European Commission

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Pepino Mosaic Virus, EU strain, mild isolate Abp1
Pepino Mosaic Virus, CH2 strain, mild isolate Abp2
Product data: AbioProtect®

Volume 3 – Annex B.9 Effects on non-target organisms

Rapporteur Member State: Spain

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B.9 EFFECTS ON NON-TARGET ORGANISMS

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; which include plant viruses only. It is widespread in Europe EPPO A2 list no. 3691[1]; regulated pest in the EU based on emergency decision 2004/200/EC and in fact is a major disease in greenhouse tomato crops worldwide. Currently, two major genotypes or strain groups are distinguished in Europe: European (EU) that predominated initially in European tomato crops since 2004 and Peru, CH2.Iisolates of strain group EU seems to be replaced by, and/or to occur increasingly in mixed infections with, strain CH2 in Spain. This latter genotype, first identified from tomato seeds originating from Chile, is genetically very distinct (79% identity) from the EU strains.

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, are 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 high technology greenhouse 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.

The active agents Abp1 and Abp2 and the MPCP Abioprotect, contain nicotine as an impurity that would affect non-target organism. Therefore, the nicotine concentration in PepMV, CH2 strain, mild isolate Abp2 has been determined (please refers to volume 4, part . C.1.4.2.). Nicotine is present at very low levels, ranging from 0.007 mg/kg to 0.111 mg/kg with a medium of 0.050 mg/kg nicotine (Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-).

The use of AbioProtect® as MPP in a single application on the seedlings (BBCH 13-15) is not expected to affect the level of natural occurrence of nicotine in tomato plants, tomato fruits nor the level of exposure of birds, bees, non-target arthropods, earthworms, and soil microorganisms.

Direct exposure to impurity nicotine present in Abioprotect MPP to birds, bees, non-target arthropods, earthworms, and soil microorganisms is not considered relevant due to: (1) The information provided in Volume 4, C.1.4.2 has demonstrated nicotine natural occurrence will not increase significantly after Abiprotect application in tomato plants or tomato fruit. (2) For the proposed use pattern in tomato crop in permanent greenhouses.

Release of nicotine 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.

As regards of the exposure of the impurity nicotine included in Abioprotect to non-target organisms (birds, bees, non-target arthropods, earthworms, and soil microorganisms) was not considered relevant. Therefore, a low risk was concluded for non-target organisms.

Mode of action in target host plants

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

[1] [1]*EPPO A2 list no. 3691.*

silencing and coat-protein-mediated resistance (Volume 9-B.2.2.2). 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 belongs to the *Alphaflexiviridae* family order Tymovirales that include plant viruses only. For this virus family a qualified presumption of safety has been found at the European level (EFSA BIOHAZ Panel, 2013)[2]. Plant viruses can only reproduce in their host plant living cells. PepMV is transmitted mechanically very efficiently in tomato by standard crop handling through contaminated tools, hands and clothing and by direct plant-to-plant contact (Spence *et al.*, 2006; Van der Vlugt, 2009; Wright and Mumford, 1999). Plant viruses enter cells only through wounds made mechanically or by vectors or by deposition into an ovule by an infected pollen grain (Agrios, 2005).

A focused search for scientific peer review literature on pathogenicity of plant viruses to animals or humans has been conducted and is included (Document K-MA 5.2.5, Hernando, 2017). This search has not retrieved any relevant summary report related to any effect of PepMV, Potexvirus or other members of the family *Alphaflexiviridae* on humans or mammals. For other plant virus families, such search has pointed out that some authors have reported the presence of RNA from pepper mild mottle virus (PMMoV) and pepper mottle virus (PMV) in human feces (Colson *et al.*, 2010; Zhang *et al.*, 2006) and in human serum (Tobacco mosaic virus, TMV) (Liu *et al.*, 2013). However, no information or cases on multiplication of these or other plant viruses in vertebrate or human tissues has been reported in the scientific literature.
Furthermore, the cell culture studies described in Document K-MA 5.2.4/01 (Žegura and Novak, 2017) and Document K-MA 5.2.4/02 (Žegura *et al.,* 2017) did not show any effect on cell viability and proliferation attributable to PepMV, besides there is no indication of infectivity or multiplication on PepMV in human cells.

Vectors (mainly insect species) appear to be affected by plant viruses. In specific vector-plant virus relations the plant virus affects the behavior or development of vector species in order to optimize its spread to other plants. Although bumblebees can spread PepMV mechanically no specific vector-plant virus relation is known and PepMV is not known to be harmful to bumblebees or any other insects.

Besides, the risk to birds, fish, aquatic invertebrates, bees and other non-target arthropods, earthworms and soil microorganisms of the use of PepMV European (EU) genotype (mild isolates VX1 and VC1) (EFSA, 2017a; EFSA, 2017b)[3][4] and PepMV Chilean (CH2) genotype (mild isolate 1906) (EFSA, 2015)[5] in greenhouses was concluded as low by the European Food Safety Authority.

The results of the search for scientific peer review literature supports the general assumption that plant viruses are considered to be pathogenic to plant species only and not towards other organisms.

It is therefore concluded that potential risk of PepMV virus in general and PepMV EU strain, mild isolate Abp1 and PepMV CH2 strain, mild isolate Abp2, in particular, towards animals is negligible.

Based on the representative use in permanent glasshouses, birds and mammals, free living arthropods and soil dwelling organisms are not expected to be exposed to PepMV strains Abp1 and Abp2. Therefore, all study summaries on birds and mammals and soil dwelling organisms have been evaluated by the RMS according to current guidelines and guidance.

[2] *EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013. Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). EFSA Journal 2013; 11(11):3449, 107 pp. doi:10.2903/j.efsa.2013.3449.*

[3] *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.*

[4] *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.*

[5] *EFSAEFSA (European Food Safety Authority), 2015. Conclusion on the peer review of the pesticide risk assessment of the active substance Pepino mosaic virus strain CH2 isolate 1906. EFSA Journal 2015;13(1):3977, 25 pp. doi:10.2903/j.efsa.2015.3977.*

GAP TABLE: DETAILS OF ALL NATIONAL GAPS WITHIN EACH ZONE

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

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

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

Remarks:

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

B.9.1 EFFECTS ON BIRDS (OECD IIM 8.1, IIIM 10.1, IIIM 10.3)

Not relevant please refer to MA 9.

RMS comments:
Since the product is a plant virus that is applied in high technology tomato cultures in permanent greenhouses the release of PepMV isolates Abp1 and Abp2 to the environment through air and soil is limited.
The virus does not survive more than 60 days outside host plant (it is broken down by UV light), therefore, exposure of birds is considered remote.
Exposure to birds through drinking water as a result of exposure of surface water to recirculation water, is also considered low according to persistence in soil and leachate water study submitted by the applicant. (KMA6.2.4, Pratts 2017-a and KMA6.2.5, Pratts 2017-b). There is no need to determine the risk or effects of the MPCAs virus PepMV strains Abp1 and Abp2 on birds.

No study was available on birds.

B.9.1.1 Toxicity to birds

RMS comments:
Due to the physical and biological characteristics of the MPAs Abp1 and Abp2 refers in (B2.8.) there is no production of toxins/metabolites.

No studies on birds were submitted on toxins/metabolites from MPP AbioProtect®

B.9.1.2 Infectiveness to birds

RMS comments:
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.
To confirmed literature review, the company provided two analytical studies on cell culture described in Document K-MA 5.2.4/01 (Žegura and Novak, 2017) and Document K-MA 5.2.4/02 (Žegura et al., 2017). The studies did not show any effect on cell viability and proliferation attributable to PepMV, besides there is no indication of infectivity or multiplication on PepMV EU strain, mild isolate Abp1 and PepMV CH2 strain, mild isolate Abp2 in human cells.

No studies on the infectivity to birds were submitted by the applicant.

B.9.1.3 Pathogenicity to birds

RMS comments:
Plant pathogenic viruses are generally considered to be pathogenic towards plant species only and not towards other organisms. Birds exposure to plant pathogenic viruses is enormous and birds illnesses caused by plant pathogenic viruses have not been described. Until very recently the scientific community has not investigate the possible health risk to animals caused by plant pathogenic viruses.

The applicant refers to Document K-MA 5.2.5, Hernando, 2017. Plant viruses are not related with any animal or human pathogen because they only reproduce in living plant cells, therefore, plant viruses cannot replicate in humans or other animals, largely due to the lack of specific receptors for recognition and entry into host cells. Like all viruses (both plant and animal/human pathogenic) pepino Mosaic Virus strains Abp1 and Abp2, can only reproduce inside its host cells.

A focused search for scientific peer review literature on pathogenicity of plant viruses to birds and mammals has been conducted and is included (Document K-MA 5.2.5, Hernando, 2017). This search has not retrieved any relevant summary report related to any effect of PepMV, Potexvirus or other members of the family

Alphaflexiviridae on birds or mammals. For other plant virus families, such search has pointed out that some authors have reported the presence of RNA from pepper mild mottle virus (PMMoV) and pepper mottle virus (PMV) in human feces (Colson et al., 2010; Zhang et al., 2006) and in human serum (Tobacco mosaic virus, TMV) (Liu et al., 2013). However, no information or cases on multiplication of these or other plant viruses in vertebrate or human tissues has been reported in the scientific literature.

No studies on the pathogenicity to birds were submitted by the applicant.

B.9.1.4 Summary of the studies on birds on toxicity, infectiveness and pathogenicity

RMS comments:
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.

There is no need to determine the risk or effects of the MPCAs virus PepMV strains Abp1 and Abp2 on birds.

- **MPP AbioProtect®has no relevant effect on birds, please refer to MA 9.**

B.9.2 EFFECTS ON AQUATIC ORGANISMS (OECD IIM 8.2, IIM 8.3, IIM 8.4, IIM 8.5, IIIM 10.2)

PepMV has been found to be spread by recirculating water from plant to plant (Schwarz *et al.*, 2010), and to be able to survive and be transmitted in water (Mehle *et al.,* 2014). Recently other authors have shown that PepMV dispersal could be prevented using a sensor based disinfectant (Bandte *et al.*, 2016). Although recirculating water of greenhouses must be disinfected before discharge in the environment, emission to surface water cannot be excluded as the water can be drained in emergencies. According to the GEP persistence in water study performed (Document K-MP 6.2/04, Prats, 2017a) the formulation containing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 mild isolate Abp2 (AbioProtect®), has no persistency in the leachate from tomato plants treated with it. Hence, there is no risk of PepMV infection with this leachate. Therefore, it is not persistent in water and no relevant effect on aquatic organism is expected.

All this information, together with the fact that PepMV is widespread in Europe, indicates that introduction of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 in (high technology green house) tomato crops is not expected to affect the level of natural occurrence of the virus and therefore the risk to aquatic organisms is considered negligible.

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 a 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 (Prats, 2017a). Furthermore, as viruses have no metabolism of their own are not able to produce secondary metabolites. Thus, the risk of the product Abioprotec to aquatics organisms is negligible and impact of water treatment processes on the active substance and its metabolites in water abstracted for drinking water is not foreseen.

B.9.2.1 Effects on fish (OECD IIM 8.2, IIIM 10.2)

A focussed search for scientific peer review literature on potential effects of PepMV and other plant viruses to fish and other animals has been conducted and is included (Document K-MA 5.2.5, Hernando, 2017).

This search identified 30 summary records potentially referring to the effects of PepMV, Potexvirus or other members of the family *Alphaflexiviridae* on fish. However, after rapid assessment none of this 30 summary records were found to be relevant for the potential effect on fish of PepMV or other closely related plant viruses, supporting

the general assumption that plant pathogenic viruses are not pathogenic to animals or humans and so are not pathogenic to fish.

Besides, the risk to fish of the use of PepMV (mild isolates VX1 and VC1) EU genotypes, same as Abp1 isolate, and of PepMV (mild isolates 1906) CH2 genotype, same as Abp2 isolate, in permanent greenhouses was concluded as low by the EFSA[3,4,5].

Therefore, it can be concluded that the potential risk of PepMV in general and PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 in particular towards fish is negligible.

B.9.2.1.1 Toxicity to fish

RMS comments:
Due to the physical and biological characteristics of the MPAs Abp1 and Abp2 refers in (Vol. 3 B 2.5) there is no production of toxins/metabolites. No studies on fish were submitted on toxins/metabolites from MPCAs Abp1 and Abp2.
Besides, the risk to fish of the use of PepMV (mild isolates CH2, VX1 and VC1) in permanent greenhouses was concluded as low by the EFSA[3,4,5].

B.9.2.1.2 Infectiveness to fish

RMS comments:
No studies investigating the infectiveness to fish was submitted by the applicant.
Since no clearance was investigated it is not possible to draw conclusions on the infectivity of Abp1 and Abp2 to fish.
Besides, the risk to fish of the use of PepMV (mild isolates CH2, VX1 and VC1) in permanent greenhouses was concluded as low by the EFSA[3,4,5].

B.9.2.1.3 Pathogenicity to fish

RMS comments:
No studies investigating the pathogenicity to fish was submitted by the applicant.
Since no clearance was investigated it is not possible to draw conclusions on the infectivity of Abp1 and Abp2 to fish.
Besides, the risk to fish of the use of PepMV (mild isolates CH2, VX1 and VC1) in permanent greenhouses was concluded as low by the EFSA[3,4,5].

B.9.2.1.4 Summary of the studies on fishes on toxicity, infectiveness and pathogenicity

RMS comments:
No specific studies on the toxicity, infectiveness or pathogenicity towards fishes 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 fishes 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 water and soil is limited.

- **MPP AbioProtect®has no relevant effect on fishes, please refer to MA9.**

B.9.2.2 Effects on freshwater invertebrates (OECD IIM 8.3, IIIM 10.2)

A focused search for scientific peer review literature on potential effects of PepMV and other plant viruses to freshwater invertebrates and other non-target organisms has been conducted and is included (Document K-MA 5.2.5, Hernando, 2017).
This search identified 31 summary records potentially referring to the effects of PepMV, Potexvirus or other members of the family *Alphaflexiviridae* on aquatic organisms. Rapid assessment on the relevance of such summary records on the potential effects on fresh water invertebrates determined that none of those 31 summary records was consider relevant for the effect of PepMV or any plant virus closely related to it on fresh water invertebrates. Supporting the general assumption that plant pathogenic viruses are only pathogenic to plants of their host species.

Besides, the risk to aquatic invertebrates of the use of PepMV (mild isolates VX1 and VC1) in greenhouses was concluded as low by the EFSA[3,4,5].

Therefore, it can be concluded that the potential risk of PepMV in general and PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 in particular, towards aquatic organism is negligible.

B.9.2.2.1 Toxicity to freshwater invertebrates

RMS comments:
Due to the physical and biological characteristics and the mode of action of the MPAs Abp1 and Abp2 refers in sections (B.2.2 and B.2.8) there is no production of toxins/metabolites. No studies freshwater invertebrates were submitted on toxins/metabolites from MPCAs Abp1 and Abp2.
Besides, the risk to fish of the use of PepMV (mild isolates CH2, VX1 and VC1) in permanent greenhouses was concluded as low by the EFS[2,3,4].

B.9.2.2.2 Infectiveness to freshwater invertebrates

RMS comments:
No studies investigating the Infectiveness to to freshwater invertebrates was submitted by the applicant.
Since no clearance was investigated it is not possible to draw conclusions on the infectivity of Abp1 and Abp2 to fish.
Besides, the risk to to freshwater invertebrates of the use of PepMV (mild isolates CH2, VX1 and VC1) in permanent greenhouses was concluded as low by the EFSA[3,4,5].

B.9.2.2.3 Pathogenicity to freshwater invertebrates

RMS comments:
No studies investigating the pathogenicity to to freshwater invertebrates was submitted by the applicant.
Since no clearance was investigated it is not possible to draw conclusions on the pathogenicity of Abp1 and Abp2 to fish.
Besides, the risk to to freshwater invertebrates of the use of PepMV (mild isolates CH2, VX1 and VC1) in permanent greenhouses was concluded as low by the EFSA[3,4,5].

B.9.2.2.4 Summary of the studies on freshwater invertebrates on toxicity, infectiveness and pathogenicity

RMS comments:
No specific studies on the toxicity, infectiveness or pathogenicity towards aquatic organisms was submitted by the applicant. The applicant refers to general information in B.9 and Document K-MA 5.2.5, Hernando, 2017.
Based on these documents, no data is required. 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 aquatic organism in paper review.

Since the product is a plant virus that is applied in tomato cultures in greenhouses the release of Pepino mosaic virus strains Abp1 and Abp2 to the environment through water, air and soil is limited.
There is no need to determine the risk or effects of the MPCAs virus PepMV strains Abp1 and Abp2 on aquatic organisms.

- **MPP AbioProtect® has no relevant effect on aquatic organisms, please refer to MA 9.**

B.9.2.3 Effects on algae growth

The focussed search for scientific peer review literature (Document K-MA 5.2.5, Hernando, 2017) already mentioned included search terms with the aim to identify documents providing information on potential effects of PepMV and other plant viruses to algae and other aquatic plants.

In total 27 summary records of those identify could be related with algae and other aquatic plants. After rapid assessment on the relevance of these 27 summary records none of them was considered relevant for this purpose as none of them addressed the issue of potential effects of PepMV or other related plant pathogenic viruses on algae growth or on other aquatic plants. Supporting the general assumption that plant pathogenic viruses are only pathogenic to plants of their host species. As PepMV host range is mainly restricted to **RMS added:** *aerial plants, especially the Solanaceae family,* no effects on algae growth or in any other aquatic plant is expected.

Besides, EFSA concluded that the risk from the representative use of PepMV (mild isolates VX1 and VC1) to algae and aquatic plants is low EFSA[3,4,5]

Nonetheless a GLP study on the potential toxicity of the preparation AbioProtect® and its components to the green alga *Pseudokirchneriella subcapitata* has been conducted and is reported below.

The **RMS** has also compile the summary below incorporating the material and methods, conclusions and relevant excerpts from the study on algae growth provided by the applicant, Schuster, 2017a:

Reference	**K-MA 8.2.3/01 Shuster 2017a.** AbioProtect® and its components PepMV-Abp1 and PepMV-Abp2: Toxicity to the Single Cell Green Alga *Pseudokirchneriella subcapitata Hindák* under Laboratory Conditions. Schuster (2017a). (Unpublished report). Study code: S17-03474.
Guideline	OECD 201 (2006, corrected 2011)
GLP	The study was conducted according to GLP principles/regulations. Certified laboratory.
Objectives	The objective of this study was to determine the effects of AbioProtect® and its components PepMV-Abp1 and PepMV-Abp2 on the growth of the single cell green alga *Pseudokirchneriella subcapitata* and to determine the no observed effect concentration (NOEC).
Material and methods	Six replicates per treatment. Number of assays 1.
Test material	AbioProtect®, batch number: L-AB01-160517, active ingredients (a.i.): Pepino mosaic virus European (EU) strain, isolate Abp1, content of a.i. (analysed): $1.91x10^6$ genome copies/µL (at 64 mg/mL); Pepino mosaic virus Chilean (CH2) strain, isolate Abp2, content of a.i. (analysed): $7.68x10^6$ genome copies/µL (at 64 mg/mL).
Test species	Algae *Pseudokirchneriella subcapitata Hindák*).
Number of test algae	0.5×10^4 cells/mL.
Treatments	The test organism was exposed to 100 mg/L of tested item concentration, 100 mg/L plant extract control and control under defined conditions in a synthetic growth medium for 72 h. Six replicates were used for the control, the plant extract control and 100 mg/L tested item concentration.
Duration	72h hours.
Test conditions	pH= 7.45-8.12 ; Temperature =22.7 – 22.9 ºC; light intensity 88.8 $\mu Em^{-2}s^{-1}$ at cell culture level, within± 15 % of variation as specified by OECD 201.
Evaluation	-Physical assessment: pH, Temperature (ºC), light intensity ($\mu Em^{-2}s^{-1}$). -Toxicity: As the % of inhibition growth rate and % inhibition of yield. Daily fluorescence measure (670nm) with fluorescence microplate reader (infinite

	200Pro). -Morphological appearance of algae cells after 72h.
Deviations from guideline	No

Results and conclusion

Biomass: Cell numbers, measured in the controls between 0 h and 72h, were found to increase by a factor of 126.33 for the control, which exceeds the threshold of 16. It corresponds to a growth rate of 1.61 d-1.

Coefficient of Variation (section by section): The mean coefficient of variation for the section-by-section specific growth rates (hours 0 - 24, 24 - 48 and 48 - 72) in the control cultures was 15 % for the control and did not exceed 35 %.
Coefficient of Variation (average growth): The coefficient of variation of average growth in replicate control cultures was 3.9% for the control and did not exceed 7 % for the whole test period.

No statistically significant inhibitory effects on any parameter (growth rate and yield) were observed at 100 mg/L (nominal) test item concentration at test end. Thus the overall LOEC (Lowest Observed Effect Concentration) was not determinable and the overall NOEC was observed to be at 100 mg/L (nominal) corresponding to $1.5x10^7$ PepMV genome copies/L ($2.99x10^6$ genome copies of PepMV-Abp1/L+$1.2x10^7$ genome copies of PepMV-Abp2/L).

Therefore, according to the results of the present study there is no effect on algae growth of AbioProtect® or its components, PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2.

RMS comments and conclusions:
The study investigates the effects of the Abioprotect (Abp1+Abp2) as a formulated product combination of the two virus strains, but not Abp1 and Abp2 themselves.

Growth stimulation (negative inhibition) have been observed for test item and for plant extract control. The addition of plant extract would be a nutrient stimulating growth factors with the test material to the minimal medium used. Nevertheless, percentage of inhibition of growth rate is higher in Abioprotect (Abp1+Abp2) product (-2.1%) compare with the plant extract control (-07%).With no statistical data it is not possible to say if these growth stimulation are significant. The RMS sustain that potential effects of Abioprotect (Abp1+Abp2) to algae are most likely due to the increasing algae growth. However, results are unconcluded since it is unclear the significant of statistic data.
RMS suggest to include a negative control or reference substance in the assay for the inhibition growth of the algae. It would be also convenient to ha a second confirmed experiment.

The applicant says that morphology of the algae cells was observed microscopically at test end and the cells were considered normal for all treatment groups. There is no data photos or references criteria to sustain the affirmation.

The applicant doesn´t confirm the presence of Abp1 and Abp2 at the end of the studio or in the green algae cells, that for, there is no confirmation of virus infectivity or pathogenicity to green algae.
Although, the RMS is not agree with the specific study Document K-MA 8.2.3-01 (Schuster, 2017a) confirms there is no virulence or transmission of the virus to algae.

The risk from the representative use of Pepino mosaic virus EU strains Abp1 isolate and CH2 strain, Abp2 isolate to algae is unclear. The applicant need to deeply explain Document K-MA 8.2.3-01. (Statistical data, viable virus presence at the end of the assay and virus presence on green algae cells).

Complete report of the study is included in Document K-MA 8.2.3-01 (Schuster 2017a).

B.9.2.4 Effects on plants other than algae (OECD IIM 8.5, IIIM 10.2)

Please refer to information provided in point MA 9.2.3 above showing that the information retrieved from the focused search on scientific literature conducted supports the general assumption that plant pathogenic viruses are only pathogenic to plants of their host species. As PepMV host range is mainly restricted to the *Solanaceae* family, no effects on aquatic plants is expected, assumption that is also supported by EFSA[3,4,5].

Nonetheless a GLP study Document Report K-MA 8.2.4/01 (Shuster, 2017b) on the potential toxicity of the preparation AbioProtect® and its components to the Duckweed *Lemna gibba* has been conducted.

The **RMS** has also compile the summary below incorporating the material and methods, conclusions and relevant excerpts from the study on algae growth provided by the applicant, Schuster, 2017b:

Reference	**K-MA 8.2.4/01 Schuster 2017b.** AbioProtect® and its components PepjMV-Abp1 and PepMV-Abp2: Toxicity to the freshwater aquatic plant *Lemna gibba* (Duckweed) under Laboratory Conditions, (Shuster, 2017b) (Unpublished report). Study code: S17-03475.
Guideline	OECD 221 (2006)
GLP	The study was conducted according to GLP principles/regulations. Certified laboratory.
Objectives	The aim of this study was to determine the effects of AbioProtect® and its components PepMV-Abp1 and PepMV-Abp2 on the growth of the *Lemna gibba* and to determine the no observed effect concentration (NOEC), where possible.
Material and methods	
Test material	Six replicates per treatment. Number of assays 1. AbioProtect®, batch number: L-AB01-160517 (PepMV-Abp1 batch L-12-060217-ABP-1-C and PepMV-Abp2 batch L-12-060217-ABP2-C). Control item: tomato watery leaf extracts non-infected with PepMV batch L12-060217.
Test species	freshwater aquatic plant *Lemna gibba G3* (Duckweed)
Number of tested plants	
Treatments	100mg/L test item; 100mg/L plant extract control; control
Duration	7 days
Test conditions	pH= 7.40-7.42 ; Temperature =23.3-24.0 ºC; light intensity 7380 lux.
Evaluation	- Biology assessment. - Physical assessment. - Growth rate and dry weight of frond numbers. - Yield rate and dry weight of frond number. - Doubling time of frond numbers.
Deviations from guideline	None

Results and conclusion

The doubling time of frond numbers in the control should be less than 2.5 days (< 60 hours). The test is valid as the doubling time of frond numbers in the control was 2.03 days (corresponding to 48.7 hours).
No statistically significant inhibitory effects on any parameter (growth rate, and yield) for fronds and dry weight were observed at 100 mg/L (nominal) test item concentration at test end.
No observations of any morphological differences between fronds of the exposures compared to fronds of the control were made at any day of assessment.

No statistically significant inhibitory effects on any parameter (growth rate and yield) for fronds and dry weight were observed at 100 mg/L (nominal) test item concentration at test end. Thus the overall LOEC (Lowest Observed Effect Concentration) was not determinable and the overall NOEC was observed to be at 100 mg/L (nominal) corresponding to $1.5x10^7$ PepMV genome copies/L ($2.99x10^6$ genome copies of PepMV-Abp1/L plus $1.2x10^7$ genome copies of PepMV-Abp2/L).

Therefore, according to the result of the present study there is no effect of AbioProtect® or its components, PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, on plants other than algae.

Complete report of the study is included in Document K-MA 8.2.4-01 (Schuster, 2017b).

RMS added:
The applicant says that morphology of the plants were observed at test end and the plants were considered normal for all treatment groups. It is not clear if there were no change in plant development, frond size, necrosis and any additional observations of test media or other abnormalities. There is no data photos or references criteria to sustain the affirmation. Nevertheless, the plant yield and growth rate were normal.

The applicant doesn´t confirm the presence of Abp1 and Abp2 at the end of the studio or in plant (by molecular hybridization), that for, there is no confirmation of virus infectivity or pathogenicity, even there is no symptoms.
RMS suggest to include a negative control or reference substance in the assay for the inhibition growth of the algae. It would be also convenient to have a second confirmed experiment.
The evaluation contains Abioprotect product. There is no information of Abp1 and abp2 MPCAs separately.
Despite the missing information, the specific study on toxicity to the duckweed plant under laboratory conditions (Document Report K-MA 8.2.4-02) does confirm there is no virulence or transmission of the virus to aquatic plants.
The risk from the representative use of Pepino mosaic virus strains EU isolate Abp1 and CH2, isolate Abp2 to algae is low.
The risk from the representative use of Pepino mosaic virus EU strains Abp1 isolate and CH2 strain, Abp2 isolate to aquatic plants is low.

B.9.2.5 Summary of the studies on aquatic organisms toxicity, infectiveness and pathogenicity

RMS-recommendations:
The RMS considers this acceptable since Abp1 and Abp2 have a specific hosts restricted to aerial plants, range mainly the Solanaceae family. No effects on aquatic plants is expected.
Since the product is a plant virus that is applied in tomato cultures in greenhouses the release of Pepino mosaic virus strains Abp1 and Abp2 to the environment through water and soil is limited.

The low risk effect of the MPCAs virus PepMV strains Abp1 and Abp2 on aquatic plants was confirmed in two specific GLP studies; on toxicity to the Single Cell Green Alga Pseudokirchneriella subcapitata Hindák (Document Report K-MA 8.2.3-01) and on Toxicity to the freshwater aquatic plant Lemna gibba green (Document Report K-MA 8.2.4-01).

The no risk from the use of PepMV viruses EU strains Abp1 isolate and CH2 strain, Abp2 isolate to aquatic plants is clear.

- **MPP AbioProtect® has no relevant effect on green algae either in freshwater aquatic plants.**

B.9.3 EFFECTS ON BEES

Bumble bees are used for pollination in tomato crops. Several authors have studied the role of bumblebees in PepMV transmission. Lacasa *et al.*, 2003 found that bumblebees could disperse PepMV. Later other three publications show that bumblebees can transmit PepMV (Shipp *et al.,* 2008; Stobbs *et al.*, 2009; Stobbs and Greig, 2014); however, a specific PepMV-bumblebee vector relation does not appear to exist. These publications do not mention any adverse effects on bumblebees. No reports on negative effects of PepMV on bumblebees were found.

PepMV belongs to the order Tymovirales that include plant viruses only. PepMV is very efficiently transmitted mechanically in tomato by standard crop handling through contaminated tools, hands and clothing and by direct plant-to-plant contact (Spence *et al.,* 2006; Van der Vlugt, 2009; Wright and Mumford, 1999). Plant viruses enter cells only through wounds made mechanically or by vectors or by deposition into an ovule by an infected pollen grain (Agrios, 2005).

Plant pathogenic viruses are generally considered to be pathogenic towards plant species only and not towards other organisms, like insects. Insect exposure to plant pathogenic viruses is enormous and insect diseases caused by plant pathogenic viruses are unknown. However, some vectors (mainly insect species) appear to be affected by plant viruses. Most hits on insects in the literature search (Document K-MA 5.2.5, Hernando, 2017) describe specific vector-plant virus relations in which behaviour or development of vector species is affected by the plant virus, in order to optimize spread of the virus to other plants. One plant-infecting virus tomato spotted wilt virus (TSWV), belonging to genus Tospovirus within the family *Bunyaviridae* of ambisense ssRNA viruses (that mainly contains animal pathogenic viruses), which is transmitted by *Frankliniella occidentalis* is known to cause a mild infection on its main insect vector (De Medeiros *et al*, 2005). Contrary to TSWV, PepMV belongs to a family of plant pathogenic viruses only. It is therefore concluded that potential risk of PepMV towards bees is negligible.
Li *et al.* (2014) reported that a plant virus, tobacco ring spot virus (TRSV) belonging to the virus family of *Secoviridae* (positive-sense ssRNA viruses, order Picornavirales), replicates in honey bees and that the prevalence of this virus was high in weak colonies. However this work has been criticized as lacking conclusive evidence (Miller

et al., 2014) and further research is required as a negative impact of the virus on colony survival was suggested, but not demonstrated yet.

Besides, the risk to bees of the use of PepMV (mild isolates VX1 and VC1) in greenhouses was concluded as low by the EFSA[3,4,5].

Therefore, it can be concluded that the potential risk of PepMV in general and PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 in particular towards bees is negligible.

B.9.3.1 Toxicity to bees

Not relevant. Please refer to B9.

RMS added:
There were no effects observed in the available toxicity studies on earthworms. However, the available studies are not designed to distinguish from toxicity to honeybees. Even so, it is reasonable to assume that Ab1 and Abp2 are not toxic to bees.

B.9.3.2 Infectiveness to bees

RMS added:
There were no effects observed in the available Infectiveness studies on bees. Bumblebees can transmit the virus mechanically (Lacasa et al., 2003; Shipp et al., 2008; Stobbs et al., 2009), however the main risk from bumble bees is associated with spread within an infected greenhouse or within a dense tomato production area, not with large-distance spread. Hence, it is reasonable to assume that Ab1 and Abp2 are not infective to bees.

B.9.3.3 Pathogenicity to bees

RMS added:
There were no effects observed in the pathogenicity studies on bees. However, the available studies are not designed to distinguish infectiveness from pathogenicity to honeybees. Hence, it is reasonable to assume that Ab1 and Abp2 are not pathogenic to bees.

B.9.3.4 Summary and risk assessment to bees

RMS added:
Some information was available on bumble bees from a literature study where bumble bees were used as vectoring agent. This study indicated no visible effects on bumble bee colonies after two weeks foraging on treated crops. The exposure of the bees was proven by isolating the virus from worker bees. Based on experimental collusions base on B2.5. (Stobbs and Greig, 2014).

- **MPP AbioProtect® has no relevant effect on bees.**

B.9.4 EFFECTS ON ARTHROPODS OTHER THAN BEES (OECD IIM 8.8, IIIM 10.4)

The focused search on scientific peer review literature (Document K-MA 5.2.5, Hernando, 2017) found a summary record studding the possibility that PepMV could be transmitted by whiteflies without reporting any negative effect on the insect (Noël *et al.,* 2014).

B.9.4.1 Toxicity to arthropods other than bees

RMS added:

A summary of the available studies on non-target arthropods other than bees is presented in Vol 2section B.2.5. Infectiveness, dispersal and colonization ability**.** There were no effects observed in the available toxicity studies. Hence, it is reasonable to assume that Abp1 and Abp2 are not toxic to arthropods other than bees.

B.9.4.2 Infectiveness to arthropods other than bees

RMS added:
It is also reported in literature (not attached documents) that can be transmitted by aphis e.g. Myzus persicae, (Noël et al., 2015). Transmission of PepMv by flywhite seems to be low according to Noël et al., 2014. Two experiments were conducted to investigate the transmission of the PepMV y the greenhouse whitefly (Trialeurodes vaporariorum) from tomato to tomato. The results confirmed the low transmission role of flywhite, showed that the number of PepMV particles carried on whitefly bodies was low, with an average occurrence of 1.33 on the 55 whiteflies tested after the insects were in contact with infected plants for 5 days. This low occurrence was confirmed by observation under microscope, which showed an absence of PepMV-contaminated tomato sap on the insect bodies, A summary of the available studies on non-target arthropods other than bees is presented in Vol 2section B.2.5. Infectiveness, dispersal and colonization ability, suggesting that PepMV transmission by whiteflies could occur when they feed on the plant.

B.9.4.3 Pathogenicity to arthropods other than bees

RMS added*:*
There were no effects observed in the available pathogenicity studies. Hence, it is reasonable to assume that Abp1 and Abp2 are not pathogen to arthropods other than bees.

B.9.4.4 Summary and risk assessment for non-target arthropod species other than bees

RMS added*:*
A summary of an available study on non-target arthropods other than bees is presented in Vol 3 section 2.9.4. refers to Whitefly transsmission, Noël et al., 2014.
A data gap was set for the assessment of the Abp1 and Abp2 on non-target arthropod. Nevertheless, based on the information provided bellow, additional studies are considered not required.

- **MPP AbioProtect® has no relevant effect on non-target arthropod species other than bees**

B.9.5 EFFECTS ON EARTHWORMS (OECD IIM 8.9, IIIM 10.5)

Not relevant. Please refer to B9.

B.9.5.1 Toxicity to earthworms

RMS added*:*
There were no effects observed in the available toxicity studies on earthworms. Hence, it is reasonable to assume that Ab1 and Abp2 are not toxic to earthworms.

B.9.5.2 Infectiveness to earthworms

RMS added*:*

There were no effects observed in the available Infectiveness studies on earthworms. Hence, it is reasonable to assume that Ab1 and Abp2 are not infective to earthworms.

B.9.5.3 Pathogenicity to earthworms

RMS added*:*
There were no effects observed in the available pathogenicity studies on earthworms. Hence, it is reasonable to assume that Ab1 and Abp2 are not pathogenic to earthworms.

B.9.5.4 Summary and risk assessment to earthworms

RMS added*:*
A data gap was set for the assessment of the toxicity, infectiveness and pathogenicity of soil macrofauna. No requirement needed because of mode of action and biological characteristics of the microorganism Apb1 and Abp2.

- **MPP AbioProtect® has no relevant effect on earthworms**

B.9.6 EFFECTS ON NON-TARGET SOIL MICRO-ORGANISMS (OECD IIM 8.10, IIIM 10.6)

Not relevant please refer to MA 9.

RMS added*:*
The focused search on scientific peer review literature (Document K-MA 5.2.5, Hernando, 2017) found a summary record studying the possibility that PepMV could be transmitted by the soil fungus Olpidium virulentus (Alfaro-Fernández et al., 2010) without reporting any negative effect on the soil fungus.
The focused search on scientific peer review literature (Document K-MA 5.2.5, Hernando, 2017) found a summary record studying the possibility that PepMV could be transmitted by the soil fungus Olpidium virulentus (Alfaro-Fernández et al., 2010) without reporting any negative effect on the soil fungus.

Reference:	Alfaro-Fernández *et al.*, 2010. Transmission of Pepino mosaic virus by the Fungal Vector *Olpidium virulentus.*
Report No.:	Journal of Phytopathology 158- 4.
Guideline:	Not applicable
GLP:	Not applicable
Abstract	Transmission of Pepino mosaic virus (PepMV) by the fungal vector *Olpidium virulentus* was studied in two experiments. Two characterized cultures of the fungus were used as stock cultures for the assay: culture A was from lettuce roots collected in Castellón (Spain), and culture B was from tomato roots collected in Murcia (Spain). These fungal cultures were maintained in their original host and irrigated with sterile water. The drainage water collected from irrigating these stock cultures was used for watering PepMV - infected and noninfected tomato plants to constitute the acquisition–source plants of the assay, which were divided into six different plots: plants containing fungal culture A (non - infected and PepMV - infected); plants containing fungal culture B (noninfected and PepMV - infected); PepMV - infected plants without the fungus; and plants non - infected either with PepMV and the fungus. Thirty - six healthy plants grouped into six plots, which constituted the virus acquisition–transmission plants of the assay, were irrigated with different drainage waters obtained by watering the different plots of the acquisition–source plants. PepMV was only transmitted to plants irrigated with the drainage water collected from PepMVinfected plants whose roots contained the fungal culture B from tomato with a transmission rate of 8%. No infection was detected in plants irrigated with the drainage water collected from plots with only a fungus or virus infection. Both the virus and fungus were detected in water samples collected from the drainage water of the acquisition–source plants of the assay.

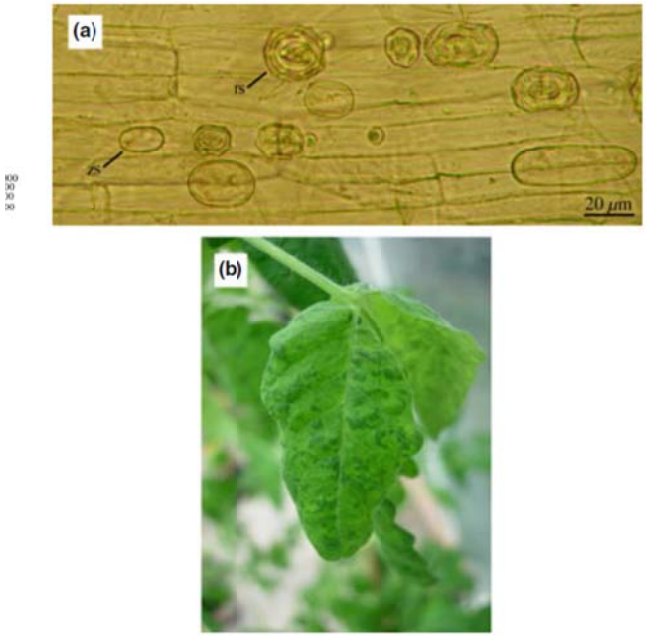

Figure B9.6-1. Transmission of PepMV by *Olpidium virulentus*. (a) Stellate resting spores characteristic of O. *brassicae sl*, and zoosporangia. (b) The typical green mosaic and bubbling symptoms on the leaves of one plant associated with PepMV infection 1 month after the beginning of the inoculative irrigation with the drainage water of the P0 plants (Alfaro-Fernández *et al.*, 2010).

	Expt. 1					Expt. 2					
	PepMV[b]			*Olpidium* spp		PepMV[b]			*Olpidium* sp.		
				Microscopic observation					Microscopic observation		
Plot[a]	Symptoms observation	DAS-ELISA	RT-PCR	Presence	Monitoring[c]	Symptoms observation	DAS-ELISA	RT-PCR	Presence	Monitoring[c]	PCR[d]
AP_1	0/6	0/6	0/6	6/6	rs = 2, zs = 1	0/6	0/6	0/6	6/6	rs = 2, zs = 2	6/6 (*O. vir*)
An_1	0/6	0/6	0/6	6/6	rs = 2, zs = 1	0/6	0/6	0/6	6/6	rs = 2, zs = 2	6/6 (*O. vir*)
BP_1	1/6	1/6	1/6	6/6	rs = 3, zs = 2	0/6	0/6	1/6	6/6	rs = 2, zs = 2	6/6 (*O. vir*)
Bn_1	0/6	0/6	0/6	6/6	rs = 2, zs = 2	0/6	0/6	0/6	6/6	rs = 2, zs = 2	6/6 (*O. vir*)
HP_1	0/6	0/6	0/6	0/6	rs = 0, zs = 0	0/6	0/6	0/6	0/6	rs = 0, zs = 0	0/6
Hn_1	0/6	0/6	0/6	0/6	rs = 0, zs = 0	0/6	0/6	0/6	0/6	rs = 0, zs = 0	0/6

[a]Plants P1 (acquisition–transmission plants) which were grouped into different plots: AP1, acquisition–transmission plants irrigated with the drainage water collected from the irrigation of AP0, which were infected with both *O. virulentus* culture A and PepMV; An1, acquisition–transmission plants irrigated with the drainage water collected from the irrigation of An0, which were infected only with *O. virulentus* culture A; BP1, acquisition–transmission plants irrigated with the drainage water collected from the irrigation of BP0, which were infected with both O. virulentus culture B and PepMV; Bn1, acquisition–transmission plants irrigated with the drainage water collected from the irrigation o Bn0, which were infected only with O. virulentus culture B; HP1, acquisition–transmission plants irrigated with the drainage water collected from the irrigation of HP0, which were infected only with PepMV; Hn1, acquisition–transmission plants irrigated with the drainage water collected from the irrigation of Hn0, which were free of O. virulentus and PepMV infection.
[b]Number of positive plants/total number of plants analyzed.
[c]Monitoring of *Olpidium* spp. structures, resting spores (rs) or zoosporangia (zs), present in the roots of the plants by light microscopic observation following the scale: no fungal structure = 0; range 0–100 rs, zs = 1; range 101–1000 rs, zs = 2; more than 1001 rs, zs = 3.
[d]O. vir, resulted in the amplicon that correspond to O. virulentus (579 bp).

Table B9.6-1 Transmission of PepMV by *Olpidium virulentus*. Results of the analysis performed with the acquisition–transmission plants (P1) to detect the possible transmission of PepMV and to confirm the presence of *O. virulentus* (Alfaro-Fernández *et al.*, 2010).

RMS comments and conclusions:
The root-infecting parasitic fungus O. virulentus can facilitate PepMV transmission (Alfaro-Fernández et al., 2010). These transmission assays (table B9.6-1) demonstrated the possibility of PepMV transmission by O. virulentus collected from tomato crops. PepMV was only transmitted to plants irrigated with the drainage water collected from PepMV-infected plants whose roots contained the fungal culture (Figure B9.6-1) from tomato with a transmission rate of 8%. No infection was detected in plants irrigated with the drainage water collected from plots with only a

fungus or virus infection. Both the virus and fungus were detected in water samples collected from the drainage water of the acquisition–source plants of the assay (Figure B9.6-1). These transmission assays demonstrated the possibility of PepMV transmission by O. virulentus collected from tomato crops.

RMS comments and conclusion
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. Hence the RMS does not consider additional studies on soil microflora necessary.

- **MPP AbioProtect® has no relevant effect on soil non-target microbiota.**

B.9.7 REFERENCES RELIED ON

The applicant has provided summaries and results of the scientific peer-review open literature, on the active substance and its relevant metabolites dealing with side-effects on health, the environment and non-target species and published within the last 10 years before the date of submission of the dossier. There is no information whether this literature search was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009".

The literature search provided was conducted in accordance to the guidelines set up in document European Food Safety Authority; Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (OJ L 309, 24.11.2009, p.1-50), (EFSA Journal 2011; 9(2):2092. [49pp.]. doi:10.2903/j.efsa.2011.209)2. Full details and justification of how the literature search was performed could be found in Document K-MA 5.2.5 Hernando 2017.

References list ordered by data point

Data point	Author(s)	Year	Title Source (where different from company) Company, Report Number GLP or GEP status Published or not	Data Protection claimed Y/N	Justification if data protection is claimed	Owner
MA 9 MA 8.3	Spence N.J., Basham J., Mumford R.A., Hayman G., Edmondson R., Jones D.R.	2006	Effect of *Pepino mosaic virus* on the yield and quality of glasshouse-grown tomatoes in the UK. Plant Pathology 55:595-606. DOI: 10.1111/j.1365-3059.2006.01406.x. No GLP Published	N		
MA 9 MA 8.3	Van der Vlugt R.	2009	*Pepino mosaic virus*. Hellenic Plant Protection Journal 2:47-56 No GLP Published	N		
MA 8 MA 8.3	Wright D., Mumford R.	1999	*Pepino mosaic Potexvirus* (PepMV): first records in tomato in the United Kingdom Central Science Laboratory. No GLP Published	N		
MA 9 MA 8.3	Agrios	2005	Plant diseases caused by viruses. Plant Pathology. Fifth Edition. Chapter 14, pp 722-820 (pp731) Elsevier Academia Press. No GLP Published	N		
MA 9 MA 9.2.1 MA 9.2.2 MA 9.2.3 MA 8.3 MA 8.4 MA 8.6	Hernando Y. **K-MA 5.2-5**	2017	Focused search of scientific peer review literature for *Pepino mosaic virus*. CEBAS-CSIC, Spain No GLP Not published			
MA 9	Colson P., Richet	2010	*Pepper mild mottle virus*, a plant virus associated	N		

Data point	Author(s)	Year	Title Source (where different from company) Company, Report Number GLP or GEP status Published or not	Data Protection claimed Y/N	Justification if data protection is claimed	Owner
	H., Desnues C., Balique F., Moal V., Grob J.-J., Berbis P., Lecoq H., Harlé J.-R., Berland Y		with specific immune responses, fever, abdominal pains, and pruritus in humans. PloS one 5:e10041. No GLP Published			
MA 9	Zhang T., Breitbart M., Lee W.H., Run J.-Q., Wei C.L., Soh S.W.L., Hibberd M.L., Liu E.T., Rohwer F., Ruan Y.	2006	RNA viral community in human feces: prevalence of plant pathogenic viruses. PLoS biology 4:e3. No GLP Published	N		
MA 9	Liu R., Vaishnav R.A., Roberts A.M., Friedland R.P.	2013	Humans have antibodies against a plant virus: evidence from *Tobacco mosaic virus*. PloS one 8:e60621. No GLP Published	N		
MA 9.2	Žegura B., Novak M., Kogovšek P. **K-MA5.2.4-02**	2017	Infectivity and replication of *Pepino mosaic virus* (PepMV, EU, strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) in human alveolar epithelial cells type 2 A549. Department of genetic toxicology and cancer biology. National Institute of Biology. Slovenia. Report number: 10G003-2017 GLP like protocols Not published	Y	Proprietary information	Abiopep S.L.
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			Not published			
MA 9.2.3	Schuster K-MA 8.2.3-01	2017a	AbioProtect® and its components PepMV-Abp1 and PepMV-Abp2: Toxicity to the single cell green alga Pseudokirchneriella subcapitata Hindák under laboratory conditions. Eurofins Agroscience Services EcoChem, Germany. Report number: S17-03474 GLP Not published	Y	Proprietary information	Abiopep S.L.
MA 8.2.4	Schuster K-MA 8.2.4-01	2017b	AbioProtect® and its components PepMV-Abp1 and PepMV-Abp2: Toxicity to the duckweed Lemna gibba under laboratory conditions (Acute Test – Static). Eurofins Agroscience Services EcoChem, Germany. Report number: S17-03475 GLP Not published	Y	Proprietary information	Abiopep S.L.
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