European Commission

Draft Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

Pepino Mosaic Virus, EU strain, mild isolate Abp1
Pepino Mosaic Virus, CH2 strain, mild isolate Abp2

List of Endpoints

Rapporteur Member State: Spain

July 2019

Version History

When	What
	Completeness check report of the dossier submitted by the notifier
March 2019	DAR submitted to the Notifier. Reception of comments
July 2019	DAR revised
July 2019	DAR submitted to EFSA

APPENDIX II: LIST OF ENDPOINTS

Appendix II.1: Chapter 1 (identity, biological properties, details of uses, further information and proposed classification and labelling)

Identity, Biological properties, Details of uses, Further information, and Proposed Classification and Labelling

Active microorganism	PepMV, EU strain, mild isolate Abp1 PepMV, CH2 strain, mild isolate Abp2
Function	Elicitor: control of PepMV aggressive isolates by cross protection after virus inoculation.

Rapporteur Member State:	Spain
Co-rapporteur Member State	

Identity of the Microbial Pest control Agent / Active substance (OECD data point IIM 1)

Name of the organism	*Pepino mosaic virus* (PepMV)
Taxonomy	Family: Alphaflexiviridae Genus: Potexvirus Order: Tymovirales
Species, subspecies, strain	PepMV, European (EU) strain, mild isolate Abp1 PepMV, Chilean (CH2) strain, mild isolate Abp2
Identification detection	Sequencing of complete genome: PepMV genome is composed of a 6,410 nucleotide long, ss(+) RNA with 5 Open Reading Frames (ORF): ORF1 encodes a 164-k Da RNA dependent RNA polymerase ORF2-4 form the PepMV triple gene block (TGB, 1, 2, 3) ORF5 codes for a 25-k Da coat protein (CP) Molecular hybridization. Real time quantitative PCR (RT-qPCR) Sequencing of a fragment encompassing part of the TGB2 and 3 and CP encoding regions (942-3 nt). To unambiguously identify PepMV, EU strain, mild isolate Abp1 and PepMV, EU strain, mild isolate Abp2 the sequence of this genomic fragment has to have an identity of at least certain percentage to the corresponding reference sequences. Bioassay (infectivity in tomato)
Culture collection	PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 are deposited in the German Collection of Micro-organisms and Cell Cultures Leibniz-Institut (DSMZ) GmbH, with reference numbers: - DSM32069 (PepMV-Abp1) - DSM32070 (PepMV-Abp2)
Minimum and maximum concentration of the MPCA used for manufacture of the formulated product	AbioProtect® end use product contains a min of $5x10^{11}$ PepMV genome copies /L: With a min of $2.5x10^{11}$ PepMV, EU strain, mild isolate Abp1 genome copies/L. In the representative batches analyzed the content of PepMV, EU strain, mild isolate Abp1 ranged from $5.43x10^{11}$ to a maximum of $3.21x10^{12}$ genome copies/L, always above the minimum set up content of $2.5x10^{11}$ genome copies/L. And, with a min of $2.5x10^{11}$ PepMV, CH2 strain, mild isolate Abp2 genome copies/L. In the representative batches analyzed the content of

	PepMV, CH2 strain, mild isolate Abp2 ranged from $1.48x10^{12}$ to a maximum of $7.68x10^{12}$ genome /L, always above the minimum set up content of $2.5x10^{11}$ genome copies/L.
Identity and content of relevant impurities, additives, contaminating organisms in the technical grade of MPCA:	None No metabolites are produced by PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 Contaminating microorganisms are determined by ISO standard methods. 18 Viruses and viroids potentially infecting tomato were evaluated by molecular hybridization. The presence of 36 phytopathogenic bacteria and fungi were evaluated by qPCR and bioassay to dischard any necrotic or yellowing PepMV variants or other PepMV strains. The absence of potential human pathogens is also analysed by ISO methods. In case a human pathogen is detected in a batch, such batch would be destroyed. The impurity nicotine is present at very low levels in the active substances. Nicotine medium content in five independent batches of PepMV, EU strain, mild isolate Abp1 and of PepMV, CH2 strain, mild isolate Abp2, was 0.027 mg/kg for isolate Abp1 and 0.050 mg/kg for isolate Abp2.
Is the MCPA genetically modified; if so provide type of modification	PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 are natural wild type microorganisms.

Biological properties of the microorganism (OECD data point IIM 2)

Origin and natural occurrence	PepMV, EU strain, mild isolate Abp1 was isolated from a commercial tomato crop in Murcia (Spain) in 2001. PepMV, CH2 strain, mild isolate Abp2 was isolated from a commercial tomato crop in Murcia (Spain) in 2007. Both isolates were characterised and their biological properties further studied.
Background level	Background levels are not known but the virus replicates in plants of the *Solanaceae* family and can survive for short times on plants of other botanical families as well.
Target organism(s)	All other isolates of PepMV in tomatoes (*Solanum lycopersicum*)
Mode of action	Cross-protection
Host specificity	Restricted to plants of the *Solanaceae* family
Life cycle	It starts by penetration of the virion into the cytoplasm of plant cells through wounds caused by mechanical damage to the cuticle and cell wall, or through the stomata. The next phase is the partial or complete removal of the coat protein shell of the virion in the cytoplasm. Then the cell mediates expression of the viral genome by providing a translation apparatus producing viral proteins that are required for completion of the virus life cycle. The next step is movement of the virus into neighbouring cells. Virions are transported into neighbouring cells through small channels called plasmodesmata that form connections between cells. The time between initial infection of one or a few cells and systemic infection of the plant varies from a few days to a few weeks depending on the virus, host plant, and environmental conditions. Transmission of the virus from one plant to another completes the virus life cycle.
Infectivity, dispersal and colonization ability	PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, prevent other PepMV isolates from entering into the crop.

	PepMV is highly infective to plants of the *Solanaceae* family. PepMV might survive or replicate also in plants of other botanical families without causing adverse effects.
Relationships with known plant, animal or human pathogens	PepMV is closely related to *Narcisus mosaic virus* (NMV), *Scallion virus X* (SVX), *Cymbidium mosaic virus* (CymMV) and *Potato aucuba mosaic virus* (PAMV). The highest overall nucleotide identities are with NMV and CymMV. Plant viruses are not related with any animal or human pathogen because they only reproduce in living plant cells. They cannot replicate in humans or other animals, largely due to the lack of specific receptors for recognition and entry into host cells.
Genetic stability	As with all RNA viruses mutation can occur. Risk management procedures are in place to prevent the occurrence of virulent isolates in the end product.
Information on the production of relevant metabolites (especially toxins)	Viruses do not produce metabolites, as they do not have metabolism of their own. The PepMV complete viral genome sequence is known and the five encoded typical Potexvirus proteins are well understood. None of these proteins show any homology to known human or animal toxins. It can therefore be stated with certainty that PepMV does not produce toxins, not even after infecting the plant host cell.
Resistance/sensitivity to antibiotics/ antimicrobial agents used in human or veterinary medicine	Not relevant for plant viruses. Viruses are not metabolically active and cannot produce antimicrobial substances; they are not sensitive to antibiotics and therefore cannot become resistant to these substances or spread resistance.

Appendix II.2: Chapter 2 (Methods of analysis)

Analytical methods for the micro-organism (OECD data point IIM 4.2, 4.3 and IIIM 5.3)

Manufactured microorganism (principle of method)	The Microbial Pest Control Product (MPCP) AbioProtect® is formulated with equivalent amounts of two Microbial Pest Control Agents (MPCA), PepMV EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. The production of each MPCA starts using pure inocula of the viral isolates, which are then amplified separately through a maximum of two rounds of amplification passages to get enough inoculum. Since the purity of the inoculum is critical to ensure the effectiveness and safety of AbioProtect®, no more than two rounds of amplification are ever made, it always begins with the maximum guaranteed material, and the whole process is carried out in confined facilities under quality control measures.
Impurities and contaminating microorganism in manufactured material (principle of method)	The virus does not produce metabolites. 18 Viruses and viroids potentially infecting tomato were evaluated by molecular hybridization. The presence of 36 phytopathogenic bacteria and fungi were evaluated by qPCR and bioassay to dischard any necrotic or yellowing PepMV variants or other PepMV strains. The absence of potential human pathogens is also analysed by ISO methods. In case a human pathogen is detected in a batch, such batch would be destroyed. The impurity nicotine is present at very low levels in the active substances. Nicotine medium content in five independent batches of PepMV, EU strain, mild isolate Abp1 and of PepMV, CH2 strain, mild isolate Abp2, was 0.027 mg/kg for isolate Abp1 and 0.050 mg/kg for isolate

	Abp2. Primer and probes design, in addition to real time PCR optimization were conducted with the requirements stablished by UNE-EN ISO 9001:2015, Certificate No. EC-9505/18
Microbial pest control product (principle of method)	PepMV Abp1 and PepMV Abp2 are identified and discriminated at strain level by molecular hybridisation using RNA digoxygenin-labelled probes complementary to nucleotides 1388-1711 (Abp1) and 1411-1891 (Abp2) Both probes were tested for background and cross detection showing their specificity for the corresponding virus isolate and lack of cross reaction with heterologous RNA. Molecular hybridisation using specific probes is a scientifically validated method that when conducted with the proper controls and scientific rigour allows to identify and fully discriminate the presence of nucleic acids of the virus strain of interest against other strains of the same virus, as well as other viruses, viroid, bacterium, fungi and oomycetes. Specific primers pair for PepMV Abp1 unambiguous identification and specific primers for PepMV Abp2 unambiguous identification were also applyed. The indicated primers pairs are used to quantify and fully discriminate PepMV Abp1 and PepMV Abp2 by RT-qPCR which is a scientifically validated procedure, conducted with the proper primers and controls, and performed with scientific rigour to guaranty specificity. The method to identify and fully discriminate PepMV Abp1 and Abp2 at strain level was fully confirmed by testing variants of PepMV as well as other viruses, viroid, bacterium, fungi and oomycetes.

Analytical methods for residues (viable and non-viable) in exposed compartments and organisms (OECD data point IIM 4.5)

Of the active microorganism (principle of method)	Not necessary
Of relevant impurities (principle of method)	Not necessary (no metabolites)

Appendix II.3: Chapter 3 (Further information, Efficacy)

Effectiveness (Regulation (EU) N° 284/2013, Annex Part A, point 6.2)

Effectiveness	Pepino mosaic virus (PepMV), European strain (EU), mild isolate Abp1 and Pepino mosaic virus (PepMV), Chilean strain (CH2), mild isolate Abp2 are recommended for use as a preventive treatment in greenhouse tomato crops to protect them from infection by aggressive isolates of the EU strain of PepMV and CH2 strain of PepMV, respectively. Both mild isolates function as elicitors: control of PepMV aggressive isolates by cross-protection after virus inoculation. The mode of action of cross-protection has been explained in a relatively complete general manner by a model based on a combination of RNA silencing and coat-protein-mediated resistance. Mild isolates will induce in tomato crop a symptomless

	infection without damage to the fruit, while an aggressive isolate will induce symptoms leading to economic losses in the crop. Both mild isolates Abp1 and Abp2 provides wide spectrum protection against PepMV aggressive isolates from both the CH2 and EU strain, as well as from other strains of PepMV, it is effective against all isolates of PepMV that are known to be present in Europe. The treatment can only be applied successfully on healthy tomato plants therefore treatment is done on tomato seedlings directly from the nursery and with the corresponding phytosanitary passport certifying absence of PepMV infection.

Adverse effects on field crops (Regulation (EU) N° 284/2013, Annex Part A, point 6.4)

Adverse effects on field crops	No adverse effects on field crops have been observed. As the mode of action is based on cross-protection in the tomato crop against aggressive isolates of PepMV the possibility of development of resistance is not relevant.

Observations on other undesirable or unintended side-effects (Regulation (EU) N° 284/2013, Annex Part A, point 6.5)

Observations on other undesirable or unintended side effects	-Non target organisms: see Ecotoxicology Section. -Pepino mosaic virus (PepMV), European strain (EU), mild isolate Abp1 and Pepino mosaic virus (PepMV), Chilean strain (CH2), mild isolate Abp2 are two new microbial active ingredients. Under the proposed directions of use of the GAP, there is not risk of PepMV infection to succeeding crops. There are no indications that the plant protection product could affect other plants, including adjacent crops. Impact on treated plants or plant products to be used for propagation is not relevant. There are not effects on the incident of other non-target organisms or environmental effects have been observed.

Appendix II.4: Chapter 4 (Toxicology)

(Regulation (EU) N° 283/2013, Annex Part A, point 5 and Regulation (EU) N° 284/2013, Annex Part A, point 7)

Medical data (including medical surveillance on manufacturing plant personnel) (MA 5.1.1)	Routine exposure of personnel, laboratory researchers, as well as consumers of tomatoes affected with PepMV, has not resulted in any known adverse effects of toxicological significance, and no literature is available in the public domain supporting evidence for toxicity potential of plant viruses in general or PepMV specifically to humans and mammals. A medical officer annually examined the involved personnel (up to 13 persons over 3 years) by performance of standard medical tests and confirmed in writing that

	exposures of workers to PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, during the years 2014–2016 did not result in any known incidence of adverse health effect, including hypersensitivity or chronic sensitization
Sensitisation (MA 5.2.1 & MP 7.2.3)	Regular exposure of farm and research personnel to PepMV did not result in any known incidence of hypersensitivity or chronic sensitisation. In the acute dermal toxicity studies on the formulation signs of erythema and oedema were assessed. No erythema and oedema were observed However, according to Regulation (EC) 283/2013, the formulation AbioProtect® should be regarded as potential sensitiser
Acute oral infectivity, toxicity and pathogenicity (MA 5.2.2.1 & MP 7.1.1)	LD_{50} oral rat > 2000 mg/kg bw corresponding to $6.32x10^{10}$ genome copies of PepMV/kg bw that is the sum of $1.7x10^{10}$ genome copies of Abp1/kg bw and $4.62x10^{10}$ genome copies of Abp2/kg bw. During the observation period, neither test item-related mortality nor toxic signs were recorded in animals treated with a dose of 2000 mg/kg bw (p.o.). PepMV, potexviruses or other members of the family Alphaflexiviridae do not have any effect on humans or mammals, and on the light of the results of the acute oral toxicity study, a report on the pathogenicity and infectivity by oral route of the active substances was not consider relevant.
Acute intratracheal/inhalation infectivity, toxicity and pathogenicity (MA 5.2.2.2 & MP 7.1.2)	LC_{50} > 5.02 mg/L air that corresponds with 3.39×10^8 PepMV genome copies/L air (1.46×10^8 genome copies/L air of Abp1 and 1.93×10^8 genome copies/L air of Abp2) No mortality was recorded during the study period. The main clinical signs observed after finishing exposure were chromorrhinorrhea, chromodacryorrhea, soiled coat, piloerection and breathing difficulty. All these signs were transient and most of them were not present the day after exposure. From study day 3 to the end of the 14-day observation period the animals exhibited a normal behaviour and no clinical signs related test item exposure were recorded with the exception of an isolated nasal discharge in one female animal. PepMV, potexviruses or other members of the family Alphaflexiviridae do not have any effect on humans or mammals, and on the light of the results of the acute oral toxicity study, a report on the pathogenicity and infectivity by inhalation of the active substances was not consider relevant.
Acute intravenous/intraperitonial infectivity (MA 5.2.2.3)	Instead of an intravenous toxicity study, an acute dermal toxicity study was performed because this type of exposure was considered to be more likely to happen in practice. The family Alphaflexiviridae, to which PepMV belongs, is recommended for the QPS list and all the scientific evidence supports the general assumption that PepMV, potexviruses or other members of the family

	Alphaflexiviridae do not have any effect on humans or mammals. Therefore, an intraperitoneal/subcutaneous single dose study is not considered relevant.
Genotoxicity (MA 5.2.3)	*In vitro* study: Bacterial reverse mutation test. (Ames test) *Salmonella typhimurium strains* TA98, TA100, TA102, TA1535 and TA1537 were exposed to the test item at 5 concentrations (100-50-25-12.5-6.25 µL/plate) with and without metabolic activation system (S9) under the direct incorporation and the pre-incubation procedures. No test item related cytotoxic activity was observed at any of the concentrations tested. PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 components of the test item AbioProtect® were NON-MUTAGENIC / NON PRO-MUTAGENIC in this bacterial test system, either in the presence or absence of metabolic activation in the strains tested at an exposure dose range of 6.25–100 µL/plate. A test on clastogenicity and gene mutation in mammalian cells were considered not relevant
Cell culture study (MA 5.2.4)	<u>Cell viability assay:</u> *Assay with the mixture of isolates Abp1 and Abp2 :* Reference tomato leaves extract not containing PepMV and tomato leaves extract containing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 at 2.5, 5, 10 and 20 v/v% decreased the viability of A549 cells after 24 hours of exposure to a similar extent, while lower concentrations (0.15625, 0.3125, 0.625, 1.25 v/v%) had no statistically significant effect on the viability of A549 cells. *Assay with the isolates Abp1 and Abp2 separately* *Abp2* The results showed a statistically significant decrease of the viability of A549 cells at concentrations > 10 v/v % in both the reference extract not containing PepMV and the extract containing PepMV, CH2 strain, mild isolate Abp2. *Abp1* The extract containing PepMV, EU strain, mild isolate Abp1 decreased the viability of A549 cells to a higher extent compared to reference extract not containing PepMV that was statistically different at concentrations ≥ 1.25 v/v%. <u>Cell proliferation assay</u>: *Assay with the mixture of isolates Abp1 and Abp2 :* Statistically significant differences between the reference tomato leaves extract not containing PepMV and tomato leaves extract containing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 were observed after 24 and 48 hours, while after 72 hours of exposure no statistically significant differences between test items were determined. *Assay with the isolates Abp1 and Abp2 separately* *Abp2*

	No statistically significant differences between reference extract not containing PepMV and the extract containing PepMV, CH2 strain, mild isolate Abp2were observed at any treatment time (24, 48 and 72 hours). *Abp1* Statistically significant differences between the reference extract not containing PepMV and the extract containing PepMV, EU strain, mild isolate Abp1 were observed after 24 and 48 hours, while after 72 hours of exposure no statistically significant differences between these two test items were determined. The likely reason for cytotoxic activity and anti-proliferative activity is the presence of biologically active natural constituents and metabolic products in the plant extracts and not the presence of Pepino mosaic virus (PepMV) as all test items decreased cell proliferation to a similar extent after prolonged treatment. <u>Infectivity of cells and replication assay</u> There are no indications of the infectivity or the replication of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 in human alveolar epithelial cells type 2 A549.
Information on short-term toxicity and pathogenicity (MA 5.2.5)	No acute toxicity or mutagenicity or cell infectivity was observed in the acute toxicity, mutagenicity and cell infectivity studies. Further studies including short-term toxicity and pathogenicity studies are not considered relevant.
Dermal toxicity (MP 7.1.3)	LD_{50} dermal rat > 2000 mg/kg bw that corresponds with $6.32x10^{10}$ genome copies of PepMV/kg bw, that is the sum of $1.7x10^{10}$ genome copies of Abp1/kg bw and $4.62x10^{10}$ genome copies of Abp2/kg bw. During the observation period, neither test item-related mortality nor severe toxic signs were recorded in animals administered topically with a dose of 2000 mg/kg.
Specific toxicity, pathogenicity and infectivity (MA 5.3)	Not required
In vivo studies in somatic cells (MA 5.4)	Not required
Genotoxicity – *in vivo* studies in germ cells (MA 5.5)	Not required

values

AOEL	<u>Technical Grade Active Ingredient (TGAI):</u> As no exposure models exist for microbials, setting an AOEL would be of low relevance to the risk assessment.	
ADI	Not required as oral exposure is not expected	
ARfD	Not required as oral exposure is not expected	

Exposure

Exposure (operator, workers, bystander, consumer) (MA 6.1 & MP 7.3, 8.0)	Operators:	Mixing/loading: PPE (coveralls, respiratory equipment and gloves) are recommended. Spray application: PPE (coveralls, respiratory equipment and gloves) are recommended.
	Workers:	Spray application: PPE (coveralls, respiratory equipment and gloves) are recommended.
	Bystanders:	Not applicable

Appendix II.5: Chapter 5 (Residues)

Residues in or on treated products, food and feed (Annex IIM 6; IIIM 8)

Viable residues	No risk for consumer is expected since plant viruses like Pepino Mosaic Virus are ubiquitous in plants and fruits and there are no documented causes of harmful effects in humans. Viruses are not able to produce metabolites.
Non-viable residues	No risk for consumer is expected since the assessment shows that the exposure to the impurity nicotine is well below the ADI and ARfD.

Appendix II.6: Chapter 6: Fate and behavior in the environment (Regulation (EU) N° 283/2013, Annex Part B, point 7 and Regulation (EU) N° 284/2013, Annex Part B, point 9)

Persistence and multiplication in soil, water and air	*Pepino mosaic virus*, EU strain and CH2 strain are endemic in tomato culture in most European countries. The presence of EU strain, mild isolate Abp1 and CH2 strain, mild isolate Abp2 does not pose an additional risk. Plant virus does not replicate outside the plant cell. The virus has limited stability outside host, does hardly replicate in plants other than hosts (*Solanaceae*). The virus does not have a cellular structure and does not produce metabolites. Based on experimental results, it can be concluded that the virus is no persistent in soil, water and air when the product AbioProtect® is used as a plant vaccine in protected tomato crops.
Mobility	It is unlikely that Pepino Mosaic Virus will be mobile in the environment via soil or air. Limited mobility in water (recirculation water in greenhouses). No risk of PepMV infection from recirculating water in greenhouses. Not relevant.

PEC soil

Microorganism	PepMV, EU strain, mild isolate Abp1 PepMV, CH2 strain, mild isolate Abp2
Method of calculation	

Application data	Crop: Tomato Depth of soil layer: 5cm Soil bulk density: 1.5g/cm3 plant interception: 50% Number of applications: 1 Interval (d): - BBCH: 13 Application rate(s): 8 L/ha, equal to a range from 51.2 to 512 g product/ha (>$4x10^{12}$ genome copies/ha).

PECsoil	mg product/kg soil	Genome copies/ha
Initial	0.34 mg AbioProtect®/kg	>$0.26x10^{3}$ genome copies of PepMV/kg soil: - >$0.13x10^{3}$ genome copies of PepMV, EU strain, mild isolate Abp1/kg of soil, and - >$0.13x10^{3}$ genome copies of PepMV, CH2 strain, mild isolate Abp2/kg of soil.

PEC surface water

Microorganism Method of calculation	PepMV, EU strain, mild isolate Abp1 PepMV, CH2 strain, mild isolate Abp2 Drift from a greenhouse is assumed to be 0.1%
Application rate	8 L/ha, equal to a range from 51.2 to 512 g/ha (>$4x10^{12}$ genome copies/ha).

PECsw	µg product/L water	Genome copies/L water
Initial	$17.06x10^{-2}$ µg Abioprotect®/L water	$1.33x10^{6}$ genome copies of PepMV/L water: - $6.66x10^{6}$ genome copies of PepMV, EU strain, mild isolate Abp1/L water, and - $6.66x10^{6}$ genome copies of PepMV, CH2 strain, mild isolate Abp2/L water.

Appendix II.7: Chapter 7 (Effects on non-target organisms)

Effects on non-target organisms (Regulation (EU) 283/2013, Annex Part B, point 8 and Regulation (EU) 284/2013 Annex Part B, point 10; OECD IIM point 8 & IIIM point 10)

Effects on birds or other terrestrial invertebrates (MA 8.1 & MP 10.1; OECD IIM 8.1 & IIIM 10.1)

Application rate (kg MPCA/ha)	Crop	Category (e.g. insectivorous bird)	Time-scale	Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)
No studies provided. Given the nature of the MCPAs (plant virus) no toxicity or infectivity has to be expected in vertebrates. The levels of natural occurrence of CH2 and EU strains ranged from between 10^{3} – 10^{5} genome copies/g dry plant.				

Effects on aquatic organisms (MA 8.2 & MP 10.2; OECD IIM 8.2, 8.3 & IIIM 10.2)

Group	Test substance	Time-scale	Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)
No studies provided. Given the nature of the MCPAs (plant virus) no toxicity or infectivity has to be expected in fish or aquatic invertebrates.			

EFFECTS ON ALGAE: (species, growth, growth rate, capacity to recover) (MA 8.2.3 & MP 10.2; OECD IIM 8.4. & IIIM 10.2)	Given the nature of MPCAs (plant virus) no toxicity or infectivity is expected in algae and has been corroborated in a GLP study on the toxicity to the algae *Pseudokirchneriella subcapitata* NOEC<100 mg/L (nominal) corresponding to 1.5x107 PepMV genome copies/L ($2.99x10^6$ genome copies of PepMV-Abp1/L+$1.2x10^7$ genome copies of PepMV-Abp2/L).
EFFECTS ON AQUATIC PLANTS: (species, growth, growth rate, capacity to recover) (MA 8.2.4 & MP 10.2; OECD IIM 8.5 & IIIM 10.2)	Given the nature of MPCAs (plant virus) limited infectivity has to be expected with no adverse effects in plants other than *Solanaceae,* no toxicity is expected and has been corroborated in a GLP study on the toxicity to the dunkweed *Lemna gibba.* NOEC <100 mg/L (nominal) corresponding to 1.5x107 PepMV genome copies/L ($2.99x10^6$ genome copies of PepMV-Abp1/L+$1.2x10^7$ genome copies of PepMV-Abp2/L).

Effects on bees (MA 8.3 & MP 10.3; OECD IIM 8.7 & IIIM 10.3)

Application rate (kg MPCA/ha)	Crop	Route	Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)
Laboratory test			
No studies provided. Given the nature of the MCPAs (plant virus) no toxicity or infectivity has to be expected in bees. Given the timing of application (BBCH 13-15) very low exposure is expected for bees.			

Effects on terrestrial arthropods other than bees (MA 8.4 & MP 10.4; OECD IIM 8.8 & IIIM 10.4)

Species	Stage	Test substance	Route	Dose (kg MPCA/ha)	Toxicity, infectivity and pathogenicity (endpoint, value or other description of effects)
Laboratory test					
No studies provided. Given the nature of the MCPAs (plant virus) no toxicity or infectivity has to be expected in other arthropod species.					

Effects on other terrestrial invertebrates (MA 8.5 & MP 10.5; OECD data point IIM 8.9.2 & IIIM 10.5)

Toxicity, infectivity and pathogenicity: (endpoint value or other description of effects)	No studies provided. Given the nature of the MPCAs no toxicity or infectivity has to be expected in non-arthropod invertebrates.
Further information	None.

Effects on soil microorganisms (MA 8.6 & MP 10.6; OECD data point IIM 8.10 & IIIM 10.6)

No studies provided. Given the nature of the MPCAs no toxicity or infectivity has to be expected in non-target soil microorganisms.

Additional studies (MA 8.7 & MP 10.7; OECD data point IIM 8.11 & IIIM 10.7)

None.

GAP TABLE: DETAILS OF ALL NATIONAL GAPS WITHIN EACH ZONE

MPCP/PPP (product name/code) **AbioProtect®** **Formulation: Type:** **SC**[(a-b)]
MPCA: active ingredient 1 **PepMV, EU strain, mild isolate Abp1** **Conc. of as 1:** **at least 2.5 x 10^{11} genome copies/L**
MPCA: active ingredient 2 **PepMV, CH2 strain, mild isolate Abp2** **Conc. of as 2:** **at least 2.5 x 10^{11} genome copies/L**

Zone(s): **EU** **Professional use** ☒ **Non professional use** ☐

1	2	3	4	5	7	8	9	10	11	12	13	14
					Application			**Application rate per treatment**				
Use-No	**Member state(s)**	**Crop and/ or situation** (crop destination/purpose of crop) (c)	**F G or I** (d)	**Pests or Group of pests controlled** Additionally: developmental stages of the pest or pest group (e)	Method Kind (f-g)	Timing/ Growth stage of crop & season (h)	Max number (min interval between applications) a) per use b) per crop/ season	kg, L product /ha a) max rate per appl. b) max. total rate per crop/season (i)	kg, L a.s /ha a) max rate per appl. b) max. total rate per crop/season	Water L/ha min/ max	**PHI (days)** (j)	**Remarks** e.g. g. safener/synergist per ha (k)
1	All	*Solanum lycopersicum* (tomato) (LYPES)	G	Pepino mosaic virus (PEPMVO, PepMV)	Low volume spraying (aerial spraying with an airbrush 75 psi/ 5171.07 mbar/ 517.10 kPa)	Seedlings immediately before planting (BBCH 13-15) Jan-Dec	a) 1 per use b) 1 per crop cycle	a) 0.1–1.6 L/ha (0.05-0.8 L/ha PepMV Abp1 and 0.05-0.8 L/ha of PepMV Abp2) b) 0.1 – 1.6 L/ha per crop cycle	At least 1.25 – 2.0 x 10^{12} genome copies/ha of Abp1 and At least 1.25-2.0 x 10^{12} genome copies/ha of Abp2	4–7.84 L/ha	NA	-

Remarks:

a) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR).
b) GCPF Codes - GIFAP Technical Monograph No 2, 1989.
c) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure).
d) Outdoor or field use (F), glasshouse application (G) or indoor application (I).
e) e.g. biting and suckling insects, soil born insects, foliar fungi, weeds.
f) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench.
g) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated.
h) Growth stage at last treatment (BBCH Monograph, Growth stages of mono- and dicotyledoneous plants, 2º edit 2001, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application.
i) The minimum and maximum number of application possible under practical conditions of use must be provided.
j) PHI - minimum pre-harvest interval.
k) Remarks may include: Extent of use/economic importance/restrictions.