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.6

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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INTRODUCTION

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

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

The cross-protection effect and thus the actual activity is obtained by infection of the plants with the mild isolates of the virus: PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. Viral cross-protection in plants is known as an acquired immunity phenomenon, where a mild virus isolate can protect plants against economic damage caused by a severe challenge isolate of the same virus. The mode of action of cross-protection has been explained in a relatively complete general manner by a model based on a combination of RNA silencing and coat-protein-mediated resistance. Mild isolates will induce in tomato crop a symptomless infection without damage to the fruit, while an aggressive isolate will induce symptoms leading to economic losses in the crop.

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

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

Gap table: Details of all national gaps within each zone

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

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

1	2	3	4	5	7	8	9	10	11	12	13	14
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)	**Application** Method Kind (f-g)	Timing/ Growth stage of crop & season (h)	Max number (min interval between applications) a) per use b) per crop/ season	**Application rate per treatment** 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.6. EFFECTS ON HUMAN HEALTH

B.6.1. BASIC ACUTE TOXICITY STUDIES

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

B.6.1.1. Acute oral toxicity

Reference	[redacted], 2017a
Study	Evaluation of the acute oral toxicity of the test item AbioProtect® (and its components Abp1 and Abp2) in female Sprague-Dawley rats by the acute toxic class method (OECD nº 423) Unpublished report B-02315
Guidelines	OECD guideline Nº 423 (Adopted 17th December 2001)
Deviations	No
GLP	Yes
Acceptability	Yes

Materials and Methods

Test substance	AbioProtect® batch number L-AB01-311016: Abp1 (EU genotype) batch number L-7-311016-ABP1-C and Abp2 (CH2 genotype) batch number L-7-311016-ABP2-C. Number of viral copies/µL: 6.32 x 10^6 (1.70 x 10^6 of Abp1 and 4.62 x 10^6 of Abp2) The test item is a solution freshly prepared from harvested infected tomato (*Solanum lycopersicum*) leaves, at a concentration of 200 mg/mL of the infected plant material in water. Those tomato leaves are infected with naturally occurring mild isolates of Pepino mosaic virus (PepMV) Abp1 and Abp2.
Test animals	3 female Sprague-Dawley rats per dose level.
Method	Limit test, since available information of the test item suggested that mortality was unlikely at the highest starting dose level (2000 mg/kg body weight). No formulation was needed since the test item was supplied ready to use at a concentration of 200 mg (vegetal mass)/mL in sterile water. This method was based on a stepwise procedure with the use of 3 animals of a single sex per dose level. Accordingly, for each step, a total of 3 female rats were administered orally with the test item AbioProtect® (Abp1 and Abp2). Animals were fasted overnight prior to dosing (only food was withheld, not water), and for 3-4 hours after test item administration. The starting dose was 2000 mg/kg and the administration volume for oral gavage was 10 mL/kg. Clinical observations in response to treatment were performed 30 minutes, 1h, 2h, and 4h post-administration and once daily thereafter during the 14-day observation period.

Results and Conclusion

A. Results

No test item-related mortality was recorded on study day 4, 72 hours after treatment, in animals from dose step 1 (group A). An additional group of 3 animals were orally administered with the same dose (group B - dose step 2) in order to confirm the results of dose step 1. Since test item neither caused mortality nor toxic signs in either of the groups administered with a dose of 2000 mg/kg bw (p.o.) no further steps were needed.

Animals were observed daily for a period of 14 days for mortality and clinical signs. Body weight was also recorded on a weekly basis during the observation period. After the 14-day observation period, animals were sacrificed and subjected to gross necropsy.

During the observation period, neither test item-related mortality nor toxic signs were recorded in animals from dose step 1 and 2 treated with a dose of 2000 mg/kg bw (p.o.).

After test item administration, none of the animals from dose step 1 and 2 showed weight loss during the study period. According to values provided by the animal supplier, the increment of body weight was within the expected range for animals of this strain and sex. No other clinical signs were observed in any of the remaining animals from dose step 1 and 2 treated with 2000 mg/kg bw (p.o.) (group A and B).

All the animals from group A and B were sacrificed 14 days after test item administration (study day 15 and 18, respectively) and a gross necropsy was performed on all animals. Necropsies did not reveal any relevant finding or morphological change in the evaluated tissues or organs.

B. Conclusion

It can be concluded that, according to the results obtained in this study and under the assayed experimental conditions, the test item AbioProtect® (and its components PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) could be considered Unclassified according to Regulation 1272/2008 criteria, although the active substance is a microorganism and therefore the criteria for chemicals classification does not apply.

Under the conditions of the study the acute oral lethal dose (LD_{50}) of the test item was found to be higher than 2000 mg/kg of body weight in female Sprague-Dawley rats.

LD_{50} oral rat > 2000 mg/kg bw => $6.32x10^{10}$ genome copies of PepMV/kg bw.

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

RMS comments and conclusion

The study is considered acceptable for the evaluation of acute oral toxicity. This study was also presented for the evaluation of the oral acute toxicity of the active substances.

B.6.1.2. Acute inhalation toxicity

Reference	[redacted] 2017
Study	Acute inhalation toxicity of test item AbioProtect® (and its components Abp1 and Abp2) in Sprague Dawley rats: OECD Nº403. Unpublished report B-02317.
Guidelines	OECD guideline Nº 403 (Adopted 7th September 2009)
Deviations	No
GLP	Yes
Acceptability	Yes

Materials and Methods

Test substance	AbioProtect® batch number L-AB02-241116: Abp1 (EU genotype) batch number L-9-241116-ABP1-C and Abp2 (CH2 genotype) batch number L-9-241116-ABP2-C. Number of viral copies/µL: 1.35 x 10^7 (5.84 x 10^6 of Abp1 and 7.72 x 10^6 of Abp2) The test item is a solution freshly prepared from harvested infected tomato (*Solanum lycopersicum*) leaves, at a concentration of 200 mg/mL of the infected plant material in water. Those tomato leaves are infected with naturally occurring mild isolates of Pepino mosaic virus (PepMV) Abp1 and Abp2.

Test animals	3 female and 3 male Sprague-Dawley rats.
Method	Limit test: Traditional protocol A group of 3 male and 3 female Sprague Dawley rats was exposed by nose-only, flow-past inhalation to AbioProtect® (and its components Abp1 and Abp2) at a mean concentration of 5.02 mg/L air for 4 hours. This concentration was found to be the limit concentration for aerosol according to the limit test of the guideline OECD Nº 403. During an observation period of 14 days following the end of exposure, clinical observations and body weight were recorded in order to characterise the toxicological effects of the aerosol. Body weight was recorded just before starting exposure (study day 1), on study days 2, 4, 8 and immediately before sacrifice and gross necropsy on study day 15 during which descriptions of all macroscopic abnormalities were recorded.

Results and Conclusion

A. Results

Geometric Standard Deviation (GSD) on one of the particle size distribution determinations was 4.55 which is above the target range (1.5 to 3). Nevertheless, this value was considered to be acceptable taking into account that more than 56 % of particles were below upper limit of 4µm. Hence, the particle size distributions obtained were considered to be respirable to rats and appropriate for acute inhalation toxicity testing. The ranges of aerosol concentration, temperature, relative humidity and airflow rate were considered satisfactory for a study of this type.

No mortality was recorded during the study period.

The main clinical signs observed after finishing exposure were chromorrhinorrhea, chromodacryorrhea, soiled coat, piloerection and breathing difficulty. All these signs were transient and most of them were not present the day after exposure. From study day 3 to the end of the 14 day observation period the animals exhibited a normal behaviour and no clinical signs related test item exposure were recorded with the exception of an isolated nasal discharge in one female animal.

A transient and marginal body weight loss of approximately 4.5 % in males and 1 % in females was observed in the majority of animals from exposure day to day 2 of the study. Thereafter, body weight increased gradually in all animals except for one female in which a body weight decrease of ~6 % was observed until study day 8. Mean body weight gains over the 14 day observation period of approximately 17 % and 5 % were recorded for males and two out of three females respectively.

Upon terminal necropsy, red lungs were observed in one male animal and a red spot in the left lung was observed in another male animal. In addition, red enlarged mandibular lymph nodes were presented in all animals. These findings were considered to be related to test item exposure.

B. Conclusion

It is concluded that, under these experimental conditions, the LC_{50} of AbioProtect® (and its components PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) was greater than 5.02 mg/L air (gravimetric aerosol concentration) and it can be considered not classified, based on the CLP (Regulation 1272/2008) classification criteria, although classification criteria for chemicals does not apply since it is a microorganism.

LC50 > 5.02 mg/L air

RMS comments and conclusion

The study is considered acceptable for evaluation of acute inhalation toxicity. This study was also presented for the evaluation of the acute inhalation toxicity of the active substance.

B.6.1.3. Acute percutaneous toxicity

Reference	[redacted] 2017b
Study	Evaluation of the acute dermal toxicity of the test item AbioProtect® (and its components Abp1 and Abp2) in female and male Sprague-Dawley rats (OECD nº 402) Unpublished report B-02316.
Guidelines	OECD guideline Nº 402 (*RMS comment: The study was performed before the update of the guideline which was adopted October 9, 2017*)
Deviations	No
GLP	Yes
Acceptability	Yes

Materials and Methods

Test substance	AbioProtect® batch number L-AB01-311016: Abp1 (EU genotype) batch number L-7-311016-ABP1-C and Abp2 (CH2 genotype) batch number L-7-311016-ABP2-C. Number of viral copies/µL: 6.32 x 10^6 (1.70 x 10^6 of Abp1 and 4.62 x 10^6 of Abp2) The test item is a solution freshly prepared from harvested infected tomato (*Solanum lycopersicum*) leaves, at a concentration of 200 mg/mL of the infected plant material in water. Those tomato leaves are infected with naturally occurring mild isolates of Pepino mosaic virus (PepMV) Abp1 and Abp2.
Test animals	14 Sprague-Dawley rats (5 males and 5 females; plus 3 females and 1 male in a second application round)
Method	A limit test at one dose level of 2000 mg/kg bw was carried out in a group of 5 male and 5 female animals. Approximately 24 hours before the test, fur was removed from the dorsal area of the trunk of the animals by shaving (no less than 10% of the body surface area was cleared for application of the test substance). Animals from both sexes were administered topically on the back skin with a dose of 2000 mg/kg of test item AbioProtect® (Abp1 and Abp2) for 24 hours in order to determine the toxic effects and mortality rates. On study day 15, a 2nd round of 4 rats [(n=3 females and 1 male were topically administered with the same dose of test item (2000 mg/kg) for a 24-hour period. Animals were monitored for clinical signs and mortality once daily during the 14-day observation period. All surviving animals were sacrificed 14 days after test item administration and a gross necropsy was performed on all animals.

Results and Conclusion

A. Results

During the observation period, neither test item-related mortality nor severe toxic signs were recorded in animals from experimental group A (1st and 2nd round, ID1 to ID14) administered topically with a dose of 2000 mg/kg.

Animals ID3, ID8 and ID9 showed occasional chromorrhinorrhea (abnormal porphyrin secretion) at several time-points throughout the observation period. Animal ID3 and ID9 presented with transient chromorrhinorrhea at the end of the observation period, on study day 14 and 15, respectively. This abnormal porphyrin secretion was also observed in animal ID8 on study days 9, 10, 12, 13 and 14.

No other clinical signs were observed in any of the remaining animals treated with 2000 mg/kg of test item.

After test item administration, none of the animals from group A (1st round, ID1 to ID10) showed weight loss during the study period. According to values provided by the animal supplier, the increment of body weight was within the expected range for animals of this strain and sex. Animals from the 2nd round (ID11 to ID14) did not

show the expected weekly weight gain for animals of this strain and sex, during the first week after test item administration. While weekly body weight gain was normalized in animals from the 2nd round during the second week of the 14-day observation period.

All the animals from group A (1st and 2nd round) were sacrificed 14 days after test item administration (study day 15 and 29, respectively) and a gross necropsy was performed on all animals. Necropsies did not reveal any relevant finding or morphological change in the evaluated tissues or organs.

B. Conclusion

It can be concluded that, according to the results obtained in this study and under the assayed experimental conditions, the dermal LD_{50} for the test item AbioProtect® (and its components PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Apb2) is established to be > 2000 mg/kg body weight.

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

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

RMS comments and conclusion

The study is considered acceptable for evaluation of acute percutaneous toxicity.

B.6.2. ADDITIONAL ACUTE TOXICITY STUDIES

B.6.2.1. Skin irritation

In the acute dermal toxicity studies on the formulation (B.6.1.3) signs of erythema and oedema were assessed. No erythema and oedema were observed. On the basis of these studies, the test material does not warrant classification as being a skin irritant.

RMS comments and conclusions

The skin irritation study has not been performed, based on the results of the acute dermal toxicity study and because the preparation does not include co-formulants therefore a test is not required.

B.6.2.2. Eye irritation

The formulation AbioProtect® is formulated without co-formulants, the microorganisms contained in it are not eye irritant and severe effects on the eyes are not likely. However, to avoid any risk of potential eye sensitisation the use of protective eye equipment might be recommended.

RMS comments and conclusions

The applicant's conclusion is considered acceptable.

B.6.2.3. Skin sensitisation

According to Regulation (EC) 283/2013, all microorganisms should be regarded as potential sensitisers thus the formulation AbioProtect® should be regarded as potential sensitiser. The following warning phrase is applicable "Microorganisms may have the potential to provoke sensitising reactions".

RMS comments and conclusions

The formulation does not contain co-formulants so a skin sensitisation study is not required but contains microorganisms so it is considered as potential sensitiser. The applicant's conclusion is considered acceptable.

B.6.3. DATA ON EXPOSURE

Clinical cases and poisoning incidents did not occur in the laboratories and facilities of the applicant. There are no indications for a toxic potential regarding to the information of the medical record of the employees involved in the manufacture of AbioProtect® and its components PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 (Cabezas, 2017).

The available information on the mammalian toxicity of the formulation AbioProtect® and its components, indicates that the use of AbioProtect® in the manner proposed presents no systemic hazard for operators or others who may handle treated plants and an exposure assessment is not required.

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

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

RMS comments and conclusions
The applicant's conclusion is considered acceptable.

B.6.4. AVAILABLE TOXICOLOGICAL DATA RELATING TO NON-ACTIVE SUBSTANCES

The preparation AbioProtect® is formulated as a suspension concentrate of tomato plants extract infected with equivalent amounts of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. The formulation does not contain any other non-active substances.

Other inert ingredients are tomato plant extract and water.

Nicotine (Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-) has been reported to be present in plants of the *Solanaceae* family, including tomato (Sheen 1988, Siegmund et al. 1999). Sheen (1988) reported nicotine content of 2.31 ppm in tomato fruits, 2.82 ppm in tomato leaves and 2.77 ppm in tomato stem. However the author indicates that as the determinations were done in a moisture free basis, nicotine content in a fresh weight basis will only be a fraction of 1 ppm. He also suggested that the nicotine presence in tomato might have a biosynthetic origin.

More recently (Siegmund et al. 1999) analysed the presence of nicotine in different products of plants from the *Solanaceae* family, reporting a content of 2-4 µg/kg (2-4 x 10^{-3} ppm) in fresh tomato fruits. These authors calculated the dietary nicotine intake due to the consumption of potatoes, fresh tomatoes, tomato paste, aubergines and tea to be of 1.4 µg/day (of those 0.089 µg/day could be due to the consumption of fresh tomatoes).

Fresh tomato fruits are largely consumed by humans, according to FAO, Europeans consume 31 kg of tomatoes per year, while global per capita consumption is 20 kg per year (http://www.agribenchmark.org/agri-benchmark/did-you-know/einzelansicht/artikel// tomatoes-are.html), as indicated above; a dietary nicotine intake due to consumption of products from plants of the Solanaceae family of 1.4 µg/day has being calculated (Siegmund et al. 1999).

Considering all the information indicated above, the use of AbioProtect® and its components (PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2), as a plant protection product in a single application on the seedlings (BBCH 13-15) is not expected to affect the level of natural occurrence of nicotine in tomato fruits nor the level of exposure of humans to nicotine. Nonetheless, the nicotine content in PepMV, EU strain, mild isolate Abp1 and in PepMV, CH2 strain, mild isolate Abp2 has been determined as indicated below.

Nicotine content

Nicotine content in five independent batches of PepMV, EU strain, mild isolate Abp1 and of PepMV, CH2 strain, mild isolate Abp2 were determined by a validated analysis method by high performance liquid chromatography coupled with triple quadrupole mass spectrometry (HPLC/QqQ) with a quantification limit of 0.003 mg/kg by an external independent laboratory as reported by Veiga et al (2018). The nicotine content of the five independent batches of PepMV, EU strain, mild isolate Abp1 and of PepMV, CH2 strain, mild isolate Abp2 are summarised in **Table B.6.4.1**. The results show that there is very low content of nicotine in the active

substances (PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2), and as AbioProtect® is a suspension concentrate (SC) formulated with equivalent amounts of each of the active substances, it has a very low content of nicotine.

Table B.6.4.1. Nicotine content in five independent batches of PepMV, EU strain, mild isolate Abp1 and of PepMV, CH2 strain, mild isolate Abp2

Test or study	Validated method	Test Material	Finding	Reference
Nicotine	HPLC-QqQ	PepMV, EU strain, mild isolate Abp1, batches:	Mean: 0.027mg/L	Pineda, 2018a,b, c,d,e
		L-5-041017-Abp1-C	0.011 mg/kg	
		L-6-111017-Abp1-C	0.013 mg/kg	
		L-7-251017-Abp1-C	< 0.003 mg/kg	
		L-8-081117-Abp1-C	0.005 mg/kg	
		L-9-221117-Abp1-C	0.102 mg/kg	
Nicotine	HPLC-QqQ	PepMV, CH2 strain, mild isolate Abp2, batches:	Mean: 0.050 mg/L	Pineda, 2018f,g,h,i, j
		L-5-041017-Abp2C	0.063 mg/kg	
		L-6-111017-Abp2C	0.057 mg/kg	
		L-7-251017-Abp2-C	0.111 mg/kg	
		L-8-081117-Abp2-C	0.012 mg/kg	
		L-9-221117-Abp2-C	0.007 mg/kg	

According to the results presented in **Table B.6.4.1**, the impurity nicotine is present in the active substance PepMV, EU strain, mild isolate Abp1 at very low levels, ranging from < 0.003 mg/kg to 0.102 mg/kg, with a mean value of 0.027 mg/kg as its density is 0.9979 g/mL (Baños 2016), the mean content of nicotine in the five batches of this active substance is 0.027 mg/L. In the case of the active substance PepMV, CH2 strain, mild isolate Abp2 the impurity nicotine is present at very low levels, ranging from 0.007 mg/kg to 0.111 mg/kg with a mean value of 0.050 mg/kg, as its density is 1.0007 g/mL (Baños 2016), the mean content of nicotine in the five batches of the active substance analysed is of 0.050 mg/L. Therefore it could be concluded that the impurity nicotine is present at very low levels in the active substances (PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) or the formulation AbioProtect®.

Exposure assessment

Dermal absorption

According to the EFSA Guidance on Dermal absorption, 2017, since no studies have been performed, the default dermal absorption values will be established. These dermal absorption values are: 10% for the concentrate and 50% for the diluted product since the preparation is a suspension concentrate.

Operator exposure

To further guaranty that the use of AbioProtect® in the manner proposed presents no systemic hazard for operators or others who may handle the product an exposure assessment on nicotine present in the formulation has being conducted.

The maximum nicotine content in the five batches of PepMV, EU strain, mild isolate Abp1 analysed is 0.102 mg/L with a mean content of 0.027 mg/L. While the maximum nicotine content in the five batches of PepMV, CH2 strain, mild isolate Abp2 analysed is 0.111 mg/L, with a mean content of 0.05 mg/L. AbioProtect® is formulated with equivalent amounts of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, the maximum application rate is 1.6 L of AbioProtect® (0.8 L of each active substance) in a final volume of 8 L, which leads to a maximum of 0.1704 mg of nicotine /ha (0.1065 mg/L), in the worst possible scenario. Therefore an exposure assessment is conducted based on this data. Usage information pertinent to operator exposure is summarized in **Table B.6.4.2**.

Table B.6.4.2: AbioProtect® user information pertinent for operator exposure

	Application rate (product/ha)	Maximum potential (nicotine kg/ha)	Spray dilution	Application equipment
Tomato	1.6 L/ha	1.704×10^{-7}	8 L	Hand sprayer

Nicotine has not been approved as an active substance in Europe (SANCO/2686/08 – rev. 0, 8 August 2008). EFSA published a statement on the toxicological assessment of nicotine in 2010 (EFSA Journal 2010; 8(10): 1835 (www.efsa.europa.eu/en/efsajournal/doc/1835.pdf)). In its statement, EFSA proposed an LOAEL of 3.5 µg/kg b.w for nicotine obtained from Lindgren et al (1999). Based on this LOAEL of 3.5 µg/kg b.w., EFSA established an acute reference dose (ARfD) of 0.0008 mg/kg body weight.

Operator exposure assessment has been performed considering the following parameters:

-Dermal absorption values (10% (concentrate) and 50% (diluted), values by default according EFSA Guidance, 2012).

-Using AOEM for mixing/loading and EUROPOEM II database for application in greenhouses scenario.

The area treated was established at 1 ha/day, at the maximum dose of 1.6 L/ha in a spray volume of 4 L/ha during 8 h, as "worst case". Operator exposure values were compared to the ARfD of 0.8 µg/kg b.w. In the greenhouse there is inhalation exposure in addition to exposure by dermal route, while outdoors it is usually negligible. Results are summarised in **Table B.6.4.3**

Table B.6.4.3. Estimated operator exposure to nicotine during mixing and loading, AOEM model

Model	Method of application	Crop	Active substance	Dose (L pf/ha)	Volume (L/ha)	PPE		% AOEL
						Mixing/ loading	Application	
AOEM model for mixing & loading and EUROPOEM II database for application 1 ha/day 60 kg operator	Hand held application in greenhouse to low crops	Tomato	Nicotine	1,6	4	Chemical protective gloves and suitable chemical protective coverall type 6	Suitable chemical protective coverall type 6	**62.13**
	Hand held application in greenhouse to high crops							**62.24**

According to AOEM model for mixing and loading and EUROPOEM II database for application, the exposure estimate using the product AbioProtect® by handheld equipment in greenhouse to low and high crops are acceptable with the use of chemical protective gloves and workwear during mixing/loading and workwear during application.

Conclusion operator exposure

There are no detectable risks to operators arising from the proposed use of AbioProtect®.

RMS comments and conclusions

The exposure of operators to nicotine during mixing and loading has been included by the RMS. The assessment of the exposure to operators is considered acceptable.

Bystander and resident exposure

Since AbioProtect® (components PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) is to be used only in greenhouses, bystander and resident exposure is not relevant. Bystanders and resident should not be entering the greenhouse during/after ventilation.

Worker exposure

Worker exposure has been estimated using the EFSA model, taking into account an inhalation factor of 0.15 ha/h x 10^{-3} ("worst case"), an activity duration of 8 and 2 hours, a transfer coefficient of 2500 cm^2/h for tomato, according EFSA 2014 guideline, and one application. Results are summarised in **Table B.6.4.4.**

Table B.6.4.4. Estimated worker exposure to nicotine, EFSA model.

Crop	Transfer coefficient (cm^2/h)	Reentry work duration (h)	Application ratio		Applications		Active Substance	% AOEL
			Dose (L pf/ha)	Volume (L/ha)	Nº	Interval		
Tomato	2500	8	1.6	4	1	-	Nicotine	**0.01**
		2						**0**

Table B.6.4.4 shows the estimated worker exposure based on nicotine content of 0.1065 mg/L (1.704 x 10^{-7} kg/ha) in the formulation, indicating that worker exposure to nicotine after application of AbioProtect® (components PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) is negligible and therefore there is no risk to worker due to the exposure of nicotine after application of AbioProtect®.

Operator exposure for spray applications, AOEM model (only mixing/loading).

Application rate of active substance	0.00000017	kg a.s./ha	*i_AppRate*
Assumed area treated	1	ha/day	*d_AreaTreated*
Amount of active substance applied	0.00000017	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	10.00%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	50.00%		*i_AbsorInuse*
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.		
Indoor or Outdoor application	Outdoor		
Application method	Upward spraying		
Application equipment	Manual-Knapsack		
Season	not relevant		

	Exposure values	µg exposure/day mixed and loaded		Reference	Comment
		75th centile	95th centile		
Mixing and loading	Hands	9495	25482	AOEM	
	Body	803	2787	AOEM	
	Head	5	11	AOEM	
	Protected hands (gloves)	18	164	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	25	103	AOEM	
	Protected head (hood and face shield)	5	11	AOEM	
	Inhalation	25	26	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

1. Total

	Without RPE/PPE	With RPE/PPE
Longer term		
Total systemic exposure from mixing, loading and application (mg a.s./day)	3.7350412	0.0298000
Total systemic exposure from mixing, loading and application per kg body weight (mg/kg bw/day)	0.0622507	0.0004967
% of RVNAS	7781.34%	62.08%

Operator exposure for spray applications, EUROPOEM II model (application).

PRODUCT	**AbioProtect**
ACTIVE SUBSTANCE	**Nicotine**
Concentration (g/L o g/kg)	0.0001065
Application rate (L/ha o kg/ha)	1.6
Maximum application rate of active substance (kg/ha)	$1.704x10^{-07}$
Minimum volume water for application	4
Work ratio (ha/day)	1
Corporal weight (kg)	60
ARfD (mg/kg p.c./día)	0.0008
Dermal absorption (%)	50
Inhalatory exposure (%)	100
%AOEL (mixing/loading)	62.08

HIGH CROPS		
852	0.000145181	0.00
72	$1.22688x10^{-05}$	0.00
0.770	$1.31208x10^{-07}$	0.00
Dermal (mg/kg sa)		0.00
Inhalatory (mg/kg sa)		0.00
Total exposure (mg/kg sa)		0.00
Operator exposure (mg/kg pc/día)		0.000001
	%AOEL (application)	**0.16**
	%AOEL (mixing/loading)	**62.08**
	%AOEL TOTAL	**62.24**

LOW CROPS		
196	$3.33984x10^{-05}$	0.00
57.8	$9.84912x10^{-06}$	0.00
0.443	$7.54872x10^{-08}$	0.00
Dermal (mg/kg sa)		0.00
Inhalatory (mg/kg sa)		0.00
Total exposure (mg/kg sa)		0.00
Operator exposure (mg/kg pc/día)		0.000000
	%AOEL (application)	**0.05**
	%AOEL (mixing/loading)	**62.08**
	%AOEL TOTAL	**62.13**

Calculation of worker exposure during re-entry tasks

PRODUCT:	AbioProtect	**Dermal absorption:**	50	%
Substance Active:	Nicotine	**Concentration:**	0.00011	g i.a./kg o L pf
Crop:	Tomato	**AOEL:**	0.0008	mg i.a./pc/day

DERMAL EXPOSURE

DF_{50} (default)	30	days	
DFR (default)	3	ug/cm^2	
Dose	1.6	Kg o L pf/ha	
Application rate	1.704x10^{-07}	Kg i.a./ha	
Nº of applications	1	FACTOR	
Interval between applications		1.00	
Re-entry delay	0	1.00	
Dissipation coefficient	1.00		
DFR	5.112x10^{-07}	ug/cm^2/day	
TC	2500	cm^2/h	
T	8	h	
Dermal exposure	0.00	mg i.a./day	
Gloves	NO	mg i.a./day	
Absorbed dose	5.112x10^{-06}	mg i.a./day	

INHALATORY EXPOSURE

Application rate	1.704x10^{-07}	Kg i.a./ha

Specific tasks

Re-entry to the greenhouses after high application.

Exposure Factor	0.15	mg i.a./kg i.a./day
Duration	8	h
Inhalatory exposure	2.045x10^{-07}	mg i.a./day
Absorbed dose	2.045x10^{-07}	mg i.a./day

Total potential exposure	1.043x10^{-05}	mg i.a./day
Total absorbed dose	5.316x10^{-06}	mg i.a./day
Corporal weight	60	Kg
Systemic exposure	**0.00000**	**mg i.a./pc/day**
Proportional AOEL	**0.01%**	

Calculation of worker exposure during re-entry tasks

PRODUCT:	AbioProtect	**Dermal absorption:**	50	%
Substance Active:	Nicotine	**Concentration:**	0.00011	g i.a./kg o L pf
Crop:	Tomato	**AOEL:**	0.0008	mg i.a./pc/day

DERMAL EXPOSURE

DF_{50} (default)	30	days	
DFR (default)	3	ug/cm^2	
Dose	1.6	Kg o L pf/ha	
Application rate	1.704x10^{-07}	Kg i.a./ha	
Nº of applications	1	FACTOR	
Interval between applications			1.00
Re-entry delay	0		1.00
Dissipation coefficient	1.00		
DFR	5.112x10^{-07}	ug/cm^2/day	
TC	2500	cm^2/h	
T	2	h	
Dermal exposure	0.00	mg i.a./day	
Gloves	NO	mg i.a./day	
Absorbed dose	1.278x10^{-06}	mg i.a./day	

INHALATORY EXPOSURE

Application rate	1.704x10^{-07}	Kg i.a./ha

Specific tasks

Re-entry to the greenhouses after high application.

Exposure Factor	0.15	mg i.a./kg i.a./day
Duration	2	h
Inhalatory exposure	5.112x10^{-08}	mg i.a./day
Absorbed dose	5.112x10^{-08}	mg i.a./day

Total potential exposure	2.607x10^{-06}	mg i.a./day	
Total absorbed dose	1.329x10^{-06}	mg i.a./day	
Corporal weight	60	Kg	
Systemic exposure	**0.00000**	**mg i.a./pc/day**	
Proportional AOEL	**0.00%**		

B.6.5. SUPPLEMENTARY STUDIES FOR COMBINATIONS OF PLANT PROTECTION PRODUCTS

AbioProtect® is not applied with any other plant protection products or with any tank mix.

B.6.6. SUMMARY AND EVALUATION OF HEALTH EFFECTS

The acute toxicity studies on the formulation AbioProtect® including acute oral toxicity, acute inhalation toxicity and acute dermal toxicity have shown that it is not toxic and could be considered not classified according to CLP classification criteria. Furthermore, the studies on the effect of the PepMV isolates of the formulation AbioProtect® on cell viability and proliferation as well as on infectivity and replication on human alveolar epithelial cell type 2 A 549 have concluded that PepMV does not have any effect.

No additional acute toxicity studies have been conducted, nonetheless the formulation AbioProtect® should be regarded as potential sensitiser as according to Regulation (EC) 283/2013, all microorganisms should be regarded as potential sensitisers. As the product is classified as a potential sensitiser, the use of PPE is recommended as a precautionary measure. The following warning phrase is applicable “Microorganisms may have the potential to provoke sensitising reactions”. AbioProtect® does not warrant classification as being a skin irritant. It is not consider as eye irritant although to avoid any risk of potential eye sensitisation the used of protective eye equipment might be recommended.

There are not clinical cases and poisoning incidents or indications for a toxic potential regarding the information of the medical record of the employees involved in the manufacture of AbioProtect® and its components. All the available information indicates that the use of AbioProtect® in the manner proposed presents no systemic hazard for operators or others who may handle treated plants and an exposure assessment is not required.

The formulation AbioProtect® does not contain any non-active substances and is not applied in combination with any other plant protection products or with any tank mix.

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

It could be concluded that the use of AbioProtect® as a Microbial Plan Control Product in greenhouse (protected) tomato crops does not pose any risk to human and animals and therefore there is no risk to operators, workers, bystanders or residents.

B.6.7. REFERENCES RELIED ON

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
B6.1.1	[redacted]	2017a	Evaluation of the acute oral toxicity of the test item AbioProtect® (and its components Abp1 and Abp2) in female Sprague-Dawley rats by the acute toxic class method (OECD nº 423)". [redacted] Report number B-02315. GLP Not published	Y	Y	Proprietary information	Abiopep S.L.
B6.1.2	[redacted]	2017	Acute inhalation toxicity of test item AbioProtect® (and its components Abp1 and Abp2) in Sprague Dawley rats: OECD Nº403. [redacted] Report number: B-02317. GLP Not published	Y	Y	Proprietary information	Abiopep S.L.
B6.1.3	[redacted]	2017b	Evaluation of the acute dermal toxicity of the test item AbioProtect® (and its components Abp1 and Abp2) in female and male Sprague-Dawley rats (OECD nº 402)". [redacted] Report number: B-02316. GLP Not published	Y	Y	Proprietary information	Abiopep S.L.
B6.3	Cabezas J.	2017	Study on the potential hazards to humans of using *Pepino mosaic virus* (PepMV) as a microbial biopesticide in greenhouse tomato crops. Instituto Murciano de Investigación Biosanitaria Virgen de la Arrixaca, Spain. Not GLP Not published	N	Y	Proprietary information	Abiopep S.L.
B6.4-01	Sheen S.J.	1988	Detection of nicotine in foods and plant materials. Journal of Food Science,	N	N		

			53 (5) 1572-1573 No GLP Published				
B6.4-02	Siegmund B. et al.	1999	Determination of the Nicotine Content of Various Edible Nightshades (Solanaceae) and Their Products and Estimation of the Associated Dietary Nicotine Intake Journal of Agricultural and Food Chemistry 47(8), 3113–3120 DOI: 10.1021/jf990089w No GLP Published	N	N		
B6.4-03	Veiga J.M. et al.	2018	Validation report Laboratorio Químico-Microbiológico No GLP Not published	N	Y	Proprietary information	Abiopep S.L.
B6.4-04	Pineda J.L.	2018a	Assay report: Chemical determination: Nicotine. Active substance PepMV, EU strain, mild isolate Abp1. Batch L-5-041017-Abp1-C Laboratorio Químico-Microbiológico JN/MUR/16215/18 No GLP Not published	N	Y	Proprietary information	Abiopep S.L.
B6.4-05	Pineda J.L.	2018b	Assay report: Chemical determination: Nicotine. Active substance PepMV, EU strain, mild isolate Abp1. Batch L-6-111017-Abp1-C Laboratorio Químico-Microbiológico JN/MUR/16216/18 No GLP Not published	N	Y	Proprietary information	Abiopep S.L.
B6.4-06	Pineda J.L.	2018c	Assay report: Chemical determination: Nicotine. Active substance PepMV, EU strain, mild isolate Abp1. Batch L-7-251017-Abp1-C Laboratorio Químico-Microbiológico JN/MUR/16217/18 No GLP Not published	N	Y	Proprietary information	Abiopep S.L.
B6.4-07	Pineda J.L.	2018d	Assay report: Chemical determination: Nicotine. Active substance PepMV, EU strain, mild isolate	N	Y	Proprietary information	Abiopep S.L.

			Abp1. Batch L-8-081117-Abp1-C Laboratorio Químico-Microbiológico JN/MUR/16218/18 MO00 No GLP Not published				
B6.4-08	Pineda J.L.	2018e	Assay report: Chemical determination: Nicotine. Active substance PepMV, EU strain, mild isolate Abp1. Batch L-9-221117-Abp1-C Laboratorio Químico-Microbiológico JN/MUR/16219/18 No GLP Not published	N	Y	Proprietary information	Abiopep S.L.
B6.4-09	Pineda J.L.	2018f	Assay report: Chemical determination: Nicotine. Active substance PepMV, CH strain, mild isolate Abp2. Batch L-5-041017-Abp2-C Laboratorio Químico-Microbiológico JN/MUR/16220/18 No GLP Not published	N	Y	Proprietary information	Abiopep S.L.
B6.4-10	Pineda J.L.	2018g	Assay report: Chemical determination: Nicotine. Active substance PepMV, CH strain, mild isolate Abp2. Batch L-6-111017-Abp2-C Laboratorio Químico-Microbiológico JN/MUR/16222/18 No GLP Not published	N	Y	Proprietary information	Abiopep S.L.
B6.4-11	Pineda J.L.	2018h	Assay report: Chemical determination: Nicotine. Active substance PepMV, CH strain, mild isolate Abp2. Batch L-7-251017-Abp2-C Laboratorio Químico-Microbiológico JN/MUR/16223/18 No GLP Not published	N	Y	Proprietary information	Abiopep S.L.
B6.4-12	Pineda J.L.	2018i	Assay report: Chemical determination: Nicotine. Active substance PepMV, CH strain, mild isolate Abp2. Batch L-8-081117-Abp2-C Laboratorio Químico-Microbiológico	N	Y	Proprietary information	Abiopep S.L.

			JN/MUR/16224/18 No GLP Not published				
B6.4-13	Pineda J.L.	2018f	Assay report: Chemical determination: Nicotine. Active substance PepMV, CH strain, mild isolate Abp2. Batch L-9-221117-Abp2-C Laboratorio Químico-Microbiológico JN/MUR/16225/18 No GLP Not published	N	Y	Proprietary information	Abiopep S.L.
B6.4-14	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.
B6.4-15	MacBride J.S. et al.	1988	Green tobacco sickness. Tobacco control 7: 294-298 No GLP Published	N	N		
B6.4-16	Lindgren M. et al.	1999	Electroencephalographic effects of intravenous nicotine – a dose-response study. Psychopharmacology 145: 342 DOI: 10.1007/s002130051067 No GLP Published		N		