European Commission

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

Pepino Mosaic Virus, EU strain, mild isolate Abp1
Pepino Mosaic Virus, CH2 strain, mild isolate Abp2
Active organism data

Volume 3 – Annex B.1 Identity

Rapporteur Member State: Spain

July 2019

Version History

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

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B.1. IDENTITY OF THE MICROORGANISM

B.1.1. APPLICANT

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B.1.2. PRODUCER

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B.1.3. NAME AND SPECIES DESCRIPTION, STRAIN CHARACTERIZATION

B.1.3.1 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.

B.1.3.2 Scientific name and taxonomic grouping, i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the microorganism.

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

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.

B.1.3.3 Test procedures and criteria used for identification at strain level

The microorganism (PepMV) is well characterised at the molecular level. PepMV forms flexuous rod-like virus particles (508 nm long) which contain a ss(+)RNA genome of about 6.4 kb (6,410nt). The PepMV genome encodes five proteins: a protein involved in virus replication (RdRp); three proteins involved in cell-to-cell movement, encoded by overlapping genes organized into a triple gene block (proteins TGB1, TGB2, and TGB3); and the coat protein (CP) (Gómez et al., (2009).

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.

To unambiguously identify PepMV, EU strain, mild isolate Abp1 the identity of this fragment to the reference sequence for Abp1 has to be above 99.5 %.

To unambiguously identify PepMV, CH2 strain, mild isolate Abp2 the identity of this fragment to the reference sequence for Abp2 has to be above 99.5 %.

There are also other good technologies for identification of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 including molecular hybridisation, reverse transcription (RT) and polymerase chain reaction (PCR), real time (quantitative) one-step RT-PCR (RT-qPCR) and by a bioassay.

Further details are reported in confidential information, Volume 4, Annex C.

B.1.3.4 Common name or alternative and superseded names and code names used during the development.

There is no common name for the organism.

B.1.3.5 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.

B.1.4 SPECIFICATION OF THE MATERIAL USED FOR MANUFACTURING OF FORMULATED PRODUCTS

B.1.4.1 Content of the microorganism

As PepMV is a plant virus, which can only replicate in living plant cells, it can only be produced in plants. Tomato (Solanum lycopersicum) is the most suitable host for PepMV, so production of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, is done in tomato plants. Large-scale purification of virus particles from tomato leaves is not feasible because of (1) the very time-consuming procedures that have only been developed for diagnostic and research purposes on a laboratory scale, (2) the required use of chemicals, (3) the relatively low efficiency of methods and (4) the negative impact of purification on the viability and stability of the virus.

Although each MPCA is the virus particles, it is produced in tomato, therefore the MPCA is contained in a technical concentrate (TK) of watery extracts of tomato leaves, with a high content of particles of PepMV, EU strain, mild isolate Abp1 (MPCA technical 1) or of PepMV, CH2 strain, mild isolate Abp2 (MPCA technical 2).

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®).

Inert ingredients consist of tomato leaves extracts and water.

RMS Assessment

The applicant must set a minimum value for the content of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, isolate Abp2 in both MPCA technical (see details in confidential Annex C for both isolates).

B.1.4.2 Identity and content of impurities, additives, contaminating microorganisms

The whole set of tomato leaves harvested from a production cycle (batch) of each MPCA are processed by homogenization in water to obtain the corresponding MPCA technical. The whole production process is done with much care under controlled conditions to guarantee absence of contaminating microorganisms; controls are implemented to exclude the presence of any potential harmful microorganism.

During production plants are randomly sampled (1:50) from every production cycle of each MPCA, and analysed by molecular hybridisation with DIG-labelled RNA specific probes for each isolate, PepMV, EU strain, mild isolate Abp1, and PepMV, CH2 strain, mild isolate Abp2. Hybridization is conducted with standard molecular biology techniques, with the proper controls and under conditions to avoid false positives and ambiguous results (details in Agüero, 2017a).

The content of potential contaminating microorganisms is determined in every batch of each MPCA by RT-qPCR. To do so, nucleic acids are extracted from every batch of each MPCA technical, and besides using them to quantify the amount of PepMV genome copies/µL are used to analyse the presence of extraneous microorganisms, including the presence of contaminants potentially pathogen for tomato, such as bacteria and fungi, as well as viruses and viroids.

The tests for pathogenic bacteria and fungi are conducted by an external contractor, which has set up the procedure to test the presence/absence and semi-quantification of 36 phytopathogenic bacteria and fungi. If any one of these phytopathogenic fungi or bacteria were identified in a batch at a potentially dangerous concentration, the whole batch would be discarded (details in Agüero, 2017a and de Gea, 2017).

Also, the presence/absence of 14 viruses and 4 viroids potentially infecting tomato is analysed in every batch of each MPCA technical, the nucleic acids extracted are used in a molecular hybridization analyses with a RNA polyprobe designed to detect those viruses and viroids. If any batch of each MPCA technical is positive for any of the 14 viruses and 4 viroids potentially infecting tomato such batch is discarded (details in Agüero, 2017a).

Furthermore, every batch of the formulation containing each MPCA is tested for human pathogens by certified methods according to UNE-EN ISO/IEC 17025:2005, with the limits indicated in data point 1.4.3 (Analytical profile of batches). An external contractor conducts the tests. The results of testing five representative batches of MPCP showed no detectable levels of human pathogens (Salmonella, listeria, Escherichia coli, faecal coliform or aerobic count) (details Inglés, 2017). If any of these human pathogens were detected in any batch such batch would be destroyed.

The end product AbioProtect® (MPCP) was determined to be formulated with PepMV, EU strain, mild isolate Abp1 (MPCA technical 1) and PepMV, CH2 strain, mild isolate Abp2 (MPCA technical 2), and did not contain any other microorganism (details in confidential part)

Neither the MPCP nor any of the MPCA have chemical impurities that are relevant for human health and/or the environment, and there are not any additives in their formulation.

The content of PepMV, EU strain, mild isolate Abp1 in the MPCA technical 1 is at least $2.5x10^{12}$ genome copies/L and could be up to $3.21x10^{13}$ genome copies/L.

The content of PepMV, CH2 strain, mild isolate Abp2 in the MPCA technical 2 is at least $2.5x10^{12}$ genome copies/L and could be up to $7.68x10^{13}$ genome copies/L.

B.1.4.3 Analytical profile of batches

The content of five representative batches of recent production is included and summarized in confidential part.

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 according to the reference standard UNE-EN ISO/IEC 17025:2005:

- 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).

B.1.5 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.

Data point	**Author(s)**	**Year**	**Title** **Company Report No** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study** **Y/N**	**Data Protec-tion Claimed** **Y/N**	**Justification if data protection is claimed**	**Owner***
B.1.3.1	Bussas V.	2015a	Receipt of deposit Nº DSM 32069 of microorganism PepMV-Abp1. Budapest treaty on the international recognition of the deposit if microorganisms for the purposes of patent procedures. DSMZ, Germany. Report number: DSM 32069 GLP: no Unpublished	N	N	Proprietary information	Abiopep S.L.
B.1.3.1	Bussas V.	2015b	Receipt of deposit Nº DSM 32070 of microorganism PepMV-Abp2. Budapest treaty on the international recognition of the deposit if microorganisms for the purposes of patent procedures. DSMZ, Germany. Report Number: DSM 32070 GLP: no Unpublished	N	N	Proprietary information	Abiopep S.L.
B.1.3.2	Jones R.A.C., Koenig R., Lesemann D.	1980	Pepino mosaic virus, a new potexvirus from pepino (Solanum muricatum). Annals of Applied Biology 94:61-68. GLP: no Published	N	N		LIT
B.1.3.3	Gómez P., Sempere R., Elena S.F., Aranda M.A.	2009	Mixed infections of Pepino mosaic virus strains modulate the evolutionary dynamics of this emergent virus. Journal of Virology 83:12378-12387. GLP: no Published	N	N		LIT

Data point	Author(s)	Year	Title Company Report No Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Data Protec-tion Claimed Y/N	Justification if data protection is claimed	Owner*
B.1.3.4 B.1.4.1 B.1.4.2	Agüero J.	2017a	Method of production of the Microbial Pest Control Agents (MPCAs) PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 and manufacturing of the Microbial Pest Control Product (MPCP) AbioProtect®. Abiopep S.L., Spain. GLP: No Unpublished	N	N	Proprietary information	Abiopep S.L.
B.1.3.6	Aguilar J., Hernandez-Gallardo M., Cenis J., Lacasa A., Aranda M.	2002	Complete sequence of the Pepino mosaic virus RNA genome. Archives of virology 147:2009-2015. GLP: no Published	N	N		LIT
B.1.4.3	Aranda, M.	2016b	Method for the absolute quantification of PepMV genome copies by real time one-step RT-PCR in watery extracts of tomato plants. CEBAS-CSIC, Spain. GLP: No Published	N	N	Proprietary information	Abiopep S.L.
B.1.3.6	Cotillon A.C., Girard M., Ducouret S.	2002	Complete nucleotide sequence of the genomic RNA of a French isolate of Pepino mosaic virus (PepMV). Archives of Virology 147:2231-2238. DOI: 10.1007/s00705-002-0873-8. GLP: No Published	N	N		LIT
B.1.4.2	Baños M.	2016	Physico-Chemical characterization of technical Abp1 and Abp2 and formulation AbioProtect® Laboratorios Munuera S.L., Spain. Report number: 16-4951-01 GLP : Yes Unpublished	N	Y	Proprietary information	Abiopep S.L.
B.1.4.2	de Gea A.	2017	Analyses of phytopathogenic fungi and bacteria in tomato plant extract (Solanum lycopersicum) from the active substances, PepMV-Abp1 and PepMV-Abp2, of the Plant Protection Product AbiopProtect®. Report number : IP-17-2223/1; IP-17-2224/1; IP-15-2225/1; IP-17-2226/1; IP-17-2227/1 and IP-17-2228/1. Microgaia Biotech S.L., Spain. GLP: No Unpublished	N	N	Proprietary information	Abiopep S.L.

Data point	**Author(s)**	**Year**	**Title** **Company Report No** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study** **Y/N**	**Data Protec-tion Claimed** **Y/N**	**Justification if data protection is claimed**	**Owner***
B.1.4.3	Inglés M.J.	2017	Reports of research on Salmonella, Listeria, Escherichia coli, faecal coliform or aerobic count of 5 batches of Abioprotect®. Report number: M-17-0306/1; M-17-0307/1; IP-17-0308/1; IP-17-0309/1 and IP-17-0310/1. Microgaia Biotech S.L. GLP: No Unpublished	N	N	Proprietary information	Abiopep S.L.

*LIT: LITERATURE