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.6 Toxicology

Rapporteur Member State: Spain

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B.6. EFFECTS ON HUMAN HEALTH

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.

B.6.1. TIER I

B.6.1.1. Basic information

Plant viruses are ubiquitous in plants and fruits, and as a consequence people continuously consume them. Cases of plant viruses causing diseases in humans have never been documented and none of them so far is known as pathogen to animals and human beings. The occurrence of the mutations required for a change in host range (e.g. from plants to animals or humans) would be huge and may become a limiting factor for the introduction phase of disease emergence; thus, the barriers imposed by the host to a new viral pathogen may render establishment extremely unlikely.

PepMV belongs to the *Alphaflexiviridae* family (genus *Potexvirus*). The EFSA Panel on Biological Hazards (2013)[1] concludes that: "No scientific or other evidence was found that alphaflexiviruses (Family *Alphaflexiviridae*) or members thereof such as from the genus Potexvirus (Adams et al., 2011) have any negative effect on animals and humans to date. Viruses of this family have been reported from a wide range of herbaceous and woody plants, both mono-and dicotyledons. Species of this virus family are mostly plant-specific and are transmitted either mechanically or through insect vectors from plant to plant. In terms of safety, the familiarity principle was taken into account as well, in that these viruses have been part of the food and feed of animals and humans since plant material was part of the food package. The major component of an alphaflexivirus (e.g. PepMV), the coat protein, was tested computationally in 2013 against the UniRef100 plant database (Suzek et al., 2007) and did not show any homology to known toxins. None of the hits were related to the search terms 'disease' or 'toxins'. No other negative impacts of alphaflexiviruses, more specifically potexviruses such as PepMV (genus *Potexvirus*) on humans or animals have been reported to date. Hence it was agreed that the family *Alphaflexiviridae*, as the highest taxonomic unit, is recommended for the QPS list[1]. A recent study found that the overall fold of PepMV CP resembles that of nucleoproteins (NPs) from the genus *Phlebovirus* (family *Bunyaviridae*), a group of enveloped (-)ssRNA viruses which infect animals. The main difference between potexvirus CP and phlebovirus NP is in their C-terminal extensions, which appear to

[1] 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

determine the characteristics of the distinct multimeric assemblies – a flexuous, helical rod or a loose ribonucleoprotein (Agirrezabala et al., 2015).

Interactions of some viruses with humans have been reported for other plant virus families (Colson et al., 2010; Liu et al., 2013). For example, *Pepper mild mottle virus* (PMMoV), a member of the *Virgaviridae* family, is present in stools from healthy individuals, but it is found associated with higher frequency in individuals with clinical symptoms (fever and abdominal pain) (Colson et al., 2010). Such patients appear to have a higher specific immune response to PMMoV (seropositivity). Recently, Liu et al. (2013) argued that antibodies against *Tobacco mosaic virus* (TMV, *Virgaviridae*) in humans, e.g. as a long-term consequence of smoking, interact with the human TOMM40L protein through a conserved amino acid stretch between TMV and TOMM40L. Such TMV antibodies are involved in the emergence of autoimmune diseases. However, a direct causal relationship between a plant virus and disease in humans, such as virus replication in cells or pathology, has not been demonstrated.

The complete viral genome sequence of PepMV is known and many functions of the five-encoded typical potexvirus proteins are well understood. None of these proteins show any homology to known human or animal toxins. It can therefore be stated with certainty that PepMV does not produce toxins, not even after infecting with the plant host cell. In addition, the fact that the majority of the tomatoes available on the European markets are infected with a broad variety of PepMV isolates since the early 2000s indicates that PepMV does not pose any threat to human health (Werkman and Sansford, 2010).

B.6.1.1.1. Medical data

Routine exposure of personnel, laboratory researchers, as well as consumers of tomatoes affected with PepMV, has not resulted in any known adverse effects of toxicological significance, and no literature is available in the public domain supporting evidence for toxicity potential of plant viruses in general or PepMV specifically to humans and mammals.

B.6.1.1.2. Medical surveillance on manufacturing plant personnel

A medical officer annually examined the involved personnel (up to 13 persons over 3 years) by performance of standard medical tests and confirmed in writing that exposures of workers to PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, during the years 2014–2016 did not result in any known incidence of adverse health effect, including hypersensitivity or chronic sensitization (Cabezas, 2017).

After expanded literature search, it can be concluded that no studies reported adverse effects including allergic reactions (neither by skin contact nor by oral intake or inhalation) in humans who were in close contact with PepMV (Cabezas, 2017).

Although routine exposure of personnel, laboratory researchers, as well as consumers of tomatoes affected with PepMV, has not resulted in any known adverse effects of toxicological significance, and although no literature is available in the public domain supporting evidence for toxicity potential of plant viruses in general or PepMV specifically to humans and mammals, a number of toxicological and genotoxicity studies have been conducted with the tomato watery leaf extract containing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. None of these studies revealed any hazardous effects on human or animal health.

B.6.1.1.3. Sensitisation/ allergenicity observations, if appropriate

There is no document in the scientific literature reporting on sensitisation or allergic reactions to plant viruses. Since many viruses are pathogenic, and will invoke a normal immune response, and are quite extensively studied, it is extremely unlikely that sensitisation to plant viruses, if extant, would have gone totally unnoticed. A prerequisite for sensitisation is the infection of the host by the virus, which does not take place in the case of plant viruses (Cabezas, 2017). Particularly, as regards to PepMV, regular exposure of farm and research personnel did not result in any known incidence of hypersensitivity or chronic sensitisation. Nevertheless, according to Regulation (EC) 283/2013, all microorganisms should be regarded as potential sensitisers.

B.6.1.1.4. Direct observations e.g. clinical cases

Information regarding exposure of working personnel (farmers, technicians and researchers) to PepMV during experiments at CEBAS-CSIC and Abiopep is summarized below (Cabezas, 2017).

PepMV, EU strain, mild isolate Abp1 was first isolated in 2001 from a commercial tomato crop. The fruits of this crop had been sold through the commercial chain. No adverse health effects were reported throughout the whole period after consumption of the harvested crops. Similarly, exposure of the workers to the isolate did not exert any adverse health effects.

Research on PepMV using PepMV, EU strain, mild isolate Abp1 was conducted at CSIC first at EE La Mayora, and later at CEBAS-CSIC. No adverse health effects were observed among workers (technical and scientific personnel) being exposed to the isolate.

Since the introduction of PepMV in tomato crops in the Region of Murcia in 2001, tomato crops have been regularly infected with PepMV. Initially from the EU strain and after 2003-2004 from both the CH2 strain and the EU strain. Those tomatoes had been sold through different chains during several seasons. No adverse health effects were ever reported related with consumption of the harvested crops or with exposure of the workers to the virus. No adverse health effects related to the virus were ever detected.

Since 2014 until 2017, several field trials have been conducted using both PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. No adverse health effects were observed among operators and workers

AbioProtect® the MPCP formulated with both PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 was first registered in Spain in 2014 according to Orden APA/1470/2007 number 2536, followed by a registration according to RD 951/2014 until October 2015. Therefore, it was used as a preventive treatment against infection by aggressive isolates of PepMV from both the EU and the CH2 strains in commercial tomato greenhouses in Spain since 2014 until October 2015. The fruits of these crops were sold through different chains. No adverse health effects were ever reported related with consumption of the harvested crops or with exposure of the workers to the virus.

In 2016 a temporary exemption provided for in Article 53 of Regulation (EC) 1107/2009 have been granted for the use of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, for the protection of greenhouse tomato cultivation against damage by aggressive PepMV from both the EU strain and the CH2 strain, and especially adapted for the specific phytosanitary situation in Spain. According to this exemption, AbioProtect® was used extensively for the treatment of commercial tomato crops. The fruits of these crops were sold through different chains. No adverse health effects were ever reported related with consumption of the harvested crops or with exposure of the workers to the virus.

A medical officer examined annually the involved personnel by performance of standard medical tests. A confirmation of the aforementioned results by the medical officers is provided supporting the claims that exposure of workers to PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain mild isolate Abp2, during the years 2014-2016 did not result in any incidence of adverse health effects including hypersensitivity or chronic sensitisation.

RMS comment and conclusions

It is concluded that no adverse reactions in Abiopep personnel involved in research, production and application were reported as a result of exposure to PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2.

The study is considered acceptable regarding medical surveillance on manufacturing plant personnel. The report (Cabezas 2017) included additional information regarding exposure of working personnel (farmers, technicians and researchers) to PepMV during experiments at CEBAS-CSIC and no adverse health effects were observed, although without certification of a medical officer.

B.6.1.2. Basic studies

RMS Introductory comment

The studies performed by the applicant to assess the effects on human health of the active substances PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 were carried out employing both isolates together in the preparation AbioProtect® that is a suspension concentrate formulated with equivalent amounts of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. Althought the studies could have been performed with the isolates separately, both isolates could be considered equivalent in terms of their potential effects on human health and there are no reasons to expect any difference between both regarding human health. This approach enables to reduce the number of studies with vertebrate animals in compliance with provisions in article 8 of Regulation CE 1107/2009 regarding avoidance of animal testing and duplication of studies in vertebrate animals, while results of tests with both active substances are sound and fully applicable for each one independently. In fact, conducting the study for both active substances together

can be considered equivalent to the study for one although at a doubled dose. Other inert ingredients are tomato plant extract and water.

Nonetheless to further support the assertion that both isolates could be considered equivalent in terms of their potential effects on human health, new cell cultures studies on the effects of each PepMV isolate independently on cell viability and proliferation on human alveolar epithelial cells, as well as studies on infectivity or replication of each PepMV isolate on such cells have been performed and included in this assessment report.

B.6.1.2.1. Sensitization

As plant pathogens can induce expression of defence or pathogenesis-related proteins, including identified allergens, Welter et al. (2013) hypothesised that PepMV infection would result in the expression of allergens leading to a higher allergenic potential of tomato fruits. Transcript level analyses showed differential expression of 17 known and putative tomato fruit allergen encoding genes at early and late time points after PepMV inoculation, but no general induction was detected. Immunoblot analyses were conducted and IgEs from a serum pool of tomato allergic subjects reacted with 20 proteins, of which ten have not yet been described. In parallel, skin prick tests with a group of tomato allergic subjects did not show a general difference between PepMV infected and non-infected tomato fruits and basophil activation tests confirmed these results. In summary, PepMV infection of tomato plants can lead to long-lasting upregulation of particular allergens in fruits, but the hypothesis that this results in a higher allergenic potential of the fruits proved invalid (Welter et al. 2013).

Regular exposure of farm and research personnel to PepMV did not result in any known incidence of hypersensitivity or chronic sensitisation. The application of the MPCP Abiopep takes place immediately only before planting, in seedlings (planting time (BBCH 13-15)).Taken all together, it is unlikely that PepMV may provoke sensitisation and consequently, allergic reactions to humans.

However, it is customary to classify and label microorganisms for sensitisation by default in the EU. As according to Regulation (EC) 283/2013, all microorganisms should be regarded as potential sensitisers.

RMS comments and conclusion

The available scientific literature has not reported the incidence of hypersensitivity due to PepMV, nevertheless, following the Regulation (EC) 283/2013, all microorganisms should be considered potential sensitizers.

B.6.1.2.2. Acute toxicity, pathogenicity and infectiveness

B.6.1.2.2.1. Acute oral toxicity, pathogenicity and infectiveness

B.6.1.2.2.1-01 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 step.
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 items PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, contained in the formulation AbioProtect®, could be considered Unclassified according to Regulation 1272/2008 criteria, although the active substances are microorganisms and therefore the criteria for chemicals classification does not apply.

Under the conditions of the study the acute oral lethal dose (LD_{50}) was found to be higher than 2000 mg/kg of body weight in female Sprague-Dawley rats for AbioProtect® containing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2

PepMV, EU strain, mild isolate Abp1:

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.

PepMV, CH2 strain, mild isolate Abp2:

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.

B.6.1.2.2.1-02 Pathogenicity by oral route

RMS comments and conclusion

The applicant has not presented a report where the pathogenicity of the active substance is evaluated. The applicant has stated that as the family Alphaflexiviridae, to which PepMV belongs, is recommended for the QPS[1] list and all the scientific evidence supports the general assumption that PepMV, potexviruses or other members of the family Alphaflexiviridae do not have any effect on humans or mammals, and on the light of the results of the acute oral toxicity study, a report on the pathogenicity by oral route of the active substances is not consider relevant.

The non-submission of a test report by the applicant is accepted.

B.6.1.2.2.1-03 Infectiveness by oral route

RMS comments and conclusion

The applicant has not presented a report where the infectiveness of the active substances is evaluated. The applicant has stated that as the family Alphaflexiviridae, to which PepMV belongs, is recommended for the QPS list and all the scientific evidence supports the general assumption that PepMV, potexviruses or other members of the family Alphaflexiviridae do not have any effect on humans or mammals, and on the light of the results of the acute oral toxicity study a report on the infectiveness by oral route of the active substances is not consider relevant.

The non-submission of a test report by the applicant is accepted.

B.6.1.2.2.2. Acute inhalation toxicity, pathogenicity and infectiveness

B.6.1.2.2.2-01 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

[1] 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

	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 can be concluded that, according to the results obtained in this study and under the assayed experimental conditions, that the LC_{50} for the test items PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, in the formulation AbioProtect® was greater than 5.02 mg/L air (gravimetric aerosol concentration) and they can be considered not classified, based on the CLP (Regulation 1272/2008) classification criteria, although classification criteria for chemicals does not apply since they are microorganisms.

PepMV, EU strain, mild isolate Abp1:

LC_{50} > 5.02 mg/L air => $3.39x10^8$ genome copies of PepMV/L air

LC_{50} > $3.39x10^8$ genome copies of PepMV/L air = > $1.46x10^8$ genome copies of Abp1/L air + > $1.93x10^8$ genome copies of Abp2/L air.

PepMV, CH2 strain, mild isolate Abp2:

LC_{50} > 5.02 mg/L air => $3.39x10^8$ genome copies of PepMV/L air

LC_{50} > $3.39x10^8$ genome copies of PepMV/L air = > $1.46x10^8$ genome copies of Abp1/L air + > $1.93x10^8$ genome copies of Abp2/L air.

RMS comments and conclusion

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

B.6.1.2.2.2-02 Pathogenicity by inhalation

RMS comments and conclusion

The applicant has not presented a report where the pathogenicity of the active substance is evaluated. The applicant has stated that as the family Alphaflexiviridae, to which PepMV belongs, is recommended for the QPS list and all the scientific evidence supports the general assumption that PepMV, potexviruses or other members of the family Alphaflexiviridae do not have any effect on humans or mammals, and on the light of the results of the acute inhalation toxicity study a report on the pathogenicity by inhalation of the active substances is not consider relevant.

The non-submission of a test report by the applicant is accepted.

B.6.1.2.2.1-03 Infectiveness by inhalation

RMS comments and conclusion

The applicant has not presented a report where the infectiveness of the active substance is evaluated. The applicant has stated that as the family Alphaflexiviridae, to which PepMV belongs, is recommended for the QPS list and all the scientific evidence supports the general assumption that PepMV, potexviruses or other members of the family Alphaflexiviridae do not have any effect on humans or mammals, and on the light of the results of the acute inhalation toxicity study a report on the infectiveness by inhalation of the active substances is not consider relevant.

The non-submission of a test report by the applicant is accepted.

B.6.1.2.2.3. Intraperitoneal/subcutaneous single dose

B.6.1.2.2.3.-01 Acute dermal toxicity test

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: 6.32×10^6 (1.70×10^6 of Abp1 and 4.62×10^6 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 PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Apb2 in the formulation AbioProtect® is established to be > 2000 mg/kg body weight.

PepMV, EU strain, mild isolate Abp1:

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

LD_{50} dermal 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

PepMV, CH2 strain, mild isolate Abp2:

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

LD_{50} dermal 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 applicant submitted an acute dermal toxicity study instead of the intra-peritoneal injection that is always required for all microorganisms (Regulation (EU) no 283/2013/EC). Although it is indicated that intra-peritoneal injection can be replaced by subcutaneous injection following expert's judgement if the maximum temperature for growth and multiplication is lower than 37°C the applicant decided to carry out an acute dermal toxicity test because it was considered that it would be the most likely route of exposure. Furthermore, they state that PepMV is a plant virus, which lacks of structures and mechanisms to infect mammalian cells, so it was considered not relevant to measure the presence of the microorganism in tissues, organs and body fluids.

Although this study is adequate for the evaluation of the preparation product it is not considered acceptable for the active substance. However the applicant provided two cell culture studies one on cell viability and proliferation and other on infectivity and replication of both PepMV isolates in human alveolar epithelial cells (see B6.1.2.4./01 and B6.1.2.4/02 below) and both studies showed that there is no effect of the active substances on the preparation on cell viability and proliferation or on infectivity and replication of human alveolar epithelial cells. Besides, the family Alphaflexiviridae, to which PepMV belongs, is recommended for the QPS list and all the scientific evidence supports the general assumption that PepMV, potexviruses or other members of the family Alphaflexiviridae do not have any effect on humans or mammals. Therefore, an intraperitoneal/subcutaneous single dose study is not considered relevant.

B.6.1.2.3. Genotoxicity testing

B.6.1.2.3.1. In vitro studies

B.6.1.2.3.1-01 Bacterial Reverse Mutation Test

Reference	Gómez and Calvo (2017)
Study	Bacterial Reverse Mutation Test (OECD Guideline 471). AbioProtect® (Abp1 and Abp2) Unpublished report B-02316. Ames test.
Guidelines	OECD guideline Nº 471 (Adopted 21st July 1997)
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.
Controls **Negative (solvent)** **Positive (-S9)** **Positive (+S9)**	Water Sodium azide for *S. typhimurium* TA100 and TA1535 2-nitrofluorene for *S. typhimurium* TA98 Mitomycin C for *S. typhimurium* TA102 9-acrinidine for *S. typhimurium* TA1537 2-amino-anthracene for *S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537
Test microorganisms	*Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537

Metabolic activation (S9)	Commercially available post-mitochondrial fraction (S9) from livers of rodents treated with the enzyme-inducing agent Aroclor
Test Method	Ames test. Cytotoxicity evaluation of the test item and its components was performed in the *S. typhimurium* TA100 strain by the direct incorporation procedure with 10 concentrations based on the solubility profile of the test item (100, 50, 25 12.5. 6.25, 3.13, 1.56, 0.78, 0.39 and 0.20 µL/plate). Five *S. typhimurium* strains (TA98, TA100, TA102, TA1535, TA1537) were exposed to the test item at 5 concentrations (100-50-25-12.5-6.25 µL/plate) with and without metabolic activation system (S9) under the direct incorporation and the pre-incubation procedures. In the direct incorporation procedure the mixture was immediately poured over a minimal agar medium plate whereas in the pre-incubation procedure, the mixture was incubated for 20 min at 37ºC prior to be poured over the minimal agar medium plate. Plates were incubated for 48h at 37ºC and colonies were counted. The assay was performed by triplicate along with vehicle and reference item controls. Each bacterial strain culture was mixed with the test item either with metabolic activation system mix (S9) or without metabolic activation system mix (PBS was used instead).

Results and Conclusion

A. Results

In the preliminary cytotoxicity evaluation of the test item with *S. typhimurium* TA100 strain by the direct incorporation procedure at concentrations ranging from 0.20 to 100 µL/plate no test item related cytotoxic activity was observed at any of the concentrations tested.

Table B.6.1.2.3.1-01 Results of the Ames test by the direct incorporation procedure. Number of revertants per plate

Salmonella Strain	TA98		TA100		TA102		TA1535		TA1537	
Metabolic activation (S9)	without	with	without	with	without	with	without	with	without	with
Solvent control	23.0	28.3	87.3	93.0	350.7	352.0	20.7	12.0	6.3	6.7
Test item										
100.00 µL/plate	21.7	27.0	83.0	98.3	271.0	390.0	20.0	19.3	6.3	5.7
50.00 µL/plate	25.3	25.0	71.3	101.7	267.7	407.7	18.7	14.0	6.7	6.3
25.00 µL/plate	23.7	27.3	70.7	82.7	289.0	413.0	20.7	21.3	8.3	6.0
12.50 µL/plate	23.7	26.0	92.0	89.7	257.0	364.0	12.0	19.0	7.7	5.7
6.25 µL/plate	23.0	23.0	78.0	90.7	263.0	375.7	10.7	11.3	7.0	4.0
Positive control	340.7	676.0	769.0	1544.7	1162.0	2193.3	989.7	374.0	175.0	197.0

Table B.6.1.2.3.1-02 Results of the Ames test by the preincubation procedure. Number of revertants per plate.

Salmonella Strain	TA98		TA100		TA102		TA1535		TA1537	
Metabolic activation (S9)	without	with	without	with	without	with	without	with	without	with
Solvent control	30.7	27.0	83.7	106.3	411.3	378.7	16.7	16.7	7.0	6.3
Test item										
100.00 µL/plate	23.0	28.3	83.7	94.3	382.7	352.0	14.7	17.0	5.3	6.7
50.00 µL/plate	21.7	27.0	105.3	98.7	381.3	369.3	14.7	15.7	7.3	10.3
25.00 µL/plate	20.3	25.7	86.3	96.3	373.7	370.3	20.7	12.7	6.0	7.0
12.50 µL/plate	18.3	29.0	86.7	90.0	355.3	361.0	17.3	15.0	6.3	7.3
6.25 µL/plate	20.7	25.7	81.0	77.0	337.0	237.3	15.3	16.3	5.3	6.7
Positive control	472.3	511.7	932.7	1336.7	1323.3	1913.0	984.0	331.3	190.3	172.7

No dose response for the test item AbioProtect® and its components (Abp1 and Abp2) was observed in any of the tested bacterial strains (**Tables B.6.1.2.3.1-01 and B.6.1.2.3.1-02**)

Overall interpretation of the study results suggests that the test item and/or its components do not induce point mutations or frame-shifts in the genome of the bacterial salmonella strains with or without metabolic activation, regardless of the procedure.

B. Conclusion

It was concluded that under the test conditions PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 components of the test item AbioProtect® were NON-MUTAGENIC / NON PRO-MUTAGENIC in this bacterial test system, either in the presence or absence of metabolic activation in the strains tested at an exposure dose range of 6.25–100 µL/plate.

RMS comments and conclusion

The study is considered acceptable for in vitro mutagenicity tests.

B.6.1.2.3.1-02 Test for clastogenicity in mammalian cells

RMS comment and conclusion

The applicant has not presented a report where the test for clastogenicity in mammalian cells is carried out. The applicant states that PepMV is a plant virus which lacks structures and mechanisms to infect mammalian cells. According to the studies conducted, on the bases of the scientific evidence on the microorganism and considering that the family Alphaflexiviridae, to which PepMV belongs, is recommended for the QPS list, a test for clastogenicity in mammalian cells is considered not relevant.

The non-submission of a test report by the applicant is accepted, although it would have been advisable to have it performed.

B.6.1.2.3.1-03 Test gene mutation in mammalian cells

RMS comment and conclusion

The applicant has not presented a report where the test for gene mutation in mammalian cells is carried out. The applicant states that, as PepMV is a plant virus which lacks structures and mechanisms to infect mammalian cells, a test for gene mutation in mammalian cells is considered not relevant.

The non-submission of a test report by the applicant is accepted.

B.6.1.2.4. Cell culture study

B.6.1.2.4-01/1 Cell viability and proliferation assays with both isolates together

Reference	Žegura and Novak (2017a)
Study	The effect of Tomato leaves extract infected with naturally occurring mild isolates of Pepino mosaic virus (PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) on viability and proliferation of human alveolar epithelial cells type 2 A549 determined with the MTT assay. Unpublished report 10G002-2017
Guidelines	Not included. Procedure SOP 10G-Pos03-02 (MTT assay)
Deviations	-
GLP	No *RMS comment: The laboratory where the test was carried out was granted a certificate for a quality management system to the ISO 9001:2008 standard*
Acceptability	Additional information

Materials and Methods

Control test item	Tomato leaves extract (*S. lycopersicum*) not containing PepMV.

Test item	Tomato leaves extract (*S. lycopersicum*) infected with the naturally occurring mild isolates PepMV-Abp1 (EU strain) and PepMV-Abp2 (CH2 strain). AbioProtect® batch number L-AB02-060217. Abp1 batch number L-12-060217-ABP1-C and Abp2 batch number L-12-060217-ABP2-C.
Test cell line	Human alveolar epithelial cell type 2 A549
Method	*Cell viability assay*: the A549 cells were treated with various concentrations (0.15625, 0.3125, 0.625, 1.25, 2.5, 5, 10 and 20 v/v%) of the reference tomato leaves extract not containing PepMV and tomato leaves extract containing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 for 24 hours. After 24-hour treatment, the viability of A549 cells was determined with the MTT assay. The experiment was repeated three times with five replicates for each concentration. *Cell proliferation assay*: the A549 cells were treated with various concentrations (0.15625, 0.3125, 0.625, 1.25, 2.5 v/v%) of the reference tomato leaves extract not containing PepMV and tomato leaves extract containing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 for 24, 48 and 72 hours. After each treatment time, cell viability was measured with the MTT assay. The experiment was repeated three times with five replicates for each concentration.

Results and Conclusion

A. Results

Cell viability assay: the results showed that the reference tomato leaves extract not containing PepMV and tomato leaves extract containing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 at 2.5, 5, 10 and 20 v/v% decreased the viability of A549 cells after 24 hours of exposure to a similar extent, while lower concentrations (0.15625, 0.3125, 0.625, 1.25 v/v%) had no statistically significant effect on the viability of A549 cells.

Cell proliferation assay: the results showed that the reference tomato leaves extract not containing PepMV and the tomato leaves extract containing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 statistically significantly decreased A549 cell proliferation at 2.5 v/v% after all treatment times (24, 48 and 72 hours). Statistically significant differences between the reference tomato leaves extract not containing PepMV and tomato leaves extract containing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 were observed after 24 and 48 hours, while after 72 hours of exposure no statistically significant differences between test items were determined.

B. Conclusion

According to the results of the viability and proliferation assays we can conclude that in human alveolar epithelial cells type 2 A549 the reference tomato leaves extract not containing PepMV and tomato leaves extract containing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 decreased cell viability and affected the proliferation to a similar extent. The likely reason for cytotoxic activity and anti-proliferative activity of test items is the presence of biologically active natural constituents and metabolic products in the plant extracts and not the presence of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2.

RMS comments and conclusion

The study is considered acceptable for the evaluation of cell viability and cell proliferation.

B.6.1.2.4-01/2 Cell viability and proliferation assays with each isolate separately

Reference	Žegura and Novak (2019a)
Study	The effect of Tomato leaves extract infected with naturally occurring mild isolates of Pepino mosaic virus (PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) on viability and proliferation of human alveolar epithelial cells type 2 A549 determined with the MTT assay. Unpublished report 10G007-2019

Guidelines	Not included. Procedure SOP 10G-Pos03-02 (MTT assay)
Deviations	-
GLP	No *RMS comment: The laboratory where the test was carried out was granted a certificate for a quality management system to the ISO 9001:2008 standard*
Acceptability	Additional information

Materials and Methods

Control test item	Tomato leaves extract (*S. lycopersicum*) not containing PepMV.
Test items	Tomato leaves extract (*S. lycopersicum*) infected with the naturally occurring mild isolate PepMV, EU strain, mild isolate Abp1. Abp1 batch number: L-TO-01-ABP1-C Tomato leaves extract (*S. lycopersicum*) infected with the naturally occurring mild isolate PepMV, CH2 strain, mild isolate Abp2. Abp2 batch number: L-TO-02-ABP2-C
Test cell line	Human alveolar epithelial cell type 2 A549
Method	Cell viability assay: the A549 cells were treated with various concentrations (0.15625, 0.3125, 0.625, 1.25, 2.5, 5, 10 and 20 v/v%) of the reference tomato leaves extract not containing PepMV and tomato leaves extracts containing PepMV, EU strain, mild isolate Abp1 or PepMV, CH2 strain, mild isolate Abp2 for 24 hours. After 24-hour treatment, the viability of A549 cells was determined with the MTT assay. The experiment was repeated three times with five replicates for each concentration. Cell proliferation assay: the A549 cells were treated with various concentrations (0.15625, 0.3125, 0.625, 1.25, 2.5 v/v%) of the reference tomato leaves extract not containing PepMV and tomato leaves extract containing PepMV, EU strain, mild isolate Abp1 or PepMV, CH2 strain, mild isolate Abp2 for 24, 48 and 72 hours. After each treatment time, cell viability was measured with the MTT assay. The experiment was repeated three times with five replicates for each concentration.

Results and Conclusion

A. Results

Cell viability assay: the results showed that the reference tomato leaves extract not containing PepMV and tomato leaves extract containing PepMV, CH2 strain, mild isolate Abp2 decreased the viability of A549 cells at concentrations > 10 v/v% after 24 hours of exposure to a similar extent, while lower concentrations (≤5 v/v%) had no statistically significant effect on the viability of A549 cells.

The tomato leaves extract containing PepMV, EU strain, mild isolate Abp1 decreased the viability of A549 cells to a higher extent compared to reference tomato leaves extract not containing PepMV that was statistically different at concentration ≥ 1.25 v/v%.

Cell proliferation assay: the results showed that after 24hours of exposure the reference tomato leaves extract not containing PepMV and the tomato leaves extract containing PepMV, CH2 strain, mild isolate Abp2 had no effect on the viability of A549 cells, while tomato leaves extract containing PepMV, EU strain, mild isolate Abp1 significantly decreased A549 cell proliferation at 5 v/v%. After 48 hours of exposure, the reference extract not containing PepMV significantly decreased cell viability at concentrations ≥ 1.25 v/v% compared to non-treated A549 cells, the extract containing PepMV, EU strain, mild isolate Abp1 significantly decreased cell viability at concentrations ≥ 0.625 v/v % and PepMV, CH2 strain, mild isolate Abp2 significantly decreased cell viability at concentrations ≥ 0.3125 v/v %. After 72 hours of exposure, all test items significantly decreased cell viability at ≥ 0.3125 v/v % compared to non-treated A549 cells.

Statistically significant differences between the reference tomato leaves extract not containing PepMV and tomato leaves extracts containing PepMV, EU strain, mild isolate Abp1 were observed after 24and 48 hours, while after 72 hours of exposure no statistically significant differences between these two test items were determined.

No statistically significant differences between the reference tomato leaves extract not containing PepMV and tomato leaves extracts containing PepMV, CH2 strain, mild isolate Abp2 were observed at any treatment time (24, 48 and 72 hours).

B. Conclusion

According to the results of the MTT assays we can conclude that in human alveolar epithelial cells type 2 A549 tomato leaves extract PepMV, CH2 strain, mild isolate Abp2 and reference tomato leaves extract not containing PepMV, decreased cell viability at concentration above 10 v/v % to a similar extend.

Tomato leaves extract containing PepMV, EU strain, mild isolate Abp1 decreased cell viability at concentration above 1.25 v/v %; however the viability was decreased by more than 30%, which is considered as cytotoxic effect only at concentration ≥ 5 v/v %. Thus, we can conclude that the likely reason for cytotoxic activity of test items is the presence of biologically active natural constituents and metabolic products in the plant extracts and not the presence of PepMV.

All three test items had anti-proliferative effect on human alveolar epithelial cells type 2 A549 at the concentration ≥ 0.625 v/v %. A statistically significant difference in the anti-proliferative effect between PepMV, EU strain, mild isolate Abp1 and reference tomato leaves extract not containing PepMV was determined after 24 and 48 hours at the concentration 5 v/v % but not after 72 hours of exposure.

Thus, is it unlikely that the anti-proliferative effect observed in the present study was due to Pepino mosaic virus especially as a comparable decrease in cell viability was detected also in the extract that did not contain Pepino mosaic virus and differences between isolates were observed during the first 48 hours but not after 72 hours.

RMS comments and conclusion

The study is considered acceptable for the evaluation of cell viability and cell proliferation.

B.6.1.2.4-02/1 Cell infectivity and replication assays with both isolates together

Reference	Žegura and Novak (2017b)
Study	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. Unpublished report 10G003-2017
Guidelines	Not included. Procedures SOP 10G-Pos04-03, SOP 2D-Pos37-03, SOP 2D-Pos51-03
Deviations	No
GLP	No *RMS comment: The laboratory where the test was carried out was granted a certificate for a quality management system to the ISO 9001:2008 standard*
Acceptability	Yes

Materials and Methods

Test item	Tomato leaves extract (*S. lycopersicum*) infected with the naturally occurring mild isolates PepMV-Abp1 (EU strain) and PepMV-Abp2 (CH2 strain). AbioProtect® batch number L-AB02-060217. Abp1 batch number L-12-060217-ABP1-C and Abp2 batch number L-12-060217-ABP2-C.
Test cell line	Human alveolar epithelial cell type 2 A549
Method	The A549 cells were exposed to 1.25 v/v % of tomato leaves extract containing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2for 48 hours in cell growth media. The following controls were included: non-treated cells as negative control (NT), non-treated cells to which PepMV tomato leaves extract at 1.25 v/v% (NTPep) was added prior to total RNA extraction to show that the presence of A549 cells does not inhibit the detection of PepMV and PepMV tomato leaves extract at 1.25 v/v% in growth media without A549 cells (T0) to check for the adsorption of the virus to the plastics (cell culture plate). After the 48 hours incubation period, all exposed samples of cells were detached from the

	cell culture plate to the liquid media by tripsinization, washed with 1xPBS buffer (2 times) and re-plated 3 times (3 passages) each time after 48 hours incubation. After each passage, the cells from each treatment were counted, and half of cells were plated for next passage and half stored for total RNA extraction. Same protocol was applied also for treatment without cells.

Results and Conclusion

A. Results

Presence of PepMV was tested by RT-qPCR (reverse transcription - real-time polymerase chain reaction). To verify the efficiency of the RNA extraction and cell growth, human glyceraldehide3-phosphatedehydrogenase (GAPDH) gene was used as control gene in RT-qPCR experiments.

In test item (tomato leaves extract with PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) treated cells, a significantly lower amount of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 were detected after each passage. After the last passage, the virus was not detected in two out of three replicates, showing that PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 did not multiply in human alveolar epithelial cells type 2 A549. Because of the significant decrease in viral concentration between subsequent passages, it is very unlikely that the virus entered the cells.

B. Conclusion

There are no indications of the infectivity or the replication of the naturally occurring PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 from the tomato (*S. lycopersicum*) leaves extract in human alveolar epithelial cells type 2 A549 in vitro.

RMS comments and conclusion

The study is considered acceptable for the evaluation of the replication of test substance in human cells.

B.6.1.2.4-02/2 Cell infectivity and replication assays with each isolate separately

Reference	Žegura, Novak and Kogovsek (2019b)
Study	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. Unpublished report 10G008-2019
Guidelines	Not included. Procedures SOP 10G-Pos04-03, SOP 2D-Pos37-03, SOP 2D-Pos51-04
Deviations	No
GLP	No *RMS comment: The laboratory where the test was carried out was granted a certificate for a quality management system to the ISO 9001:2008 standard*
Acceptability	Yes

Materials and Methods

Test item	Tomato leaves extract (*S. lycopersicum*) infected with the naturally occurring mild isolate PepMV, EU strain, mild isolate Abp1. Abp1 batch number: L-TO-01-ABP1-C Tomato leaves extract (*S. lycopersicum*) infected with the naturally occurring mild isolate PepMV, CH2 strain, mild isolate Abp2. Abp2 batch number: L-TO-02-ABP2-C
Test cell line	Human alveolar epithelial cell type 2 A549
Method	The A549 cells were exposed to 1.25 v/v % of tomato leaves extract containing PepMV, EU strain, mild isolate Abp1 or PepMV, CH2 strain, mild isolate Abp2 for 48 hours in cell growth media. The following controls were included: non-treated cells as negative control (NT), non-treated cells to which PepMV tomato leaves extract at 1.25 v/v% (NTPep) was added prior to total RNA extraction to show that the presence of A549 cells

	does not inhibit the detection of PepMV and PepMV tomato leaves extract at 1.25 v/v% in growth media without A549 cells (T0) to check for the adsorption of the virus to the plastics (cell culture plate). After the 48 hours incubation period, all exposed samples of cells were detached from the cell culture plate to the liquid media by tripsinization, washed with 1xPBS buffer (2 times) and re-plated 3 times (3 passages) each time after 48 hours incubation. After each passage, the cells from each treatment were counted, and half of cells were plated for next passage and half stored for total RNA extraction. Same protocol was applied also for treatment without cells.

Results and Conclusion

A. Results

Presence of PepMV was tested by RT-qPCR (reverse transcription - real-time polymerase chain reaction). To verify the efficiency of the RNA extraction and cell growth, human glyceraldehiden 3-phosphatedehydrogenase (GAPDH) gene was used as control gene in RT-qPCR experiments.

In cells treated with each test item, PepMV, EU strain, mild isolate Abp1 tomato leaves extract and PepMV, CH2 strain, mild isolate Abp2 tomato leaves extract, a significantly lower amount of PepMV RNA was detected after first passage. After second passage, the PepMV RNA was not detected in any replicate, showing that PepMV did not multiply in human alveolar epithelial cells type 2 A549. Because of the significant decrease in viral concentration between subsequent passages, it is very unlikely that the virus entered the cells.

B. Conclusion

In test items PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 treated cells, a significantly lower amount of PepMV was detected after each passage. After the third passage, in all parallels both virus isolates were not detected anymore, showing that PepMV isolates did not multiply in human alveolar epithelial cells type 2 A594. Because of the significant decrease in viral concentration, it is very unlikely that the virus isolated entered the cells. Importantly, a similar decreased in viral amount was obtained for both PepMV tomato leaves extract treated wells without cells. These results indicate unspecific carry-over of PepMV on cell culture plate or via tomato leaves extract and buffer leftovers.
There are not indications of infectivity or replication of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 from the tomato leaves extract.

RMS comments and conclusion
The study is considered acceptable for the evaluation of infectivity and replication of naturally occurring mild isolates 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.

B.6.1.2.5. Information on short term toxicity and pathogenicity

No acute toxicity or mutagenicity or cell infectivity was observed in the acute toxicity, mutagenicity and cell infectivity studies.
As PepMV not always cause visible symptoms in tomatoes (Hanssen et al., 2008), high and chronic exposure of humans towards this virus is expected. Nevertheless, there are no published reports of adverse effects on humans after exposure to PepMV (B6.1.1.2). Due to the mode of action as cross protection effect, actual activity is obtained by infection of the plants with mild isolates of the virus Abp1 and Abp2. Therefore, mild isolates Abp1 and Abp2 induce in tomato plants a symptomless infection with no damage to the fruit. Abp1 and Abp2 do not cause visible symptoms in tomatoes, therefore, high and chronic exposure of humans towards this virus is expected. Nevertheless, there are no published reports of adverse effects on humans after exposure to PepMV.
lant pathogenic viruses are generally considered to be pathogenic towards plant species only and not towards other organisms, like humans. Human exposure to plant pathogenic viruses is enormous and human illnesses caused by plant pathogenic viruses have not been described.
A search for scientific peer review literature on pathogenicity of plant viruses to animals or humans has been conducted and is included (Hernando, 2017). Such search has pointed out that some authors have reported the presence of RNA from plant virus in human faeces. In this regard, Zhang et al. (2006) showed that some RNA viruses isolated from healthy human faeces were plant RNA viruses. A large and diverse community of plant

RNA viruses exemplified by pepper mottle virus (PMMV) was found in two-thirds of individuals tested from different continents. Moreover, it appeared that the RNA viral community in human faeces was dynamic and could cause infection to host plants. The authors concluded that foods might be the major source of faecal borne RNA viruses (Zhang et al., 2006). Other authors (Colson et al., 2010) showed that PMMoV was present in stools from healthy individuals, but it was found associated with higher frequency in individuals with clinical symptoms (fever and abdominal pain). However, these authors consider that the symptoms might not be caused by consumption of PMMoV but by consumption of spicy foods. In another publication, Liu et al. (2013) conducted a study to determine whether exposure to tobacco products induces an immune response to TMV in humans. They argued that antibodies against TMV in humans, e.g. as a long-term consequence of smoking, interact with the human TOMM40L protein through a conserved amino acid stretch between TMV and TOMM40L. Such TMV antibodies are involved in the emergence of autoimmune diseases and the authors discussed that people who smoke cigarettes or other tobacco products experience a lower risk of developing Parkinson's disease, but the mechanism by which this occurs is unclear. However, no information or cases on multiplication of these or other plant viruses in mammal or human tissues has been reported in the scientific literature.

Furthermore, the cell culture study (B.6.1.2.4-01/1) reported by Žegura et al (2017a) did not show any effect on cell viability and proliferation attributable to PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 as the decrease in cell viability and cell proliferation was similar between the tomato extract infected with both PepMV mild isolates and the non-infected tomato extract. In the cell culture study on cell viability and proliferation carried out with each PepMV isolate separately (B.6.1.2.4-01/2) reported by Zegura et al (2019a) PepMV, EU strain, mild isolate Abp1 showed a slightly more cytotoxic effect for A549 cells in the viability assay. A statistically significant difference in the anti-proliferative effect between PepMV, EU strain, mild isolate Abp1 and reference tomato leaves extract not containing PepMV was determined after 24 and 48 hours at the concentration 5 v/v % but not after 72 hours of exposure. No statistically significant differences between the reference tomato leaves extract not containing PepMV and tomato leaves extracts containing PepMV, CH2 strain, mild isolate Abp2 were observed at any treatment time (24, 48 and 72 hours).The likely reason for cytotoxic activity and anti-proliferative activity of test items is the presence of biologically active natural constituents and metabolic products in the plant extracts and not the presence of PepMV.

The analysis of the infectivity and multiplication in human cells of both PepMV isolates together (B.6.1.2.4-02/1) reported by Žegura et al, (2017b) or tested individually (B6.1.2.4.02/2, Zegura et al, 2019b) has shown that there are no indications of any infectivity or multiplication of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, Abp2 in human cells.
All this information supports the general assumption that plant pathogenic viruses including PepMV are considered to be pathogenic towards plant species only and not towards other organisms, like humans. Moreover, the family *Alphaflexiviridae*, to which PepMV belongs, is recommended for the QPS list (EFSA, 2013)[1], 11(11): 3449).
Everything indicates that potential risk of PepMV towards humans is low. Further studies including short-term toxicity and pathogenicity studies with PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 are therefore not considered relevant.

RMS comments and conclusion
Taking into consideration that no acute toxicity or mutagenicity or cell infectivity was observed for PepMV it is acceptable that short term toxicity tests are not considered relevant.

B.6.1.2.5.1. Health effects after repeated inhalatory exposure.
According to the indications in point B.6.1.2.5 above, any relevant health effects after repeated inhalatory exposure are not expected.

B.6.1.2.6. First aid measures medical treatment.

Likely direct or indirect adverse effects:

- *May cause sensitization or an allergic reaction.*

[1] 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

First aid measures:

- *Inhalation: assure fresh air for breathing, keep individual calm and at rest. If you feel unwell, seek medical advice*
- *Skin contact: rinse immediately with plenty of water NO scrubbing. Take a shower for about 15 minutes. Remove contaminated shoes and clothing.*
- *Eye exposure: ALWAYS check for and remove contact lenses, wash eyes with plenty of water with eye lids open for at least 15 minutes*
- *Mouth contact or Ingestion: rinse mouth with plenty of water. Do NOT induce vomiting unless told to do so by poison control center operator or health care professional.*
- *If irritation or other symptoms develops, persists or worsens seek medical advice, bring packaging or label whenever possible.*

NEVER LEAVE THE AFFECTED INDIVIDUAL UNATTENDED!

Advice for medical and healthcare personnel:

- *Monitor vital signs and provide symptomatic and supportive treatment.*
- *Evaluate indication of activated charcoal.*

WHEN ASKING FOR MEDICAL ADVICE KEEP PACKAGING OR LABEL AT HAND AND CALL YOUR LOCAL POISON CONTROL CENTER

END OF TIER I

B.6.2. TIER II

B.6.2.1. Specific toxicity, pathogenicity and infectiveness studies

As the studies on toxicity, pathogenicity and infectiveness conducted have shown that PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 will not cause any health effects further data are not required.

B.6.2.2. *In vivo* studies in somatic cells

According to the results of the several studies conducted (B.6.1.2) further data are not required.

B.6.2.3. Genotoxicity- *In vivo* studies in germ cells

According to the results of the genotoxicity test (B6.1.2.3.1-01), PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 are considered non-mutagenic / non-promutagenic so no further data are required.

END OF TIER II

B.6.3. SUMMARY OF MAMMALIAN TOXICITY, PATHOGENICITY AND INFECTIVENESS AND OVERALL EVALUATION OF THE ACTIVE MICRO-ORGANISM

Table 6.3 Overview of the available data

Study	Test material	Species	Result	References
Acute oral toxicity	PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 (Abioprotect ®)	Rat	LD_{50} > 2000 mg/kg bw LD_{50} > $1.7x10^{10}$ genome copies/kg bw of Abp1 + $4.62x10^{10}$ genome copies/kg bw of Abp2	B.6.1.2.2.1-01 ([redacted] 2017a)
Acute inhalation toxicity	PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 (Abioprotect ®)	Rat	LC_{50} > 5.02 mg/L air LC_{50} > $1.46x10^{8}$ genome copies/L air of Abp1 + $1.93x10^{8}$ genome copies/L air of Abp2	B.6.1.2.2.2-01 ([redacted] 2017)
Acute dermal toxicity	PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 (Abioprotect ®)	Rat	LD_{50} > 2000 mg/kg bw LD_{50} > $1.7x10^{10}$ genome copies/kg bw of Abp1 + $4.62x10^{10}$ genome copies/kg bw of Abp2	B.6.1.2.2.3 -01 ([redacted] 2017b)
Bacterial Reverse Mutation Test	PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 (Abioprotect ®)	*Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537	Non-mutagenic / non pro-mutagenic	B.6.1.2.3.1-01 (Gomez and Calvo 2017)
Cell culture studies	PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 (Abioprotect ®)	Human alveolar epithelial cells type A549	The likely reason for cytotoxic activity and anti-proliferative activity of test items is the presence of biologically active natural constituents and metabolic products in the plant extracts and not the presence of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 No infectivity or replication in human alveolar epithelial cells A549	B.6.1.2.4-01/1 (Žegura 2017a) B.6.1.2.4-01/2 (Žegura 2019a) B.6.1.2.4-02/1 (Žegura 2017b) B.6.1.2.4-02/2 (Žegura 2019b)

The acute toxicity studies, including acute oral toxicity, acute inhalation toxicity and acute dermal toxicity, which were carried out employing PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 together in the preparation AbioProtect® that is a suspension concentrate formulated with equivalent amounts of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, have shown that both isolates are not toxic and could be considered as not classified according to CLP (Regulation 1272/2008) criteria. Nevertheless, since the active substances are microorganisms, classification criteria for chemicals does not apply. 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.

Regular exposure of farm and research personnel to PepMV did not result in any known incidence of hypersensitivity or chronic sensitisation. Taken all together, it is unlikely that PepMV may provoke sensitisation and consequently, allergic reactions to humans. Nonetheless, Regulation (EC) 283/2013 states that all microorganisms should be regarded as potential sensitisers. The following warning phrase might be applicable to PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 “Microorganisms may have the potential to provoke sensitising reactions”. PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 do not warrant classification as being a skin irritants. They are not considered 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 PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. All the available information indicates that the use PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 in the manner proposed present no systemic hazard for operators or others who may handle treated plants and an exposure assessment is not required. Therefore, it is considered that an estimation of operators' exposure is not relevant. Furthermore, in other cases of PepMV mild isolates approved as active substances according to regulation (CE) 1107/2009, regulations (EU) 2015/1176, 2017/406 and 2017/408, no AOEL, ADI, and ARfD have been determined.

It could be concluded that the use of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 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. Furthermore, exposure to animals or humans does not have any implications for vaccination or serological monitoring.

B.6.4 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
B6.1.1-01	Adams M., et al	2011	Family *Alphaflexiviridae*, in: A. King, et al. (Eds.), Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses, Elsevier Academic Press. pp. 904-909. No GLP Published	N	N		
B6.1.1-02	Agirrezabala X. et al	2015	The near-atomic cryoEM structure of a flexible filamentous plant virus shows homology of its coat protein with nucleoproteins of animal viruses. DOI: 10.7554/eLife.11795. No GLP Published	N	N		
B6.1.1-03	Suzek B.E. et al	2007	UniRef: comprehensive and non-redundant UniProt reference clusters. Bioinformatics 23:1282-1288. DOI: 10.1093/bioinformatics/btm098. No GLP Published	N	N		
B6.1.1-04	Colson P. et al.	2010	*Pepper mild mottle virus,* a plant virus associated with specific immune responses, fever, abdominal pains, and pruritus in humans. PloS one 5:e10041. No GLP Published	N	N		
B6.1.1-05	Liu R. et al	2013	Humans have antibodies against a plant virus: evidence from *Tobacco mosaic virus.* PloS one 8:e60621. No GLP Published	N	N		
B6.1.1-06	Werkman A., Sansford C.	2010	Pest Risk Analysis for *Pepino mosaic virus* for the EU. Deliverable Report 4.3. EU Sixth Framework Project Project PEPEIRA. No GLP Published	N	N		
B6.1.1.2	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. No GLP Mot published	N	Y	Proprietary information	Abiopep S.L.
B6.1.2.1	Welter S. et al	2013	*Pepino mosaic virus* infection of tomato affects allergen expression, but not the allergenic potential of fruits.	N	N		

			PloS one 8:e65116. No GLP Published				
B6.1.2.2.1-01	[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.
B6.1.2.2.2-01	[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-02137. GLP Not published	Y	Y	Proprietary information	Abiopep S.L.
B6.1.2.2.3-01	[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.
B6.1.2.3.1-01	Gómez R., Calvo F.	2017	Bacterial Reverse Mutation Test (OECD Guideline 471). AbioProtect® (Abp1 and Abp2). Vivotecnia Research S.L., Spain. Report number: B-02314 GLP Not published	N	Y	Proprietary information	Abiopep S.L.
B6.1.2.4-01/1	Žegura B., Novak M.	2017a	The effect of tomato leaves extract infected with naturally ocurring mild isolates of *Pepino mosaic virus* (PepMV, EU, strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) on viablility and proliferation of human alveolar epithelial cells type 2 A549 determined with the MTT assay. Department of genetic toxicology and cancer biology. National Institute of Biology. Slovenia. Report number: 10G002-2017 GLP like protocols Not published	N	Y	Proprietary information	Abiopep S.L.
B6.1.2.4-01/2	Žegura B., Novak M.	2019a	The effect of tomato leaves extract infected with naturally ocurring mild isolates of *Pepino mosaic virus* (PepMV, EU, strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) independently on viablility and proliferation of human alveolar epithelial cells type 2 A549 determined with the MTT assay. Department of genetic toxicology and cancer biology. National Institute of Biology. Slovenia. Report number: 10G007-2019 GLP like protocols	N	Y	Proprietary information	Abiopep S.L.

			Not published				
B6.1.2.4-02/1	Žegura B. et al	2017b	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	N	Y	Proprietary information	Abiopep S.L.
B6.1.2.4-02/2	Žegura B., et al.	2019b	Infectivity and replication of *Pepino mosaic virus* (PepMV, EU, strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) independently in human alveolar epithelial cells type 2 A549. Department of genetic toxicology and cancer biology. National Institute of Biology. Slovenia. Report number: 10G008-2019 GLP like protocols Not published	N	Y	Proprietary information	Abiopep S.L.
B6.1.2.5-01	Hanssen I.M. et al	2008	Genetic characterization of *Pepino mosaic virus* isolates from Belgian greenhouse tomatoes reveals genetic recombination. Eur J Plant Pathol 121. DOI: 10.1007/s10658-007-9255-0. No GLP Published	N	N		
B6.1.2.5-02	Hernando Y.	2017	Focused search of scientific peer review open literature for *Pepino mosaic virus.* CEBAS-CSIC, Murcia. Spain No GLP Not published	N	N		
B6.1.2.5-03	Zhang T., et al	2006	RNA viral community in human feces: prevalence of plant pathogenic viruses. PLoS biology 4:e3. No GLP Published	N	N		