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.5 Analytical methods

Rapporteur Member State: Spain

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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

Table of contents

B.5. ANALYTICAL METHODS

B.5.1 METHODS FOR THE ANALYSIS OF THE MICROORGANISM AS MANUFACTURED

Methods for the identification of the micro-organism

Four main *Pepino mosaic virus* (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% (see ref C.5 in Vol 4). More recently Moreno-Pérez et al. (2014) reported the occurrence in wild tomatoes of isolates belonging to a new PepMV genotype, not yet reported in domestic tomato and named the South Peruvian genotype (PES) (Moreno-Pérez et al., 2014).

The EU and LP genotypes have a nucleotide sequence identity of 96 % and cluster phylogenetically. The CH2 genotype is more distant and has a nucleotide sequence identity of 78.47 % with the EU genotype, of 78.45 % with the LP and of around 77.8 % with the PES genotype. The US1 genotype has a sequence identity of 78.34 % with the CH2 genotype and of 81.89 % with the EU genotype. Table B.5.1.1/01 shows the nucleotide identity between the main strains of PepMV.

Table B.5.1.1/01. Nucleotide identity (%) between the complete genome sequences of isolates of reference from the main strains of PepMV.

Accesion No.	Strain	LP	US1	PES	EU
DQ000985.1	**CH2**	78,45	78,34	77,82	78,47
AJ606361.1	**LP**		81,85	81,71	96,14
AY509926.1	**US1**			86,09	81,89
HG313805.1	**PES**				81,61
FJ940223.1	**EU**				
Source of isolates of reference: CH2 (Ling, 2007), LP (ref C.6 in Vol 4), US1 (Maroon-Lango et al., 2005), PES (Moreno-Pérez et al., 2014), EU (Van Der Vlugt et al., 2002).					

Several molecular assays (molecular hybridization, ELISA, conventional RT-PCR, RT-PCR followed by RFLP, and real time quantitative RT-qPCR) have been developed to discriminate between those different strains or genotypes. Abiopep uses a combination of two scientifically validated assays to identify the microorganism and discriminate between PepMV genotypes or strains. Further details about these analytical methods are described in confidential information, Volume 4, Annex C.

Methods for providing information on possible variability of seed stock/ active microorganism.

Every production cycle is started with the material lyophilized and characterized (from both PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) limiting the risk of variability of the microorganism. However, since the active microorganisms are ssRNA virus, and RNA viruses are known to have a relatively high mutation rate, several adequate methods are in place to assay the possible variability of the seed stock material Abiopep uses a scientifically validated method for such checks (details in confidential information, Volume 4, Annex C).

Methods to differentiate a mutant of the micro-organism from the parent wild strain

A mutant can be discriminated from the parent by determination of the nucleotide sequence of part of the genome (see confidential information, Volume 4, Annex C for details).

Methods to establish the purity of seed stock from which batches are produced and methods to control purity.

PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 are kept in lyophilized material. This material is revived to produce the original pure inoculum for each production cycle. Scientifically validated methods are used to establish and control purity of seed stock. Only the material that complies with the identity control criteria is stored as seed stock and used for production (described in detail confidential information, Volume 4, Annex C).

Methods to determine the content of the microorganism in the manufactured material used for production of plant protection product and methods to show that contaminating microorganisms are controlled to an acceptable level.

Quantification of the amount of PepMV, EU strain, mild isolate Abp1 and PepMV CH2 strain, mild isolate Abp2 is done with adequate scientifically validated methodology (details in confidential information, Volume 4, Annex C).

Plant viruses are not related to any animal or human pathogen. Plant viruses are harmless to humans and other animals because they only reproduce in plant living cells. The production of every batch of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 is done with much care, and the presence of human pathogens is mostly unlikely, nonetheless the presence of human pathogens in the product is regularly checked with validated methods as described in B.5.1.7. More details in confidential information, Volume 4, Annex C.

Methods for the determination of relevant impurities in the manufactured material.

PepMV as other plant viruses is not related to any animal or human pathogen. Plant viruses are harmless to humans and other animals because they only reproduce in plant living cells. They do not have a cellular structure and do not produce metabolites; they could be only produced in plant living cells. PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 are produced in tomato plants.

During the production cycle, the presence of microorganisms potentially harmful for tomato is determined with scientifically validated methods as described in confidential information, Volume 4, Annex C. If any potentially harmful microorganism for tomato is identified in enough quantities to generate an infection the whole batch is destroyed.

Due to the manufacturing method, any other relevant impurities are not expected, as the other ingredients consist of tomato leaves and water.

Methods to control the absence and to quantify the possible presence of any mammalian pathogen.

To control the absence of any human and mammalian pathogens samples are taken from every production batch and analyzed by certified and validated methods according to the reference standard UNE-EN ISO/IEC 17025:2005. An external contractor conducts this test. The criteria used in these controls are:

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

If any batch is found not to fulfil these requirements such batch is destroyed.

The methods to control the absence of any human and mammalian pathogens used do not interfere with methods for analysis of pathogens in drinking water.
More details in confidential information, Volume 4, Annex C.

Methods to determine storage stability, shelf-life of the microorganism, if appropriate

The storage stability and shelf life could be determined experimentally after storage of the microorganisms and subsequent evaluation as detailed in confidential information, Volume 4, Annex C.

B.5.2 METHODS TO DETERMINE AND QUANTIFIED RESIDUES (VIABLE OR NON-VIABLE)

B.5.2.1 The active microorganism(s)

Residue analytical methods for PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 in food, or feed or in environmental matrices like water, air and soil are consider not necessary since:

- The virus is already naturally present on tomato plants in greenhouses today.

- The virus does not multiply outside its plant host. The virus only survives short periods outside the host cell since it is broken down by proteases, RNAses and UV light. However, some authors have reported that it can persist in dried plant sap 4 weeks at 5 ºC, 2 weeks at 15ºC and only 4 days at 25ºC (O'Neill et al., 2003). PepMV does not remain infectious in water at 20ºC more than 3 weeks (Mehle et al., 2014).

- Persistence in water has been evaluated in a GEP trial and found that PepMV was not persistent in the leachate from the tomato plants treated with PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. Therefore, there is no risk of infection from this leachate (see Document K-MP 6.2/04, Prats, 2017a).

- The persistence in soil has been experimentally evaluated in a GEP trial and found the PepMV was not persistent in the soil from tomato plants treated with PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. Therefore, there is no risk of infection from this soil to subsequent crops (see Document K-MA 6.2/05, Prats, 2017b).

B.5.2.2 Relevant metabolites (especially toxins)

Viruses have no metabolism of their own and are therefore not able to produce secondary metabolites. Because of the above reasons, the risk to consumers is negligible and determination and quantification of the residues and metabolites is considered not relevant in this case.

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	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
B.5.1.1	Ling K.S.	2007	Molecular characterization of two *Pepino mosaic virus* variants from imported tomato seed reveals high levels of sequence identity between Chilean and US isolates. Virus Genes 34. DOI: 10.1007/s11262-006-0003-x. No GLP Published	N	N		LIT*
B.5.1.1	Maroon-Lango C.J., Guaragna M.A., Jordan R.L., Hammond J., Bandla M., Marquardt S.K.	2005	Two unique US isolates of *Pepino mosaic virus* from a limited source of pooled tomato tissue are distinct from a third (European-like) US isolate. Archives of Virology 150:1187-1201. DOI: 10.1007/s00705-005-0495-z. No GLP Published	N	N		LIT
B.5.1.1	Moreno-Pérez M.G., Pagán I., Aragón-Caballero L., Cáceres F., Fraile A., García-Arenal F.	2014	Ecological and genetic determinants of *Pepino mosaic virus* emergence. Journal of virology 88:3359-3368. No GLP Published	N	N		LIT
B.5.1.1	Van Der Vlugt R.A.A., Cuperus C., Vink J., Stijger I.C.M.M., Lesemann D.E., Verhoeven J.T.J., Roenhorst J.W.	2002	Identification and characterization of Pepino mosaic potexvirus in tomato. EPPO Bulletin 32:503-508. DOI:10.1046/j.1365-2338.2002.00598.x. No GLP Published	N	N		LIT
B.5.2.1	Mehle N., Gutiérrez-	2014	Survival and transmission of *Potato virus Y*, *Pepino mosaic*	N	N		LIT

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
	Aguirre I., Prezelj N., Delić D., Vidic U., Ravnikar M.		*virus*, and Potato Spindle Tuber Viroid in Water. Applied and Environmental Microbiology 80:1455-1462. DOI: 10.1128/aem.03349-13. Not GLP Published				
B.5.2.1	O'Neil T., Spence N., Mumford R., Skelton A.	2003	Final Report on project PC 181: Protected tomato: sources, survival and disinfection of *Pepino mosaic virus* (PepMV), ADAS/CSL, UK. No GLP Published	N	N		LIT
B.5.2.1	Prats C.	2017a	Field study to evaluate the crop safety and the efficacy of the Plant Protection Product (PPP) AbioProtect®, and its components or agents (PPA1 and PPA2), for the control of PepMV in tomato crop (Southern Spain, 2016). Agrocolor S.L., Spain Report Number ACEX/1274/AB GEP Not published	N	Y	Proprietary information	Abiopep S.L.
B.5.2.1	Prats C.	2017b	Field study to evaluate the crop safety and the efficacy of the Plant Protection Product (PPP) AbioProtect®, and its components or agents (PPA1 and PPA2), for the control of PepMV in tomato crop (Southeast Spain, 2016). Agrocolor S.L., Spain Report number: ACEX/1277/AB GEP Not published	N	Y	Proprietary information	Abiopep S.L.

*LIT: LITERATURE