxicity of metabolites of potential concern and MoC, and where appropriate, in case no US EPA test guideline is available, test guidelines as described in Part A of Regulation (EU) No 283/2013 may be used upon adaptation.

When testing is required, the scope for replacement, reduction and refinement of animal tests should be taken into account, which is strongly promoted in Regulation (EU) No 283/2013 and Regulation (EC) No 1107/2009.

Furthermore, point 4.2 of the introduction to the Annex to Regulation (EU) No 283/2013 emphasises that the active substance as manufactured should be used in studies (i.e., the MPCA-AM). When different test material (e.g. active substance manufactured in the laboratory or in a pilot plant production system) is used, a justification should be provided that the test material is essentially the same for toxicological testing and assessment.

Please note that in certain cases studies may be carried out by using the PPP containing the micro-organisms under assessment. It is recommendable to discuss such option with RMS and EFSA during pre-submission meetings.

Good laboratory practice (GLP)
All experimental data for the assessment for human and animal health should be GLP-compliant, as laid down in the introduction to the Annex of Regulation (EU) No 283/2013. Please note that this also includes information submitted in other sections of the dossier, but used for the assessment of human and animal health, such as antimicrobial resistance or growth temperature of the micro-organism.

Classification
The provisions of the CLP Regulation (Regulation (EC) No 1272/2008) are not applicable to micro-organisms and thus the micro-organism cannot be classified or labelled under the current classification and labelling system.

Dismissal of data-provision
Please note that not submitting data for a particular data requirement is not acceptable without further justification.

A.5.1 Medical data

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 5.1
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.5.1
Relevant approval criterion	Reg (EC) No 1107/2009, Annex II, point 3.6.6
Eligible for substantiated dismissal of data-provision:	No

Purpose of this point:

Information related to any symptoms of infection or pathogenicity caused by the microbial active substance that may be available from medical reports or from case reports should be reported. Information on the effectiveness of first aid and therapeutic measures should be submitted as well.

Conditionality: Listing antimicrobial agents with effectiveness against the micro-organism is

[49] https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-885-microbial-pesticide-test-guidelines

not required for viruses.

Required information:

Therapeutic and first aid measures

In addition to therapeutic and first aid measures, a list must be provided with antimicrobial agents with effectiveness against the micro-organism to ensure the availability of sufficient therapeutic measures in the event of opportunistic infections. For further guidance, please refer to data requirement 2.9 and the 'Guidance on the approval and low-risk criteria linked to antimicrobial resistance' (SANTE/2020/12260). Furthermore, where relevant, antagonists should be listed in case of identification of MoC in 2.8.

It is important to specify that the information on antimicrobials to be used as therapeutic measures and to be provided under this point is different than the information to be provided under point 2.8 on the production of 'relevant antimicrobials agents' (i.e., antimicrobials which are also used as therapeutic and first aid measures in infections - (see 'Guidance on the risk assessment of metabolites produced by micro-organisms used as plant protection active substances', SANCO/2020/12258).

Information on presence of transferrable, acquired, and functional antimicrobial resistance genes and phenotypic antimicrobial resistance of the micro-organism should be provided under data requirement 2.9.

Medical surveillance

Reports on occupational health surveillance programmes should include detailed information on the design of the programme as well as on frequency, level and duration of exposure to the micro-organism and personal protective equipment, if applicable. These reports must include data from persons exposed in manufacturing plants or after application of the micro-organism (e.g. in efficacy trials). Available information on (the absence of) the sensitisation and allergenic response from workers, e.g. in the manufacturing plants, agricultural and research workers, must be provided as well. These records provide useful information, particularly as there are no validated methods for testing of sensitisation in animals.

Information on sensitisation and allergenicity

In compliance with Regulation (EU) No 283/2013 and the uniform principles from Regulation (EU) No 546/2011, all micro-organisms must be regarded as potential sensitisers until validated tests for investigating sensitisation are available[50]. Please note that currently (October 2023), work is ongoing on a possible amendment of Regulation (EU) 547/2011[51] on the labelling requirements for plant protection products; additional provisions and attribution criteria may be provided on precautionary phrases to communicate the sensitisation potential of micro-organisms on the labels for hazard communication.

If a study to evaluate sensitisation potential of micro-organisms is performed, or a study report retrieved from e.g. the literature search, the results of this study, either positive or negative,

[50] Regulation (EU) No 546/2011 point 2.5.1.4. states the following: "*All micro-organisms shall be regarded as potential sensitizers in the absence of validated test for investigating sensitisation. Authorisations granted shall therefore specify, as a non-specific risk mitigation measure, that personal protective equipment (e.g. masks) shall be worn, taking into account the conditions of use, and that the exposure via inhalation to the plant protection product containing a micro-organism shall be minimized (…)".*

[51] Commission Regulation (EU) No 547/2011 of 8 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards labelling requirements for plant protection products (OJ L 155, 11.6.2011, p. 176). N.b., at the time of publication of these EN, an amendment of this Regulation is under discussion; please always refer to the most updated consolidated version.

should be interpreted with caution since the current sensitisation test guidelines are not validated for micro-organisms.

Direct observations
Clinical case reports and epidemiological studies of the micro-organism and related micro-organisms should be considered for the assessment of infectivity and pathogenicity in humans, including immunodeficient subjects or patients under treatment with immunosuppressant agents.

A.5.2 Assessment on potential infectivity and pathogenicity of the micro-organism to humans

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 5.2
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.5.1
Relevant approval criterion:	Reg (EC) No 1107/2009, Annex II, point 3.6.6
Eligible for substantiated dismissal of data-provision:	No

Purpose of this point:

The outcome of the weight of evidence approach provided for this point is used to determine which information should be included under data requirements 5.3 and 5.4. To this end, available information on infectivity and pathogenicity should be combined in a weight of evidence approach to provide a robust conclusion to either exclude infectivity and pathogenicity of the micro-organism or to provide information on (specific) data needed to conclude on the assessment of infectivity and pathogenicity.

Assessment principle:
A weight of evidence approach can be applied in order to evaluate whether the possible non-submission of certain studies required in points 5.3.1 and 5.4 of Part B of the Annex Part B to Regulation (EU) No 283/2013 is justified. Information such as provided under points 2.1, 2.3, 2.4, 2.6 and 5.1, public literature and QPS can be used in a weight of evidence approach to demonstrate absence of infectivity and pathogenicity to humans as regards dietary and/or non-dietary exposure pathways (n.b., QPS approach is relevant only for dietary exposure). The body of knowledge should be sufficient to provide a robust conclusion. The evaluation of the body of knowledge and whether it is sufficient to demonstrate absence of infectivity and pathogenicity of the micro-organism will be based on case-by-case expert judgement. If the applicant cannot demonstrate absence of infectivity and pathogenicity based on a weight of evidence approach, studies, data and information must be provided.

Considerations:

- As indicated in Regulation (EU) No 546/2011: *'Replication temperatures may be different from mammalian body temperature, possibly indicating low likelihood of persistence and multiplication in the host. However, temperature adaptation may occur, and this parameter alone shall not be considered sufficient to conclude on persistence and multiplication of the micro-organism in the host.'* Furthermore, growth temperature data is less relevant for e.g. eye and skin infections. When data on growth temperature is used in the weight of evidence approach under this point, please note that the experimental data should be GLP-compliant.

- Suitability of the test model: for micro-organisms, infectivity and pathogenicity tests on animals may not be suitable for extrapolation to humans due to differences between humans and test animals (e.g. immune system, microbiome). Furthermore, micro-organisms might have a narrow host range, hence it cannot always be assumed that a

micro-organism that does not cause disease in the animals tested has the same result in humans for example. Please provide adequate justification for the suitability of the test model.

A.5.3 Infectivity and pathogenicity studies on the micro-organism

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B,
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.5.1
Relevant approval criterion:	Reg (EC) No 1107/2009, Annex II, point 3.6.6
Eligible for substantiated dismissal of data-provision:	Yes (see text)

Purpose of this point:

The information on experimental infectivity and pathogenicity studies are only needed in case infectivity and pathogenicity of the micro-organism to humans cannot be excluded based on the body of knowledge on the micro-organism.

Conditionality: Infectivity and pathogenicity studies with the micro-organisms are only needed when infectivity and pathogenicity of the micro-organism to humans cannot be excluded based on the body of knowledge on the micro-organism (see point A.5.2). Please note that also when experimental studies are needed, the body of knowledge should be used as a starting point to determine which studies are relevant for the micro-organism. Especially for those micro-organisms for which there are indications of infectivity and pathogenicity based on their relationship to known pathogens, information on these related pathogens is needed to determine the approach to exclude infectivity and pathogenicity in the micro-organism under assessment. This approach may be based on in silico methods (e.g., excluding the presence of known virulence factors – see point A.2.6), in vitro methods (e.g., cell culture studies, see A.5.3.2 and A.5.4) or in vivo methods (A.5.3.1).

A.5.3.1 Infectivity and pathogenicity

If testing is required, then consider CC-MPCA point 5.3.1, the recommendations specified under the point “hazard testing” in the introduction of A.5 and the following points:

- Suitability of the test model
  For micro-organisms, infectivity and pathogenicity tests on animals may not be suitable for extrapolation to humans due to differences between humans and test animals (e.g. immune system, microbiome, host range; see point 5.2). Please provide adequate justification for the suitability of the test model.

- Observation period and clearance
  Please consider an observation period allowing a suitable assessment of the clearance of the micro-organism in the host. The choice of appropriate timing of the observational period may be based on available information such as biological properties of the micro-organism or other relevant available information. Slow clearance is known for some species (for example *Bacillus thuringiensis*) and the given observation period of 21 days in the test guidelines might not be sufficient to observe reduction of CFUs. For these species a longer observation period is appropriate.

- Dose level
  According to OPPTS guidelines (see CC-MPCA point 5.3.1), a single dose level of at least 10^8 CFU of the micro-organism per test animal should be used for oral and

intratracheal studies, and 10^7 CFU for injection studies. If the minimum dose level is not used, a justification must be provided (e.g., low concentration of the micro-organism in the MPCA-AM, leading to exceeding the maximum volume of liquid that can be administered at one time, or, where no maximum volume is specified in the guidelines, leading to suffocation or other undesirable effects not of relevance to the risk assessment).

- Exposure route
  The most appropriate exposure route for infectivity and pathogenicity studies (e.g., oral exposure; intratracheal/intranasal exposure; intravenous, intraperitoneal or subcutaneous single exposure exposure) should be determined based on the body of knowledge of the micro-organism and relevant exposure routes due to intended uses. The choice for the exposure route used for testing should be justified accordingly.

  - Intratracheal/ intranasal infectivity and pathogenicity
    Intratracheal/ intranasal infectivity and pathogenicity can be tested either through inhalation or intratracheal single exposure. While intratracheal exposure ensures high exposure of the test animal to the micro-organism, the exposure due to inhalation often is too low due to a low concentration of micro-organisms in the atmosphere and a large particle size. Furthermore, the viability of the micro-organism can be affected by nebulisation and this should therefore be quantified as part of the experimental test. Due to these considerations inhalation exposure is normally not recommended for micro-organisms and, in case, an intratracheal study is preferred.
    In intratracheal studies unspecific effects can occur which are caused by the administration of a material directly into the lungs. Therefore, it is important to include a suitable negative control in the study, e.g. inactived (e.g., autoclaved) material. It may also be useful to perform a preliminary test to establish a suitable dose level, especially if the concentration of the micro-organism in the material is relatively low, or if the test material contains a high amount of intert material that may cause suffocation.

  - Intravenous, intraperitoneal or subcutaneous single exposure
    In addition to the oral and intratracheal study, an intravenous, intraperitoneal or subcutaneous injection study can be considered. The subcutaneous injection may be preferred if the maximum growth temperature is lower than 37 °C as the micro-organism may in this case be more likely to cause infections in the skin rather than deep tissue. Intravenous and intraperitoneal injection studies are unrealistic exposure routes and should only be performed if justified, e.g. if unexpected adverse effects occur in the acute oral or intratracheal study.

A.5.3.2 Cell culture study

The data requirements state that for intracellular replicating micro-organisms, such as viruses and viroids, or in some cases for bacteria and protozoa, a cell culture study should be carried out. A dismissal of data-provision may apply when infectivity and proliferation in homoeothermic (warm-blooded) organisms can be excluded based on the body of knowledge on the micro-organism (e.g., it applies to baculoviruses based on the known host range which excludes homoeothermic organisms).

A cell culture study gives information on the ability of a micro-organism to infect, replicate in, transform or cause toxicity in human cells. A virus which is infective to humans under any circumstances cannot be approved. The selection for cell or tissue cultures of a specific organ should be based on the expected target organ upon infection. If human cells or tissue cultures

of the specific organ are not available, other mammalian cells and tissue cultures can be used.

A.5.4 Specific infectivity and pathogenicity studies on the micro-organism

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B,
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.1
Relevant decision making criterion	Reg (EU) No 546/2011, Annex, Part B, 2.5.1
Relevant approval criterion:	Reg (EC) No 1107/2009, Annex II, point 3.6.6
Eligible for substantiated dismissal of data-provision:	Yes (see text)

Purpose of this point:

Provide further information on infectivity and pathogenicity of the micro-organism to humans if there are indications of infectivity, pathogenicity or any other adverse effect.

Conditionality: If infectivity and pathogenicity of the micro-organism to humans is excluded based on the information provided under points 5.1, 5.2 and 5.3 further testing is not required.

Testing: if testing is required, then consider CC-MPCA Section 5. However, in many cases test guidelines may not be available. Before performing such studies it is highly recommended that the applicant must seek the agreement of the competent authority on the approach including type of study.

Assessment principle: The evaluation of specific infectivity and pathogenicity studies on the micro-organism will be based on case-by-case expert judgement.

An example of a specific infectivity and pathogenicity study on the micro-organism to be included in this Section is a cell culture study conducted with the micro-organism (e.g. bacteria) to assess the germination behaviour of spores upon exposure to intestinal cells showing if vegetative cells start to grow and produce harmful secondary metabolites. It is important to consider that unclear indication of infectivity or pathogenicity do not trigger a repeated dose study, as the ability to cause an infection is not influenced by repetitions of single exposure events.

A.5.5 Information and toxicity studies on metabolites

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 5.5
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.5.1
Eligible for substantiated dismissal of data-provision:	No

Purpose of this point:

Provide all the information on toxicity of secondary metabolites produced by the micro-organism to humans which is used for point 2.8 of Regulation (EU) No 283/2013 to identify or exclude secondary metabolites as being of concern.

A.5.5.1 Information on metabolites

While the information submitted for point 2.8 of Regulation (EU) No 283/2013 should consist of a summary and conclusion of the assessment of secondary metabolites produced by the micro-organism, for the current point all underlying information for the hazard identification and characterisation of secondary metabolites which are relevant specifically for the assessment of the effects on human and animal health should be included. This is reflected by the text in the data requirements (i.e., point 5.5.1 of Regulation (EU) No 283/2013): *'Information (e.g.*

scientific literature, studies results) on the toxicological characterisation of the metabolites and the related identified hazards to human and animal health, collected or generated with the aim to identify the metabolites of concern, or to exclude them as being of concern…'.

The information included in the dossier for point 2.8 related to human health should therefore be also based upon the information included in Section 5. Please refer to point A.2.8 of these EN for information on the assessment of secondary metabolites produced by the micro-organism according to the 'Guidance on the risk assessment of secondary metabolites produced by micro-organisms used as plant protection active substances, SANCO/2020/12258', including a template for an overview table for microbial secondary metabolites. Please see Figure A.2.8-01 for a visual representation of correspondences of the Stages of SANCO/2020/12258 with the data requirements of the Regulation (EU) No 283/2013.

For organisms which have not been extensively studied, the absence of indications for MoC in scientific literature is not sufficient to conclude on the absence of a foreseeable risk to human health due to secondary metabolites produced by the micro-organism. Therefore, for these less studied micro-organisms more experimental data is needed. This experimental data may comprise (geno-)testing with crude extracts and WGS analysis for the presence of genetic material coding for known toxins or medically important antimicrobials. Also in this case, it is highly recommended to reach agreement on these tests with the competent authority beforehand.

For those secondary metabolites produced by the micro-organism for which a hazard to human or animal health is identified, information on human exposure should be provided as described under points A.6 (residues in or on treated products, food and feed) and A.7.2 (fate and behaviour of metabolite(s) of concern).

A.5.5.2 Additional toxicity studies on metabolites of concern

For extensively studied micro-organisms, in this point information is only needed in case a MoC which causes a hazard to humans or animals has been identified and reference values for toxicity cannot be set based on already available information (including TTC values) or need further investigation. Studies must be performed based on a case-by-case approach (for example short-term toxicity studies and genotoxicity studies) and using the requirements set out in Part A for the specific type of study using relevant fractions of the MPCA-AM, or the pure MoC, if possible. It is highly recommended to reach agreement on these tests with the competent authority beforehand.

Testing: if testing is required, then consider CC-MPCA, Section 5.

Considerations related to testing: In addition to the recommendations specified under the section "hazard testing", it is highly recommended to consider the following:

- Genotoxicity
  For secondary metabolites suspected to be of genotoxic concern from the scientific literature and following (Q)SAR prediction and read-across, and when their exposure is exceeding 0.0025 µg/kg[52] bw per day, it seems appropriate to conduct the testing battery which should include as a minimum two *in vitro* tests, covering all three genetic endpoints, i.e. gene mutations, structural and numerical chromosomal aberrations. The specific secondary metabolite could be tested in purified form using the same test methods as for chemical active substances. However, since micro-organisms may

[52] TTC-value relevant to such substances.

produce a large array of secondary metabolites, testing of a crude extract (i.e. the chemical constituents of the MPCA-AM with cell walls etc., removed) could be considered if absolutely necessary due to lack of other information, for instance when the micro-organism has not been extensively studied, as indicated in 'Guidance on the risk assessment of secondary metabolites produced by micro-organisms used as plant protection active substances', SANCO/2020/12258 and Regulation (EU) No 283/2013. In these cases, it is highly recommended to discuss the relevance and design of such tests with RMS and EFSA during pre-submission meetings. In such a test, the study design needs to be carefully considered as the concentrations of each component can be expected to be low and a component with a low genotoxic potential would thus not be detected in the test.
When performing genotoxicity studies with a crude extract it is important to avoid interference by constituents in the test samples such as provision of nutrients by lysates (e.g. histidine), growth factors that may produce abnormal growth, inhibitors of DNA synthesis leading to growth inhibition, enzymatic activity that could mimic endogenous activity in the test organism (e.g. kinase or phosphokinase activity in the TK+/- or HPRT assays), the occurrence of potentially active constituents as bound or complexed forms, or intracellular molecules with nuclease or proteolytic activity from *in vitro* lysates that would not normally have access to mammalian cell *in vivo* (MacGregor, 2005)[53]. If a positive result has been obtained with an *in vitro* study an a suitable *in vivo* follow-up genotoxicity study is required. When any results of an *in vivo* in somatic cells is positive, in vivo testing for germ cell effect may be justified. The recommended methods are the same as for chemicals, please refer to Commission Communication C/2023/6245 Part A point 5.4.

- Cytotoxicity studies conducted with for example the fermentation broth of the MCPA-AM are not considered sufficient to exclude the toxicity of metabolites of (potential) concern identified in 2.8. As these secondary metabolites may not be formed during the laboratory test or in very low quantity and therefore the possible adverse effects of the secondary metabolites may not be reliably covered by *in vitro* laboratory studies. In addition, when an effect on viability of cells is observed in the study it is not clear which secondary metabolite is responsible for the response.

A.6 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED

As micro-organisms which are pathogenic to humans cannot be approved, consumer exposure to the micro-organism itself is not relevant for the risk assessment. This Section therefore focuses on consumer exposure to metabolites of (potential) concern. Any adverse effect to humans caused by the micro-organism itself should be addressed in Section 5: Effects on human health.

While the information submitted for point 2.8 of Regulation (EU) No 283/2013 should consist of a summary and conclusion of the assessment of secondary metabolites produced by the micro-organism, for the current point all underlying information for the consumer exposure assessment for secondary metabolites which are relevant specifically for the assessment of the effects on human and animal health should be included. Qualitative or semi-quantitative information on consumer exposure should be included under point 6.1, while quantitative information should be included under point 6.2. Please see Figure A.2.8-01 for a visual representation of correspondences of the Stages of SANCO/2020/12258 with the data requirements of the Regulation (EU) No 283/2013.

[53] MacGregor, JT. Genetic Toxicity Assessment of Microbial Pesticides: Needs and Recommended approaches. Report to OECD. December 2005.

Please note that while consumer exposure to the micro-organism itself is normally not considered relevant, information on the absence or density of the micro-organism on edible parts of treated crops can be used to support the assessment.

Literature search
According to the introduction to the Annex to Regulation (EU) No 283/2013, all available relevant data from the scientific peer reviewed and open literature on the micro-organism should be provided. The literature search should be carried out in accordance with the Literature GD. Literature retrieved from this search should be reported in the relevant sections of the dossier. When a literature search is conducted it is important to correspondingly consider previous/alternate taxonomic names for the organism in question which may have been used in past publications. The search strategy reported by Hackl et al. (2015)[54] and Mudgal (2013)[55] might be helpful for consideration of search terms in the literature search.

Conditionality
Please note that not submitting data for a particular data requirement is not acceptable without further justification.

A.6.1 Estimation of consumer exposure to residues

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 6.1
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.2
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.5.2
Relevant approval criterion:	Reg (EC) No 1107/2009, Annex II, point 3.1
Eligible for substantiated dismissal of data-provision:	Yes (see text)

Purpose of this point:
Qualitative or semi-quantitative information on consumer exposure to residues of metabolites of potential concern is used to determine that there is no harmful effect to human or animal health arising from residues. In this way, metabolites of potential concern for human or animal health are either determined to be not of concern (no further assessment needed) or of concern (in which case a quantitative assessment is needed; see A.6.2).

Conditionality: If there are no metabolites of potential concern (no hazard) for human and animal health identified under point 5.5 and summarised under point 2.8 no further information than a justification for not providing data is required for this point.

Assessment principle:
Information should be provided for metabolites of potential concern for human or animal health (identified based on information provided under point 5.5.1 and summarised under point 2.8) to be able to perform an indicative consumer risk assessment (i.e., a qualitative or semi-quantitative assessment). Regulation (EU) No 283/2013 provides multiple methods to estimate the exposure to secondary metabolites, for which a hazard to human or animal health was identified, considering the intended use:

- *'…a calculation of the expected residue levels of these metabolites on edible parts of treated crops using worst-case estimates, taking into account the*

[54] Hackl, E; Pacher-Zavisin, M; Sedman L.; Arthaber, S; Bernkopf, U.; Brader G; Gorfer, M; Mitter, B; Mitropoulou, A; Schmoll, M; Van Hoesel, W; Wischnitzky, E; Sessitsch, A. EFSA Supporting Publication 2015:EN-801.
[55] Mudgal, S; De Toni, A; Tostivint, C; Hokkanen, H; Chandler, D. EFSA Supporting Publications 2013:EN-518.

critical good agricultural practice(s), ecology of the micro-organism, such as its lifestyle (e. g. saprophytic, parasitic, endophytic), host range, life cycle, population growth requirements and the conditions which trigger the production and the properties of the metabolite for which a hazard to human health was identified.'

When an endophytic lifestyle has been demonstrated for the micro-organism, it must be considered that many micro-organisms can grow endophytically and that this lifestyle does not constitute a hazard in itself as population densities and secondary metabolite concentrations for endophytic micro-organisms are commonly low.

- *'…direct measurements of the metabolite, e.g. to show the absence of the metabolite on edible parts at time of harvest. When determining the need for direct measurements, the possibility and relevance of exposure to the metabolite produced after application on the edible parts (in-situ production) shall be taken into consideration. This may include a comparison between the background level of the metabolite and the elevated level of it due to treatment with the plant protection product containing the active substance.'*

- *'…direct measurements of the density of the micro-organism on edible parts of treated crops, e.g. if it cannot be adequately justified that in-situ production of the metabolite is not relevant for the consumers. Such measurements shall be performed under normal conditions of use and in accordance with good agricultural practice.'*

  In case experimental data to demonstrate the absence of the micro-organism on edible crops is used, for instance, to demonstrate the absence of consumer exposure to the secondary metabolite, it is needed to do so for all growth stages of the edible part – not only at the time of harvest. Especially if plating methods are used as detection method, the absence of live micro-organisms is not sufficient to demonstrate the absence of the secondary metabolite, as the secondary metabolite may have been produced at an earlier growth stage and remained on the edible part.

- Where relevant, adequate justification for read-across must be provided.

When using information on the natural background levels of the secondary metabolite or the microbial species, please note that natural occurrence in itself is not sufficient to determine on the absence of harmful effects, as the natural presence of a secondary metabolite may result in harmful effects. Therefore, information on natural occurrence should always be used in combination with information on the (absence of) harmful effects.

If the metabolite of potential concern is present in the MCPA-AM, a consumer risk assessment should be provided based on the maximum level (mean value found in the batch analysis + three times standard deviation) at which the secondary metabolite may be present in the product (see also P.1.4, 'Specification of the microbial pest control agent as manufactured'). As described in SANCO/2020/12258 (Stage 3, Step 14) for hazards arising from human dietary exposure, a worst-case theoretical estimate of the residue can be made by assuming that, upon application, the entire product-borne amount of the metabolite of concern will end up on the edible parts. With data on crop yields, a theoretical estimate of the residue can be calculated, by taking the lowest mean crop yield for the EU in the last five years (a low level of crop yield from a possible range should be used to give a worst-case estimate of the residue,

since the aim should be to assess the highest likely residues that could arise following the intended use). Together with the application rate (e.g., CFU/kg per ha) and the secondary metabolite concentration (in mg/ha), the maximal residue of the secondary metabolite in µg/kg crop can be calculated. With this worst-case approach, dietary uptake from a given crop can be compared with available health-based reference values, such as the Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD), with natural exposure levels, or with the Threshold of Toxicological Concern (TTC) when no other reference values are available. Furthermore, the expected consumer exposure to these residues can be estimated using EFSA's Pesticide Residue Intake Model (PRIMo).

A.6.2 Data generation on residues

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 6.2
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.2
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.5.2
Relevant decision making criterion:	Reg (EC) No 1107/2009, Annex II, point 3.1
Eligible for substantiated dismissal of data-provision:	Yes (see text)

Purpose of this point:

Provide data on residues of MoC for which a hazard to humans or animals has been identified in case where the substantiated estimation in point 6.1 (SANCO/2020/12258, step 14) does not demonstrate an acceptable risk to consumers (SANCO/2020/12258, stage 4, step 16)

Conditionality: As described in the introduction of Section 6 in Regulation (EU) No 283/2013: *'Data on residues as required in point 6.2 shall be provided, unless:*

— based on a weight of evidence approach concerning the information submitted in accordance with Sections 2, 3, 5 and 7, it can be justified that possible metabolites of concern identified (see point 2.8) are not hazardous to humans as a result of the intended use,

— it is possible to conclude, through estimation of consumer exposure to residues of metabolites for which a hazard to human health was identified (see point 5.5.1) that the risk for consumers is acceptable, or

— the micro-organism is a virus.'

Testing: if testing is required, then consider Commission Communication C/2023/6245 Part A Section 6.

Assessment principle:
Full residue data, as required for chemicals, are rarely needed because in general sufficient information is available to address the concerns. However, if significant quantities of the MoC are foreseen (for example due to high concentrations in the MPCA-AM) and risk to humans and animals cannot be excluded based on information provided under points 5.5.1 and 6.1, and summarised under point 2.8, relevant studies of a data package on residues as provided in Section 6 of Part A may be required.

To determine concentrations of MoC, edible parts that have been treated with the PPP in accordance with representative use can be chemically analysed. By determining the concentration of MoC in this way, both the exposure resulting from the presence of the secondary metabolite in the product and from *in situ* production are covered. If quantitative measurements of MoC are performed, storage stability data for the measured MoC must be

provided. The expected consumer exposure to these residues can be estimated using EFSA's Pesticide Residue Intake Model (PRIMo) and this can then be compared with the health-based reference values mentioned above.

For further guidance, please refer to Annex II of the 'Guidance on the risk assessment of metabolites produced by micro-organisms used as plant protection active substances', SANCO/2020/12258.

A.7 ENVIRONMENTAL OCCURRENCE OF THE MICRO-ORGANISM, INCLUDING FATE AND BEHAVIOUR OF METABOLITE(S) OF CONCERN

The aim of this Section 7 is to provide information on the occurrence of the micro-organism in the relevant environmental compartments (e.g. soil and/or surface water, according to the hazards identified under Section 8) and to assess the potential exposure of non-target organisms to the micro-organism, and where relevant the potential exposure of humans non-target organisms to metabolites of concern. Please note that while this Section addresses the environmental occurrence of the micro-organism which is the active substance (*i.e.*, the strain/isolate under evaluation), information on the natural occurrence of related organisms (*e.g.*, at species level) should be provided under point 2.1.2.

Information on the fate and behaviour of secondary metabolites may be needed either to exclude secondary metabolites from being of concern, or to perform a risk assessment for MoC. The method by which environmental concentrations are determined depends on whether the secondary metabolite is present in the MPCA-AM (hence in the PPP), or produced *in situ* upon application. For secondary metabolites which are present in the MPCA-AM and for which *in situ* production is not relevant, calculation models can be used (point 7.2.1). When *in situ* production may be relevant, calculation models may not be relevant and a tiered approach to assess exposure of humans, non-target organisms and the environment can be followed. First, a qualitative exposure assessment is performed based on the available information on the ecology of the micro-organisms and information on the secondary metabolite (point 7.2.2). If both the calculation of environmental concentrations using calculation models and the qualitative assessment are not sufficient to exclude this secondary metabolite from being of concern, a quantitative assessment for the MoC is needed (point 7.2.3). As calculation models are not appropriate for MoC at this stage of the assessment (see point 7.2.1), experimental data on concentration of the secondary metabolite in relevant environmental compartments is needed.

It should be also noted that the Regulation (EU) No 283/2013 does not always oblige the applicant to fulfil the data requirements related to the hazard first, and those related to the exposure later. In certain cases, the applicant may prefer proving absence of exposure first (i.e. to the secondary metabolites or to the micro-organism), in order to dismiss provision of hazard-related data. However, this possibility does not apply to data provision concerning hazards potentially caused by the micro-organism to humans (Section 5) and to terrestrial vertebrates (point 8.1); in these cases, absence of exposure does not allow dismissal of data-provision.
Please note that while information submitted under point 2.8 of Part B of the Annex to Regulation (EU) 283/2013 should consist of a summary and conclusion of the assessment of secondary metabolites produced by the micro-organism, all underlying information for the environmental exposure assessment of secondary metabolites should be included under point 7.2.

A.7.1 Environmental occurrence of the micro-organism

Corresponding Annex point:	**Reg (EU) No 283/2013, Annex, Part B, 7.1**
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.6.1

Purpose of this point:

The purpose of this point is to provide information on the environmental occurrence of the micro-organism to be able to assess the potential exposure of humans and non-target organisms to the micro-organism in the environment.

Information on the environmental occurrence of the micro-organism is only needed in case a hazard for humans or non-target organisms caused by this micro-organism has been identified. In case a hazard is identified through ecotoxicological testing on soil-living or aquatic organisms, a tiered approach is followed under this Section. As a first step, PED must be calculated based on the proposed use (points 7.1.1.1 and 7.1.1.2; see also Section 8). In case further information on the environmental occurrence of the strain is needed because risks are still observed after exposure to densities of the micro-organism calculated with the PED, as a second step qualitative information is used (point 7.1.3). If this information based on modelling, calculations and qualitative information is not sufficient to conclude the risk assessment, quantitative information on the population densities of the micro-organism is needed (point 7.1.4).

According to point 7.1 of Part B of the Annex to Regulation (EU) No 283/2013, the data provision on environmental occurrence of the micro-organism does not depend on data on possible effects on human health, because micro-organisms cannot be approved if they are pathogenic to humans regardless the level of exposure.

In this Section, specific additional information is required for micro-organisms which are pathogenic to target or non-target organisms (point 7.1.2) and which do not occur in Europe at the relevant highest taxonomic level. This is because pathogenic micro-organisms may multiply in their host organism and therefore, in contrast to non-pathogenic micro-organisms that usually decrease upon application[56], calculated PED values may not be a conservative estimation of exposure of NTOs as they may be exposed to infected host organisms. Therefore, the exposure assessment for pathogenic micro-organisms which do not occur in the relevant European environments differs in two main points from the assessment for non-pathogenic organisms. Firstly, the primary exposure of NTOs to pathogenic micro-organisms can never be completely excluded based on the proposed use and needs to be assessed because the release of pathogenic micro-organisms can in principle spread and cause disease in populations of NTOs[57]. In addition, the secondary exposure of NTOs to the micro-organism can also occur via an exposure to host organisms infected with the pathogenic organisms: therefore, an estimation of the expected development of this infection is also needed.

An overview of the line of thought for exposure assessment is given in the scheme below:

[56] Köhl et al., 2019 (p. 474): BioControl (2019) 64:469–487 https://doi.org/10.1007/s10526-019-09964-y

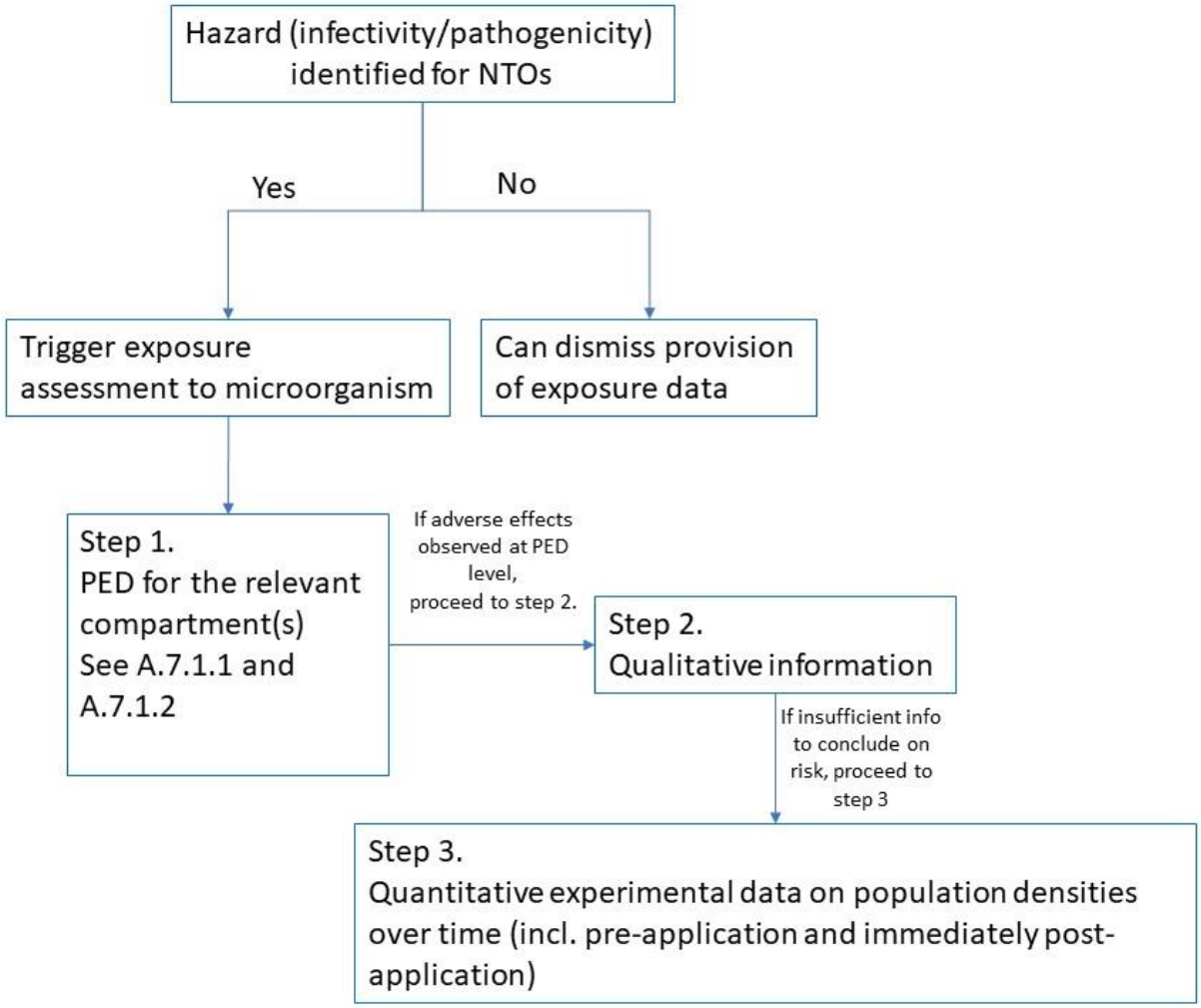

Figure A.7-01: Graphical representation of the exposure assessment approach.

A.7.1.1 Predicted environmental density

Assessment principle: In case a hazard for NTOs has been identified (see Section A.8), PED values must be determined for the relevant environmental compartments (soil and/or surface water). For groundwater, no PED value for the micro-organism is needed because the relevant risk assessment would concern human exposure through dietary pathway, and as micro-organisms cannot be approved if they are pathogenic to humans this exposure information would be superfluous.

In contrast to qualitative (A.7.1.3) and quantitative exposure assessments (A.7.1.4) where the approach is targeted (e.g. based on biological properties of the micro-organism, and focused on certain environmental compartments such as plant surfaces), PED calculation cannot be excluded for the environmental compartments soil and surface water as a “bulk”.

Until a new relevant guidance document is endorsed at EU level or produced by EFSA, PED values must be calculated using the formula provided below (A.7.1.1.1, A.7.1.1.2) based on the proposed use. The exposure of soil and surface water differs for field and protected crops. The “EFSA Guidance Document on clustering and ranking of emissions of active substances of plant protection products and transformation products of these active substances from protected crops (greenhouses and crops grown under cover) to relevant environmental

compartments" can be consulted to determine if the proposed use should be determined as a field or protected use is relevant. However, please note that the calculation methods described in this guidance document do not apply to micro-organisms.

Background information on the nature of the assumptions and methods used for the PED calculations are provided in CC-MPCA, points 7.1.1.1 and 7.1.1.2. It should be noted that these calculations provide in most cases an unrealistic worst-case initial predicted density when multiple applications are considered and especially in case of multiple crop cycles per year; these calculations do not take into account unfavourable conditions that will most likely rapidly and negatively impact the population density for most micro-organisms (*e.g.,* temperature, lack of nutrients, competition).

A.7.1.1.1 PEDSOIL

The method to calculate the PED in soil is based on a worst-case scenario in which all applications of a crop cycle (in case of multiple crop cycles per year) or year (in case of permanent crops such as fruit trees) are summed and no decline of the population density upon application is assumed (n.b., as explained in the previous point, this assumption may be not applicable for infective/pathogenic micro-organisms which can multiply in the host). The application rate used in PED calculations is calculated based on the use rate of the product (*e.g.*, L/ha) and the maximum content of the micro-organism in the product based on the specification (*i.e.*, the highest concentration of the range of values). Please consider also relevant guidance documents listed under CC-MPCA, points 7.1.1.1. No crop interception is assumed for micro-organisms; the full amount applied is assumed to reach the soil. Assuming the amount of the micro-organism being expressed in CFU (but it applies to any other metric that is compatible with the micro-organism's specification), this results in the following formula to calculate the initial PED value in soil:

$$\mathrm{PED_{SOIL}\ as}\ \frac{\mathrm{CFU}}{\mathrm{kg_{dry\ soil}}} = \frac{\mathrm{Application\ rate} \cdot\ n}{10000 \cdot \mathrm{d} \cdot \rho}$$

Where:

- the application rate (expressed in CFU/ha) is the unit of product applied per application multiplied by the maximum concentration of the micro-organism in the product (as given in the specification)
- *n* is the number of applications per crop cycle/year
- 10 000 as the conversion factor from hectare to m^2
- d as the depth of soil layer (default of 0.05 m)
- ρ as the density of the soil[58]

The concept of accumulation in soil as for persistent chemical substances does not apply to micro-organisms. Therefore, only the initial PED values in soil as described above are relevant for the risk assessment.

For uses in <u>permanent</u> greenhouses and indoor uses no PED_{SOIL} is required.

A.7.1.1.2 PED_{SW}

[58] Please refer to soil density values indicated in Table 1, Section 2.2. of the EFSA Guidance document for predicting environmental concentrations of active substances of plant protection products and transformation products of these active substances in soil (EFSA Journal 2017;15(10):4982).

As for the calculation of the PED in soil, the method to calculate the PED for surface waters is based on a worst-case approach. Note that for field uses run-off, drainage and aerial deposition are in principle not considered as relevant route of contamination into the water bodies for microbial active substance.

$$\mathrm{PED_{SW}\ as}\ \frac{\mathrm{CFU}}{\mathrm{L}} = \frac{\mathrm{Application\ rate} \cdot\ n\ \cdot (\frac{\mathrm{D}}{100})}{10000 \cdot \mathrm{Vd}}$$

Where:

- the application rate (expressed in CFU/ha) is the unit of product applied per application multiplied by the maximum concentration of the micro-organism in the product (as given in the specification)
- *n* is the number of applications per crop cycle/year
- D is the percentage of drift (field uses) or emission (protected uses)
- 10 000 as the conversion factor from hectare to m^2
- Vd as the volume of the standard ditch per surface area (L/m^2)

The BBA drift[59] values in combination with the volumetry of the FOCUS standard ditch should be used (*i.e.*, 300 L/m^2) for field uses including uses in non-permanent greenhouses.

For permanent greenhouse uses of micro-organisms, an appropriate emission percentage should be used in combination with the volumetry of the FOCUS standard ditch, in order to cover all relevant emission routes of microbial active substances. As an exception to this rule, for granular soil-incorporated applications of micro-organisms in permanent greenhouses[60], no emission to surface water is assumed (as proposed by EFSA as part of the peer review of *Metarhizium brunneum* BIPESCO 5/F52[61]).

A.7.1.2 Exposure to micro-organisms known to be pathogenic either for plants or for other organisms.

Assessment principle: While for non-pathogenic micro-organisms the PED values can be used as a conservative estimation of the environmental occurrence of the micro-organism upon application, this assumption may not hold for pathogenic micro-organisms not occurring in the relevant European environments, for which the PED value may not be the worst-case exposure scenario as local and temporary proliferation may occur. These pathogenic micro-organisms may proliferate in their host organisms resulting in highly localised (*i.e.*, in their host) higher population densities of the micro-organism. Therefore, in addition to providing information on PED values in soil and surface water, information is needed on the likelihood and level of exposure of NTOs to the micro-organism via infected host organisms. This information may be provided based on biological properties of the active substance or relevant literature data or studies (from *e.g.* Section 2 or 8). For example, if the micro-organism is an entomopathogen which can infect larvae of a certain species of beetle, this information may be required for the risk assessment for insectivorous NTOs.

[59] Rautmann D, Streloke M, Winkler R. (2001), New basic drift values in the authorisation procedure for plant protection products, In: Forster R, Streloke M. Workshop on Risk Assessment and Risk Mitigation Measures in the Context of the Authorisation of Plant Protection Products (WORMM), Mitt. Biol. Bundesanst. Land-Forstwirtsch. Berlin-Dahlem, Heft 381 pp.

[60] N.b., this exception does not apply to micro-organisms not occurring in the relevant European environments at the relevant highest taxonomic level and which are known to be pathogenic either for plants or for other organisms.

[61] https://www.efsa.europa.eu/en/efsajournal/pub/6274

Please note that for pathogenic micro-organisms, exposure to NTOs is in principle assumed regardless of the proposed use, even in case PED values in both soil and surface water equal zero.

A.7.1.3 Qualitative exposure assessment of the micro-organism

In principle information on the environmental occurrence of the micro-organism upon application is needed if a hazard has been identified for humans or NTO. This hazard can consist of adverse effects on NTOs due to the micro-organism itself (pathogenicity and infectivity) or to humans and NTOs due to *in situ* production of toxic secondary metabolites. In the latter case, the qualitative exposure assessment to the micro-organism is used to inform the qualitative exposure assessment to the secondary metabolite (A.7.2.2). However, in certain cases and as described in the introduction to this Section A.7, the applicant may decide proving absence of exposure first in order to dismiss the provision of hazard-related data.

Assessment principle: In contrast to the calculation of PED values, which are calculated for the environmental compartments soil and surface water, the qualitative exposure assessment should follow a targeted approach. For example, if a hazard has been identified due to pathogenicity of the micro-organism to tree-dwelling caterpillars, information on the population density of the micro-organism in soil or surface water may be irrelevant. In contrast, information on the population density on the leaves on which the caterpillar feeds may be relevant. As such, the first step to provide information for the qualitative exposure assessment is to identify the exposure routes for NTOs to which the hazard applies. Next, the environmental compartments relevant for this exposure route should be identified. Please note that these compartments may be highly specific, such as infected insects, flowers or the rhizosphere.

As indicated in the data requirements, the weight of evidence approach for the qualitative exposure assessment as a rule draws heavily on information on the biological properties of the micro-organism (e.g. ecology, growth requirements). In addition, in certain cases a semi-quantitative approach can be followed using experimental data, for example by using information generated during efficacy trials. In this way and where relevant, it may for example be possible to demonstrate the absence of the micro-organism in edible parts of the plant for micro-organisms which are applied as seed or soil treatment or as a foliar application during early growth stages.

In addition to information on the ecology of the micro-organism itself, information on the natural occurrence of closely related micro-organisms can be included. When using information of closely related micro-organisms, a justification should be provided as to why this information is relevant for the micro-organism – a close phylogenetic relationship in itself is not sufficient as justification. For example, when a hazard has been identified for a NTO due to pathogenicity, information on the comparability of the virulence traits (*e.g.*, host range) is needed when information on the natural occurrence of closely related micro-organisms is used for the risk assessment.

A.7.1.4 Experimental exposure assessment

Experimental (quantitative) data on the exposure of humans or NTO to the micro-organism is needed in case the information considered under points 7.1.1 to 7.1.3 and 7.2 is not sufficient to conclude on the risk caused by the identified hazard. This may also be needed if the hazard is due to *in situ* production of toxic secondary metabolites. In the latter case, the experimental quantitative exposure assessment to the micro-organism is used to inform the exposure

assessment to the secondary metabolite (A.7.2.2 and A.7.2.3).

Assessment principle: The quantitative exposure assessment should follow the same targeted approach as described in A.7.1.3. For the relevant environmental compartments, experimental data on the population density of the micro-organism should be provided in a time course including pre-application (*i.e.,* not the natural background of closely-related strains) and immediately post-application. The length of the time course and the sampling frequency should be set so as to be able to assess the potential decline of the population density upon application.

The relevance of the experimental conditions for the risk assessment should be justified. This justification may include information on the choice of crop, soil and climatic region. For post-harvest application information on storage conditions may be relevant. Furthermore, information on relevant environmental parameters should be provided, such as humidity, pH, temperature, salinity, as these parameters may have a large effect on the population dynamics of the micro-organism. In case the experimental data are extrapolated to other uses of the micro-organism, a justification for this read-across should be provided.

A.7.2 Fate and behaviour of metabolite(s) of concern

Corresponding Annex point:	Reg (EU) No 283/2013, Annex, Part B, 7.2
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.6.2
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.6.1 to 2.6.3
Purpose of this point:	
The purpose of this point is to provide information on the exposure of humans and the environment to a secondary metabolite for which a hazard is identified, to determine whether the secondary metabolite is or not a MoC, and if so, to perform a quantitative risk assessment.	

As described in the ‘Guidance on the risk assessment of secondary metabolites produced by micro-organisms used as plant protection active substances’ (SANCO/2020/12258 – see Step 14 in particular), if information on the fate and behaviour of secondary metabolite(s) allows to conclude that the secondary metabolite is not of concern, no further assessment is needed; however, if the secondary metabolite is considered a MoC a quantitative risk assessment is required (see Stage 4 of SANCO/2020/12258). Please see Figure A.2.8-01 for a visual representation of correspondences of the Stages of SANCO/2020/12258 with the data requirements of the Regulation (EU) No 283/2013.

In case of secondary metabolites which are hazardous to humans or non-target organisms, calculation of PEC values or a qualitative exposure assessment may be needed to verify whether they are MoC.

For MoC to which surface water or groundwater is exposed, it should be demonstrated that the level of contamination of surface water and groundwater does not exceed the concentrations relevant for the water framework directive and the drinking water directive, as provided for in the decision-making criteria 2.6.1 of the Part B of the Annex to the Regulation (EU) No 546/2011. Please note that for secondary metabolite for which a hazard has been identified but it was demonstrated not to be a MoC (e.g. based on a qualitative risk assessment), this information is not needed.

No PEC information is required for secondary metabolites for which no hazard is identified, or for secondary metabolites for which hazard is identified but are not present in the MPCA-AM (even if *in situ* production is known to occur, or its occurrence cannot be excluded).

A.7.2.1 Predicted environmental concentration

The need for PEC values of secondary metabolites present in the product is triggered by the approach described in the metabolite guidance. Please note that this approach to calculate PEC values cannot be used for secondary metabolites which are produced *in situ* upon application. To provide information on the exposure to secondary metabolites which are produced *in situ*, a qualitative or quantitative exposure assessment is needed, as described in A.7.2.2 and A.7.2.3, respectively.

Assessment principle: As a first step, the environmental compartments which are relevant for the exposure to the secondary metabolite of either humans or the NTO(s) for which a hazard has been identified should be determined. In case the secondary metabolite is a medically important antimicrobial, all environmental compartments (soil, surface water and groundwater) are considered to be relevant (SANCO/2020/12258, Step 14).

For secondary metabolites present in the product, the pesticide fate models developed for chemical active substances (FOCUS DG SANTE[62]) should be used. If data on the physical-chemical parameters that are required for the models are lacking, conservative default values may be used in line with standard approaches for the assessment of chemical actives. Aside from the EFSA guidances, ECHA guidances may also be used[63] but keep in mind that these values are normalised for 12°C.

A.7.2.2 Qualitative exposure assessment

Purpose of this point: Information on the environmental concentrations of a secondary metabolite is only needed if a hazard (toxicity) for this secondary metabolite has been identified for humans or non-target organisms. Based on the qualitative assessment, the secondary metabolite can either be demonstrated not to be of concern, or to be of concern. In the latter case, a quantitative assessment is needed for this MoC (see A.7.2.1 and A.7.2.3). For secondary metabolites which are also present in the product at the time of application, the qualitative assessment is used to determine if *in situ* production of the secondary metabolite is relevant for the risk assessment.

The qualitative exposure assessment of secondary metabolites follows the same targeted approach as described for the qualitative exposure assessment to the micro-organism (see A.7.1.3). Please note that the environmental compartments which are relevant for the assessment may differ for the micro-organism and its secondary metabolite, as secondary metabolites may be mobile in the environment upon production.

Assessment principle: For the qualitative exposure assessment for a secondary metabolite, the information on the environmental occurrence of the micro-organism is used (see A.7.1). In addition, available information on the levels at which the secondary metabolite can be produced by the micro-organism and the environmental conditions needed for this production should be included (see A.2.8). Furthermore, available information on the fate and behaviour of the secondary metabolite itself should be used. This includes information on the stability and the adsorption of the secondary metabolite.

As for the qualitative exposure assessment for the micro-organism, the qualitative exposure assessment for secondary metabolites may include information on the natural background

[63] e.g., https://echa.europa.eu/documents/10162/13632/information_requirements_r16_en.pdf/b9f0f406-ff5f-4315-908e-e5f83115d6af

levels of the secondary metabolite, if available. As for metabolite of potential concern and MoC it is highly likely that they are produced by other micro-organisms (which are not necessarily closely related), information on the natural occurrence of other producers of the secondary metabolite may also be included. Therefore, the natural background concentrations should be used in a weight of evidence approach in which also information on the (absence of) effects due to the natural exposure is included.

A.7.2.3 Experimental exposure assessment

Purpose of this point: Experimental (quantitative) data on the exposure of humans or NTOs to the metabolite of concern is needed in case the information provided under points 7.2.1 and 7.2.2 is not sufficient to conclude on the risk caused by the identified hazard.

Assessment principle: As mentioned for the qualitative exposure assessment for secondary metabolites, this experimental exposure assessment should follow a targeted approach (*i.e.*, addressing those environmental compartments which are relevant for the exposure route of humans or NTOs for which a hazard was identified for this metabolite of concern). In case the relevant environmental compartment is soil, surface water or groundwater, the study should be conducted in accordance with the provisions for this study as described in Part A of the data requirements (*i.e.*, the environmental fate and behaviour of chemical substances). For those cases where the environmental compartment which is relevant for the exposure to the secondary metabolite is the same environmental compartment in which the micro-organism is present, information on the population density of the micro-organism should be provided in accordance with point 7.1.4.

Similar to the experimental exposure assessment for the micro-organisms, the relevance of the experimental conditions for the risk assessment should be justified. This justification may include information on the choice of crop, soil and climatic region. For post-harvest application information on storage conditions may be relevant. Furthermore, information on relevant environmental parameters should be provided, such as humidity, pH, temperature, salinity, as these parameters may have a large effect on the population dynamics of the micro-organism. In case the experimental data are extrapolated to other uses of the micro-organism, a justification for this read-across should be provided.

Please note that for all metabolites of concern it should be demonstrated that the level of contamination of surface water and groundwater does not exceed the concentrations relevant for the water framework directive and the drinking water directive. However, while for metabolites of concern for which *in situ* production is not relevant the approach for chemical substances can be followed, a different approach is needed for secondary metabolites which are produced *in situ*. It is expected that these uniform principles are mainly relevant in case the metabolite of concern is present in relevant quantities in the product, not for *in situ* produced secondary metabolites. However, a justification should be provided and in certain cases agreement with the competent authority on the approach may be sought prior to dossier submission.

A.8 ECOTOXICOLOGICAL STUDIES

Scope

As mentioned in Regulation (EU) No 283/2013, the information provided by the applicant should be sufficient to:

- *“decide whether or not the micro-organism can be approved,*

- *specify appropriate conditions or restrictions to be associated with any approval,*
- *permit an evaluation of short- and long-term risks for non-target species - populations, communities, and processes, as appropriate, and*
- *specify any precautions deemed necessary for the protection of non-target species*".

According to Regulation (EU) No 283/2013, "*special attention shall be paid to microbial species which are not known to occur in the relevant European environments. The information provided shall be sufficient to determine the physiological and ecological host range (in conjunction with the analysis of key biological traits of the micro-organisms) in order to assess impacts on non-target organisms"*.

As a result of the risk assessment conducted in the ecotoxicology Section, a safe use should be concluded for the products placed on the market and thus their application does not result in unacceptable effects to non-target organisms.

Please note that with regard to the literature search, it should be conducted encompassing all trophic levels, as listed in the data requirements. For example, the search strategy should include the name of the species of the micro-organism, the terms "infectivity", "pathogenicity" or others relevant to other adverse effects, and relevant taxa concerning: e.g. birds, mammals, reptiles, amphibians, fish, daphnia, bees, pollinators, arthropods and beneficials. Effects on algae, aquatic and terrestrial plants should be included for the organisms that are closely related to plant pathogens or with known herbicidal mode of action. Please also note that in some situations the search strategy might need to be adapted, for instance to the nature or mode of action of the micro-organism or the genus level be included.

Conditional requirement / justifications needed
According to the points from 8.1 to 8.6, for all the NTO, a summary of the biology and ecology of the microorganisms and the potential infectivity and pathogenicity towards NTO deriving from these biological characteristics, must always be provided. This summary must be based on the body of knowledge, such as on the information already provided under Sections 1, 2, 3 and 7, and on other information which may be retrieved from any other reliable source (e.g., uses of the micro-organism in food and feed).

If pathogenicity/infectivity studies are required based on the data requirements (i.e, the relevant point from 8.1 to 8.6 of the Annex Part B to Regulation (EU) No 283/2013,), ecotoxicological tests must be performed using the relevant guidance documents and test methods (e.g., those referred to in the CC-MPCA). The uniform principles and data requirements follow the risk principle (i.e., risk = hazard × exposure) and allow for justifications based either exposure or hazard. As indicated in Section A.7, an applicant may prefer firstly assessing possible hazards for NTO, in order to not provide data related to exposure to the micro-organism or the MoC if absence of hazards is justified. However, the Regulation (EU) No 283/2013 still allows the applicant, in certain cases, to prove absence of exposure first (i.e. to the secondary metabolites or to the micro-organism), in order to dismiss the provision of data under this Section 8 (n.b., absence of exposure does not allow data-waiving concerning hazards potentially caused by the micro-organism to terrestrial vertebrates; information on the potential infectivity and pathogenicity of the micro-organism to terrestrial vertebrates must always be provided). An overview of the lines of thought for dismiss the performance of hazard testing for NTOs (including terrestrial vertebrates), is shown in Figure A.8-01.

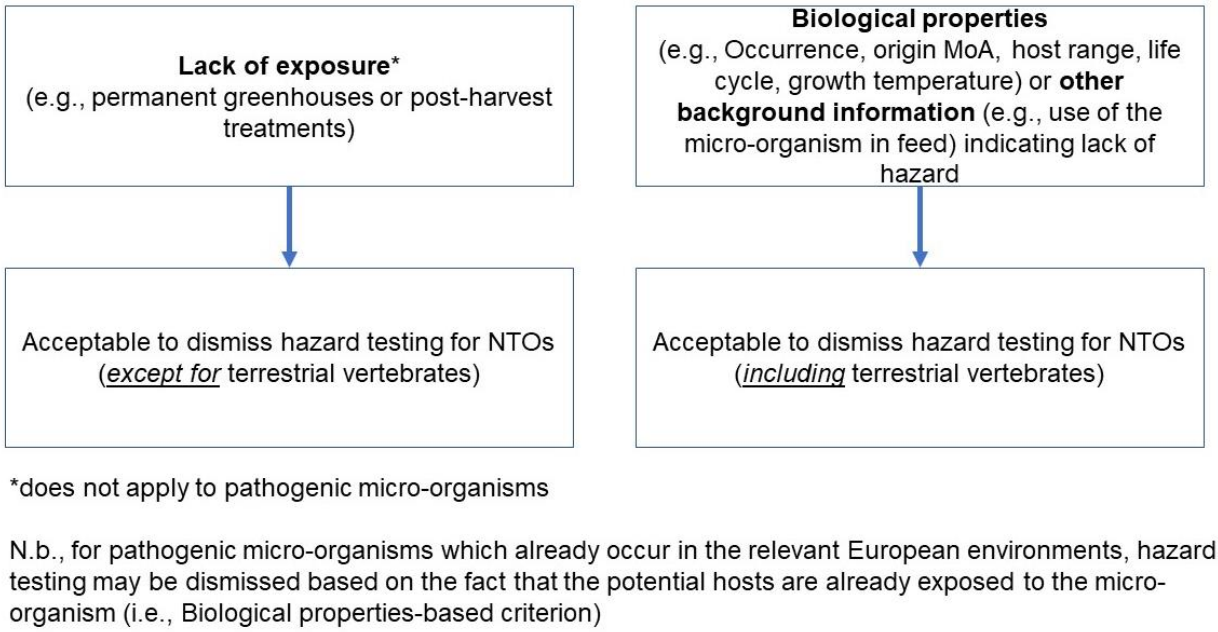

Figure A.8-01: Graphical representation of how dismissing provision of data concerning ecotoxicological studies.

If effects are observed in preliminary tests on a particular NTO species (or in the preliminary information provided in the summary on potential infectivity and pathogenicity of the micro-organism to a particular NTO species), further information on exposure and hazard characterisation is needed for the risk assessment for that specific NTO species.

Justifications for dismissing ecotoxicological testing/data requirements:

a) For all the NTO, no tests are required if, based on the available data on the micro-organism at the most relevant taxonomic level in the peer-reviewed literature (e.g., including the risk assessment in other regulations outside EU, the use of the micro-organism in food, feed, or other sectors), and/or information on the biological properties of the micro-organism (e.g., mode of action and host range, growth temperature, natural occurrence of the species, ecology and life-cycle, fate and behaviour for qualitative exposure estimations (see Fate Section, point 7.1.3)), an assessment of the hazard to specific NTOs can be performed.

b) When it can be determined that there will be no exposure from the proposed use(s) there is no need to provide data on potential hazards (n.b., it does not apply for terrestrial vertebrates). Commonly, it may be argued that there is no exposure of a certain organism based upon the type of proposed use (e.g., in greenhouses, post-harvest treatment). The “EFSA Guidance Document on clustering and ranking of emissions of active substances of plant protection products and transformation products of these active substances from protected crops (greenhouses and crops grown under cover) to relevant environmental compartments” provides definitions for different types of protected crops as well as guidance on deriving exposure estimations for different types of environmental compartments. Following the publication of this guidance, it was considered necessary to address the ecotoxicological risk assessment for organisms for which the exposure is not covered by the Guidance on protected crops. Therefore, this topic was discussed in the general ecotoxicology meeting,

Pesticide Peer Review Meeting 133 in September 2015 [64].

Please note that while certain justifications for absence of exposure may be appropriate for non-pathogenic micro-organisms (e.g., only applied in permanent greenhouses), for pathogenic micro-organisms which do not occur in the relevant European environments at the relevant highest taxonomic level the phrasing of the uniform criterion '*where the possibility of being exposed cannot be excluded*' should be considered[65]. For example, for a plant-pathogenic micro-organism for which approval is only sought for greenhouse uses, invoking absence of exposure to non-target plants will not be acceptable, as any release of the plant-pathogen into the environment (e.g., ventilation of greenhouses) may lead to unacceptable effects on non-target plants. Another example may concern arthropods' pathogens intended to be applied only in the greenhouse, for which data on non-target arthropods are needed to assess possible unwanted effects on beneficial arthropods eventually used in the greenhouse, and on other non-target arthropods in case of possible release in the environment via ventilation. However, for pathogenic micro-organisms which already occur in Europe at the relevant highest taxonomic level, it may be considered that exposure of possible host organisms already takes place. Hence, a justification for not generating further data may be based on this argument, with the legal justification provided by point 1.5 of the Introduction to the Annex of Regulation (EU) No 283/2013.

When justifying no exposure to specific organisms (as in the greenhouse example above), the following must be considered:

According to point 7.1.2 of Regulation (EU) 283/2013 "*For micro-organisms not occurring in the relevant European environments at the relevant highest taxonomic level and which are known to be pathogenic either for plants or for other organisms (see points 2.2 and 2.3), the host organisms in which proliferation of the micro-organism is expected shall be indicated. If non-target organisms indicated under Section 8 may be exposed to the host organisms colonised by the pathogen, information on the likelihood and, if applicable, level of exposure shall be provided".*

Hence, for micro-organisms which preliminary information indicates a pathogenic MoA against a certain group on NTO, possible justification on dismissing data provision based on exposure should take into consideration relevant host densities[66].

Please note that '*the relevant highest taxonomic level'* is meant to refer to either the strain, species or genus level, depending on the 'similarity' of the micro-organism strain with regard to the naturally occurring strains/ species or genera in the EU. Information on closely related micro-organisms can be addressed via identity, e.g. (phylo)genetic/molecular) similarity analyses and their biological properties, including the comparability of the virulence traits (*e.g.*, host range; see Sections 1, 2 and 7).

[64] EFSA Supporting publication 2015:EN-924, Outcome of pesticides peer review meeting on recurring issues in ecotoxicology.

[65] Please note that this stricter approach on pathogenic micro-organisms not occurring in European environment is also justified by the introduction to Section 8 of the Annex Part B to Regulation (EU) No 283/2013: "*Special attention shall be paid to microbial species which are not known to occur in the relevant European environments. The information provided shall be sufficient to (…) assess impacts on non-target organisms.".*

[66] Please note that this specification on dismissing data provision based on exposure refers to "preliminary indication of pathogenicity" because in case the summary on potential infectivity and pathogenicity of the micro-organism would allow to conclude on pathogenicity (or its absence) of the micro-organisms to the NTO, the hazard-based dismissal criterion applies.

Hazard testing

When discussing hazard testing for a micro-organism as an active substance, it is important to clearly understand what this encompasses. According to the definition provided in Regulation (EU) 283/2013,

> ***'Microbial Pest Control Agent as manufactured**' ('**MPCA-AM**') means the outcome of the manufacturing process of the micro-organism(s) intended to be used as active substance in plant protection products, consisting of the micro-organism(s) and any additives, metabolites (including metabolites of concern), chemical impurities (including relevant impurities), contaminating micro-organisms (including relevant contaminating micro-organisms) and the spent medium/rest fraction resulting from the manufacturing process or, in case of a continuous manufacturing processes where a strict separation between the manufacturing of the micro-organism(s) and the production process of the plant protection product is not possible, a non-isolated intermediate*". The MPCA-AM may thus be a complex mixture of a living organism and chemicals, including the metabolites produced by the micro-organism.'

Where there is a predicted exposure to non-target organisms from the intended use of the micro-organism, its secondary metabolites and impurities, as mentioned in the introduction to the Annex to Regulation (EU) No 283/2013, the information provided by the applicant should be sufficient to assess the foreseeable risk to non-target organisms from exposure to the micro-organism and relevant associated metabolites of concern.

According to the Introduction to the Annex to Regulation (EU) 283/2013, all available relevant data from the scientific peer reviewed and open literature on the micro-organism should be provided. The literature search should be carried out in accordance with the relevant guidance document listed under the section "General test methods and guidance documents" of the CC-MPCA. See point A.3.5 for more information. Literature retrieved from this search should be reported in the relevant ecotoxicological points of the dossier.

If data provision cannot be dismissed, and the information retrieved from the peer-reviewed literature provided does not allow to assess possible adverse on NTO, the applicant should consider generating new data (see the text below for more specific information on testing).

With regard to the test material, point 4.2 of the Introduction to the Annex to Regulation (EU) No 283/2013 emphasises that the MCPA-AM must be used in studies (i.e., the MPCA-AM). When a different test material (e.g. active substance manufactured in the laboratory or in a pilot plant production system) is used, a justification for equivalence of the MPCA-AM (as in full scale production) and the lab or pilot batches tested in ecotoxicological studies and used for the environmental assessment is needed. For this the relevant guidance document listed under Section 1 of the CC-MPCA can be used. Please note that in certain cases studies may be carried out by using the PPP containing the micro-organisms under assessment. It is recommendable to discuss to discuss such option with RMS and EFSA during pre-submission meetings.

In general, GLP studies are preferred, but other scientifically sound studies can also be accepted. In point 3.2 of Commission Regulation (EU) No 283/2013, it is stated that by way of derogation from point 3.1 (i.e., conducting tests in accordance with the principles laid down in Directive 2004/10/EC) for a.s consisting of micro-organisms, tests done to obtain data on safety with respect to aspects other than human health may be conducted by official or officially recognised testing facilities or organisations which satisfy at least the requirements under points 3.2 and 3.3 of Introduction of the Annex to (EU) 284/2013. In order to avoid conducting extra vertebrate studies, studies that are not fully compliant with GLP or current test methods must be considered in the data package if they were conducted in accordance to

the test guidelines in place at the moment when the studies were conducted, however, when conducting new studies it is recommended to follow GLP.

The CC-MPCA provides a list of test methods and guidance documents relevant to the implementation of Regulation (EU) No 283/2013. The document lists OECD guidelines, US Environmental Protection Agency (US EPA) OCSPP 885 test guidelines and the approach used by Canada's Pest Management Regulatory Agency (PMRA). The PMRA recommendations are, in fact, a combination of PMRA's microbial registration guidelines, US EPA test guidelines and detailed study descriptions of OECD test guidelines. It is important to note that there are fundamental differences between chemical and micro-organism based active substances. Micro-organisms are living organisms, and thus a specific approach is required when conducting ecotoxicological studies. This fundamental difference should be reflected in testing by assessing the infectivity and pathogenicity of the organism in question. Therefore, testing a micro-organism -based active substance by using a standard OECD test guideline which is intended for "conventional" chemical compounds generally will not adequately address the potential for infectivity and pathogenicity. On the other hand, the US EPA OCSPP guidelines were written to test a variety of micro-organisms at very high level of exposure intended to account for the potential field exposure and thus include the possible threshold for infection of test organism. Testing at these high levels, however, can result in challenges to testing, for example due to hydrophobicity or increased turbidity in test media, which complicate the interpretations of the study results.

Micro-organisms which are infectious can invade, evade the immune system of a host, persist in a viable state in the host or even subsequently multiply in tissues and organs over an extended period of time, with or without causing a disease.

A complete definition of pathogenicity is given in the PMRA Guideline, namely "*Pathogenicity refers to the ability of a micro-organism to infect a host (e.g., a test organism), establish itself and multiply there, and subsequently inflict injury or damage that might or might not lead to death. The effect on the host might be sublethal or lethal, and depends on the virulence of the pathogen (i.e., the micro-organism) as well as on host resistance or susceptibility*". […]

In order to ascertain whether the effects seen in tests are due to pathogenicity or due to toxicity, appropriate controls (i.e., sterile filtrate and non-infectious, attenuated controls) should always be included. An attenuated control consists of the micro-organism whose cell integrity was preserved, but which was inactivated (e.g. by using heat or UV lights). The micro-organism has thus lost its viability and the capacity to infect a non-target organism and potentially cause pathogenicity (i.e., disease). Please note that the heating procedure often used in attenuated ('autoclaved') controls may alter the physical nature and ecotoxicological properties of the test item, which complicates interpretation of test data (Karaoglan B, 2022[67]) (as shown in the Figure A.8-02).

[67] Karaoglan B. (2022) Aquatic Safety Studies with Microbial Pesticides – Retrospective analysis and recent advancements, OECD Conference on Innovating Microbial Pesticide Testing.

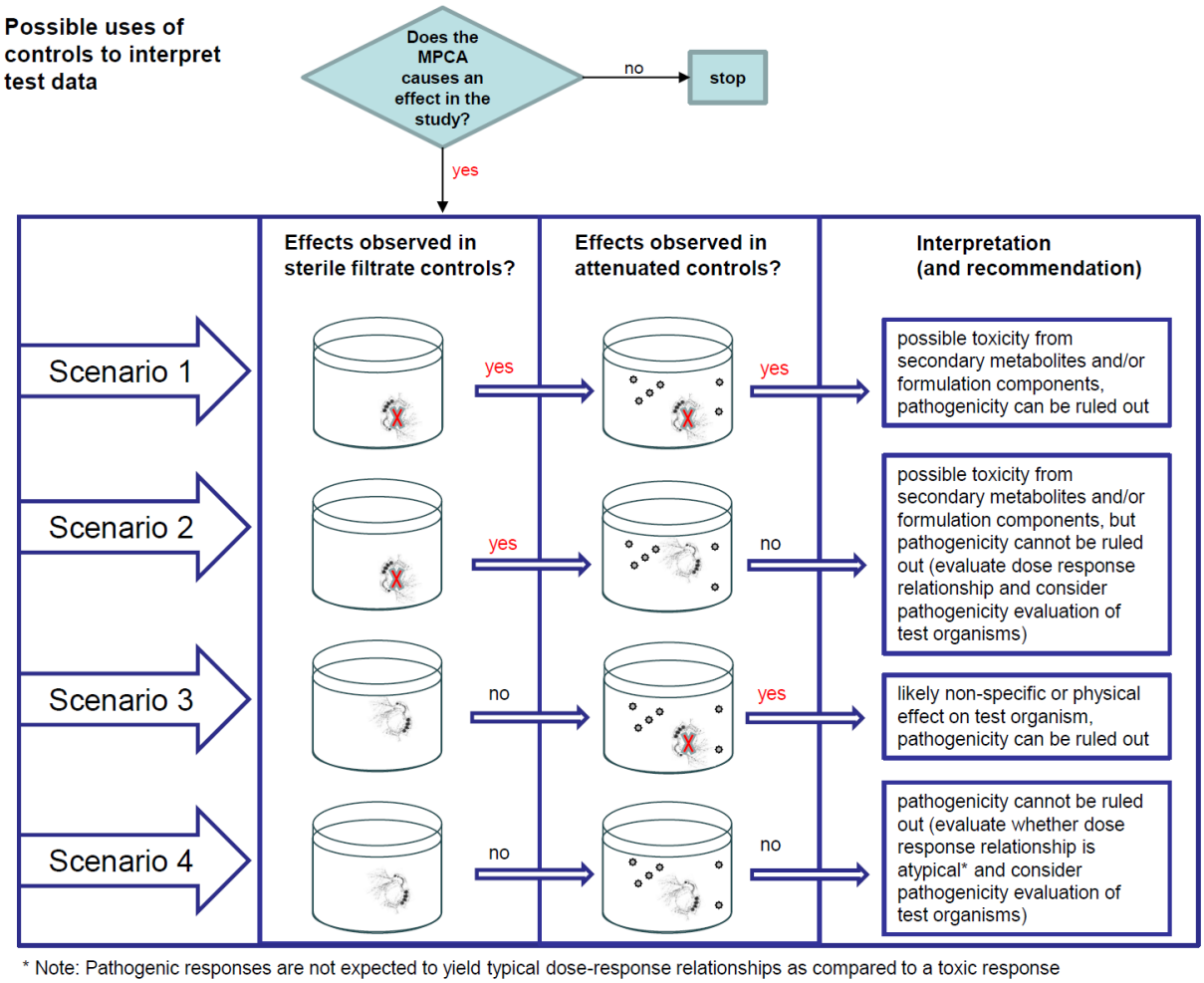

Figure A.8-02: Graphical representation of possible uses of controls to interpret test data.

A sterile filtrate control consists of the MHC of the micro-organism which was filtered to remove all the suspended solids (i.e., suspended particles associated with the inactivated micro-organism and any other particles from the test materials) from the sample. This control determines whether the soluble secondary metabolites initially produced by the micro-organism prior to inactivation, and any other chemicals that are heat stable, are responsible for the effects seen in the test. Currently (October 2023), work is ongoing at OECD on guidance documents on microbial species used in plant protection.

As opposed to toxicity, pathogenicity does not follow a log dose-response curve and it is not particularly dependent upon the initial introduced concentration of the test material, in this case the micro-organism. While a chemical can be diluted to less harmful concentrations, a micro-organism cannot be diluted in the same manner. If a micro-organism is pathogenic, the initial concentration is not paramount for risk assessment and risk management purposes, as the micro-organism will multiply in the host and cause sub-lethal or lethal effects over time. Considering this, if the applicant decides to use methods designed for assessing toxicity of chemical substances, it is important to adapt these methods (e.g., increase study duration, include appropriate controls) so that these infective or pathogenic effects can be better captured. It is also recommended to discuss such adaptation with RMS and EFSA during pre-submission meetings.

The choice of the appropriate non-target test organism is another important aspect when

conducting tests with micro-organisms. Some of the standard test organisms were selected based on their sensitivity to chemicals (i.e., tier 1 NTAs), their usefulness in the agriculture (i.e., honey bees, NTAs) and are considered representative for certain taxonomic groups. In the case of micro-organisms, however, the biological characteristics (e.g., entomopathogens) can be a trigger for which taxonomic groups might be expected to be impacted by the application of the micro-organism in the plant protection framework. Therefore, it is important to determine the host range, as well as the most important route of exposure (e.g., contact for entomopathogenic fungi).

A.8.1 Effects on terrestrial vertebrates

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 8.1
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.7.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.7.1
Eligible for substantiated dismissal of data provision:	Yes (see the point above on data waiving)

Purpose of this point:

Provide information on the infectivity and pathogenicity of the micro-organism, and on the toxicity of its secondary metabolites and impurities to birds, mammals, amphibians and reptiles. Please note that this point can be addressed with the information available in the peer-reviewed and public literature. In the context of the 3Rs (i.e., Replacement, Reduction and Refinement), the vertebrate testing should be avoided, when possible.

Testing: if testing is required, then consider CC-MPCA point 8.1.
Note: Please note the OECD test with amphibians, were validated for investigation of thyroid active chemicals (e.g. OECD 248 XETA assay), substances active within hypothalamic-pituitary-thyroid (HTP) axis (e.g. OECD 231 AMA assay), and adverse effects on endocrine-relevant endpoints (e.g. OECD 241 LAGDA assay). Please note that these tests are not per se suitable to assess infectivity and pathogenicity in terrestrial amphibians. There are currently no test guidelines validated for reptiles. Nevertheless, should there be a concern for amphibians or reptiles (i.e., based on the micro-organism in question and/or indicative literature data), the applicant is encouraged to discuss the options for testing and risk assessment with the RMS and EFSA during pre-submission meetings.

Considerations related to testing: In addition to the recommendations specified under the section “A.8 hazard testing”, it is highly recommended to:

- Perform the gross necropsy, including investigations of the presence of the micro-organism.
- For pathogenic micro-organisms or viruses (e.g., entomopathogens) that are expected to multiply in the environment following an application according to the GAP, the oral dose administered in the studies should be at least the concentration/density possible in the field, e.g., taking into account the numbers of maximally infected insects that the terrestrial vertebrates may ingest on a daily basis in case of acute exposure. The oral dose might be justified based on the information submitted under fate Section, points 7.1.1. and 7.1.2.

A.8.2 Effects on aquatic organisms

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 8.2
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.7.2
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.7.2
Eligible for substantiated dismissal of data provision:	Yes (see the point above on dismissal of data provision)

Purpose of this point:

Provide information on the infectivity and pathogenicity of the micro-organism, and on the toxicity of its secondary metabolites and impurities to fish, aquatic invertebrates, algae and aquatic macrophytes. Please note that the data requirement for fish can be addressed with the information available in the peer-reviewed and public literature. In the context of the 3Rs, the vertebrate testing should be avoided, when possible.

Testing: if testing is required, then consider CC-MPCA point 8.2.

Considerations related to testing: In addition to the recommendations specified under the section "A.8 hazard testing", the following are also highly recommended:

- Perform gross necropsy in fish
- Studies with algae and macrophytes are required in cases where the micro-organism is known to have an herbicidal mode of action or to be closely related to a plant pathogen.
- In order to ensure that the test organisms are sufficiently exposed, the test item concentration must be verified throughout the study period.
- Testing at MHC as recommended in the US EPA OCSPP guideline can result in turbidity of the aqueous medium. The turbidity can cause oxygen depletion in the test system as well as physical effects on test organisms. These effects are unrelated to the infectivity and pathogenicity of the micro-organism. Before conducting these tests, it is recommended to consult the Environment and Climate Change Canada (2016) Guidance document for testing the pathogenicity and toxicity of new microbial substances to aquatic and terrestrial organisms (EPS1/RM/44)[68] for additional guidance on testing.

A.8.3 Effects on bees

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 8.3
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.7.3
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.7.3
Eligible for substantiated dismissal of data provision:	Yes (see the point above on dismissal of data provision)

Purpose of this point:

Provide information on the infectivity and pathogenicity of the micro-organism, and on the toxicity of its secondary metabolites and impurities to bees including adult and larval stages

Testing: if testing is required, then consider CC-MPCA point 8.3.
Please note that for the uses in permanent greenhouses, exposure of pollinators introduced is considered relevant.

Considerations related to testing: In addition to the recommendations specified under the section "A.8 hazard testing", it is highly recommended to:

- Conduct the studies at the maximum recommended application rate
- Verify the exposure. For instance, for oral honey bee larva studies the need for providing

[68] https://publications.gc.ca/site/eng/9.827958/publication.html

royal jelly in the diet might present a challenge as royal jelly is known to have antimicrobial effects and the exposure is hence expected to be lower. As the presence of royal jelly is a realistic scenario this phenomenon is not to be avoided. The 'stability' of the micro-organims in the diet may be characterized with appropriate pre-testing analytical work (e.g., qPCR and/or plating techniques). This will allow a more quantitative exposure estimate.

A.8.4 Effects on non-target arthropods other than bees

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 8.4
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.7.4
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.7.4
Eligible for substantiated dismissal of data provision:	Yes (see the point above on dismissal of data provision)
Purpose of this point:	
Provide information on the infectivity and pathogenicity of the micro-organism, and on the toxicity of its secondary metabolites and impurities to non-target arthropods other than bees.	

Testing: if testing is required, then consider CC-MPCA point 8.4.
Please note that for the uses in permanent greenhouses, exposure of natural enemies (of insect pests) introduced as part- of IPM programmes is considered relevant.

According to Regulation (EU) No 283/2013, Annex, Part B, 8.4, "*If studies are required, they shall be performed on two arthropod species other than bees playing a role in biological control and comprising different taxonomic groups (orders), where possible, for which agreed testing protocols are available, and the applicant shall provide a justification for number and taxonomy of the tested species. Moreover, these tests may require conditions affecting growth or viability of the micro-organism.*
Where adverse effects are observed in such studies, further relevant studies (e.g. extended laboratory tests or field studies under representative conditions in accordance with the proposed conditions of use) shall be performed".

Considerations related to testing: In addition to the recommendations specified under the section "A.8 hazard testing", it is highly recommended to:

- Conduct the studies at the maximum recommended application rate;
- Verify the exposure rate;
- Consider the contact and oral route in the case of entomopathogenic fungi and bacteria, respectively;
- Consider the life-cycle of the non-target organism and whether it makes sense to use the organism in testing for pathogenicity.

A.8.5 Effects on non-target meso- and macro-organisms in soil

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 8.5
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.7.5
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.7.5
Eligible for substantiated dismissal of data provision:	Yes (see the point above on dismissal of data provision)
Purpose of this point:	
Provide information on the infectivity and pathogenicity of the micro-organism, and on the toxicity of its secondary metabolites and impurities to non-target meso- and macro-organisms in the soil.	

Testing: if testing is required, then consider CC-MPCA point 8.5.

According to Regulation (EU) No 283/2013, Annex, Part B, 8.5, "*If studies are required, they shall be performed on two non-target meso- and macro-organisms species chosen based on the biological properties of the micro-organism under evaluation, where possible, for which agreed testing protocols are available.*
Where adverse effects are observed in such studies, further relevant studies (e.g. under representative conditions in accordance with the proposed conditions of use) shall be performed".

Considerations related to testing: In addition to the recommendations specified under the section "A.8 hazard testing", it is highly recommended to:

- Conduct the studies at the maximum recommended application rate
- Verify the exposure rate

A.8.6 Effects on non-target terrestrial plants

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 8.6
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.7.6
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.7.6
Eligible for substantiated dismissal of data provision:	Yes (see the point above on dismissal of data provision)

Purpose of this point:
Provide information on the infectivity and pathogenicity of the micro-organism and on the toxicity of its secondary metabolites and impurities to non-target terrestrial plants. These effects should be addressed if the MPCA-AM has a herbicidal mode of action or is known to be closely related to a plant pathogen.

Testing: if testing is required, then consider CC-MPCA point 8.6.
Considerations related to testing: In addition to the recommendations specified under the section A.8 "hazard testing", it is highly recommended to conduct the studies at the maximum recommended application rate for the spray application and 10^6 CFU/g soil (dw), or 1000 times the expected concentration in the soil, as indicated in the relevant guidance document listed in CC-MPCA Section 8, which currently (October 2023) is SANCO/12117/2012.

A.8.7 Additional studies on the micro-organism

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 8.7
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.7.1-1.7.6
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.7.1-2.7.6
Eligible for substantiated dismissal of data provision:	Not applicable

Purpose of this point:
To provide additional information on:
- the infectivity and pathogenicity of the micro-organism which is the active substance, and
- on the toxicity of its secondary metabolites and impurities of the MPCA-AM

to species of non-target organisms different than those assessed in points 8.1 to 8.6, if adverse effects were identified based on information provided under points 8.1 to 8.6.

This information may concern data submitted under Section 2 (for example host range, growth requirements, relationship to pathogens to non-target organisms, information on metabolites of concern), Section 3 (for example function and target organisms, literature data), Section 5 (for example, infectivity and pathogenicity studies in mammals, secondary metabolites toxicity studies conducted in mammals), and Section 7 (environmental exposure data). Information from approval under other regulations outside the EU can be submitted under this data point.

A.8.8 Information and toxicity studies on metabolites

Corresponding data requirement:	Reg (EU) No 283/2013, Annex, Part B, 8.8
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part A, 1.5.2.1-1.5.2.6
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part A, 2.5.2.1-2.5.2.6
Eligible for substantiated dismissal of data provision:	Not applicable
Purpose of this point:	
Provide all the information on toxicity of secondary metabolites produced by the micro-organism to NTO which is used for point 2.8 of Regulation (EU) No 283/2013 to identify or exclude the secondary metabolites as being of concern.	

Please see Figure A.2.8-01 for a visual representation of correspondences of the Stages of SANCO/2020/12258 with the data requirements of the Regulation (EU) No 283/2013.

A.8.8.1 Information on metabolites

While the information submitted for point 2.8 of Regulation (EU) No 283/2013 should consist of a summary and conclusion of the assessment of secondary metabolites produced by the micro-organism, for the current point all underlying information for the hazard identification and characterisation of secondary metabolites which are relevant specifically for the assessment of the effects on NTO should be included. This is reflected by the text in the data requirements (i.e., point 8.8.1 of Regulation (EU) No 283/2013): "*Information (e.g. scientific literature, studies results) on the toxicological characterization of the metabolites and the related identified hazards relevant to non-target organisms, collected or generated with the aim to identify the metabolites of concern, or to exclude them as being of concern, shall be submitted. […]*

The information included in the dossier for point 2.8 related to effects on NTO should therefore also be based upon the information included in the current section. Please refer to point A.2.8 of these explanatory notes for information on the assessment of secondary metabolites produced by the micro-organism according to the 'Guidance on the risk assessment of secondary metabolites produced by micro-organisms used as plant protection active substances, SANCO/2020/12258', including a template for an overview table for microbial secondary metabolites.

For those secondary metabolites produced by the micro-organism for which a hazard to NTO is identified, information on human exposure should be provided as described under point A.7.2 (fate and behaviour of metabolite(s) of concern).

A.8.8.2 Additional toxicity studies on metabolites of concern

For extensively studied micro-organisms, in this section information is only needed in case a metabolite of concern which causes a hazard to NTO has been identified and reference values for toxicity cannot be set based on already available information (including TTC values) or need further investigation. Studies must be performed based on a case-by-case approach (for example short-term toxicity studies and genotoxicity studies) and using the requirements set

out in Part A for the specific type of study using relevant fractions of the MPCA-AM. It is highly recommended to reach agreement on these tests with the RMS beforehand.

For organisms which have not been extensively studied, the absence of indications for MoC in scientific literature is not sufficient to conclude on the absence of a foreseeable risk to NTO due to secondary metabolites produced by the micro-organism. Therefore, for these less studied micro-organisms more experimental data is needed.

Explanatory notes on the data requirements concerning the plant protection product

The Explanatory Notes provided under this section refers to the data requirements laid down in Part B of the Annex to the Regulation (EU) No 284/2013, relevant to dossiers submitted in the context of:

- an application for approval of active substances that are micro-organisms (i.e., with respect to one or more representative uses), and
- an application for authorisation of a PPP.

P.1 IDENTITY OF THE APPLICANT, IDENTITY OF THE PLANT PROTECTION PRODUCT AND MANUFACTURING INFORMATION

P.1.1 Applicant

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 1.1
Relevant evaluation criterion:	-
Relevant decision making criterion:	-
GLP-compliance:	Not relevant
Purpose of this point:	

The applicant must be identified as an entity addressing all issues relating to the plant protection product, either directly or through a notified representative.

Conditionality
Not relevant.

Confidentiality
No confidentiality can be claimed for the identity of the applicant.

P.1.2 Producer of the preparation and the micro-organism(s)

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 1.2
Relevant evaluation criterion:	-
Relevant decision making criterion:	-
GLP-compliance:	Not relevant
Purpose of this point:	

The producer acts as contact point with regard to the production of the preparation.

Conditionality
Not relevant.

Confidentiality
Confidentiality can be claimed for the identity of the producer and the location of the plant where the preparation is produced, as this information complies with the criteria in Regulation (EC) No 1107/2009, Art. 63.

P.1.3 Trade name or proposed trade name, and producer's development code number of the preparation if appropriate

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 1.3
Relevant evaluation criterion:	-
Relevant decision making criterion:	-
GLP-compliance:	Not relevant

Purpose of this point:

The trade name provides a unique identifier relating to the product's authorisation. The development code number is associated with a specific compositional version of the product, and is required to keep track of any preparation changes throughout the dossier history.

Conditionality
Not relevant.

Confidentiality
Not relevant.

P.1.4 Detailed quantitative and qualitative information on the composition of the preparation

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 1.4
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.1.3
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.1.2
GLP-compliance:	5-BA data on contaminating micro-organisms, metabolites of concern, and relevant impurities must be produced under GLP.

Purpose of this point:

The information on the composition of the preparation includes the PPP-specification (derived or newly established) and a detailed description of components that have been added during the formulation process (co-formulants, all other active substances, and safeners / synergists). This data includes all compositional parameters of the PPP that are necessary to unambiguously identify the product, and that are furthermore critical to the multiple purposes of the product-level assessment.

Conditionality
Not relevant.

Confidentiality
Quantitative and qualitative information on the composition of the preparation that relates to the micro-organism, claimed active metabolites (if present), relevant impurities (either originating from the MPCA-AM or from co-formulants), contaminating micro-organisms, or MoC(s) cannot be claimed as confidential.

(i). ***Background information*** *Identification at strain level of the micro-organism which is the active substance*

Please refer to A.1.3.

(ii). *Defining the specification for the preparation*

The PPP specification is the product-level counterpart of the MPCA-AM specification and

should include all elements that have been specified for the MPCA-AM. In general, the PPP-specification can be derived from the MPCA-AM specification by simple calculation (see 'Ideal derivative' below), but other approaches may be chosen when appropriate, i.e., when derivation by calculation is not practical (see 'Non-ideal derivative'), or when there is no MPCA-AM specification in the first place (see 'Non-derivative'). Derivation approaches cannot be used for the relevant contaminating micro-organisms, which are separately covered on the product-level in the storage stability test (see P.2.6.2, 'Effects of temperature and packaging; Main long-term test – custom temperature').
Given the fact that the micro-organism is a living entity, some unforeseen, baseline variation in the content of the micro-organism itself, and in that of any associated claimed active metabolites and MoCs may occur.

IDEAL DERIVATIVE
In general, an MPCA-AM specification is available for the microbial active substance, and the PPP is an ideal derivative of the MPCA-AM. In other words, the specification elements that have been established for the MPCA-AM (see A.1.4.1 and A.1.4.2) are not intrinsically affected by the formulation process and their content can be translated to the PPP-level by simple calculation:

$C_{EA} \times C_{AP} = C_{EP}$

Where 'C_{EA}' represents the content of a given element in the MPCA-AM, 'C_{AP}' is the content of MPCA-AM in the PPP, and 'C_{EP}' is the content of the respective element in the product.

In this way, corresponding ranges (including min. and/or max. limits, wherever available) are derived for the PPP for all established elements. For ideal derivatives, the specification ranges should however be sufficiently broad to allow for this.

NON-IDEAL DERIVATIVE
In some cases, the content of the micro-organism in particular may not be so easily translated from the MPCA-AM specification, as the micro-organism has been substantially affected by the formulation process and any resulting changes to the matrix. As discussed in A.2.4, shifts in pH, temperature, osmotic pressure, or chemical composition of the environment, that commonly occur during preparation, may significantly affect the capacity of spores to form colonies or to germinate – and the subsequent enumeration results in terms of CFU or viable spores, respectively. In addition, but more subtle, the generally higher, co-formulant-enhanced dispersibility potential of the PPP may prevent spore aggregation to a higher degree than is the case in the MPCA-AM. As a result, spores tend to be more clumped in the latter and may generate biased CFU-enumeration outcomes, as one clump of spores only counts as one colony.
Any mismatch between MPCA-AM and PPP is likely to become apparent when comparing the CFU-count in the storage stability test with the PPP-limits derived under the assumption of ideal derivation (see above). If not, the applicant will in any case be aware of any mismatches from archived quality control-data. Depending on the severity (as evidenced by the percentage of product batches falling outside of the specified limits), it may be warranted to perform a separate 5-BA on the PPP to determine a representative product-level range for the micro-organism only (all other specification elements are simply calculated assuming ideal derivation).
Of course, the amended range must, on PPP-level, comply with the criteria described in A.1.4.3 – the most important of which is in this context that the minimum will guarantee minimal effectiveness and the maximum safe use.
If needed, i.e., when the required quality cannot be guaranteed for product output, adaptations to the production process may be necessary.

NON-DERIVATIVE
Whenever no MPCA-AM specification is available (see A.1.4, 'Conditionality') there is nothing from which an PPP specification can be derived in the first place. In this case, an PPP-dedicated specification needs to be established from scratch (e.g. from a 5-BA, as GLP is not strictly required for all specification elements).
The approach and conditions are essentially the same as those described for MPCA-AMs in A.1.4. A notable difference is that PPPs may be mixtures of micro-organisms, whereas this is more of a theoretical option for MPCA-AMs (unless the MPCA-AM is a consortium). An PPP specification that is newly established must cover all relevant specification elements for all micro-organisms included in the mixture. If the elements cannot be characterized for a separate micro-organisms, due to interference with other micro-organisms in the mixture on that particular element, it is desirable to establish an MPCA-AM specification anyway (provided that this is possible).

(iii). Composition of the preparation in terms of co-formulants, other active substances, and safeners and synergists

All ingredients in the preparation, i.e., active substances, co-formulants, and safeners and synergists, must be described in terms of identity (see Regulation (EU) No 284/2013, Part B, 1.4 for details) and gravimetric content. Content ranges are not allowed, as these would enable significantly different recipes (with potentially different properties) for the same PPP.
Formally, Regulation (EU) No 284/2013 approves of min. and max. contents for the MPCA-AM only, to enable additional stretching of the micro-organism's specified range. As the CA-advised, one-log-unit broad micro-organism-range (see A.1.4.3) already represents the practical maximum, more flexibility is deemed unnecessary. Besides, the MPCA-AM is likely to affect the rheological parameters of the preparation; modifying its content in the PPP may therefore affect relevant physical properties.

(iv). Co-formulant function

No specific reading necessary for this point.

(v). Relevant contaminating micro-organisms

As mentioned under P.1.4 (ii) 'Ideal derivative', these will be addressed in the course of storage stability testing (see P.2.6.2).

P.1.5 Physical state and nature of the preparation

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 1.5
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.1.3
Relevant decision making criterion:	-
GLP-compliance:	Not relevant

Purpose of this point:
The definition of preparation type is a determinant of the technical characteristics that need to be investigated and of the exposure context. The assigned preparation type must align with the physical and compositional background of the product and with its intended use.

Conditionality
Not relevant.

Confidentiality

Not relevant.

P.1.6 Method of production of the preparation and quality control

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 1.6
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.1.4
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.1.3 and 2.1.5
GLP-compliance:	Not relevant
Purpose of this point:	

The formulation process must, *mutatis mutandis*, be a consistent description of the process from the end of the MPCA-AM manufacturing process in terms of control, efficiency, hygiene, and monitoring.

Conditionality
Not relevant.

Confidentiality
Confidentiality can be claimed for details of the formulation process that comply with the criteria in Regulation (EC) No 1107/2009, Art. 63.

Background information
The formulation process must be described in detail so that it covers the consecutive order of component addition and corresponding conditions under which they are added.
Describe quality control steps implemented in the formulation process as outlined for the manufacturing process under A.1.5.1, 'The essential process checkup; quality control', with regard to the placement of quality control-steps in the process, methodology, and criteria.

P.1.7 Packaging and compatibility of the preparation with proposed packaging materials

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 1.7
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.2.1
Relevant decision making criterion:	-
GLP-compliance:	Not relevant
Purpose of this point:	

The specifications of the PPP's commercial packaging must be described in sufficient detail to allow (i) evaluation against European and possible national requirements regarding handling / storage / transport / disposal, (ii) verification of the equivalence with the packaging material tested in the storage stability test, and (iii) support of packaging extrapolation.

Conditionality
Not relevant.

Confidentiality
Not relevant.

P.2 PHYSICAL, CHEMICAL AND TECHNICAL PROPERTIES OF THE PLANT PROTECTION PRODUCT

P.2.1 Appearance (colour and odour)

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 2.1
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.2.2
Relevant decision making criterion:	-
GLP-compliance:	Not relevant

Purpose of this point:

Clear characteristics are established for the PPP that may be confirmed by simple visual and olfactory assessment. These may serve to identify the PPP at a glance, and possibly any obvious product defects.

Conditionality

Not relevant.

Confidentiality

Not relevant.

P.2.2 Explosivity and oxidising properties

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 2.2
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.2.2
Relevant decision making criterion:	-
GLP-compliance:	Only relevant for experimental data

Purpose of this point:

Any tendency of the PPP to explode or to exhibit oxidizing behaviour must be correctly assessed to avoid accidental combustion.

Conditionality

In all conceivable cases, data related to this point may not be provided if the lack of explosive and oxidizing behaviour of the preparation has been reasonably substantiated at component-level.

Confidentiality

Not relevant.

P.2.3 Flash point and other indications of flammability or spontaneous ignition

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 2.3
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.2.2
Relevant decision making criterion:	-
GLP-compliance:	Only relevant for experimental data

Purpose of this point:

Any capability of the PPP to burn must be correctly assessed to avoid accidental ignition.

Conditionality

In all conceivable cases, data related to this point may not be provided if the lack of flammable and self-heating behaviour of the preparation has been reasonably substantiated at component-level. A notable exception is flammability of powdered preparations, which may not always be easily put aside by theoretical argumentation. In some cases, testing – according to recommended methodology and in compliance with GLP-criteria – may be preferable.

Confidentiality

Not relevant.

P.2.4 Acidity, alkalinity and if necessary pH value

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 2.4
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.2.2
Relevant decision making criterion:	-
GLP-compliance:	Not relevant
Purpose of this point:	

The PPP's pH must be established. The pH value is a robust and convenient indicator of any unintended changes to the preparation and of any tendency of the PPP towards corrosiveness.

Conditionality
No data are required for solid or non-aqueous products that will not be applied as aqueous dilutions.

Confidentiality
Not relevant.

Background information
No specific reading necessary. pH is always required, and the necessity to determine respectively acidity and alkalinity is triggered by a pH below 4 or above 10.

P.2.5 Viscosity and surface tension

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 2.5
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.2.2
Relevant decision making criterion:	-
GLP-compliance:	Only relevant for viscosity testing, when the PPP will be classified as aspiration hazard
Purpose of this point:	

The viscosity is a determinant of H304-classification of the PPP, in case it consists of ≥ 10% of components that are classified as aspiration hazards themselves.
Based on the surface tension, it is established whether GAP-proposed dilutions of the PPP can be considered surface active or not. Surface activity is required for spreading over and penetrating surfaces and is therefore a relevant parameter for product efficacy.

Conditionality
Surface tension is only relevant for PPPs that will be applied to the crop by spraying.

Confidentiality
Not relevant.

Background information
To allow efficient assessment of the surface activity of the product within the in-use range, the surface tension needs to be determined at the highest dilution. If the surface tension is below 60 mN/m water at that level, the product can be considered surface active at all intended dilutions.

P.2.6 Storage stability and shelf life

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 2.6

Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.2.1
Relevant decision making criterion:	-
GLP-compliance:	Only required for storage stability data relating to contaminating micro-organisms, as well as relevant impurities, and MoCs if expected to be present in the PPP

Purpose of this point:

The PPP must be evidenced to retain its critical performance parameters, i.e., (i) viability of the micro-organism(s), (ii) content of any secondary metabolites claimed to contribute to the plant protection action, (iii) absence or acceptable quantities of microbial contamination, (iv) acceptable contents of any defined MoCs and other relevant impurities, (v) packaging integrity, and (vi) acceptable technical properties, under relevant storage conditions. Furthermore, the PPP's stability under the influence of environmental parameters must be demonstrated.

Conditionality

The contaminating micro-organisms should be determined before and after storage, unless a reasoned case can be made that these are unlikely to be introduced or grow during storage.

Confidentiality

Not relevant.

Background information

P.2.6.1 Use concentration

The in-use concentration range must be indicated in appropriate terms (generally in % v/v or % w/v for liquid and solid preparations, respectively). The range should be covered in the tests conducted for the relevant technical properties.

P.2.6.2 Effects of temperature and packaging

In principle, a storage stability test is considered successful when, for the full duration of the test, (i) all relevant specification elements remain within their established ranges, (ii) the proposed packaging retains its integrity, and (iii) the technical properties associated with the respective preparation type stay within acceptable limits.

For PPPs, three types of storage stability tests are recognised (i.e., high temperature, low temperature, custom temperature), each intended to address a particular feature of the shelf-life. Other factors that may potentially affect stability are discussed under P.2.6.3.

SHORT-TERM TEST – HIGH TEMPERATURE

'Accelerated storage stability tests' can in most cases not be considered to support a provisional long-term shelf-life for PPPs. Despite this, the tests can however serve a useful purpose in PPP-context as 'high temperature storage stability test'; a successful 18-week long test at 30°C provides sufficient evidence that the respective PPP may likely retain its efficacy when stored in a non-temperature controlled environment throughout a typical summer in the Northern, Central, and Southern Zone. Also, the test reasonably covers for any inevitable short-term high temperature exposure of the product during application in hot weather.

For PPPs whose principal efficacy is caused by the activity of one or more claimed active metabolites, and for which the viable fraction is of minor direct or indirect importance, the additional 'accelerated storage'-functionality is regarded in the same way as for conventional chemical PPPs and may thus be used to support a provisional long-term shelf-life when the main long-term test is not yet available. In this case, the test must be carried out in appropriate commercial packaging. Also, the extant data package must at least contain adequate pre-storage data on contaminating micro-organism-screening; again, as it concerns viable components, post-high temperature storage screening is not supported. Except for the obvious

differences, a high temperature test is performed preferably in the same way as the main long-term test (see below). Please note that at the time of publication of these EN, a new FAO manual for microbial pesticides is under drafting, and may also contain useful indication on this matter.

SHORT-TERM TEST – LOW TEMPERATURE

The low temperature stability test is intended to assess the stability of liquid preparation types after exposure to frost. The test is mandatory from a practical perspective when the intended shelf-life for a liquid preparation demands storage at a temperature close to 0°C, at which unintentional freezing of the PPP due to temperature fluctuations cannot be ruled out.
In other situations, submission of the test is not tightly enforced; its absence may effectively be covered by a recommendation for the label: '*protect from frost*'.

MAIN LONG-TERM TEST – CUSTOM TEMPERATURE

The main long-term test may be carried out at any temperature that is favorable for the PPP and practical for the seller/end-user, and may continue for as long as the applicant deems feasible. A shelf-life will be established based on any set of conditional parameters (temperature, duration, packaging) for which complete and acceptable data have been presented. There is no limit to the amount of shelf-lives that may be assigned to a given PPP.
The test report must include pre -and post-storage data on micro-organism (and if relevant, claimed active metabolite) content, packaging integrity, physical/chemical/technical properties required for the respective preparation-type, contaminating micro-organism-screening, and, if relevant, on MoCs and relevant impurities. Specification elements, like the micro-organism, claimed active metabolites, and components of concern (relevant impurities, MoCs, and contaminating micro-organism) are expressed in line with the established specification.
Because stability and temperature resistance of a viable active are not always as reliable as would benefit long-term planning, inclusion of fully supportive interim timepoints may turn out to be hugely advantageous. The Competent Authority maintains a pragmatic opinion on the status of interim reports; as long as submission of a final version is guaranteed – i.e., by provision of a study plan that states a clear finalisation date – interim data are used without any special reserve. Regarding GLP-status (whenever relevant), an interim report that (i) has been produced by a lab whose GLP-status could be verified, (ii) includes a GLP-statement from the study director and a QA-statement from the QA-officer, and (iii) has an unmistakable appearance of an interim report, is considered to be GLP-compliant. Alternatively, the interim report may be drafted as a final version, whereas the actual final version may be submitted as an amendment to the final report.
Last, some leniency is allowed with regard to submission of long-term storage stability data for the context of an approval dossier; the data predominantly relate to the product-level and may therefore for the largest part be evaluated in the course of the plant protection product assessment (both at authorisation level, and approval level when the plant protection product concerns the representative use). As long-term stability of the microbial substance itself is considered vital for approval, post-storage data relating to micro-organism -and claimed active metabolite contents are required at the substance-level, whereas phys/chem/tech properties may be addressed at a later stage.

P.2.6.3 Other factors affecting stability

Under A.2.4, the relevant conditions are discussed that are required for growth and proliferation of the micro-organism. In some cases, the micro-organism's sensitivity to factors such as UV, humidity, pH, temperature, and osmotic potential may interfere with the context of (effective) use. In some cases, preparation design may serve to mitigate such interferences (e.g., by adding a solar protectant to a product containing a UV-sensitive species, that is nonetheless intended to be applied via foliar spray). The effectivity of such solutions must be evidenced, by rationale or simple test, e.g., in which two preparations (one containing a

protectant and one that does not) may be exposed to a corresponding limiting factor, followed by a comparison of micro-organism-viability in the two media.

Specific recommendations can be proposed (e.g. on protection from UV light or humidity, on not storing at elevated temperatures).

Note that the scope of this point is limited to stability-decreasing factors and corresponding mitigators.

P.2.7 Technical characteristics of the plant protection product

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 2.7
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.2.2
Relevant decision making criterion:	-
GLP-compliance:	Not relevant
Purpose of this point:	

Technical characteristics need to remain within acceptance limits to ensure convenient and effective use of the PPP. Suboptimal behaviour under relevant conditions must be identified and resolved.

Conditionality
Substantiated dismissal of data provision may be accepted when the context of use of a product would render a particular technical property irrelevant.

Confidentiality
Not relevant.

Background information
Regulation (EU) No 284/2013, 2.7 states which technical characteristics must be investigated for which preparation type. Furthermore, CC-PPP provides information on recommended methodologies and guidance documents with which to assay these characteristics. Additionally, the FAO Manual includes preparation type-specific information on characteristics that need to be checked post-storage. Last, the respective CIPAC (-or equivalent) sources provide detailed guidance as to how tests are to be conducted.
The Competent Authority maintains a very limited degree of specific reading that provides any additional depth to the existing framework.

Currently, only suspensibility and spontaneity of dispersion require an alternative approach for micro-organisms. More than for chemicals, the distribution of the active substance is detached from the distribution of weight in the solution. Consequently, reporting suspensibility / spontaneity on a gravimetric basis for PPPs does not allow a clear assessment of this property. The test results should therefore be presented as percentages derived from CFU-counts (or, of course, any other metric that is compatible with the micro-organism's specification) in the respective solution samples.
In case of multiple micro-organisms in the PPP, the distribution of several of them should be assessed, as their dispersibility is not necessarily equivalent.

P.2.8 Physical and chemical compatibility with other plant protection products including plant protection products with which its use is to be authorised

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 2.8
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.2.3

Relevant decision making criterion:	-
GLP-compliance:	Not relevant
Purpose of this point:	
Mixing of the micro-organism with other products or adjuvants must not disturb the physical and chemical properties that are critical for the particular PPP to a degree that the overall plant protection action may be affected. This point serves to investigate the compatibility between proposed mixing partners.	

Conditionality
Data related to this point must be provided in case tank mixing partners have been defined on the label. Otherwise, this point can be considered not relevant.

Confidentiality
Not relevant.

Background information
No specific reading available.

P.2.9 Adherence and distribution to seeds

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 2.9
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.2.2.2
Relevant decision making criterion:	-
GLP-compliance:	Not relevant
Purpose of this point:	
Tests submitted under this point must demonstrate that the PPP-coating created around treated seeds contains sufficient, and sufficiently constant amounts of the micro-organism(s) to ensure the level of plant protection action intended for the application. In addition, test results must show that the coating is tough enough to stick to the seeds during representative seed handling.	

Conditionality
Data related to this point must be provided in case the GAP includes applications as seed treatment. Otherwise, this point can be considered not relevant.

Confidentiality
Not relevant.

Background information
Dedicated methods CIPAC MT 175 (seed loading and uniformity of distribution) and MT 194 (adherence to seed) provide sufficient practical information to carry out the respective tests. Furthermore, the guidance document on phys/chem/tech properties, SANCO/10473/2003, presents some additional notes under 2.10 of that document, especially regarding seed types that are not explicitly covered by the CIPAC-methodology, and representative seed treatment procedures.
As these sources are not specifically tailored for Part B active substances, some considerations must be added for the particular context of this EN.

<u>REPRESENTATIVENESS OF THE SEED TREATMENT TECHNIQUE</u>
The technique used to treat seeds (e.g., seed dressing, film coating, pelleting, slurry coating) is a major determinant of the overall beneficial effect for the plant that is achieved by this particular mode of application. As a rule, the technique used to generate test batches of treated seeds should be representative of the actual, commercial-scale seed treatment

technique.
Preparations based on microbial active substances are generally less compatible with default seed coating processes, mainly due to a higher tendency towards inhomogeneity, and a narrower choice range of coating-enhancing formulants that are suited for PPP. As a consequence, some modifications may be required to the process to secure the intended quality in terms of adherence, loading, and distribution. For the sake of representativeness, any process modifications need to be described and employed in the treatment of seed batches that are submitted to testing.

TROUBLESHOOTING DATA
Seed treatment with PPPs is challenging and includes multiple steps that are critical to ensure effective application. Whenever submitted test data demonstrates unacceptable performance, it is often difficult to pinpoint a causative. To enable a more targeted evaluation, the test report must include the following data (that are mostly expected to be available anyway):

- the specification of the PPP-batches used in the treatment process;
- pre-treatment of seed if relevant;
- the critical process conditions, i.e., the dilution factor of the product used to produce the slurry (in L product per L solvent), the composition of the solvent, the seed: slurry ratio (in g seed per L of slurry), the process temperature and duration;
- the post-process conditions, i.e., duration and conditions during drying and subsequent storage of the coated seeds – especially with regard to stressants (see A.2.4 and P.2.6.3).

DEFINITION OF TEST CRITERIA
Worst case testing – As the number of crops or crop groups for which seed treatment is proposed, and the range of dilution factors for the slurry that is stated per crop easily lead to a large number of conditional combinations, testing only needs to cover one (if possible) or more (if needed) worst case scenarios.
What can be interpreted as worst case for seed type depends on the tested parameter; (i) smooth seeds are reasonably considered worst case regarding adhesion, (ii) smaller seeds may be worst case with regard to loading capacity per seed, and (iii) irregularly shaped seeds with a variable size distribution are worst case regarding distribution.
For dilution factors, the most diluted slurry according to GAP-specifications is pragmatically considered worst case, due to expected less favorable rheological conditions, and a lower overall loading.
The selected scenario(s) should be justified in perspective of the preceding information.

Compliance with GAP-specified loading – Pre -and post-agitation seed loading must be expressed in a way that is compatible with the GAP (usually as CFUs/g of seed), and need to fall within the GAP-specified range. Seed treatment parameters must be determined pre- and post-storage.

P.3 DATA ON APPLICATION

The information provided in this Section is essential for the risk assessment as they indicate both qualitative and quantitative information on the proposed uses (in accordance with GAP).

The information to be provided under this Section is normally distributed in the PPP dossiers among different Sections (e.g., P.3.1, P.3.3 to P.3.7, and P.3.9 in the GAP table, P.3.2 in the Efficacy Section, and P.3.8 on draft national label). The requirements laid down under this Section function as a descriptive indication for the dossier – without this indication it may not be clear why certain information is included in other sections of the dossier.

P.3.1 Field of use envisaged

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 3.1
Relevant evaluation criterion:	The information provided in this Section forms the basis of the risk assessment. Specific evaluation and decision making criteria are therefore referred to in other sections of the risk assessment (e.g. on impact on human and animal health and effects on non-target organisms).
Relevant decision making criterion:	

Purpose of this point:

The indication of the field of use of the plant protection product can be employed to determine the appropriate exposure scenario for the risk assessments for humans, animals and the environment (if required)

Assessment principle:

The field(s) of use for the PPP, existing (in case of renewal) and proposed, can be specified from among the following:

- agriculture, horticulture, forestry, or viticulture,
- protected crops (e.g. in greenhouses)
- non-cultivated areas,
- home gardening,
- houseplants,
- stored food/feed items,
- other (needs to be specified).

If amateur/non-professional use is intended (whether or not in addition to professional use), this should be clearly indicated.

Under A.3.2. the field of use is specified for the intended use of the micro-organism, while under this data requirement the field use for the representative PPP (active substance) or PPP to be registered (product) should be listed. Note that the field(s) of use may deviate between products in case of more than one representative PPP (e.g. when the dossier for the active substance approval of the micro-organism is initiated by a task force, each submitting the data for their own representative PPP preparation). In the case of a PPP registration, the field(s) of use may be more extensive than indicated previously for the representative product for active substance approval. However, all fields of use should be covered by the risk assessments performed in other sections.

Reading of the framework in specific cases

Protected crops

Note that for the protected crops, a distinction should be made in the PPP dossier between crops cultivated in permanent greenhouses (high- and low-tech) and crops cultivated in non-permanent structures (e.g. plastic walk-in tunnels). The first will be evaluated interzonally, while for the latter a zonal registration dossier should be prepared (in line with the Agreement of the Interzonal Steering Committee and applicable as from June 1st 2022).

For more information regarding different types of protection see A.3.2, Field of use envisioned includes also a reference to the “EFSA Guidance Document on clustering and ranking of emissions of active substances of plant protection products and transformation products of these active substances from protected crops (greenhouses and crops grown under cover) to relevant environmental compartments” EFSA Journal 2014; 12(3):3615.

P.3.2 Mode of action on the target organism

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 3.2
Relevant evaluation criterion:	The information provided in this Section forms the basis of the risk and efficacy assessment. Relevant evaluation and decision making criteria are therefore referred to in other sections of the risk and efficacy assessment.
Relevant decision making criterion:	

Purpose of this point:

In addition to the mode of action of the micro-organism on the target organism that has already been extensively described in accordance with point 2.3 of Part B of the Annex to Regulation (EU) No 283/2013, any information on the MoA regarding additional components in the PPP that may have an effect (e.g effects of co-formulants on efficacy, human and animal health or the environment) should also be considered.

Assessment principle:

A concise summary/conclusion of the information provided in accordance with point 2.3 of Part B of the Annex to Regulation (EU) No 283/2013 (as described under point A.2.3) needs to be provided for PPP registration. It is essential to include this information in the dRR, as the MoA plays an important role in the efficacy assessment. However, reference can be made to the active substance dossier if needed. Possibility of extrapolation may, for instance, depend on the MoA. For more detailed explanation regarding extrapolation see P.6.3 on testing effectiveness.

In addition to the MoA of the micro-organism on the target organism, other components of the PPP (e.g. co-formulants, including also other micro-organisms or chemical active substances) may trigger significant difference in the mode of action described for the single active substance. Hence, also for these components information on the mode of action on the target organism(s) should be provided. More details on the efficacy principles concerning co-formulated products are provided under point P.6.1 on “Preliminary data”.

P.3.3 Function, target organisms and plants or plants products to be protected and possible risk mitigation measures

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 3.3
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.3.1
	Reg (EU) No 546/2011, Annex, Part B, 1.3.2
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.3.1.1

Purpose of this point:

The function, target organisms and plant or plant products to be protected needs to be specified. This is not only essential information for the assessment of efficacy, but also the risk assessment on human health and the environment.

Assessment principle:

It should be indicated why the micro-organism will be applied as active substance for plant protection. The biological function can be specified as one or several of the following:

- control of bacteria,
- control of fungi,
- control of viruses,
- control of insects,
- control of mites,
- control of molluscs,
- control of nematodes,

- control of plants,
- other (must be specified).

In addition, details on the target organism(s) are needed to provide an overview of specific crop/pest combinations. This also includes information regarding occurrence and agro-economical relevance of the pest. One requirement is documentation needed to assess whether significant damage to plants or plant products or loss of yield occur due to the pest. Thereafter, the evaluation of all aspects of efficacy (See P6.3) will indicate the benefit of the product in reducing this damage, in line with point 1.3.2 of Part B of Annex to regulation (EU) No 546/2011. Agro-economical relevance of target organism(s) may differ among concerned MS. Therefore, the information provided here can also be used to support the conclusion on which conditions will be considered worst case for the proposed claim (and hence should preferably be included within the efficacy tests). Note that no authorisation can be granted for micro-organisms targeting target organism(s) that are not considered harmful for the crop or plant products to be protected. Likewise, authorisation cannot be granted neither for those uses targeting pests that are not considered causing a problem to the crop or plant products claimed to be protected (e.g. against a target-organism that does not occur in the zone where authorisation is requested).

Furthermore, information should be provided on the plant crops, crop groups, or plant products that are intended for the plant protection use. To avoid misinterpretation of ambiguous terms (e.g. ornamentals can encompass different plant groups in different Member States) EPPO codes and scientific names for the intended crops, crop groups or plant products should be used. If relevant, the crop destination or purpose of the crop can be added (e.g. oilseed rape can be cultivated as oilseed crop but also as green manure crop, poppy seeds can be cultivated as oilseed crop, but also as herb seed crop, for potatoes there is a difference between seed, ware and starch potatoes). When the proposed uses are limited to a specific subset of crop uses (e.g. only for seed production, or fodder), this should be clearly indicated.

If relevant also the part of the plant that will be used may be indicated (e.g. for medical crops roots, leaves or seeds). To avoid misunderstandings, crop definition from EPPO may be used[69], and it is essential to indicate the proposed uses as clearly as possible.

The information provided here should be in line with the information provided in the table of intended uses as presented in appendix 1 of the template for dRR B0 for product approvals. In case of active substance approval: Document D is generated by the report generator tool in IUCLID based on the information included in the relevant documents (see IUCLID Microbial active substances manual for additional details).

Information on possible risk mitigation measures must also be provided by the applicant, if applicable, under this point. Risk mitigation measures must be considered either “non-specific” (e.g. masks to protect operators and workers from inhalation of dusts), or “specific”. Note that the proposal for risk mitigation measures should follow from, and be supported by, the data provided under the other sections (e.g. P.7 on effects on human health, P.8 on residues, P.9 on fate and behaviour in the environment and P.10 on the effect on non-target organisms), as also discussed below under point P.3.9.

P.3.4 Application rate

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 3.4
Relevant evaluation criterion:	Reg (EU) No 546/2011, General Introduction, point 2.4

[69] https://gd.eppo.int/taxon/3CRODK

Relevant decision making criterion:	The information provided in this Section forms the basis of the risk and efficacy assessment. Relevant decision making criteria are therefore referred to in other sections of the risk assessment.

Purpose of this point:

The application rate of the PPP (for each use, using the most relevant units) is essential information for all other aspects of the risk and efficacy assessment (physical, chemical and technical properties of the PPP, and the risk assessment on human and animal health or the environment).

Assessment principle:

For each method of application and each use, the rate of application per unit treated, in terms of g, kg, ml, or l for the plant protection product and in terms of appropriate units for the micro-organism (e.g. number of active units, CFU or IU per volume or weight), must be provided. For protected crops and home gardening use rates must be expressed in g or kg/100 m^2, or g or kg/m^3, ml or l/100 m^2, or ml or l/m^3.

A correctly indicated application rate is essential information for all other aspects of the risk assessment (efficacy, physical, chemical and technical properties of the PPP, and the risk assessment on human and animal health or the environment).

EPPO standard PP1/239[70] on "Dose expression for plant protection products" explains in detail the dose expression for plant protection products. and guidance can also be found in Appendix 1 of the dRR B0 template.

The GAP table is based on the min. max. or mean CFU per unit formulated product.

For micro-organisms, in addition to the rate of application in kg or l product/ha, the number of CFU, IU, or OB (or other relevant units) per ha should be indicated. As PPP based on micro-organisms can be more variable in composition than conventional chemical products, it should be indicated what the range of CFU, IU, or OB (or other relevant units) in the formulated product is. Furthermore, it is highly recommended to explain in detail which numbers of this range were used to generate the GAP table.

Besides the range in content of the micro-organism, PPPs based on micro-organisms can also have a range in their application rate (for instance 0.5 to 1 l product/ha, depending on e.g disease pressure) and a range in water spray volume to be used (if applicable). As a result, when calculating the numbers of e.g. CFU/ha, these numbers can deviate substantially when based on the minimum amount of CFU in the formulated product compared to the maximum amount of CFU.

It is considered extremely helpful if information on the minimal and maximal amount of CFU/ha is provided. This information can be included as "Remarks on application rate" in IUCLID (See IUCLID Microbial active substances manual[71], paragraph 3.1 "Use of the plant protection product (GAP)"). Including these details prevents misunderstanding and unnecessary re-calculations of alternative approaches.

It should be noted that, while from an efficacy point of view the minimum amount (dose /ha applied) is the most relevant, the maximum amount (dose /ha applied) is the most informative value to assess negative side-effects on human and animal health or the environment. The mean (nominal or average) of the 5-batch analysis provided for the formulated product is an arbitrary value, and as such not meaningful.

Reading of the framework in specific cases

[70] https://pp1.eppo.int/standards/PP1-239-3

[71] https://doi.org/10.5281/zenodo.4773526

Dose rate expressions (list is not exhaustive)

Dose rates in high growing crops – Because of historical reasons, many different dose-rates systems exist on national labels for high growing crops, this greatly complicates the writing and evaluation of dossiers.

For the central registration zone dates have been set for introduction of LWA as the mandatory dose expression system in pome fruits, grapevine and high growing (fruiting) vegetables. All trials carried out in these crops after 1 January 2018 must be planned and carried out on the basis of LWA. Furthermore, as from 1 January 2020 all dossiers submitted under Article 33 of Reg (EC) No 1107/2009 must be supported by trials planned and carried out based on LWA as the efficacy unit of dose expression. The dose rate in LWA should be included in the GAP table. It is important to note that the rate per unit of surface area (e.g. kg/ha) should always be included as well, as it is required for risk assessments. The dose rate in LWA is needed for the efficacy Section only. EPPO standard PP1/239 on "Dose expression for plant protection products" explains the principle and necessity of LWA in more detail.

Seed treatment – Typical dose rate for seed treatment is given in a relevant unit per number of seeds or 100 kg seeds. The sowing densities of a number of main EU crops can be found in Lucchesi V. et al., (2016)[72]. For crops not present in this publication, national documents may be consulted.

Incorporation – For incorporation (e.g. in potting soil), the dose expression can be provided in kg or l product/m^3. In that case an estimation of the amount of m^3 potting soil per ha should be provided, unless it can be reasoned that there is no emission to soil, surface water of groundwater (e.g. for granular soil incorporation applications in greenhouses)

Row, strip or spot treatment – For row or strip treatment, the percentage of treated area should be indicated.

P.3.5 Content of micro-organism in material used (e.g. in the diluted spray, baits or treated seed)

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 3.5
Relevant evaluation criterion:	Reg (EU) No 546/2011, General Introduction, point 2.4
Relevant decision making criterion:	The information provided in this Section forms the basis of the risk assessment. Relevant decision making criteria are therefore referred to in other sections of the risk assessment.

Purpose of this point:

The content of the micro-organisms in the material used needs to be reported, using appropriate units, such as number of active units (e.g. CFU, IU, OB, or other) per volume or weight formulated product, including information regarding water spray volumes used (if relevant) or amount of micro-organisms per number of seeds (in case of seed treatment). This information is essential for all aspects of the risk assessment (efficacy, physical, chemical and technical properties of the PPP, and the risk assessment on human and animal health or the environment), as it forms the basis for the calculation of the amount of CFU (or other relevant unit) applied per ha.

Assessment principle:

[72] Results of the EPPO Survey on dose expression for seed treatment and authorized dose for plant protection products in general, Lucchesi et al. (2016), 46 (3), 618–624, https://doi.org/10.1111/epp.12342 (see in particular the supplemental documents).

Content of the micro-organism in formulated product
The content (min.-max.) of the micro-organism in the formulated product will be the basis of the calculation of the amount of micro-organism applied per ha (or other relevant unit) and is thus important for the evaluation of efficacy and the risk assessment on human and animal health or the environment. As described earlier under P.3.4, it will be highly appreciated to (briefly) explain whether the calculated GAP values are based on the minimal or maximum amount of CFU (or other relevant unit) in the formulated product.

Water spray volume
Water spray volumes (if relevant) should be indicated here. Water spray volume is not only relevant for efficacy, but also for human toxicology and/or ecotoxicology (the most diluted version and the most concentrated uses of the product should be indicated), fate and behaviour in the environment (to assess spray drift if required), but also to correctly assess physical, chemical and technical properties of the product.

Seed treatment
As indicated above under P.3.4., the amount of micro-organisms (using the appropriate unit) should be indicated per number of seeds or 100 kg seeds. For instance, this is important for the risk assessment of ecotoxicology (e.g. for birds and/or mammals who may potentially eat these treated seeds). Note that for seed treatment, the volume of diluent (slurry volume used for the coating itself) should be specified.

Dipping of flower bulb and flower tuber crops
Fluid uptake during dipping application and planting density per ha, resulting in the actual amount (in the appropriate unit) applied per ha, should be provided.

P.3.6 Method of application

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 3.6
Relevant evaluation criterion:	Reg (EU) No 546/2011, General Introduction, point 2.4
Relevant decision making criterion:	The information provided in this Section forms the basis of the risk assessment. Relevant decision making criteria are therefore referred to in other sections of the risk assessment.

Purpose of this point:

The method of application of the PPP is used for instance to determine the exposure to humans or the environment (if required).

Assessment principle:
The proposed method of application needs to be described, indicating the type or equipment to be used, if any, as well as the type and volume of diluent to be used per unit of area of application, or volume of plant protection product. Examples of methods are (but not limited to): high volume spraying, low volume spraying, spreading, dusting, drench, drilling, etc. It should also be specified where the application will be performed, e.g. overall, broadcast, row, individual plant, between the plants etc. (see also the example provided for application rate of row treatment under P.3.4). When a tank mix is recommended or required for the PPP, this should be clearly specified (as this information must be considered by other aspects of the efficacy and risk assessment).

Information on the method of application is essential information for the risk assessment as for instance the use of machinery that has the potential to generate drift during spray application, might pose a higher risk for bystanders. This is only relevant in case potential hazards for

humans are indicated for the PPP. In contrast, the risk for bystanders may be negligible for e.g. paint application on tree trunks for the use in forestry.

P.3.7 Number and timing of applications on the same crop, duration of protection and waiting period(s)

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 3.7
Relevant evaluation criterion:	Reg (EU) No 546/2011, General Introduction, point 2.4
Relevant decision making criterion:	The information provided in this Section forms the basis of the risk assessment. Relevant decision making criteria are therefore referred to in other sections of the risk assessment.

Purpose of this point:

The maximum number of applications to be used on the same crop provides information on the maximum amount of product that can be used on a crop and is needed for the risk assessment of several aspects (e.g. residue and the effects on non-target organisms). If applicable, the interval between applications (in days) needs to be provided.

Also, the timing/growth stages of the crops to be protected and the duration of protection is essential information for various aspects of the risk assessment (e.g., residue, efficacy, effects on non-target organisms). The information on susceptible stages of the target organism (provided under point A.2.3.), used together with information on timing on application provided under this point, may be needed in the efficacy assessment.

Assessment principle:

The maximum number of applications on the same crop should be indicated. When the application of the micro-organism acts *via* an inoculative approach (where the micro-organism is expected to multiply and maintain its presence), a lower number of applications is envisioned compared to an inundative approach (where directly a high number of micro-organism is applied to promote a rapid control of pests over the short term). If several crop cycles are envisioned during the growing season, this should be clearly indicated (as this determines the max amount of product that may be used on a yearly basis at the same location).

Information regarding the number of applications, in combination with interval and the growth stage of the crop provides essential information for the assessment of residues. Analogously, information on growth stage of the crop provides information to correctly assess exposure of non-target organisms (e.g. pollinators and application during flowering). Growth stages are indicated as BBCH stages (if applicable), e.g. as described by Meier, Uwe (2018)[73]. Note that it is highly recommended to also provide months of application, as BBCH-stages will be achieved in individual member states in different time frames.

Information on the development stage of target organisms may support the assessment of efficacy.

P.3.8 Proposed instructions for use

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 3.8
Relevant evaluation criterion:	-
Relevant decision making criterion:	Reg (EU) No 546/2011, General Introduction, point 3.6
Purpose of this point:	

[73] Growth stages of mono- and dicotyledonous plants: BBCH Monograph. Quedlingburg: Open Agrar Repositorium https://www.openagrar.de/receive/openagrar_mods_00042351

The proposed instructions for use of the plant protection product to be printed on labels and leaflets need to be provided.

Assessment principle:
A draft label needs to be submitted, in accordance with the provision of Reg (EU) 547/2011 and the information provided in the GAP table.

For the representative product used during active substance approval, a general draft label can be drawn up. In contrast, for product registration, when drafting the label, national requirements of individual Member States should be taken into account. For specific details contact can be sought with relevant CA prior to submission.

P.3.9 Safety intervals and other precautions to protect human health, animal health and the environment

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 3.9
Relevant evaluation criterion:	Reg (EU) No 546/2011, General Introduction, point 2.4
Relevant decision making criterion:	The information provided in this Section forms the basis of the risk assessment. Relevant decision making criteria are therefore referred to in other sections of the risk assessment.

Purpose of this point:
Information provided here would support the risk assessors in evaluating possible hazards linked to human and animal health, residues, and non-target organisms.

Assessment principle:
The information provided needs to follow from, and be supported by, the data provided for the micro-organism(s) and that provided under Sections 7 to 10.

(i) Where relevant pre-harvest intervals, re-entry periods or withholding periods necessary to minimise the presence of residues in or on crops, plants and plant products, or in treated areas or spaces, with a view to protecting humans or livestock, needs to be indicated e.g.:

- pre-harvest interval (in days) for each relevant crop,

- re-entry period (in days) for livestock, to areas to be grazed,

- re-entry period (in hours or days) for humans to crops, buildings or spaces treated,

- withholding period (in days) for animal feedingstuffs and for post-harvest uses,

- waiting period (in days), between application and handling treated products,

- waiting period (in days), between last application and sowing or planting succeeding crops.

(ii) Where necessary, in the light of the test results, information on any specific agricultural, plant health or environmental conditions under which the plant protection product may, or may not, be used should be indicated.

P.4 FURTHER INFORMATION ON THE PLANT PROTECTION PRODUCT

P.4.1 Procedures for cleaning and decontaminating of application equipment

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 4.1
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.3.3(i)
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.3.2.8
GLP-compliance:	Not required for method validation
Purpose of this point:	
Cleaning / decontaminating procedures must be sufficiently effective to avoid an impact on efficacy and to prevent crop damage caused by carry-over of residual PPP that may be present in application equipments and protective clothing.	

Confidentiality
Not relevant.

Background information
Adequate cleaning and decontaminating procedures (for both application equipment and protective clothing) needs to be described.

EPPO standard PP1/292[74] on “Cleaning pesticide application equipment (PAE) – efficacy aspects” describes methods that can be used to examine whether cleaning procedures are sufficient to ensure that residues of PPPs do not remain in the PAE after cleaning.

If significant (>50%) phytotoxicity is observed, further testing is required. According to EPPO standard PP1/292 dose-response relationships should be established. However, when the observed phytotoxicity is not due to a chemical component such as a co-formulant, a different approach may be required. Hence, for micro-organisms, in case of phytotoxicity, it will be necessary to deviate from EPPO standard PP1/292 and, for instance, to demonstrate with small scale testing that appropriate cleaning procedures are sufficient.

It should be noted though, that for the majority of micro-organisms severe phytotoxicity symptoms are not reasonably expected.

The word “decontamination” suggests complete disinfection. However, adequate cleaning and decontaminating procedures should always be viewed in light of the risk assessment. In the absence of demonstrated negative effects of the micro-organism, the survival of a single cell or spore may not be considered a potential risk. Nonetheless, if required, adequate cleaning and decontaminating procedures need to be provided. However, accurate washing may already be sufficient in some cases.

P.4.2 Recommended methods and precautions concerning: handling, storage, transport, fire or use

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 4.2
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.1.3 (d)
Relevant decision making criterion:	-
GLP-compliance:	Not required for method validation
Purpose of this point:	
Precautionary methods must be defined for safe operation in the context of handling, storage, transport, fire, or use.	

Conditionality
Not relevant.

[74] https://pp1.eppo.int/standards/PP1-292-1

Confidentiality
Not relevant.

P.4.3 Measures in case of accident

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 4.3
Relevant evaluation criterion:	-
Relevant decision making criterion:	-
GLP-compliance:	Not required for method validation
Purpose of this point:	
Practical response actions must be defined to mitigate the effects of PPP-related accidents (i.e., spillage, contamination, damage to packaging, calamities, injury).	

Conditionality
Not relevant.

Confidentiality
Not relevant.

P.4.4 Procedures for destruction or decontamination of the plant protection product and its packaging

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 4.4
Relevant evaluation criterion:	-
Relevant decision making criterion:	-
GLP-compliance:	Not required for method validation
Purpose of this point:	
Controlled measures to dispose of the product and its packaging must be evidenced to adhere to principles of environmental friendliness, economy, and practicality.	

Conditionality
Not relevant.

Confidentiality
Not relevant.

P.5 ANALYTICAL METHODS

P.5.1 Methods for the analysis of the preparation

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 5.1
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.4.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.4.1
GLP-compliance:	Not required for method validation
Purpose of this point:	
The differences between PPP and MPCA-AM may necessitate adaptation of the analytical methodology evaluated under A.4.1, to maintain functionality. Under this point, the need for such adaptations is discussed, and effectuated adaptations are described and evaluated.	

Conditionality
Whenever MPCA-AM and PPP are the same (or at least bridgeable for analytical purposes) and the required method validations have already been provided under A.4.1, no data are

required in addressal of this point.

Confidentiality
Not relevant.

Background information

IDENTIFICATION METHODS FOR THE PRODUCT LEVEL
The product level methods are essentially the same as those described for the substance level. Please refer to A.4.1.

METHODS TO DETERMINE THE CONTENT OF SPECIFICATION ELEMENTS IN THE PPP
The largest part of the information provided under A.4.1 applies *as is* for the PPP. Excepting the cases in which there is no distinction between the two, MPCA-AM and PPP are different matrices for the purpose of analytical method performance, as (i) concentrations of specification elements are generally lower in the PPP due to dilution during preparation, (ii) the components added during preparation may interfere with the analysis of the specification elements (chemical constituents may affect the analysis of claimed active metabolites, MoCs and other relevant impurities, whereas additional micro-organisms may complicate easy distinction during enumeration), and (iii) the physically different PPP may necessitate alternative sample preparation.

Micro-organism
Depending on the nature of the difference between MPCA-AM and PPP, translation of the enumeration method validated for the MPCA-AM may not require a full re-evaluation for the PPP;
'Specificity' only needs amendment when the PPP contains more microbial active substances than are present in the MPCA-AM, as it needs elaboration on how the additional micro-organisms may be distinguished from those that were already present.
'Accuracy' needs no further addressal, once stability of the PPP, as supported by e.g., suspensibility or dispersion stability, has been adequately evidenced.
Last, 'linearity' and 'precision' are not reasonably expected to be affected by changes to the matrix brought about by the formulation process.

Chemical components
In principle, the chemical components of the PPP (claimed active metabolites, relevant impurities, additives and MoCs) are dealt with under the assessment of the active substance, and relevant methods are validated in that context. Indeed, point 5.1 of Part B of the Annex to Regulation (EU) No 284/2013 does not directly provide for such requirement.

However, point 5.1 of Part B of the Annex to Regulation (EU) No 284/2013 provides also for a description of methods used to determine the storage stability and shelf life of the PPP. Hence, a method for a chemical component in the PPP must be separately validated, unless:

- it can be demonstrated that the storage period does not affect the composition of the chemical components of the PPP (which in principle is the case for impurities and additives), and
- the manufacturing process is a continuous one (in which case the MPCA-AM exists only in a hypothetical stage),

these methods must be separately validated for the PPP.

Obviously, the PPP-level method for claimed active metabolite determination must be sufficiently sensitive to allow for any formulation process-related dilution of the matrix.
According to SANCO/3030/99 the LOQ for relevant impurities must also be derived for the

PPP, based on the maximum limit of the substances in the MPCA-AM and the content of MPCA-AM in the PPP. Whenever this would result in a level that would be too low to measure, validation must be performed at the lowest possible level. In that case, the nature of the technical limitations that necessitate a higher LOQ must be described, and the LOQ's fitness for purpose with regard to relevant thresholds must be evidenced.

METHODS TO DETECT AND ENUMERATE RELEVANT CONTAMINATING MICRO-ORGANISMS
The same approach employed to fulfill the point A.4.1(f) can be used.

METHODS TO DETERMINE THE STORAGE STABILITY AND SHELF LIFE
No remarks needed.

P.5.2 Methods to determine and quantify residues

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 5.2
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.4.2
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.4.2
GLP-compliance:	Not required for method validation

Purpose of this point:

The method used to determine and quantify densities of the micro-organism and residues, as provided for under A.4.2 may be required, unless the information already submitted in accordance with point 4.2 of Part B of the Annex to Regulation (EU) No 283/2013 is sufficient for the PPP also.

Conditionality
Not relevant.

Confidentiality
Not relevant.

Background information
Please refer to A.4.2.

P.6 EFFICACY DATA

Active substance approval
According to Regulation (EC) No 1107/2009, ANNEX II, 3.2, an active substance, alone or associated with a safener or synergist, must only be approved where it has been established for one or more representative uses that the PPP, when applied under the proposed conditions, is sufficiently effective. The relevant guidance document listed under Section 6 of the CC-PPP, describes how and why efficacy needs to be addressed for the approval of new active substances. Typically, only a few representative trials may suffice. While it is not mandatory to submit individual trial reports, it will be highly appreciated by the CA to do so. Furthermore, in addition to the obtained efficacy across all tests, it is advisable to add also the minimum and maximum efficacy that is obtained in individual trials, as this may give information on the variability of the efficacy of the representative product and/or micro-organism. Although it is noted (and fully acceptable when properly explained) that due to their nature and/or MoA, PPPs based on micro-organisms may have a more variable effect than PPPs based on chemicals. For the principles of determining acceptable efficacy is referred to the relevant section below, which is applicable for both active substance approval and PPP registration.

For renewals of active substances the draft Guidance document on the renewal of approval of active substances to be assessed in compliance with Regulation (EU) No 844/2012 (the

Renewal Regulation) can be referred to (SANCO/2012/11251). Considering that PPPs containing the active substance have already been evaluated previously, effectiveness does not need to be re-evaluated for active substance renewal. An overview of representative uses and all supported uses already authorized in Member States should be provided.

Representative product during active substance approval versus PPP registration
For the approval of new active substances, evidence must be submitted to demonstrate that the dose(s) proposed is/are sufficiently effective and selective and broadly speaking appropriate. In other words, the proposed dose rate for the intended use(s) of the representative product should be realistic. These intended use(s) should encompass the "worst case" GAP. Confirming the dose rate indicated in the GAP table is of vital importance, since the risk assessments on human health, fate and behaviour in the environment, and the effect on non-target organisms in the active substance dossier are based on this dose rate. Typically, for active substance approval a limited number of representative trials are acceptable to support the dose rate of the representative product. In contrast, during PPP registration, efficacy needs to be demonstrated for all intended uses by a full data-package for each climatic zone. This can result in >100 efficacy trials when many crop-pest combinations are applied. Therefore, in this Section a distinction will be made between the representative product for active substance approval and PPP registration, where relevant.

Guidance documents/standards
Standards for the efficacy evaluation of PPP are provided by the EPPO. These standards encompass general standards, which cover general aspects of the efficacy evaluation, and specific standards (covering one type of PPP, e.g. fungicide or herbicide, and often for a specific crop-pest combination). The general standards are freely available in the EPPO database on PP1 Standards[75]. In these EN several of these standards (but not all) will be discussed briefly in the appropriate context.

Regarding PPPs based on micro-organisms, special attention should be paid to the following three standards, as these include considerations that are specific for PPPs based on micro-organisms.

EPPO standard PP1/276[76] on the "Principles of efficacy evaluation for microbial plant protection products" refers to PPPs based on micro-organisms, including microbial products that are not necessarily low-risk. Relevant for all PPPs based on micro-organisms.

EPPO standard PP1/296 on "The evaluation for low-risk plant protection products" contains essential information on reduced data and efficacy requirements for low-risk products and should be taken into account when writing a dossier for a low-risk PPP. This standard discusses both microbial and non-microbial low-risk (chemical) products. Relevant for low-risk PPPs based on micro-organisms.

EPPO standard PP1/319 on the "General principles for efficacy evaluation of plant protection products with a mode of action as plant defense inducers" refers to PPPs based on plant defense inducers or elicitors that induce plant defenses as MoA. This standard includes both micro-organisms and other type of elicitors (e.g. chemical, or in-activated micro-organisms). Relevant for PPPs based on micro-organisms that have as MoA the induction of plant defense.

P.6.1 Preliminary tests

[75] https://pp1.eppo.int/

[76] https://pp1.eppo.int/standards/PP1-276-1

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 6.1
Relevant evaluation criterion:	-
Relevant decision making criterion:	-

Purpose of this point:

The information provided under this point may support the understanding of the biological properties, MoA and the dose-range finding of the PPP.

Preliminary tests (for the representative PPP for active substance approval or PPP to be registered) may consists of laboratory, greenhouse and field studies. In case of combination of several active substances, safeners and/or synergist, information should be provided on the ratio that is envisioned. In case of bridging a PPP to another preparation (e.g. a previous preparation or other registered PPPs) based on the same active substance, this Section should contain tests demonstrating compatibility between the different preparations. If preliminary data is not deemed necessary (e.g. when the micro-organism has already been used in plant protection for a long time and it is the sole active component in the (representative) PPP), a justification should be provided.

Assessment principle:

According to EPPO standard PP1/276 on the “Principles of efficacy evaluation for microbial plant protection products” and the EPPO standard PP1/296 on the “Principles of efficacy evaluation for low-risk plant protection products”, data from other sources (e.g. published papers, laboratory studies) may be used to supplement the efficacy data. This data can consist of information regarding the MoA, susceptibility of the target pests or hosts, dose response behaviour, and/or the effect on environmental, agronomic and other factors of the product. Data from well-designed small-scale laboratory and/or growth chamber studies can provide data to reduce the number of field/glasshouse trials to test effectiveness (further discussed under point P.6.3). Despite it is preferrable performing studies under GEP certification, non-GEP data may be also acceptable. This supportive data can be submitted under preliminary tests.

Furthermore, preliminary tests (consisting e.g. of laboratory, greenhouse or field studies) should be provided in the following situations:

Co-formulation, active substance approval

For the active substance approval, it should be demonstrated that the representative PPP is efficacious on itself (unless the micro-organism is applied for as part of a qualitatively defined combinations of strains, e.g. a consortia). The possibility that the effectiveness is derived from other components in the preparation and not from the active substance itself should be excluded. It can occur that components are added to the preparation (e.g. as preservatives) that may directly contribute to the effectiveness of the formulated product. In some cases it may be possible to theoretically exclude (justification) that these components will contribute to the effectiveness of the representative formulated product (e.g. in case when the proposed dose rate is much lower than expected to be efficacious for the co-formulant, or when the proposed function is not similar etc.). If it cannot be adequately justified that the additive/co-formulant does not contribute significantly to the effectiveness of the representative product, then this should be demonstrated in efficacy trials (e.g. by testing the efficacy of the preparation in the presence and absence of the active substance for which approval is sought). The principle described here is similar to the principle for the evaluation of chemical active substances.

Co-formulation with more than one active substance, PPP registration

For PPP registration of PPPs based on co-formulated mixtures with more than one active

substance, a justification for the ratio of active substances within the mixture should be provided (in line with EPPO standard PP1/225[77]). In addition, a rationale behind the inclusion of each active substance should be provided. Examples of potential advantages and disadvantages of mixtures with respect to effectiveness and other considerations regarding these mixtures are provided in EPPO standard PP1/306[78] on the "General principles for the development of co-formulated mixtures of plant protection products". The principles described here are similar to the principles for the evaluation of PPPs based on chemical active substances.

Using different preparations, active substance approval
For active substance approval it can occur that efficacy data is (partly) based on preparations under development. This should be clearly indicated. An explanation of the differences of the used preparation(s) relative to the final representative product should be provided. It should be noted that when only preparations under development are used, evaluation of efficacy may not be possible.

Bridging, PPP registration
In case a biological significant change in the composition of PPPs is made it should be demonstrated (by reasoned case and/or data, depending on the nature of the change) that efficacy of the new preparation is comparable to the previous preparation. Similar is the development of a new product, which is to be based on the principle of comparing with, and "bridging" to an existing preparation based on the same active substance. Details on the data required are described in EPPO standard PP1/307[79] on "Efficacy considerations and data generation when making changes to the chemical composition or preparation type or plant protections products". The principles described here are similar to the principles applied for the evaluation of PPPs based on chemical active substances.

Absence of data
If preliminary data is not deemed necessary (e.g. when the micro-organism has already been used in plant protection for a long time and is the sole active component in the (representative) PPP, a justification must be provided to explain the absence of preliminary data.

P.6.2 Minimum effective dose

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 6.2
Relevant evaluation criterion:	
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.3.1.2
Purpose of this point:	

It should be justified what is the minimum effective dose that is still sufficiently effective for the intended use(s). This is required to prevent unnecessary overdosing of PPPs, to reduce the exposure to PPPs in the environment.

Assessment principle:
The MED needs to be reported. EPPO standard PP1/225 on the "Minimum effective dose", described the requirements for efficacy testing to establish the MED. However, especially for PPPs based on micro-organisms, there are several aspects that should be taken into consideration when addressing the MED (some are described in EPPO standard PP1/276 on the "Principles of efficacy evaluation for microbial plant protection products"):

(a) If the micro-organism species already occurs naturally in the EU environment, the

[77] https://pp1.eppo.int/standards/PP1-225-2
[78] https://pp1.eppo.int/standards/PP1-306-1
[79] https://pp1.eppo.int/standards/PP1-307-2

concern of reducing exposure to this micro-organism in the environment may be less critical (even more so when the PPP is considered as low risk);

(b) Micro-organisms are capable of replication and may therefore multiply, rendering the concept of a MED both less relevant and more difficult to establish.

(c) PPP efficacy may be more affected to environmental conditions compared to chemical products, and hence efficacy data generated more variable. Hence, a dose response may be more difficult to obtain.

(d) Whereas for chemical PPPs a more linear dose response is expected, the dose response for microbial PPPs may have a logarithmic nature (hence, applying twice as much product, may not be sufficient to trigger a double response).

Therefore, due to the nature of PPPs based on micro-organisms, field testing to address the MED may not be necessary. Nonetheless, an appropriate explanation for the proposed dose remains required, including a justification in the eventuality of the absence of field data. Explanations can include information regarding the MoA and any other information provided like preliminary tests or literature (as discussed previously in point P.6.1.).

The principles regarding the MED of PPPs based on micro-organisms, are applicable for both active substance approval and PPP registration, with the distinction that for active substance approval, this is not mandatory. Nevertheless, it is considered useful to include lower dose rates in the tests submitted to support active substance approval.

P.6.3 Testing effectiveness

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 6.3
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.3.3 Reg (EU) No 546/2011, Annex, Part B, 1.3.4
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.3.1.3 Reg (EU) No 546/2011, Annex, Part B, 2.3.1.4 Reg (EU) No 546/2011, Annex, Part B, 2.3.1.5

Purpose of this point:

In order to assess the efficacy of the PPP (i.e., the overall effect of the PPP including both positive and negative effects), it is important to know its effectiveness also (i.e., the positive effects regarding the desired plant protection activity). Effectiveness should be demonstrated to be beneficial under the agricultural, plant health and environmental (including climatic) conditions in the area of proposed use. In addition to confirmation of the claimed protection of the PPP, testing effectiveness is also essential to avoid unnecessary exposure to PPPs in the environment.

Assessment principle:

As indicated prior, under the Introduction of point P.6, the purpose of the efficacy consideration for active substance approval compared to PPP registration is not the same. While for the first the principal objective is to confirm that the dose rate is realistic, for the second, for all proposed uses it should be demonstrated (or made plausible via extrapolation) that effectiveness is sufficient, when applied under the relevant climatic and agronomical conditions.

Number of trials, PPP registration

Sufficient data needs to be provided to permit the evaluation on the level, duration, and consistency of intended effects of the PPP. The EPPO standard PP1/226[80] on the “Number of efficacy trials”, describes the number of trials that is required to assess efficacy, taking into consideration e.g. crop and pest status (major or minor), supporting evidence, and extrapolation possibilities (see below for a more detailed explanation regarding extrapolation).

[80] https://pp1.eppo.int/standards/PP1-226-3

It should be noted that for low risk PPPs the number of trials considered as full data package may be lower than for chemical-based PPPs (both for PPPs based on micro-organisms and chemical active substances). This is described in the EPPO standard PP1/296 on the "Principles of efficacy evaluation for low-risk plant protection products".

Especially for micro-organisms non-GEP trials can be used as supportive information (e.g. on the MoA, susceptibility of the target pests or hosts, dose response, and/or the effect on environmental, agronomic and other factors of the product). This information provides support to reduce the number of large scale GEP certified field trials (in line with EPPO standard PP1/276 on the "Principles of efficacy evaluation for microbial plant protection products" and EPPO standard PP1/296 on the "Principles of efficacy evaluation for low-risk plant protection products"). Especially for low-risk products, non-GEP trial data may also be acceptable to test effectiveness, if scientifically sound and in line with other applicable and relevant EPPO standards. It should be noted though, that data protection can only be granted for GEP/GLP certified trials and not for non-GEP trial data. Non-GEP trial data can be used as supporting information and can in this way lower the number of required GEP-certified trials.

Acceptable efficacy, active substance approval and PPP registration
The EPPO standard PP1/214[81] on the "Principles of acceptable efficacy", describes how to determine whether the efficacy of a PPP is acceptable for the purposes of registration, taking into consideration both the positive effects of the treatment and possible negative effects (e.g. development of resistance, phytotoxicity, reduction on yield, etc). The net result of the positive and negative effects should be of sufficient overall agricultural benefit to justify the use of the PPP.

In line with EPPO standard PP1/276 on the "Principles of efficacy evaluation for microbial plant protection products" and EPPO standard PP1/296 on the "Principles of efficacy evaluation for low-risk plant protection products", it is generally accepted that PPPs based on micro-organisms can have a lower and/or more variable efficacy than PPPs based on chemical active substances. For PPPs based on micro-organisms, the observed effects in the trials should (on average) at least be significantly higher than those observed in the untreated control, and when possible similar to suitable reference products. For PPPs based on micro-organisms, similar microbial products are most appropriate as reference products. However, when not possible, a conventional chemical product should be included (and under this aspect it can be fully acceptable when the level of control of the PPP based on micro-organisms will fall below that of the chemical product). This reference then acts as control for the success or failure of the trial.

A lower level of benefit obtained by the use of PPPs based on micro-organisms can still be acceptable, when taking into consideration their advantages. PPPs based on micro-organisms, especially those that are considered low-risk, may have the following advantages (list not exhaustive): (a) they can often be used over a wider range of growth stages of the crop (due to a shorter or complete absence of a pre-harvest interval (PHI), (b) they often are (better) compatible with Integrated Pest Management (IPM) or organic farming, (c) they may have a lower probability of developing resistance in the target organisms and can therefore their use be integrated in a resistance management strategy, (d) they may have fewer undesirable effects of the PPP (e.g. on beneficial organisms), and (e) there may be less need for specific mitigation measures. To take into consideration possible benefits (other than the claimed protection) these should be well explained in the dossier. Often, PPPs based on micro-organisms, are used as a component of an IPM programme. As this program is designed to lower the pest populations by all available means that are ecologically justified, a

[81] https://pp1.eppo.int/standards/PP1-214-4

moderate effectiveness of the PPP based on micro-organisms may still be of use within such a program (as pest pressure is kept low). See also point P.6.7 on “Compatibility in plant protection programmes”.

Plant protection exerted by PPPs based on micro-organisms can often be more variable, as micro-organisms may be more susceptible to unfavourable environmental conditions (e.g. too hot, cold or dry for the micro-organism, resulting in a reduced performance of the PPP). For PPPs based on micro-organisms, this variability is more acceptable than for PPPs based on chemical active substances, as long as the reasons for inconsistencies in pest control by the PPP are explained and justified. If adequately justified, recommendations can be proposed for the user to ensure the PPP will be applied under conditions that may provide optimal efficacy (in line with (EU) No 546/2011, Annex, Part B, 2.3.1.5). If a PPP performs variable and there is no sound explanation that can enable the situations to be identified were effective control might be expected, authorisation might be refused until a robust demonstration or explanation of the factors affecting performance are provided.

Extrapolations, PPP registration
Extrapolation is based on the principle that certain groups of pests or groups of crops are considered to be more or less equivalent in relation to efficacy. Extrapolation may be used to extend an accepted plant protection claim to additional crops or pests in the absence of specific data, or used to allow a more reduced data package. Regular extrapolation principles (also applicable for conventional chemical products) are described in EPPO standard PP1/257[82] on the “Efficacy and crop safety extrapolations for minor uses”. As of the time in which these EN are developed, these extrapolations are currently only applicable to crops or pests with a minor use status . Extrapolations are either based on Extrapolation tables[83] provided by EPPO, or on expert judgement.

The above-mentioned extrapolation tables have mostly been written for conventional crop protection products. For PPPs based on micro-organisms, extrapolation by expert judgement may be possible based on the mode of action of the micro-organism, the biology of the target pest or disease, and the micro-organism itself. The EPPO standard PP1/296 on the “Principles of efficacy evaluation for low-risk plant protection products” provides detailed information regarding extrapolation possibilities for low-risk products:

- “*If a product has a **direct mode of action** which is pest dependent the crop may be of less relevance. Extrapolation from data on a major pest in a major crop to the same pest in other major and minor crops may be possible depending on the quality of the existing data*”.
- “*For low-risk products with an **indirect mode of action** the claimed pest may be less relevant. (…) In this case efficacy trials can be conducted on a limited number of claimed pests and extrapolation to other claimed and relevant pests may be possible*”.

While described in the EPPO standard PP1/296 on the “Principles of efficacy evaluation for low-risk plant protection products”, these extrapolations are applicable for all PPPs based on micro-organisms, with the distinction that for low-risk products also extrapolation towards major uses is allowed. However, such extrapolations (of major uses) should be made, where applicable, at Member State level, until a harmonised approach for major use extrapolations is agreed upon.
For PPP with a MoA as plant defense inducers, the EPPO standard PP1/319 provides guidance on how to test efficacy. If properly motivated some of these principles may also be applied to PPPs based on micro-organisms with other MoAs.

For all proposed extrapolations, it is important that *e.g.,* the MoA of the active substance and

[82] https://pp1.eppo.int/standards/PP1-257-2

[83] https://www.eppo.int/ACTIVITIES/plant_protection_products/extrapolation_tables

the reasoning behind the extrapolations are well explained within the dossier, possibly supported by literature studies. Furthermore, it is preferrable to submit a full data package on one crop and extrapolate the results (including full justification specifying the crop structure and cropping practice) to other crops than to submit 1-2 trials on individual crop-pest combinations (especially taking into consideration that efficacy of PPPs based on micro-organisms may be more variable). Hence, the choice of crop-pest combinations that will be tested in the efficacy trials, should be considered carefully. What is the most difficult target-organism to control? Which crop should be tested? And what are the worst-case conditions for the chosen crop-pest combination?

P.6.4 Information on possible development of resistance in target organisms

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 6.4
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Introduction, 2.1 Reg (EU) No 546/2011, Annex, Part B, 1.3.4(d) Reg (EU) No 546/2011, Annex, Part B, 1.3.11
Relevant decision-making criterion:	Reg (EU) No 546/2011, Annex, Introduction, 3.3

Purpose of this point:

Information on possible development of resistance in target organisms is essential to ensure a lasting efficacy of the micro-organism used in the plant protection product(s).

If there are reasons to believe that the use of the plant protection product may lead to resistance in certain target organisms, this must be addressed. If data is available from literature or experimental studies, but does not refer to the organisms claimed as the target one, this data can still be provided as it may support the evaluation of the possibility of resistance in the target organism. If relevant resistance management strategies are applicable to minimised the risk of occurrence of resistance in the target populations, these must be described under this point.

Assessment principle:

The assessment principles described earlier under A.3.4 are also applicable here. Where A.3.4. focusses only on the inherent risk of the micro-organism to trigger the development of resistance in the target organism, here under P.6.4 also the inherent risk of the claimed target organisms, and the agronomic risk deriving from the conditions of use of the product can be taken into account. Steps of the resistance risk assessment and resistance risk management are described in EPPO standard PP1/213[84] on “Resistance risk analysis”.

As indicated earlier, EPPO standard PP1/276 on the “Principles of efficacy evaluation for microbial plant protection products”, makes a clear distinction between micro-organisms with a direct MoA and micro-organisms with an indirect MoA in respect to the risk of inducing the development of resistance in the target organism. Micro-organisms with an indirect MoA (e.g. host plant defence induction or competition for nutrients) have less risk of inducing resistance development in target organisms in the current state of knowledge. This is because there is no direct selection pressure on the target organism. In such cases this data point can be addressed with a justification.

Only when there is reason to believe that the use of the PPP may lead to resistance in certain target organisms, which is more likely for micro-organisms with a direct MoA on the target organisms, a full resistance risk assessment, following EPPO standard PP1/213 on “Resistance risk analysis” is required.

[84] https://pp1.eppo.int/standards/PP1-213-4

Nonetheless, in general, micro-organisms used as active substances often use completely new MoAs compared to chemical active substances and can therefore be beneficial for resistance management purposes.

Resistance may be also of less relevance when the activity of the micro-organism is based on multiple MoA.

It should be noted that at the moment of renewal of approval/authorisation for active substance/PPP, the information submitted under the point 6.4 “Information on possible development of resistance in target organisms” should be updated with new information on occurrence of resistance (if available), and such information should be re-evaluated.

P.6.5 Adverse effects on treated crops

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 6.5
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.3.5
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.3.2.1
	Reg (EU) No 546/2011, Annex, Part B, 2.3.2.2
	Reg (EU) No 546/2011, Annex, Part B, 2.3.2.3
	Reg (EU) No 546/2011, Annex, Part B, 2.3.2.4

Purpose of this point:

Absence of unacceptable effects on treated plants or plant products should be demonstrated or adequately justified. This is essential as the use of the PPP should have a sufficient overall agricultural benefit (which is the net results of the positive and negative effects). If adverse effects are expected, appropriate limitations of use must be put on the label.

EPPO standard PP1/135[85] on “Phytotoxicity assessment” provides detailed information on how phytotoxicity of PPPs to treated plants or plant products (including propagating material) can be accurately assessed and recorded. This standard also describes the effects on quantity and quality of the yield. This standard is relevant for points P.6.5.1 to P.6.5.5. described below.

Note that crop safety is also addressed in Extrapolation tables[86] provided by EPPO (to be read in conjuncture with in EPPO standard PP1/257 on the “Efficacy and crop safety extrapolations for minor uses”).

Proposed labels of PPPs may include recommendations or requirements for the use with other PPPs and/or adjuvants as a tank mix. In these cases, the points discussed under P.6.5.1 till P.6.5.5 regarding adverse effects on treated crops apply in relation to the information provided for the tank mix (as specified in Regulation (EU) No 546/2011, Annex, Part B, 2.3.2.7). Therefore, recommended and required tank mixes should be clearly indicated under method of application (as discussed under point P.3.6).

P.6.5.1 Phytotoxicity to target plants (including different cultivars) or to target plant products

Purpose of this point:

It should be demonstrated that there will be no relevant phytotoxic effects on the treated plants or plant product. If phytotoxic effects are expected, limitations of uses will be proposed on the label to mitigate these adverse effects.

[85] https://pp1.eppo.int/standards/PP1-135-4

[86] https://www.eppo.int/ACTIVITIES/plant_protection_products/extrapolation_tables

Assessment principle:
For PPPs based on micro-organsims, in most cases it may be sufficient to assess phytotoxicity in the efficacy trials. This is because the majority of PPPs based on micro-organisms (currently) have a function as insecticide or fungicide, and for these types of products phytotoxicity can be firstly assessed in the efficacy trials and only when phytotoxicity symptoms are observed further testing is needed (similar to conventional chemical products). Further testing would for instance include using twice the recommended dose rate in efficacy or sensitivity trials. When negative effects are considered unimportant in comparison with the benefits or of a transient nature, there should be supportive evidence (e.g. by submitting yield measurements demonstrating that the observed negative effects does not affect yield or by submitting data demonstrating improved quality of the treated plant or plant product). Based on the results, an appropriate warning can be placed on the label (e.g. to alert the user that phytotoxicity symptoms are of a transient nature).

Reading of the framework in specific cases

Cases where phytotoxicity testing in efficacy trials is not sufficient
In line with EPPO standard PP1/135 on "Phytotoxicity assessment", there are several exceptions, were addressing phytotoxicity in the regular effectiveness trials may not be sufficient and specific selectivity trials are required (trials in the absence of pests, and typically including different varieties of the treated crops). Nonetheless, when phytotoxic effects are observed in the effectiveness efficacy trials, they should still be accurately assessed and recorded, as this information will supplement the phytotoxicity assessment done in the selectivity trials.

Herbicides – For herbicides specific selectivity trials are required in which also a double (2N) dose rate needs to be tested. Furthermore, selectivity trials should be set up with a number of different cultivars. This would include common varieties, but also those known to be sensitive.

Growth regulators – For growth regulators, doses higher than the intended dose (e.g. 2N dose rate) should be tested to determine the margin of crop safety.

Seed treatment – For seed treatment specific selectivity trials are required in which germination is tested, which includes usually at least 3 common cultivars. See EPPO standard PP1/135 on "Phytotoxicity assessment" for further information regarding the timing between seed treatment and these phytotoxicity trials.

P.6.5.2 Effects on the yield of treated plant or plant products

Purpose of this point:
It should be demonstrated, or adequately justified, that there will be no negative effect on yield at harvest due to the use of the PPP, unless a possible reduction in yield is compensated for by other advantages besides the plant protection action, such as an enhancement of the quality of the treated plants or plant products.

Assessment principle:
For PPPs based on micro-organisms used as fungicides or insecticides, as for conventional chemical products, in the absence of phytotoxicity in the efficacy trials, assessing yield in selectivity tests may not be required (note that yield specific parameters may also already be included for testing effectiveness, when required by any of the EPPO standards for specific crop-pest combinations, which may provide additional information). Only for herbicides and growth regulators, yield should be assessed in selectivity trials.

P.6.5.3 Effects on the quality of plants or plant products

Purpose of this point:
It should be demonstrated, or adequately justified, that there will be no unacceptable adverse effects on the quality of treated plants or plant products.

Assessment principle:
The criteria for assessing quality of yield are generally crop-specific and can be found in specific EPPO standards.

For certain crops there may be need to address taint. EPPO standard PP1/242[87] on "Taint test" gives further guidance on making relevant cases and where data may be required. If taint is observed, this may be indicator, for instance, of spoilage of edible parts. This would then eventually also provide information for the risk assessment related to residues of chemical components of the PPP (i.e. co-formulants, metabolites of concern and relevant impurities), or effects on human health.

P.6.5.4 Effects on the transformation process

Purpose of this point:
Under the conditions described in Annex Part B, Regulation (EU) No 284/2013, point 6.5.4, tests are required to exclude that the use of the PPP has a negative effect on intended transformation processes.

Assessment principle:
EPPO standard PP1/243[88] on "Effects of plant protection products on transformation processes" describes when transformation should be addressed.

Testing the effects on actual transformation processes is only necessary as a last resort.

If not sufficiently addressed, the use of the PPP on plant or plant product intended for being used in transformation processes must be excluded from the label.

P.6.5.5 Impact on treated plants or plant propagating material

Purpose of this point:
It should be demonstrated, or adequately justified, that there will be no unacceptable adverse effects on treated plants or plant products used for propagation or reproduction, such as effects on viability, germination, sprouting, rooting and establishment, except where proposed label specifies that the plant protection product will not be applied to plants or plant products to be used for propagation or reproduction.

Assessment principle:
Propagating material may include (depending on the crop): seeds, cuttings, runners, tubers, or bulbs and corms. EPPO standard PP1/135 on "Phytotoxicity assessment" describes the circumstances under which data on plant parts for propagation are required. As stated by EPPO standard PP1/276: "*for fungicidal and insecticidal products, data are generally not required unless the product has systemic activity, is applied close to harvest, and phytotoxicity effects have been observed on some of the tested crops. For most microbial products therefore generally a reasoned case may suffice in lieu of data, which should include*

[87] https://pp1.eppo.int/standards/PP1-242-2

[88] https://pp1.eppo.int/standards/PP1-243-2

reference to the phytotoxicity Assessment".

P.6.6 Observations on undesirable or unintended side-effects on succeeding crops and other plants

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 6.6
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.3.5 Reg (EU) No 546/2011, Annex, Part B, 1.3.8
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.3.2.5

Purpose of this point:

Absence of unacceptable effects on succeeding crops and/or adjacent crops should be demonstrated or adequately justified. Possible negative effects on succeeding crops must also be taken into account when defining the net result of positive and negative effects of the use of PPP. If adverse effects are expected, appropriate limitations of use must be put on the label.

Proposed labels of PPPs may include recommendations or requirements for the use with other PPPs and/or adjuvants as a tank mix. In these cases, the points discussed under P.6.6.1 and P.6.6.2 regarding undesirable effects on succeeding and/or adjacent crops apply in relation to the information provided for the tank mix (as specified in Regulation (EU) No 546/2011, Annex, Part B, 2.3.2.7). Therefore, mandatory tank mixes should be clearly indicated under method of application (as discussed under point P.3.6).

P.6.6.1 Impact on succeeding crops

Purpose of this point:
Absence of unacceptable effects on succeeding crops should be demonstrated or adequately justified. If adverse effects on succeeding crops are expected, appropriate limitations of use will be put on the label (e.g. by warning the user about growing certain susceptible succeeding crops after use of the PPP).

Assessment principle:
In most cases, for micro-organisms it may be sufficient to make a reasoned case based on the results from the crop safety assessment and possible occurrence of the microbial species in EU environments relevant to agriculture (see EPPO standard PP1/276).

For PPP based on micro-organisms, data to assess the impact on succeeding crops should be submitted in case the micro-organism is a plant pathogen, or in case metabolites of concern for which a hazard to plants were identified, and for which it is demonstrated retention in soil or in plant materials up to sowing or planting time of succeeding crops.

EPPO standard PP1/207[89] on "Effects on succeeding crops" explains how and why the effects on succeeding crops (including replacement crops) should be assessed. It should be noted though that this standard is more appropriate for chemical active substances than for micro-organisms though, as within this standard the decision-support scheme on the extent of testing needed starts with $PEC_{soil\ actual}$ and TER values. While for micro-organisms for instance no PEC values are required for metabolites of concern that are produced *in situ* and are not present in the PPP. Furthermore, for micro-organisms that are plant pathogenic, the host range and the population density of the micro-organism in specific environmental compartments (PED values, see also A.7.1) will be the most relevant characteristics of the micro-organism to assess the impact on succeeding crops. Nonetheless, the general principles that are discussed within EPPO standard PP1/207 on "Effects on succeeding crops"

[89] https://pp1.eppo.int/standards/PP1-207-2

are still applicable for micro-organisms.

P.6.6.2 Impact on other plants, including adjacent crops

Purpose of this point:
Absence of unacceptable effects on adjacent crops should be demonstrated or adequately justified. If adverse effects on adjacent crops are expected, appropriate limitations of use must be put on the label (e.g. by warning the user about the use of the PPP when certain susceptible crops are grown in the vicinity).

Assessment principle:
In most cases, for micro-organisms it may be sufficient to make a reasoned case based on the results from the crop safety assessment and possible occurrence of the microbial species in EU environments relevant to agriculture (see EPPO standard PP1/276). Only when there are indications that other plants than the intended target plants (including adjacent plants) could be negatively affected (e.g. as discussed under P.10.6 on "Effects on non-target terrestrial plants") and that the PPP could affect these plants via drift, further testing may be required. As stated by EPPO standard PP1/276: "*small scale screening tests against a range of appropriate plant species may be sufficient to demonstrate safety of formulated products to adjacent crops*. The general principles are described in the EPPO standard PP1/256[90] on "Effects on adjacent crops", although some points of this standard are more applicable for chemical active substances.

If safety cannot be made plausible for certain adjacent crops, then it should be specified on the label that the plant protection product should not be applied when these specific adjacent crops are present.

P.6.7 Compatibility in plant protection programmes

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 6.7
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.3.9 Reg (EU) No 546/2011, Annex, Part B, 1.3.7
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.1.3.6 Reg (EU) No 546/2011, Annex, Part B, 2.3.2.7

Purpose of this point:
As PPPs based on micro-organisms will be used predominantly within a programme (e.g., an IPM programme), it should be assessed whether the PPP is compatible with other available plant protection methods that are likely included within such a program (taking into consideration the field of use and intended target organism(s), especially when these are required for the conditions of use on the proposed label. This includes that the potential effects (e.g. antagonism, fungicidal effects) of other PPPs (used within a tank mix or in sequence) on the activity of the micro-organism should be evaluated. In addition, potential negative effects of the micro-organism on beneficial organisms (e.g. natural enemies) should be evaluated.

Assessment principle:
PPPs based on micro-organism are predominantly used within a programme (e.g., an IPM programme), in line with Directive 2009/128/EC[91] establishing a framework for Community action to achieve the sustainable use of pesticides. As defined by the European Commission

[90] https://pp1.eppo.int/standards/PP1-256-1

[91] Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides (OJ L 309, 24.11.2009, p. 71).

(see Integrated Pest Management (IPM) (europa.eu)[92]), the IPM strategy means careful consideration of all available plant protection methods as subsequent integration of appropriate measures that discourage the development of populations of harmful organisms. This, to keep the use of PPPs and other forms of intervention to levels that are economically and ecologically justified and to reduce or minimise risks to human health and the environment. 'Integrated pest management' emphasises the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms.

IPM may encompass the following methods (list not exhaustive): use of crop rotation, use of resistant/tolerant cultivars, use of certified disease-free seed or planting material, monitoring of harmful organisms, use of biological control (which includes PPPs based on micro-organisms and other types of biopesticides, but also for instance the release of natural enemies) and lastly (if still required), the use of chemical control.

PPPs based on micro-organisms used in tank mix or spray sequence.
For PPPs based on micro-organisms it may be needed to include on the product label requirements for the use conditions with other PPPs in tank mix, spray sequences or other relevant types of applications to ensure control of the target organisms throughout the growing season.

In case the PPP based on micro-organisms is envisioned to be used with other PPPs in tank mix, spray sequence of other relevant types of application, information should be provided to address the potential effects (e.g. antagonism, fungicidal effects) on the activity of the micro-organism after mixing, spraying in sequence, or employing other relevant types of applications with other PPPs. For instance, applying a fungicide shortly after a PPP based on a micro-organism, which happens to be a fungus, may have potential adverse effects on the activity of the micro-organism. In that case, appropriate label recommendations (e.g. intervals between application of the PPP and other products) may need to be specified to avoid these potential negative effects. As is the case for all label recommendations, these should be supported by appropriate information (e.g. justification).

For PPP based on micro-organisms, known incompatibilities with other PPPs must be reported on the label. A general precautionary statement must be proposed on the label, alerting the user about possible loss of efficacy of the micro-organism due to interaction in tank mix, spray sequence or other relevant types of applications with PPPs other than those indicated on the label.

Potential adverse effects on beneficial organisms
The use of natural enemies to reduce the population of harmful organisms is an important strategy of IPM. These natural enemies can be released on purpose, but also specific measures can be taken to promote the conservation of specific natural enemies already present within the agricultural set-up (for instance by planting specific plants on the border of the field that can be used as refugees of natural enemies e.g. by providing shelter or food). In this light, potential adverse effects on natural enemies should be discussed. This can be done by taking into account the host range of the micro-organism (as discussed under point A.2.3), by referring to the assessment on the effects on bees or non-target arthropods other than bees (as discussed under A.8.3, P10.3 A.8.4, and P10.4), and/or by providing any other relevant information.

Note that among the specific EPPO standards there are 4 specific standards that deal with

[92] https://food.ec.europa.eu/plants/pesticides/sustainable-use-pesticides/integrated-pest-management-ipm_en

side effects of PPP to beneficial organisms (including natural enemies like parasitic wasps and predatory mites). These are EPPO standard PP1/142[93] on the "Side effects on *Encarsia formosa*", EPPO standard PP1/151[94] on the "Side effects on *Phytoseiulus persimilis*", EPPO standard PP1/180[95] on the "Side effects on *Trichogramma cacoeciae*", and EPPO standard PP1/170[96] on the "Side effects on honeybees. However, it should be noted that these standards predominantly describe how to set-up (small scale) trials to assess the effects. Therefore, these standards might be more relevant for PPPs based on chemical active substances. For PPPs based on micro-organisms a similar approach, as taken for micro-organisms in the risk assessment for non-target organisms (see A.8 and P.10), may be more relevant, which follows the risk principle (i.e., risk = hazard x exposure) and allow for dismissal of provision of either exposure-related data or hazard-related data, if the absence of the other can be concluded. Based on the body of knowledge on the micro-organism (e.g MoA, host range) absence of a hazard on beneficial organisms may be concluded.

P.7 EFFECT ON HUMAN HEALTH

Scope

According to the Introduction to Section 7 of Regulation (EU) No 284/2013:

'The information provided shall be sufficient to allow an evaluation of the risks to human health associated with the use of the plant protection products (e.g. operators, workers, bystanders, residents and consumers), the risks for human health handling treated crops, as well as the risk for human health and animals arising from residual traces remaining in food, feed and water. In addition, the information provided shall be sufficient to:

— Permit a decision to be made as to whether, or not, the plant protection product may be authorised,

— specify appropriate conditions or restrictions to be associated with any authorisation,

— specify hazard and precautionary statements for the protection of human health, animal health and the environment to be included on packaging (containers),

— identify relevant first aid measures as well as appropriate diagnostic and therapeutic measures to be followed in the event of infection or another adverse effect in humans.'

Hazard testing

According to the introduction of Section 7 in the revised Regulation (EU) No 284/2013 the infectivity and pathogenicity of the micro-organism have already been assessed in Section 5 of Part B of the Annex to Regulation (EU) No 283/2013. Therefore, the purpose of this Section is the following:

'This Section identifies the relevant additional tests to be carried out to determine the classification and labelling of the plant protection product and the acceptability of the risks related to its use. In some cases, already existing information on toxicity of co-formulants and other non-active ingredients of the plant protection product may be sufficient to conclude on the toxicity of the plant protection product.'

[93] https://pp1.eppo.int/standards/PP1-142-2

[94] https://pp1.eppo.int/standards/PP1-151-2

[95] https://pp1.eppo.int/standards/PP1-180-2

[96] https://pp1.eppo.int/standards/PP1-170-4

Therefore, medical data and an assessment of potential toxicity of the PPP by using the weight of evidence approach must be provided first. The assessment of these data demonstrates whether or not sufficient information is available to classify the plant protection product in accordance with Regulation (EC) No 1272/2008 with regard to toxicity to humans and whether or not acute toxicity studies on animals as described in points 7.3.1 to 7.3.6 of Regulation (EU) No 284/2013 are needed.

Information on toxicity of MoC, co-formulants, safeners, synergists, and relevant impurities must be assessed also using a weight of evidence approach as explained further in the text under point 1.5.1.3 of Regulation (EU) No 546/2011 and data requirement 7.3 of Regulation (EU) No 284/2013. The explicit mentioning of the weight of evidence approach is an important update in the revised data requirements and supports the 3-R principle for replacement, reduction and refinement of animal use.

When testing is required, take into account the scope for replacement, reduction and refinement of animal tests which is strongly promoted in Regulation (EU) No 284/2013 and Regulation (EC) No 1107/2009.

Furthermore, it is important to take into account point 4.1 of the Introduction to the Annex to Regulation (EU) No 284/2013 on test material used in studies:

'Due to the influence that impurities and other components can have on toxicological and ecotoxicological behaviour, a detailed description (specification) of the test material used shall be provided for each study submitted. Studies shall be conducted using the plant protection product to be authorised or bridging principles may be applied, for example, by using a study on a plant protection product with a comparable/equivalent composition. A detailed description of the composition used shall be provided.'

Good laboratory practice (GLP)
All experimental data for the assessment for human and animal health should be GLP-compliant, as laid down in the Introduction to the Annex to Regulation (EU) No 284/2013.

Data conditionality
Please note that not submitting data for a particular data requirement is not acceptable without further justification.

P.7.1 Medical data

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 7.1
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.5.1
Eligible for substantiated dismissal of data provision:	No
Purpose of this point:	
Provide information on possible adverse effect on human health, including sensitisation and allergenic response of humans exposed to the plant protection product.	

Please refer to A.5.1 for more information.

P.7.2 Assessment of potential toxicity of the plant protection product

Corresponding data requirement:	**Reg (EU) No 284/2013, Annex, Part B, 7.2**

Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.5.1
Eligible for substantiated dismissal of data provision:	No

Purpose of this point:

Combine available information on toxicity, such as from published literature, medical information, Integrated Approach to Testing and Assessment (IATA), results of CLP calculation rules or studies conducted with the plant protection product in accordance with Regulation (EC) No 1272/2008, or bridging data from similar plant protection products in a weight of evidence approach which may provide robust and reliable scientific indication on the toxicity of relevant chemical substances contained in the PPP, and be used for classification and labelling.

Information provided under Sections P.2, P.3, P.4 and point P.7.1 may be used in a weight of evidence approach to determine whether potential toxicity of the PPP is to be expected, and be used for classification and labelling.

Assessment principle:
A weight of evidence approach must be applied in order to evaluate whether the possible non-submission of certain studies required in points 7.3.1 to 7.3.6 of Part B of the Annex to Regulation (EU) No 284/2013 is justified. Although, the provisions of the CLP Regulation (Regulation (EC) No 1272/2008) cannot be used for the micro-organisms, the chemical constituents in a plant protection product and the results obtained from testing of the product, containing the micro-organisms, may trigger classification and labelling according to the CLP Regulation and other specific labelling requirements can apply.

P.7.3 Acute toxicity

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 7.3
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.5.1
Eligible for substantiated dismissal of data provision:	Yes (see text)

Purpose of this point:

Provide information on the acute toxicity of the PPP to humans.

Conditionality: Please also refer to the data requirement explained under P.7.2.

Testing: if testing is required, then consider the CC-PPP, Section 7.

Considerations related to testing: In addition to the recommendations specified under the section “hazard testing”, it is highly recommended to consider the following points indicated in “Specific information regarding sensitisation”.

Specific information regarding sensitisation
In compliance with Regulation (EU) No 283/2013 and the uniform principles from Regulation (EU) No 546/2011, all micro-organisms must be regarded as potential sensitisers until validated tests for investigating sensitisation are available[97]. Please note that currently

[97] Regulation (EU) No 546/2011 point 2.5.1.4. states the following: “*All micro-organisms shall be regarded as potential sensitizers in the absence of validated test for investigating sensitisation. Authorisations granted shall therefore specify, as a non-specific risk mitigation measure, that personal protective equipment (e.g. masks) shall be worn, taking into account the conditions of use, and that the exposure via inhalation to the plant protection product containing a micro-organism shall be minimized (…)”.*

(October 2023), work is ongoing on a possible amendment of Regulation (EU) 547/2011 on the labelling requirements for plant protection products; additional provisions and attribution criteria may be provided on precautionary phrases to communicate the sensitisation potential of micro-organisms on the labels for hazard communication.

Unless information can be provided to allow an assessment to be conducted on the skin sensitisation properties of the plant protection product from the available information regarding its chemical components (i.e., co-formulants, metabolites of concern and relevant impurities) as set out in point 7.2, a test for skin sensitisation when available, must be carried out in accordance with the most appropriate guidelines. The test must provide the potential for skin sensitisation of the chemical components. Standard OECD tests with the PPP are sufficient to evaluate the sensitising potential of the combination of co-formulants, safeners, synergists, metabolites of concern and relevant impurities.

P.7.4 Additional toxicity information

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 7.4
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.5.1
Eligible for substantiated dismissal of data provision:	Yes (see text)
Purpose of this point:	
Provide additional information on toxicity of the PPP to humans.	

Conditionality: When the toxicity of the PPP is sufficiently addressed under the data requirement 7.3 no further information is required besides a justification for not submitting data.

Assessment principle:
The evaluation/risk assessment of additional toxicity information on the PPP will be based on expert judgement case-by-case.

Testing: if testing is required, then the particular parameters to be investigated and the objectives to be achieved are considered on expert judgement case-by-case.

P.7.5 Data on exposure

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 7.5
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.5.1
Eligible for substantiated dismissal of data provision:	No
Purpose of this point:	
Provide information or generate data on non-dietary exposure of operator, worker, bystander and residents to the PPP and the components which may be toxicologically relevant (e.g. metabolites of concern, relevant impurities, safeners, synergists, co-formulants), under the proposed conditions of use, including a realistic worst-case exposure scenario.	

Results from exposure monitoring during production and use of the plant protection product must be submitted.

The information and data referred to in this point must provide the basis for the selection of

appropriate protective measures including personal protective equipment to be used by operators and workers and other appropriate risk mitigation measures (e.g. for bystanders and residents) and to be specified on the label. A risk assessment should be provided (qualitative for the micro-organism regarding sensitisation, in case no reference value was derived, and quantitative for compound where a reference value was derived, e.g. metabolites of concern, relevant impurities, safeners, synergists).

Conditionality: In most cases no reference values are set for micro-organisms and therefore no quantitative exposure assessment is required. A qualitative exposure/risk assessment is required for the sensitisation potential of the micro-organism. A quantitative exposure/risk assessment is required for e.g. metabolites of concern or relevant impurities for which a hazard was identified for humans or animals.

Assessment principle:
A risk assessment should be provided for the micro-organism regarding sensitisation as well as for components which may be toxicologically relevant (e.g. MoC, impurities, safeners, synergists). The exposure assessment for the components which may be toxicologically relevant (e.g. MoC, relevant impurities, safeners, synergists) must include a quantitative exposure assessment considering dermal absorption/default data. The risk assessment should be considered as the basis for the selection of appropriate protective measures including personal protective equipment to be used by operators and workers and other appropriate risk mitigation measures (e.g. for bystanders and residents) and to be specified on the label (n.b., information on risk mitigation measures, if applicable, must be provided under point B.3.3).

Risk assessment to the micro-organism:
In the absence of appropriate test methods all micro-organisms are currently assumed to have the potential to cause sensitisation reactions in humans. Therefore, the use of adequate personal protective equipment for operators has to be considered where appropriate. Operator exposure may occur during mixing/loading and application. In case of a powder preparation (but not for liquid preparations or granule preparation which are nearly dust-free) respiratory protective equipment is required for the operator during mixing and loading of the PPP. No substantial inhalation exposure to the micro-organism is to be expected in certain cases, but using respiratory protection equipment as a non-specific risk mitigation measure is still recommendable, especially for greenhouse uses where substantial inhalation exposure could be expected during spray application.

Risk assessment to metabolites of concern:
According to the Regulation (EU) No 546/2011:

'Metabolism is inherent of all living organisms. If secondary metabolites that are known to be hazardous to humans or other non-target organisms have been identified during the assessment of the micro-organism, the evaluation of a plant protection product containing this micro-organism shall include an assessment of the risk due to exposures to such metabolites expected from the intended use.'

The exposure assessment for MoC for which a hazard has been identified for human or animal health should consider both the presence of the secondary metabolite in the product and in situ production (see points A.6.1 and A.7.2). Exposure resulting from the presence of the MoC in the PPP can be assessed in the same way as for chemical PPP. The amount of the MoC in the PPP can be used as input parameter in a model (please see CC-MPCA), where possible (e.g., where reference values are established for that MoC). This would address the risk to the operator, bystander, resident, and worker. Since generally no specific dermal absorption values will be available, default values should be used. Exposure resulting from *in situ*

production of the metabolite of concern can be assessed by an alternative approach as explained in the metabolite guidance in step 14: '…*the concentration of metabolite formed under production promoting conditions which can be used as a maximum for metabolite production. Examples of such conditions favourable for metabolite production may be laboratory conditions which can be justified to be conditions which maximize the formation of the metabolite (e.g., nutrient-rich medium), or during interaction of the micro-organisms with the target or host organism. Information on population dynamics of the micro-organism in the relevant environmental compartment can then be used to infer information on the maximal metabolite production in the relevant environmental compartment upon application and to which the relevant non-target organisms (i.e., the organism for which the hazard is identified) might be exposed.'*

For further guidance, please refer to Annex II of the 'Guidance on the risk assessment of secondary metabolites produced by micro-organisms used as plant protection active substances', SANCO/2020/12258.

P.7.6 Available toxicological data relating to non-active substances

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 7.6
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.5.1
Eligible for substantiated dismissal of data provision:	No
Purpose of this point:	
To provide information on each co-formulant, safener and synergist present in the PPP.	

All available information of each co-formulant, safener and synergist present in the PPP will be evaluated and be used for classification and labelling. The criteria used for classification and labelling of a mixture are described in the Annex to Regulation (EC) No 1272/2008. Please note that a List of co-formulants which are not accepted for inclusion in plant protection products is kept updated in the Annex III to Reg (EC) No 1107/2009.

P.7.7 Supplementary studies for combinations of plant protection products

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 7.7
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.5.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.5.1
Eligible for substantiated dismissal of data provision:	Yes (see text)
Purpose of this point:	
Provide information on the synergistic or additive toxicological effects of the combination of plant protection products.	

Conditionality: In certain cases it may be necessary to carry out additional studies for combination of PPP where the product label includes requirements for use of the PPP with other PPP and/or with adjuvants as a tank mix. Currently, PPP are hardly used in combination with other PPP and/or with adjuvants, therefore a statement to indicate that the PPP will not be used in combination with other PPP and/or adjuvants will be sufficient to address this data requirement.

Assessments principle:
The evaluation/risk assessment of combination of PPP and/or adjuvants will be based on

expert judgement case-by-case.

Testing: if testing is required, then the specific parameters to be investigated and the objectives to be achieved are considered on expert judgement case-by-case.

P.8 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED

Most of the data and information on residues is generated at active substance level under the data requirements of Section 6 of Part B set in Annex to Regulation (EU) No 283/2013. The applicant is requested to provide justification that the data and information provided in the active substance assessment report is already sufficient for a risk assessment for the PPP. If not, the route of assessment as provided by the data requirements Regulation (EU) No 283/2013 can be followed to provide a new risk-envelope assessment (Section A.6 of these EN).

P.9 FATE AND BEHAVIOUR IN THE ENVIRONMENT

Most of the fate and behaviour assessment is performed on active substance assessment level under the data requirements set in the Annex to the Regulation (EU) No 283/2013. The applicant is requested to provide justification that the data and information provided in the active substance assessment report is already sufficient for a risk assessment for the PPP. If not, the route of assessment as provided by Section 7 of the data requirements Regulation (EU) No 283/2013 can be followed to provide a new risk-envelope assessment (the A-part of these EN).

P.10 EFFECT ON NON-TARGET ORGANISMS

Scope
According to the Introduction (vii) to Section 10 of Regulation (EU) 284/2013:

> *" The information provided for the plant protection product, together with other relevant information, and that provided for the micro-organism (including possible metabolites of concern as identified in point 2.8 of Part B of the Annex to Regulation (EU) No 283/2013) shall be sufficient to:*
>
> *— specify the hazard symbols, the indications of danger and relevant risk and safety phrases or the pictograms, signal words, relevant hazard and precautionary statements for the protection of the environment to be mentioned on packaging (containers),*
>
> *— permit an evaluation of the short- and long-term risks for non-target species – populations, communities, and processes as appropriate,*
>
> *— permit an evaluation whether special precautions are necessary for the protection of non-target species"*

Data conditionality
For all the non-target organism' groups, points 10.1 to 10.5 of Regulation (EU) 284/2013 mention that "*The same information submitted on the micro-organism (and/or on a plant protection product containing that active substance with respect to a representative use), as detailed in points* [ed.8.1-8.6], *8.7 and 8.8 of Part B of the Annex to Regulation (EU) No 283/2013 shall be provided for the plant protection product subject of the application, unless the applicant can:*

— justify the applicability and relevance of the outcome of the assessment made on the same data submitted for the micro-organism approval (and/or for a plant protection product containing that active substance with respect to a representative use),

— predict the effects of the plant protection product on the basis of the data available for the co-formulants (e.g. qualitative and quantitative composition), as well as for the micro-organism and possible metabolites of concern (based on data submitted in accordance with Section 8 of Part B of the Annex to Regulation (EU) No 283/2013 for the approval of the micro-organism(s) in the plant protection product), or"

Data provision and risk assessment concerning infectivity, pathogenicity and toxicity of MoC are supposed to be already covered by Section 8 of Annex B to Regulation (EU) No 283/2013, at least as concerns the exposure scenario(s) related to the representative use(s) described in the dossiers submitted for an approval of an active substance. At PPP-authorisation level, new information on infectivity, pathogenicity and toxicity of MoC may be needed only if different intended uses imply different (and relevant) exposure routes, or if different co-formulants are included in the preparation compared to representative PPP assessed at the time of approval of the active substance.

As mentioned as well under Section A.8, it is not considered necessary to conduct studies with the non-target organisms if the applicant can justify based on the data submitted under the Section environmental occurrence of the micro-organisms that no exposure on non-target organisms to PPP will occur. However, as a generic risk mitigation measure approach, appropriate labelling (e.g., EUH401, P501, P391) must be applied as provided for by Regulation (EC) 1272/2008 and Regulation (EU) 547/2011.

Hazard testing

Therefore, in addition to the points mentioned in Section A.8, the following aspects are important when considering the effects of the PPP on the non-target organisms:

- If testing is needed, it may be acceptable to conduct tests directly using the PPP to identify possible adverse effects caused by infectivity and pathogenicity of the micro-organism, and toxicity of chemical components of the PPP. Absence of effects observed may already exclude concerns for a certain non-target organism, while further investigation (e.g. by testing the micro-organism separately from the chemical preparation) may be required if effects are observed.
- Based on these requirements, it is considered necessary that a sound justification should be provided in the case the applicant wishes to dismiss the performance of studies with the preparation. Data on preparation are not required if the preparation consists solely of the micro-organism, for example rice grains coated with the micro-organism, or the MPCA-AM formulated without any co-formulants, safeners and synergists.
    - When co-formulants are present, it is not acceptable dismissing the performance of preparation testing based upon the fact that the co-formulants are inert and thus non-hazardous. For example, in the 10 days oral chronic bees study, certain additives can increase the mortality and even reduce the feed consumption of bees. In other cases, co-formulants may have physical mechanisms of action relevant for some non-target organism groups. The focus NTOs for physical mechanisms of action are bees, foliar and soil arthropods, and soil meso- and macro-fauna[98,99,100]. However, aquatic

[98] Straw E. A. *et al.* (2022) "Inert" ingredients are understudied, potentially dangerous to bees and deserve more research attention, Proc. R. Soc. B 289: 20212353.

[99] Karise R. and Mänd M. (2015) Recent insights into sublethal effects of pesticides on insect respiratory physiology, Insect Physiology, 2015:5, 31-39.

organisms may also suffer from physical MoAs, and should be addressed on a case-by-case basis, depending upon the ingredient and the physical/chemical properties thereof. In all cases vertebrate testing is to be stringently avoided. Further justification might include a reference to the EFSA conclusion comparing the current application rates/predicted exposure levels to the application rates/predicted exposure levels in the EFSA conclusion, if these co-formulants are registered as PPP. For oily co-formulants (or actives), a physical mode of action via suffocation is generally considered to be possible. For example, for oil dispersion (OD) preparations, physical effects on NTAs and bees are expected, please refer to the FAO/WHO[101] for definitions of different type of preparations. It is noted that oily active substances generally have a physical mode of action, i.e., insects are killed because an oil film is formed on their body, which prevents them from breathing. The available NTA studies usually are performed with exposure to dried residues. The tested exposure scenarios therefore reflect introduction of species after the product has dried, which is relevant for organisms hiding under leaves or entering from off-field areas. The studies do not cover the direct effect of the application, i.e., when arthropods are oversprayed or come in contact with the wet oil spray, which based on the mode of action are considered the routes of exposure with the highest risk. The standard studies in fact can be considered as 'aged residue' studies (i.e., with an ageing time of 1-2 hours). For the in-field risk assessment, this is acceptable, however for the off-field risk assessment aged residue studies are not acceptable. Therefore, for oily active substances the relevance of the submitted studies may be a point of discussion in the risk assessment for non-target arthropods. The consequence for the risk assessment will be a case-by-case decision, ranging from an uncertainty analysis to the request for new studies (e.g., lab studies with overspray, or field studies). It should be noted that the same line of reasoning may apply to active substances with a mode of action aimed at suffocation of the target organisms, and to PPP with a high percentage of oily components.

- If one or more of the co-formulants is classified, and this/these co-formulant(s) is/are present at relevant levels in the preparation according to the CLP Regulation, this/these co-formulant(s) will contribute to the classification of the PPP.

According to point 4.1 of the Introduction to the Annex to Regulation (EC) 284/2013, in the case that a study conducted with another preparation than the one to be assessed is submitted, a bridging statement must be provided in order to assess if the preparations are comparable. For this, the relevant guidance document on the assessment of the equivalence of technical materials listed in Section 1 of the CC-PPP can be used.

If adverse effects are observed in the studies used for the risk assessment and additionally the risk is unacceptable, additional information is needed on the exposure or the hazard characterisation' (e.g., field trials).

Risk assessment

In regard to the evaluation of impact of the micro-organism and PPP on non-target organisms, Regulation (EU) 546/2011 specifies which information should be considered when evaluating the risk to non-target organisms:

"— micro-organisms are living organisms capable of replication that may be naturally

[100] Karise R, Muljar R, Smagghe G, et al. Sublethal effects of kaolin and the biopesticides Prestop-Mix and BotaniGard on metabolic rate, water loss and longevity in bumble bees (Bombus terrestris). J Pest Sci. Epub 2015 Feb 2. https://doi.org/10.1007/s10340-015-0649-z

[101] https://www.fao.org/publications/card/en/c/CB8401EN

present in high numbers in the environment, and the specific micro-organism under assessment may already be occurring in relevant European environments at a relevant taxonomic level,

— the biological properties and the mode of action of a micro-organism are the first and crucial step in the evaluation process, because they define which are the relevant aspects and elements on which the evaluation should focus, and also which aspects are not relevant for a robust informed decision making,

— extensive information on the micro-organism under assessment (at the relevant taxonomic level) may be available in the public domain (e.g. history of use, peer-reviewed scientific literature). Best use of this information shall be made. Where applicable, regulatory experimental studies may be needed to determine the specific properties of the micro-organism under evaluation.

Metabolism is inherent of all living organisms. If secondary metabolites that are known to be hazardous to humans or other non-target organisms have been identified during the assessment of the micro-organism, the evaluation of a plant protection product containing this micro-organism shall include an assessment of the risk due to exposures to such metabolites expected from the intended use".

In short, the risk assessment may take into consideration the following information:

- MoA and other biological properties,
- Survival and dispersal of the active micro-organism in the environment,
- Its ecological niche,
- The natural background level of the active micro-organism, where it is indigenous,
- other authorised uses of the PPP in the area of envisaged use containing the same active substance or which give rise to the same residues,
- Studies on toxicity, pathogenicity and infectivity,
- type of application.

As of the date of publication of these EN, no Guidance Document for the environmental risk assessment of micro-organisms has been established in EU-context. During expert meetings on general issues on the risk assessment for micro-organisms in 2007 and 2009 (the 'List 4 meeting' and PRAPeR M2 resp.) it was agreed that initial off-crop exposure densities in soil and water could be determined using the worst-case approach (see fate Section for further considerations).

For any given environmental compartment, the risk characterisation should, when possible, contain a comparison of the predicted exposure with the available effect values from effect studies with the micro-organism, to differenciate between possible pathogenic effects of the micro-organism from possible toxic effects of the PPP chemical components. However, when such a comparison is made no assessment factors are available to decide whether the risk is acceptable or not. The assessment factors used for chemical substances are not validated for micro-organisms, and are only used for relevant secondary metabolites/toxins, according to the decision criteria in Regulation (EU) 546/2011.
Therefore, in most cases the risk assessment for the micro-organism will consist of a qualitative or semi-quantitative evaluation of the likelihood that an adverse effect will occur under the expected conditions of exposure. For these reasons, it is recommended to use the approach of PRAPeR M2 and derive a margin of safety (i.e., MoS) by calculating a quotient of the endpoint divided by the estimated exposure. Endpoints, for instance, may be expressed in CFU/L of the feeding solution to test bees, or CFU/kg soil to test soil organisms. Based on this evaluation it can be decided whether the risk is acceptable or not using a weight-of-evidence

considering the mode of action, information on the ecology of the micro-organism in question and the assumptions used for the calculating the exposure.

For further guidance please refer to the relevant guidance documents and test methods listed under the CC-PPP.

P.10.1 Effects on terrestrial vertebrates

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 10.1
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.7.1
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.7.1
Eligible for substantiated dismissal of data provision:	Yes (see introduction to Section P.10 on data waiving)
Purpose of this point:	
Provide information on the infectivity and pathogenicity of the PPP to birds, mammals, amphibians and reptiles.	

Conditionality: See introduction to Section P.10 and Section A.8.

Testing: if testing is required, then consider Section 10 of the CC-MPCP (n.b., which refers to the relevant points under Section 8 of the CC-MPCA and Section 10 of the Communication relevant to the implementation of Part A of the Annex to Commission Regulation (EU) No 284/2013).

According to point 10.1 of Part B of the Annex to Regulation (EU) 284/2013, "*If generation of data is required based on the provisions laid down under this point, relevant studies shall be performed and they shall provide LD50 values and include gross pathological findings. The studies may be conducted on the species used in the studies referred to in point 8.1 of Part B of the Annex to Regulation (EU) No 283/2013".*

Considerations related to testing: See the section on "Consideration related to testing" under point A.8.1.

Risk assessment/Risk evaluation:

a) Risk due to the micro-organism and its potential to infect and multiply in the host: The use of the chemical "Guidance for the risk assessment for birds and mammals" (EFSA 2009) is considered less relevant for assessing solely the effects of the micro-organism, since exposure parameters in this Guidance (e.g. DT50, RUD) are based on chemical databases. It should instead be considered the recommendation provided in the scope section included in the introduction to P.10, and in point 1.7.1 (a) of Regulation (EU) No 546/2011, Annex, Part B.
b) Risk due to toxic effects of PPP, refer to Regulation (EU) No 546/2011, Annex, Part B, 1.7.1 (b).

Decision-making:

Based on the information provided by the applicant, the Member State should conclude on whether there might be unacceptable effects on terrestrial vertebrates following the intended use of PPP. According to the Regulation (EU) No 546/2011, Annex, Part B, 2.7.1, no authorisation must be granted:

(a) if the micro-organism is pathogenic to terrestrial vertebrates,

(b) in case of toxic effects of the plant protection product, if the acute and short-term toxicity/exposure ratio for terrestrial vertebrates is less than 10 on the basis of LD50 (acute dietary risk assessment) or the long-term toxicity/exposure ratio is less than 5, unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact occurs, directly or indirectly, after use of the plant protection product in accordance with the proposed conditions of use.

P.10.2 Effects on aquatic organisms

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 10.2
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.7.2
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.7.2
Eligible for substantiated dismissal of data provision:	Yes (see introduction to Section P.10 on dismissal of data provision)
Purpose of this point:	
Provide information on the infectivity and pathogenicity of the PPP to fish, aquatic invertebrates, algae and aquatic macrophytes.	

Conditional/Waiving: See introduction to Section P.10 and Section A.8.

Testing: if testing is required, then consider Section 10 of the CC-MPCP (n.b., which refers to the relevant points under Section 8 of the CC-MPCA and Section 10 of the Communication relevant to the implementation of Part A of the Annex to Commission Regulation (EU) No 284/2013).

According to point 10.2.1 of Part B of the Annex to Regulation (EU) 284/2013, in case of investigating the effects on fish,
"*If generation of data is required based on the provisions laid down under this point, relevant studies shall be performed and they shall provide LD50 values, and shall include gross pathological findings. The studies may be conducted on the species used in the studies referred to in point 8.2.1 of Part B of the Annex to Regulation (EU) No 283/2013*".
For aquatic invertebrates, algae and aquatic plants, in case the data requirement cannot be dismissed, generation of data is required.

Considerations related to testing: See the section on "Consideration related to testing" under point A.8.2.

Risk assessment/Risk evaluation:

a) Risk due to the micro-organism and its potential to infect and multiply in the host: the risk assessment scheme described in the "Guidance on tiered risk assessment for PPPs for aquatic organisms in edge-of-field surface waters" (EFSA Journal 2013; 11(7):3290) is considered less relevant for assessing solely the effects of the micro-organism. It should instead be considered the recommendation provided in the scope section included in the introduction to P.10, and in point 1.7.2 (a) of Regulation (EU) No 546/2011, Annex, Part B,.
b) Risk due to toxic effects of PPP, refer to Regulation (EU) No 546/2011, Annex, Part B, 1.7.2 (b).

Decision-making:

Based on the information provided by the applicant, the Member State should conclude on whether there might be unacceptable effects on aquatic organisms following the intended use of PPP. According to the Regulation (EU) No 546/2011, Annex, Part B, 2.7.2, no authorisation must be granted:

(a) if the micro-organism is pathogenic to aquatic organisms, unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact on aquatic organism populations would occur after use of the plant protection product in accordance with the proposed conditions of use; or

(b) in case of toxic effects of the plant protection product if the:
— toxicity/exposure ratio for fish and Daphnia is less than 100 for acute exposure and less than 10 for long- term exposure, or

— algal growth inhibition/exposure ratio is less than 10,

unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact on exposed species occurs, directly or indirectly, after use of the plant protection product in accordance with the proposed conditions of use.

P.10.3 Effects on bees

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 10.3
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.7.3
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.7.3
Eligible for substantiated dismissal of data provision:	Yes (see introduction to Section P.10 on data dismissal of data provision)
Purpose of this point:	
Provide information on the infectivity and pathogenicity of the PPP to bees.	

Conditionality: See introduction to Section P.10 and Section A.8.

Testing: if testing is required, then consider Section 10 of the CC-MPCP (n.b., which refers to the relevant points under Section 8 of the CC-MPCA and Section 10 of the Communication relevant to the implementation of Part A of the Annex to Commission Regulation (EU) No 284/2013).

According to point10.3 of Part B of the Annex to Regulation (EU) 284/2013, in case the data requirement cannot be dismissed, generation of data is required.

Considerations related to testing: See the section on “Consideration related to testing” under point A.8.3.

Risk assessment/Risk evaluation:

a) Risk due to the micro-organism and its potential to infect and multiply in the host: the risk assessment schemes described for chemical active substances are considered less relevant for assessing solely the effects of the micro-organism. The recommendation provided in the scope section included in the introduction to P.10, and in point 1.7.3 (a)of Regulation (EU) No 546/2011, Annex, Part B should be considered instead.

b) Risk due to toxic effects of PPP, refer to Regulation (EU) No 546/2011, Annex, Part B, 1.7.3 (b).

Decision-making:

Based on the information provided by the applicant, the Member State should conclude on whether there might be unacceptable effects on bees following the intended use of PPP. According to the Regulation (EU) No 546/2011, Annex, Part B, 2.7.3, no authorisation must be granted:

a) if the micro-organism is pathogenic to bees under the proposed conditions of use, unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact is expected to occur to the populations of bees after use of the plant protection product in accordance with the proposed conditions of use; or

b) in case of toxic effects of the plant protection product, as defined in the decision-making principles of point 2.5.2.3 of Part A.

P.10.4 Effects on non-target arthropods other than bees

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 10.4
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.7.4
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.7.4
Eligible for substantiated dismissal of data provision:	Yes (see introduction to Section P.10 on dismissal of data provision)
Purpose of this point:	
Provide information on the infectivity and pathogenicity of the PPP to non-target arthropods other than bees.	

Conditionality: See introduction to Section P.10 and Section A.8.

Testing: if testing is required, then consider Section 10 of the CC-MPCP (n.b., which refers to the relevant points under Section 8 of the CC-MPCA and Section 10 of the Communication relevant to the implementation of Part A of the Annex to Commission Regulation (EU) No 284/2013).

According to point 10.3 of Part B of the Annex to Regulation (EU) 284/2013, in case the data requirement cannot be dismissed, generation of data is required "*Analyses might include further studies on additional species, or higher tier studies such as studies on selected non-target organisms using the formulated plant protection product. The choice of non-target arthropods test species playing an important role in integrated pest management may be based on several factors, such as biological properties of the micro-organism and the intended use (e.g. crop type).*"

Considerations related to testing: See the section on "Consideration related to testing" under point A.8.3.

Risk assessment/Risk evaluation:

a) Risk due to the micro-organism and its potential to infect and multiply in the host: the risk assessment schemes described in the "Guidance Document on Terrestrial Ecotoxicology" (Sanco/10329/2002), which follows the recommendations of the

ESCORT 2 workshop are considered less relevant for assessing solely the effects of the micro-organism. It should instead be considered the recommendation provided in the scope section included in the introduction to P.10, and in point 1.7.4 (a) of Regulation (EU) No 546/2011, Annex, Part B.

b) Risk due to toxic effects of PPP, refer to Regulation (EU) No 546/2011, Annex, Part B, 1.7.4 (b).

Decision-making:

Based on the information provided by the applicant, the Member State should conclude on whether there might be unacceptable effects on non-target arthropods other than bees following the intended use of PPP. According to the (EU) No 546/2011, Annex, Part B, 2.7.4, no authorisation must be granted:

a) if the micro-organism is pathogenic to arthropods other than bees, unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact is expected to occur to the populations of arthropods other than bees after use of the plant protection product in accordance with the proposed conditions of use; or

b) in case of toxic effects of the plant protection product, as defined in the decision-making principles of point 2.5.2.4 of Part A, unless it is clearly established through an appropriate risk assessment that under field conditions there is no unacceptable impact on arthropods other than bees after use of the plant protection product in accordance with the proposed conditions of use. Any claims for selectivity and proposals for use in integrated pest management systems shall be substantiated by appropriate data.

P.10.5 Effects on non-target meso- and macro-organisms in soil

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 10.5
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.7.5
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.7.5
Eligible for substantiated dismissal of data provision:	Yes (see introduction to Section P.10 on dismissal of data provision)
Purpose of this point:	
Provide information on the infectivity and pathogenicity of the PPP to non-target meso- and macro-organisms in soil	

Conditionality: See introduction to Section P.10 and Section A.8.

Testing: if testing is required, then consider Section 10 of the CC-MPCP (n.b., which refers to the relevant points under Section 8 of the CC-MPCA and Section 10 of the Communication relevant to the implementation of Part A of the Annex to Commission Regulation (EU) No 284/2013).

Considerations related to testing: See the section on “Consideration related to testing” under point A.8.5.

Risk assessment/Risk evaluation:

a) Risk due to the micro-organism and its potential to infect and multiply in the host: the risk assessment schemes described in the “Guidance Document on Terrestrial Ecotoxicology” (Sanco/10329/2002) are considered less relevant for assessing solely

the effects of the micro-organism. It should instead be considered the recommendation provided in the scope section included in the introduction to P.10, and in point 1.7.5 (a) of Regulation (EU) No 546/2011, Annex, Part B.

b) Risk due to toxic effects of PPP, refer to Regulation (EU) No 546/2011, Annex, Part B, 1.7.5 (b).

Decision-making:

Based on the information provided by the applicant, the Member State should conclude on whether there might be unacceptable effects non-target meso- and macro-organisms in soil following the intended use of PPP. According to the Regulation (EU) No 546/2011, Annex, Part B, 2.7.5, no authorisation must be granted:

a) if the micro-organism is pathogenic to meso- and macro-organisms in soil, unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact on soil meso- and macro-organism populations occurs after use of the plant protection product in accordance with the proposed conditions of use; or

(bin the case of toxic effects of the plant protection product, if the acute toxicity/exposure ratio for meso- and macro-organisms in soil is less than 10 or the long-term toxicity/exposure ratio is less than 5, unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact on soil meso- and macro-organism populations occur after use of the plant protection product in accordance with the proposed conditions of use.

P.10.6 Effects on non-target terrestrial plants

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 10.6
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.7.6
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.7.6
Eligible for substantiated dismissal of data provision:	Yes (see introduction to Section P.10 on dismissal of data provision)
Purpose of this point:	
Provide information on the infectivity and pathogenicity of the PPP to non-target terrestrial plants.	

Conditionality: See introduction to Section P.10 and Section A.8.

Testing: if testing is required, then consider Section 10 of the CC-MPCP (n.b., which refers to the relevant points under Section 8 of the CC-MPCA and Section 10 of the Communication relevant to the implementation of Part A of the Annex to Commission Regulation (EU) No 284/2013).

Considerations related to testing: See the section on “Consideration related to testing” under point A.8.6.

Risk assessment/Risk evaluation:

a) Risk due to the micro-organism and its potential to infect and multiply in the host: the risk assessment schemes described in the “Guidance Document on Terrestrial Ecotoxicology” (Sanco/10329/2002) are considered less relevant for assessing solely the effects of the micro-organism. It should instead be considered instead the

recommendation provided in the scope section included in the introduction to P.10, and in point 1.7.6 (a) of Regulation (EU) No 546/2011, Annex, Part B.

b) Risk due to toxic effects of PPP, refer to Regulation (EU) No 546/2011, Annex, Part B, 1.7.6 (b).

Decision-making:

Based on the information provided by the applicant, the Member State should conclude on whether there might be unacceptable effects on non-target terrestrial plants following the intended use of PPP. According to the Regulation (EU) No 546/2011, Annex, Part B, 2.7.6, no authorisation must be granted:

"If the micro-organism has an herbicidal mode of action or it is closely related to a known plant pathogen, and there is a possibility of terrestrial plants being exposed to the micro-organism according to the consideration done under point 1.6, no authorisation shall be granted if the micro-organism is pathogenic to, or the plant protection product has toxic effects on, terrestrial plants. This criterion applies unless it is clearly established through an appropriate risk assessment that, under field conditions, no unacceptable impact on non-target terrestrial plant populations occurs after use of the plant protection product in accordance with the proposed conditions of use".

P.10.7 Additional toxicity studies

Corresponding data requirement:	Reg (EU) No 284/2013, Annex, Part B, 10.7
Relevant evaluation criterion:	Reg (EU) No 546/2011, Annex, Part B, 1.7.1-1.7.6
Relevant decision making criterion:	Reg (EU) No 546/2011, Annex, Part B, 2.7.1-2.7.6
Eligible for substantiated dismissal of data provision:	Not applicable

Purpose of this point:

According to Regulation (EU) 284/2013, Annex, Part B, 10.7 "*Further data may be submitted or additional toxicity studies performed, if tests required in points 10.1 to 10.6 have shown adverse effects in one or more non-target organisms and the risk is considered not acceptable. The type of study to be performed must be chosen based on the effects and the affected non-target organism(s) observed in the studies required in points 10.1 to 10.6 and during efficacy testing, and may have to include also further studies on additional non-target species*".

APPENDIX I: OVERVIEW TABLE IN SUPPORT OF THE METABOLITE ASSESSMENT ACCORDING TO SANCO/2020/12258

	Stage 1		Stage 2					Stage 3				Stage 4
Metabolite identifier[1)]	**Active substance (Y/N)**	**Claimed active metabolite (Y/N/?)**	**Verification of MoPC-status**				**Outcome chemical analysis[4)]**	**Relevant exposed group[5)]**	**MoC (Y/N)**	**Ref. values (TOX) and endpoints (ECOTOX)**	**Exposure level**	**Unacceptable risk (Y/N)**
			Toxic / antimicrobial effect observed, test species, and strain[2)]	**Potential relevance for micro-organism [3)]**	**WGS-evidenced (Y/N)**	**MoPC (Y/N/?)**						
Name, CAS, and/or IUPAC	Y/N	Y/N/?	Study 1: Effect / test species / strain Study 2, etc…	Metabolite / Effect	Y/N	Y/N/?	MPCA-AM: Y/N or max. PPP: Y/N or max. Induced: Y/N	TOX; TOX.. / ECOTOX; ECOTOX..	Y/N	TOX; TOX.. / ECOTOX; ECOTOX..	TOX; TOX.. / ECOTOX; ECOTOX..	TOX; TOX.. / ECOTOX; ECOTOX..
The row below presents the SANCO/2020/12258 step-numbers associated with the respective table column												
1, 3, 7, 10	**1**	**1**	**3, 4, 6, 10, 12, 18**	**4, 6, 8, 10, 12**	**9, 10, 12**	**11**	**7, 12**	**13, 14**	**15**	**14, 17, 19**	**14, 16**	**20**

[1)] Typically the name that is unambiguously used throughout the dossier to refer to the metabolite.
[2)] For each relevant study (author and year are entered on the 'Study x'-position) the nature of the observed toxic / antimicrobial effect (? = data unavailable; null = no effect observed; ACU = acute toxicity; CYT = cytotoxicity; MUT = mutagenicity; GEN = genotoxicity; CAR = carcinogenicity; REP = reprotoxicity; NEU = neurotoxicity; AM = antimicrobial activity), the test species (or at least a detailed description of the exposed organism / material), and the name of the strain for which the effect has been observed (could be the micro-organism itself, a closely related strain, or both) is stated.
[3)] In this column, the potential relevance of an identified metabolite and observed effect is made explicit for the micro-organism in particular. If the potential relevance is confirmed for the metabolite or the effect, 'Y' is entered on the respective position in the cell. In case non-relevance is established, an 'N' is added.
[4)] This column states whether or not a metabolite has been detected in the MPCA-AM or PPP. Whenever relevant for the assessment, the 5-BA-established max. content (max.; average + 3xSD) for a metabolite is entered for the MPCA-AM (if available) and the PPP (either measured or derived).
[5)] The following codes may be used to refer to any relevant exposed group. For TOX: OP (operators), WO (workers), BY (bystanders), RE (residents), and CO (consumers). For ECOTOX: MAM (mammals), BRD (birds), REP (reptiles), AMP (amphibians), FSH (fish), AQI (aquatic invertebrates), ALG (algae), AQM (aquatic macrophytes), BEE (bees), ART (non-target arthropods other than bees), MMO (non-target meso- and macro-organisms in soil), and PLA (non-target terrestrial plants). When proposed use does not lead to exposure of any of these groups, add '-'.