Evaluation Manual for the Authorisation of Biopesticides according to Regulation (EC) No 1107/2009

Micro-organisms, Botanicals, and Semiochemicals

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ctgb

Board for the Authorisation of Plant Protection Products and Biocides

Biopesticides Evaluation Manual
Micro-organisms, Botanicals, and Semiochemicals

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Document history

Biopesticides Evaluation Manual Micro-organisms, Botanicals, and Semiochemicals			
Version	**Date**	**Amended paragraph(s)**	**General description of changes**
1.0	July 2017		**All aspects:** Initial Biopesticides EM
1.1	June 2018	1.4.1 (2.5.1)*, 1.11 (2.12), 2.7 (3.7), 3.6 (4.6)	**Efficacy:** Implementation of new EPPO standard "*principles of efficacy evaluation for low-risk plant protection products"* PP1/(296). (All efficacy-related paragraphs updated).
1.2	July 2021	2.12, 3.7, 4.6	**Efficacy:** - Implementation of new EPPO standard "*General principles for efficacy evaluation of plant protection products with a mode of action as plant defence inducers"* PP1/(319). - An update for the efficacy section on semiochemicals was also made as several EPPO standards for this section were released or updated. (PP1/264(2), PP1/314(1), PP1/323(1)).
		1, 2.1, 2.2, 2.3, 2.6, 2.7, 3.2	**Chemistry:** - Rework of the General Introduction to align with Green Deal principles and to expand EM functionality. - Full refurbishment of the Chemistry content for micro-organisms to reflect current evaluation systematic. - Preliminary addition to Botanicals-section: an alternative to the pragmatic approach regarding characterization of Group 3 botanicals (see SANCO/11470/2012 – rev.8, section 7) is presented. Use of a broader spectrum of scientifically supported methods for UVCB-characterization is now actively encouraged by Ctgb.
		2.4.6	**Human toxicology:** Adding information on new Guidance Document to Assess Antimicrobial Resistance of Micro-organisms used as Plant Protection Product.
1.3	November 2021	2.4.5, 2.8.1, 2.8.3, 2.9.2, 2.10, 2.10.1, 2.11, 2.12	**All aspects:** Addition of explanatory notes and textual adjustments to align the EM with 'Metabolite GD' SANCO/2020/12258.
		2.1, 2.2, 2.3.2, 2.6.2, 2.7.1, 2.7.2	**Chemistry:** Minor fixes and amendments to content added during the V.1.2-update.
		2.8.1, 2.8.3	**Human toxicology:** Adding information on the implication of precautionary warning phrases on the low-risk status of the active substance and product.
1.4	July 2022	2.11.1, 2.11.2	**Ecotox:** Update on formulation data requirements; addition on test guidelines

* Due to version 1.2 insertions, the paragraph numbering has been altered. For ease of reference, the current numbers of the passages altered during the 1.1 update, are provided between brackets.

1. General introduction

1.1 Regulatory framework and outline of the Biopesticides Evaluation Manual

In this Manual we consider *biopesticides*, i.e., plant protection products (PPPs) that contain micro-organisms (including viruses), botanicals, or semiochemicals as active substance. Due to their inherent differences with conventional chemical active substances, these groups of substances have customized data requirements and guidances, which logically justifies a separate Evaluation Manual.

It is important to note that, in reference to biopesticides, Regulation (EC) No 1107/2009 only distinguishes between microbial and chemical active substances, and low-risk and non-low-risk substances and products; the text makes no mention of the term biopesticides.
Although the domain of biopesticides is a great repository of low-risk products, the two concepts are not synonymous. A biopesticide is not necessarily considered as 'low-risk' according to Art. 47 of the Regulation, and not all products based on chemical active substances are automatically ineligible for a low-risk status.

This Biopesticides Evaluation Manual (Biopesticides EM) particularly describes the Dutch evaluation of biopesticides in the EU framework under (EC) No 1107/2009. Naturally, the EU Data Requirements, Uniform Principles, and relevant guidances provide the abstract foundation to this EM. The actual outline of the evaluation is however mostly shaped by the interpretation of these strata of rules and guidelines.
Ideally, interpretation is a constantly evolving product of growing experience, critical reflection and, to an increasing extent, necessity (see 1.2 below); it adds a less solidified layer to the core framework and thus allows budding innovation to take root, and amendments to be easier to integrate. The purpose of the EM is to keep track of the ongoing developmental process thus avoiding something necessarily progressive to become unnecessarily elusive.

The perspectives described in this EM can be used for both the approval procedure for microbial, botanical, and semiochemical active substances, and for zonal and interzonal applications for the authorisation of biopesticidal products (i.e., Core registration reports).

The layout of the Biopesticides EM follows the regulatory division applied to biopesticides and presents three sections, dealing with microbial, botanical, and semiochemical active substances, respectively.

1.2 Strategic shifts prompted by the European Commission's Green Deal

Since the last update of this EM, the European Commission has set out the course towards achieving the goals of the European Green Deal. To underline its importance: the Green Deal is a massively spirited charge toward securing a sustainable future and a higher level of well-being for all. Not only does the initiative serve most of the EU's raisons d'être set out in the Treaty of Lisbon, it also reflects a powerful Zeitgeist that is steadily gaining momentum.

A prominent aspiration of the Deal is a significant reduction of the use of chemical pesticides within this decade. As such, the new course set out by the Commission directly affects the position that Competent Authorities should hold towards biopesticides in particular. After all, a substantial phaseout of chemical PPPs will leave a gap in the crop protection puzzle that requires filling up with at least several pieces, one of which is decidedly biopesticides-shaped.

It is acknowledged, however, that the current assessment practice regarding biopesticides is not in the right gear to serve the acute objectives of the Green Deal, that is, by facilitating registration of ever more innovative, efficaceous, and safe biopesticides.

Of course, there are multiple impeding factors, not all of which are even causally related to the assessment. Even then, laying down a perfectly balanced set of interpretations on evaluation systematics would not straighten out all bumps in the procedure.
Still, multifaceted challenges are most frequently tackled with the necessary kit of partial solutions.

Therefore to begin with, a principal issue that is within the actual scope of this EM is the absence of a truly comprehensive, clear-cut, and unambiguous set of guidelines for any of the three biopesticide groups currently recognized. Too often, the incompleteness, incompatibility, and non-specificity of the allotted framework results in substantial variation among dossiers, a lot of which is a product of necessary *ad hoc* choices from both applicants and evaluators.
For the current EM-version, Ctgb started out to define a more predictable direction for the micro-organisms-related specification-, product properties-, and analytical methods-sections, as these had been lacking in sufficiently detailed substantiation for some time.

When proving effective, these introductory amendments may prelude a possible revaluation of the EM as a unique supporting document with the potential to fulfill a broader range of functions critical to the improvement of biopesticide assessment that the higher-tier regulatory texts could *by design* not address – at least not in a responsive, specific, and, if needed, purposefully aligned way.

2. Micro-organisms

2.1 Introduction with regard to new terminology

At the time of preparing this third EM-upgrade, the main Regulations that set out the Uniform Principles and the Data Requirements for microbial active substances in specific are undergoing revision. Among other things, the terminology used to refer to any component that may be present in the microbial active substance as manufactured, or its derived product, will undergo cleanup. The change is mainly intended to increase transparency by phasing out ambiguous terms, such as 'MPCA', 'degradation product', 'significant contaminant', etc., and to dramatically reduce the roughly 60 terms that are currently used in (EU) No 283/2013 and 284/2013 to indicate any associated components.
Ctgb believes that the new terminology allows a more systematic understanding of the specification of micro-organisms and facilitates clear communication on this core feature. For the EM-section relating to the specification, some revised terms have therefore been adopted ahead of the formal processes. As a caveat, it needs mentioning that on this point the sections in the current version are possibly not yet aligned; that will be done once the new Regulations have entered into force.
As an accompanying reassurance, the change is rather intuitive and not overly drastic, and may well go largely unnoticed, so that confusion due to section-incompatibility is not expected.

2.2 General introduction into micro-organisms

According to Regulation (EC) No 1107/2009, the term 'micro-organism' covers any microbiological entity, including lower fungi and viruses, cellular or non-cellular, capable of replication or of transferring genetic material.
Approval of microbial active substances is done on strain/isolate level, except for baculoviruses[1] which are approved on species level.

Due to the fundamental difference between chemical and microbial active substances, hazards arising from the use of either type of substance are not necessarily of the same nature. The inevitable distinction in their assessment is reflected by the separation of the data requirements laid down in Commission Regulation (EU) No 283/2013 (active substances) and Commission Regulation (EU) No 284/2013 (PPPs); Part A deals with chemical substances, whereas Part B is dedicated to microbial active substances. Likewise, the uniform principles for the evaluation and authorisation of plant protection products, Commission Regulation (EU) No 546/2011, are divided into a Part I for chemical products and a Part II for products containing micro-organisms.

Furthermore, SANCO/12545/2014 – rev.3 provides guidance on how to prepare a dossier for the (renewal of) approval of a microbial active substance.
Formats of dossiers for zonal approval of microbial PPPs must be compliant with the current templates.

2.2.1 Specific introductory points (283/2013 and 284/2013, Introduction)

For all micro-organisms that are subject to application, all available relevant knowledge and information in literature should be provided. The literature search should be carried out in accordance with the EFSA Guidance on the submission of scientific peer-reviewed open literature (EFSA Journal 2011; 9(2): 2092). 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 also take into account previous taxonomic names which may have been used in past publications.

[1] A separate Guidance Document is available on how new isolates of baculovirus species can be evaluated and added to the already approved isolates. Please refer to SANCO/0253/2008 – rev.2.

According to (EU) No 283/2013, Part B, Introductory point (iv) and (EU) No 284/2013, Part B, Introductory point (v), the test material used in any study included in a dossier must be fully characterized in accordance with the corresponding specification (see also 2.3.2). In addition, batch number, batch weight/volume, and manufacturing date must be provided.

2.3 Identity and specification of the micro-organism

Identity and specification are at the source of the dossier; they set out the direction of the downstream assessment, both in terms of efficacy (active components) and risk (components of concern). They establish (i) the traits based on which a given material can be unambiguously recognized as the active substance, (ii) the identity of any relevant components that are inevitably associated with (the manufacturing of) the active substance, and (iii) the proportions of both the active and the undesirable constituents of the manufactured material.

2.3.1 Name and species description, strain characterisation (283/2013; 1.3)

Each microbial active substance must be identified and named at the strain level.

Strain level identification should be carried out using the best available technology. The appropriate test procedures and criteria used for identification must be provided; nowadays DNA/RNA sequencing is considered the most appropriate procedure.

Taxonomy can change in time due to the transition to DNA sequence analysis for use in systematics, the names of micro-organisms may change as well as the species affiliation. When a literature search is conducted it is important to also take into account previous taxonomic names which may have been used in past publications.

2.3.2 Specification of the material as manufactured and of the product (283/2013; 1.4 and 284/2013; 1.4, respectively)

Regulation (EU) No 283/2013, Part B, 1.4 provides a principal outline of the elements that need to be covered by the specification established for a given microbial active substance as manufactured (henceforth: MASAM). To a lesser degree, (EU) No 284/2013, Part B, 1.4 does the same for the microbial PPP (henceforth: mPPP).
Directly below, under ‘Specification elements’, the core features of both these ‘specification-levels’ are outlined point by point. Subsequently, the drafting of the MASAM -and mPPP specification is highlighted in ‘Establishing a MASAM specification based on the 5-batch analysis and ‘Deriving an mPPP specification’, respectively.

<u>Specification elements</u>

In general, the following must be characterized for the MASAM:

1. Primarily, a specification must provide clear information on the content of the **active component**, i.e., the component that actually brings about the desired plant protective effect. In most cases, this will be the microbial active substance itself, but it is also possible that a major metabolite fulfills this role. In this latter case, the dossier should include the relevant Part A-type data[2] (see 283/2013, Introduction, point viii, and SANCO/2020/12258). Still, in cases where the micro-organism itself does not significantly contribute to the overall functionality, its contents must still be included in the specification. Here, it is important to add that the contents of the active components as included in the MASAM specification do not directly relate to efficacy (except of

[2] In case of ‘active metabolites’, it is advisable to consult with Ctgb prior to drafting a ‘Part A’ dossier, as would be required for those instances. Ctgb holds the viewpoint that, whenever a metabolite can be considered the active substance, there is no need for submitting a separate dossier for this component. Rather – in the interest of avoiding unreasonable burdening and maintaining an ‘independent dossier’ philosophy – all Annex points relevant to the context are to be united in one dossier.

course, when MASAM and mPPP are the same); only the mPPP specification provides efficacious ranges. Rather, the active component ranges established for the MASAM serve as input for the risk assessment and as a basis for evaluation of technical equivalence, according to SANCO/12823/2012 – rev.4.
As stated in the Regulation, '*the content shall be expressed in appropriate terms*', which means that, when the content of the active component is expressed in these terms, it most closely correlates with its efficacy – and thus, its quality of manufacture.

2. Further, the specification must provide an adequate inventory of **components that are of concern to human and/or environmental health**. Within this group, three types of components are recognized: contaminating micro-organisms, metabolites of concern, and relevant impurities.
   For contaminating micro-organisms, it needs to be verified whether a context-dependent set of pathogens remains within thresholds maintained by OECD. Relevant species and limits are provided in SANCO/12116/2012 – rev.0.
   Metabolites of concern are eventually established following the procedure described under 2.4.5 (see below), according to SANCO/2020/12258 on the risk assessment of metabolites produced by micro-organisms.
   To identify relevant impurities, clues may be gathered from starting materials, the manufacturing process, and additives – in short, all parameters that may unintentionally introduce (chemical) impurities to the manufactured material.
   Ctgb holds that screening beyond this practical approach may be exclusively necessary in reasoned cases. But even then, investigations must retain their targeted character. An unfocused 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, as per (EU) 283/2013, Part A, 1.11, is considered unjustifiably inefficient for micro-organisms.
3. Last, any **additives** must be characterized in terms of chemical identity, content range, and function.

Whenever the mPPP is not equal to the MASAM (i.e., when a separate formulation process can be distinguished) a product-level specification needs to be established. By practical interpretation of (EU) No 284/2013, it must include the following relevant features:

1. The content of the micro-organism, expressed in weight or volume per weight or volume of product. Also, in line with the MASAM specification presented above, minimum and maximum contents need to be specified for all active components, in appropriate terms relative to the product.
2. The same set of contaminating micro-organisms that has been monitored in the MASAM must be assayed for the product, before and after storage at the conditions relevant to the intended shelf-life. Similarly, metabolites of concern need to be determined pre -and post-storage to show their compliance with acceptable limits derived from the MASAM specification.
   Relevant impurities, if present, are considered to have ended up in the MASAM during the manufacturing process; their levels are generally not expected to increase during formulation or storage, so, in principle, no pre -and post-storage monitoring is required for these substances.
3. Co-formulants must be listed, along with their content, identity (IUPAC name(s) and CAS number(s) of its constituent(s), trade name), and function.

To determine the content of most of the elements of the MASAM -and mPPP specifications described above, appropriate analytical methodology is required. Please refer to section 2.7 – 'Analytical methods' for further information on this point.

Establishing a MASAM specification based on the 5-batch analysis

As already noted above, an important aspect of the MASAM specification is that it reflects the variation in the output of the relevant manufacturing process[3]. After all, the main purpose of this specification level is to provide the risk-related assessments with realistic ranges for all relevant specification elements and to set a reference quality for production of the material.

The 5-batch analysis is a pragmatic way to get an indication of these ranges; five batches[4] produced (i) within five years before dossier submission, (ii) within a time window that is sufficiently representative of the manufacturing calendar, and (iii) according to the relevant process, shall be characterized in agreement with the principles outlined under 'Specification elements'.

In general, for the active components, the lower limit (or minimum) of the range is determined by subtracting three standard deviations from the 5-result average. The upper limit (or maximum) is calculated by adding three standard deviations to the average. 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.
When the active substance content is expressed in terms of microbial units, e.g., colony forming units (CFUs), spores, or virus particles per gravi -or volumetric unit, an alternative approach may be justified, depending on the context. For instance, when the content-variation among the five assayed batches is, by chance, too low and therefore results in a tight range that is not reasonably expected to capture actual inter-batch variation, a one order of magnitude range – which is commonly accepted in microbiology – could be superimposed over the initial range, mindful of considerations of both efficacy (on the min. side) and risk (on the max. side).
Establishing an even broader range is not desirable, as this would at some point result in non-trivial performance differences between minimally and maximally specified batches.

When activity is expressed in terms of biopotency, the above-mentioned 'average plus or minus three standard deviations' approach may be less appropriate and another way to define a range that covers the biopotency in 99 % of the cases may be warranted (*this note on biopotency is only relevant when MASAM and product are the same, as biopotency should only be derived for the product*).

Next, it must be shown for all batches included in the analysis that the levels of contaminating micro-organisms relevant to the context are within OECD safe-limits as adopted by SANCO/12116/2012 – rev.0.

All identified metabolites of potential concern must be quantified to establish their status as either a metabolite of concern, or as substance that requires no further assessment. Metabolites of concern will be included in the specification at a level equal to the 5-result average plus three standard deviations. Relevant impurities will be treated analogously.

[3] Here, 'relevant manufacturing process' is defined as the actual process employed in the manufacture of the microbial active substance that will eventually end up in products to be marketed in the EU. Beside its technical characteristics, production scale (pilot or industrial) and plant location are key process identifiers. In case at least one of these three determinants changes, technical equivalence to the already evaluated process must be demonstrated according to SANCO/12823/2012 – rev.4.
[4] 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.

Although for the active components themselves, GLP-compliance of the batch data is not strictly enforced, it is mandatory for the data relating to contaminating micro-organisms, metabolites of concern, and relevant impurities.

Deriving an mPPP specification
(*N.B. This subsection is only relevant for micro-organisms for which the MASAM and the product are different entities*).

The main aspect of the mPPP specification is that it defines a minimum active component limit (in appropriate terms) at which the product is still sufficiently effective. Typically, this limit is at least equal to the one characterized for the batch used in the critical field trials[5]. In certain cases, subsidence below the minimum may be accepted, but requires evaluation by the Efficacy expert. However, given the overarching context regarding biopesticides (see 1.2), which emphasizes the critical role of their efficacy, underachievement should certainly not be facilitated. Instead, applicants should be encouraged to address the cause of 'falling out of spec.' in a more upstream fashion (e.g. by increasing a.s. stability or by boosting a.s. content). In rarer cases, stretching the maximum limit may be necessary to allow for increase of the active component, e.g. due to substantial growth of the micro-organism during storage; here, the risk assessment may need amendment to cover for this eventuality.
Unlike the MASAM specification, the mPPP specification does not incorporate any specific information on contaminating micro-organisms. This feature is addressed in the storage stability test (see 2.6.2, 'Storage stability and shelf-life').
Incidentally, the mPPP specification includes critical limits for any identified metabolites of concern and relevant impurities, mainly for monitoring purposes.

By default, an mPPP specification is derived from the MASAM specification based on simple calculation, using the contents of the established elements in the MASAM and the MASAM-content in the product, following:

$C_{EM} \times C_{MP} = C_{EP}$

Where 'C_{EM}' represents the content of a given element in the MASAM (e.g. in 'spores/g'), 'C_{MP}' is the content of MASAM in the product (e.g. in 'g/kg'), and 'C_{EP}' is the content of the respective element in the product (e.g. in 'spores/kg').

Especially for any elements of a chemical nature (like active metabolites, metabolites of concern, and relevant impurities), this approach should suffice to express the representative levels of the established elements in relation to the product. Some relevant elements may – due to their conditional dependence[6] – however not be so easily translated from the MASAM specification, as their contents may have been affected by changes induced by the formulation process.
Depending on the degree of mismatch, it may be warranted to perform a separate 5-batch

[5] It is generally noticed, that efficacy trial reports often lack a detailed description of the employed test batch in terms of appropriately expressed active substance-contents. These data are nevertheless highly relevant for the assessment, and are moreover required as per Regulation (see 2.2.1 'Specific introductory points).
[6] Elements whose quantification is insensitive to any physical and chemical changes of the environment (like those occuring during the formulation process) are considered *non-conditionally dependent*. For instance, upon formulation, spores are not reasonably expected to change in any way that would affect their countability; their content only changes due to dilution in co-formulants. The same applies to all elements of a chemical nature (metabolites of concern and relevant impurities).
In contrast, quantification of conditionally dependent elements may be affected by a shift in pH, temperature, osmotic pressure, or chemical composition of the environment. These factors may significantly affect the capacity of spores to form colonies. As such, the content of colony forming units (CFUs) in the product may not always be derivable from the MASAM specification.

analysis on the product, only to determine a representative product-level range for these elements in particular.

Being conditionally dependent by nature, biopotency should only be established for the product, by product-level 5-batch analysis. As mentioned above, the intention is to define a statistically sound range that covers the biopotency in 99 % of the cases.
As it may furthermore not be simply assumed that biopotency is equal for all intended target species stated in the GAP, a rationale that substantiates the choice for a particular species is highly appreciated, of course, unless only one species will be targeted. With regard to this point, Ctgb is opined that arguments of economy outweigh those of complete elucidation: one 5-batch biopotency analysis performed for one – preferably 'toughest case' – species suffices. For the GAP-species whose sensitivity to the active component is not formally established by 5-batch analysis, submission of 'less formalized' background data, that are likely available anyway, is desirable.

2.4 Biological properties of the micro-organism

2.4.1 Origin and natural occurrence (283/2013; 2.1.2)

This section should be a summary of the information on the origin and natural occurence of the micro-organism given in the section Fate and behaviour in the environment. It should include the following information:

- The geographical region and the place in the ecosystem (e.g. host plant, host animal, or soil from which the micro-organism was isolated).
- Information on the geographical range and habitat of the strain and species.
- Information on the natural abundance (prior to application) of the species/strain in natural systems, if available.

2.4.2 Infectiveness, dispersal and colonisation ability (283/2013; 2.5)

This section should be a summary of the information on the infectiveness, dispersal and colonisation ability of the micro-organism given in the section Fate and behaviour in the environment. It should include the following information:

- Information on possible dispersal routes of the micro-organism (via air as dust particle or aerosols, with host vectors etc.) under typical environmental condition.

- The persistence of the micro-organism and information on its life cycle under the typical environmental conditions of use must be indicated. In addition, any particular sensitivity of the micro-organism to certain environmental conditions (UV light, temperature, pH, humidity, nutrition requirements etc.) should be provided.

- Information on the growth of the specific strain at different temperatures.

2.4.3 Relationship to known plant or animal or human pathogens (283/2013; 2.6)

The possible existence of one or more species of the genus of the active and/or, where relevant, contaminating micro-organisms known to be pathogenic to humans, animals, plants or other non-target species and the type of disease caused by them must be indicated. It must be stated whether it is possible, and if so, by which means to clearly distinguish the active micro-organism from the pathogenic species. When appropriate, particularly with regard to detection techniques, reference can be made to sections on identification and quality control. Appropriate scientific literature on related pathogens should be cited.

2.4.4 Genetic stability and factors affecting it (283/2013; 2.7)

This section should be a summary of the information on the genetic stability of the micro-organism and factors affecting it given in the section Fate and behaviour in the environment.

2.4.5 Information on the production of metabolites (especially toxins) (283/2013; 2.8)

Microbial metabolites are intermediates or products in the metabolism of a micro-organism and must not be confused with the metabolites that are the result of degradation of a chemical active substance.
Micro-organisms produce a wide range of metabolites – mostly as a result of entering a new phase in their life cycle or as a response to environmental conditions – in order to regulate their own growth, to control competitors, or to aid organisms beneficial to them. For some of these purposes, micro-organisms may produce hazardous metabolites/toxins or antimicrobial agents that, in the context of (EC) No 1107/2009 require assessment.

These relevant metabolic products will be examined according to the systematic established in (EU) No 283/2013 and, in particular, to the step-wise process set out in the dedicated Guidance on the risk assessment of metabolites produced by micro-organisms used as plant protection active substances – SANCO/2020/12258.

For the risk assessment, the approach described in this Guidance document firstly intends to identify all potentially hazardous and/or antimicrobial metabolites that may be produced by the micro-organism, i.e., the metabolites of potential concern (Stage 2).
To do this, three separate lists are to be maintained[7].
The first inventorizes metabolites that have been identified for the strain under assessment and (closely) related strains, and attempts to connect them with a scientifically supported hazardous effect.
The second list includes hazardous effects, possibly related to metabolite action, observed in toxicology studies performed with the micro-organism.
The last provides a listing of toxicology studies in which no effect was established.

Completion of the lists notably relies on literature searches performed according to EFSA Guidelines[8], and verification with (i) toxicity studies that investigate the relationship between established candidate metabolites and hazardous effects, and (ii) focused analytical screening for candidate metabolite in MASAM and/or mPPP. Further suppositions of metabolite production, e.g., hinted at by data on closely related strains, may be checked by genomic analysis. Additional context included in the step-wise process may be provided by a described history of safe use.

Next, the assessment focuses on establishing the identified metabolites of potential concern to be either ‘of concern’ or ‘of no concern’, by clearing up their exposure profile (Stage 3) and by performing a risk assessment for metabolites that may be produced under relevant *in situ* circumstances and/or whose presence in the mPPP has been confirmed (Stage 4).
For metabolites that are identified as a hazard, it may be necessary to determine the risk route by means of a qualitative or (semi-)quantitative assessment using reference values when

[7] N.B. At present, Ctgb is developing a pilot version of the Overview table that the Guidance frequently refers to for the purpose of keeping track of the metabolite assessment. Given its likely ability to significantly aid the metabolite assessment’s transparency and to facilitate communication among assessors and applicants alike, expedited distribution of the table is desirable. As such, once sufficiently examined by Ctgb, the Overview table will be shared in this Evaluation Manual for perusal in assessment reports – at least until a more formalized, EU-reviewed tool has been made available.
[8] Refer to EFSA Journal 2011;9(2):2092 – Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009.

needed and/or available (see further the paragraphs on effects on human health, fate and behaviour in the environment and effects on non-target organisms).

2.4.6 Antibiotics and other anti-microbial agents (283/2013; 2.9)

Three issues should be addressed. First, many micro-organisms produce some antimicrobial metabolites. Interference with the use of these metabolites in human or veterinary medicine must be avoided at any stage of the development of a microbial product. The level of production of any known antibiotics used in human or veterinary medicine by the micro-organism must be indicated.

In addition, 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.Information on the stability, in terms of genetic transfer, is of particular interest if these genes are carried on mobile genetic elements, since this may be of medical relevance. Principles apply to active substances consisting of living/inactivated/dead bacteria, because genetic material and in particular antimicrobial resistency genes (AMR) genes can be present. Screening for the presence of known AMR genes within the Whole Genome Sequencing (WGS) data and screening of mobile genetic elements in case a resistance gene has been identified must be provided. Strains with transferable resistance may not be approved, unless the applicant demonstrates that the identified genes of concern are not present in the product (e.g. via an appropriate PCR protocol) and thus there is no hazard and no risk to be expected from the living/inactivated/dead bacterium.
For **fungi** there is no need to assess the potential transfer of genes for resistance to antimicrobials. In general, the possibility for horizontal gene transfer in all eukaryotes is very limited and resistance is multifactorial, not coded by single genes and not associated with specific mechanisms, as described for bacteria (for instance through plasmid exchange). So, it is of low concern that genes that code for antimicrobial resistance in fungal agents used in biocontrol will transfer any resistance to other organisms.

Viruses excluding bacteriophages have not been reported in the scientific literature as contributor to the AMR concern.

At last 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.

A Guidance is yet available (SANTE/2020/12260, d.d. 23 October 2020). This Guidance document explains how to assess antimicrobial resistance of micro-organism, as well as the risk of increasing the spread of antimicrobial resistance of human and veterinary concern, in relation to the approval criteria and the low-risk criteria set under Regulation (EC) 1107/2009.

2.5 Further information on the micro-organism

2.5.1 Information on the occurrence or possible occurrence of the development of resistance of target organism(s) (283/2013; 3.5)

Low-risk PPPs often have novel modes of action that do not show cross-resistance with existing products, as such they can offer advantages to resistance management. It is however possible for pests or pathogens to develop resistance to certain low-risk products. Resistance management therefore needs to be addressed.

Resistance risk depends for a large part on the mode of action. As stated in EPPO PP1(276(1) micro-organisms with an indirect mode of action (e.g. host plant defence induction or

competition for nutrients) are often not at risk of resistance development in target organisms. In such cases this data point can be addressed with a statement. Micro-organisms with a direct mode of action (for example infection of the target organism, or production of a toxin) can be at risk of resistance development, and several such cases are known from practice. In these cases the EPPO standard for resistance risk analysis should be followed. Please refer to EPPO standard PP1/213(4) (Resistance risk analysis)

It should be noted that most micro-organisms and other low-risk products are not listed in the FRAC or IRAC mode of action classifications. Therefore, it is important to clearly describe the mode of action and the current resistance situation, preferably with references to scientific literature.

In some cases target organisms may develop resistance to some strains of a micro-organism, but not to other strains of the same species. This differs from conventional PPPs where often cross resistance exists between many active substances.

2.6 Properties of the mPPP

2.6.1 Composition, type, and function (284/2013; 1.4, 1.5, and 1.6)

The content of the MASAM in the mPPP must be reported in % w/w (for liquid preparations also in g/L), and all established elements must be stated in line with the corresponding mPPP specification (please refer to 2.3.2, 'Deriving an mPPP specification' for more details).

Co-formulants in the mPPP are characterized in terms of content, identity (IUPAC name(s) and CAS number(s) of its constituent(s), trade name), and function.

Next, a formulation type must be assigned that appropriately reflects the physical state and use of the preparation.

In analogy, the biological function of the mPPP needs to align with its principal mode of action.

2.6.2 Physical, chemical and technical properties of mPPPs (284/2013; 2)

Storage stability and shelf-life

For mPPP, three types of storage stability tests are recognized, each intended to address a particular feature of the shelf-life.

Short-term test – high temperature

In accordance with OECD Series on Pesticides No. 85[9], Ctgb holds that for mPPPs, 'accelerated storage stability tests' can in most cases not be considered to support a provisional shelf-life. Despite this, the tests can however serve a useful purpose in mPPP-context as 'high temperature storage stability test'; a successful 18-week long test at 30 °C provides sufficient evidence that the respective mPPP may likely retain its efficacy when stored in a non-temperature controlled environment throughout a typical summer in the Northern -and Central Zone. Also, the test reasonably covers for any inevitable short-term high temperature exposure of the product during application in hot weather.

For mPPPs whose principal efficacy is caused by an active metabolite, 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,

[9] OECD Environment Directorate (2016), Guidance document on storage stability of microbial pest control products, Series on Pesticides No. 85, Paris, France.

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 in the same way as the main long-term test (see below).

Short-term test – low temperature
The low temperature stability test according to CIPAC MT 39.3 is intended to assess the stability of liquid formulation types after exposure to frost. Ctgb holds that 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 mPPP 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 favourable for the mPPP 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-lifes that may be assigned to a given mPPP.
The test report must include pre -and post-storage data on active component content, packaging integrity, physical/chemical/technical properties required for the respective formulation-type, contaminating micro-organism-screening, and, if relevant, on metabolites of concern, and relevant impurities. Specification elements, like the active component and components of concern, are expressed in line with the mPPP 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. Ctgb maintains a pragmatic opinion on the status of interim reports; as long as submission of a final version is guaranteed – usually by provision of a study plan that states a clear finalization date – interim data are used without any special reserve. Regarding GLP-status, 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. Here, it must be emphasized that interpretations of GLP-issues relating to interims are known to vary among Competent Authorities. It is advisable to keep track of extant opinions, whenever relevant.
Last, Ctgb does not require submission of a full long-term storage stability study 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 product assessment. As stability of the microbial substance itself must however be demonstrated for approval, post-storage data relating to active component contents are already required at the substance-level.

<u>Physical hazards</u>
All properties required for CLP labelling shall be addressed, although, given the nature of regular mPPPs, the corresponding Annex points can often be addressed by a waiver. A notable exception is flammability, which, especially for powdered formulations, may not be easily put aside by theoretical argumentation. By default, Ctgb simply advises testing of flammability for dry formulation types, according to recommended methodology, and in compliance with GLP-criteria.

Physical, chemical, and technical properties
As for context-related test requirements, recommended methodologies, and acceptability thresholds, Ctgb maintains no specific interpretation that provides any additional depth to the existing framework. Please refer to (EU) No 284/2013, Part B, Section 2, the FAO Pesticide Specifications Manual, and respective CIPAC (-or equivalent) sources regarding methodological guidance.

2.7 Analytical methods

2.7.1 Methods for the analysis of the micro-organism as manufactured (283/2013; 4.1)

The data package containing the required analytical methodology must primarily support the established specification elements (see 2.3.2). As such, the specifications define for the largest part which methods are required within the given context. Below, all method types categorized by (EU) No 283/2013 are briefly discussed in relation to applying perspectives held by Ctgb.

Method for the identification of the micro-organism
The method to identify the micro-organism should be capable of identifying the micro-organism at strain level.

Method for providing information on possible variability of seed stock/active micro-organism
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 produce.

Methods to differentiate a mutant of the micro-organism from the parent wild strain
Already included in the broad quality assurance check (see 'Method for providing information on possible variability of seed stock/active micro-organism' above).

Methods for the establishment of purity of seed stock from which batches are produced and methods to control that purity
Already included in the broad quality assurance check (see 'Method for providing information on possible variability of seed stock/active micro-organism' above).

Methods to determine the content or degree of activity of the active components
Based on current experience, Ctgb recognizes the following main approaches to quantify the content of the active component, and/or its activity:

Spore/particle counting and enumeration of colony -or plaque forming units
Bacterial and fungal spores, and virus particles[10] are generally counted in a haemocytometer. Micro-organisms that rely on their colony-forming capacities to be effective are transferred to the appropriate type of nutrient agar, incubated, and enumerated colony by colony. 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,

[10] Often, expressing the microbial active substance in terms of spores or virus particles per g or L of matrix may not be meaningful in relation to activity. Still the spore content may be a useful metric in the context of the technical equivalence assessment insofar it may be an indication of quality of the produced material. Especially for viruses, the virus particle content is commonly required to derive 'concentrations in appropriate terms' (e.g. in virus particles per g of diet) for the dosing groups in the bioassay (see 'Bioassays' in this subsection).

linearity, accuracy, and precision, but leaves the actual acceptance thresholds undefined. Methods that have been validated according to SANCO/3030/99 – rev.4 and 5, at least for linearity and precision, are commonly encountered. The document is however intended for evaluation of analytical chemistry methods, and is not suitable for the microbiological methods discussed here. Nevertheless, as the SANCO/3030/99-criteria are on the stringent side for the microbial context, compliance automatically means acceptability for enumeration methods, again, at least with regard to linearity and precision.
To provide a degree of systemization, the following pragmatic rules may be considered until a micro-organism-dedicated guideline has been adopted:

- *Specificity:* the morphological characteristics based on which the micro-organism is identified during counting must be described;
- *Linearity:* given the limitations enforced by statistics on the one hand and practicability on the other, the haemocytometer -and plate count range roughly covers a factor of 10. 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 fit for purposeness;
- *Accuracy:* a possibly critical feature affecting the method's accuracy is the dilution 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. To gauge the extent of this error, a final dilution must be prepared by consistently drawing material from the lower tenth of the vessel, whereas the other is produced by sampling the upper tenth during every step. Both synchronous measurements must be performed three times.
- *Precision:* Precision data must include at least 5 independent determinations 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 fit for purposeness.

Bioassays
Limited by practicality, specifications can do little more than capture a rough abstraction of the multifaceted reality of a micro-organism's efficacy. 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.
Factually being a toxicity test that is carried out with a micro-organism instead of a chemical substance, a bioassay includes exposure of test organisms to a range of dosing levels, plotting of response against dose, and subsequent derivation of a median lethal dose (LD_{50}).
Currently, tests are far from being standardized, which limits the evaluation efficiency. Furthermore, test outcomes are reported in various ways, often non-intuitive and difficult to untangle. Recognizing the fact that bioassays could present an ultimately representative tool to establish the effectiveness of an mPPP (note that for some micro-organisms, they present the *only* tool) and that, heretofore, they seem to have missed the evaluator's attention they deserved, Ctgb presents the following considerations that may both give direction to the evaluation, and produce a better referenced and more communicable metric.

- First, it is important to have the test batch characterized in a way proportional 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 diet).
- The amount of diet material per organism must be such that a non-negligible fraction will be consumed, in order to minimize bias due to the invariably inhomogeneous

distribution of the active component in the food.

- Next, the test species must be equal to the mPPP's intended target species. In case the GAP includes more than one species, the least sensitive species should be selected, assuming that information may be available to support this choice. The tests must be performed with healthy individuals, i.e., specimens without apparent defects.
- 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 diet. 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.
  Ideally, the LD_{50} coincides with the group in the middle, mortality in the lowest and highest dose groups, and the control group is about 15, 90, and 10 %, respectively. 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.

Chromatographic methods
When the actual active component produced by a microbial active substance is compatible with conventional chromatographic methods, validation must be compliant with the criteria set out in SANCO/3030/99 – rev.5.

Other methods
Currently, there are no known micro-organisms, other than most strains of *Bacillus thuringiensis*, whose activity cannot be adequately quantified via at least one of the approaches mentioned above. *B. thuringiensis*' massive multi-kDa crystal proteins may be determined using SDS-PAGE. Future cases may necessitate the use of other custom methods. As no dedicated guidelines are in place, the method evaluation will focus on the universal quality criteria, i.e., specificity, linearity, accuracy, and repeatability, stated in (EU) No 283/2013, Part B, Section 4.

Methods for the determination of metabolites of (potential) concern and (potentially) relevant impurities
Once it has been established that the microbial active substance is capable of producing metabolites of potential concern, these components must be included in the 5-batch analysis. Although methods intended for the quantification of metabolites of potential concern are validated on a fit for purpose basis, methods for the determination of established metabolites of concern shall be compliant with SANCO/3030/99 – rev.5 criteria.
The same applies for (potentially) relevant impurities.

Methods to show that contaminating micro-organisms are controlled to an acceptable level
Beside safe limits and context-dependent information on relevant contaminating species, SANCO/12116/2012 – rev.0 provides guidance on recommended methodology. Having drawn its inspiration from food/feed-legislation, the SANCO-document typically advises use of internationally standardized reference methods (e.g. FDA BAM, USDA MLG, AOAC, and ISO), commonly employed in screening of food and feed. Whereas the SANCO-recommended methods still only include the more traditional plating methods that were the norm at the time of drafting, the food/feed-framework has evolved in the meantime to allow the use of more innovative, alternative methods – 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, Ctgb deems that any method that is in compliance with ISO 16140-2 criteria, is considered acceptable for mPPP-screening purposes.

Methods to determine storage stability of the micro-organism
Here, 'storage stability' is interpreted as the ability of a microbial active substance to maintain its activity over a longer period at which it is stored in certain packaging at a practical temperature. The required analytical methodology is the same as that described under 'Methods to determine the content or degree of activity of the active components'. The context of storage stability testing is furthermore described in detail under 2.6.2.

2.7.2 Methods for the analysis of the preparation (284/2013; 5.1)
The largest part of the information provided under 2.7.1 applies *as is* for the mPPP. Excepting the cases in which there is no distinction between the two, MASAM and mPPP are different matrices in the sense that method performance may be affected. For the non-viable components that require analysis, methods must be separately validated for the product. Formally and analogous to chemical PPP practice, the threshold values established for metabolites of concern and relevant impurities are corrected for 'dilution' occurring during the formulation process, resulting in a lower quantification limit (LOQ) for the components in the product. Given the contextual difference – for micro-organisms, components of concern are not monitored to uncover unwarranted variation in manufacturing output, but to directly ensure safe use – LOQs for MASAM and mPPP are often equalized.

Translation of the enumeration method validated for the MASAM may not require a full re-evaluation for the mPPP; 'specificity' only needs amendment once the product contains more than one microbial active substance, as it needs elaboration how the multiple actives may be distinguished from one another. 'Accuracy' needs no further addressal, once stability of the mPPP, as supported by e.g. suspensibility or dispersion stability, has been adequately evidenced. 'Linearity' and 'precision' are not reasonably expected to be affected by changes to the matrix brought about by the formulation process.

2.7.3 Methods to determine and quantify residues (283/2013; 4.2 and 284/2013; 5.2)
As yet, there is no approved microbial active substance for which a Residue Definition for monitoring has been defined. It is foreseen that, if this should nonetheless happen, the components included will likely be chemical substances for which validation criteria are already available (see SANTE/2020/12830 – rev.1).
Monitoring of viable residues is reasonably expected to pose practical problems; the development of a well-founded viewpoint would require some careful reflection. At this moment however, Ctgb is doubtful whether such an investment would ever pay off.

2.8 Effects on human health
Hazards arising from micro-organisms should be assessed differently from chemicals. Micro-organisms are unlikely to be toxic in themselves but they may produce toxic metabolites.

Micro-organisms also have the potential to replicate and therefore their ability to cause infection or pathogenicity must be carefully assessed. They may also have the potential to cause sensitising reactions and non-specific effects such as an inflammatory response after exposure via inhalation.

The typical OECD test guidelines are not tailored towards micro-organisms. Pending the acceptance of specific guidelines at international level, the information required shall be generated using available test guidelines that may be accepted by the Competent Authority (e.g. US EPA's microbial pesticide test guidelines). Where appropriate if no US EPA test guideline is available, test guidelines as described in Part A of Commission Regulation (EU) No 283/2013 could be adapted in such a way that they are appropriate for micro-organisms.

For all studies actual achieved dose in colony forming units per kg body weight (CFU/kg bw), as well as in other appropriate units, must be reported.
Evaluation of micro-organisms is carried out in a tier-wise manner with the first tier consisting of basic information and basic studies and the second tier consisting of additional studies if the first tier tests have shown adverse health effects.

2.8.1 Active substance: Tier 1: Basic information and basic studies

Basic information (283/2013 ; 5.1)
Information related to 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.
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. Preferably, 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 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.

Clinical case reports and epidemiological studies of the active micro-organism or of any taxonomically related strains and species should be considered to assess whether the active micro-organism is known to cause infection and pathogenicity in humans. If the micro-organism in the study is a different species than the micro-organism being assessed, it is important to clarify what distinguishes the two and whether it is likely that the active micro-organism could exhibit the same properties. For such an analysis, information on the biological properties of the micro-organism such as growth requirements and the presence of genes encoding known toxins may be useful. If the pathogenic species requires significantly different growth conditions or is taxonomically not closely related, that could be indications of a lower risk of pathogenicity associated with the active micro-organism.

Basic studies (283/2013 ; 5.2)
Sensitisation (283/2013; 5.2.1)
Although the data requirements do request a sensitisation study, there are currently no validated methods to evaluate sensitisation potential of micro-organisms. Consequently, no study is required. If a study is carried out the results of this study, either positive or negative, should be interpreted with caution since the current dermal sensitisation studies are not validated for micro-organisms.
In compliance with the Uniform Principles from Regulation (EU) 546/2011, all micro-organisms shall be regarded as potential sensitizers in the absence of validated tests for investigating

sensitisation. The precautionary warning phrase 'Micro-organisms may have the potential to provoke sensitising reactions' is included on mPPP-labels for all micro-organisms in the product. Consequently it is considered as a general precautionary measure and not as the result of a risk assessment. Therefore, this sentence does not preclude micro-organisms being considered as 'low-risk' substances (see excerpt[11] from the 'Background document for the purpose of a possible amendment of the current low-risk criteria', SANTE/11953/2015 – rev.5). In case there is clear evidence in literature that a component from the microbial active substance is a respiratory sensitiser (H334), conventional classification applies instead of the warning phrase (see also 2.8.2).

Acute toxicity, pathogenicity and infectiveness (283/2013; 5.2.2)
Studies on acute oral and inhalation toxicity, pathogenicity and infectiveness must be reported.

Inhalation toxicity can be tested either through inhalation or intratracheal exposure. Intratracheal exposure would ensure adequate exposure of the test animal to the micro-organisms. For the inhalation exposure, generally the concentration of micro-organisms in the atmosphere becomes too low and the particle size distribution is too high when administered via inhalation. Further, the viability can be affected due to shear forces from nebulisation. Most vegetative microbes, particularly Gram-negatives, suffer considerable damage (about 95% are killed) while gram positives are less sensitive and most spores survive. Fungi are difficult to get into respirable aerosols without significant loss in viability because of their size. Due to these considerations inhalation exposure is normally not recommended for micro-organisms and an intratracheal study is preferred.

In addition to the oral and inhalation study, an intraperitoneal injection study is required. However, expert judgement may be exercised to evaluate whether subcutaneous injection is preferred instead of intraperitoneal injection if the maximum growth temperature and multiplication is lower than 37 degrees. This is because in those cases the micro-organism would be more likely to cause infections in the skin rather than deep tissue infections.

All acute toxicity, pathogenicity and infectiveness studies should be carried out in accordance with GLP and the US EPA guidelines (OPPTS, series 885).

Genotoxicity (283/2013; 5.2.3)
It is considered unlikely that the micro-organisms themselves can cause a genotoxic effect. Genotoxicity testing however may be relevant for metabolites. The specific metabolite could be tested in purified form using the same test methods as for chemical active substances. However, since micro-organisms may produce a large array of metabolites, testing of a crude extract (i.e. the chemical constituents of the TGAI with cell walls etc., removed) could be considered. 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, growth inhibition of DNA synthesis,

[11] SANTE/11953/2015 – rev.5 states the following: '*In compliance with the Uniform Principles from Regulation (EU) 546/2011, all micro-organisms shall be regarded as potential sensitizers in the absence of validated test for investigating sensitisation. The warning sentence "Micro-organisms may have the potential to provoke sensitising reactions" is included for all micro-organisms in the label of plant protection products containing micro-organisms.* ***Consequently it is considered as a general precautionary measure and not as the result of a risk assessment.*** *Therefore, this sentence does not preclude micro-organisms being considered as 'low-risk' substances'.*

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[12]).
In the case of a virus the risk of insertion mutagenesis in mammal cells and the risk of carcinogenicity has to be discussed.

Cell culture study (283/2013; 5.2.4)
A cell culture study gives information on the ability of a micro-organism to infect, replicate in, transform or cause toxicity in the cell system. The data requirements state that for intracellular replicating micro-organisms, such as viruses, viroids of specific bacteria and protozoa, a cell culture study should be carried out.

The study shall be performed in human cell or tissue cultures of different organs. Selection can be based on expected target organs after infection. If human cell or tissue cultures of specific organs are not available, other mammal cell and tissue cultures can be used.

OPPTS guideline 885.3500 states that if the data show that the viral pest control agent preparation is toxic to any of the test cell cultures, but does not infect, replicate in or transform any of the cell cultures, further information may be required to identify the toxic components of the preparation. Moreover, an acute toxicity study may be required with the toxic components.

Information on short-term toxicity and pathogenicity (283/2013; 5.2.5)
If adverse effects have been observed in the acute toxicity, pathogenicity and infectivity studies than further testing may be necessary to clarify the nature and severity of effects that may result from repeated administration of the microbial active substance.

Relevant metabolites
As described under 2.4.5, 'Information on the production of metabolites (especially toxins)', for metabolites that are identified as a hazard, it may be necessary to determine the expected risk by means of a qualitative or (semi-)quantitative assessment using reference values when needed and/or available. The metabolites of concern and the endpoints from the respective tests should be presented in an Overview table – for which a template will be available in the near future (see explanation under Footnote 7 of this EM). In some cases, additional endpoints are needed (see Stage 4, Step 18) that are derived from newly performed toxicity studies. These endpoints should be presented in the Overview table as well. A quantitative assessment comparable to the assessment performed for chemical active substances is required in cases where non-relevance cannot be confirmed.

For detailed information on the decision tree, 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'.

2.8.2 Active substance: Tier 2 studies
Specific toxicity, pathogenicity and infectiveness studies (283/2013; 5.3)
In certain cases, it may be necessary to carry out additional studies to further clarify the adverse human effects. In particular, if results from earlier studies indicate that the micro-organism may cause long-term health effects, studies on chronic toxicity, pathogenicity and

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

infectiveness, carcinogenicity and reproductive toxicity must be carried out. Micro-organisms infective to human cell lines may also need further investigations.

Before performing such studies it is recommended that the applicant shall seek the agreement of the competent authorities on the type of study to be performed.

Genotoxicity - In vivo studies in somatic cells (283/2013; 5.4)
If a positive result has been obtained with an *in vitro* study an *in vivo* genotoxicity study is required. The recommended methods are the same as for chemicals.

Genotoxicity – In vivo studies in germ cells (283/2013; 5.5)
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.

2.8.3 Product

Basic acute toxicity studies (284/2013; 7.1)
Instead of carrying out the basic acute toxicity studies it would also be possible to address the need for classification and labelling of the product by using the calculation rules in accordance with Regulation (EC) No 1272/2008.

Additional acute toxicity studies (284/2013; 7.2)
Skin -and eye irritation studies are required in accordance with the OECD test guidelines. Instead of carrying out these studies it would also be possible to address the need for classification and labelling of the product by using the calculation rules in accordance with Regulation (EC) No 1272/2008.

As there are currently no validated methods to evaluate sensitisation potential of micro-organisms, no study for skin -and respiratory sensitisation is required. To address the potential sensitising properties of co-formulants, the calculation rules in accordance Regulation (EC) No 1272/2008 can be used.

If based on the co-formulants no classification for sensitisation (H317) is needed, the following precautionary warning phrase should be included on the label:
‘Contains [*name micro-organism*]: Micro-organisms may have the potential to provoke sensitising reactions’.

In case (i) there is clear evidence in literature that a component from the microbial active substance is a respiratory sensitiser (H334), (ii) the product is liable to labelling according to CLP calculation rules, and (iii) inhalatory exposure is expected, the product shall be classified H334.
In case (i) the allergic potency of the responsible proteins will be strongly reduced, (ii) the exposure related to recommended use of the mPPP is negligible when compared to that pertaining the use by e.g. bakers, or (iii) no allergic reactions are expected for operators, H334-classification is not considered necessary. In this case, only a precautionary warning phrase focused on exposure by inhalation for the usual component of living organisms is considered to be necessary. In that case, the following ‘precautionary warning phrase’ will be added to the Dutch label (WG/GA):

‘Contains [name of usual component of living organism]: [name of usual component of living organism] may have the potential to provoke sensitising reactions and allergy or asthma symptoms or breathing difficulties if inhaled’.

This precautionary warning phrase has been derived from the standard precautionary warning phrase used for all micro-organisms in accordance with the PRAPeR Expert Meeting on micro-organisms in June 2009: 'Contains [*name micro-organism*]: Micro-organisms may have the potential to provoke sensitising reactions'.

The two precautionary warning phrases are considered as general preemptive measures and not as results of a risk assessment. These sentences do not preclude a potential low-risk status of the micro-organism or accompanying components of living organisms with an allergic potential. Also, any specific risk mitigation measures related to these two precautionary warning phrases do not rule out the mPPP being considered as a 'low-risk' product.
In general, classification based on the presence of a co-formulant excludes the product's 'low-risk' status, as risk mitigation measures (restriction sentences for conditions for safe use) are needed. This is also valid for the prescription of risk mitigation measures based on the risk assessment performed for the micro-organism or its metabolites.

Data on exposure (284/2013; 7.3)
Exposure to the micro-organism:
In most cases no reference values are set for micro-organism and therefore no quantitative exposure assessment is required.

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 user may be assumed to wear protective clothing (PPE). However, it should be noted that with regard to PPE there is no harmonized approach possible due to national requirements. Some Member States require respiratory protective equipment (RPE) for certain types of products (e.g. mixing and loading of powders) or type of application (indoor) while other Member States always prescribe RPE for all micro-organisms. In the Netherlands RPE is required for powder formulations but not for liquid formulations or granule formulation which are nearly dust-free.

Exposure to hazardous metabolites:
The exposure assessment should include any hazardous metabolites/toxins that are either present in the product or produced *in situ.* The relevance of *in situ* production should, according to SANCO/2020/12258, be based on 'whether the production is limited due to energy/resources contraints and therefore triggered only under certain conditions and rapidly decreasing under environmental and agricultural conditions'. If quantitative data in the product is available for a hazardous metabolite, exposure may be assessed in the same way as for chemical plant protection products. The level of the metabolite in the product can be used as input parameter in the model. 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.
For worker exposure some additional argumentation may be needed to show that the relevant metabolite is not expected to increase on the crops after application. Generally, the information that is provided in the residue section can be useful to address this concern.

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.

Supplementary information for combination of plant protection products (284/2013; 7.5)
In certain cases it may be necessary to carry out additional studies for combination of plant protection products where the product label includes requirements for use of the plant protection product with other plant protection products and/or with adjuvants as a tank mix. However, this is not often the case for microbial plant protection products.

2.9 Residues in or on treated products

Information should be provided that allow an evaluation to be made regarding the risk arising from exposure to the micro-organism and its residual traces and relevant metabolites (toxins) remaining in or on plant or plant products.

To evaluate the risk arising from residues, exposure data on levels of exposure to the residue may not be required where it can be justified that the micro-organism and its metabolites are not hazardous to humans in the concentrations that could occur as a result of authorised use.

2.9.1 Persistence and likelihood of multiplication in or on crops, feedingstuff or foodstuffs (283/2013; 6.1)

The persistence of toxic metabolites, where relevant, and the likelihood of persistence and multiplication of the micro-organism in or on treated articles, food or feedingstuffs must be addressed.

2.9.2 Further information required (283/2013; 6.2)

Non-viable residues (283/2013; 6.2.1)

Non-viable residues could be non-viable micro-organisms or metabolites/toxins produced by the active micro-organism either during fermentation or during growth of the active micro-organism after application. Information on levels of non-viable residues in or on the crop is required when the following applies:

- metabolites or other chemical substances of concern are present in the product; and/or
- metabolites of concern are expected to be produced by the micro-organism in or on the crop

If a metabolite of concern has been identified, this should be addressed in the consumer risk assessment taking into account the two points above. When a concern exists that, due to *in situ* production, metabolite concentrations are above the expected natural background level, the performance of field trials to measure the concentration of the metabolite of potential concern shall be carefully checked.

If the metabolite of concern is present in the MASAM, a consumer risk assessment should be provided for the maximum level that the metabolite may be present in the product (see also 2.3.2, ‘Specification of the material as manufactured and of the product’). 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 (CFU/kg per ha) and the metabolite concentration (in mg/ha), the maximal residue of the 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).

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

metabolite of concern are foreseen and risk to humans cannot be excluded, residue studies may be required.
To determine concentrations of metabolites, edible parts that have been treated with the mPPP in accordance with representative use (as part of efficacy trials performed with the representative formulation) can be chemically analysed. By determining the concentration of metabolites, both the exposure resulting from the presence of the metabolite in the product and from *in situ* production are covered. 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.

Viable residues (283/2013; 6.2.2)
If the information on persistence and multiplication indicate that persistence of relevant amounts of the micro-organism may occur than possible risk to humans and/or animals must be investigated, unless it can be justified that the micro-organism are not hazardous to humans in the concentrations that could occur as a result of the authorised use.

2.10 Fate and behaviour in the environment

The basis for the assessment of the environmental fate and behaviour of a micro-organism is information regarding its origin and the properties, and regarding the survival of both the micro-organism and its potential residual metabolites after application.

The assessment of the environmental fate and behaviour therefore partly relies on information that is also required in Section 2 of the assessment dossier, reflecting the data requirements on the biological properties (2.1-2.9). To avoid duplication within the dossier, it is preferred to provide the full description of the paragraphs from Section 2 listed below related to the biological properties of the micro-organism as part of the current section (using the same headers). A summary of this information should be provided in Section 2, along with a reference to the current section. The paragraphs from Section 2 that should be described here, are (first number between brackets refers to the numbering used in this document):

- **Origin and natural occurrence (1.3.1; 283/2013; 2.1.2)**
  The geographical region and the place in the ecosystem (e.g. host plant, host animal, or soil from which the micro-organism was isolated) must be stated. Information must be provided on the geographical range and habitat of the strain and species. Moreover, if information is available on the natural abundance (prior to application) of the species/strain in natural systems, this information should be provided to support the environmental evaluation.

- **Infectiveness, dispersal and colonisation ability (1.3.2; 283/2013; 2.5)**
  Information on possible dispersal routes of the micro-organism (via air as dust particle or aerosols, with host vectors etc.) under typical environmental condition should be reported.

  The persistence of the micro-organism and information on its life cycle under the typical environmental conditions of use must be indicated. In addition, any particular sensitivity of the micro-organism to certain environmental conditions (UV light, temperature, pH, humidity, nutrition requirements etc.) should be provided.

Growth temperature:
The growth temperature provides information which is relevant to human health risk and some other non-target animals such as mammals and birds. If the growth temperature is comparable to human body temperature, this may indicate a potential for infection. In contrast, a growth temperature incompatible with human body temperature could indicate a low concern for infectivity in humans. Therefore, a study on the growth of the specific strain of micro-organism should be provided. If the growth temperature data is used to waive infectivity/pathogenicity studies than the growth temperature study should be carried out under GLP. Please note that the growth temperature data is not sufficient for waiving all toxicological infectivity/pathogenicity studies.

- **Genetic stability and factors affecting it (1.3.4; 283/2013; 2.7)**
  Information on genetic stability (e.g. mutation rate of traits related to the mode of action or uptake of exogenous genetic material) under the environmental conditions of proposed use must be provided.
  In addition, if the micro-organism contains plasmids or other mobile genetic elements known to be involved in pesticidal activity, pathogenicity, toxicity, resistance etc., the stability of the encoded traits shall be indicated.

Metabolites of concern
As mentioned under 2.4.5, 'Information on the production of metabolites (especially toxins)', hazardous metabolites are characterised according to (EU) No 283/2013 and by using the step-by-step process provided in the Guidance on the risk assessment of metabolites produced by micro-organisms used as plant protection active substances, SANCO/2020/12258.

For metabolites that are identified as a hazard, it may be necessary to determine the exposure route by means of a qualitative assessment using peer-reviewed literature. If this justification is sufficient and no concern is identified, no quantitative assessment is required. However, if according to Step 14 of the Guidance, the metabolite is considered to be of concern, a quantitive assessment is required in accordance with the current guidance for fate modelling. If the quantitative or semi-qualitative assessment described in steps 14 and 16 are not sufficient or do not propose an acceptable risk, or the metabolite is persistent and will significantly add to the natural background level data requirements conform part A of the regulation 283/2013 are necessary.

The quantitive assessment is required for the relevant environmental compartments of exposure. This exposure depends on the intended use, the concentration of the relevant metabolite in the technical grade MPCA and the *in situ* production of the relevant metabolite. Examples for relevance of compartments are provided in the SANCO/2020/12258 Guidance in Step 13.1. The information on the environmental fate and behaviour is required if the exposure of environmental compartments to the relevant metabolite cannot be excluded.

In contrast to chemical pesticides, no standard OECD test guidances are currently available for micro-organisms to provide data for the assessment of environmental fate and behaviour. As an alternative, OPPTS guidelines from the US Environmental Protection Agency can be used for the assessment dossier. In addition, all relevant scientific, peer-reviewed, open literature should be provided in the application.

To determine Predicted Environmental Concentrations (PEC) in relevant environmental compartments for relevant non-target organisms (i.e., non-target organisms for which a hazard is identified), the pesticide fate models developed for chemical active substances can be used (see FOCUS DG SANTE). When physical-chemical parameters needed as input for these

models are not available, conservative default values should be used as prescribed by the respective guidance documents.

2.10.1 Persistence and multiplication (283/2013; 7.1)

The persistence and multiplication of the micro-organism is assessed in three environmental compartments (soil, water and air) as described below, unless it can be justified that exposure of a specific environmental compartment is unlikely to occur. During the assessment, special attention is paid to the competitiveness of the micro-organism in question and to its population dynamics upon application of the biopesticide. The persistence and multiplication of the micro-organism is evaluated within the context of the ecology of the micro-organism based on information provided in section 2 on biological properties.

For each of the three compartments (soil, water and air) information is required to determine if it is expected that the micro-organism and relevant metabolites/toxins persist in the environment in concentrations considerably higher than the natural background levels, taking into account repeated applications over the years. A methodology to determine the natural background levels is suggested in Scheepmaker and Butt (2010)[13]. If the micro-organism is expected to be persistent, then a robust risk assessment should be provided to show that the risks from accumulated plateau concentrations are acceptable (Uniform Principles; point 2.7.7 of Commission Regulation (EU) 546/2011).

Soil (283/2013; 7.1.1)

If there is no expected exposure of soil to the micro-organism due to the use of the representative formulation according to the proposed use, a clear statement should be provided on why exposure to soil does not occur. In all other cases, the information as described below should be provided.

To assess the environmental fate and behaviour of micro-organisms in soil, the test guidelines for chemical pesticides (described in Part A of EU Regulation 283/2013) should be adapted in such a way that they are appropriate for micro-organisms. This means that the viability and population dynamics of the micro-organism upon application must be reported in several cultivated and uncultivated soils that are typical of the various EU regions where use exists or is anticipated, or in other media (e.g. rockwool) in which use is intended. The data should include population numbers of the micro-organism before application and during a time period of sufficient length after applications (including just after application). The method of quantification (e.g. counting of CFUs, or copy numbers) should be specific enough to draw conclusions about the dynamics of the applied test organism. Note that data from both laboratory and field tests can be used.
In addition, the initial predicted environmental density in soil (PEDsoil,initial) upon application of the representative formulation should be determined. This value can be calculated with the method described below.

PEDsoil

The method to calculate the Predicted Environmental Density (PED) in soil is based on a worst-case scenario. The application rate in CFU/ha and the total amount of applications per year is used to determine the initial PEDsoil. All applications are dosed at once, no degradation and growth is taken into account and no crop interception is taken into account.

PEDsoil (CFU/ kg dry soil) = AR x n per Y/ 10.000 x d x ρ

[13] Scheepmaker JWA, Butt TM (2010). Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil in relation with risk assessment and in accordance with EU regulations. Biocontrol Science and Technology 20, 503-552.

- AR is application rate (CFU/ha; assuming the highest concentration of the micro-organism according to the mPPP specification)
- n per Y is number of applications per year
- 10.0000 is the conversion factor from ha to m^2
- d is the thickness of the soil layer (default of 0.05 m)
- ρ is the density of soil (default of 1500 kg/m^3)

Water (283/2013; 7.1.2)

If there is no expected exposure of surface water to the micro-organism due to the use of the representative formulation according to the proposed use, a clear statement should be provided on why exposure to surface water does not occur. In all other cases, the information as described below should be provided.

The viability and proliferation of the micro-organism in natural water/sediment systems has to be addressed under both dark and illuminated conditions. The data should include population numbers of the micro-organism before application and during a time period of sufficient length after application (including just after application). The method of quantification (e.g. counting of CFUs, or copy numbers) should be specific enough to draw conclusions about the dynamics of the applied test organism. Note that data from both laboratory and field tests can be used. When data is missing for either dark or illuminated conditions, a statement should be included as to if and why the results for the one condition can be used for the both conditions.

The Initial Predicted Environmental Density in surface water (PEDsw,initial) upon application of the representative formulation should be provided. This value can be calculated with the method described below.

PEDsw

The method to calculate the PEDsw is a worst-case application scenario. The application rate in CFU/ha and the total amount of applications per year is required to estimate the PEDsw. All applications are dosed at once, no degradation and growth is taken into account.

PEDsw (CFU/L) = AR x n per Y x (D/100) / (10.000 x Vd)

- AR is application rate (CFU/ha)
- n per Y is number of applications per year
- D drift percentage
- 100 conversion of percentage
- 10.0000 is the conversion from ha to m^2
- Vd is volume of the standard ditch per m^2

Ctgb uses the BBA drift values[14] in combination with the TOXSWA standard ditch (30 cm deep with a slope of 45 degrees and volume of 210 L/m^2) to determine the PEDsw values for micro-organisms. For greenhouse uses of micro-organisms, Ctgb uses an emission percentage of 0.1%.

Air (283/2013; 7.1.3)

In case of particular concerns for operator, worker or bystander exposure, information on the concentrations in air should be provided.

[14] Ganzelmeier H, Rautmann D, Drift values according to the BBA (Federal Biological Agency of Agriculture and Forestry, Germany) 2000: Bekanntmachung des Verzeichnisses risikomindernder Anwendungsbedingungen für Nichtzielorganismen. Bundesanzeiger 100: 9878-9880.

2.10.2 Mobility (283/2013; 7.2)

The possible dispersal of the micro-organism and its degradation products in relevant environmental compartments has to be evaluated, unless it can be justified that exposure of the particular environmental compartments to the micro-organism is unlikely to occur. For each of the compartments which are exposed to the micro-organism upon application, information should be provided on the mobility of the micro-organism (e.g., dispersal of dormant stages, rain-splash dispersal).

In addition, information should be provided to demonstrate that the use of the micro-organism, under the proposed conditions of use, does not have any harmful effects on groundwater.

If the micro-organism poses a possible hazard to humans, animals or the environment, the applicant and the competent authority should first come to an agreement on which studies should be performed to provide sufficient information on the mobility of the micro-organism.

2.10.3 Additional information required regarding the uniform principles for evaluation and authorisation of plant protection products

1) No authorisation shall be granted if contamination of ground water, surface water or drinking water expected as a result of the use of a plant protection product under the proposed conditions of use, may cause interference with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC (point 2.7.2 of 546/2011).

If a route of exposure of ground water, surface water or drinking water upon application exists, information should be provided to demonstrate that there is no interference of the micro-organism or its residues with the analytical systems for the control of the quality of drinking water.

2) No authorisation shall be granted if it is known that transfer of genetic material from the micro-organism to other organisms, may lead to unacceptable effects on the environment (point 2.7.5 of 546/2011).

This information should be provided under point 2.7 of 283/2013 and does not need to be addressed in the environmental fate and behaviour section.

Additional information on the environmental risk assessment is for example available in the OECD Guidance to the environmental safety evaluation of microbial biocontrol agents (OECD Series on Pesticides No. 67) and EFSA literature review on microbial organisms used in plant protection products[15].

2.11 Effects on non-target organisms

2.11.1 Data requirements

Pending the acceptance of specific guidelines at international level, the information required for the risk assessment on non-target organisms shall be generated using available test guidelines accepted by the Competent Authority, i.e., US EPA's microbial pesticide test guidelines. The US EPA test guidelines do not require dose-response testing in the first Tier level, but instead a maximum hazard dose is tested, which is based on a safety factor times the maximum predicted environmental exposure. Where appropriate or if no US EPA test guideline is available, test guidelines as described for the data requirements in Part A of Commission Regulation (EU) No 283/2013 could be adapted in such a way that they are appropriate for micro-organisms (the relevant test guidelines are included in in Commission

[15] Mudgal S, De Toni A, Tostivint C, Hokkanen H, Chandler D (2013). Scientific support, literature review and data collection on microbial organisms used as active substance in plant protection products – Lot 1 Environmental risk characterisation. EFSA supporting publication 2013:EN-518.

Communication 2013/C 95/01 and Commission Communication 2013/C 95/02). Adaptation is for example relevant with respect to the test duration, which in the acute OECD guidelines usually is too short for investigating infectivity. Useful guidance for such adaptations and microbial testing in general is given by the Canadian authorities, who published a Guidance document for testing the pathogenicity and toxicity of new microbial substances to aquatic and terrestrial organisms in 2016. The guidance document is much more detailed regarding test methods than the US EPA guidelines and consultation is highly recommended when drafting study plans.

Testing shall include viable and, if appropriate, non-viable micro-organisms, and a blank control. In general, GLP studies are preferred, but peer reviewed, scientifically sound studies can also be accepted. In section 3 of Commission Regulation (EU) No 283/2013, it is stated that by way of derogation from point 3.1 (conducting tests under GLP) for the a.s consisting of micro-organisms and viruses, tests done to obtain data on safety with respect to other aspects 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 284/2013, meaning organisations with qualified personnel and suitable testing equipment.

Tests must be performed unless it can be justified that non-target organisms will not be exposed. When according to the applicant a certain study is not necessary, a relevant scientific justification can be provided for the non-submission of the particular study.

The data requirements for micro-organisms in Commission Regulations (EU) No 283/2013 and 284/2013 ask for information on toxicity, infectiveness and pathogenicity (except when stated otherwise) on the following non-target organisms:

- Birds
- Aquatic organisms:
  - Fish
  - freshwater invertebrates
  - algae (effects on algal growth, growth rate and capacity to recover)
  - plants other than algae (any effects)
- Bees
- Arthropods other than bees
- Earthworms
- Non-target soil micro-organisms (impact on relevant non-target micro-organisms and on their predators)

The Part B of Commission Regulation (EU) No 284/2013 refers to plant protection products containing micro-organisms including viruses.

According to the introduction to Section 10 “Effects on non-target organisms”, (i) “ *The information provided, taken together with that for the micro-organism(s), must be sufficient to permit an assessment of the impact on non-target species (flora and fauna), of the plant protection product, when used as proposed”* and (vi) “ *For the estimation of exposure of organisms all relevant information on the plant protection product and on the micro-organism must be taken into account. Where relevant the parameters provided for in this Section shall be used. Where it appears from available data that the plant protection product has a stronger effect than the micro-organism, the data on effects on non target organisms of the plant protection product have to be used for the calculation of relevant effect/exposure ratios”.*

Furthermore, in the introduction of each sub-section it is stated that “The *same information as provided in point 8 of Part B of the Annex to Regulation (EU) No 283/2013 has to be reported*

where it is not possible to predict the effects of the plant protection product on the basis of the data available for the micro-organism, unless it can be justified that exposure of birds is unlikely to occur"

Based on these requirements, the Ctgb is of opinion that the justification for not providing formulation data should be always provided. Data on formulation is not required if the formulation is a mixture of a micro-organism and water.

In cases where inert ingredients are used, a formulation testing waiver based upon the fact that the co-formulants are "inert" and thus non-hazardous is not accepted without further justification, as these may nevertheless have physical mechanisms of action relevant for some non-target organism groups. 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 plant protection products.

The focus NTOs for physical mechanisms of action should be bees, foliar and soil arthropods, and soil meso- and macro-fauna[16,17]. However, aquatic 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.

When oily co-formulants are included, generally a physical mode of action via suffocation is expected. For conventional chemicals, Chapter 7 version 2.4 of the Ctgb Evaluation Manual presents the approaches for testing and risk assessment for oily active substances and for products with a high concentration of oily components. The excerpt from the Evaluation Manual is included here and considered to be equally applicable to plant protection products based upon micro-organisms and containing a high concentration of oily components:

"*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 hrs). 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: - other a.s. with a mode of action aimed at suffocation of the target organisms, and - products with a high percentage of oily components*".

The choice of the appropriate test organism shall be based on the identity of the micro-organism (including the host-specificity, mode of action and ecology of the organism).

[16] 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

[17] Karise R. and Mand M., 2015, Recent insights into sublethal effects of pesticides on insect respiratory physiology, Insect Physiology, 2015:5, 3

Furthermore, the focus non-target organisms are also dependent on application method (e.g.for soil applications, overspray/dust is not relevant).

As described in more detail under 2.4.5, 'Information on the production of metabolites (especially toxins)', hazardous metabolites are assessed according to the data requirements of (EU) No 283/2013 and particularly by following the systematic presented in the Guidance on the risk assessment of metabolites produced by micro-organisms used as plant protection active substances, SANCO/2020/12258.
For metabolites of potential concern that are identified as a hazard (either through analysis of literature data and/or by running laboratory studies with the MASAM), it may be necessary to determine if they are of concern for the risk assessment. This step is conducted for the metabolites present in the product and also metabolites formed *in situ*. The relevance of *in situ* production should be based, according to the Guidance document on " *information on the proposed use, the ecology of the micro-organism including the environmental conditions which trigger the production of the metabolite, and the properties of the metabolite may be used* ". Justification of natural production should be considered (see Stage 3, Step 14).

2.11.2 Risk assessment

An active micro-organism may give rise to risks because of its potential to infect and multiply in host systems, or due to its ability to produce relevant toxic metabolites during the production of the MCPA and/or in contact with the (non-)target organism. Therefore, the risk for non-target organisms should be assessed, unless it can be demonstrated that non-target organisms will not be exposed.

For the environmental risk assessment, information obtained by the characterisation and identification of a micro-organism forms the starting point. This information is obtained in the sections on "Identity, Biological properties and Further information on the micro-organism" (section 1-3 in the data requirements). Additional useful information may be found in the section on environmental fate and behaviour (section 7) and residues in plants (section 6). The proposed manner of use defines the nature and extent of potential exposure.

In short, the risk evaluation should take into consideration the following information:

- Mode of action 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
- Where relevant, other authorised uses of the plant protection product 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

No Guidance Document for the environmental risk assessment 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 'chemical' approach, but using a crop interception value of 0% for predicted densities in soil and using BBA (Ganzelmeier and Rautmann) drift values in combination with an 'all at once' worst-case loading approach for predicted densities in water (see section 1.9 for further considerations).

The use of the chemical Guidance for the risk assessment for birds and mammals (EFSA 2009) is considered less relevant, since exposure parameters in this Guidance (e.g. DT50, RUD) are based on chemical databases.

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. 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 metabolites/toxins, according to the decision criteria in Regulation (EU) No 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, the Ctgb is using the approach of PRAPeR M2 and derives a margin of safety (i.e., MoS) by comparing the endpoint with exposure. Based on this evaluation it is decided whether the risk is acceptable or not by considering the mode of action, information of the ecology of the micro-organisms and the assumptions used for the calculating the exposure will need to be considered in a weight-of-evidence in order to conclude if a risk is expected.
For further guidance it can be referred to the OECD Guidance to the environmental safety evaluation of microbial biocontrol agents (OECD Series on Pesticides No. 67).

Relevant information from the open literature can be found in an EFSA literature review on microbial organisms used in plant protection products[18].
For a general discussion and working approach on metabolites/toxins, please refer to section 2.4.5 'Information on the production of metabolites (especially toxins)' in this Evaluation Manual.

Specifically for the ecotoxicology section, information that can be used to determine the expected exposure of different non-target species to the relevant metabolites includes:

a) The concentration of the metabolite of potential concern in the MASAM and/or mPPP.
b) The *in situ* production of the metabolite of potential concern (e.g., by determining quantities of metabolites in insects in the case of entomopathogenic fungi).
c) For exposure of the environment, relevant information includes:
    - Degradation of the metabolite in the relevant environmental compartments.
    - Uptake potential of the metabolite
    - In the case of non-target species, the information on the type of application (e.g., F, G and/or I) and MoA can help to determine which non-target species will be exposed to the metabolite.

2.12 Efficacy

The data requirements for efficacy for a low-risk product can differ markedly from those for a conventional product. At the start of the efficacy evaluation the status of the product (low-risk or not) is however not known with certainty. In some cases a product based on a low-risk substance may not receive low-risk status as mitigation measures need to be prescribed due to the risk assessment.

In most cases however the outcome of the evaluation should be predictable. When in doubt the applicant is advised to contact the Ctgb to discuss the possible low-risk status of the product, and the approach for the efficacy dossier.

Description of the product and mode of action.
Microbial products may require specific environmental conditions to reach optimal

[18] Mudgal S, De Toni A, Tostivint C, Hokkanen H, Chandler D (2013). Scientific support, literature review and data collection on microbial organisms used as active substance in plant protection products – Lot 1 Environmental risk characterisation. EFSA supporting publication 2013:EN-518.

effectiveness, or may have other characteristics that need to be understood when evaluating their effectiveness. In addition the evaluation of these products depends for a large part on the mode of action of the active substance. To facilitate evaluation of the dossier by the ZRMS and concerned member states it is very important to clearly describe the micro-organism and its mode of action. If the micro-organism has multiple modes of action an attempt should be made to clarify which mode(s) of action are the most relevant.
In addition, (part of) the plant protecting action of a micro-organism may be caused by a metabolites. Elucidating the mode of action of such metabolites is equally critical to the downstream assessment (see SANCO/2020/12258 for addressal of these cases).

Specific guidance is available for certain modes of action. There is a general EPPO standard for plant protection products with a predominant mode of action as plant defence inducers (elicitors), PP1/(319). Preliminary and supporting trials for these types of product can differ from other modes of action.

2.12.1 Evaluation of the efficacy dossier

General EPPO standards

Because of the lower associated risk, there is more room for flexibility regarding the level of effectiveness and variability for low-risk microbial products. In addition there are other characteristics that differ from conventional products. To address these issues EPPO has drafted a specific standard on the principles of efficacy evaluation for low-risk plant protection products, PP1/(296). This standard contains essential information on reduced data and efficacy requirements for these types of products and should be taken into account when writing a dossier for a low-risk product. This evaluation manual does not repeat the content of this EPPO standard, but provides some further context.

The low-risk standard PP1/(296) is also used for non-microbial low-risk products, and therefore does not go into much detail on specific characteristics of micro-organisms. For biopesticide products based on micro-organisms another standard is available (*Principles of efficacy evaluation for microbial plant protection products* EPPO PP1(276)), this standard is also relevant for microbial products that are not low-risk.

Specific EPPO standards

The EPPO standards database includes many standards on specific plant pathogen combinations. It should be noted that these have mostly been written with conventional products in mind. As low-risk products often have novel application methods, label claims or modes of action, existing standards may not be fully relevant.

In principle EPPO standards should be followed, and trials should be performed according to GEP. When deviating from GEP and/or EPPO standards, the applicant should give a clear justification for the use of alternative (trial) data. Valid data from other sources, e.g. published papers and laboratory studies, may be used to supplement this data.

IPM and spray programmes.

Biopesticides are often used in an integrated pest management system. Most EPPO standards however assume that only one product is used in a testing programme, as multiple products may complicate the interpretation of trial results. It is relevant to note that the new EPPO standard for plant defence inducers/elicitors (PP1/(319)) includes several paragraphs with guidance on how to test efficacy in mixtures, or in spray programmes with other products. If properly motivated some of these principles may also apply to other low-risk products.

2.12.2 Extrapolations

The aforementioned EPPO standard PP1/(296) provides guidance on data requirements for low-risk products. It should be noted that when this standard is followed a robust dataset and number of trials is still required even if requirements are reduced (refer to the standard for details). Low-risk products have a major advantage however in the extent of extrapolations that are possible. As a result, a low-risk product may end up with a much wider label claim compared to a conventional product with a similar initial claim supported by trials.

For a more detailed description please refer to chapter 9 (extrapolation possibilities for effectiveness of PP1/196, in addition some further context is provided below, consisting of an explanation of extrapolations in general, followed by a section specifically for low-risk products.

Principles of extrapolation
The regular extrapolation principles (non low-risk) are described in EPPO Standard PP 1/257 “Efficacy and crop safety extrapolations for minor uses”. Extrapolations are either based on extrapolation tables, or on expert judgement. Extrapolation tables that can be used are available from the EPPO website. For the Dutch situation additional possibilities exist; Dutch national extrapolation tables are available in our Evaluation Manual as an appendix of Chapter 8 Efficacy (also available in English). This national document has not been approved by EPPO, however it can be reffered to using expert judgement.

It should also be noted that the Netherlands take a flexible approach to the requirement in PP 1/257 that extrapolations are from major to minor crops only. For Dutch labels extrapolations may also be possible to major crops.

Extrapolation for low-risk products
The above-mentioned extrapolation tables have mostly been written for conventional crop protection products. For low-risk products different extrapolations may be possible using expert judgement. The possibility for extra extrapolations depend for a large part on the mode of action of the micro-organism, the biology of the target pest or disease, and the micro-organism itself.

It is therefore important that the applicant clearly describes the mode of action of the active substance and the reasoning behind the extrapolations, and if possible provides literature studies that support these extrapolations. Where multiple modes of action are claimed the relative importance of the different modes of action should be described.It is advisable to contact Ctgb or schedule a pre-submission meeting (PSM) if more information is required.

2.12.3 Resistance management
For information on the evaluation of the occurrence or possible occurrence of the development of resistance of target organisms please refer to paragraph 1.4.1 in this document

3. Botanicals

3.1 Introduction

The relevant EU Guidance document for botanicals is the Guidance document on botanical active substances, SANCO/11470/2012. In this guidance, a botanical active substance is defined as follows:

A 'botanical active substance' consists of one or more components found in plants and obtained by subjecting plants or parts of plants of the same species to a process such as pressing, milling, crushing, distillation and/or extractions. The process may include further concentration, purification and/or blending, provided that the chemical nature of the components is not intentionally modified/altered by chemical and/or microbial processes.

The botanical active substances that are covered by the guidance are:

- Plant powders
- Unprocessed plant extracts
- Processed plant extracts
- Highly refined plant extracts
- Complex mixtures of plant extracts

Not included in the guidance are extracts from genetically modified organisms and chemically derived analogues of plant extracts (which can be referred to as mimics, natural-identical synthesized molecules and biosimilars).

The approval criteria and legal frame work for botanical active substances are also described in the guidance document. In principle, plant protection products (PPPs) containing botanical active substances have to be approved under Regulation (EC) No 1107/2009 and a dossier has to be compiled according to the data requirements as laid down in parts A of Regulation (EU) No 283/2013 (active substance) and Regulation (EU) No 284/2013 (plant protection product). The evaluation should take into account the uniform principles for the evaluation and authorisation of plant protection products as described in Commission Regulation (EU) No 546/2011.

For many botanicals there is a long historical use and exposure is known. If the use and exposure are documented in peer reviewed open literature or from other reliable sources these data can be used for the dossier. In case a botanical is used in another regulatory context than the approval for the use as active substance in a PPP, such as in human nutrition, animal feeding, cosmetics, as a fertiliser, in pharmacopoeia, or as a biocide, these data may also be used for the dossier.

For all botanicals that are subject to application all available relevant knowledge and information in literature should be provided. The literature search should be carried out in accordance with the EFSA Guidance on the submission of scientific peer-reviewed open literature (EFSA Journal 2011; 9(2): 2092). Literature retrieved from this search should be reported in the relevant sections of the dossier.

3.2 Physical chemical properties and analytical methods

The Method of manufacture should be reported in detail. All steps should be described including, amount of solvent, pressure, temperature, time, type of filter/distillation/extraction.

The 5-batch analysis has to reflect the practical production. Therefore, It should contain a number of products taken from at least 2 crop seasons and, if applicable, several sources of starting material.

Especially for Group 3 botanicals (i.e., '*Botanical active substances that are not based on a material with an established specification*'; see guidance document SANCO/11470/2012 – rev.8, Section 7), the criteria for characterization of the botanical extract may in specific cases be disproportionately difficult, if not impossible, to satisfy.
The approach presented in the guidance requires the identification and quantification of at least 80 % w/w of the extract. This pragmatic threshold solely exists because there has been no way to characterize a complex mixture to a degree that only its active components and components of concern are identified – note here that the regulatory framework has no interest for constituents that are neither active nor (eco)toxicologically relevant.
Due to scientific developments in the field of metabolomics, there may now however be a way to address the analytical core challenge involved – notably in cases where proportionally applied conventional methodology is not fit for purpose. Although the existing framework does not yet accommodate these methods, Ctgb holds that only familiarization and experience-building could enable eventual implementation. Addition of these innovative methods is therefore encouraged, if only for the fact that there is no other feasible way to obtain the data they promise to generate.
In this, Ctgb maintains an assertive interpretation of EFSA's opninion on the use of 'omics methods in risk asessment, that recognizes the methods' indispensable value in the field of risk assessment, and identifies the need to employ them in order to generate confidence in the support they may provide.

For the determination of the phys-chem properties of both the botanical and the plant protection product, referral is made to the regular Evaluation Manual (chapter 2). Preferably, all phys-chem data for the main components should be submitted. Especially since botanicals are often complex mixtures, and it may not be feasible to test all properties of the complete mixture.

All properties required for CLP labelling should be addressed; for products containing a botanical, these points can often be covered by a waiver. For the technical properties a waiver is not allowed.

3.3 Mammalian toxicology

Botanical active substances are not per se non-toxic and it should be ensured that the botanical active substance does not lead to any harmful effects on the health of operators, worker, bystanders or residents under the conditions of use.

Botanical active substances should not lead to any harmful effects. The approach to address this depends on the intended use with corresponding exposure levels and whether there is information available on the botanical active substance from document uses, e.g. biocide, food or medicinal use, which may be relevant for the exposure assessment. The guidance document on botanical active substances states that reference values and good quality assessments from other regulatory frameworks may be taken into account if the basis for the derivation of these thresholds can be assessed. In accordance with Regulation (EU) 1107/2009 the deliberate administration of an active substance to humans with the purpose of determining no effect levels is prohibited. However, if data is available from e.g. clinical studies if the botanical active substance is used in human medicine these data are considered acceptable.

When available data on background exposure show similar exposure levels compared to the plant protection use than further animal testing should be avoided. This would be the case when similar exposures to known levels of the botanical active substance by the same routes have occurred in large population groups for many years without adverse effects being

reported e.g. in epidemiological studies. It is advised to discuss the proposed background exposure level with the rapporteur member state prior to submission of the dossier. When comparing the exposure levels from the plant protection use to the background exposure all relevant exposure groups, i.e. operators, workers, bystanders and residents, should be taken into account. To this end typical exposure models, such as the EFSA AOEM, can be applied as is done for chemical active substances.

For botanical active substances lacking a substantially reported history of use or for botanical active substances whose intended use levels will significantly exceed historical use or background exposure levels the assessment has to rely on basically the same set of data as for synthesized chemical active substances (default approach) with options for scientifically justified deviations from data requirements. At the moment there is no EU agreement on what the minimum data requirements would be for botanical active substances for which background exposure levels are exceeded or not available. It is recommended to discuss the test strategy with the rapporteur member state prior to dossier submission.

In case the botanical active substance contains components of concern with known toxic properties the significance of overall exposure to the component of concern should be assessed and compared with existing health-based guidance values. If no specific health based reference value is available consideration of exposure to the component of concern in relation to the Threshold of Toxicological Concern (TTC) values may also be helpful to avoid unnecessary animal testing.

3.4 Residues

Assessment of the possible residues of botanical active substance is required to make sure that there is no risk for the consumers after the exposure via food to the plant protection product containing a botanical active substance.

In particular cases, evaluation of residues could be waived, based on relevant argumentation provided by the applicant:

It is acknowledge that if the proposed botanical active substance is considered to be the same material that is reasonably expected to be or to become a component of food, this provides considerable reassurance for consumer exposure. The applicant is asked to provide a reasoned case/evidence to the way the material complies with relevant food legislation, confirming that technical material is the same as that is supplied to the food industry. The same applies to “feed”.

For many botanical active substances, residue data may not be required if it has been determined that detectable residues on the consumable commodity are unlikely to occur, or that residue levels are unlikely to exceed natural exposure and when the residues are not of toxicological concern. The (scientific) rationale including a comparison between the residue levels arising from the use as a plant protection product and levels from the naturally occurrence or other use, should be demonstrated by the applicant.

Food or feed

In case of botanical active substances listed as food and feed (Annex I of Regulation No 396/2005), information on nature and magnitude of residues is usually not necessary. For those botanicals, normally no MRLs are set and they are included in Annex IV of Regulation (EC) No 396/2005).

Not food or feed

Though occurring naturally, for botanical active substances as a minimum, information on the

nature and magnitude of residues on plants and processed products is needed for the consumer risk assessment. Further information e.g. concerning the nature and magnitude of residues in livestock or in succeeding crops may often be addressed by a reasoned case.

If MRLs are in place or needed, residue data will be needed to show compliance with these MRLs. If relevant toxicological endpoints are established and residues on food and/or feed cannot be excluded, a consumer risk assessment will be required. It is advised to discuss this approach at an early stage with the rapporteur member state.

3.5 Fate and behaviour

The aim of the environmental risk assessment is to ensure that botanical active substances for use in plant protection products do not have any unacceptable effects on the environment. Botanical active substances are not *per se* non-toxic and often risk mitigation measures may be necessary to avoid risk for the environment.

The application of the guidance to specific cases will depend on the nature of the botanical active substance, its intended uses, exposure levels and whether there is information on the botanical active substance from documented use (e.g. as plant protection product, biocide, in food or medicine) these may be relevant for the exposure and effect assessment.

In the fate and behaviour assessment the aim is to identify areas of potential unacceptable effect on the environment and to assess whether the exposure levels do not result in unacceptable effects. The natural occurring exposure levels should be compared with the exposure levels resulting from use of the product in the different environmental compartments (i.e., soil, water and air).

Components from botanical active substances occur naturally in plants and it is to be anticipated that there will be common pathways for their breakdown and decomposition in plants and the environment. Further guidance on the assessment of fate and behaviour of botanical active substances in the environment may be developed by the European Food Safety Authority in the future.

For the application of botanicals in protected crops an emission percentage of 0.1% should be used, as the "Guidance document on clustering and ranking emission of active substrates of plant protection products and transformation products from protected crops (greenhouses and crops grown under cover) to relevant environmental compartments" has no scenario for botanical active substances. Alternatively, if the botanical consists of components with known endpoints, the greenhouse emission model (GEM) should be used for the assessment.

3.6 Effects on non-target species

The aim of the ecotoxicological risk assessment is to ensure that botanical active substances for use in plant protection products do not have any acute or long-term unacceptable effects on non-target species. Botanical active substances are not *per se* non-toxic to non-target organisms, therefore risk mitigation measures may be necessary.

The risk from botanical active substances used in plant protection products can be considered acceptable if the estimated exposure is lower or similar to the natural exposure and no unacceptable effects occur on the non-target organisms. If the estimated exposure is higher than the natural exposure, additional data must be submitted to assess the possible effects on the non-target organisms. The guidance document on botanical ative substances does not provide a general testing strategy, but instead recommends that applicants propose a relevant testing strategy in line with the mode of action, proposed use(s) and the relevant exposure situation, avoiding animal testing when unnecessary.

3.7 Efficacy

For efficacy it is important to know if a botanical is low-risk or not. Botanical products that do not receive low-risk status should be evaluated as conventional products.The data requirements for efficacy for a low-risk product can differ markedly from those for a conventional product and are described here. At the start of the efficacy evaluation the status of the product (low-risk or not) is however not known with certainty. In some cases a product based on a low-risk substance may not receive low-risk status as mitigation measures need to be prescribed due to the risk assessment.

In most cases however the outcome of the evaluation should be predictable. Where there is doubt the applicant is advised to contact the Ctgb to discuss the low-risk status of the product, and the approach for the efficacy dossier.

Efficacy evaluation

General EPPO standards

Because of the lower associated risk, there is more room for flexibility regarding the level of effectiveness and variability for low-risk products. In addition there are other characteristics that differ from conventional products. To address these issues EPPO has drafted a specific standard on the principles of efficacy evaluation for low-risk PPPs, PP1/(296). This standard contains essential information on reduced data and efficacy requirements for these types of products and should be taken into account when writing a dossier for a low-risk botanical. This evaluation manual does not repeat the content of this EPPO standard, but provides some further context.

In addition to this standard, specific guidance exists for products with a predominant mode of action as plant defence inducers (elicitors), PP1/(319). Preliminary and supporting trials for these types of product can differ from other modes of action.

Specific EPPO standards

The EPPO standards database includes many standards on specific plant pathogen combinations. It should be noted that these have mostly been written with conventional products in mind. However as the mode of action and method of application of botanicals is usually quite similar to conventional products, these should in most cases be usefull for botanicals.

In principle EPPO standards should be followed, and trials should be performed according to GEP. When deviating from GEP and/or EPPO standards, the applicant should give a clear justification for the use of alternative (trial) data. Valid data from other sources, e.g. published papers and laboratory studies, may be used to supplement this data.

IPM and spray programmes.

Biopesticides are often used in an integrated pest management system. Most EPPO standards however assume that only one product is used in a testing programme, as multiple products may complicate the interpretation of trial results. It is relevant to note that the new EPPO standard for plant defence inducers/elicitors (PP1/(319)) includes several paragraphs with guidance on how to test efficacy in mixtures, or in spray programmes with other products. If properly motivated some of these principles may also apply to other low-risk products.

Extrapolations

The aforementioned EPPO standard PP1/(296) provides guidance on lower data requirements for low-risk products. It should be noted that when this standard is followed a robust dataset and number of trials is still required even if requirements are reduced (refer to the standard for details). Low-risk products have a major advantage however in the extent of extrapolations

that are possible. As a result, a low-risk product may end up with a much wider label claim compared to a conventional product with a similar initial claim supported by trials.

For a more detailed description please refer to chapter 9 (extrapolation possibilities for effectiveness) of PP1/196. In addition some further context is provided below, consisting of an explanation of extrapolations in general, followed by a section specifically for low-risk products.

Principles of extrapolation
The regular extrapolation principles (non low-risk) are described in EPPO Standard PP 1/257 “Efficacy and crop safety extrapolations for minor uses”. Extrapolations are either based on extrapolation tables, or on expert judgement. Extrapolation tables that can be used are available from the EPPO website. For the Dutch situation additional possibilities exist; Dutch national extrapolation tables are available in our Evaluation Manual as an appendix of Chapter 8 Efficacy (also available in English). This national document has not been approved by EPPO, however it can be reffered to using expert judgement.

It should also be noted that the Netherlands take a flexible approach to the requirement in PP 1/257 that extrapolations are from major to minor crops only. For Dutch labels extrapolations may also be possible to major crops.

Extrapolation for low-risk products
The above-mentioned extrapolation tables have mostly been written for conventional crop protection products. For low-risk botanicals different extrapolations may be possible using expert judgement. The possibility for extra extrapolations depend for a large part on the mode of action of the botanical. It is therefore important that the applicant clearly describes the mode of action of the active substance and the reasoning behind the extrapolations.
Where multiple modes of action are claimed the relative importance of the different modes of action should be described.`It is advisable to contact Ctgb or schedule a PSM if more information is required.

Resistance management
For information on the evaluation of the occurrence or possible occurrence of the development of resistance of target organisms please refer to paragraph 1.4.1 in this document

4. Semiochemicals

4.1 Introduction

The information in this section is taken from the EU guidance document on semiochemical active substance and plant protection products (SANTE/12815/2014 – rev.5.2).
Semiochemical active substances have to be approved under Regulation (EC) No 1107/2009 and a dossier has to be compiled according to the data requirements as laid down in Part A to Regulation (EU) No 283/2013 (active substance) and Part A to Regulation (EU) No 284/2013 (Plant Protection Product; PPP). The evaluation should take into account the uniform principles for the evaluation and authorisation of plant protection products as described in Commission Regulation (EU) No 546/2011.

Semiochemicals are substances or mixtures of substances emitted by plants, animals, and other organisms that evoke a behavioural or physiological response in individuals of the same or other species. To be used as PPP, semiochemicals can for example be used in conjunction with dispensing devices, as granular product, or as seed treatment product.
Different types of semiochemicals are:

- Allelochemicals produced by individuals of one species that modify the behaviour of individuals of a different species (i.e. an interspecific effect). They include allomones (emitting species benefits), kairomones (receptor species benefits) and synomones (both species benefit).
- Pheromones produced by individuals of a species that modify the behaviour of other individuals of the same species (i.e. an intraspecific effect; for example straight-chained lepidopteran pheromones).

In plant protection products or biocides these semiochemicals can have different functions.

(Determined in the Netherlands: C-302.I.5)
Semiochemicals can act as:

- *Attractant*
- *Repellent*
- *Disruptor (for example mating disruptors)*

Depending on the function, and the target organism, either an authorisation as a plant protection product, a biocide, or no authorisation is required. The following decision table can be used.

Decision table for semiochemical products.

Types of semiochemicals in the product	***Extra function of the product***	***Plant Protection Product (PPP)***	***Product to protect against harmful organisms or to prevent effects of these organisms (BPR)***
Attractant	*None*	*Authorisation is not required*	*No authorisation required**
	Mechanical control	*Authorisation is not required*	*BPR authorisation required***
	Insecticide or other kind of active ingredient	*PPP authorisation is required (semiochemical is not an active ingredient)*	*BPR authorisation requried*
Repellent	-	*PPP authorisation is required (semiochemical is an active ingredient)*	*BPR authorisation required*

(mating) Disruptor	-	*PPP authorisation is required (semiochemical is an active ingredient)*	*BPR authorisation required*

** Including traps for insects and pests without the intention to influence the population.*
*** The intention is to limit the population.*

Calculations of the natural exposure levels are provided in the EU guidance document that also provides methods to compare naturally occurring exposures levels with levels achieved by a product containing semiochemical. When based on the use of plant protection product a similar exposure is achieved (within one order of magnitude by the same route) to the natural exposure level of the semiochemical, the risk characterisation is concluded. No further information is needed with the exception of identity, characterisation and analytical methods. If the exposure levels of the product are higher than the naturally exposure the guidance document provides per application method the data required. The exposure and requirement per aspect will be briefly described in the following paragraphs.

The OECD Series on Pesticides Number 12 Guidance for registration requirements for pheromones and other semiochemicals used for arthropod pest control will be updated with the EU guidance document on semiochemicals which will result in an update OECD guidance document. The update of the OECD guidance is expected to be available end 2017.

The guidance document on the assessment of new substances falling into the group of Straight Chain Lepidopteran Pheromones (SCLPs) (SANCO/5272/2009 – rev.3) can be used in order to add a new SCLP to the group of approved active substances, using a simplified procedure for the assessment. Currently this GD is being updated to be in line with current legislation and the EU guidance on semiochemicals. The list of SCLPs already approved can be found in the Commission review report (SANCO/2633/08 – rev.14).

For all semiochemicals that are subject to application all available relevant knowledge and information in literature should be provided. The literature search should be carried out in accordance with the EFSA Guidance on the submission of scientific peer-reviewed open literature (EFSA Journal 2011; 9(2): 2092). Literature retrieved from this search should be reported in the relevant sections of the dossier.

4.2 Identity, physical chemical properties

For the determination of the phys-chem properties of both the semiochemical and the plant protection product, referral is made to the regular Evaluation Manual (chapter 2). All properties required for CLP labelling should be addressed. For the technical properties a waiver is not allowed.

4.3 Mammalian toxicology

For semiochemicals it should be ensured that they do not have any harmful effects on the health of consumers (see section 3.4), operators, workers, bystanders or residents.

To ensure this the semiochemicals can be divided into two groups.
- Group 1: the only exposure route is via the vapour phase, e.g. retrievable dispensers (category 1A and 1B), non-retrievable dispensers (category 2A) and dosable matrix (category 2B). In addition, the exposure (by the same route) caused by the plant protection product use is within one order of magnitude to natural exposure levels of the semiochemical.
- Group 2: the exposure cannot be related to a natural background exposure level further hazard identification.

For group 1 no further hazard or risk characterisation would be required. However, for group 2 further hazard identification and risk assessment would be needed.

For the hazard identification the dossier should comply with the data requirements as laid down in Part A to Regulation (EU) No. 283/2013. In general data requirements can be fulfilled by submitting studies, a reasoned approach and/or relevant literature. Reference values or good quality assessments from other regulatory frameworks may be taken into account if the basis for the derivation of these thresholds can be assessed and any data access issues have been addressed by the applicant. Extrapolating from one semiochemical active substance to another (read-across) will be considered when accompanied by evidence of comparable relevant properties. This approach has been followed for the well-defined group of SCLPs. If no information from other regulatory frameworks or structurally similar semiochemicals is available further toxicity testing may be required to derive reference values.

For the exposure assessment the following exposure scenarios should be taken into account:

	Retrievable dispensers		**Non-retrievable application techniques**				
	Passive	**Active**	**Passive dispensers**	**Dosable matrix**	**Capsule suspension**	**Granular application**	**Seed treatment**
	1A	**1B**	**2A**	**2B**	**2C**	**2D**	**2E**
Operator exposure contact	Y	N	Y	Y	Y	Y	Y
Operator exposure inhalation	Y	N	Y	Y	Y	Y	Y
Worker exposure contact	Y	N	Y	Y	Y	Y	N
Worker exposure inhalation	Y	Y	Y	Y	Y	Y	N
Bystander exposure contact	N	N	N	N	Y	Y	N
Bystander exposure inhalation	Y	Y	Y	Y	Y	Y	Y
Resident exposure contact	N	N	N	N	Y	Y	N
Resident exposure inhalation	Y	Y	Y	Y	Y	Y	Y

Y = Yes, N = No

Vapour exposure can be estimated using the approach described in the guidance document on semiochemicals (Chapter 6, step II). For other exposure routes standard approaches as is done for chemicals, e.g. the EFSA AOEM, can be applied.

4.4 Residues and MRLs in or on treated products, food and feed

Assessment of the possible residues of semiochemicals active substance is required to make sure that there is no risk of the consumers after the exposure via food to the plant protection product containing a semiochemical active substance.

In general, for semiochemicals residue data may not be required if it has been determined that quantifiable residues (limit of quantification according to Regulation (EC) No 396/2005) on the consumable commodity are unlikely to occur or that reside levels are unlikely to exceed natural exposure levels during outbreaks of the pest (see Section 2.4.1 of Guidance Document on Annex IV; SANCO/11188/2013 – rev.2 or later). This can be demonstrated by a scientific rationale. In this case, an application for inclusion in Annex IV of Regulation (EC) No 396/2005 should be done by the applicant and the same time as it is applied for the approval of the

active substance.

When the exposure route for the commodity is by the vapour phase only or when those conditions are not fulfilled it is referred to Guidance document on semiochemical active substances and plant protection products.

If MRLs are in place or needed, residue data addressing the data requirements will be needed to show compliance with these MRLs or to propose new MRLs.

4.5 Environmental fate and behaviour and Effects on non-target species

As described in the introduction when use of the plant protection product results in similar exposure (within one order of magnitude by the same route) to the natural exposure level of the semiochemical, the risk characterisation is concluded for the aspects fate and behaviour and effects on non-target organisms. No further information is needed. The guidance document provides information and examples how to compare natural background exposure with the product exposure.

If the exposure levels of the product are higher than the naturally occurring exposure levels the following exposure routes for the environment and non-target species should be taken into account.

The following table is taken from the guidance document.

Table 3.5-01 Compartment for which exposure is expected

	Retrievable dispensers		Non-retrievable application techniques				
	Passive	Active	Passive dispensers	Dosable matrix	Capsule suspension	Granular application	Seed treatment
	1A	1B	2A	2B	2C	2D	2E
Soil	N	N	N	N	Y	Y	Y
Groundwater	N	N	N	N	Y	Y	Y
Surface water	Y*	Y*	Y*	Y*	Y	Y	Y
Sediment	Y*	Y*	Y*	Y*	Y*	Y*	N
Air	Y	Y	Y	Y	Y	Y	Y
Birds and mammals	Y	Y	Y	Y	Y	Y	Y
Aquatic organisms	Y*	Y*	Y*	Y*	Y	Y	Y
Reptiles and amphibians	Y*	Y*	Y*	Y*	Y	Y	Y
Non target athropods (above ground)	Y	Y	Y	Y	Y	Y	Y**
Soil invertebrates	N	N	N	N	Y	Y	Y
Pollinators	Y	Y	Y	Y	Y	Y	Y

Y = Yes; N = No
* FOCUS (2008) air guidance regarding short range deposition estimations to surface water bodies should be followed.
** Unless information is provided that the active substance is not systemic so not taken up by the roots (e.g. use of the Briggs equation to calculate transpiration stream concentration factor on the transpiration stream concentration).

The guidance document on botanical active substances does not provide a general testing strategy for non-target organisms, but instead recommends that applicants propose a relevant testing strategy in line with the mode of action, proposed use(s) and the relevant exposure situation, avoiding animal testing when unnecessary.

4.6 Efficacy evaluation of semiochemicals

The data requirements for efficacy for a low-risk product can differ markedly from those for a conventional product. At the start of the efficacy evaluation the status of the product (low-risk or not) may however not yet be known. In some cases a product based on a low-risk substance may not receive low-risk status. In most cases however this should be predictable. Where there is doubt the applicant is advised to contact the Ctgb to discuss the low-risk status of the product, and the approach for the efficacy dossier.

Evaluation of the efficacy dossier

General EPPO standards

An EU guidance document on semiochemical actives and plant protection products is available (SANTE/12815/2014 – rev.5.2). This document also covers some efficacy aspects. For more detailed guidance on efficacy requirements however, reference should be made to EPPO standards.

Because of the lower associated risk, there is more room for flexibility regarding the level of effectiveness and variability for low-risk products. In addition there are other characteristics that differ from conventional products. To address these issues EPPO has drafted a specific standard on the principles of efficacy evaluation for low-risk plant protection products, EPPO standard PP1/(296). This standard contains essential information on reduced data and efficacy requirements for these types of products and should be taken into account when writing a dossier for a low-risk product This evaluation manual does not repeat the content of this EPPO standard, but provides some further context.

The low-risk standard PP1/(296) is also used for low-risk products that are not semiochemicals. As such it does not go into much detail about pheromone specific issues, and partly refers to other standards on this subject.

Guidance specific for mating disruption pheromones is available in EPPO standard PP1/264(2). Many of the principles in this standard are also relevant for semiochemicals that have a different mode of action than mating disruption.
Trial setup may depend for a large part on behaviour and mobility of pests, and how these are affected by the semiochemical. It is possible that for a certain pheromone only incomplete guidance is available. It is advisable to contact Ctgb or schedule a PSM if more information is required. When deviating from GEP and/or EPPO standards, the applicant should give a clear justification for the use of alternative (trial) data. Valid data from other sources, e.g. published papers and laboratory studies, may be used to supplement this data.

In addition to this general standard on mating disruptors several specific EPPO standards are available. These should be used for products where the relevant uses are claimed:
PP1/314(1); *Evaluation of mating disruption techniques against Lepidopteran pests in grapevine, pome and stone fruits under field conditions.*
PP1/323(1); *Evaluation of mating disruption techniques against Lepidopteran pests in grapevine, pome and stone fruits under semi-field conditions.*

Extrapolations

Pheromones are very different from conventional products and as such, conventional extrapolation tables are not relevant. Semiochemicals are often pest specific and act by modifying behaviour. The plant species is not relevant in relation to the product's performance. For that reason extrapolation is possible to other crops in which the same pest appears. In the case of semiochemicals that have multiple targets, extrapolation to a group of related species may be possible if properly motivated.