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Guidance Document for the Regulatory Framework for the Microorganism Group: Bacteriophages

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GUIDANCE DOCUMENT FOR THE REGULATORY FRAMEWORK FOR THE MICROORGANISM GROUP: BACTERIOPHAGES

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Foreword

This Guidance Document is intended to provide guidance to industry and regulatory authorities, on regulatory approaches that can be taken for assessment and approval of bacteriophages to be used as plant protection products. Bacteriophages (or phages) are a group of microorganisms that are viruses specifically infective to bacteria, which can be considered a niche use.

This Guidance Document is an output of the OECD Expert Group on BioPesticides (EGBP), former known as BioPesticides Steering Group (BPSG), established by the Working Party on Pesticides in 1999 to help member countries to harmonise methods and approaches used to assess biological pesticides and to improve the efficiency of regulatory procedures. The first tasks the EGBP undertook were: (i) reviewing the regulatory data requirements for three categories of biopesticide (microbials, pheromones and invertebrates); and (ii) developing formats for dossiers and monographs for microbials, pheromones, and other semiochemicals. After tasks were concluded, the EGBP concentrated efforts on addressing the scientific and technical issues that act as barriers to the efficient regulation of biological pesticides by organising seminars and following up on the resulting recommendations. One of the recommendations of the 2017 OECD EGBP seminar on "Niche uses of highly specific biocontrol products" was development of overview documents for certain groups of microorganisms used as biopesticides (e.g., baculoviruses, bacteriophages).

The initial draft of this document was developed by a consultant (Roma L Gwynn) and was overseen by the EGBP with special input from the Expert Group members Chantal Arar (France), Anne Steenbergh (The Netherlands), and additional regulatory or technical experts Jonas Ptasinski (Canada), Clara Torres-Barceló (France) and Cécile van der Vlugt-Bergmans (The Netherlands).

The draft guidance was sent to the EGBP for comments on three occasions: June 2019, June 2020 and November 2021. The draft guidance was revised, based on comments received, and a group formed by government and academic experts finalised the document. The final draft was sent to the Working Party on Pesticides for comments and subsequent approval in March 2022. The document was approved by the Working Party on Pesticides in April 2022.

This document is being published under the responsibility of the Chemicals and Biotechnology Committee (CBC), which has agreed that it be declassified and made available to the public.

Table of Contents

1. INTRODUCTION

1. As a general principle, the level of hazard posed for health and environmental protection must be assessed to allow the product to be used in plant protection. Microorganisms in general can be approved for plant protection and there are some provisions and guidance available to support regulation of these microorganisms for this use (e.g. developed and published by OECD, EU, EPA, FAO). Bacteriophages (or phages) are a group of microorganisms that are viruses specifically infective to bacteria only, which can be considered a niche use. Currently, the available guidance for microorganisms for use in plant protection, does not directly address bacteriophages. This document is intended to provide guidance to both industry and regulatory authorities, in the context of the regulatory approach that can be taken for assessment and approval of bacteriophages to be used in plant protection products. This guidance document is applicable only in the framework of application for authorisations for plant protection products.

2. BACKGROUND

2. Plant pathogenic bacterial species in agriculture cause significant economic damage to a large number of crops during cultivation and storage that can be difficult to control. Bacteria represent ~15% of damaging plant pathogens that are seed borne or transfer directly on vegetative material (Annex 1). There are at least 38 different plant pathogenic species. Despite the importance of bacterial phytopathogens to agriculture, there are few plant protection products on the market and even fewer biocontrol products. A type of biocontrol organism that is active against bacterial plant pathogens are bacteriophages.

3. Bacteriophages are viruses and the most abundant organisms in the world found in a wide range of substrates such as soil, water, plants, and animals. They act as antibacterial agents, with different bacteriophages being specific to different hosts, often down to the level of bacterial strain within a species. For almost every bacterial species, at least one bacteriophage exists that can specifically infect that particular bacterial group.

4. While there are many approved uses of bacteriophages in food and feed processing, there are only six examples of bacteriophages approved for use in plant protection in OECD countries specifically USA and Canada; these applications were supported by the IR4 Project[1] in USA.

5. Within most OECD Member Countries, bacteria and fungi used as plant protection products are approved at strain level and the current practice is to consider each new strain on its own merits for registration. As for baculoviruses (OECD Series on Pesticide number 20), the strain concept is not fully applicable to bacteriophages. They consist of a mixture of different, often very similar genotypes and they are approved at species level. This guidance document addresses this aspect.

[1] The IR-4 Project was established in 1963 by the U.S. Department of Agriculture to address the challenges faced by the specialty crop farmers.

6. This Guidance Document is applicable for all bacteriophages intended to be used for plant protection.

7. For all applications and evaluation, there will be value in preparing an application and evaluation report using the data requirements indicated in the Annex 2, to this guidance document.

2.1. Definitions

8. In the framework of this Guidance Document the following definitions apply.

Bacterial strain

9. A strain is a genetic variant of an organism in its taxonomic rank (species) that is made up of the descendants of a single isolation in pure culture and usually is made up of a succession of cultures ultimately derived from an initial single colony.

Bacteriophage

10. A bacteriophage is a type of virus that infects bacteria.

Contaminant (microbial)

11. A pathogenic/infective micro-organism unintentionally present in the technical grade ingredient.

Infectivity/infectiveness

12. The ability of a micro-organism to cause an infection. An infection may or may not result in overt disease.

Material for production

13. Material for production is considered to be all ingredients used for the manufacturing of the technical grade active ingredient.

Metabolite

14. A metabolite is a general term to refer to any substance produced by a microorganism. Viruses are unable to produce metabolites. For this document, metabolite refers only to secondary metabolites (i.e. metabolites which are not essential for basic life processes of the microorganism).

Microorganisms

15. Microbiological entities, cellular or non-cellular, capable of replication and/or of transferring genetic material. The definition applies to, but is not limited to, bacteria, fungi, protozoa, viruses and viroids; nematodes are not included.

Pathogenicity

16. Ability of a micro-organism to inflict injury and damage to a host upon infection; it depends on host resistance or susceptibility. A pathogen is determined by three characteristics: invasiveness, infectivity and pathogenic potential. Invasiveness is the ability of the organism to spread to adjacent or other tissues. Infectivity is the ability of the organism to establish a focal point of infection. Pathogenic potential refers to the degree that the pathogen causes morbid symptoms.

Phage mixture

17. A specified combination of bacteriophages also commonly referred to as a 'cocktail'.

Relevant metabolite

18. A relevant metabolite is a metabolite of concern for human or animal health and/or the environment. It may pose a risk by its properties and its concentration. Therefore, some toxins are considered relevant metabolites.

Technical grade active ingredient (TGAI)

19. TGAI is the outcome of the manufacturing process of the micro-organism(s) intended to be used as active substance in plant protection products. It consists of the micro-organism(s) and any additives, metabolites product, chemical impurities, contaminating micro-organisms and the spent medium/rest fraction resulting from the production process.

Temperate phage

20. A phage type that is capable of performing a lytic and lysogenic cycle. In the lysogenic cycle, temperate phages do not kill the host but they insert their DNA into the host cell, either as a free plasmid or integrated into the chromosome, and are thus called "prophages".

Toxin

21. A toxin is a (organic) substance that is produced in nature and is able to injure or cause damage in a living organism.

Virus isolate

22. A virus isolate is a pure clone (genetically identical) derived from a wild population of a microorganism.

Virus species

23. According to the International Committee on Taxonomy of Viruses (ICTV, http://ictv.global), a species is the lowest taxonomic level in the hierarchy approved by the ICTV. Two phages are assigned to the same species if their genomes are more than 95% identical at the nucleotide level over their full genome length, tested reciprocally (Turner, et al. 2021).

Virulent phage

24. A phage type that is only capable of the lytic cycle, where it is multiplied by the host metabolic machinery and causes lysis of the bacteria it infects upon release of new phages.

2.2. Scientific and regulatory consideration

25. Bacteriophages were discovered 100 years ago and are the most abundant and diverse entities in the world estimated to be 10^{31} on Earth and abundant in a wide range of ecological niches such as soil, water (marine and fresh), plants and animals (Comeau *et al.* 2008). They are present in significant numbers in drinking water, on food and as normal commensals of humans and animals. Humans and animals naturally ingest large numbers of bacteriophages, with no known adverse effects.

26. By nature, bacteriophages are capable of infecting only bacteria (prokaryotes) and as such are obligate intracellular parasites of bacteria and reproduce by using their bacterial host's biosynthetic pathways. They are not capable of infecting eukaryotic

cells so they are not capable of infecting either animals, plants, or fungi. Bacteriophages are typically host specific, with host range limited to one or a few species of the same genus or often, one or a few strains of the same species. Their extreme specificity means that they will not infect any bacteria other than their host. For almost every bacterial species, there is at least one bacteriophage that can specifically infect that particular bacteria species or group.

27. In a comprehensive review of bacteriophages (Kimura et al. 2008), the authors note that viruses are not functionally-active outside of their host cells. Furthermore, since they are not in themselves metabolically active, bacteriophages are incapable of producing any metabolic products (including toxins) outside their hosts. Nowadays, research advances such as next generation sequencing allowing the full characterisation of phages, and the discovery of new bacterial defence systems against phages, will certainly help in the understanding and selection of efficient phage biocontrol agents for plant protection.

Commercial development

28. The use of virulent bacteriophages as biocontrol agents has great potential, based on their long history of safe use, relatively easy scale-up and handling, and their highly-specific antimicrobial activity. Since their discovery in the early 1900's, bacteriophages have attracted interest as antimicrobial agents against human bacterial pathogens and they have been used in such therapeutic situation for over 80 years. This interest has been revived recently due to the threat of antibiotic-resistant bacteria and there are a number of commercially-available bacteriophages-based products targeting human diseases. Other areas of application include water and food safety (as biocides), crop protection, food processing, cosmetics and animal health. In the area of food safety, the concept of combating pathogens in food by means of bacteriophages can be addressed at all stages of production throughout the entire food chain and indeed, bacteriophages have been used for over 10 years to control *Listeria* in meat, poultry, and fish. Some bacteriophages have been granted GRAS status in the USA (GRAS Notice 672) and their use approved for a number of food processing and packing industries, in addition to agricultural applications.

29. Despite the potential of bacteriophages for the control of plant pathogens, their commercial potential has not been fulfilled to date. Commercial developments face technical barriers of accommodating bacterial variation on both a geographical and temporal level. Bacteriophages are highly specific, which is a commercial disadvantage when a disease is often caused by a large range of bacterial strains and frequently by different bacterial species. Commercial developments, however, are developing technological approaches to address this such as the use of mixtures of bacteriophage isolates (also called phage cocktails).

30. The initial stage in developing a bacteriophages-based plant protection product (PPP) involves the isolation and enrichment of bacteriophages using enrichment techniques from a range of potential environmental sources (e.g. soil or plant material) with the host plant-pathogenic bacteria.

31. For large scale production, bacteriophages are typically co-cultured with their bacterial host in liquid medium. Fermentation or other batch-culture systems are usually used, allowing the control of the relevant growth parameters for optimal production. Once produced, bacteriophages are concentrated and purified; removing excess nutrients and the cell debris which remains following bacterial lysis. Individual bacteriophage isolates are produced separately and if required, mixed in defined ratios to increase the product's efficacy against a wide range of bacterial strains.

Regulatory precedence for bacteriophages

32. In the EU, EFSA has completed a comprehensive review of bacteriophages technology for use in food of animal origin including animal carcases, meat products and dairy products (EFSA, 2009, 2012). The principal debate in the EU centres around whether bacteriophages are able to prevent recontamination of food. EFSA's BIOHAZ Panel concluded that under specific conditions, bacteriophages may be very effective in the elimination of specific pathogens from foods. However, based on data currently available in peer-reviewed scientific literature, the Panel could not conclude whether bacteriophages can protect against bacteria in case the food becomes re-contaminated. Some relevant conclusions of this review were that "Bacteriophages in the environment behave as inert particles and tend to persist longer than their hosts. However, their long-term antibacterial activity is compromised on dry surfaces." and "The persistence in/on food varies with each bacteriophage, and with the conditions of application, including dose and physical and chemical factors associated with the food matrix."

33. EFSA's focus to date has been on the application of bacteriophages to food for combating human pathogens, mainly on food of animal origin, although also on vegetables in relation to Listeria. There has been no published consideration of the technology's potential for controlling bacterial pathogens of plants and plant products, either as processing aids or plant protection products.

34. Currently, there are few examples of bacteriophages that have been developed and registered as PPP (Table 1).

Table 1: Examples of bacteriophages registered as PPP in the USA and Canada.

Registering authority	Product name/producer	Target diseases	Target species	Registration details
EPA US	Agriphage (Omnilytics Inc., USA)	Bacterial spot of tomatoes & peppers Bacterial speck of tomatoes	*Xanthomonas campestris* pv. *vesicatoria* & *Pseudomonas syringae* pv. tomato	67986-1 (December 2005, amended June 2006 & October 2011)
EPA US	Agriphage CMM (Omnilytics Inc., USA)	Bacterial canker of tomatoes	*Clavibacter michiganensis* subsp. *michiganensis*	67986-6 (September 2011)
PMRA Canada	Agriphage CMM (Omnilytics Inc., USA)	Bacterial canker of tomatoes	*Clavibacter michiganensis* subsp. *michiganensis*	RD2012-21 (January 2012)
EPA US	AgriPhage-Citrus canker OmniLytics Inc.	*Xanthomonas citri* subsp. *citri*	*Xanthomonas citri* subsp. *citri*	67986-9 (September 2018)
EPA US	AgriPhage-Fire Blight OmniLytics Inc	*Erwinia amylovora*	*Erwinia amylovora*	67986-8 (February 2020)
EPA US	XylPhi-PD Otsuka Pharmaceutical Co., Ltd.	*Xylella fastidiosa*	*Xylella fastidiosa*	92918-1 (April 2019)

35. Both the EPA and PMRA have followed a similar route in determining the suitability of bacteriophages for plant protection, particularly concerning their apparent safety and very low toxicity profiles, based both on data either from scientific peer-reviewed literature or submitted by the applicant.

36. The conclusion of PMRA review of bacteriophages of *Clavibacter michiganensis* (subsp. *michiganensis*) was "By nature, bacteriophages are viruses that are only capable of infecting bacteria. Bacteriophages are not capable of infecting animals, plants, or fungi and are not capable of producing any toxins outside their hosts because they are not metabolically active. Bacteriophages rely on the bacterial host's metabolism for reproduction and survival. Bacteriophages themselves are not considered to be toxic. Also, since the host bacterium, *C. michiganensis* subsp. *michiganensis*, does not produce toxins nor is it otherwise considered to be harmful to humans, the infection of these bacteria by bacteriophages of *Clavibacter michiganensis* (subsp. *michiganensis*) will not alter the bacterial population in a way that could be harmful to humans. Although the relative exposure of people to bacteriophages of *Clavibacter michiganensis* (subsp. *michiganensis*) may increase from the use of AgriPhage-CMM, there have been no reports of adverse effects or incidents resulting from the direct exposure to naturally occurring bacteriophages."

37. EPA granted an exemption from the requirement of a tolerance for residues of Agriphage CMM bacteriophages in or on tomato when applied as a bactericide in accordance with good agricultural practices. They concluded : "there is a reasonable certainty that no harm will result to the U.S. population from aggregate exposure to residues of the lytic bacteriophages of *Clavibacter michiganensis* subspecies *michiganensis* produced in *Clavibacter michiganensis* subspecies *michiganensis*."

38. EPA granted an exemption from the requirement of a tolerance for residues of Agriphage-Citrus Canker noting that "the available data demonstrated that bacteriophages active against *Xanthomonas citri* subsp. *citri* are not anticipated to be toxic, pathogenic, or infective *via* any route of exposure. Furthermore, humans, including infants and children, have been exposed to bacteriophages through food and water, where they are commonly found, with no known adverse effects. Although there may be some exposure to residues of bacteriophages active against *Xanthomonas citri* subsp. *citri* that are used on citrus fruit in accordance with label directions and good agricultural practices, there is a lack of concern due to the lack of potential for adverse effects. EPA also determined that retention of the Food Quality Protection Act (FQPA) safety factor was not necessary as part of the qualitative assessment conducted for bacteriophages active against *Xanthomonas citri* subsp. *citri".* And "Based upon its evaluation, EPA concludes that there is a reasonable certainty that no harm will result to the U.S. population, including infants and children, from aggregate exposure to residues of bacteriophages active against *Xanthomonas citri* subsp. *citri.* Therefore, an exemption from the requirement of a tolerance is established for residues of lytic bacteriophages active against *Xanthomonas citri* subsp. *citri* that are produced in *Xanthomonas citri* subsp. *citri* in or on food commodities included in the fruit, citrus groups 10 and 10-10, when used in accordance with label directions and good agricultural practices."

39. In a consistent approach, EPA granted an exemption from the requirement of a tolerance for residues of the products Agriphage-Fire Blight and XylPhi-PD for a similar rationale, respectively.

2.3. Proposal for a regulatory approach for bacteriophages for use in plant protection

40. Reference is made to successful registrations of bacteriophages-based products for the control of plant bacterial pathogens in the USA and Canada. Within these, the applications provided few studies and addressed the data requirements by waiver or

reasoned case. The relevant authorities EPA and PMRA separately concluded that the products, when used in accordance with good agricultural practice, presented minimal or negligible risks to operators, consumers or the environment.

41. Currently, the available regulatory guidance for microorganisms do not specifically refer to bacteriophages. The intention of this document is to close this gap. A guidance document on a comparable organism, namely baculovirus, is available in the EU 'Guidance document on the assessment of new isolates of baculovirus species' (SANCO/0253/2008 rev. 2)'. Baculoviruses are natural pathogens of insects and other arthropods and in this document, baculoviruses are considered a special regulatory case.

42. As for baculoviruses, the characteristics of bacteriophages warrant that a special regulatory case can be made:

a. Bacteriophages are typically host specific, with host range limited to one or a few species of the same genus or often, one or a few isolate of the same species. Larger host ranges covering different genera are rare. Bacteriophages probably represent the most specific plant protection agents, biologicals and chemicals taken together. They are more specific than baculoviruses, which can infect several species within the same family of lepidopterous insects (OECD, 2002).

b. Bacteriophages are ubiquitous but are specific to bacteria.

c. Bacteriophages are not infective for mammals and replication does not occur in mammalian cells.

d. Due to their intrinsic specificity, effects on non-target host species are not expected

e. No pathogenic, genotoxic, mutagenic, or carcinogenic effects of bacteriophages have ever been observed in mammals.

f. Bacteriophages do not produce metabolites since they have no independent metabolism, and therefore, are not considered to be toxic.

g. Most bacteriophages are highly sensitive to environmental factors such as UV light or pH and are naturally degraded after days, weeks or a couple of months maximum (Iriarte *et al*. 2012). In contrast, baculoviruses can survive for many years in soil (OECD 2002).

43. The above listed properties warrant a special regulatory approach regarding the data requirements for bacteriophages due to the absence of certain hazards linked to the use of bacteriophages in plant protection. In addition, a special regulatory process may aid the sustainable employability of bacteriophages in plant protection. As for chemical substances (antibiotics), target bacteria can develop resistance against bacteriophages. However, in contrast with chemical substances, bacteriophages have the potential to adapt to overcome these resistances. In fact, bacteria and their viruses are continuously coevolving which has been ongoing for billions of years.

44. The ability of bacteriophages to overcome bacterial resistance can be exploited for plant protection in two ways. Firstly, mixtures of isolates can be used which have efficacy against different variants of the target organisms. These mixtures may be adapted depending on the genotypes and phenotypes of the target organism to control, which may change over time or differ per geographical region. Secondly, by co-culturing bacteria and bacteriophages, the capacity of bacteriophages to rapidly adapt to a bacterial resistance can be exploited to develop new mixtures to overcome any observed resistance to bacteriophages. For both these approaches to increase and maintain efficacy a regulatory process which is designed to allow for flexibility in the

exact mixture of isolates of a certain species of bacteriophages will aid the sustainable employment of bacteriophages.

45. The possibility for a customised flexible approach for the regulatory procedures of microorganisms is for example described in the guideline of the European Commission on the taxonomic level of micro-organisms[2], which states that for species which are known to be relatively homogenous and well-studied it may be decided that certain questions may be handled on a species level rather than on strain level. Using this approach, strain-specific information is needed only for certain data requirements. This approach was put to practice in the EU procedure SANCO/0253/2008 rev. 2 of the assessment of baculovirus isolates. While for the first isolate within a species a full active substance assessment is needed, subsequent to the approval of this reference isolate, other isolates can be approved following an assessment of information on the new isolate only for a subset of the data requirements and by comparing this information to that of the reference isolate.

46. Even more flexibility is provided by the regulatory approach taken by PMRA Canada for bacteriophages. For a phage product that has acquired regulatory approval, additional clones of phage may be added without oversight from the regulator so long as a set of criteria are met. These criteria would depend on specific characteristics of the host bacteria and phage clones and could include:

a. whole genome sequencing;

b. confirmation by genomic analysis that the life-cycle is strictly virulent;

c. confirmation that the host range is specific to the target bacterium;

47. However, any change in the strains of the bacterial host used to manufacture phage mixtures would require regulatory oversight.

3. IDENTITY

3.1. Taxonomy

48. Bacteriophages taxonomy is complex and there is considerable debate about this subject until recent detailed genome-based guidelines have been established by the ICTV (Turner *et al.* 2021). Historically, bacteriophages were classified by morphotype and host genus (Kimura *et al.* 2008). Phages are viruses with a DNA or RNA genome encapsulated in a protein capsid, which is sometimes completed with a tail and more or less complex appendages (Torres-Barceló, 2018). Over 96% of currently known bacteriophages are 'tailed' with linear, double-stranded DNA, belonging to the Order Caudovirales. This Order is divided into three families, based on morphology (tail type) and nucleic acid structure: Myoviridae with a contractile tail, Siphoviridae with a long non-contractile tail and Podoviridae with a short non-contractile tail. Siphoviridae are the most numerous and comprise 61% of the tailed bacterial virus. While most bacteriophages are Caudovirales, this document can be relevant to phage belonging to other orders. However, it is noted that taxonomy is always updated and this categorisation can change.

49. The ICTV has been charged with the task of developing, refining, and maintaining a universal virus taxonomy (Lefkowitz, *et al.*, 2018). The ICTV charge

[2] Guideline developed within the Standing Committee on the Food Chain and Animal Health on the taxonomic level of micro-organisms to be included in Annex I to Directive 91/414/EEC; Sanco/10754/2005 rev. 5

extends not only to developing the guidelines for naming of taxa, but to establishing guidelines for taxonomic classification of viruses, and approving the proposed taxonomy and names before they become official. This group confirms that 'phages' are 'viruses'.

50. According to the ICTV, two phages are assigned to the same species if their genomes are more than 95% identical at the nucleotide level over their full genome length, tested reciprocally" (Turner, et al. 2021).

51. Virus taxonomy is being continually updated. Current (2019) virus taxonomy indicates that there are 55 orders (containing 168 families) and at least 6590 species categorised. The ICTV, however, is considered to be far behind in its classification and this is particularly the case for bacteriophages since new bacteriophages are being discovered on a daily basis; hence, bacteriophage classification is always going to be open-ended, with the ICTV being significantly behind in its classification schedule. Many gaps exist; for example, more often than not, bacteriophages will be grouped within an order, family or sub-family based on similarities to other members but not be assigned to a genus.

52. As indicated by the ICTV, taxonomic guidelines and methodological tools are improving and increasing. Inferring genetic distances and phylogeny of phage genomes is now relatively straightforward. The criteria necessary for delimitation of phage species and genera is now clearly established by the ICTV based on the genome content (Morau et al., 2020; Meier-Kolthoff and Göker, 2017; Adriaenssens and Rodney Brister, 2017). For bacteriophages used in plant protection products, the association of the bacteriophages with the plant pathogen hosts is the important and most relevant relationship for taxonomic classification and is therefore commonly used.

53. *Methods for Identification of Bacteriophages* (Ackermann, 2011): The commonest approaches examine both morphology and nucleic acid composition. Morphological studies commonly rely on transmission electron microscopy to distinguish shape, presence/absence of tails, envelopes, features such as spindles/base plates and symmetry. This is usually the first approach to attribute bacteriophages to morphological families.

54. Nucleic acids studies include:

a. Genome composition (ssRNA, dsRNA, ssDNA, dsDNA, linear or circular, molecular weight). This is generally non-specific for the classification of most bacteriophages as all of the tailed phage (Caudovirales) have double-stranded DNA. It can, however, confirm morphological designation.

b. Comparison of specific gene sequences e.g. polymerases or terminases; is useful for some small groups but not across larger taxonomic groups and hence is considered unsuitable for identification.

c. Whole Genome sequences is the commonest method, focussing on genome organisation, gene number, presence/absence/type of "modules" (genes required for a specific function e.g. replication, tail proteins, lysis genes) etc.

55. The complication with bacteriophages identification is that the majority (70-80%) of bacteriophages genes are of unknown function. Although a number of bacteriophages genome sequences are deposited in international libraries, little other information is usually included with them. As with taxonomy, this fact is also rapidly improving, with the development of new and more adapted tools to annotate phage genes, e.g. Phrogs (Terzian *et al*. 2021), Phanotate (MacNeir *et al.* 2019), Phaster (Arndt *et al*. 2016) and Prokka (Seeman, 2014). Phage genomes range in size from 3.4 kb to

almost 500 kb, and unlike bacteria, there is no single gene (e.g. 16S rRNA) conserved in all phage genomes (Keen, 2015). While having a big genome size range, the average genome size of most phages stands around 30-60 kb (Dion *et al.* 2020)

Regulatory consideration and evaluation

56. Bacteriophages for plant protection will be mixtures of isolates that may need to be changed to overcome bacterial resistance and to manage regional variations in the host plant pathogens. This concept of bacteriophage isolate mixtures was accepted as a principle in the evaluation and approval decisions made by EPA and PMRA for the active substance 'Bacteriophages of *Clavibacter michiganensis* (subsp. *michiganensis*)'. Further, to have products that are effective against several target species, mixtures can be assembled of isolates from different bacteriophages species.

57. As the genome of bacteriophages is small, it is technically feasible to sequence and obtain a whole genome analysis and thus classify the bacteriophages species. According to good scientific practice, each new bacteriophage isolate and each associated host plant pathogen used to produce the phage isolate should be placed into an international collection.

3.2. Specification of active substance and product

58. A traditional approach to quantification of bacteriophages is to use plaque counts on agar plates seeded with host bacteria in which bacteriophages can propagate (Anderson *et al.* 2011). One commonly used method is proposed by Kropinski *et al*. (2008) in which the determination of the functional concentration of bacterial virus particles (titre), usually expressed as plaque-forming units (PFU)/mL, is the fundamental protocol for those guidance with bacteriophages. Quantifying culturable phages underestimates the number of phage present in a suspension, whereas molecular techniques can include non-viable phage and may have more precision. Also, quantification based on molecular techniques (e.g., qPCR) of phages does not depend on the bacterial host, which reduces measurement variation and promotes standardization of procedures. A combination of traditional plating approaches and molecular techniques may be used if feasible and needed.

Regulatory consideration and evaluation

59. The specification of the concentration of a bacteriophage isolate in a preparation can be done following published methods of in vitro counting of plaques on agar plates. Alternatively, molecular quantification by qPCR based on the phage genome can be assessed.

3.3. Purity (microbial contaminants)

60. Contaminating microorganisms, e.g. pathogenic microorganisms, could be unintentionally present in the technical grade active ingredient.

Regulatory consideration and evaluation

61. No additional criteria are needed for bacteriophages compared to other microorganisms and the OECD Issue paper on microbial contaminant limits for microbial pest control products Series on Pesticides No. 65 should be followed. In addition, it may be useful to also confirm that the host bacteria used for the production of the phages are absent.

4. BIOLOGICAL PROPERTIES

4.1. Origin, biogeography and habitat

62. For bacteriophages to be used for plant protection, they will have necessarily co-evolved with their host plant pathogenic bacteria therefore will have their origins in, and be isolated from, bacteria associated with agriculture. Due to the specificity of the relationship between bacteriophages and host being at isolate level it is expected that there will be multiple phages in different locations to obtain the correct bacteriophages isolates mixture to offer sufficient coverage of the host bacterial pathogens.

Regulatory consideration and evaluation

63. A description of the sources of isolation of both the bacteriophage isolates and their host bacterial pathogens should be provided.

4.2. Natural occurrence and geographical distribution

64. Because bacteriophages are obligate intracellular parasites, the natural occurrence and distribution of bacteriophages is dependent on the occurrence of the host bacterium (Kimura et al. 2008). Bacteriophages can be naturally present in high densities in soils. Densities of soil bacteriophages in bulk soil determined with several indicator strains of bacteria (e.g., Bacillus or Actinomycete strains) range from 7×10^2 to 4×10^7 pfu/g soil (Marsh and Wellington, 1994).

Regulatory consideration and evaluation

65. Information on the natural occurrence and geographical distribution may be provided, for example if available in peer-reviewed scientific literature.

4.3. Mode of action

66. During a lytic life cycle, phages attach to receptors on the surface of bacteria and inject their genomes into the bacterial cell. Subsequently, the bacterial metabolism produces viral proteins and genomes, which are assembled into viral particles. The infected cell is lysed and the virus particles are released.

Regulatory consideration and evaluation

67. The lytic life cycle causes lysis of the target plant-pathogenic bacterium (see ‘Life cycle of phages’). As a consequence, phages cause lytic plaques in a bacterial lawn in solid microbiology media or inhibit bacterial growth in liquid media, observed when followed by optical density measurements. Evidence of either these phenomena may be provided.

4.4. Host specificity range and effects on other species than the target organism(s)

68. Bacteriophages are typically host specific, with host range limited to one or a few species of the same genus or often, one or a few strains of the same species. Their extreme specificity means that they will not infect any bacteria (for example, beneficial soil and plant bacteria) other than their host. The nature of obligate parasites is that they are specific to their hosts and susceptible to change in their host genotype as the host

attempts to evolve resistance to their parasites: as the host evolves so does a successful parasite.

69. *Specificity to host:* Although phages are very specific, they may target several bacterial strains from one species or even from different species when they are closely related (Göller, *et al*. 2021). Bacterial plant pathogens of the same species can have many strains and isolates that are associated with different plant varieties and with geographical location. One bacteriophage isolate may affect one or more plant pathogen host isolates but rarely all. Therefore, the active substance in plant protection will necessarily be a mixture of bacteriophage isolates in order to target several strains of a bacterial pathogen. This ensures that the active substance and end-use product provides effective disease reduction of the plant pathogen across crop species and varieties and over a wider geographical and cropping area.

Regulatory consideration and evaluation

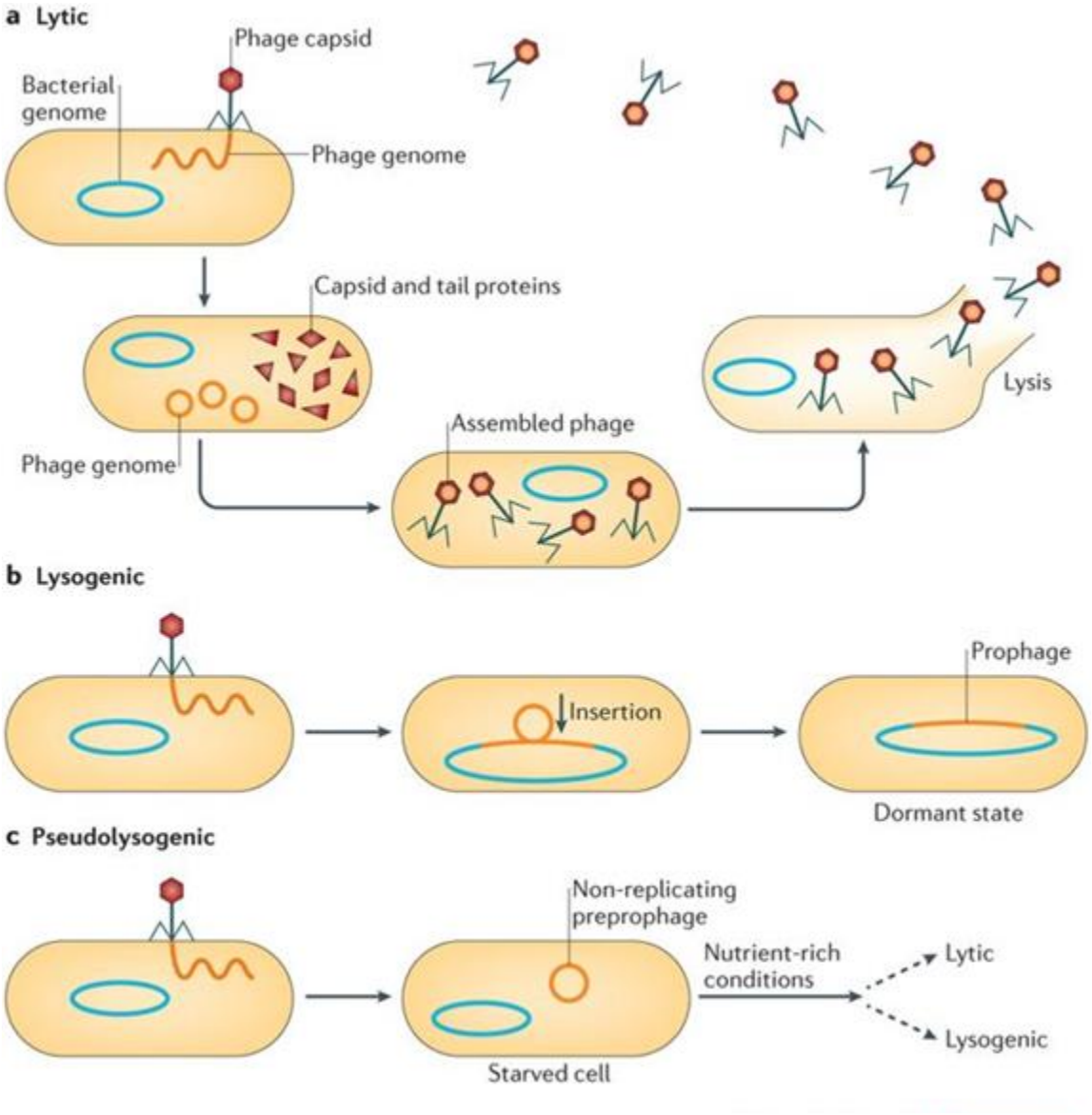
70. Host range specificity against the targeted bacterial pathogen should be provided with subsequent testing against closely related species.

4.5. Life cycle of phages

71. The life-cycle of bacteriophages has been described many times. For example Torres-Barceló (2018) provides the following information: Phages attach to specific receptors on the surfaces of bacteria (more than one in many cases) and subsequently inject their genomes into the bacterial cells, after which one of two outcomes may occur. The first is the manipulation of the bacterial molecular machinery to produce viral proteins and copy the viral genome. Subsequently, the viral particles are assembled and the bacterial cell is lysed, releasing numerous new phages. This is the case for **virulent** phages (Fig. 1), which only perform lytic cycles, and as a result form a clear halos (plaques) in bacterial lawns. The second possibility is the lysogenic cycle, where phage insert their DNA into the host cell (now called a "prophage"), either as a free plasmid or integrated into the chromosome. Prophages can have profound effects on their host physiology and pathogenic potential. In general, some prophages can carry virulence-related products, such as toxins. Other prophage derived genes play a role in antibiotic resistance, by increasing the tolerance and/or resistance levels of the host bacterium. Virulent phages do not enter into this lysogenic state. Phages able to perform lysogeny are called **temperate** (Fig. 1). If bacteria reproduce, the daughter cells will also carry the prophage. Phages with the ability to undergo this lysogenic cycle must encode specific proteins, such as a transcriptional repressor, and if integrated, a so-called integrase.

72. Antimicrobial resistance genes (AMRGs) in bacterial chromosomes or plasmids can be mobilized by temperate phages as a consequence of the inaccurate excision of the prophage, which can lead to the capture of the flanking genes adjacent to the phage integration point. The probability that the transferred genes are antibiotic resistance-related is very low. However, the possibility of temperate phages spreading AMRGs among bacteria is certainly a cause for public health concern.

Figure 1. Schema of the type of phages life-cycle, Feiner, et al (2015).



Regulatory consideration and evaluation

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

4.6. Relationship to animal or human pathogens

74. By nature, bacteriophages are viruses that are capable of infecting only bacteria (prokaryotes). They are not capable of infecting eukaryotic cells.

Regulatory consideration and evaluation

75. Bacteriophages are not capable of infecting eukaryotic cells. This is further confirmed by the specificity of bacteriophages to its host plant pathogen bacteria.

4.7. Genetic stability

76. Bacteriophages are specific to their host isolates and they are cultured with their host bacteria. They are potentially susceptible to changes in both their own and their host's genotype. Therefore, it is good practice to conserve both the bacteriophages and its host in a long-term storage facility at a suitable temperature for the isolate (e.g. 4ºC for the virus and -80ºC for the bacteria). New preparations are generated from the stored material 'mother stock' to maintain genetic stability.

Regulatory consideration and evaluation

77. Both the bacteriophage isolates and their host plant pathogen isolates should be stored in a suitable or low temperature facility to maintain their genetic stability.

4.8. Gene transfer (transduction)

78. The process by which a virus introduces foreign DNA into a bacterial cell is called transduction. Temperate phages can integrate their DNA into the bacterial host genome, resulting in the presence of a prophage in the bacterial genome. Prophages may contain genetic elements which are undesired from a risk assessment perspective. In contrast, virulent phages are incapable of forming prophages.

Regulatory consideration and evaluation

79. Due to their intrinsic differences, the potential hazards connected to the use of bacteriophages in plant protection differ for those bacteriophages which are capable of a lysogenic cycle (temperate phages) and those bacteriophages which are not capable of a lysogenic cycle (virulent phages). Temperate phages may pose a hazard due to the introduction of antimicrobial-resistance genes or genes coding for virulence factors (such as toxins) into bacteria of the environment. To exclude the hazard of these genes transferring horizontally into environmental bacteria, information should be provided to demonstrate that the bacteriophages proposed for plant protection performs an exclusively lytic pathway (i.e., is incapable of the lysogenic cycle). In case it cannot be adequately demonstrated that the bacteriophages are incapable of the lysogenic cycle, the suitability of this phage for plant protection may be questioned. As a minimum, information should be provided to demonstrate that the occurrence of a lysogenic cycle of the phage does not pose hazards due to gene transfer or lysogenic conversion. To demonstrate the absence of hazards due to gene transfer in case of a lysogenic pathway, the genome of the bacteriophages and/or the bacterial host used for production may be screened. Bacteriophage isolates for which it cannot be demonstrated to have an exclusively lytic pathway (i.e., may be capable of lysogeny) and their hosts used for production should be screened to demonstrate the absence of relevant antimicrobial

resistance genes and genes coding for relevant virulence factors and toxins in order to be considered for use in a plant protection product.

4.9. Information on metabolites

80. Bacteriophages themselves do not produce metabolites. When bacteriophages are manufactured in a bacterial host that is capable of producing toxic metabolites, there is the potential for these toxins to be present in the end-use product.

Regulatory consideration and evaluation

81. In order to mitigate the risk of the presence of bacterial toxins in the end-use product, analytical methods must be employed by the manufacturer to confirm the absence of relevant toxins in the end-use product. Which toxins should be included in these analyses should be based on the knowledge on the bacterial host. Alternately, it could be shown that the bacterial host (identified to the strain level) is not capable of producing toxic metabolites by preforming sequencing analysis of its genomic content.

4.10. Antimicrobial substances

82. Phages are incapable of producing metabolites including antimicrobial agents. When bacteriophages are manufactured in a bacterial host that is known to produce relevant amounts of antibiotics in culture, there is the potential for these antibiotics to be present in the end-use product.

Regulatory consideration and evaluation

83. Information on the production of antimicrobial substances by phage is not relevant because phage are incapable of producing metabolites. Where relevant, analytical methods must be employed by the manufacturer to confirm the absence of relevant amounts of antimicrobial agents in the end-use product.

4.11. Resistance/sensitivity to antibiotics

84. Phages, being viruses, are not sensitive to antibiotics. Information on antiviral agents is not considered relevant, as phage are incapable of infection eukaryotic cells.

Regulatory consideration and evaluation

85. Information on the production of antimicrobial substances is not relevant because phage are incapable of producing metabolites. Information on the sensitivity of phage to antimicrobials is also irrelevant since phage are incapable of infecting eukaryotic cells.

5. EFFICACY (Further information on the micro-organism)

86. Information on the occurrence or possible occurrence of the development of resistance of the target organism(s).

87. To manage the potential of evolution of resistance and the specificity of bacteriophage active substances, bacteriophages for plant protection will be mixtures of isolates that may need to be changed. Further, to have products that are effective against several target species, mixtures can be composed of isolates from different

bacteriophage species that have efficacy against one or more bacterial plant pathogenic hosts. The mixtures of isolates may need to be altered to manage regional variations in the epidemiology of the host plant pathogen.

Regulatory consideration and evaluation

88. Information on the potential of the targeted plant pathogen to develop resistance to the bacteriophages mixture may be provided. If relevant, information on resistance management may be provided.

5.1. Trials data

89. Efficacy data will be required as per country or regional requirements (development of EPPO guidance for trials with bacteriophages would be supportive).

5.2. Adverse effects on crop plants

90. The specificity of bacteriophages to its host plant pathogen bacteria means that adverse effects on the crop plant are not expected, no studies are required.

6. ANALYTICAL METHODS AND QUALITY CONTROL

6.1. Quality control methods

91. The bacterial host for production may harbour prophages or other phages than the active substance. These phages may be released during the manufacturing of the active substance.

Regulatory consideration and evaluation

92. In case of concerns on the presence of temperate bacteriophages that may emerge from the genome of the production host, quality assurance of the final bacteriophages preparation should be provided (e.g. by sequencing the production batch of individual phage). Additionally, prophage content should be checked in the production host by sequencing, and if possible avoided by using prophage-free strains (Arndt *et al*. 2016).

6.2. Methods to determine storage stability of the microorganism

Regulatory consideration and evaluation

93. As for many microorganisms, bacteriophages are sensitive to temperature and will be inactivated by elevated temperature. Therefore, there is no usefulness in running an accelerated storage stability study. A long-term storage stability study will be required, at an appropriate temperature, including if frozen, following OECD Guidance document on storage stability of microbial pest control products (Series on Pesticides No. 54).

7. HUMAN HEALTH

94. Bacteriophages are not capable of infecting eukaryotic cells and are therefore not capable of being pathogenic to organisms other than their specific bacteria. Bacteriophages occupy the same niches as their host bacteria so humans are constantly exposed to bacterial virus naturally through their own internal and external resident microbiota. It is generally accepted that bacteriophages are not toxic to humans or other mammals. Based on the host specificity of bacteriophages, the potential toxicity or pathogenicity to humans from direct exposure a bacterial virus used in plant protection products is considered negligible.

95. A summary and evaluation of available data with view on sensitization could be useful to harmonize the assessment and necessity of risk mitigation measures (RMM) to protect from an exposure to prevent sensitisation.

Regulatory consideration and evaluation

96. For active substances based on bacteriophages, there is no requirement to provide information on the infectivity and pathogenicity of the phage for human health.

8. RESIDUES

Regulatory consideration and evaluation

97. As there is no human toxicity risk and it can be confirmed that there are no relevant toxins in the product resulting from the host bacteria used for production, there is negligible risk from exposure to bacteriophages used as plant protection on edible plant products.

9. FATE AND BEHAVIOUR IN THE ENVIRONMENT

98. The coexistence of bacteria and their phages in the same habitats is a common occurrence and is explained using the theory of coevolution. (Kimura *et al.* 2008). Bacteriophages are very specific to their bacterial hosts.

99. Furthermore, bacteriophages are not capable of infecting eukaryotic cells and are therefore not capable of being pathogenic to organisms other than their specific bacteria.

100. The environmental densities of bacteriophages used in plant protection products upon application are determined by the presence of the host plant-pathogenic bacteria.

Regulatory consideration and evaluation

101. For active substances based on bacteriophages, there is no requirement to provide environmental fate data for the phage. As bacteriophages are not pathogenic or toxic to eukaryotic organisms, they cannot cause harm so their fate and behaviour in the environment is not relevant for the risk assessment.

10. EFFECTS ON NON-TARGET ORGANISMS

102. Bacteriophages are not capable of infecting eukaryotic cells and are therefore not capable of being pathogenic or toxic to organisms other than their specific bacteria. In addition, non-target organisms are constantly exposed to bacteriophages as they are ubiquitous in the environment. Based on the host specificity of bacteriophages, the potential toxicity or pathogenicity to eukaryotic non-target organisms from direct exposure to bacteriophages used in plant protection products is considered non-existent.

Regulatory consideration and evaluation

103. For active substances based on bacteriophages, there is no requirement to provide in vivo non-target organism data in eukaryotes for the phage.

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ANNEX 1: BACTERIAL PLANT PATHOGENS CAUSING SIGNIFICANT ECONOMIC DAMAGE

Seed borne diseases

Crop	Disease
Wheat	*Pseudomonas syringae* pv. *syringae, Xanthomonas campestris* pv. *translucens*
Maize	*Pantoea stewartii* subsp. *stewartii, Clavibacter michiganensis* subsp. *Nebraskensis*
Rice	*X. oryzae* pv. *oryzae, X. oryzae* pv. *oryzicola, Acidovorax oryzae*
Bean	*P. syringae* pv. *phaseolicola, Curtobacterium flaccumfaciens* pv. *flaccumfaciens, Xanthomonas campestris* pv. *phaseoli* and *X. fuscans* var. *fuscans*
Soybean	*P. syringae* pv. *glycinea*
Chickpea	*Rhodococcus fascians*
Cereals, grasses	*Rathayibacter* sp.
Alfalfa	*C. michiganensis subsp. insidiosus*
Tomato, pepper	*Pseudomonas syringae* pv. *tomato* (tomato), *P. syringae* pv. *syringae, Xanthomonas spp., Clavibacter michiganensis* subsp. *Michiganensis*
Carrot	*Xanthomonas campestris* pv. *carotae*
Onion	*Pantoea ananatis, Burkholderia cepacia*
Crucifers	*Xanthomonas campestris* pv. *campestris, P. syringae* pv. *alisalensis* (broccoli), *Pseudomonas* spp. (crucifers)
Cucurbits	*P. syringae* pv. *lachrymans, Acidovorax citrulli*
Lettuce	*Xanthomonas campestris* pv. *vitians*

Vegetative transferred diseases*

Crop	Disease
Potato	*Clavibacter michiganensis* subsp. *sepedonicus, Ralstonia solanacearum, Streptomyces scabies, Candidatus Liberibacter* sp., *Pectobacterium/Dickeya* spp.
Citrus	*Candidatus Liberibacter asiaticus, Xylella fastidiosa* subsp. *pauca, Xanthomonas citri*
Strawberry	*X. fragariae*
Grape, almond	*Xylella fastidiosa* subsp. *fastidiosa,*
Pear, apple, quince	*Pectobacterium amylovora*
Sugarcane	*Leifsonia xyli* subsp. *xyli, Xanthomonas albilineans*
Cassava	*Xanthomonas campestris* pv. *cassavae*
Banana	*X. campestris* pv. *musacearum*
Roses, ornamentals	*Agrobacterium tumefaciens*

* Propagation production systems offer ideal conditions for bacterial reproduction and spread

ANNEX 2: EXAMPLE OF DATA REQUIREMENTS FOR BACTERIOPHAGES

The tests listed in this Annex are generally conducted with the MPCA (i.e., the microbial pest control agent, which for this Annex refers to the bacteriophages under assessment) itself but depending on the type of MPCA, its production method, stability and/or formulation, testing may be done only on the technical grade active ingredient (TGAI; which is the microbial active substance as manufactured) or MPCP (microbial pest control product), as appropriate. A reasoned case may be made for the non-submission of some studies or data and addressed instead by provision of scientific information from good quality sources.

If the technical grade active ingredient contains relevant (toxic) secondary compounds (metabolites) then applicable data requirements for chemical pesticides may need to also be fulfilled.

Microbial active substance: Identity, composition, physical and chemical properties

Codes used: CR = conditionally required, R = required, NR = not required or not relevant

OECD code	Information, test or study on the Active Substance (technical)	Study required	Test material	Notes
1	**Identity of micro-organism (Microbial Pest Control Active (MCPA))**			
1.1	**Applicant (name, address, contact, telephone and telefax numbers)**	R	NR	
1.2	**Manufacturer(s) (name, address, contact, telephone and telefax numbers)**	R	NR	
1.3	**Scientific information**			
1.3.1	Scientific name of micro-organism to species level or a level sufficient to show taxonomic relation to known micro-organisms, especially pathogens	R	MPCA	
1.3.2	Accession no. of sample in a recognised culture collection	R	MPCA	
1.3.3	Test procedures and criteria, using best available technology, to characterise the isolate(s);	R	MPCA	
1.3.4	For mutant or genetically-modified virus, indicate all known differences between the modified virus and the parent wild virus	CR	NR	
1.3.5	Include any trade names, common names, developmental code names	R	MPCA	
1.3.6	Indigenous or non-indigenous at the species level to the intended area of application.	R	MPCA	
1.4	**Specification of the material used for manufacturing of formulated products**			
1.4.1	Content of the micro-organism: Concentration of micro-organism (and metabolite, if appropriate) in terms of g/kg or g/L (for US and Canada, also in % w/w) and cfu	R	MPCA	
1.4.2	Identity and content of impurities, additives, contaminating micro-organisms. Composition of microbial material used for manufacture of end use products in terms of g/kg or g/L (for US and Canada also in % w/w) for each active ingredient.	R	MPCA	
1.4.4	Quality control data (measures of quality criteria) from 3 - 5 production batches, including storage stability data. If the Technical Grade of MPCA is a stage in a continuous production process of an end-use product, this information should be provided for the entire production process.	R	MPCA	
1.4.5	The formation, presence and/or impact of unintentional ingredients	R	MPCA	

OECD code	Information, test or study on the Active Substance (technical)	Study required	Test material	Notes
1.4.6	Physical and chemical properties, if MPCA is produced as a manufacturing product that is stored prior to formulation of end-use products: physical state; density; viscosity or surface tension; explosivity, corrosive character, oxidising properties; technical characteristics as appropriate	R	MPCA	
1.4.7	International regulatory status of micro-organism	R	MPCA	
1.4.8	Sample of MPCA, analytical standard of metabolite (and reference substances for the relevant impurities - EU only): if requested	CR	MPCA	
1.5	**Patent status information**			
2	**Biological Properties of the micro-organisms (MCPA)**			
2.1	**Origin of the isolate; method of isolation; preservation and maintenance of isolate(s) during development; historical information on testing and use of the isolate(s); history of use of closely related isolate(s) or species; Description of any unusual morphological, physiological, pesticidal or resistance characteristics of the MPCA which differ from classical description of the species**			
2.1.1	**Historical background**	R	MPCA	
2.2	**Origin and natural occurrence.** *(including geographic distribution, hosts, habitat, ecological niche, level of natural occurrence)*	R	MPCA	
2.3	**Information on target organism(s)**			
2.3.1	Description of the target organism(s)	R	MPCA	
2.3.2	Information on mode of action, kind of antagonism to target host, infective/toxic dose, transmissibility	R	MPCA	
2.4	**Host specificity range and effects on species other than the target harmful organisms**	R	MPCA	For host specificity only
2.5	**Development stages/life-cycle of the micro-organism, Infectiveness, dispersal and colonisation ability**	R	MPCA	Life-cycle only, indicating lytic pathway
2.6.	**Information of the production of metabolites (especially toxins)**	NR	NR	
2.7	**Relationships to known plant or animal or human pathogens.**			
2.7.1	Among closely related species, provide information on pathogenicity to plants, animals or humans	NR	NR	
2.7.2	Among closely related species, provide information on formation of toxic metabolites: structure, stability, conditions under which they are formed, mode of action	NR	NR	
2.8	**Physiological properties, especially effect of environmental parameters on growth, infectivity, dispersal and colonisation ability: temperature, pH, redox potential, humidity, light, nutritional requirements**	CR	MPCA	
2.9	**Description of any plasmids or other extra chromosomal genetic elements involved in pesticidal activity, pathogenicity, toxicity, etc.**	R	MPCA	
2.10	**Genetic stability and factors affecting it (mutation rate of traits related to the mode of action).**	CR	MPCA	Information on stability
2.11	**Detailed discussion of relationship of micro-organism to any known human dermatophyte (see point 5.2)**	NR	NR	
2.12	**Antibiotics and other antimicrobial agents**	NR	NR	
3	**Further information on the micro-organism (MCPA)**			
3.1	**Function, e.g. fungicide**	R	MPCA	
3.2	Placeholder			
3.3	**Field of use, e.g. forestry**	R	MPCA	
3.4	**Crops or products to be protected or treated.**			
3.4.1	Details of existing and intended uses (crops, groups of crops, plants or plant products treated or protected)	R	MPCA	

OECD code	Information, test or study on the Active Substance (technical)	Study required	Test material	Notes
3.4.2	Details of harmful organisms against which protection is afforded	R	MPCA	
3.4.3	Effects achieved e.g. sprout suppression	R	MPCA	
3.5	**Information on mode of action and metabolites**			
3.5.1	Statement of the mode of action of the MPCA	NR	NR	
3.5.2	Details of active metabolites (especially toxins) and degradation products	NR	NR	
3.6	**Information on the occurrence or possible occurrence of the development of resistance of the target organism(s)**	R	MPCA	
3.7	**Recommended methods and precautions concerning handling, storage, transport or fire.**	R	MPCA	
3.8	**Procedures for destruction or decontamination.**	R	MPCA	
3.9	**Measures in case of an accident**	R	MPCA	
3.10	**Other/special studies**	NR	NR	
3.11	**Crops or products to be protected or treated**	R	MPCA	
3.12	**Measures in case of an accident**	R	MPCA	
4	**Analytical methods and validation**			
4.1	**Methods for the analysis of the micro-organism as manufactured**	R	MPCA	
4.2	**Methods to determine and quantify residues (viable and non-viable)**	NR	NR	
4.3	**Quality control and post-registration monitoring methods**			
4.3.1	Methods to detect, isolate, and enumerate the micro-organism	R	MPCA	QC only
4.3.2	Methods to differentiate a mutant or genetically-modified micro-organism from the parent micro-organism.	CR	NR	
4.3.3	Methods to detect spontaneous change in major characteristics of micro-organism.	NR	NR	
4.3.4	Methods to define content of micro-organism in appropriate terms (same as IIM 1.4.1), incl. standardisation, sensitivity, reproducibility, statistical validity, and representative data to validate the bioassay.	R	MPCA	QC only
4.3.5	Methods to show control to a specified and acceptable level, of microbial impurities and of any other impurities of toxicological concern, including toxic metabolites, which are known or suspected to be present at any stage of the manufacturing process.	R	MPCA	QC only
4.3.6	Methods to show presence of any human and mammalian pathogens.	R	MPCA	QC only
4.4	**Storage stability test, data and determination of shelf life, if MPCA is stored**	CR	MPCA	
4.5	**Post-registration monitoring methods to determine and quantify residues of viable or non-viable micro-organism and metabolites (especially toxins)**			
4.5.1	Food (where relevant)	NR	NR	
4.5.2	Feed (where relevant)	NR	NR	
4.5.3	Animal tissue (where relevant)	NR	NR	
4.5.4	Soil (where relevant)	NR	NR	
4.5.5	Water (where relevant)	NR	NR	
4.5.6	Air (where relevant)	NR	NR	
4.5.7	Analytical methods for amount or activity of proteinaceous products (where relevant)	NR	NR	
5	**Effect on human health**			
	Basic information.			
5.1	Medical data	NR	NR	
5.2	Occupational health surveillance report on workers during production and testing of MPCA, including information on: see IIM 5.2.1 to 5.2.4. Published reports of adverse effects, especially reports of clinical cases and follow up studies. Proposed first aid measures and medical treatment.	CR	MPCA	

OECD code	Information, test or study on the Active Substance (technical)	Study required	Test material	Notes
5.2.1	The sensitisation and allergenic response of workers	CR	MPCA	
5.2.2	Details on any occurrence of hypersensitivity and chronic sensitisation	NR	NR	
5.2.3	Any significant clinical findings related to exposure, with special attention to those whose susceptibility may be affected.	NR	NR	
5.2.4	Published reports of adverse effects, especially reports of clinical cases and follow up studies; list databases and key words used in a literature search.	NR	NR	
5.2.5	Proposed first aid measures and medical treatment	CR	MPCA	
5.3	**Basic studies**			
5.3.1	Sensitisation properties	NR	NR	
5.3.2	Acute oral infectivity, toxicity and pathogenicity	NR	NR	
5.3.3	Acute intratracheal/inhalation infectivity, toxicity and pathogenicity	NR	NR	
5.3.4	Acute intravenous/intraperitoneal infectivity	NR	NR	
5.3.5	Genotoxic potential	NR	NR	
5.3.6	Cell culture study, for viruses and viroids or specific bacteria and protozoa with intracellular replication	NR	NR	
5.3.7	Short-term toxicity (including inhalatory short-term toxicity), pathogenicity, infectivity	NR	NR	
5.4	**Toxicity studies on metabolites**			
5.5	**Other/special studies**			
5.5.1	Specific toxicity, pathogenicity and infectiveness studies	NR	NR	
5.5.2	In vivo studies in somatic cells	NR	NR	
5.5.3	Genotoxicity - In vivo studies in germ cells	NR	NR	
5.6	**Summary of mammalian toxicity, pathogenicity and infectiveness and overall evaluation**	CR	MPCA	
6	**Metabolism and residues data**			
6.1	**Rationale for waiver of residue data based on information showing that MPCA is not hazardous to mammals, i.e. lack of potential for a known mammalian toxin and negative result from the acute oral toxicity test.**	NR	NR	
6.2	**Rationale for waiver based on a substantiated estimation that MPCA is unlikely to occur on treated food/feed stuffs in concentrations considerably higher than under natural conditions.**	NR	NR	
6.3	**Persistence and likelihood of multiplication in or on crops, feeding stuffs or foodstuffs**	NR	NR	
6.4	**Further information required**			
6.4.1	Non-viable residues	NR	NR	
6.4.2	Viable residues	NR	NR	
6.5	**Summary and evaluation of residue behaviour resulting from data submitted under points 6.1 and 6.2**	NR	NR	
7	**Fate and behaviour in the environment**			
7.1	**Persistence and multiplication**			
7.1.1	Soil	NR	NR	
7.1.2	Water	NR	NR	
7.1.3	Air	NR	NR	
7.2	**Other/special studies**	NR	NR	
8	**Effects on non-target organisms**			
8.1	**Effects on birds**	NR	NR	
	Effects on aquatic organisms			
8.2	Effects on fish	NR	NR	
8.3	Effects on freshwater invertebrates	NR	NR	
8.4	Effects on algae growth	NR	NR	
8.5	Effects on aquatic plants other than algae	NR	NR	
8.6	Effects on terrestrial plants	NR	NR	
8.7	**Effects on bees**	NR	NR	
8.8	**Effects on arthropods other than bees**	NR	NR	

OECD code	Information, test or study on the Active Substance (technical)	Study required	Test material	Notes
8.9	**Other terrestrial invertebrates**	NR	NR	
8.9.1	**Effects on earthworms**	NR	NR	
8.9.2	**Effects on other terrestrial invertebrates**	NR	NR	
8.10	**Effects on non-target soil micro-organisms**	NR	NR	
8.11	**Additional studies**	NR	NR	
9	**Summary and evaluation of environmental impact:**			
9.1	**- addressing distribution and fate of MPCA**	NR	NR	
9.2	**- identifying non-target species at risk and the extent of their exposure**	NR	NR	
9.3	**- identifying precautions necessary to minimise environmental contamination and to protect non-target species.**	NR	NR	

Microbial product: recommended data requirements for registration of the formulated products (MPCP)

Codes used: CR = conditionally required, R = required, NR = not required or not relevant

Data point	Information, test or study of the product	Study required	Test substance	Notes
1	**Identity of the Microbial Pest Control Product**			
1.1	**Applicant (name, address, contact, telephone and telefax numbers)**	R	MPCP	
1.2	**Manufacturer(s) of the preparation and producer of the microbial pest control agent**			
1.2.1	Manufacturer(s) of the preparation (name, address, contact, telephone and telefax numbers)	R	MPCP	
1.2.2	Producer of the microbial pest control agent (name, address, contact, telephone and telefax numbers)	R	MPCP	
1.3	**Trade name or proposed trade name and manufacturers code number(s), for the preparation and similar preparations (differences to be specified)**	R	MPCP	
1.4	**Placeholder**			
1.5	**Physical state of MPCP (Crop Life formulation type)**	R	MPCP	
1.6	**Function (herbicide, insecticide, etc.)**			
1.6.1	Biological function category and field of use category, using terms defined by each country, e.g. "control of weeds" for "forestry"	R	MPCP	
1.7	**Other/special studies**			
1.7.1	Concentration of MPCA in MPCP, measured in terms of g/kg or g/L of the MPCP and in cfu: indicate scientific name and isolate(s) designation.	R	MPCP	
1.7.2	Composition in terms of g/kg or g/L and % w/w of each ingredient in MPCP, including technical grade, additives, microbial and non-microbial impurities.	R	MPCP	
1.7.3	Quality criteria for the production and storage of the MPCP, including range of content of MPCA, presence of human or non-target animal pathogens, maximum acceptable level for microbial impurities and known mammalian toxins.	R	MPCP	
1.7.4	Quality control data (measures of quality criteria) from 3 - 5 production batches, including product stored for duration of shelf life if it is metabolically active.	R	MPCP	
1.7.5	The formation, presence and/or impact of unintentional ingredients (theoretical discussion).	R	MPCP	
2	**Physical, chemical and technical properties of the MPCP**			
2.1	**Appearance**	R	MPCP	
2.2	**Storage stability and shelf-life**	R		
2.3	**Explosivity, oxidising properties, flash point, flammability, spontaneous ignition, acidity, alkalinity, pH, viscosity, surface tension**			
2.3.1	Explosivity, oxidising properties: as appropriate	CR	MPCP	

2.3.2	Flash point, flammability, spontaneous ignition: as appropriate	CR	MPCP	
2.3.3	Acidity, alkalinity, pH: as appropriate	CR	MPCP	
2.3.4	Viscosity, surface tension: as appropriate	CR	MPCP	
2.4	**Technical characteristics of the MPCP - as appropriate**			
2.4.1	Wettability	CR	MPCP	
2.4.2	Persistent foaming	CR	MPCP	
2.4.3	Suspensibility, suspension stability	CR	MPCP	
2.4.4	Dry sieve test and wet sieve test	CR	MPCP	
2.4.5	Particle size distribution (dustable and wettable powders, granules), content of dust/fines (granules), attrition and friability (granules)	CR	MPCP	
2.4.6	Emulsifiability, re-emulsifiability, emulsion stability	CR	MPCP	
2.4.7	Flowability, pourability (rinsability), dustability	CR	MPCP	
2.5	**Density**	CR	MPCP	
2.6	**Distribution and adherence to seeds**	CR	MPCP	
2.7	**Summary and evaluation of data on properties of the MPCP**			
3	**Data on application**			
3.1	**Pest to be controlled, crop to be protected, available information on mode of action (site of uptake, toxic/competitive effect, is micro-organism transmitted or translocated to another part of plant?)**	R	MPCP	
3.2	**Available information on the development of resistance in target pest and appropriate mitigation strategy.**	CR	MPCP	
3.3	**Application rate in terms of mass/vol of MPCP per unit area/volume (e.g. kg/ha). Content of micro-organism in material used (diluted spray, bait, treated seed).**	R	MPCP	
3.4	**Application rate in terms of units of micro-organism per unit area/volume**	R	MPCP	
3.5	**Method of application (incl. type of equipment and volume of diluent)**	R	MPCP	
3.6	**Number, timing and conditions of applications, related to: host/pest phenology, duration of protection, application of other pesticides, pre-harvest interval**	R	MPCP	
3.7	**Precautions to avoid phytotoxic/ phytopathogenic effects on protected crop or on succeeding crops, if appropriate**	CR	MPCP	
3.8	**Proposed instructions for use as printed, or to be printed, on labels**	R	MPCP	
4	**Further information on the plant protection product**			
4.1	**Packaging: description**	R	MPCP	
4.2	**Specifications of packaging and measures of its suitability**	R	MPCP	
4.3	**Label instructions regarding cleaning equipment and protective clothing**	CR	MPCP	
4.4	**Procedures to clean equipment and protective clothing; measures of their effectiveness**	CR	MPCP	
4.5	**Necessary waiting periods for re-entry; recommended protective measures to reduce occupational exposure**	CR	MPCP	
4.6	**Label instructions regarding: safe handling and storage**	R	MPCP	
4.7	**Recommendations regarding: handling, storage, transport, fire: specify risks, specify procedures to minimise hazards and the generation of waste.**	CR	MPCP	
4.8	**Label instructions regarding: clean-up of spills**	CR	MPCP	
4.9	**Detailed procedures in case of accident to: contain a spill, decontaminate an area or vehicle, dispose of adsorbents and packaging, protect workers and bystanders, first aid.**	CR	MPCP	
4.10	**Procedures for destruction/disposal of MPCP and its packaging**	CR	MPCP	
5	**Methods of analysis**			
5.1	**Quality control and post-registration monitoring methods**			

5.1.1	Methods to differentiate a mutant or genetically-modified micro-organism from the parent isolate(s).	CR	NR	
5.1.2	Methods to detect spontaneous change in major characteristics of micro-organism.	NR	NR	
5.1.3	Methods to define content of micro-organism in appropriate terms	R	MPCP	
5.1.4	Methods to identify contaminant micro-organisms in MPCP	CR	MPCP	
5.1.5	Methods to show control to a specified and acceptable level, of microbial impurities	R	MPCP	
5.1.6	Methods to show presence of any human and mammalian pathogens.	CR	MPCP	
5.2	**Storage stability test and determination of shelf life (methods of analysis)**	CR	MPCP	
5.3	**Production process for MPCP**	R	MPCP	
5.4	**Method for determination of residues**	NR	NR	
6	**Efficacy data**	R	MPCP	
7	**Toxicological studies and exposure data and information for MPCP**			
7.1	**Basic studies**			
7.1.1	Acute oral toxicity	CR	MPCP	
7.1.2	Acute percutaneous (dermal) toxicity	CR	MPCP	
7.1.3	Acute inhalation toxicity to rats	CR	MPCP	
7.1.4	Skin irritation	CR	MPCP	
7.1.5	Eye irritation	CR	MPCP	
7.1.6	Skin sensitization	CR	MPCP	
7.2	**Operator, bystander and worker exposure - monitoring**	CR	MPCP	
7.3	**Operator and bystander exposure - hypersensitivity**	CR	MPCP	
7.4	**Safety data sheet for each additive**	CR	MPCP	
7.5	**Supplementary information**	CR	MPCP	
7.6	**Summary and evaluation of all health effects**	CR	MPCP	
8	**Metabolism and residues data**			
8.1	Residues in/on food for MPCP	NR	NR	
9	**Fate and behaviour in the environment**			
9.1	**Sufficient information on the origin, properties, survival and residual metabolites of the micro-organism to assess its fate and behaviour in the environment**			
9.1.1	Persistance and mobility in soil	NR	NR	
9.1.2	In water	NR	NR	
9.1.3	In air	NR	NR	
9.2	**Other special studies**	NR	NR	
10	**Rationale to waive additional testing, based on adequacy of information provided for MPCA, to permit an assessment of the impact of the MPCP on non-target organisms.**			
10.1	**Effects on birds**	CR	MPCP	
10.2	**Effects on aquatic organisms**	CR	MPCP	
10.3	**Effects on bees**	CR	MPCP	
10.4	**Effects on terrestrial arthropods other than bees**	CR	MPCP	
10.5	**Effects on earthworms**	CR	MPCP	
10.6	**Effects on soil micro-organisms**	CR	MPCP	
10.7	**Additional studies**	CR	MPCP	
11	**Summary and evaluation of environmental impact: summarise all data relevant to environmental impact and assess environmental risk by:**			
11.1	**Addressing distribution and fate of MPCP**	NR	NR	
11.2	**Identifying non-target species at risk and the extent of their exposure**	CR	NR	
11.3	**Identifying precautions necessary to minimise environmental contamination and to protect non-target species**	CR	NR	