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

Microorganisms, Botanicals and Semiochemicals

version 1.1; July 2018

ctgb

Board for the Authorisation of plant protection products and biocides

Biopesticides - Plant Protection Products
Microorganisms, Botanicals and Semiochemicals

Changes in the Evaluation Manual Biopesticides

Evaluation Manual Biopesticides Microorganisms, Botanicals, Semiochemicals			
Version	**Date**	**Paragraph**	**Changes**
1.0	July 2017		Initial biopesticides E.M.
1.1	June 2018	1.4.1 1.11 2.7 3.6	implementation of new EPPO standard "*principles of efficacy evaluation for low risk plant protection products"* PP1/(296). (All efficacy related paragraphs updated.)

General introduction

In this Manual we consider *biopesticides* plant protection products that contain microorganisms (including viruses), botanicals, or semiochemicals as active ingredient. These groups of ingredients have different data requirements and guidances, which justifies a separate Evaluation Manual.

This Biopesticides Evaluation Manual (Biopesticides E.M.) describes in more detail the data requirements and risk assessment for biopesticides. Especially for botanicals and semiochemicals this Biopesticide E.M. should be read in conjunction with the Evaluation Manual EU part and national elements, which is dealing with the conventional (chemical) plant protection products, i.e. part A of the data requirements.

This Biopesticides E.M. describes the Dutch evaluation of biopesticides in the EU framework under Regulation (EC) No 1107/2009. This Evaluation Manual addresses the evaluation of biopesticides based on the data requirements and uniform principles,. Mainly those issues that need further explanation are addressed. Where needed, important information from the Regulations or additional explanations and interpretations are provided.

The risk assessment described in this E.M. can be used both for the approval procedure for microorganisms, botanicals, and semiochemicals as active substance, as well as for zonal and interzonal applications for the authorization of biopesticides (i.e. core registration reports).

The Biopesticides E.M. is divided in three sections:
Section 1 describes the risk assessment and data requirements for the use of microorganisms as active substance. Section 2 deals with botanicals. In section 3, the evaluation of semiochemicals is described.

If the active substance used in the plant protection product has been registered under the plant protection framework in other non-EU countries, or under a different regulatory framework, the dossier and evaluation should preferably be made available.

Article 51 authorisations are authorised on a national level and may also be relevant for low risk products. For the Netherlands more information about article 51 authorisations can be found on the minor use section of the Ctgb website.

1. Biopesticides based on Microorganisms

1.1 Introduction

Under Regulation (EC) No 1107/2009 a microorganism is defined as any microbiological entity, including lower fungi and viruses, cellular or non-cellular, capable of replication or of transferring genetic material.

Due to the ability of microorganisms to proliferate, there is a clear difference between chemical active substance and microbial active substance. Hazards arising from microbial active substances are not necessarily of the same nature as chemicals and these differences should be taken into account in the assessment.

The approval of microbial active substances is done on strain/isolate level. The exception to this is the group of **Baculoviruses** which have been approved on species level. A separate Guidance Document is available on how new isolates of Baculovirus species can be evaluated and added to the already approved isolates (SANCO/0253/2008 rev. 2).

For all microorganisms 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.

The data requirements are laid down in Part B of Commission Regulation (EU) No 283/2013 for active substances and in Part B of Commission Regulation (EU) No 284/2013 for plant protection products (PPP) based on microorganisms. The uniform principles for the evaluation and authorisation of plant protection products are described in Commission Regulation (EU) No 546/2011.

The Guidance Document on dossier preparation describes how the applicant should submit a dossier for the approval or the renewal of approval of an active substance which is a microorganism to comply with the Table of Contents described in Part B of the Annex to Regulation (EU) No 283/2013 and Part B of the Annex to Regulation (EU) No 284/2013.

For the submission of dossiers for zonal approval of plant protection products containing microorganisms, the microbial dRR formats should be used.

A guidance for applicants on preparing dossiers for the approval of a microbial active substance (SANCE/12545/2014 rev 2) is available on the EU website.

1.2 Identity of the microorganism

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

Each microbial active substance should 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 microorganisms 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.

1.2.2 Specification of the material used for manufacturing of formulated products (283/2013; 1.4)

A Guidance on the Assessment of the equivalence of technical grade active ingredient for identical microbial strain or isolates (SANCO/12823/2012 rev 4) is available.

Content of the microorganism (283/2013;1.4.1)
The minimum content of the microorganism should be reported. Appropriate terms that are relevant to microorganism, e.g. colony forming units (CFU) per volume or weight, should be applied. Information of a maximum content should also be reported if concern for human health or the environment exists due to exposure to the microorganism or if relevant metabolites are produced.

Identity and content of impurities, additives, contaminating microorganisms (283/2013;1.4.2)
It should be shown that the level and nature of contaminating microorganisms are within the acceptable limits as stated in the OECD issue paper on microbial contaminant limits for microbial pest control products. Batch analysis should be provided to show that the TGAI complies with the OECD issue paper. This should be done under GLP.

If relevant metabolites are formed by the microorganism they shall be identified and characterised at different states or growth stage of the microorganism.

If present, additives (eg. organic solvents) contaminating the microorganisms have to be identified and quantified.

Analytical profile of batches
In principle five representative batches from recent and current industrial scale production of the microorganism shall be analysed for content of pure microorganism, impurities, additives and relevant metabolites, as appropriate. Submission of less than 5 batches should be justified.

The requirements in part A 1.11 regarding determination of all components in quantities of 1 g/kg or more and at least 980 g/kg of the material to be analysed is considered not appropriate for microorganisms. Relevant impurities and relevant metabolites need to be addressed.

Where the information provided relates to a pilot plant production system, the information required shall again be provided once industrial scale production methods and procedures have stabilised. Where available, industrial scale data shall be provided before approval under Regulation (EC) No 1107/2009. Where data on industrial scale production are not available, a justification shall be provided.

1.3 Biological properties of the microorganism

1.3.1 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 microorganism 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 microorganism 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.

1.3.2 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 microorganism given in the section Fate and behaviour in the environment. It should include the following information:

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

- The persistence of the microorganism and information on its life cycle under the typical environmental conditions of use must be indicated. In addition, any particular sensitivity of the microorganism 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.

1.3.3 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 microorganisms 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 microorganism 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.

1.3.4 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 microorganism and factors affecting it given in the section Fate and behaviour in the environment.

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

Microbial metabolites are the intermediates or products of the metabolism of a microorganism and are not to be confused with the metabolites that are the result of degradation of a chemical active substance. Microorganisms produce a wide range of metabolites, mostly as a result of growth or as a response to environmental conditions in order to regulate their own growth, control competitors or to foster other organisms beneficial to them. In this process microorganisms can also produce toxins.

The interpretation of the data requirements for metabolites/toxins is still an issue of considerable debate in the EU. Guidance is not yet available. The recent Commission Review Reports indicate that requesting information on all possible metabolites, which might (hypothetically) be produced under relevant environmental conditions, go beyond the concept of "foreseeable risk" and the scope of the data requirements according to Regulation (EU) No 544/2011 part B and in particular the requirements of paragraph 2.8.

The first step regarding microbial metabolites for the risk assessment is to determine if the microorganism is expected to produce metabolites of potential concern for humans and/or the environment. Ctgb will refer to these metabolites as *relevant metabolites* in order to distinguish them from the thousands of non-harmful metabolites that can be produced by microorganisms (see Figure below). The term relevant metabolites does not a priori imply that

they are of concern to humans and/or the environment, as this depends on the exposure. Information on the ability of a microorganism to produce a relevant of metabolite should be deduced for example from:

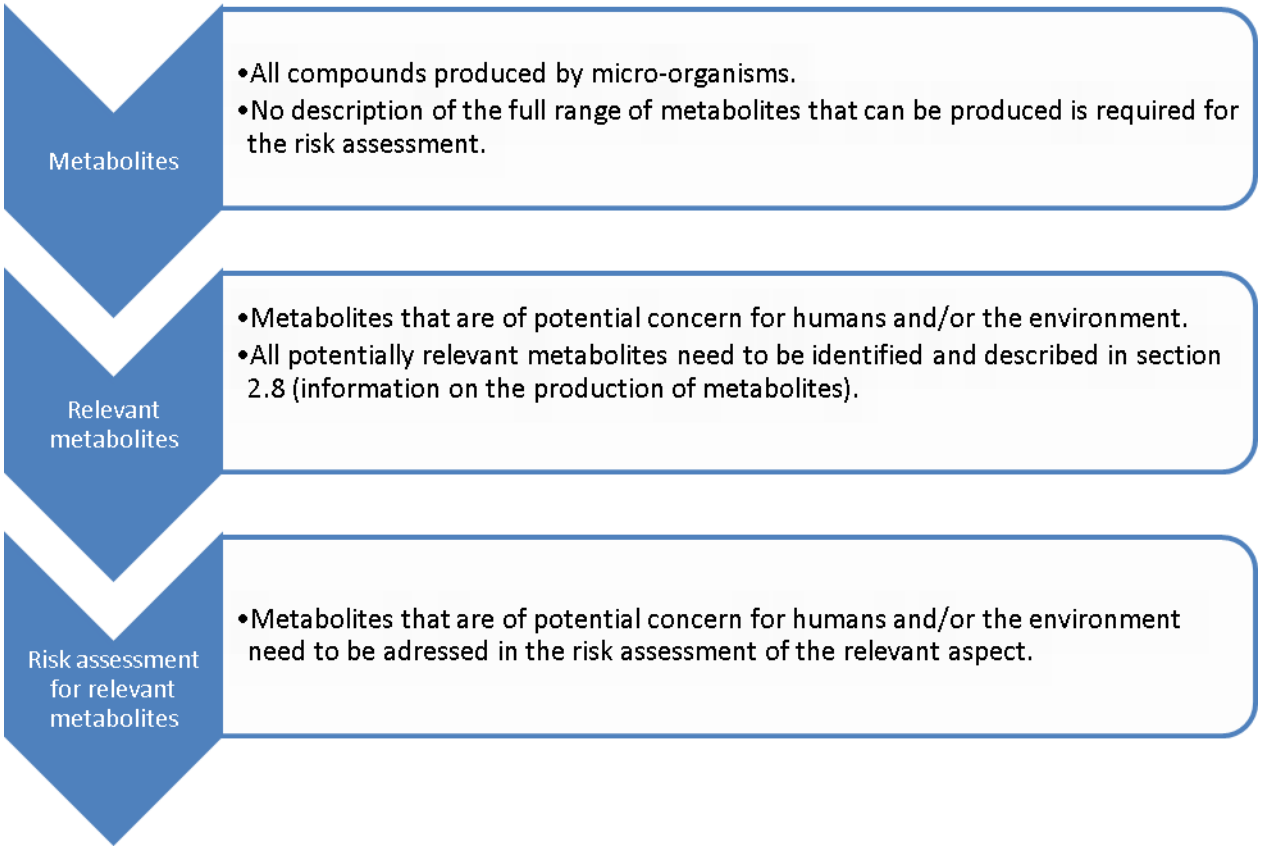
(a) presence of relevant metabolites in the MPCA and/or MPCP
(b) (eco)toxicity studies;(either guideline studies or information from published scientific literature.)
(c) information e.g. in published scientific literature on:

- biological properties of the microorganism;
- relationship to known plant, animal or human pathogens and the potential of related species and strains to produce relevant metabolites/toxin. The text under the data requirement states that if other strains belonging to the same microbial species as the strain applied for are known to produce relevant metabolites with unacceptable effect to human health and/or the environment, the nature and structure of this substance, its presence inside or outside the cell and its stability, its mode of action (MoA) (including external and internal factors of the microorganism necessary to action) as well as its effect on humans, animals or other non-target species shall be provided. However, if it can be proven that the microorganism for approval does not have the genes to produce these metabolites, no additional information on these metabolites should be needed.
- MoA.

When on the basis of this information relevant metabolites are identified for the MPCA, the expected exposure of humans and the environment must be assessed in the risk assessment in the relevant sections. In these sections it should be assessed if these relevant metabolites have adverse effects on humans and/or the environment at the expected exposure levels. This expected exposure consist of both the concentration of the potentially relevant metabolite in the product, as well as the *in situ* production of the potentially relevant metabolite in the environment. Note that as the exposure differs per aspect, the relevance of a particular metabolite can also differ per aspect; for example a metabolite that is relevant for the ecotoxicological assessment may not be relevant for the assessment of human toxicology.

For MPCA’s that have been on the market for several years and for which no adverse effects have been demonstrated for humans and the environment, Ctgb is of the opinion that the *in situ* formation of potentially relevant metabolites is of no concern as they are unlikely to be produced at relevant concentrations. However, since there is no EU harmonization on the issue of relevant metabolites further information is generally requested.

Therefore, whether or not a metabolite is considered to be a relevant metabolite, should be stated in the respective sections. More information on how to address the relevant metabolites in the risk assessment is included in each specific section in this Evaluation Manual.



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

Two issues should be addressed. First, many microorganisms 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 biocidal product. The level of production of any known antibiotics used in human or veterinary medicine by the microorganism must be indicated.

In addition, information on the microorganism's resistance or sensitivity to antibiotics or other antimicrobial agents must be provided. 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.

1.4 Further information on the microorganism (283/2013; 3)

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

Low risk plant protection products 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) microorganisms 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. Microorganisms 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 microorganisms 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 microorganism, but not to other strains of the same species. This differs from conventional plant protection products where often cross resistance exists between many active substances.

1.5 Properties plant protection product

1.5.1 Content of the microorganism and co-formulants in the plant protection product (284/2013; 1.4)

The content of the microorganism in the plant protection product should be reported in % w/w and in the appropriate units (eg. CFU/kg, spores/kg, IU/g). For liquid preparations, the content should also be reported in g/L.

For co-formulants in the plant protection product, referral is made to the regular evaluation manual (Chapter 2).

When the production process is a continuous process of an end-use product the five batch analysis can be provided for the product instead of the MPCA. The investigation should include the content of the microorganisms as well as to show that the product complies with OECD issue paper on microbial contaminants. This should be done under GLP.

1.5.2 Phys-Chem and technical properties of plant protection products containing microorganisms (284/2013; 2)

For the determination of the phys-chem properties referral is made to the regular Evaluation Manual EU part (chapter 2).

The accelerated storage stability study does not have to be performed if the microorganisms are not compatible with higher temperatures. The shelf life study may be performed at lower temperatures. In this case, the label should include the correct storage temperature.
The contaminating microorganisms should be determined before and after storage unless a reasoned case can be made that these contaminants cannot be formed during storage. The correct, commercial packaging type should be used in the storage stability study and should be indicated.

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

1.6 Analytical methods (283/2013; 4)

1.6.1 Methods for the analysis of the microorganism as manufactured (283/2013; 4.1)

The following analytical methods should be provided:

- Method for the identification of the microorganism
- Method for providing information on possible variability of seed stock/active microorganism

- Methods to differentiate a mutant of the microorganism from the parent wild strain
- Methods for the establishment of purity of seed stock from which batches are produced and methods to control that purity
- Methods to determine the content of the microorganism in the manufactured material used for the production of formulated products and methods to show that contaminating microorganisms are controlled to an acceptable level
- Methods for the determination of relevant impurities in the manufactured material
- Methods to control the absences and to quantify (with appropriate limits of determination) the possible presence of any human and mammalian pathogens
- Methods to determine storage stability, shelf-life of the microorganism, if appropriate

The method to identify the microorganism should be capable of identifying the microorganism at strain level.

The method to quantify the content of the microorganism in the manufactured material should be a validated method. In addition, the method to show that contaminating microorganisms are controlled to an acceptable level should be validated.

If the microorganism has the potential to produce a relevant metabolite with harmful effects to human health and/or the environment a validated method should be provided which can detect and quantify this relevant metabolite.

1.7 Effects on human health (283/2013; 5)

Hazards arising from microorganisms should be assessed differently from chemicals. Microorganisms are unlikely to be toxic in themselves but they may produce toxic metabolites. Microorganisms 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 microorganisms. Pending the acceptance of specific guidelines at international level, the information required shall be generated using available test guidelines accepted by the competent authority (e.g. US EPA microbial pesticide test guidelines: https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-885-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 microorganisms.

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 microorganisms 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.

1.7.1 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 microorganism. Preferably, these reports must include data from persons exposed in manufacturing plants or after application of the microorganism (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 microorganism or of any taxonomically related strains and species should be considered to assess whether the active microorganism is known to cause infection and pathogenicity in humans. If the microorganism in the study is a different species than the microorganism being assessed, it is important to clarify what distinguishes the two and whether it is likely that the active microorganism could exhibit the same properties. For such an analysis, information on the biological properties of the microorganism 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 microorganism.

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 microorganisms. 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 microorganisms. At the moment all microorganisms are regarded as potential sensitisers and the following precautionary phrase should be included on the label: Microorganisms may have the potential to provoke sensitising reactions'. In case there is clear evidence in literature that the microorganism is a respiratory sensitiser, classification applies instead of the warning phrase.

Acute toxicity, pathogenicity and infectiveness (283/2013; 5.2.2)

Studies on acute oral and inhalation toxicity, pathogenicity and infectiveness must be reported.

The inhalation toxcity can be tested either through inhalation or intratracheal exposure. Intratracheal exposure would ensure adequate exposure of the test animal to the microorganisms. For the inhalation exposure, generally the concentration of microorganisms 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 microorganisms 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 microorganism 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 microorganisms 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 biocides. However, since microorganisms 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, 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* (J.T. MacGregor, 2005[1]).

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 microorganism to infect, replicate in, transform or cause toxicity in the cell system. The data requirements state that for intracellular replicating microorganisms, 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.

1.7.2 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

[1] James T. MacGregor. Genetic Toxicity Assessment of Microbial Pesticides: Needs and Recommended approaches. Report to OECD. December 2005

adverse human effects. In particular, if results from earlier studies indicate that the microorganism may cause long-term health effects, studies on chronic toxicity, pathogenicity and infectiveness, carcinogenicity and reproductive toxicity must be carried out. Microorganisms 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.

1.7.3 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)
A skin irritation and eye irritation study is 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 microorganisms no study for skin 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 is needed the following precautionary phrase should be included on the label: Microorganisms may have the potential to provoke sensitising reactions’. In case there is clear evidence in literature that the microorganism is a respiratory sensitiser, classification applies instead of the warning phrase.

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

In the absence of appropriate test methods all microorganisms 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 microorganisms. In the Netherlands RPE is required for powder formulations but not for liquid formulations or granule formulation which are nearly dust-free.

Exposure to relevant metabolites:
The exposure assessment should include any relevant metabolites/toxins present in the product. If quantitative data is available for a relevant metabolite, the 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 and resident. Since in general 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.

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.

1.8 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 microorganism 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 microorganism and its metabolites are not hazardous to humans in the concentrations that could occur as a result of authorised use.

1.8.1 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 microorganism in or on treated articles, food or feedingstuffs must be addressed.

1.8.2 Further information required (283/2013; 6.2)

Non-viable residues (283/2013; 6.2.1)
Non-viable residues could be non-viable microorganisms or metabolites/toxins produced by the active microorganism either during fermentation or during growth of the active microorganism after application. Information on levels of non-viable residues in or on the crop is required when the following applies:

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

If a relevant has been identified this should be addressed in the consumer risk assessment taking into account the two points above.

If the relevant metabolites is present in the MPCA than a consumer risk assessment should be provided for the maximum level that the metabolite may be present in the product.

In addition, potential in situ production of the relevant metabolite needs to be addressed. Relevant information that can address this concern includes:

a) Translocation of the microorganism to the edible part of crop, e.g. for seed treatment
b) Persistence and multiplication of the microorganism on crops

c) Degradation of the relevant metabolite on crops
d) Residue data on the potentially relevant metabolite

Full residue data as required for chemicals is rarely needed as usually sufficient information is available to address the concern. However, if significant quantities of the relevant metabolite are expected and a risk to humans cannot be excluded residue studies may be required.

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

1.9 Fate and behaviour in the environment (283/2013; 7)

The basis for the assessment of the environmental fate and behaviour of a microorganism is information regarding its origin and the properties, and regarding the survival of both the microorganism 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 microorganism 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 microorganism 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 microorganism (via air as dust particle or aerosols, with host vectors etc.) under typical environmental condition should be reported.

  The persistence of the microorganism and information on its life cycle under the typical environmental conditions of use must be indicated. In addition, any particular sensitivity of the microorganism 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 microorganism 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 microorganism 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.

Relevant metabolites
All relevant metabolites of the MPCA that are identified in the section 'information on the production of metabolites' (283/2013; 2.8; see section 1.3.5 of this evaluation manual) need to be addressed in the assessment of the environmental fate and behaviour. Information on these relevant metabolites includes:

- Exposure of environmental compartments to the relevant metabolite. The exposure depends on the intended use (e.g., protected crop vs. field use, application method), the concentration of the relevant metabolite in the MPCP, and the *in situ* production of the relevant metabolite. As an example, the latter can be addressed for entomopathogenic fungi by information on quantities of the relevant metabolite in insects.
- Information on the environmental fate and behaviour of the relevant metabolite if exposure of environmental compartments to the relevant metabolite cannot be excluded. In the section 'persistence and multiplication' (1.9.1), information on the degradation rate of the relevant metabolite can be addressed. The adsorption potential of the relevant metabolite can be included in the section 'mobility' (1.9.2).

In contrast to chemical pesticides, no standard OECD test guidances are currently available for microorganisms to provide data for the assessment of environmental fate and behaviour. As an alternative, OPPTS guidelines from the US Environmental Protection Agency (https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-885-microbial-pesticide-test-guidelines) can be used for the assessment dossier. In addition, all relevant scientific, peer-reviewed, open literature should be provided in the application.

1.9.1 Persistence and multiplication (283/2013; 7.1)

The persistence and multiplication of the microorganism 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 microorganism in question and to its population dynamics upon application of the biopesticide. The persistence and multiplication of the microorganism is evaluated within the context of the ecology of the microorganism 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 microorganism 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)[2]. If the microorganism is

[2] 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.

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).

A full assessment of the environmental fate and behaviour according to 283/2016 Part A.7 may be required for any relevant metabolites that have been identified in section 2.8 which meet all of the following criteria:

- the relevant metabolite is stable outside the microorganism
- the toxic effect of the relevant metabolite is independent of the presence of the microorganism
- the relevant metabolite is expected to occur in the environment in concentrations higher than under natural conditions

Soil (283/2013; 7.1.1)

If there is no expected exposure of soil to the microorganism 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 microorganisms 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 microorganisms. This means that the viability and population dynamics of the microorganism 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 microorganism 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

- AR is application rate (CFU/ha; assuming the highest concentration of the microorganism according to the product specifications)
- 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 microorganism 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 microorganism in natural water/sediment systems has to be addressed under both dark and illuminated conditions. The data should include population numbers of the microorganism 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[3] 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 microorganisms. For greenhouse uses of microorganisms, 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.

1.9.2 Mobility (283/2013; 7.2)

The possible dispersal of the microorganism 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 microorganism is unlikely to occur. For each of the compartments which are exposed to the microorganism upon application, information should be provided on the mobility of the microorganism (e.g., dispersal of dormant stages, rain-splash dispersal).

[3] Ganzelmeier and Rautmann 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.

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

If the microorganism 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 microorganism.

1.9.3 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 microorganism 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 microorganism 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[4].

1.10 Effects on non-target organisms

1.10.1 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 microbial pesticide test guidelines: https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-885-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 microorganisms (the relevant test guidelines are included in in Commission 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.

[4] Mudgal et al. 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 publications 2013: EN-518

Testing shall include viable and, if appropriate, non-viable microorganisms, 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 microorganisms 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 microorganisms 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 microorganisms (impact on relevant non-target microorganisms and on their predators)

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

1.10.2 Risk assessment

An active microorganism 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 microorganism forms the starting point. This information is obtained in the sections on “Identity, Biological properties and Further information on the microorganism” (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 microorganism in the environment
- Its ecological niche
- The natural background level of the active microorganism, 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 microorganisms 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 microorganism. 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 microorganisms, 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 microorganism will consist of a qualitative or semi-quantitative evaluation of the likelihood that an adverse effect will occur under the expected conditions of exposure. Based on this evaluation it is decided whether the risk is acceptable or not.

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 [5].

For a general discussion and working approach on metabolites/toxins it is referred to section 1.3.5 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 potentially relevant metabolite in the MPCA and/or MPCP.
b) The *in situ* production of the potentially relevant metabolite (e.g. by determining quantities of metabolites in insect in the case of entomopathogenic fungi).
c) For exposure of the environment, relevant information includes:
    - Degradation of the relevant metabolite in the relevant environmental compartments.
    - Adsorption potential of the relevant 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 helps to determine which non-target species will be exposed to the relevant metabolites.

[5] Mudgal et al. 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 publications 2013: EN-518

1.11 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.
Microbial products may require specific environmental conditions to reach optimal 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 memberstates it is very important to clearly describe the microorganism and its mode of action.

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 microorganisms. For biopesticide products based on microorganisms 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.

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 microorganism, the biology of the target pest or disease, and the microorganism 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 an RFM meeting 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

2. Botanicals

2.1 Introduction

The relevant EU guidance document for botanicals is the guidance document on botanical active substances. 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.

2.2 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.

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.

2.3 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.

2.4 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.

2.5 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) could be used for the assessment.

2.6 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.

2.7 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. 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. Botanical products that do not receive low risk status should be evaluated as conventional products.

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.

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 an RFM meeting 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

3. Semiochemicals

3.1 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 authorization as a plant protection product, a biocide, or no authorization 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) (SANTE/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).

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.

3.2 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.

3.3 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.

3.4 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.

3.5 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.

3.6 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

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.

Specific standards and other guidance

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.
Currently specific EPPO guidance is available for mating disruptors (EPPO standard PP1/264(1) Mating disruption pheromones). 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 an RFM meeting 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.

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.