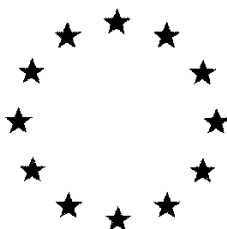


European Commission



**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
Product data: AbioProtect®
Volume 3 – Annex B.2
Physical, chemical and technical properties**

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.2. PHYSICAL AND CHEMICAL PROPERTIES OF THE PLANT PROTECTION PRODUCT SCORE 250 EC

Test or Study & Data point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
B.2.1. APPEARANCE						
Physical state, colour and odour B.2.1	Visual	AbioProtect®: (L-AB02-220716) PepMV, EU strain, mild isolate Abp1 L-2.3-240516-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 L2.3-240516-ABP2-C	Clear solution with green particles in suspension, greenish yellow colour and characteristic odour before and after 7 days at 0°C.	Acceptable	Y	Baños, 2016
B.2.2. STORAGE STABILITY AND SHELF LIFE						
Storage stability and shelf life B.2.2.1		AbioProtect®: (L-AB03-240217) (PepMV, EU strain, mild isolate Abp1 L-8-151116-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 L-8-151116-ABP2-C). PepMV, EU strain, mild isolate App1 (L-2.8-240616-Abp1-C and L-2.9-250616-Abp1-C) and PepMV, CH2 strain isolate Abp2	<p>Stable and with a shelf life of up to 35 days when stored at – 18°C and at 4 °C.</p> <p>Stable and with a shelf life of up to 1 day when stored at 20 °C.</p> <p>Stable and with a shelf life of 9 months when each MPCAs is stored at <–18 °C and defrost slowly previous to MPCP formulation.</p> <p>Product must be stored at <-18°C.</p> <p>There is no effect of air or other factors in stability.</p>	<p>Biological stability after 35days storage at -18°C was done, by detecting the presence of Abp1 and Abp2 in tomato seedlings. Plant infectivity. It is acceptable</p> <p>No information regarding the technical properties after storage up to 35 days at 4°C or -18°C (shelf life claimed by the applicant) has been reported. The RMS has considered these data are not necessary due to the specific application</p>	N	Agüero, 2017c

Test or Study & Data point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
		(L-2.8-240616-Abp2-C and L-2.9-250616-Abp2-C). AbioProtect® (L-AB04-240217 and L-AB05-240217)		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 ±2°C, kept refrigerated at 4-7°C 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		
B.2.2.2 Effect of low temperatures on stability		AbioProtect®: (L-AB02-220716) PepMV, EU strain, mild isolate Abp1 L-2.3-240516-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 L2.3-240516-ABP2-C	<0.1 % After 7 days at 0°C (% retained in 75 nm sieve) n*= 2	Not Acceptable. Suspensibility tested at the highest and lowest recommended rates of use must be performed after storage.	Y	Baños, 2016
B.2.3. EXPLOSIVITY AND OXIDISING PROPERTIES						
Explosivity B.2.3.1			Not tested, however based on its composition the formulation is not considered explosive.	Acceptable		
Oxidising properties B.2.3.2			Not tested, however based on its composition the formulation is not considered oxidizing	Acceptable		

Test or Study & Data point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
B.2.4. FLASH POINT AND OTHER INDICATIONS OF FLAMMABILITY OR SPONTANEOUS IGNITION						
Flash point, flammability, spontaneous ignition B.2.4			Not tested, however based on its composition the formulation is not considered flammable.	Acceptable		
B.2.5. ACIDITY, ALKALINITY AND IF NECESSARY PH VALUE						
Acidity, alkalinity and pH value B.2.5	CIPAC MT 75.3	AbioProtect®: (L-AB02-220716) PepMV, EU strain, mild isolate Abp1 L-2.3-240516-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 L2.3-240516-ABP2-C	5.89±0.03 (23°C) pH direct n*= 2 5.82± 0.10 (1%v/v dilution) (22.7°C) n*= 2	Acceptable	Y	Baños, 2016
B.2.6 VISCOSITY AND SURFACE TENSION						
Dynamic viscosity B.2.6.1	OECD 114 CIPAC MT 192	AbioProtect®: (L-AB02-220716) PepMV, EU strain, mild isolate Abp1 L-2.3-240516-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 L2.3-240516-ABP2-C	5.03-5.70 mPa s (mPa s at 20 ±0.2°C) n*= 2 4.20-4.77 mPa s (mPa s at 40 ±0.2°C) n*= 2	Acceptable	Y	Baños, 2016
Density B.2.6.2	CIPAC MT 3.3.2	AbioProtect®: (L-AB02-220716) PepMV, EU strain,	0.9986± 0.0002 Density at 20 °C (g/mL) n*= 2	Acceptable	Y	Baños, 2016

Test or Study & Data point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
		mild isolate Abp1 L-2.3-240516-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 L2.3-240516-ABP2-C				
B.2.7. TECHNICAL CHARACTERISTICS OF THE PLANT PROTECTION PRODUCT						
Wettability B.2.7.1			Not applicable the formulation is not a solid preparation.	Acceptable		
Persistent foaming B.2.7.2	CIPAC MT 47.2	AbioProtect®: (L-AB02-220716) PepMV, EU strain, mild isolate Abp1 L-2.3-240516-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 L2.3-240516-ABP2-C	0 mL after 10 seconds (max. volume in mL, at 5 % with Standard water D) n*= 2	Acceptable.	Y	Baños, 2016
Suspensibility and suspension stability B.2.7.3	CIPAC MT 184 (similar to MT 161) CIPAC MT 160	AbioProtect®: (L-AB02-220716) PepMV, EU strain, mild isolate Abp1 L-2.3-240516-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 L2.3-240516-ABP2-C	110.82±0.02 gravimetric in standard water D, at 30 °C and 5 %) n*= 2 110.85±0.02(gravimetric in standard water C, at 30 °C and 5 %) n*= 2	Not Acceptable. Suspensibility must be tested also at the lowest recommended rate of use after storage.	Y	Baños, 2016
Wet sieve test B.2.7.4	CIPAC MT 185 (equivalent to MT 167)	AbioProtect®: (L-AB02-220716) PepMV, EU strain, mild isolate Abp1 L-	<0.1 % (% retained in 75 nm sieve) n*= 2		Y	Baños, 2016

Test or Study & Data point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
		2.3-240516-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 L2.3-240516-ABP2-C		Acceptable.		
Particle size distribution, content of dust/fines, attrition and friability B.2.7.5			Not applicable, the formulation is not a dustable or wettable powder nor granules	Acceptable.		
Emulsifiability, re-emulsifiability, emulsion stability B.2.7.6			Not applicable, the formulation is not an emulsion and does not form emulsions	Acceptable.		
Flowability, pourability and dustability B.2.7.7	CIPAC MT 148.1	AbioProtect®: (L-AB02-220716) PepMV, EU strain, mild isolate Abp1 L-2.3-240516-ABP1-C and PepMV, CH2 strain, mild isolate Abp2 L2.3-240516-ABP2-C	Pourability 0.282±0.005 (% Formulation remaining in test cylinder) n*= 2	Acceptable.	Y	Baños, 2016

Test or Study & Data point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
B.2.8. PHYSICAL, CHEMICAL AND BIOLOGICAL COMPATIBILITY WITH OTHER PRODUCTS INCLUDING PLANT PROTECTION PRODUCTS WITH WHICH ITS USE IS TO BE AUTHORIZED						
<p>The formulation AbioProtect® is only applied by Abiopep own properly trained and qualified personnel, it is not sold to be applied by farmers or other professionals and it is applied without mixing with any other product.</p> <p>AbioProtect® contains equivalent amounts of PepMV, EU strain, mild isolate Abp1 (with a minimum content of at least 2.5×10^{11} genome copies/L) and PepMV, CH2 strain, mild isolate Abp2 (with a minimum content of at least 2.5×10^{11} genome copies/L). As PepMV is a plant virus, which can only replicate in living plant cells, it can only be produced in plants. Tomato (<i>Solanum lycopersicum</i>) 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.</p> <p>The formulation is a suspension concentrate of watery extracts of tomato leaves, containing a high content of particles of PepMV, EU strain, mild isolate Abp1 and of PepMV, CH2 strain, mild isolate Abp2. Those tomato leaves are harvested and homogenized at a concentration of 320 g of fresh leaves /L of distilled water, the amount of each isolate of PepMV in the tomato leaves batch is determined by a scientifically validated absolute quantification by real time RT-PCR (adapted from ref C2 in Vol 4) as described in Aranda, 2016b, and diluted to the application dose to guarantee a minimum content of at least 2.5×10^{11} genome copies of PepMV, EU strain, mild isolate Abp1/L, and of at least 2.5×10^{11} genome copies of PepMV, CH2 strain, mild isolate Abp2/L in the final formulation.</p>						
Physical compatibility B.2.8.1			The formulation AbioProtect® is applied alone without any tank mixes therefore the physical compatibility is considered not relevant.	Acceptable.		
Chemical compatibility B.2.8.2			The formulation AbioProtect® is applied alone without any tank mixes therefore the chemical compatibility is considered not relevant.	Acceptable.		
Biological compatibility B.2.8.3			The formulation AbioProtect® is applied alone without any tank mixes therefore the biological compatibility is considered not relevant.	Acceptable.		
B.2.9 ADHERENCE AND DISTRIBUTION TO SEEDS						
Adherence and distribution to seeds B.2.9			Not applicable, the formulation is not a preparation for seed treatment.	Acceptable.		

Test or Study & Data point	Guideline and method	Test material purity and specification	Used methods / Results	Comments (Acceptable / Non acceptable)	GLP	Reference
B.2.10 SUMMARY AND EVALUATION OF DATA PRESENTED UNDER POINTS 2.1 TO 2.9						
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 characteristics 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 °C ± 0.2, and of 4.20-4.77 mPa s at 40 °C±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 (details in Document K-MP 2.1/01, Baños, 2016).						
When stored at room temperature this biological plant protection product is only stable for just one day. When stored at <- 18°C is stable for 9 months with an estimated shelf life of 9 months (Agüero, 2017c).						

RMS Assessment:

The method to evaluate biological stability of the active substances is adequate. Stability and shelf life were tested after 35 days by inoculating mechanically tomato seedlings, with AbioProtect® stored at -18°C in water. Evaluation of the infectivity by detecting the presence of the mild isolates Abp1 and Abp2 was done 13 dpi by molecular hybridization with digoxigenin (DIG)-labelled RNA specific probes.

Classical storage stability studies (accelerated and at room temperature) are not possible and irrelevant (instability of the virus). Due to the heat sensitive nature of the active substance (virus), the storage stability was not performed at 54°C but at the following temperatures: -18°C, +4°C and 20°C. Data indicate that AbioProtect® remains viable (the test plants are infected) up to 35 days, and the product keeps the same appearance upon storage at -18 and 4°C. Upon storage at 20 °C, the test product remains viable (the test plants are infected) up to 1 day.

The other technical characteristics for a SC formulation have not been performed at -18°C or 4°C up to 35 days. These data are required in order to indicate that no particular problems are to be expected when it is used as recommended. To confirm this formulation type, pH, suspensibility, spontaneity of dispersion, pourability and wet sieve residue were deemed the most critical parameters and are required after storage according to the FAO manual (Manual on development and use of FAO and WHO specifications for pesticides, First edition, third revision, March 2016). Furthermore, a surface tension test must be performed.

Only determination of colour, odour, appearance and a wet sieve test have been performed before and after storage at 0°C for one week and are acceptable. Suspensibility was not performed. AbioProtect® is only applied by qualified Abiopep personnel, and it is always formulated the same day of application, and after formulation it is kept at 4°C. It has to be highlighted that the product is generally intended to be formulated on demand, and therefore directly used (storage at 4°C for 35 days being exceptional).

No information regarding the technical properties after storage up to 35 days at 4°C or -18°C (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 $4 \pm 2^{\circ}\text{C}$, kept refrigerated at $4\text{--}7^{\circ}\text{C}$ 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.

Although the storage stability study conducted has shown that MPCP could be stored up to 35 days at -18°C , it is recommended for use on the same day of formulation and therefore testing technical properties after storage at -18°C for 35 days should not be considered relevant in this case.

B.2.2. 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	Year	Title Doc. No., (prev. used Doc. No.), (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.2.1 B.2.2.2 B.2.5 B.2.6 B.2.7	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 Not published	N	Y	Proprietary information	Abiopep S.L.
B.2.2.1	Agüero J.	2017c	Study to evaluate the storage stability and shelf life of the Microbial Pest Control Product AbioProtect® and its components Pepino mosaic virus (PepMV), EU strain, mild isolate App1 and PepMV, CH2 strain, mild isolate Abp2. Abiopep S.L., Spain. Report number: ABP04/2017 No GLP Not published	N	N	Proprietary information	Abiopep S.L.
B.2.7	Arteseros A., Martínez M	2017	Analysis FQ three different batches of tomato plant watery extract containing two strains, PepMV-Abp1 (European genotype) and PepMV-Abp2 MV (Chilean genotype) Laboratorios IDUQC ALMABE, Spain. Report number: AP42800 No GLP Not published	N	N	Proprietary information	Abiopep S.L.
B.2.8	Aranda M.A.	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. No GLP Not published	N	N	Proprietary information	Abiopep S.L.