European Commission

Draft Renewal 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

Volume 1

Rapporteur Member State: Spain

July 2019

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

Statement of subject matter and purpose for which this report has been prepared and background information on the application

1 Statement of subject matter and purpose for which this report has been prepared and background information on the application

1.1 Context in which the renewal assessment report was prepared

1.1.1 Purpose for which the renewal assessment report was prepared

This dossier is submitted by Abiopep S.L., Spain, for the European approval of **two new microbial active ingredients (Microbial Pest Control Agents) MPCA**:

- ***Pepino mosaic virus*, European (EU) strain, mild isolate Abp1.**
- ***Pepino mosaic virus*, Chilean (CH2) strain, mild isolate Abp2.**

And the representative formulation **AbioProtect®**

Under REGULATION (EC) No 1107/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 21 October 2009 concerning the placing of plant protection products on the market.

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

The dossier is submitted to Spain, which acts a Rapporteur Member State for the EU Commission.

1.1.3 EU Regulatory history for use in Plant Protection Products

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1.1.4 Evaluations carried out under other regulatory contexts

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1.2 Applicant(s) information

Contact person: [redacted]

Address: Abiopep Plant Health S.L.
Parque Científico de Murcia,
Edif. R, 2ª. Complejo de Espinardo,
Ctra. Madrid Km 388.
E-30100 Espinardo, Murcia,
Spain

Telephone: [redacted]
[redacted]
[redacted]

1.2.1 Name and address of applicant(s) for approval of the active substance

1.2.2 Producer or producers of the active substance

Contact person: [redacted]

Address: Abiopep Plant Health S.L.

Parque Científico de Murcia,
Edif. R, 2ª. Complejo de Espinardo,
Ctra. Madrid Km 388.
E-30100 Espinardo, Murcia,
Spain
Telephone: [redacted]

1.2.3 Information relating to the collective provision of dossiers

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1.3 Identity of the micro-organism

1.3.1 Name and species description, strain characterisation	• **Pepino mosaic virus (PepMV), European (EU) strain, mild isolate Abp1.** • **Pepino mosaic virus (PepMV), Chilean (CH2) strain, mild isolate Abp2.** Species: Pepino mosaic virus (PepMV). First description: Jones et al., (1980) **Strain: European (EU) mild isolate Abp1** **Chilean (CH2) mild isolate Abp2** Genus: Potexvirus Family: Alphaflexiviridae Order: Tymovirales
1.3.1.1 Composition of material used for manufacturing of the formulated product	
Confidential information, see Vol 4.	
1.3.1.2 Accession number in culture collection	The isolates are deposited in the German Collection of Microorganisms and Cell Cultures Leibniz-Institut (DSMZ) GmbH. Inhoffenstraße 7 B-38124 Braunschweig, Germany. PepMV, EU strain, isolate Abp1. Reference number DSM32069. PepMV, CH2 strain, isolate Abp2. Reference number DSM32070.
1.3.1.3 Scientific name and taxonomic grouping, i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the micro-organism	
Taxonomy	Pepino mosaic virus (PepMV), European (EU) strain, mild isolate Abp1. Pepino mosaic virus (PepMV), Chilean (CH2) strain, mild isolate Abp2.

	Species: Pepino mosaic virus (PepMV). First description: Jones et al., (1980) Strain: European (EU) mild isolate Abp1 Chilean (CH2) mild isolate Abp2 Genus: Potexvirus Family: Alphaflexiviridae Order: Tymovirales
Indigenous or non-indigenous	PepMV, EU strain, mild isolate Abp1 originates from a natural, indigenous wild type and it is not genetically modified. PepMV, CH2 strain, mild isolate Abp2 originates from a natural, indigenous wild type and it is not genetically modified.
Wild type	PepMV, EU strain, mild isolate Abp1 originates from a natural wild type PepMV, isolated from samples taken in a commercial tomato crop in Murcia (Spain) in 2001. PepMV, CH2 strain, mild isolate Abp2 originates from a natural wild type PepMV, isolated from samples taken in a commercial tomato crop in Murcia (Spain) in 2007.
Spontaneous or induced mutant*	The strain is not described as a mutant
Genetically modified according to Directive 2001/18/EC*	No
* All known differences between the modified micro-organism and the parent wild strain must be provided	
1.3.1.4 Test procedures and criteria used for identification	
The best available technology for identification of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 is nucleotide sequencing, by sequencing a genomic fragment that includes a part of tgb2 and tgb3 genes and the complete cp gene (942 and 943 nt, respectively). In short, total RNA (RNAt) extracts are used to generate complementary DNAs (cDNAs) by reverse transcription (RT) and amplification by polymerase chain reaction (PCR) using generic primers for PepMV. PCR products are purified, ligated using the TA cloning vector pGEM-T Easy (Promega, USA) and transformed in StellarTM competent cells (Clontech Laboratories, USA) following the manufacturers' instructions. Several clones are sequenced with universal M13 primers.	
1.3.1.5 Common name or alternative and superseded names and code names used during the development	There is no common name for the organism.
1.3.1.6 Relationship to known pathogens	PepMV belongs to the order Tymovirales that include plant viruses only. All closely related species are plant pathogens. PepMV is closely related to Narcisus mosaic virus (NMV), Scallion virus X (SVX), Cymbidium mosaic virus (CymMV) and Potato aucuba mosaic virus (PAMV) (Cotillion et al., 2002). The highest overall nucleotide identities are with NMV and CymMV (Aguilar et al., 2002).

	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. Plant viruses like PepMV are ubiquitous in plants and fruits and therefore humans are continuously exposed to them.
1.3.1.7 Method of manufacture (synthesis pathway) of the active substance	Confidential information, see Volume 4.
1.3.2 Specification of the material used for manufacturing of formulated products	Confidential information, see Volume 4.
1.3.3 Content of the micro-organism	The content, of the pure microorganism PepMV, EU strain, mild isolate Abp1 is set up to be at least $2.5x10^{11}$ genome copies/L in the final formulation of the MPCP (AbioProtect®). Also, the content of the pure microorganism PepMV, CH2 strain, mild isolate Abp2 is set up to be at least $2.5x10^{11}$ genome copies/L in the final formulation of the MPCP (AbioProtect®). **The applicant must specified the content of the MPCA technical of both actives substances.**
1.3.4 Identity and content of impurities, additives, contaminating micro-organisms	
1.3.4.1 Significant impurities	No significant impurities are present.
1.3.4.2 Relevant impurities	No relevant impurities are present.
1.3.4.3 Additives	Not applicable
1.3.4.4 Contaminating micro-organisms	Five batches of the formulation containing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 were tested for determination of the following microorganisms potentially harmful for humans: - Salmonella absence in 25 mg o 25 mL (PNT-2; qPCR method conforms to ISO/IEC 17025:2005). - Listeria monocytogenes absence in 25 mg o 25 mL (PNT-06; qPCR method conforms to ISO/IEC 17025:2005). - Escherichia coli absence in 1g or mL (ISO 16649-2:2001). - Thermotolerant (faecal) coliforms < 10 CFU/g or mL (Petrifilm). - Aerobic plate count <105 CFU/g or mL (UNE-EN ISO 4833-2:2014) See Volume 4, Annex C.
1.3.5 Analytical profile of batches	Confidential information, see Volume 4.

1.4 Information on the plant protection product

1.4.1 Applicant	[redacted] Address: Abiopep S.L. Parque Científico de Murcia, Edif. R, 2ª. Complejo de Espinardo, Ctra. Madrid Km 388. E-30100 Espinardo, Murcia, Spain [redacted]
1.4.2 Producer of the plant protection product	[redacted] Address: Abiopep Plant Health S.L. Parque Científico de Murcia, Edif. R, 2ª. Complejo de Espinardo, Ctra. Madrid Km 388. E-30100 Espinardo, Murcia, Spain [redacted]
1.4.3 Current, former and proposed trade names and development code numbers	
Trade name	AbioProtect®
Code number	
1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product	
1.4.4.1 Composition of the plant protection product	Confidential information, see Volume 4.
1.4.4.2 Information on the active substance(s)	The preparation is composed by: Pepino mosaic virus (PepMV), European (EU) strain, mild isolate Abp1. Pepino mosaic virus (PepMV), Chilean (CH2) strain, mild isolate Abp2.
1.4.4.3 Information on safeners, synergists and co-formulants	Confidential information, see Volume 4.

1.4.5 Type and code of the plant protection product	Aqueous suspension concentrate [SC].
1.4.6 Function	Elicitor: control of PepMV aggressive isolates by cross-protection after virus inoculation.
1.4.7 Field of use envisaged	Use in horticulture, in protected (greenhouse) crops.
1.4.8 Effects on harmful organisms	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.

1.5 Detailed uses of the plant protection product

1.5.1 Details of representative uses

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 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:
e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR).
GCPF Codes - GIFAP Technical Monograph No 2, 1989.
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).
Outdoor or field use (F), glasshouse application (G) or indoor application (I).
e.g. biting and suckling insects, soil born insects, foliar fungi, weeds.
Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench.
Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated.
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.
The minimum and maximum number of application possible under practical conditions of use must be provided.
PHI - minimum pre-harvest interval.
Remarks may include: Extent of use/economic importance/restrictions.

1.5.2 Further information on representative uses

AbioProtect® is to be used against aggressive isolates of PepMV in greenhouse tomato crops.

It is not necessary to wait any specific interval before treatment with any chemical pesticide, the treatment is to be applied alone by Abiopep trained and qualified personnel.

Application rate: AbioProtect® is used at 5 L/10,000 plants equivalent to 1 ha in standard tomato greenhouse practice, in cases where a very high risk of PepMV infection exist the rate could reach 8 L/ha.

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

1.5.4 Overview on authorisations in EU Member States

The incidence of *Pepino mosaic virus* (PepMV) in greenhouse tomato crops has increased substantially over the last decade all across Europe and so have the problems to the tomato production induced by the corresponding disease. PepMV was first detected in tomato crops in the Netherlands in 1999, shortly after it was detected in other tomato crops in Europe. In Spain it was first detected in 2000. Studies in the genetic variability and evolution of the PepMV populations in Spain have shown that in tomato crops there is presence of EU and CH2 strains of PepMV both in single and mixed infections. This spread of PepMV is causing important economic losses to tomato producers all across Europe; therefore there is an urge to find an effective solution against the problems induced by PepMV.

In Spain the end used product AbioProtect® has been authorized under Art. 53 of Regulation (EC) 1107/2009 starting on 15th June 2016, for a period of 120 days. This Authorization was granted since:

There is no chemical or biological plant protection product approved for treatment of tomato crops against PepMV offering wide spectrum protection against isolates of both strains EU and CH2, suitable for the phytosanitary conditions of Spain, and

The local tomato sector expressed in writing the urgent need for authorization of a product against PepMV with wide spectrum protection against both EU and CH2 strains.

The authorization holder is Abiopep S.L.

Registered uses	Actual uses
Tomato in greenhouse, against PepMV of both EU and CH2 strains, 5 L/ha, 1 application per crop cycle, tomato seedlings immediately before transplanting to the greenhouse, 3-5 leaves (BBCH 13-15).	The same as registered

Level 2

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

Summary of active substance hazard and of product risk assessment

2 SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

2.1 IDENTITY

2.1.1 Identity of microorganism

The microbial pest control agents of the biological elicitor product against infections of aggressive isolates of *Pepino mosaic virus* (PepMV) in tomato protected crops named AbioProtect® are PepMV, European (EU) strain, mild isolate Abp1 and PepMV, Chilean (CH2) strain, mild isolate Abp2. PepMV is a member of the genus *Potexvirus*, family *Alphaflexiviridae*, Order *Tymovirales*.

Both PepMV mild isolates (Abp1 and Abp2) originate from natural, indigenous wild type viruses, are not genetically modified.

The isolates are deposited in the German Collection of Micro-organisms and Cell Cultures Leibniz-Institut (DSMZ) GmbH. Inhoffenstraße 7 B-38124 Braunschweig, Germany. PepMV, EU strain, isolate Abp1 with reference number DSM32069 and PepMV, CH2 strain, isolate Abp2 with reference number DSM32070.

There are several methods for detection, identification and differentiation of PepMV genotypes and identification of the isolates, including nucleotide sequence, real-time quantitative reverse transcription PCR (RT-qPCR), molecular hybridisation and a bioassay in tomato.

PepMV has a ss(+)RNA genome of about 6.4 kb, which encodes five proteins: a protein involved in virus replication (RdRp); three proteins involved in cell-to-cell movement, with overlapping genes organized into a triple gene block (proteins TGB1, TGB2, and TGB3), and the coat protein (CP). The best available technology for identification of both MPCAs is based on sequencing a genomic fragment, which includes part of the *tbg2* and *tbg3* genes and the complete *cp* gene (942 nt for Abp1 and 943 nt for Abp2). PepMV forms non-enveloped flexuous rod-like virus particles, typical of potexviruses 508 nm long x 12.5nm.

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.

To unambiguously identify PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, the identity of that fragment to the reference sequence for Abp1 or Abp2 has to be at least of a certain percentage.

The content of pure virus in PepMV, EU strain, mild isolate Abp1 technical is set up to be at least $2.5x10^{11}$ genome copies/L, and in PepMV, CH2 strain, mild isolate Abp2 technical is set up to be at least $2.5x10^{11}$ genome copies/L.

The content of the pure microorganisms PepMV, EU strain, mild isolate Abp1 is set up to be of a minimum of $2.5x10^{11}$ genome copies/L in the final formulation of the MPCP (AbioProtect®), ranging from 10 to 100 mL/L (3.2 to 32 g of the inoculated plant material). The representative batches of PepMV EU strain, mild isolate Abp1 analyzed and included in this dossier were formulated at a dosage of 100 mL/L, ranging from $5.43x10^{11}$ to $3.21x10^{12}$ genome copies/L, always above the minimum set up content of $2.5x10^{11}$ genome copies/L.

The content of the pure microorganisms PepMV, CH2 strain, mild isolate Abp2 is set up to be of a minimum of $2.5x10^{11}$ genome copies/L in the final formulation of the MPCP (AbioProtect®), ranging from 10 to 100 mL/L (3.2 to 32 g of the inoculated plant material). The representative batches of PepMV, CH2 strain, mild isolate Abp2

analyzed and included in this dossier were formulated at a dosage of 100 mL/L, ranging from $1.48x10^{12}$ to $7.68x10^{12}$ genome/L, always above the minimum set up content of $2.5x10^{11}$ genome copies/L.

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.If in a batch the presence of any of these pathogens were detected at a potential dangerous concentration the whole batch would be destroyed.

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.

Nicotine content has been determined in five independent batches of PepMV, EU strain, mild isolate Abp1 and of PepMV, CH2 strain, mild isolate Abp2, with a medium of 0.027 mg/kg for PepMV, EU strain, mild isolate Abp1 and with a medium of 0.050 mg/kg for PepMV, CH2 strain, mild isolate Abp2. Therefore it could be concluded that the impurity nicotine is present at very low levels in the active substances (PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) or the formulation AbioProtect®.

There are no impurities or additives. Inert ingredients consists of tomato leaves extract and water.

2.1.2 Identity of the microbial plant protection product

The microbial plant protection product AbioProtect® acting as an elicitor to control infection of aggressive PepMV isolates in greenhouse tomato crop is formulated to contain at least $5x10^{11}$ PepMV genome copies /L, with equivalent amounts of PepMV, EU strain, mild isolate Abp1 and of PepMV, CH2 strain, mild isolate Abp2 to guarantee a minimum content of at least $2.5x10^{11}$ genome copies of each PepMV isolate.

Other inert ingredients are tomato leaves extracts and water.

All the concerned CA data points (microbial pest control agent: PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2), and CP data points (preparation AbioProtect®) have been addressed and included in the corresponding Vol 3 CA and CP reports.

2.2 BIOLOGICAL, PHYSICAL, CHEMICAL AND TECHNICAL PROPERTIES

2.2.1 Biological properties of the microorganism

PepMV, EU strain, mild isolate Abp1 was isolated from plants of a commercial tomato crop in Murcia (Spain) in 2001. PepMV, CH2 strain, mild isolate Abp2 was isolated from plants of a commercial tomato crop in Murcia (Spain) in 2007. Both isolates were characterised and their biological properties further studied. PepMV as other plant viruses (wild types including mild strains or isolates) can be expected everywhere in plants in the environment. Viruses tend to survive only for short periods outside of host organisms in soil and water.

PepMV presence has been described in 19 countries in Europe and is included in European and Mediterranean Plant Protection Organization (EPPO) A2 list of pest recommended for regulation as quarantine pest.

PepMV mild isolates are used to protect tomato crops against infection from aggressive isolates of PepMV by cross-protection. In fact, both PepMV mild isolates of this application have been tested in cross-protection of tomato crops against aggressive isolates of PepMV, and these tests have shown that PepMV, EU strain, mild isolate Abp1 protects effectively against PepMV aggressive isolates of the EU strain and that PepMV, CH2 strain, mild isolate Abp2, protects effectively against PepMV aggressive isolates of the CH2 strain. Furthermore, cross-protection tests using both PepMV mild isolates together have shown that such combination provides wide spectrum protection against aggressive isolates of both strains as well as of other PepMV strains.

Viral cross-protection in plants is known as an acquired immunity phenomenon, where a mild virus isolate can protect plants against economic damage caused by a severe challenge isolate of the same virus. 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.

The PepMV host range is mainly restricted to plant species from the *Solanaceae* family, it is highly infectious for plants of this family. PepMV might survive or replicate also in plants of other botanical families without causing adverse effects. The virus has been detected in weeds however the exact role of these weed species in the

epidemiology of PepMV is not known. Furthermore, a study on the potential role of alternative and potential non-tomato host plants of PepMV has shown that treatment of tomato plants in a greenhouse with PepMV does not appear to affect the level of natural occurrence of the virus.

PepMV life cycle 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 or viral ribonucleoprotein complexes are transported into neighbouring cells through small channels called plasmodesmata that form connections between cells. Long-distance movement of the virus within the plant occurs through the vasculature, normally the phloem. 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.

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. Regarding human or animal pathogens, plant pathogenic viruses are generally considered to be pathogenic towards plant species only and not towards other organisms, like humans. Human exposure to plant pathogenic viruses is enormous and human illnesses caused by plant pathogenic viruses have not been reported. Multiplication of plant viruses in humans is unknown. PepMV does not produce toxins.

As with all ssRNA viruses mutation can occur. Risk management procedures are in place to prevent the occurrence of virulent isolates in the end product.

2.2.2 Physical, chemical and technical properties of the microbial plant protection product

AbioProtect® is a SC preparation containing the microbial pest control agents PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, it is not reactive, not oxidizing, not flammable. It is a greenish yellow liquid with slight suspension (turbid) and a characteristic odour. The preparation is slightly acidic, has a pH 5.89±0.03, with a dynamic viscosity of 5.03-5.70 mPa s at 20 ºC ± 0.2, and of 4.20-4.77 mPa s at 40 ºC±0.2, the persistence in foam is 0 mL after 10 seconds, it has a suspensibility of 110.82±0.02% and a spontaneity of dispersion of 110.85± 0.02%. The wet sieve test on the formulation gives a <0.1% retention and the formulation has a pourability of 0.282±0.005 %. Its density is 0.9986±0.0002 g/mL.

When stored at room temperature (20ºC) this biological plant protection product is only stable for just one day, the stability goes up to 35 days if stored at 4 ºC. When stored at <- 18ºC is stable for 9 months with a shelf life of 9 months.

No information regarding the technical properties after storage up to 35 days at 4ºC or -18ºC (shelf life claimed by the applicant) has been reported. The RMS has considered these data are not necessary due to the specific application and used of Abiopep according to the applicant:
MPCP is only applied by qualified Abiopep personnel, it is always formulated on demand after slowly defrosting the MPCAs at 4 ±2ºC, kept refrigerated at 4-7ºC until use on the same day. The technical properties have been tested in the MPCP thus formulated showing that no particular problems are to be expected when the product is used as recommended.

2.3 DATA ON APPLICATION AND EFFICACY

2.3.1 Summary of effectiveness

The efficacy of AbioProtect® and its components was tested in six greenhouse GEP certified trials in different locations in Spain from 2014-2017. The trials demonstrated the effectiveness and appropriate crop safety of AbioProtect® against infection by PepMV aggressive isolates from both the EU and the CH2 strains, in different tomato cultivars and at a dose of 5 L/10,000 plants (equivalent to 1 ha) in only one application to the tomato seedlings (BBCH stage 13 to 15) done in a close compartment at the final destination premises, right before transplanting to the commercial greenhouse tomato plot. The protection conferred with AbioProtect® treatment lasted until the end of the crop. The data were obtained in greenhouse trials and therefore are representative for the entire EU.

In all the trials the formulation has been applied at the proposed dose rate of 5 L/10,000 plants (1 ha), containing at least 5x1011 genome copies of PepMV/L (at least 2.5x1011 genome copies of PepMV, EU strain, mild isolate Abp1/L and at least 2.5x1011 genome copies of PepMV, CH2 strain, mild isolate Abp2/L).

Treatment with AbioProtect® and its components resulted in no symptoms or some mild symptoms of PepMV infection. In cases were some mild symptoms appeared those were generally transient and in most cases did not affect quality of the fruits. Moreover yield was not affected. The treatment does not pose any risk to or impact on succeeding or adjacent crops or plants, is not for use in plants to be used for propagation and has no effect on beneficial or other non-target organisms.

In conclusion AbioProtect® has proved a good performance in practice providing wide spectrum protection against infection induced by PepMV aggressive isolates in tomato, under the agricultural, plant health and environmental conditions of tomato greenhouse cultivation in EU. The dose of 5 L/ha, applied once on tomato seedlings (13-15 BBCH) gives adequate protection against PepMV aggressive isolates of both the EU and the CH2 strain.

2.3.2 Summary of information on the development of resistance

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. Please refer to Volume 3 Annex B.2 data point B.2.7 Genetic stability and factors affecting it and Volume 3 Annex B.3 (Data on application) data point B.3.5 Information on the occurrence or possible occurrence of the development of resistance of the target organism(s) for further information.

2.3.3 Summary of adverse effects on treated crops

Effects on the quality of plants or plant products

AbioProtect® and its components achieve a high efficacy against PepMV, showing no symptoms in fruits during the trials with similar data to the un-inoculated control and with clear significant differences with the challenged inoculated controls.

Effects on the transformation process

Treatment with AbioProtect® will not have any interference on transformation processes as viruses have no metabolism of their own, it does not produce residues and neither leaves residues at harvest.

Effects on the yield of treated plants or plant products

The formulation AbioProtect® and its components achieve a high efficacy against PepMV, showing no symptoms during the trials with similar data and total production (yield) to the un-inoculated control and with clear significant differences with the challenged inoculated controls.

Phytotoxicity to target plants (including different cultivars), or to target plant products

Treatment with AbioProtect® and its components resulted in no symptoms or some mild symptoms of PepMV infection. In cases where some mild symptoms appear those were generally transient and, in most cases, did not affect quality of the fruits. Moreover, yield was not affected.

2.3.4 Summary of observations on other undesirable or unintended side-effects

Impact on succeeding crops

Impact on succeeding crops was not tested in the efficacy trials. However, as the persistence in water GEP study concluded that the Plant Protection Product AbioProtect® has no persistency in the leachate from tomato plants treated with AbioProtect®, it could be concluded that there is no risk of PepMV infection with this leachate to succeeding crops.

Impact on other plants, including adjacent crops

Impact on other plants including adjacent crops was not tested as there are no indications that the plant protection product could affect adjacent crops via vapor drift.

Impact on treated plants or plant products to be used for propagation

Not relevant. The formulation AbioProtect® is not for used in plants or plants product to be use for propagation, more specifically it is not intended to be use in the production of seeds, cuttings or runners for propagation.

Effects on beneficial and other non-target organisms

In the different tests and studies conducted no effects on the incident of other non-target organisms or environmental effects have been observed.

2.4 FURTHER INFORMATION

2.4.1 Further information on the microorganism

The biological function of the PepMV mild isolates from this application is cross-protection against aggressive wild type PepMV isolates.

The microbial pest control agents (MPCAs) PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 are produced in plants by two rounds of amplification. AbioProtect® is formulated with the inocula obtained from the second round of amplification. Several quality checks are performed in every batch of each MPCA, to guarantee the identity of each isolate and to discard the appearance of potential aggressive PepMV variants. The presence of other pathogens potentially affecting tomato is also checked in every batch of each MPCA, in case of detection of a harmful microorganism to tomato at a potentially dangerous concentration the whole batch would be destroyed. Safety check is applied in every batch by determination of the presence of human pathogens.

2.4.2 EFFICACY

2.4.2.1 Dose justification and efficacy

2.4.2.1.1 Introduction

This dossier is submitted by Abiopep S.L., Spain, for the approval of two new microbial active ingredients (Microbial Pest Control Agents) MPCAs: Pepino mosaic virus (PepMV), European (EU) strain, mild isolate Abp1 and PepMV, Chilean (CH2) strain, mild isolate Abp2, under the Regulation (EC) 1107/2009 of the European Parliament.

PepMV belongs to the genus Potexvirus of the Alphaflexiviridae family; it is widespread in Europe and in fact is a major disease in greenhouse tomato crops worldwide.

The cross-protection effect and thus the actual activity is obtained by infection of the plants with the mild isolates of the virus: PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. Viral cross-protection in plants is known as an acquired immunity phenomenon, where a mild virus isolate can protect plants against economic damage caused by a severe challenge isolate of the same virus. 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.

PepMV is a plant virus, which can only replicate in living plant cells and the virus can only be produced in plants. Tomato is the most suitable host for PepMV, so production of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, is performed in tomato plants.

The preparation (Microbial Pest Control Product) MPCP AbioProtect® is a suspension concentrate formulated with equivalent amounts of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. The MPCP is envisaged as a preventive treatment in greenhouse (protective) tomato production against aggressive isolates of PepMV to be applied in a close compartment near or inside the final destination greenhouse in a single application to tomato seedlings (BBCH 13-15). Abiopep employs trained and qualified personnel to conduct product application and the product is never applied by third parties.

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 (PEPMO, PepMV)	Low volume spraying (aerial spraying with an airbrush 75 psi/ 5171.07 mbar/ 517.10 kPa)	Seedlings immediately before planting. Planting time (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.

2.4.2.2 Efficacy data

2.4.2.2.a Preliminary test

Gomez et al. (2009) showed that tomato plants infected with Pepino mosaic virus (PEPMVO, PepMV) isolates from the European (EU) strain together with isolates from the Chilean (CH2) strain (mixed infections) were symptomless. Therefore, to test the possibility of using mixed infections with mild isolates of PepMV from those two strains in cross-protection, several research trials were conducted using PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 in tomato plants against infection with an aggressive PepMV isolate.

A first small preliminary trial with 2 tomato cultivars, Pera and Kumato, was conducted in April-September 2012 in an experimental greenhouse at CEBAS-CSIC facilities in Murcia (Spain). Plants were inoculated with different treatments including mild isolate Abp1, mild isolate Abp2 and both mild isolates Abp1 and Abp2 simultaneously, as well as the corresponding controls. Treatments were later challenged with an aggressive PepMV isolate. Plant vigor and fruit production was observed and determined that the plants inoculated with both mild isolates simultaneously and subsequently challenged with the aggressive isolate, showed vigor and fruit production similar to the control without inoculation and not challenged. Results were confirmed in a second trial during April-September 2013 (a summary of those trials could be found in Aranda et al., 2016a).

Another preliminary trial to assess symptom performance was conducted in 2012 in a greenhouse in a commercial tomato production area in Región de Murcia (southeast Spain) with a history of high incidence of PepMV infections. The greenhouse was divided in two parts. The tomato seedlings for one part were inoculated with both PepMV mild isolates (Abp1 and Abp2) simultaneously at the moment of transplanting from the nursery and the tomato seedlings for the other part, were kept un-inoculated as control. Approximately 12-14 weeks after inoculation, the fruits of the un-inoculated control started to show PepMV symptoms, with a high percentage of plants affected, while the fruits of the inoculated plants remained symptomless or with very mild transient symptoms (a summary of those trials could be found in Aranda et al., 2016a).

AbioProtect® the formulation containing equivalent amounts of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, was first registered in Spain in 2014 according to Orden APA/1470/2007 number 2536, followed by a registration according to RD 951/2014 until October 2015. In 2016 a temporary exemption provided for in Article 53 of Regulation (EC) 1107/2009 have been granted for the use of AbioProtect® for the protection of greenhouse tomato cultivation against damage by aggressive PepMV from both the EU strain and the CH2 strain, and especially adapted for the specific phytosanitary situation in Spain. The application rate for those treatments during that period was set at 5 L/ha (10,000 tomato seedlings), as standard cropping practice for tomato greenhouses in southeast Spain grow on average 10,000 plants/ha.

A dose trial was conducted in 2016-2017 with GEP certification.

Report MP 6.1/01 Field study to evaluate the crop safety and the efficiency and velocity of the infection of the Plant Protection Product (PPP) AbioProtect® applied at different doses in tomato crop (Southern Spain, 2016). Prats (2017c). (Unpublished report). Study Code: ACEX/1276/AB.

Guideline: PP 1/152(3), PP 1/181(3) and PP/135 (3).

GEP: Fully GEP compliance

The aim of the study was to evaluate the efficiency and velocity of the infection of AbioProtect® applied at different doses in tomato crops, as well as to evaluate the treated crop for appearance of phytotoxicity effects.

Methodology

The trial lasted form October 10th until November 15th, 2016.

The test product, AbioProtect®, was applied at three rates (3, 5 and 8 L/ha). At a concentration of at least $5x10^{11}$ genome copies of PepMV/L (at least $2.5x10^{11}$ genome copies of PepMV, EU strain, mild isolate Abp1/L and at least $2.5x10^{11}$ genome copies of PepMV, CH2 strain, mild isolate Abp2/L)

AbioProtect® was applied with an airbrush on tomato plants before planting. Distance 25-30 cm.

Assessments of phytotoxicity were conducted at 7, 14, 21, 28 and 35 days after the application.

Analyses of AbioProtect® presence (presence of virus, PepMV-EU and PepMV-CH2 strains) were conducted in each plant throughout the trial to evaluate the efficiency and velocity of the infection of the Plant Protection Product (PPP).

Findings

No problems were encountered during application of the product under test.

No crop phytotoxicity symptoms were observed in the trial at any of the assessment timings, so the three doses of AbioProtect® were safe to the crop.

The following tables (Table MP 6.1/01 and Table MP 6.1/02) summarized the results of the trial. More information is presented in the individual trial report.

Table MP 6.1/01 Mean of % phytotoxicity on tomato (LYPES) after treatment with AbioProtect® (PepMV-EU (Abp1) and PepMV-CH2 (Abp2)). Treatment means with no letters in common are significantly different, LSD test (P≤0.05)

Trt. No.	Product	Appr ate (L fp/ ha)	A1 7DAAp	A2 14DAAp	A3 21DAAp	A4 28DAAp	A5 35DAAp
			Phytotoxicity	Phytotoxicity	Phytotoxicity	Phytotoxicity	Phytotoxicity
1	CONTROL (untreated)	-	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
2	ABIOPROTECT®	3	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
3	ABIOPROTECT®	5	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
4	ABIOPROTECT®	8	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Date type			No transf.	No transf.	No transf.	No transf.	No transf.
LSD			0.00	0.00	0.00	0.00	0.00
CV			0.0	0.0	0.0	0.0	0.0
p(F), treatments			1.0000	1.0000	1.0000	1.0000	1.0000

L fp/ha litre of formulated product /ha
DAAp: days after application

Table MP 6.1/02 Mean of % plants with presence of AbioProtect® (PepMV-EU and PepMV-CH2 strain) on tomato (LYPES). Treatment means with no letters in common are significantly different, LSD test (P≤0.05)

Tr. No	Product	App rate (L fp/ ha)	A3 21DAAp		A4 28DAAp		A5 35DAAp	
			PepMV-EU	PepMV-CH2	PepMV-EU	PepMV-CH2	PepMV-EU	PepMV-CH2
1	CONTROL (untreated)	-	0.0 b	0.0 c	0.0 b	0.0 c	0.0 b	0.0 c
2	ABIOPROTECT®	3	55.6 ab	44.4 b	79.4 a	49.2 b	81.0 a	65.1 b

3	ABIOPROTECT®	5	88.9 a	49.2 b	96.8 a	57.1 b	96.8 a	68.3 ab
4	ABIOPROTECT®	8	88.9 a	74.6 a	96.8 a	81.0 a	98.4 a	87.3 a
Date type			No transf.	No transf.	No transf.	No transf.	No transf.	No transf.
LSD			61.89	21.13	36.13	15.26	36.93	19.33
CV			46.81	22.16	23.36	14.38	23.6	15.46
p(F), treatments			0.0445	0.0028	0.0046	0.0005	0.0049	0.0009

L fp/ha litre of formulated product /ha
DAAp: days after application

Conclusions

The plant protection product AbioProtect® is safe for use in tomato crops.

The Plant Protection Product AbioProtect® showed a good efficiency and velocity of infection in the tomato plants in general.

The component Abp1 (EU strain of PepMV) showed a better efficiency of infection than the component Abp2 (CH2 strain). The high dose of AbioProtect® (T4 8 L/ha) presented the best results, followed by T3 (5 L/ha). In the case of EU strain, treatments 3 and 4 obtained similar results, but in CH2 strain treatment 4 showed an incidence of plants infected 20-25% higher than treatment 3. The low dose (T2 3 L/ha) obtained a lower incidence and velocity of infection.

Considering the results of the dose trial and according to previous experience of the Applicant, the dose for applications is set up at 5 L/ha (10,000 tomato plants) of AbioProtect®. In tomato production areas with a record of high incidence of aggressive PepMV infections it could be increased up to 8 L/ha.

2.4.2.2.b Testing effectiveness

The formulation AbioProtect®, formulated with equivalent amounts of PepMV EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, has been tested in greenhouse trials which demonstrated its effectiveness and appropriate crop safety against infection by PepMV aggressive isolates from both the EU and the CH2 strains. The trials data supporting effectiveness against this target comprise 6 trials conducted in greenhouses in different locations in Spain from 2014-2017. All trials were carried out in accordance with the principles of Good Experimental Practices (GEP), and are certified by the officially recognized organization. The trials were conducted in protected tomato crops; therefore, the data are representative for the entire EU. Further details of the individual trials conducted are provided in Table MP 6.2/01 and in the corresponding individual trials (Documents K-MP 6.2/01/02/03, /04, /05 and /06 (Céspedes, 2015b; Prats, 2017a, 2017b and 2107c).

In three of these trials the efficacy of AbioProtect® was tested against aggressive isolates of the PepMV EU strain, (isolated from a commercial greenhouse tomato crop in Alicante (Spain) on September 2015, aggressive EU) and of the PepMV CH2 strain (isolated from a commercial greenhouse tomato crop in Granada (Spain) on March 2014, aggressive CH2). Additionally, the efficacy of the separate isolates was tested against both aggressive isolates.

In one of the six trials, the efficacy of AbioProtect® was tested against the aggressive CH2 isolate of PepMV. In this trial also the efficacy of the separate isolates of PepMV was tested against the aggressive CH2 isolate.

In the remaining 2 trials the efficacy of AbioProtect® was tested against the aggressive CH2 isolate of PepMV, without testing the efficacy of the independent isolates.

In all the trials the formulation has been applied at the proposed dose rate of 5 L/ha, containing $>5x10^{11}$ genome copies of PepMV/L ($>2.5x10^{11}$ genome copies of PepMV, EU strain, mild isolate Abp1/L and $>2.5x10^{11}$ genome copies of PepMV, CH2 strain, mild isolate Abp2/L). The separate isolates Abp1 and Abp2, were each applied at a dose of $>2.5x10^{11}$ genome copies /L. In all the trials, the treatments were compared with a control treatment

that was artificially infected with either isolate aggressive EU or isolate aggressive CH2. No standard reference materials are available for this type of use and were not included.

Overall AbioProtect® and the separate active ingredients proved effective in preventing infection with the virulent isolates. PepMV related symptoms on leaves and fruits were strongly reduced in the plants infected with mild virus isolates and then challenged with the aggressive viral isolates compared with the non-treated plants. Moreover, compared to the untreated plots challenged with aggressive isolates, aggressive EU and/or aggressive CH2, the plants treated with AbioProtect® and the separate active ingredients resulted in higher fruit yield.

Material and Methods

Sites

Sites were selected on the bases of being representative of different greenhouse tomato cropping systems in Europe in general, and in Spain in particular.

Experimental details

Trials were carried out to evaluate the efficacy and crop safety of AbioProtect® and its components, PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, as a cross-protection treatment against aggressive isolates of PepMV present in Southeast Spain, when applied on tomato seedlings (BBCH 13-15) before transplanting on to the commercial tomato greenhouse. Trial plots size ranged from 300 m^2 to 800 m^2.

Formulation applied and application rates

Details of the formulations (vaccines) tested, rates and timings are indicated in Table MP 6.2/02.

Table MP 6.2/02 Formulations tested and challenge treatments applied

Product	**Active Ingredient (ai)**	**Concentration**	**Rate**	**Timing**	**Form. type**
PPA1 (Plant Protection Agent 1)	PepMV-Abp1 mild EU isolate	≥ $2.5x10^{11}$ viral genome copies/L	5 L/ha	13 BBCH	Tomato watery leaves extract containing PepMV, EU strain, mild isolate Abp1 (SC)
PPA2 (Plant Protection Agent 2)	PepMV-Abp2 mild CH2 isolate	≥ $2.5x10^{11}$ viral genome copies/L	5 L/ha	13 BBCH	Tomato watery leaves extract containing PepMV, CH2 strain, mild isolate Abp2 (SC)
AbioProtect® (PPP, Vaccine)	PepMV-Abp1 + PepMV-Abp2 (PPA1 + PPA2)	≥ $5x10^{11}$ viral genome copies/L	5 L/ha	13 BBCH	Tomato watery leaves extract containing PepMV, EU and CH2 strains, mild isolates Abp1 and Abp2(SC)
PepMV isolate (Inoculum, Challenge 1)	PepMV aggressive EU isolate	-	-	17-52 BBCH (~3 weeks after vaccine)	Inoculum containing PepMV, EU strain, aggressive isolate
PepMV isolate (Inoculum, Challenge 2)	PepMV aggressive CH2 isolate	-	-	17-52 BBCH (~3 weeks after vaccine)	Inoculum containing PepMV, CH2 strain, aggressive isolate

Application method

Due to the number of different treatments to be assessed and to avoid miss handling them; the application was done manually in most of the trials, in spite that the treatments are applied by airbrush with a pressure of 75 psi in commercial applications.

Assessment methods-crop yield

Plots were harvested by hand picking the fruit. Assessments of number and kg of fruits per plot were conducted, differentiating between total production (number of fruits/m^2 and kg/m^2) and marketable production (number of fruits/m^2, % of marketable fruits and kg/m^2).

Assessment method symptoms

Crop safety in protected tomato has been considered in all effectiveness trials by assessing PepMV symptoms. Such assessment in the crop was conducted by giving individual scores to each plant according to an appropriate severity scale. Incidence data were also obtained. Assessments interval was modified depending on the evolution of the symptoms observed.

Bright yellow mosaic was evaluated by evaluating yellowing symptoms following this severity scale:

1: No symptoms

2: Mild symptoms

3: Moderate symptoms (yellow spots; interveinal yellowing of the tips)

4: Severe symptoms (complete yellowing of at the least 2/3 leaves)

5: Very severe symptoms (complete yellowing of at the least 4/5 leaves).

Symptoms were assessed in leaves and in fruit as shown in Figures MP 6.4/01 and MP 6.5/01. Further details of individual efficacy trials are included in the individual trial reports (complete details could be found in Documents K-MP 6.2/01/02/03, /04, /05 and /06 (Céspedes, 2015b; Prats, 2017a, 2017b and 217d).

Statistical analysis

Assessment data were analysed using a two-way analysis of variance (ANOVA) on untransformed and transformed data at a 95% confidence limit.

LSD multiple comparison test was then applied to separate any treatment differences that may be implied by the ANOVA TEST at a 95% confidence level.

Analysis details included in the result tables of the individual trial reports are: co-efficient of variation (CV), least significant difference (LSD), F probability for treatments (p(F)), and data type (indicates transformation type if appropriate). Where a transformation has been carried out this is indicated in the table as follows: Detransf. (Arcsi): Arcsine square root percent - ARCSIN (SQR(X/100)); Detransf. (Sqr): Square root - SQR(X + .5); Detransf (Log): Log - LOG(X + 1).

The tabulated data presented in this document (Tables MP 6.4/01, MP 6.4/02, MP 6.5/01 and MP 6.5/02 and Figures MP 6.4/01 and MP 6.4/02 below) only represents the means of selected treatments, within an assessment. Tables of data comprising all treatment means are presented in the individual trial reports. Also plot mean data, raw data and analysis details of untransformed data are included in Appendix of each individual trial report.

2.4.2.3 Possible occurrence of the development of resistance

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. Please refer to Documents M-MA sections 2 and 3 data point MA 2.7 Genetic stability and factors affecting it and data point MA 3.5 Information on the occurrence or possible occurrence of the development of resistance of the target organism(s) for further information.

2.4.2.4 Effects on quality of plants or plant products

The quality of the plants was evaluated by assessing the presence of symptoms in the leaves and in the fruits; production was differentiated in total production and marketable production in the efficacy trials conducted.

Treatment of tomato plants with the Plan Protection Product AbioProtect® does not produce any taint or odor in the fruits of the plants treated, or in any other aspects related with the quality of the plants or of the fruits. It should be noted that it is a treatment conceived for production of premium quality fresh tomato, in which symptoms in fruits is a decrease of quality and value of the product.

According to the data obtained from the different efficacy trials it could be concluded that the formulation AbioProtect® and its components achieve a high efficacy against PepMV, showing no symptoms in fruits during

the trials with similar data to the un-inoculated control and with clear significant differences with the challenged inoculated controls.

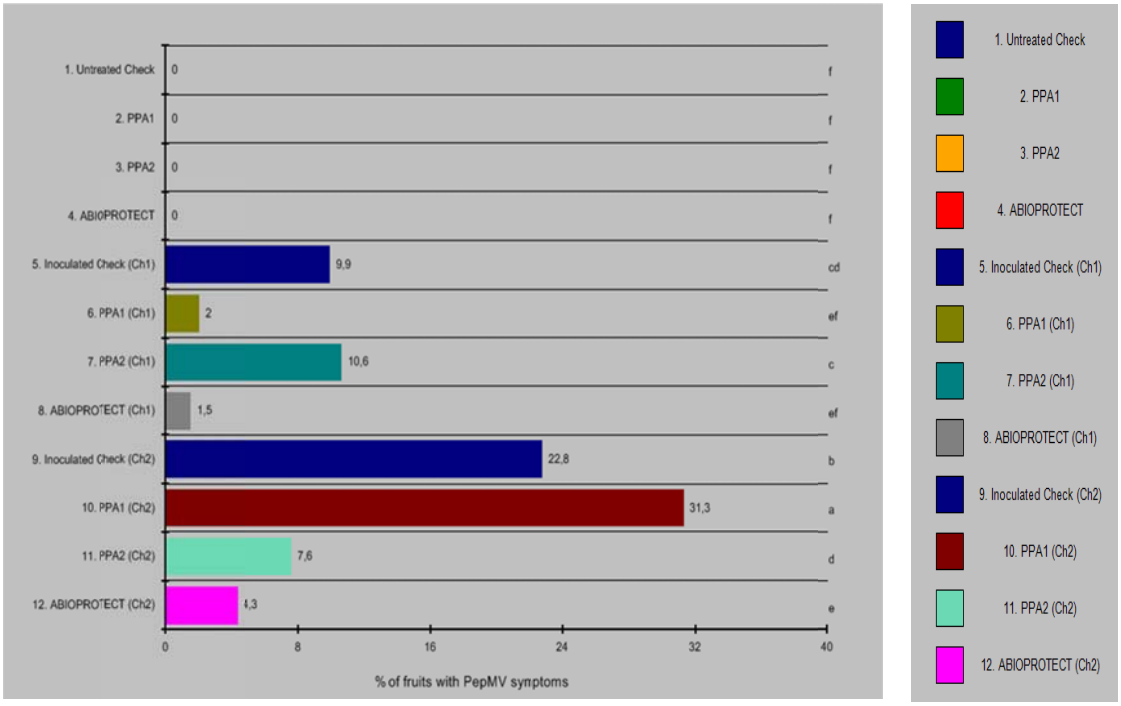
Table MP 6.4/01 and Figure MP 6.4/01 represent means of PepMV symptoms and other damage observed in fruits from a selected trial of those indicated on section MP 6.2. Tables of data comprising all treatment means for each trial are presented in the individual trial reports.

Table MP 6.4/01 Mean of PepMV symptoms and other damage observed in fruits per treatment. Treatment means with no letters in common are significantly different, LSD test (P≤0.05)

Trt. No.	Product	App rate (L fp/ha)[1]	PepMV symptoms		Other damage	
			No. of fruits	% of fruits	No. of fruits	% of fruits
1	CONTROL (untr.)	-	0.0 e	0.0 f	4.5 a	1.6 a
2	PepMV-Abp1	5	0.0 e	0.0 f	1.8 a	0.7 a
3	PepMV-Abp2	5	0.0 e	0.0 f	2.3 a	0.8 a
4	AbioProtect®	5	0.0 e	0.0 f	2.3 a	0.8 a
5	CONTROL (Ch1)[2]	-	28.5 c	9.9 cd	5.0 a	1.7 a
6	PepMV-Abp1 (Ch1)	5	6.0 de	2.0 ef	3.3 a	1.1 a
7	PepMV-Abp2 (Ch1)	5	30.3 c	10.6 c	5.0 a	1.8 a
8	AbioProtect® (Ch1)	5	4.5 de	1.5 ef	2.3 a	0.7 a
9	CONTROL (Ch2)[3]	-	62.8 b	22.8 b	6.3 a	2.2 a
10	PepMV-Abp1 (Ch2)	5	93.5 a	31.3 a	1.3 a	0.4 a
11	PepMV-Abp2 (Ch2)	5	22.3 c	7.6 d	6.3 a	2.2 a
12	AbioProtect® (Ch2)	5	12.0 d	4.3 e	4.0 a	1.4 a
Date type			No transf.	No transf.	No transf.	No transf.
LSD			9.57	2.88	3.95	1.35
CV			30.61	26.59	74.66	72.56
p(F), treatments			0.0001	0.0001	0.1383	0.1237

[1]L fp/ha: liter of formulated product per hectare.
[2]Ch1: challenge 1, EU aggressive; [3]Ch2: Challenge 2, CH2 aggressive.

Figure MP 6.4/01 Mean of % of fruits with PepMV (PEPMVO) symptoms observed per treatment. Treatment means with no letters in common are significantly different, LSD test (P≤0.05)



PPA1: PepMV-Abp1; PPA2: PepMV-Abp2, Ch1: challenge 1: EU aggressive; Ch2: Challenge 2 CH2 aggressive.
SCALE: 1: No symptoms. 2: Mild symptoms. 3: Moderate symptoms. 4: Severe symptoms. 5: Very severe symptoms.

2.4.2.5 Effects on transformation processes

Not relevant as the formulation is for use in commercial production of premium quality tomatoes for the fresh market to be consumed without any transformation process. It should be noted that in southeast Spain as well as in most countries in Europe tomato greenhouse production is a high input production only profitable when the product is market in fresh. Nonetheless treatment with AbioProtect® will not have any interference on transformation processes as viruses have no metabolism of their own, it does not produce residues and neither leaves residues at harvest.

2.4.2.6 Effects on the yield of treated plants or plants products

As already indicated, the formulation AbioProtect® and its components achieve a high efficacy against PepMV, showing no symptoms during the trials with similar data and total production (yield) to the un-inoculated control and with clear significant differences with the challenged inoculated controls.

Table MP 6.4/02 Represents mean total production and marketable production of a selected trial of those indicated in section MP 6.2. Tables of data comprising all treatment means for each trial are presented in the individual trial reports.

Table MP 6.4/02 Mean total production (fruits/m2 and kg/m2) and marketable production (fruits/m2, % of marketable fruit and kg/m2) per treatment. Treatment means with no letters in common are significantly different, LSD test (P≤0.05)

Trt. No.	Product	App (L fp/ha)[1]	Total production		Marketable production		
			Fruits/m^2	kg/m^2	Fruits/m^2	% of marketable fruit	kg/m^2
1	CONTROL (untr.)	-	57.9 a	6.66 a	57.0 ab	98.4 a	6.63 ab
2	PepMV-Abp1	5	54.1 a	5.90 a	53.8 abc	99.3 a	5.88 bcd
3	PepMV-Abp2	5	54.0 a	6.03 a	53.6 abc	99.2 a	6.01 abc
4	AbioProtect®	5	53.7 a	6.13 a	53.2 abc	99.2 a	6.11 abc
5	CONTROL (Ch1)[2]	-	57.1 a	5.98 a	50.4 c	88.3 c	5.25 de
6	PepMV-Abp1 (Ch1)	5	59.1 a	6.52 a	57.2 ab	96.8 ab	6.35 abc
7	PepMV-Abp2 (Ch1)	5	57.1 a	6.33 a	50.0 c	87.7 c	5.60 cd
8	AbioProtect® (Ch1)	5	59.6 a	6.80 a	58.3 a	97.7 ab	6.68 a
9	CONTROL (Ch2)[3]	-	55.2 a	5.76 a	41.4 d	75.1 d	4.53 ef
10	PepMV-Abp1 (Ch2)	5	59.8 a	6.37 a	40.9 d	68.3 e	4.43 f
11	PepMV-Abp2 (Ch2)	5	57.4 a	6.56 a	51.7 bc	90.2 c	6.01 a-d
12	AbioProtect® (Ch2)	5	57.3 a	6.22 a	54.1 abc	94.3 b	5.92 a-d
Date type			No transf.	No transf.	No transf.	No transf.	No transf.
LSD			6.72	0.787	6.21	3.47	0.758
CV			8.19	8.69	8.31	2.63	9.08
p(F), treatments			0.5826	0.2027	0.0001	0.0001	0.0001

[1]L fp/ha: liter of formulated product per hectare
[2]Ch1: challenge 1, EU aggressive; [3]Ch2: Challenge 2, CH2 aggressive.

2.4.2.7 Phytotoxicity to target plants or target plant products

Phytotoxicity and crop safety in protected tomato has been considered in all effectiveness trials. Treatment with AbioProtect® and its components resulted in no symptoms or some mild symptoms of PepMV infection. In cases where some mild symptoms appear those were generally transient and, in most cases, did not affect quality of the fruits. Moreover, yield was not affected.

Table MP 6.5/01, MP 6.5/02 and Figure MP 6.5/01 Represent mean of % incidence of plants affected and mean of severity of leaf symptoms of a selected trial. Tables of data comprising all treatment means for each trial are presented in the individual trial reports.

Table MP 6.5/01 Mean of % incidence in tomato plants (LYPES) per treatment. Treatment means with no letters in common are significantly different, LSD test (P≤0.05)

Trt. No.	Product	Appl rate (L fp/ha)[1]	A4 0 DBIn[4] Yellowing	A5 14 DAIn[5] Yellowing	A6 28 DAIn Yellowing	A7 42 DAIn Yellowing	A8 56 DAIn Yellowing	A9 70 DAIn Yellowing	A 17 158 DAIn Yellowing
1	CONTROL (untr.)	-	0.0 a	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c
2	PepMV-Abp1	5	0.0 a	5.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c
3	PepMV-Abp2	5	0.0 a	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c
4	AbioProtect®	5	0.0 a	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c
5	CONTROL (Ch1)[2]	-	0.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
6	PepMV-Abp1 (Ch1)[2]	5	0.0 a	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c
7	PepMV-Abp2 (Ch1)[2]	5	0.0 a	55.0 b	65.0 b	95.0 b	95.0 b	95.0 a	100.0 a
8	AbioProtect® (Ch1)[2]	5	0.0 a	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c
9	CONTROL (Ch2)[3]	-	0.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
10	PepMV-Abp1 (Ch2)[3]	5	0.0 a	85.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
11	PepMV-Abp2 (Ch2)[3]	5	0.0 a	0.0 c	0.0 c	0.0 c	0.0 c	20.0 b	20.0 b
12	AbioProtect® (Ch2)[3]	5	0.0 a	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c
Date type			No	No	No	No	No	No	No
LSD			0.00	16.81	14.24	4.17	4.17	10.69	9.63
CV			0.0	40.5	32.42	8.77	8.77	21.41	19.05
p(F), treatments			1.0000	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

[1]L fp/ha: liter of formulated product per hectare.
[2]Ch1: challenge 1, EU aggressive; [3]Ch2: Challenge 2, CH2 aggressive.
[4]DBIn: Days before inoculation, [5]DAIn: Days after inoculation.

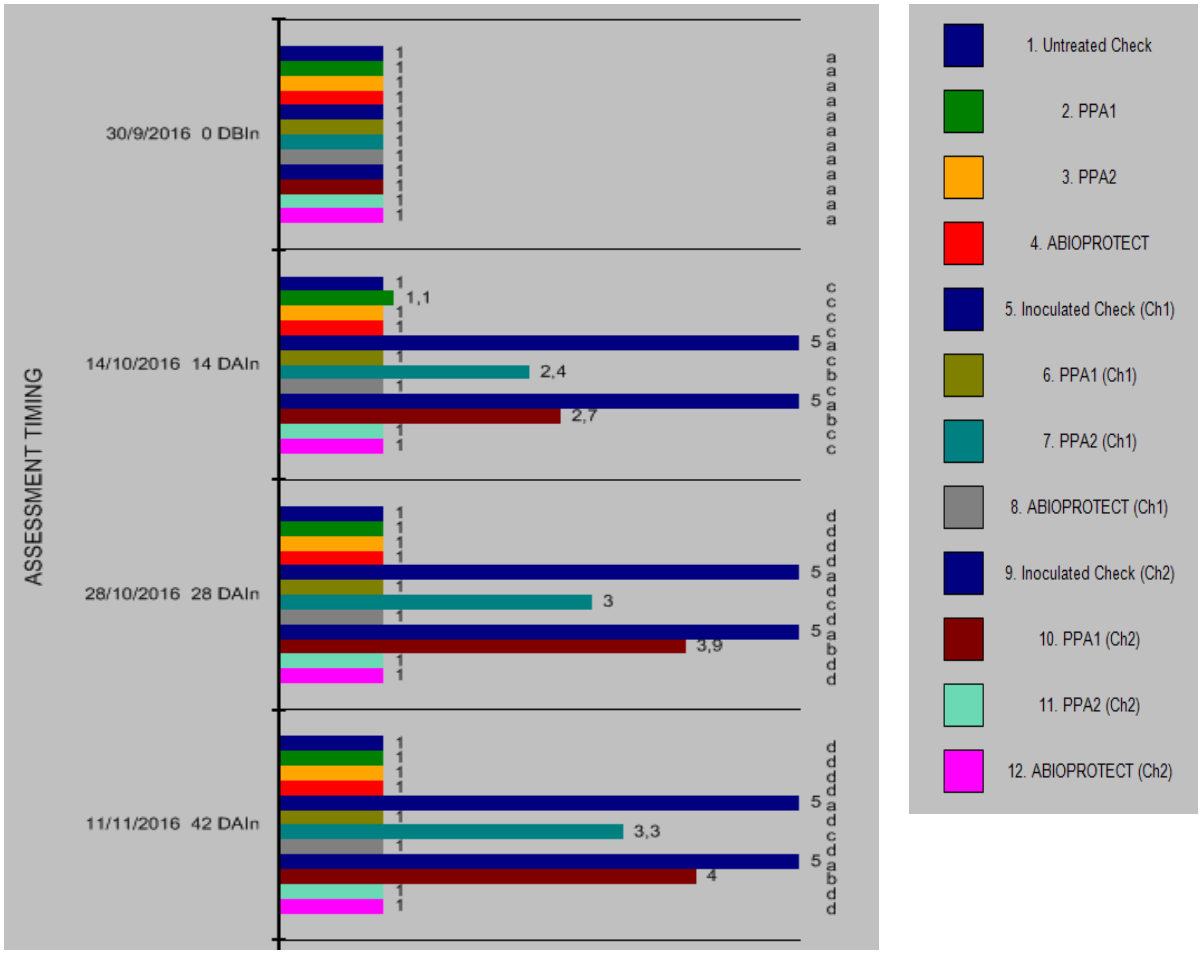
Table MP 6.5/02 Mean of severity of leaf symptoms (yellowing) per treatment according to the severity scale indicated in point MP 6.2 above. Treatment means with no letters in common are significantly different, LSD test (P≤0.05)

Trt. No.	Product	App rate (L fp/ha)[1]	A4 0 DBIn[4] Yellowing	A5 14 DAIn[5] Yellowing	A6 28 DAIn Yellowing	A7 42 DAIn Yellowing	A8 56 DAIn Yellowing	A9 70 DAIn Yellowing	A17 158 DAIn Yellowing
1	CONTROL (untr.)	-	1.0 a	1.0 c	1.0 d	1.0 d	1.0 d	1.0 e	1.0 d
2	PepMV-Abp1	5	1.0 a	1.1 c	1.0 d	1.0 d	1.0 d	1.0 e	1.0 d
3	PPA2	5	1.0 a	1.0 c	1.0 d	1.0 d	1.0 d	1.0 e	1.0 d
4	AbioProtect	5	1.0 a	1.0 c	1.0 d	1.0 d	1.0 d	1.0 e	1.0 d
5	CONTROL (Ch1)[2]	-	1.0 a	5.0 a	5.0 a	5.0 a	5.0 a	5.0 a	5.0 a

6	PepMV-Abp1 (Ch1)	5	1.0 a	1.0 c	1.0 d	1.0 d	1.0 d	1.0 e	1.0 d
7	PPA2 (Ch1)	5	1.0 a	2.4 b	3.0 c	3.3 c	3.3 c	3.3 c	3.8 b
8	AbioProtect (Ch1)	5	1.0 a	1.0 c	1.0 d	1.0 d	1.0 d	1.0 e	1.0 d
9	CONTROL (Ch2)[3]	-	1.0 a	5.0 a	5.0 a	5.0 a	5.0 a	5.0 a	5.0 a
10	PepMV-Abp1 (Ch2)	5	1.0 a	2.7 b	3.9 b	4.0 b	4.0 b	4.0 b	4.0 b
11	PPA2 (Ch2)	5	1.0 a	1.0 c	1.0 d	1.0 d	1.0 d	1.4 d	1.4 c
12	AbioProtect (Ch2)	5	1.0 a	1.0 c	1.0 d	1.0 d	1.0 d	1.0 e	1.0 d
Date type			No	No	No	No	No	No	No
LSD			0.00	0.36	0.44	0.28	0.28	0.35	0.22
CV			0.0	13.08	14.63	9.35	9.35	11.19	6.83
p(F), treatments			1.0000	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

1L fp/ha: liter of formulated product per hectare.
2Ch1: challenge 1: EU aggressive; 3Ch2: Challenge 2 CH2 aggressive.
4DBIn: Days before inoculation, 5DAIn: Days after inoculation.

Figure MP 6.5/0.1 Mean of severity of leaf symptoms (yellowing) according to the severity scale indicated in point MP 6.2 above. Treatment means with no letters in common are significantly different, LSD test (P≤0.05).



PPA1: PepMV-Abp1; PPA2: PepMV-Abp2, Ch1: challenge 1: EU aggressive; Ch2: Challenge 2 CH2 aggressive.
SCALE: 1: No symptoms. 2: Mild symptoms. 3: Moderate symptoms. 4: Severe symptoms. 5: Very severe symptoms.

2.4.2.8 Impact on succeeding crops, adjacent crops and on treated plants or planta products used for propagation

Viruses can only reproduce inside their host cells, plant viruses can only reproduce in plant living cells and PepMV can only reproduce inside its host plants. Multiplication in soil, water o air is therefore of little relevance and no undesirable or unintended side-effects have been observed.

PepMV is very efficiently mechanically transmitted; in fact, contaminated hands, clothing or tools facilitate PepMV transmission. Crop workers can transmit the virus simply by brushing against affected plants and during crop nursing activities such as pruning and harvesting (Ferguson, 2001; Van der Vlugt, 2009).

Please refer to MA 2.2 information on the target organism, and to MA 2.3 host specificity range and effect on species other than the target harmful organism (Document M-MA) for further information.

The main PepMV transmission route is mechanically. Although, some reports have analyzed the potential PepMV transmission by vectors. As this is the case of several reports studding transmission of PepMV by bumblebees (Lacasa et al. 2003; Shipp et al., 2008; Stobbs et al., 2009; Stobbs and Greig, 2014), these authors found that bumblebees could disperse PepMV, however, a specific PepMV-bumblebee vector relation does not appear to exist. Also, the possibility that PepMV could be transmitted by the soil fungus *Olpidium virulentus* has been studied at laboratory level (Alfaro-Fernández et al., 2010), however the extent of such transmission in the field remains unclear.

Impact on succeeding crops

Impact on succeeding crops was not tested in the efficacy trials. However, as the persistence in water GEP study concluded that the Plant Protection Product AbioProtect® (formulated with equivalent amounts of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) has no persistency in the leachate from tomato plants treated with AbioProtect® (see Document K-MP 6.2/04, Prats, 2017a), it could be concluded that there is no risk of PepMV infection with this leachate to succeeding crops.

Besides, the GEP studies on persistence in soil or substrate showed that PepMV is not persistence in the soil or substrate of the tomato plants treated with AbioProtect® and its components (see Documents K-MP 6.2/05, Prats, 2017b; K-MA 7.1.1/02, Céspedes, 2015a), and therefore there is no risk of PepMV infection of succeeding crops from the soil or the substrate of the plants treated.

Please refer to MA 2.3 host specificity range and effect on species other than the target harmful organism (Document M-MA) for further information.

Impact on adjacent crops

Impact on other plants including adjacent crops was not tested as there are no indications that the plant protection product could affect adjacent crops via vapor drift. Furthermore as indicated in MP 6.6.1 based on the nature of AbioProtect® and in the results of the persistence in water GEP study, the persistence in soil and substrate GEP studies (Documents K-MP 6.2/05, Prats, 2017b; K-MA 7.1.1/02, Céspedes, 2015a; K-MP 62/04, Prats, 2017a) as well as the study on the presence of PepMV in weeds and plants on the vicinity of tomato greenhouses where the formulation has been applied (Document K-MA 7.1/01, Agüero, 2017b), it could be concluded that there is no risk of PepMV infection to other plants including adjacent crops.

Please refer to MA 2.3 Host specificity range and effect on species other than the target harmful organism (Document M-MA section 2) for further information.

Effect on treated plants or plant products used for propagation

Not relevant. The formulation AbioProtect® is not for used in plants or plants product to be use for propagation, more specifically it is not intended to be use in the production of seeds, cuttings or runners for propagation.

Effects on beneficial and other non-target organisms

PepMV is ubiquitous in nature and no impact of the formulation AbioProtect® or its ingredients (PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) are expected on beneficial and other non-target organisms. In the different tests and studies conducted no effects on the incident of other non-target organisms or environmental effects have been observed. Please refer to Document M-MA section 8 and Document M-MP section 10 for further information.

2.5 METHODS OF ANALYSIS

2.5.1 Methods for the analysis of the MPCA

Adequate methodology exists for the identification of PepMV, EU strain, mild isolate Abp1, and PepMV, CH2 strain, mild isolate Abp2 in the pre-batches and final batches. Nucleotide sequence, molecular hybridization, RT-qPCR and a bioassay are available for the qualitative and quantitative determination of Abp1 and Abp2 isolates in plant extracts and formulation.

Adequate methods are also available to determine the presence of other microorganisms potentially pathogen for tomato such as bacteria, fungi, viruses and viroids.

2.5.2 Method for the analysis of the MPCP

Adequate methodology exists for the identification of PepMV in the preparation AbioProtect®; including molecular hybridization, a RT-qPCR method and a bioassay are available for the qualitative and quantitative determination of Abp1 and Abp2 isolates in the formulation. Another method for qualitative determination of PepMV isolates Abp1 and Abp2 is by nucleotide sequencing.

Methods to test the presence of human pathogens are also available and applied to test every batch of the MPCP, these methods are based on the reference standard UNE-EN ISO/IEC 17025:2005, and include:

- Salmonella absence in 25 mg o 25 mL (PNT-2; qPCR method conform ISO/IEC 17025:2005).

- Listeria monocytogenes absence in 25 mg o 25 mL (PNT-06; qPCR method conform ISO/IEC 17025:2005).

- Escherichia coli absence in 1g or mL (ISO 16649-2:2001).

- Thermotolerant (faecal) coliforms < 10 CFU/g or mL (Petrifilm).

- Aerobic plate count $<10^5$ CFU/g or mL (UNE-EN ISO 4833-2:2014).

2.6 IMPACT ON HUMAN AND ANIMAL HEALTH

The dossier of both strains (MPCAs), PepMV EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, was prepared as a whole document, due to the physico-chemical and biological properties of the mixture of both isolate is comparable to the physico-chemical and biological properties of the separated Abp1 and Abp2 isolate suspenssions. Abp1 and Abp2 isolates only differs in the target pathogen strains (CH2 and EU) of PepMV viruses (Abp1 protects tomato plants against PepMV European pathogenic strains, since Abp2 protects tomato plants against PepMV Chilean pathogenic strains). **There are common studies for both strains compiled in one DAR document for the two strains.** The main studies were made with a suspension concentrate contained at least $5x10^{11}$ PepMV genome copies /L, with equivalent amounts of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, to guarantee a minimum content of at least $2.5x10^{11}$ genome copies of each PepMV isolate. Other inert ingredients are tomato plant extract and water.

2.6.1 Effects having relevance to human and animal health arising from exposure to the microorganism or to impurities, additives, contaminating microorganisms contained in the material used for manufacturing of formulated products.

2.6.1.1 Medical data and direct observations

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.

2.6.1.2 Sensitisation/allergenicity observations

There is no document in the scientific literature reporting on sensitisation or allergic reactions to plant viruses. A prerequisite for sensitisation is the infection of the host by the virus, which does not take place in the case of plant viruses. Particularly, as regards to PepMV, regular exposure of farm and research personnel did not result in any known incidence of hypersensitivity or chronic sensitisation. The application of the MPCP Abiopep takes place immediately only before planting, in seedlings (planting time (BBCH 13-15)).

Taken all together, it is unlikely that PepMV may provoke sensitisation and consequently, allergic reactions to humans.

However, it is customary to classify and label microorganisms for sensitisation by default in the EU. As according to Regulation (EC) 283/2013, all microorganisms should be regarded as potential sensitisers.

2.6.1.3 Sensitisation studies

The applicant has not provided a sensitisation study. Nevertheless there is a published paper (Welter 2013) that hypothesised that PepMV infection would result in the expression of allergens leading to a higher allergenic potential of tomato fruits. In summary, PepMV infection of tomato plants can lead to long-lasting upregulation of particular allergens in fruits, but the hypothesis that these results in a higher allergenic potential of the fruits proved invalid.

2.6.1.4 Acute oral toxicity, pathogenicity and infectiveness

Table 2.6.1.4: Summary of acute oral toxicity studies

Test substance/ potency	**Method Guideline Acceptability**	**Species Strain Sex No./dose**	**Dose levels**	**LD_{50}**	**Observations**	**Reference**	**New data for Annex I renewal Y/N**
PepMV, EU strain, mild isolate Abp1 batch: L-7-311016-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 batch: L-7-311016-ABP2-C (Abioprotect® Batch L-AB01311016) Potency: 6.32 x 10^6 viral copies/µL (1.70x 10^6 viral copies/µL of Abp1 and 4.62 x 10^6 viral copies/µL of Abp2)	Acute Toxic Class Method Limit test OECD Guideline 423 No control group was included The observation period was 14 days	Rat Sprague-Dawley Female 3/dose	2000 mg/kg bw	LD_{50} >2000 mg/kg bw that corresponds with 6.32 × 10^{10} PepMV genome copies/kg bw (1.7 × 10^{10} genome copies/kg bw of Abp1 and 4.62 × 10^{10} genome copies/kg bw of Abp2)	No mortalities No toxic signs were recorded in animals during observation period	B.6.1.2.2.1-01 [redacted] 2017a)	Y

It can be concluded that the test items PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, contained in the formulation AbioProtect®, could be considered Unclassified according to Regulation 1272/2008 although classification criteria for chemicals does not apply since the active substances are microorganisms.

Under the conditions of the study the acute oral lethal dose (LD_{50}) was found to be higher than 2000 mg/kg of body weight in female Sprague-Dawley rats for AbioProtect® containing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2.

The Alphaflexiviridae family, 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, 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.

2.6.1.5 Acute inhalation toxicity, pathogenicity and infectiveness

Table 2.6.1.5: Summary of acute inhalation toxicity studies

Test substance/ Route	**Method Guideline**	**Species Strain Sex No./dose**	**Dose levels**	**LC_{50}**	**Observations**	**Reference**	**New data for Annex I renewal Y/N**
PepMV, EU strain, mild isolate Abp1 batch: L-9-241116-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 batch: L-9-241116-ABP2-C (Abioprotect® Batch LAB02-241116)	Acute Inhalation Test Limit test OECD Guideline 403 Nose-only, flow-past inhalation	Rat Sprague-Dawley Female 3/5.02 mg/L for 4 hours Male 3/5.02 mg/L for 4 hours	5.02 mg/L for 4 hours	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 during the study period. The main clinical signs observed after 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.	B.6.1.2.2.2-01 [redacted] 2017)	Y

It can be concluded that, according to the results obtained in this study and under the assayed experimental conditions, that the LC50 for the test items PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, in the formulation AbioProtect® was greater than 5.02 mg/L air (gravimetric aerosol concentration) and they can be considered not classified, based on the CLP (Regulation 1272/2008) classification criteria, although classification criteria for chemicals does not apply since they are microorganisms..

The Alphaflexiviridae family, 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, and on the light of the results of the acute inhalation toxicity study, a report on the pathogenicity and infectivity by inhalation of the active substances was not consider relevant.

2.6.1.6 Acute intraperitoneal/subcutaneous/intravenous toxicity, pathogenicity and infectiveness

No study was provided. An acute dermal toxicity study was carried out instead because it was considered that this type of exposure would be more likely to happen in practice.

Table 2.6.1.6: Summary of acute dermal toxicity study

Test substance/ Route	**Method Guideline**	**Species Strain Sex No./dose**	**Dose levels**	**LC_{50}**	**Observations**	**Reference**	**New data for Annex I renewal Y/N**
PepMV, EU strain, mild isolate Abp1	Acute Dermal Toxicity	Rat Sprague-	2000mg/kg	$LD_{50\ dermal\ rat}$ > 2000 mg/kg that	No mortality during the study period.	B.6.1.2.2.3 -01	Y

batch: L-7-311016-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 batch: L-7-311016-ABP2-C (Abioprotect® Batch L-AB01311016)	Test Limit test OECD Guideline 402	Dawley 1st round Female 5/2000mg/kg Male 5/2000mg/kg 2nd round Female 3/2000mg/kg Male 1/2000mg/kg		corresponds to 6.32 x 10^{10} genome copies of PepMV /kg bw ($1.7x10^{10}$ genome copies/kg bw of Abp1 + $4.62x10^{10}$ genome copies/kg bw of Abp2)	No severe toxic signs were recorded	([redacted] 2017b)	

It can be concluded that, according to the results obtained in this study and under the assayed experimental conditions, the dermal LD_{50} for PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Apb2 in the formulation AbioProtect® is established to be > 2000 mg/kg body weight. Therefore, it could be considered Unclassified according to Regulation 1272/2008 although classification criteria for chemicals does not apply since the active substances are microorganisms.

LD_{50} dermal rat > 2000 mg/kg bw = > $6.32x10^{10}$ genome copies of PepMV/kg bw.
LD_{50} dermal rat > $6.32x10^{10}$ genome copies of PepMV/kg bw = > $1.7x10^{10}$ genome copies of Abp1/kg bw + $4.62x10^{10}$ genome copies of Abp2/kg bw.

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.

2.6.1.7 Genotoxicity

Table 2.6.1.7 Summary of genotoxicity studies

Method, guideline, deviations1 if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations /Results**	**Reference**
Bacterial reverse mutation test OECD Guideline 471	PepMV, EU strain, mild isolate Abp1 batch: L-7-311016-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 batch: L-7-311016-ABP2-C (Abioprotect® Batch L-AB01311016)	Five *S. typhimurium* strains (TA98, TA100, TA102, TA1535, TA1537) were exposed to the test items at 5 concentrations (100-50-25-12.5-6.25 µL/plate) with and without metabolic activation system (S9).	No dose response for the test items PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Apb2 in the formulation AbioProtect® was observed in any of the tested bacterial strains. The test items do not induce point mutations or frame-shifts in the genome of the bacterial strains with or without metabolic activation.	B.6.1.2.3.1-01 Gomez and Calvo2017

2.6.1.8 Cell culture studies

Table 2.6.1.8 Summary of cell culture studies

Method, guideline, deviations1 if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Cell culture study 1)Cell viability assay 2)Cell proliferation assay	Abioprotect® Batch L-AB02-060217 that contains: PepMV, EU strain, mild isolate Abp1 batch: L-12-060217-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 batch: L-12-060217-ABP2-C	A549 cells (Human alveolar epithelial cells) were treated with various concentrations of PepMV with both isolates together	In human alveolar epithelial cells type 2 A549 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 Apb2 decreased cell viability and affected the proliferation to a similar extent. The likely reason for cytotoxic activity and anti-proliferative activity of test items is the presence of biologically active natural constituents and metabolic products in the plant extracts and not the presence of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Apb2	B.6.1.2.4-01/1 Žegura 2017a
Cell culture study 1)Cell viability assay 2)Cell proliferation assay	PepMV, EU strain, mild isolate Abp1. Abp1 batch number: L-TO-01-ABP1-C PepMV, CH2 strain, mild isolate Abp2. Abp2 batch number: L-TO-02-ABP2-C	A549 cells (Human alveolar epithelial cells) were treated with various concentrations of PepMV isolates separately	In human alveolar epithelial cells type 2 A549 the reference tomato leaves extract not containing PepMV and tomato leaves extract containing PepMVAbp2 separately decreased cell viability and affected the proliferation to a similar extent. 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%. Statistically significant differences between the reference tomato leaves extract not containing PepMV and tomato leaves extracts 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 of test items is the presence of biologically active natural constituents and metabolic products in the plant extracts and not the presence of PepMV	B.6.1.2.4-01/2 Žegura 2019a
Infectivity and replication of Pep MV in human alveolar epithelial cells	Abioprotect® Batch L-AB02-060217 that contains: PepMV, EU strain, mild isolate	A549 cells (Human alveolar epithelial cells) were treated with both PepMV isolates together	In tomato leaves extract (containing both isolates together) treated cells, a significantly lower amount of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Apb2 was detected after each passage. After the last passage, the	B.6.1.2.4-02/1 Žegura 2017b

Method, guideline, deviations1 if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
	Abp1 batch: L-12-060217-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 batch: L-12-060217-ABP2-C		virus was not detected in two out of three replicates, showing that PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Apb2 did not multiply in human alveolar epithelial cells type 2 A549.	
Infectivity and replication of Pep MV in human alveolar epithelial cells	PepMV, EU strain, mild isolate Abp1. Abp1 batch number: L-TO-01-ABP1-C PepMV, CH2 strain, mild isolate Abp2. Abp2 batch number: L-TO-02-ABP2-C	A549 cells (Human alveolar epithelial cells) were treated with PepMV isolates separately	In tomato leaves extracts (containing PepMV isolates separately) treated cells, a significantly lower amount of PepMV was detected after 48 hours of treatment. After the last passage, the virus was not detected showing that PepMV did not multiply in human alveolar epithelial cells type 2 A549.	B.6.1.2.4-02/2 (Žegura 2019b)

According to the results of the viability and proliferation assays we can conclude that in human alveolar epithelial cells type 2 A549 the reference tomato leaves extract not containing PepMV and tomato leaves extract containing both PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 decreased cell viability and affected the proliferation to a similar extent. The likely reason for cytotoxic activity and anti-proliferative activity of test items is the presence of biologically active natural constituents and metabolic products in the plant extracts and not the presence of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. When the viability and proliferation assays were carried out with each isolate separately, the extract containing PepMV, EU strain, mild isolate Abp1 decreased the viability of A549 cells to a higher extent compared to reference tomato leaves extract not containing PepMV that was statistically different at concentration ≥ 1.25 v/v%. On the other hand, the reference extract not containing PepMV and the extract containing PepMV, CH2 strain, mild isolate Abp2 decreased the viability of A549 cells to a similar extent.

There are no indications of the infectivity or the replication of the naturally occurring PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 from the tomato (S. lycopersicum) leaves extract in human alveolar epithelial cells type 2 A549 in vitro when tested together or separately.

2.6.1.9 Short term toxicity, pathogenicity and infectiveness

Due to the mode of action as cross protection effect, actual activity is obtained by infection of the plants with mild isolates of the virus Abp1 and Abp2. Therefore, mild isolates Abp1 and Abp2 induce in tomato plants a symptomless infection with no damage to the fruit. Abp1 and Abp2 do not cause visible symptoms in tomatoes, therefore, high and chronic exposure of humans towards this virus is expected. Nevertheless, there are no published reports of adverse effects on humans after exposure to PepMV.

2.6.1.10 Specific studies on toxicity, pathogenicity and infectiveness

Everything indicates that the potential risk of PepMV towards humans is low. Further studies including short-term toxicity and pathogenicity studies with PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 were not considered relevant.

2.6.1.11 Toxicity studies on metabolites

Viruses do not produce metabolites, as they do not have metabolism of their own. The PepMV Abp1 and Abp2 complete viral genome sequences are 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.

2.6.2 Impact on human health arising from exposure to the micro-organisms or to impurities, additives, contaminating micro-organisms contained in the material used for manufacturing of formulated products

The studies performed by the applicant to assess the effects on human health of the active substances PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 were carried out employing both isolates together in the preparation AbioProtect® that is a suspension concentrate formulated with equivalent amounts of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. The formulation does not contain any other non-active substances. Other inert ingredients are tomato plant extract and water.

Table 2.6.2 Overview of the available data

Study	Test material	Species	Result	References
Acute oral toxicity	PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 (Abioprotect ®)	Rat	LD_{50} > 2000 mg/kg bw LD_{50} > $1.7x10^{10}$ genome copies/kg bw of Abp1 + $4.62x10^{10}$ genome copies/kg bw of Abp2	B.6.1.2.2.1-01 ([redacted] 2017a)
Acute inhalation toxicity	PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 (Abioprotect ®)	Rat	LC_{50} > 5.02 mg/L air LC_{50} > $1.46x10^{8}$ genome copies/L air of Abp1 + $1.93x10^{8}$ genome copies/L air of Abp2	B.6.1.2.2.2-01 ([redacted] 2017)
Acute dermal toxicity	PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 (Abioprotect ®)	Rat	LD_{50} > 2000 mg/kg bw LD_{50} > $1.7x10^{10}$ genome copies/kg bw of Abp1 + $4.62x10^{10}$ genome copies/kg bw of Abp2	B.6.1.2.2.3 -01 ([redacted] 2017b)
Bacterial Reverse Mutation Test	PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 (Abioprotect ®)	*Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537	Non-mutagenic / non pro-mutagenic	B.6.1.2.3.1-01 (Gomez and Calvo 2017)
Cell culture studies	PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 (Abioprotect ®) PepMV, EU strain, mild isolate Abp1 PepMV, CH2 strain, mild isolate Abp2 (each isolate separately)	Human alveolar epithelial cells type A549	The likely reason for cytotoxic activity and anti-proliferative activity of test items is the presence of biologically active natural constituents and metabolic products in the plant extracts and not the presence of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 No infectivity or replication in human alveolar epithelial cells A549	B.6.1.2.4-01/1 (Žegura 2017a) B.6.1.2.4-01/2 (Žegura 2019a) B.6.1.2.4-02/1 (Žegura 2017b) B.6.1.2.4-02/2 (Žegura 2019 b)

2.6.3 Summary of product exposure and risk assessment

2.6.3.1 Exposure and personal protective equipment (PPE)

The preparation AbioProtect® is intended for professional use as a preventive treatment in greenhouse (protective) tomato production against aggressive isolates of PepMV to be applied in a close compartment near or inside the final destination greenhouse in a single application to tomato seedlings by. Abiopep employs trained and qualified personnel to conduct product application and the product is never applied by third parties.

Clinical cases and poisoning incidents did not occur in the laboratories and facilities of the applicant. There are no indications for a toxic potential regarding to the information of the medical record of the employees involved in the manufacture of AbioProtect® and its components PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2.

As the product contains microorganisms as active substances, the use of PPE is recommended as a precautionary measure

- Chemical protection gloves.
- At least, level 6 splash proof protection clothes.
- Respiratory protection: At least, level FFP2 self-filtering mask for particles or level P2 filtering mask.
- Chemical proof footwear.

2.6.3.2 Risk assessment

Nicotine is present as impurity at very low levels in the active substances (PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2). The exposure to nicotine was estimated for operators and it was far lower than the ARfD (0.8 µg/kg b.w), therefore adverse effects for operators using PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 are not expected. Bystander was considered not relevant. The estimated resident exposure was negligible and therefore, no risk to residents is expected.

2.7 RESIDUES

The preparation AbioProtect® is formulated as a suspension concentrate of tomato plant extract infected with equivalent amounts of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2.

PepMV, EU strain, mild isolate Abp1
PepMV, EU strain, mild isolate Abp1 is naturally occurring plant viruses. Viruses are not able to produce metabolites. PepMV is widespread in Europe. As PepMV does not always cause visible symptoms in tomatoes (Hanssen et al., 2008), high and chronic exposure of humans towards this virus is expected. Introduction of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, in greenhouse (protected) tomato crops is not expected to affect the level of natural occurrence of the virus. Without treatment with AbioProtect®, the crop will most probably be infected with a natural occurring mild or aggressive isolate of the virus.

Therefore, determination of residues of the components of the formulation AbioProtect®, PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 in or on treated products, food and feed is not considered relevant.

PepMV, CH2 strain, mild isolate Abp2.
PepMV, CH2 strain, mild isolate Abp2 is naturally occurring plant viruses. Viruses are not able to produce metabolites. PepMV is widespread in Europe; it is present in 19 countries. As PepMV does not always cause visible symptoms in tomatoes (Hanssen et al., 2008), high and chronic exposure of humans towards this virus is expected. Introduction of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, in greenhouse (protected) tomato crops is not expected to affect the level of natural occurrence of the virus.

Without treatment with AbioProtect®, the crop will most probably be infected with a natural occurring mild or aggressive isolate of the virus.

Therefore, determination of residues of the components of the formulation AbioProtect®, PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 in or on treated products, food and feed is not considered relevant.

As the product contains nicotine as impurity consumers may be exposed. Acute and chronic exposure to nicotine for adults, children and general populations has been estimated using the model created by EFSA including all available EU Member State diets: PRIMo (Pesticide Residue Intake Model rev. 2). The consumer exposure assessment shows that the exposure is well below the ADI and ARfD.

2.8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

Most of the information provided in the dossier for fate and behavior in the environment of AbioProtect® (PepMV, EU strain, mild isolate Abp1 + PepMV, CH2 strain, mild isolate Abp2) were taken from open literature. In addition, this information is not strain and isolate specific. Furthermore, the specific studies provided for the assessment of the active substances were conducted for the Plant Protection Product (AbioProtect®). There is not information of Abp1 and Abp2 separately.

PepMV is widespread in Europe, their presence is described in 19 countries. Four main PepMV genotypes can be distinguished, the original Peruvian genotype (LP), the European genotype (EU), the American genotype (US1), and the Chilean genotype (CH2), with an intergenotype RNA sequence identity ranging from 78% to 95%, plus the recently described South Peruvian genotype (PES) that is not yet reported in domestic tomato. The PepMV EU genotype was the first to appear in Europe, although the CH2 genotype is currently the most frequent, while isolates of the EU genotype are persisting both in single and mixed infections. Furthermore PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 are naturally occurring mild PepMV isolates, both original from Europe, therefore their introduction in (protected) tomato crops is not expected to affect the level of natural occurrence of the virus.

Transmission of the virus is expected to be very easy by plant-plant contact, plant-cultivation and equipment contact. According with the open literature PepMV can also be transmitted less efficiently by vectors (bumblebees, insects), seeds, recirculating water.

2.8.1 Summary of fate and behaviour in soil

A GEP study on the persistence of PepMV in the soil recovered from the plants treated with AbioProtect® (Prats, 2017b), as well as another GEP study on the persistence of PepMV in hydroponic grow bags where the plants treated with the preparation have grown (Céspedes, 2015a), have shown that there was no infective PepMV either in the soil or in the substrate. However, Céspedes (2015a) detected the virus in the roots remaining from the hydroponic grow bags.

Since the virus was not infective it can be concluded that there is no risk of PepMV transmission from the soil or from the substrate of the plants treated with the MPCP AbioProtect®.

2.8.2 Summary of fate and behaviour in water

Prats (2017a) studied the persistence of PepMV in recirculating water of greenhouses treated with MPCP AbioProtect®. The study was conducted with GEP, and concluded that recirculating water does not represent a risk for PepMV transmission as it has no persistency in the leachate of the plants treated with the MPCP AbioProtect®. PepMV, like all viruses (both plant and animal/human pathogenic) can only reproduce inside its host cells.

2.8.3 Summary of fate and behaviour in air

Transmission of PepMV through air is not expected as it can only reproduce inside its host plants, it is not an airborne agent and it is a sap or mechanically transmitted virus. In the greenhouse tomato cultures treated with the MPCP the release of PepMV through air is very limited. Even in the unlikely event that aerosols containing PepMV particles would be formed, exposure of the environment is not expected as the only air-exchange with the outside is located at the ventilation windows several meters above the plants. The virus does not survive long outside plant material and is degraded by UV light.

Therefore, multiplication in soil, water and air is irrelevant. Stability of virus particles outside its hosts is limited. In conclusion, persistence of PepMV in the environment is low. Transmission in agricultural environment like greenhouses however is easy by mechanical action (plant-plant contacts, and plant-tool contacts), also because of the high density of host plants. Due to the low stability of virus particles outside host plants, transmission in the natural environment is not expected to be significant. PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 particles released in the environment are expected to be in equilibrium with all naturally present strains of PepMV.

2.8.4 Summary of mobility

PepMV is unlikely to be mobile in the environment via soil, or air. The studies provided demonstrated that as the virus can infect plants through circulation water in the greenhouse (Schwarz et al., 2010), the possibility that the virus can infect host plants outside the greenhouse via drainage water was assessed (see B 8.1.2) and found that PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, have no persistency in the leachate from the tomato plants treated. Therefore, there is no risk of PepMV infection with this leachate. Besides, according to the results of the persistence in soil study there is no risk of PepMV spread from the soil or the substrate of the plants treated (see B 8.1.1).

Furthermore, as the more severe variants of PepMV are already widespread, and the above-mentioned characteristics for persistence and replication of the virus, the risk to the environment from a possible temporary increase in the natural background concentration of PepMV is expected to be negligible.

2.8.5 Effects of the micro-organism on drinking water analysis

Regarding the interference of microorganism with methods of analysis for pathogens in drinking water, the EFSA[12] concluded that PepMV isolates VX1 and VC1 are related to other plant viruses commonly found in surface water and that it is unlikely to interfere with the analytical systems intended for bacteria. Therefore, no further information or data were requested regarding the potential interference of PepMV isolates Abp1 and abp2 with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC.

PepMV, EU strain mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 are plant viruses already naturally present on tomato plants in greenhouse today. The virus does not multiply outside its plant host, it only survives short periods outside the host cell since it is broken down by proteases, RNAses and UV light. Persistence in water of the product Abioprotec has been evaluated in this GEP trial and found that PepMV was not persistent in the leachate from the tomato plants treated, concluding that there is no risk of infection from this leachate. Furthermore, as viruses have no metabolism of their own are not able to produce secondary metabolites. Thus, the risk of the product Abioprotec to consumers is negligible and impact of water treatment processes on the active substance and its metabolites in water abstracted for drinking water is not foreseen.

2.8.6 Summary of Predicted Environmental Concentrations

Predicted Environmental Concentrations in Soil (PECsoil)

The initial PEC: $PiEC_{soil} = D*(1-f_{int})/100 *d* \rho$

PiECsoil = Predicted Initial Environmental Concentration (mg Abioprotect®/kg soil);

[1] EFSA. (European Food Safety Authority), 2017a Peer review of the pesticide risk assessment of the active substance Mild *Pepino mosaic virus* isolate VX1. EFSA Journal 15:4650. DOI: doi:10.2903/j.efsa.2017.4650.
[2] EFSA. (European Food Safety Authority), 2017b Peer review of the pesticide risk assessment of the active substance Mild Pepino mosaic virus isolate VC1. EFSA Journal 15:4651. DOI: doi:10.2903/j.efsa.2017.4651.

D = dose (g/ha);
fint = crop interception (fraction);
d = depth (cm);
ρ= dry bulk density of the soil (g/cm^3).

A dry bulk density of the soil of 1.5 g/cm^3 and a dose reaching the top 5 cm of the soil (d = 5 cm) is assumed.
Interception by tomatoes at BBCH 13 is 50%.
The maximum applied dose of AbioProtect® in the greenhouse is 8 L/ha, equal to a range from 51.2 to 512 g product/ha (>4x10^{12} genome copies/ha).

PECsoil initial = 0.34 mg Abioprotect®/kg soil

Corresponding to >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.**

Predicted Environmental Concentrations in surface water (PECsw)

Predicted environmental concentration PEC for the worst-case scenario is estimated as follows:
Drift from a greenhouse is assumed to be 0.1%.
The maximum applied dose of AbioProtect® in the greenhouse is 8 L/ha, equal to a range from 51.2 to 512 g/ha (>4x10^{12} genome copies/ha).
As a result, a maximum amount per application of 0.512 g AbioProtect®/ha (corresponding to 5.12x10^{-5} g AbioProtect®/m^2, >4x10^8 genome copies of PepMV/m^2) could be considered.
This total amount is assumed to reach a standard ditch of 0.3 m depth, 1 m wide and 1 m length, which leads to:

PECsw initial = 17.06x10^{-2} µg Abioprotect®/L water

Corresponding to 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.**

2.9 EFFECTS ON NON-TARGET SPECIES

PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 are naturally occurring plant viruses, they do not have metabolism of their own and are not able to produce metabolites, therefore the risk to organisms other than plants could be excluded.

For the proposed use(s) in tomato crop in high technology glasshouses, direct exposure of terrestrial vertebrates can be considered negligible. Release of Pepino mosaic virus Abp1 abnd Abp2 to the environment through air or soil (or other means) is limited. Exposure to birds and mammals through drinking water as a result of exposure of surface water to recirculation water, is also considered low. Like all viruses (both plant and animal/human pathogenic) pepino Mosaic Virus can only reproduce inside its host.

Studies assessing the effect of the representative formulation, Abioprotect to non-target organisms are generally not available. Abioprotect was tested on green alga Pseudokirchneriella (Schuster 2017a) and water plant Lemna gibba (Schuster 2017b). 3 GPE studies on phitotoxicity to tomato plants (Prats 2017a, Prats 2017b and Prats 2017c) and one GTE study in weeds (Agüero 2017a) were also evaluated. In these studies, no effects were observed suggesting a low toxicity of the Abp1 and Abp2 and co-formulants to plants. The applicant suggests that the ingredients of the preparation Abioprotect are inert and are not expected to present any hazards to the environment. RMS agrees with the clear rationale behind this statement.

2.9.1 Summary of effects on birds (and other terrestrial vertebrates)

No specific studies on the toxicity, infectiveness or pathogenicity towards birds was submitted by the applicant. The applicant refers to general information in B.9 and Document K-MA 5.2.5, Hernando, 2017. The RMS considers this acceptable since Abp1 and Abp2 have a cross-protection (acquired immunity phenomenon on

plants) mode of action and no toxicological effect was described for birds in paper review submitted by the applicant.

Since the product is a plant virus that is applied in tomato cultures in high technology greenhouses the release of PepMVAbp1 and Abp2 to the environment through air and soil is limited.

2.9.2 Summary of effects on aquatic organisms

PepMV present in recirculation water used in greenhouses seems to be a hypothetical way for transmission to surface water. However a persistence in water GEP study has shown that PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 have no persistency in the leachate from tomato plants treated with AbioProtect®. Therefore, there is no risk of PepMV infection with this leachate.

The possibility of effects towards fish and other water invertebrates has been excluded after several searches for scientific peer review literature, concluding that the potential risk of PepMV towards fish and aquatic invertebrates in general and PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, in particular, is negligible.

Besides two GLP studies on the potential toxicity of the preparation AbioProtect® and its components to the green alga *Pseudokirchneriella* subcapitata and to the duckweed *Lemna gibba* have been conducted showing that there is no inhibitory effect on the growth of both species due to the PepMV components of AbioProtect®.

It could be concluded that the MPCP AbioProtect® and its components (PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) have no effect on aquatic species.

2.9.3 Summary of effects on bees

Bees and other pollinators might visit treated plants. However, since application is foreseen only once per crop on seedlings (BBCH 13-15), exposure of bees to PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 will be limited. In addition, since no effects of plant viruses on insects are known, the risk for bees, other pollinators and other arthropods species is considered negligible.

2.9.4 Summary of effects on earthworms

A search for scientific peer review literature on pathogenicity of PepMV to earthworms and other soil microorganisms has been conducted and no report on any negative effect has been found. A report studying the possibility of PepMV been transmitted by soil fungus *Olpidium virulentus* has been retrieved without showing any negative effect of the virus on the fungus.

Furthermore, EFSA concluded that the risk from the representative use of PepMV (mild isolates VX1 and VC1) to birds, fish, aquatic invertebrates, bees and other non-target arthropods, earthworms and soil microorganisms was considered low, and also that there is low probability for pathogenicity or infectiveness towards algae and aquatic plants. All these data and information supports that AbioProtect® and its components (PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) do not have any effect on non-target organisms.

2.9.5 Summary of effects on soil micro-organisms

No separate study was submitted on the effects on non-target soil micro-organisms bacteria and fungi. The study included under section B.9.6 investigated potential transmission effects to selected fungi O. virulentus. The conclusions from the paper indicate that the virus PepMV were not produce any harmful effect on the fungi, whether they transmitted the virus. However, it is not clear whether the tested fungi are representative for the soil microbial community. Based on the representative use in glasshouses, exposure to soils are not anticipated.

2.9.6 Summary of effects on other non-target organisms (flora and fauna)

Mammalian toxicology studies are presented and evaluated in Vol 3, section 6 (human health). No additional effect studies on non-target organisms were submitted.

Viruses such as PepMV are transmitted among plants by mechanical means and do not enter cells via specific receptors, as do animal viruses. Animal viruses enter host cells by a process called endocytosis. Plant viruses, by contrast, enter through wounds in the cell's outer coverings, e.g. through abrasions made by wind or through punctures made by insects.

2.9.7 Summary of effects on terrestrial plants

Symptomless infections of PepMV, or with mild symptoms, have been observed in weed species of different botanical families. Most of these infections were found in the vicinity of tomato greenhouses. However, no evidence was provided in these studies that the weed species plays a significant role in the epidemiology of PepMV. PepMV can survive on a broad range of crops for a short time, but efficient multiplication is only possible in *Solanaceae* crops. It was concluded from these observations that drift of aggressive or mild PepMV from greenhouses will result in a temporary population outdoor that will return to natural background levels. In this respect, no differences are expected between aggressive and mild PepMV.

2.10 SUMMARY AND EVALUATION OF ENVIRONMENTAL IMPACT

PepMV is already present in 19 countries in Europe; in fact it is a very prevalent virus in greenhouse tomato production in Europe. Transmission of the virus is expected to be very easy by plant-plant contact, plant-cultivation and equipment contact.

Agüero (2017b) studied the risk of spread of the virus from weeds in the vicinity of tomato greenhouses treated with PepMV. The risk of infection from weeds surrounding tomato found greenhouses treated with PepMV were not higher than the weeds from the greenhouses not treated. Furthermore, the treatment of tomato plants in a greenhouse with PepMV does not appear to affect the level of natural occurrence of the virus.

GEP studies to assess the risk of PepMV transmission through water (Prats, 2017a), through soil (Prats, 2017b) and through the growth substrate (Céspedes, 2015a) from tomato plants in greenhouses treated with PepMV have been conducted and the results have shown that there is no risk of PepMV infection from the leachate or from the soil or from the substrate of the plants treated.

PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 are naturally occurring plant viruses, they do not have metabolism of their own and are not able to produce metabolites, therefore the risk to organisms other than plants could be excluded. A focussed search on scientific peer review literature on the potential effects of PepMV to animal humans and non-target species has been conducted. The results from that search support the general assumption that plant pathogenic viruses are considered to be pathogenic towards plant species only and not towards other organisms, like human and other animals in general, as well as to birds, fish, aquatic invertebrates, bees and other non-target arthropods, earthworms and soil microorganisms and also that there is low probability for pathogenicity or infectiveness towards algae and aquatic plants. No toxic effect of AbioProtect® and its components (PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) to algae and aquatic plants has been detected in GLP studies.

Level 3

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

Proposed decision with respect to the application

Level 3. Summary and consideration with respect to the approval criteria of Regulation (EC) No 1107/2009
Identification of data gaps, proposed conditions, risk management measures, issues that could not be finalised and critical areas of concern
Proposed decision

3 PROPOSED DECISION WITH RESPECT TO THE APPLICATION OF THE APPROVAL OF AN ACTIVE SUBSTANCE

3.1 BACKGROUND TO THE PROPOSED DECISION

3.1.1 Proposal on acceptability against the approval criteria – Article 4 and Annex II of Regulation (EC) No 1107/2009

3.1.1.1 Article 4				
		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		*Pepino mosaic virus (PepMV) belongs to the genus Potexvirus of the Alphaflexiviridae family; with include plant virus only.* *<u>Environmental fate and behaviour:</u>* *PepMV, 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.* *The virus is very easy transmitted by plant-plant contact, plant-cultivation and equipment contact. Furthermore, can also be transmitted less efficiently by vectors (bumblebees, insects), seeds, recirculating water.* *PepMV is no persistent in soil, water and air when the product AbioProtect® is used as a plant vaccine in protected tomato crops.* *PepMV is unlikely to be mobile in the environment via soil, or air. No risk was found of PepMV infection with the leachate from the tomato plant treated with AbioProtect®.* *<u>Ecotoxicology</u>:* ***Active substances:*** - *PepMV, EU strain, mild isolate Abp1.* - *PepMV, CH2 strain, mild isolate Abp2.* *Abp1 and Abp2 are naturally occurring plant viruses, they do not have*

				metabolism of their own and are not able to produce metabolites, and therefore the risk to organisms other than plants could be excluded. - *Abp1 isolate belongs to the EU genotype, same as the aggressive strains of PepMV that cause pepino mosaic disease in tomato in Europe.* - *Abp2 belongs to the CH2 genotype, same as part of the aggressive strains of PepMV that cause pepino mosaic disease in tomato in Europe.* *Due to the mode of action as cross protection effect and thus actual activity is obtained by infection of the plants with mild isolates of the virus Abp1 and Abp2.* *Therefore, mild isolates Abp1 and Abp2 induce in tomato plants a symptomless infection with no damage to the fruit.* ***Representative uses:** Control of infectionPepino Mosaic Viruses in tomato plants.* *No risks have been identified as a result of use of the representative product Abioprotect.* *Birds and mammals infections caused by Abp1 and Abp2 seem unlikely. However, due to the representative use being treatment of tomato seedling in permanent green-houses only, this has not been further assessed and is not considered to constitute a risk.*
3.1.1.2 Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted	X		
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			*Not applicable*
3.1.1.3 Restrictions on approval				
		Yes	No	

	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.		X	
3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).			
	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.	X		*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. No MRL is required.* *As the product contains nicotine as impurity consumers may be exposed to non-viable residues. The consumer exposure assessment shows that the exposure is well below the ADI and ARfD*
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		*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, controlling 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 aggressive isolates of PepMV in indifferent tomato cultivars in several greenhouse trials, with a single application at BBCH 13-15, at a dose of 5 L/ha containing at least $5x10^{11}$ PepMV genome copies/L.* *The mode of action is based on cross-protection in the tomato crop against aggressive isolates of PepMV, therefore the possibility of development of resistance is not relevant.*
Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.			*Not applicable*
Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.	X		*The content of pure virus in PepMV, EU strain, mild isolate Abp1 technical is set up to be at least $2.5x10^{11}$ genome copies/L, and in PepMV, CH2 strain, mild isolate Abp2 technical is set up to be at least $2.5x10^{11}$ genome copies/L.*
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.			*Not applicable*
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted			*Not applicable*
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities	X		*Acceptable methods are available for the determination of virus in the MPCA and the MPCP. Adequate methods are also available to determine*

	of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.			*the presence of other microorganisms potentially pathogen. See point 2.5 in level 2.*
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.			*Not applicable, No maximum residue levels are proposed or exist for in food and feed. Also no action levels and no residue definitions are proposed or exist for soil, water and air. Consequently, analytical methods for the determination of residues are not considered necessary*
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		
Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		*All the available information indicates that the use PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 in the manner proposed present no systemic hazard for operators or others who may handle treated plants and an exposure assessment is not required.* *Therefore, no AOEL, ADI, and ARfD have been determined.*
Impact on human health – proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	*Not relevant for microorganisms.* *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 Ames Test*
Impact on human health – proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation		X	*Not relevant for microorganisms.*

	(EC) No 1272/2008, **as carcinogen category 1A or 1B.**			
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			*Not relevant*
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**		X	*Not relevant.*
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			*Not relevant for microorganisms.*
Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **carcinogenic category 2 and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties**		X	*Not relevant for microorganisms.*
ii)	It is considered that **the substance SHOULD BE classified or**		X	*Not relevant for microorganisms.*

	proposed for classification in accordance with the provisions of Regulation (EC) No 1272/2008, as **toxic for reproduction category 2 and** in addition the RMS considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**			
iii)	Linked to either i) or ii) immediately above. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			*Not relevant for microorganisms.*
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	*The criterion is not relevant for microorganisms.*
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	*The criterion is not relevant for microorganisms.*
Very persistent and very bioaccumulative substance (vPvB).				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	*The criterion is not relevant for microorganisms.*
Ecotoxicology				
		Yes	No	
	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of	X		*Due to the representative use of Abp1 and Abp1 in greenhouses exposure and consequently also the risks to birds, wild bees, wild non-target arthropods and soil dwelling organisms is considered negligible.* *See section 2.9. of this document.*

	a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.			
	It is considered that, on the basis of the assessment of Community or internationally agreed test guidelines, the substance **HAS** endocrine disrupting properties that may cause adverse effects on non-target organisms.		X	*Not relevant for virus.*
	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.	X		*Not relevant.*
	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.	X		*The representative use will result in a neglible exposure to wild honey bees.*
Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.			*No residue definition required.*
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites,	X		*The criterion is not relevant for microorganisms.*

	degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.			

3.1.2 Proposal - Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		X	*Not relevant for microorganisms.*

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** In particular it is considered that the substance **should NOT be classified or proposed for classification** in accordance with Regulation (EC) No 1272/2008 as at least one of the following: — carcinogenic, — mutagenic, — toxic to reproduction, — sensitising chemicals, — very toxic or toxic, — explosive, — corrosive. In addition it is considered that **the substance is NOT**: — persistent (half-life in soil more than 60 days), — has a bioconcentration factor higher than 100, — is deemed to be an endocrine disrupter, or — has neurotoxic or immunotoxic effects.	X		*In accordance with Commission Regulation (EU) 2017/1432, of 7 August 2017, amending Regulation (EC) No 1107/2009 of the European Parliament and the Council concerning the placing of plant protection products on the market as regards the criteria for the approval of low-risk active substances, it is considered that an active substance consisting in a micro-organism:* *5.2.1.* ***An active substance which is a micro-organism may be considered as being of low-risk unless at strain level it has demonstrated multiple resistance to anti-microbials used in human or veterinary medicine****.* *5.2.2. Baculoviruses shall be considered as being of low-risk unless at strain level they have demonstrated adverse effects on non-target insects.'* ***Multiple resistance to anti-microbials used in human or veterinary medicine is not applicable to viruses: viruses are not metabolically active and therefore cannot produce antimicrobial substances; they are not sensitive to antibiotics and therefore cannot become resistant to these substances or spread resistance.*** *The current criteria for low risk substances (Reg (EU) 1107/2009, Annex II, article 5) is not applicable for micro-organisms.* *The criteria for low risk applicable for micro-organisms are as follows:* *"An active substance which is a micro-organism may be considered as being of low-risk unless at strain level it has demonstrated multiple resistance to*

Low-risk active substances				
				anti-microbials used in human or veterinary medicine." *This criterion is fulfilled for Abp1* *This criterion is fulfilled for Abp2* *This criterion is fulfilled for AbioProtect®* *Therefore, ES position is that the active substance could be considered of low risk.* *Nevertheless, we would like to raise our concern about the following issues:* • *microorganisms per se are considered potential sensitizers, therefore, risk mitigation measures may be necessary.* • *the virus contains nicotine as an impurity derived from the manufacturing process, since the strains are isolated in tobacco leaves. Nicotine is a substance of concern from the toxicological point of view, that is why an AOEL has been established in the EU, and a risk assessment has been carried out by the RMS.*

3.1.4 List of studies to be generated, still ongoing or available but not evaluated

Data gap	**Relevance in relation to representative use(s)**	**Study status**		
		No confirmation that study available or on-going.	**Study on-going and anticipated date of completion**	**Study available but not peer-reviewed**
3.1.4.2 Biological properties of the active substance and physical, chemical and technical properties of the formulation				
B.2.7. GENETIC STABILITY AND FACTORS AFFECTING IT Genetic stability must be evaluate in a long-term study for both isolates (this information is included in Vol 4 due to strategic commercial interest of the applicant) Relevant due to possible mutation of the isolates	x	X		

3.1.5 Issues that could not be finalized

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

There are not issues that could not be finalized

Area of the risk assessment that could not be finalised on the basis of the available data	**Relevance in relation to representative use(s)**

3.1.6 Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.
An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

There are not critical areas of concern

Critical area of concern identified	**Relevance in relation to representative use(s)**
	[specify if concern relates to all or specific representative use/use scenario/product or to all uses/products]

Critical area of concern identified	**Relevance in relation to representative use(s)**

3.1.7 Overview table of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)

All columns are grey as the material tested in the toxicological studies has not been demonstrated to be representative of the technical specification.

Representative use		Use "Tomato plants" (Greenhouses) (X^1)
Operator risk	Risk identified	
	Assessment not finalised	
Worker risk	Risk identified	
	Assessment not finalised	
Bystander risk	Risk identified	
	Assessment not finalised	
Consumer risk	Risk identified	
	Assessment not finalised	
Risk to wild non target terrestrial vertebrates	Risk identified	
	Assessment not finalised	
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified	
	Assessment not finalised	
Risk to aquatic organisms	Risk identified	
	Assessment not finalised	
Groundwater exposure active substance	Legal parametric value breached	
	Assessment not finalised	
Groundwater exposure metabolites	Legal parametric value breached	
	Parametric value of 10µg/L[(a)] breached	

Representative use		Use "Tomato plants" (Greenhouses) (X^1)
	Assessment not finalised	
Comments/Remarks		

1) The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	**Justification**
	[specify the reasons why expert consultation is considered necessary]

3.1.9 Critical issues on which the Co-RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur member state. Only the points relevant for the decision making process should be listed.

Issue on which Co-RMS disagrees with RMS	**Opinion of Co-RMS**	**Opinion of RMS**

Issue on which Co-RMS disagrees with RMS	**Opinion of Co-RMS**	**Opinion of RMS**

3.2 PROPOSED DECISION

3.2.1 Proposed Decision for PepMV, EU strain, mild isolate Abp1

[redacted]

3.2.2 Proposed Decision for PepMV, CH2 strain, mild isolate Abp2

[redacted]

[redacted]

3.3 RATIONAL FOR THE CONDITIONS AND RESTRICTIONS TO BE ASSOCIATED WITH ANY APPROVAL OR AUTHORISATION(S), AS APPROPRIATE

3.3.1 Particular conditions proposed to be take into account to manage the risks identified

[redacted]	[redacted]
[redacted]	[redacted]

APPENDICES

Appendix 1 Guidance documents used in this assessment
[List of Guidance documents used in the conduct of the evaluation and risk assessment.]

Appendix 2 Reference list