European Commission

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

Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV)

Volume 1

Rapporteur Member State: Spain

April 2020

Version History

When	What
18/09/2018	Completeness check report of the dossier submitted by the notifier
December 2019	DAR submitted to the Notifier for commenting
February 2020	DAR updated with notifier comments
April 2020	DAR updated after EFSA completeness check

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Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV)

Statement of subject matter and purpose for which this report has been prepared and background information on the application

1.1 Context in which the assessment report was prepared

Active substances are approved for maximum period of 10 years under Directive 91/414/EEC. The active substance SeMNPV was under programme of renewal Regulation EU 686/2012 (AIR-III programme). According to draft working document AIR III renewal programme SANCO/2012/11284, *Spodoptera exigua* nuclear polyhedrosis virus was included in Batch 9 *" Active substance Spodoptera exigua nuclear polyhedrosis virus No application for renewal of approval has been submitted. Previous expiry date 30/11/2017"*
Commission implementing regulation (EU) No 844/2012 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 establishes in its Art 1: *"the application for the renewal of an approval of an active substance shall be submitted by a producer of the active substance to the rapporteur Member State, no later than three years before the expiry of the approval"*
The application for the renewal of the active substance *Spodoptera exigua* nuclear polyhedrosis virus was not submitted before of three years before the expiry date of the approval of the active substance SeMNPV (30/11/2017).
The applicant then have submitted an application for SeMNPV as a new active substance.

The present assessment report is prepared for the approval of the new microbial pest control agent *Spodoptera exigua* multicapsid nucleopolyhedrovirus (SeMNPV) and its representative formulation SPEXIT by Andermatt Biocontrol GmbH.
Spodoptera exigua nuclear polyhedrosis virus (SeMNPV) was included in Annex I to Directive 91/414/EEC as a new active in 01/12/2007: The (EU) No 540/2011 list of active substances approved for use in plant protection products include the active substance SeMNPV (Nº 159), with date of inclusion (1/12/2007) and expired date (30/11/2017).

The present microbial pest control agent SeMNPV was already included on Annex I (date of approval: 01/12/2007, expiration of approval: 30/11/2017) and thus evaluated by the RMS Netherlands under 91/414/EEC. Consequently, it as a new active substance, but which was already evaluated. The dossier was submitted to Spain, which acts as Rapporteur Member State for the EU-Commission for Active Ingredient / Substance approval in compliance with Regulation (EC) 1107/2009.

1.1.1 Purpose for which the assessment report was prepared

Andermatt Biocontrol GmbH submits the present assessment report for the approval of the new microbial pest control agent *S. exigua* multicapsid nucleopolyhedrovirus (SeMNPV) and its representative formulation SPEXIT.

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

Spain, acting as RMS, agreed to evaluate the dossier and prepare the RAR before the submission to the Commission and EFSA.

1.1.3 EU Regulatory history for use in Plant Protection Products

The baculovirus *S. exigua*, NPV isolate, SeNPV isolate SeMNPV-F1) was included on Annex I (date of approval: 01/12/2007, expiration of approval: 30/11/2017) (Commission Directive 2007/50/EC) and evaluated by the RMS Netherlands under 91/414/EEC. The Commission presented a Review Report (SANCO/T14/2007-rev. final 12 March 2007) in support to the consideration of Annex I inclusion.

In accordance with Article 6(2) of Directive 91/414/EEC the Netherlands received on 12 July 1996 an application from Biosys (now: Certis USA) for the inclusion of the active substance *S. exigua* nuclear polyhedrosis virus in Annex I to Directive 91/414/EEC. Commission Decision No Reasoned opinion on the review of the existing maximum residue levels (MRLs) for SeMNPV has been published by EFSA.

Consequently, SeMNPV isolate Bv0004 is a new active substance belongs to the SeMNPV baculovirus group, which was previously evaluated by EU. The dossier for the approval of SeMNPV isolate BV0004 was submitted to Spain, which acts as Rapporteur Member State for the EU-Commission for Active Ingredient / Substance approval in compliance with Regulation (EC) 1107/2009.

There are three SeMNPV isolates that were developed as a biocontrol agents and were commercialize in Europe during the last decade, including:

1) **SeNPV-F1** or Florida isolate SeU2, from the product SPOD-X was used in Europe since 2006 (Certis, USA).The SeMNPV-US2 from Florida is being used as active ingredient in the product Spod X, which is used in the USA and Thailand since 1995. This isolate contains 7 different, but closely related genotypes. *S. exigua* NPV-F1 is deposited at the American Type collection (ATCC), Manassas, Virginia 20108, USA and received SD-Number: SD-5339, designation: *S. exigua* NPV S X 121-48-12.

2) **SeMNPV-SP2**, a Spanish isolate from the product VIR-EX was used in Europe since 2008 (Biocolor, Spain). Isolate SeMNPV-SP2 contains the genotypes ALPst0935, ALPst1400 andALPst1033. These genotypes were collected from diseased *S. exigua* larvae on vegetables in greenhouses in El Ejido (Almeria), Spain, in 2002 and 2003.

3) **SeMNPV isolate Bv0004**, from the product SPEXIT was used in Europe since 2010 (Andermatt Biocontrol, Switzerland). The *S. exigua* SeMNPV isolate in use (Bv-0004) was isolated in 2000 from *S. exigua* larvae cadavers infected with nuclear polyhedrosis from a green pepper field in Huaian City, Jiangsu province, China.

SeNPV- F1 was the reference isolate (Florida isolate in Spod-X GH (Se NPV-F1), first included in ANNEX I as an active substance in 2007. Later on, in 2008 (SeMNPV-SP2) and 2010 (SeMNPV-Bv0004) were assessed for the inclusion as new isolates of SeMNPV species already included in Annex I of Council Directive 91/414/EEC, and there have been used as active ingredients until the end of the commercial period in 2017.

1.1.4 Evaluations carried out under other regulatory contexts

The RMS is not aware of any other relevant EU-evaluations of SeMNPV carried out in the framework of other relevant EU-legislation (e.g. flavourings, food additives, cosmetics).

SeMNPV was not included in the Inventory of Evaluations performed by the Joint Meeting on Pesticide Residues (JMPR).

The same product SPEXIT containing the same isolate BV-004 was approved by US EPA in October 2015. Minor label changes evaluated and approved in November 2018.
The RMS did not find any recent (less than 5 years old) evaluation of SeMNPV isolate Bv0004 from US, from, Canada or Australia.

Nevertheless, there are other *S. exigua* isolates currently on use in USA, Canada and Tanzania.

1.2 Applicant(s) information

1.2.1 Name and address of applicant(s) for approval of the active substance

Name	Andermatt Biocontrol Suisse AG (new Swiss subsidiary of Andermatt Biocontrol AG).
Address	Stahlermatten 6, CH-6146 Grossdietwil, Switzerland
Contact person	[redacted]
Phone	[redacted]
Fax	[redacted]
Email	[redacted]

1.2.2 Producer or producers of the active substance

Confidential information – see Volume 4, section C.1.1.1.

1.2.3 Information relating to the collective provision of dossiers

1.3 Identity of the microorganism

1.3.1 Name and species description, strain characterisation	*S. exigua* multicapsid nucleopolyhedrovirus belongs to the family of baculoviruses. The inclusion of other baculovirus results in this dossier is justifiable due to this family relationship. This virus acts highly specific against larvae of the beet armyworm, *S. exigua*, and is not supposed to have any harmful effects on organisms not belonging to the genus *Spodoptera*
1.3.1.1 Composition of material used for manufacturing of the formulated product	
Confidential information, see Vol 4.	
1.3.1.2 Accession number in culture collection	Reference number BV-0004. German Collection of Microorganisms and Cell Cultures (DSMZ), Mascheroder Weg 1b, D-38124 Braunschweig, Germany, deposited in 2006.
1.3.1.3 Scientific name and taxonomic grouping, i.e. family, genus, species, strain, serotype, pathovar or any other denomination relevant to the microorganism	
Taxonomy	*dsDNA virus* Order: *Unassigned* Family: Baculoviridae Genera: *Alfabaculovirus* Specie: *S. exigua multiple nucleopolyhedrovirus* (SeMNPV) Isolate: *Reference number: BV-0004*
Indigenous or non-indigenous	Indigenous, SeMNPV has been isolated from various biotopes worldwide.
Wild type	Yes, the strain was isolated from *Spodoptera exigua larvae*.

Spontaneous or induced mutant*	The strain is not described as a mutant
Genetically modified according to Directive 2001/18/EC*	No
* All known differences between the modified microorganism and the parent wild strain must be provided	
1.3.1.4 Test procedures and criteria used for identification	
Biotest, restriction endonuclease analysis (RFLP) of the viral DNA	
1.3.1.5 Common name or alternative and superseded names and code names used during the development	In the literature, the term *S. exigua* nucleopolyhedrovirus (SeNPV) is also found. No alternative or code names have been submitted. There is no common name for the organism. Other BVs isolated from *S. species*, namely *S. litura* NPV (SpltNPV), *S. terricola* NPV (SpteNPV), and *S. littoralis* NPV (SpliNPV) also were previously classified to the group II NPVs, todays, they are all classified to genera *Alphabaculoviruses*. These species are very closely related to each other, but are more distantly related to SeMNPV.
1.3.1.6 Relationship to known pathogens	SeNPV is only infective on few species within the family Noctuidae, most, if not all belonging to the genus *S.*. SeMNPV have been exclusively isolated from arthropods and not from other animals, humans or plants.
1.3.1.7 Method of manufacture (synthesis pathway) of the active substance	Confidential information, see Volume 4.
1.3.2 Specification of the material used for manufacturing of formulated products	Confidential information, see Volume 4.
1.3.3 Content of the microorganism	$3.75\ 10^{12}$ OB/L
1.3.4 Identity and content of impurities, additives, contaminating microorganisms	
1.3.4.1 Significant impurities	No significant impurities are present.
1.3.4.2 Relevant impurities	No significant impurities are present.
1.3.4.3 Additives	Not applicable
1.3.4.4 Contaminating microorganisms	The level of contaminating microorganisms falls within the limits proposed by OECD in SANCO/12116/2012-rev.0.
1.3.5 Analytical profile of batches	Confidential information, see Volume 4.

1.4 Information on the plant protection product

1.4.1 Applicant	Andermatt Biocontrol GmbH Zellerstrasse 9 79618 Rheinfelden Germany
1.4.2 Producer of the plant protection product	Confidential information, see Volume 4.
1.4.3 Current, former and proposed trade names and development code numbers	
Trade name	SPEXIT
Code number	

1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product	
1.4.4.1 Composition of the plant protection product	Confidential information, see Volume 4.
1.4.4.2 Information on the active substance(s)	Content of active substance *S. exigua* multicapsid nucleopolyhedrovirus 3.75 10^{12} OB/L. The product contains occlusion bodies (OBs).
1.4.4.3 Information on safeners, synergists and co-formulants	Confidential information, see Volume 4.
1.4.5 Type and code of the plant protection product	Aqueous suspension concentrate [SC]
1.4.6 Function	Biological insecticide for the control of *S. exigua* in protected and open field crops.
1.4.7 Field of use envisaged	Use in horticulture, in open field and protected (greenhouse) crops.
1.4.8 Effects on harmful organisms	Insecticidal mode of action in larvae of *S. exigua*.

1.5.1 Details of representative uses

1	2	3	4	5	6	7	8	9	10	11	12	13
Use-No.	Member state(s)	Crop and/ or situation (crop destination / purpose of crop)	F G or I	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application			Application rate			PHI (days)	Remarks: e.g. g safener/synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	OBs/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
1	EU	Pepper (CPSAN)	F/G	*S. exigua* (LAPHEG)	Spray	At infestation (preferably on early larva instar: L1 and L2). First treatment just before hatching)	a) 18 (6) b) 18 (6)	a) 0.2 b) 3.6	a) 7.5×10^{11} b) 1.35×10^{13}	200 / 1600	-	- 2 to 3 applications per pest generation, up to 6 generations (i.e. max. of 18 app.). -Interval between applications: min. of 6 sunny days; 2 partially sunny days = 1 sunny day
2	EU	Leafy vegetables (lettuce crops) (3LETC)	F/G	*S. exigua* (LAPHEG)	Spray	At infestation (preferably on early larva instar: L1 and L2). First treatment just before hatching)	a) 18 (6) b) 18 (6)	a) 0.2 b) 3.6	a) 7.5×10^{11} b) 1.35×10^{13}	200 / 1600	-	- 2 to 3 applications per pest generation, up to 6 generations. -Interval between applications: min. of 6 sunny days; 2 partially sunny days = 1 sunny day

1.5.2 Further information on representative uses

SPEXIT is to be used against beet armyworm *S. exigua* (Hübner) in greenhouse pepper crops and lettuces in open field.
Application rate: SPEXIT is used at 100 to 200 mL/ha at hatching of the first larvae with a water volume adjusted accordingly to leaf area index (200-600L/ha).

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

Not relevant.

1.5.4 Overview on authorisations in EU Member States

Various products, containing SeMNPV are registered in the United States and Spain by the company Andermatt Biocontrol AG (product SPEXIT), other SeMNPV products are registered in Spain by MITSUI AGRISCIENCE INTERNATIONAL S.A./B.V. (product SPOD-X) and by COEXPHAL (product VIR-EX).

Product / Code	SeMNPV isolate	Crop F/G	Country	Registration number	Product application rate per treatment (max)	Active substance application rate per treatment (max)	Number of treatments per season/crop	Active substance total dose/ha (max)
SPEXIT	SeMNPV strain BV-0004	Strawberry, lettuce, herbaceous ornamentals, woody ornamentals, cucumber, pepper, watermelon F and G	Spain	25592	0.2 L/ha	7.5×10^{11} OB/ha	3 treatments per generation	1.35×10^{13} OB/ha
SPEXIT	SeMNPV strain BV-0004	Root and tuber / bulb / leafy / *Brassica* leafy / legume / foliage of legume / fruiting / cucurbit vegetables, citrus fruits, berries and small fruits, oilseeds, cereal grains, nongrass animal feeds, herbs and spices, tree nuts, other crops, floriculture F and G	United States (State registrations: Arizona, Florida, Hawaii)	69553-4	2.5 fl oz/acre (= 182.7 ml/ha)	6.9×10^{11} OB/ha	Not limited	n/a

Level 2

Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV)

Summary of active substance hazard and of product risk assessment

2.1 IDENTITY

The company **Suisse AG (new Swiss subsidiary of Andermatt Biocontrol AG)** submitted data on *S. exigua* multicapsid nucleopolyhedrovirus (SeMNPV) and the formulated product SPEXIT to the European authorities for the evaluation of the microbial pest control agent under Regulation (EC) 1107/2009. SPEXIT is a biological insecticide formulated as suspension concentrate, containing 3.75 x 10^{12} occlusion bodies (OB) of *S. exigua* MNPV in 1 L product.

The inclusion of other baculovirus results in this dossier is justifiable due to this family relationship. This virus acts highly specific against larvae of the beet armyworm, *S. exigua*, and is not supposed to have any harmful effects on organisms not belonging to the genus Spodoptera. With regard to safety problems, it is important to note that SeMNPV and the whole group of baculoviruses are naturally present in the environment. The experience that baculoviruses present no risk to mammals and men has been confirmed by numerous studies. Their application in pest control means only a fluctuation of the virus titre in the biotope of the pest insect.

Studies performed with the products Granupom and MADEX, both containing *Cydia pomonella* granulovirus (CpGV) are considered applicable and relevant with regard to the evaluation of the formulated product SPEXIT.

2.1.1 Identity of microorganism

The microbial pest control agents of the biological insecticide product against armyworm in pepper and leafy vegetables (lettuce crops) in protected and open field crops named SPEXIT is the baculovirus SeMNPV, isolate BV-0004, is a member of the genus *Alfabaculovirus*, family, *Baculoviridae*.

The strain was deposited in 2006 in the German Collection of Microorganisms and Cell Cultures (DSMZ), Mascheroder Weg 1b, D-38124 Braunschweig, Germany, with the reference number BV-0004.

Deposit of SeMNPV in a reputable culture collection Global Catalogue of Microorganisms member collection has been confirmed by DSMZ deposit document. Once the isolate is deposited in DSMZ, is assigned a number (BV-0004).The quality and authenticity of the material is checked according to criteria of viability, purity, identity, and stability. The depositor must provide the identification at specie level information. The preservation of the MPCAs SeMNPV in DSMZ makes the isolate searchable and accessible at once.
Confirmation document regarding further descriptions of Bv-0004 isolate need to be provide: Depositor, Origen of the isolate, Host isolation, receipt and acceptance, identification of the microorganism, scientific description and proposed taxonomic designation and stability statement. Then isolate can be traced through all publications it is mentioned in, including patent files, with its assigned number (Bv-0004). This is considered a data gap.

SeMNPV isolate used in SPEXIT was originally isolated from infected *S. exigua* larvae. The virus isolate was characterised by restriction fragment analysis. The DNA sequence of a related isolate is completely determined and phylogenetic analyses revealed affiliation to Alphabaculovirus, group II NPVs within the Baculoviridae.

SeMNPV is originate from natural, indigenous wild type viruses and is not genetically modified.

SeMNPV as well as other baculoviruses is not related to any known plant pathogen or animal of human pathogens and they do not produce secondary metabolites.

Identification of SeMNPV isolate contained in SPEXIT product has been achieved to species level. However, no unequivocal identification at strain level has been presented by the applicant. According to specific consideration for BVs registrations, this is not considered a data gap.
SeMNPV isolate Bv0004 from China, contained in SPEXIT, cannot be differentiated from California isolate SeMNPV-US1.

RMS suggests the specific identification of the target strain Bv0004 from DSMZ deposit by RFLP tecniques or by the study of strain virulence, by genetic or phenotypic approach, taking into account KMA 1.3/11 report. The molecular identification of the strain Bv0004 is required in order to compare to SPEXIT product.
Biotests are conducted to see whether the efficacy of the virus is guaranteed. The activity (infectivity) of the occlusion bodies is determined by a quantitative bioassay. The identity of the virus can be checked against the parent strain by restriction endonuclease analysis of viral DNA.

The content of pure virus in SPEXIT SeMNPV, technical is set up to be at least $3.75x10^{12}$ occlusion bodies/L.

No toxins are expected in the product, as BVs do not produce any toxin.

The absence of potential human pathogens is also analysed by ISO methods. In case a human pathogen is detected in a batch, such batch would be destroyed.

2.1.2 Identity of the microbial plant protection product

The microbial pest control agents of the biological insecticide product against armyworm in pepper and leafy vegetables (lettuce crops) in protected and open field crops named SPEXIT is the baculovirus SeMNPV, isolate BV-0004, is formulated to contain at least 3.75 x 10^{12}OB/L.

The SeMNPV isolate contained in SPEXIT product was characterised by analysis of restriction fragments obtained after enzymatic digestion and compared to the California isolate SeMNPV-US1. The SeMNPV isolate contained in SPEXIT is identical to the pattern of SeMNPV-US1. Phylogenetic analysis of marker genes revealed that SeMNPV belongs to the Group II Nucleopolyhedroviruses.

All the concerned MPCA data points (microbial pest control agent: SeMNPV), and MPCP data points (microbial pest control product SPEXIT) have been addressed and included in the corresponding Vol 3 MA and MP reports.

2.2 BIOLOGICAL PROPERTIES

2.2.1 Summary of biological properties of the microorganism

SeMNPV is an important pathogen of armyworm, *S. exigua* that plays a relevant role in regulating its natural host. The baculovirus SeMNPV belongs to Group II of the genus Alphabaculovirus, differs significantly from other baculoviruses species in that it is a highly pathogenic host-specific virus.

SeMNPV and the whole group of baculoviruses are naturally present in the environment. Their application in pest control means only a fluctuation of the virus trite in the biotope of the pest insect.

The mode of action of SeMNPV is a bi-phasic infection process of the larval stages of the above cited hosts. After oral ingestion of viral occlusion bodies, the virus replicates in the midgut cells (primary infection) and then infection is spread via non-occluded viruses to other body tissues (secondary infection) leading to the insect's death.

2.2.1.1 History of the microorganism and its uses, natural occurrence and geographic distribution

SeMNPV is of particular interest for the bioinsecticide industry owing to its very high pathogenicity, fast speed of kill, and total specificity. For this reason, different isolates of SeMNPV have been produced and commercially registered in several countries for the control of *S. exigua* populations.

The selection of indigenous isolates that are suitable for development as biological control agents requires the characterization of the isolate present in each geographical region. Molecular and biological characteristics of these isolates have been widely reported. A high degree of specificity and infectivity of SeMNPV against *S. exigua* both *in vivo* and *in vitro* has been demonstrated.

The usefulness of SeMNPV in control of beet armyworm has focused interest in selecting strains with high pathogenicity and genetic stability. Different strains of naturally occurring of SeMNPV have been isolated from many geographic regions of the world. Since Steinhaus (1949) first isolated SeMNPV in California (USA), several SeMNPV isolates, including SeMNPV-SP3, SeMNPV-SP1, SeMNPV-SP2, SeMNPV-UZB, SeMNPV-TH, and SeMNPV-608 have been characterised from different regions world-wide, including the Netherlands, California, Spain, Florida, Japan, Thailand, China and Mexico. These viruses constitutes the active ingredient in a number of bioinsecticides sold in different countries for the control of *S. exigua* populations in both open-field and greenhouse crop systems, including: SPOD-X® (Certis LLC, Columbia, Maryland, USA), VIR-EX® (Biocolor, El Ejido, Almería, Spain) and SPEXIT® (Andermatt Biocontrol, Grossdietwil, Switzerland).

The *S. exigua* SeMNPV isolate in use (Bv-0004) was isolated in 2000 from *S. exigua* larval cadavers infected with nuclear polyhedrosis from a green pepper field in Huaian City, Jiangsu province, China. SeMNPV isolate Bv-0004, from the product SPEXIT was used in Europe since 2010 (Andermatt Biocontrol, Switzerland).

2.2.1.2 Description of target organism(s)

Beet armyworm, *S. exigua* (Hübner) (Lepidoptera: Noctuidae), is one of the most destructive polyphagous important pest world-wide of a wide variety of crops in sub-tropical and tropical regions and in glass houses of temperate regions feeding on crops that include corn, pea nuts, tomatoes, peppers, and sweet peppers. In recent years, it has caused serious economic losses in America, Asia, Europe, Oceania, and Africa. Currently, there are no reports on taxonomical differences between the populations of different parts of Europe. *S. exigua* (Hübner), a polyphagous noctuid pest, can be found both in open air and in greenhouse crops in the Mediterranean area, but is mostly confined to greenhouse crops in Central and Northern Europe. In the Netherlands, *S. exigua* is frequently found as a pest in sweet pepper and other greenhouse crops since 1976.

2.2.1.3 Mode of action

The feeding larva ingests the virus and the protective virus protein matrix is dissolved in the insect's midgut, releasing the virus particles. These pass through the peritrophic membrane and invade midgut cells by fusion with the microvilli. The virus particles invade the cell nuclei where they are uncoated and virus DNA is replicated. Initial replication produces non-occluded virus particles to hasten the invasion of the host insect. Later the virus particles are produced with protein matrices and remain infective when released from the dead insects.

2.2.1.4 Host specificity range and effects on species other than the target harmful organism

SeMNPV is not supposed to have any harmful effects on organisms not belonging to the *Spodoptera* genera. No virus from the family of baculoviruses are related to any animal or plant pathogen and it does not produce any metabolite. For these reasons, no harmful effects from SeMNPV on humans, other vertebrates, other non-target organisms or the environment are expected.

It is well documented the high host specificity of BVs family. Even though, there is no studios with the isolate SeMNPV-Bv0004 of the effect in non-target related arthropods (S. *litura, Agrotis ipsilon, A. segetum, Bombyx mori, Hyphantria cunea, or Stilpnotia salicis*).

There is no scientific evidences confirmed SeMNPV-Bv-0004 only infects the larvae of *S. exigua.*

It cannot be confirmed the high host specificity of *SeMNPV-Bv0004.* A data gap is therefore identified.

2.2.1.5 Development stages/life cycle of the microorganism

Occlusion bodies are ingested by the larvae. Subsequently, the occlusion bodies are dissolved in the alkaline midgut and occlusion-derived virions (ODV) are released. The nucleocapsids attach to the microvilli and enter the midgut epithelium cells. The nucleocapsids are transported to the nucleus and release their DNA, followed by DNA replication and expression. After virus replication in the midgut cells, budded virus are released through the basal lamina into the hemocoel and subsequently spread to other organs, like the fat body, epidermis, tracheal matrix, muscle, nerve, Malpighian tubules, and glandular tissues. Finally, occlusion bodies are formed, leading to hypertrophy of cells and the swollen appearance of larvae at late infection stages. Disintegration of the larval cadavers leads to release of new occlusion bodies which can again infect larvae.

In order to be effective, SeMNPV have to be ingested by larvae of the beet armyworm with the food. The virus particles dissolve under the alkaline conditions in the larval gut and liberated virions penetrate the gut cells and multiply. After most tissues are infected the larva dies. The mechanisms of host invasion, viral spread, tissue tropism and gross pathology have however been shown to be broadly common among all nucleopolyhedrosis viruses infecting Lepidopteran hosts.

Application of SeMNPV should be timed at hatching of larvae so that early-instar larvae meet the virus. The early instar larval stages of the insect life cycle are the most susceptible to infection with BVs. Following the application of BVs-based insecticides, a portion of pest population often survives to adulthood and may reproduce and lay eggs on the same, or nearby crop. During this period, the original inoculum applied to the crop is rapidly inactivated by solar ultraviolet radiation and diluted by rainfall and the growth of the plants, leaving little inoculum available to infect and control the following generation of pest larvae.

Infectiveness, dispersal and colonisation ability

BVs adopt a mixed-mode transmission strategy involving both horizontal and vertical transmission. Transmission of BVs can be horizontal among individuals through environmental contamination, or vertical from parents to offspring on or in the eggs. Upon death of an infected larva, OB are released and other larvae become infected if they ingest the OB on contaminated foliage. Vertical transmission can occurs if larvae consume occlusion bodies but pupate before death leading to sub lethally infected adults. Adults sub lethally infected as larvae might transmit virus to their offspring either on or in the eggs. This can lead to an active infection killing the offspring or potentially a covert infection that is passed on to offspring that will survive.

OB treatment results in rapid establishment of sublethal infections that persist between generations. Key pathogenicity and virulence traits of SeMNPV isolates vary according to their principal transmission strategy. Vertical transmission genotypes were generally capable of producing a high prevalence of persistent infection in adults that survived an inoculum challenge during the larva stage, since horizontal transmission genotypes tended to have higher OB pathogenicity and faster speed of kill compared to vertical transmission genotypes. The applicant did not discuss these mechanisms of transmission and dispersion and the RMS suggests it needs further investigation. **This is considered a data gap**.

2.2.1.6 Relationships to known plant or animal or human pathogens

BVs exclusively have been isolated from arthropods, primarily from the three insect orders Lepidoptera, Hymenoptera, and Diptera. In general, the host range of most BVs is restricted to one or few species of the genus or family of the host where they were originally isolated.

SeMNPV is a BV belong to the family *Baculoviridae*, which are arthropod-specific, enveloped viruses with a circular double-stranded DNA genome. SeMNPV is a naturally occurring virus worldwide and acts highly specific against larvae of the beet armyworm, *S.exigua*; therefore, the presence of SeMNPV in the environment is linked to the presence of the host, *S. exigua*. Thus, application of BVs in pest control means only a fluctuation of the virus titre in the biotope of the pest insect.

SeMNPV as well as all other BVs are not related to any known plant, animal (other than arthropods) or human pathogen.

2.2.1.7 Genetic stability and factors affecting it

The used strain of SeMNPV is maintained at –24°C since its isolation. No variation in infectivity to the host was observed. But there is no specific studies or information regarding genetic stability of SeMNPV isolate BV-0004. The possibility that the MPCP introduced BVs might recombine with indigenous strains and produce recombinants with novel characteristics is an important consideration for the introduction of SeMNPV.

It has also been reported that the passage of SeMNPV in *S. exigua* cell lines leads to the rapid accumulation of deletion mutants, some of which have been demonstrated to have defective interfering properties. The OBs of these mutants are significantly less virulent in *S. exigua* larvae. This phenomenon is due to a deletion of about 25 kbp from the SeMNPV genome. Moreover, the genotypic alterations can result in changes in phenotypic characteristics, including virus morphology and biological activity. The occurrence and accumulation of defective or mutant viruses upon passage of NPVs in cell culture have been reported in several studies. The most common mutants are few polyhedra and defective interfering particles. Both mutant viruses cause significant reduction in OB formation and virus infectivity.

Genetic stability of the target BV SeMNPV BV000-4 is unclear. No information was submitted: There is no information on genetic stability of the specific isolate SeMNPV BV-0004 during the production process, the storage or under environment condition of use. There is no information discussing whether genetic transfer may occur in soil, or any other environmental compartment. **This is considered a data gap**.

2.2.2 Summary of physical, chemical and technical properties of the plant protection product

Environmental factor that can influence in environment survival outside the host

The effect of temperature on BVs is significant regard to their stability in storage and in the field following application. Whereas expose to low temperatures has little effect on activity of the OB, expose to high temperatures would cause inactivation. 10-min expose to temperatures of 70-80ºC would expected to inactivate the virus. *In situ* studies on the effect of temperature indicate that the virus should withstand maximum temperature normally encountered in the field environment (air temperature 31ºC) or soil temperature (50ºC), for short periods.

Sunlight is considered the most important factor contributing to the inactivation of viral inclusion bodies. The ultraviolet portion of sunlight inactivates insect viruses. The sensitivity of different BVs species to short-wave UV light (254 nm) and to longer-wave UV light (285-380 nm) was observed in *in vitro* and in *situ assays.*

Humidity has less effect on stability of insect viruses than on stability of other types of pathogens. However, there may be an indirect influence by affecting chemical action on the virus, by increasing the inactivation rate by sunlight, and by washing off the virus from leaves.

pH of the soil may affect persistence of viruses. Viruses can persist in soil for longer periods. Extreme hydrogen ion concentration buffers have an adverse effect on the infectivity of BVs. There is correlation between pH and virus activity: the lower the pH, the more rapidly the virus was inactivated. In general, it is not clear whether, or how much, virus in soil is available for initiating infection in pests.

Effect of environmental substrates

Crop leaf is the most important substrate to consider due to SeMNPV target organism, beet armyworm, are leaf-eating insects. The inactivation of SeMNPV by exposure to sun light is the most important factor affecting stability of SeMNPV deposit on leaf. Inactivation by sunlight is influenced by substrate; therefore, lettuce leaf would not protect virus deposit from sunlight.

Water. While it is known that most viruses in intact occlusion bodies are reasonably stable in aqueous suspension, little is known on their persistence in natural aquatic environment. It is supposed that the pH and salt concentration

of water would influence stability. Various chemicals, as bicarbonates, carbonates, sodium, potassium and calcium found in dew on leaves could have been responsible for the effects of the dew on deposits of the BVs.

Soil. Several studios have confirmed that BVs may persist for long periods in soil. The BV activity were been reduced by the soil depth. OB appear to be adsorbed quite firmly onto soil particles and are not leached through the soil by rain. The pH of the soil may also affect persistence of the virus BVs can persist and accumulate on soil following application or natural epizootics of disease.

All information provided relate to physical, chemical and technical properties of the plant protection product are for virus MPCA in general, and some are related to BV species other than SeNPV. According to data provided, the sun light may play an importance role in the persistence and survival of SeMNPV. The RMS recommends the evaluation of UV light in the target MPCA SeMNPV isolate Bv-0004.

There is no specific storage stability studio on SPEXIT product. Considering physico-chemical properties of the formulation SPEXIT over 2 Years at 5 °C is required. This is considered a data gap.

Dispersal and Persistence in the environment outside the host

Dispersal of BVs can occur through small animals and birds (their faeces are able to contain infective viruses), predators, wind blow of dry soil and rain splash at canopy edges. Knowledge of the importance of such mechanisms is scant.

Factors affecting virus activation

Covert infections were first proposed to explain the spontaneous outbreaks of BVs disease that occurred in apparently healthy insects. Physiological stress was a major contributor to virus activation. Specifically, overcrowded rearing conditions marked changes in temperature or relative humidity. The ingestion of mildly toxic chemical compounds, parasitism or changes in nutrient availability, have all been reported as potential activators of covert disease.

The ability to respond to changes in expected host survival and anticipated reproduction, and adopt the transmission pathway that will maximize the fitness derived from each infected host, provides a unique evolutionary advantage to these viruses. As such, phenotypic plasticity in transmission strategy means that viruses with mixed-mode transmission can persist under a wider range of ecological conditions and at a higher prevalence than viruses that adopt strict single-mode transmission.

Under a persistence and dispersion point of view, It would be necessary to know the transmission strategies of SeMNPV Bv-0004 isolate.

2.2.3 Information on the production of metabolites (especially toxins)

BVs do not have any potential to form toxins and are not able to produce secondary metabolites or metabolites of concern for human health or the environment.

2.2.4 Antibiotics and other anti-microbial agents

Viruses are sensitive to disinfectants that act by a chemical or physical principle and for which resistance is not a case. Viruses can be also susceptible to virucidal or virustatic drugs acting mostly by inhibiting certain enzymes. Indeed, viruses may develop resistance to these compounds. However, the mechanism behind the occurrence of viral resistance is different from those in, e.g., bacteria and there is no evidence that resistance may be transmitted from one virus species to another.

2.3 DATA ON APPLICATION AND EFFICACY

2.3.1 Summary of effectiveness

Viral enthomopathogen biological insecticide. The active ingredient of SPEXIT® is a naturally occurring virus of *S. exigua*. It is highly selective for larvae of *S. exigua* and therefore harmless to beneficial and other non-target organisms. *S. exigua* is a polyphagous pest feeding on over 200 different crops amongst which there are: sugar beet, cabbage, lettuce, soybeans, cotton, maize, tomato, potato, legumes, citrus, strawberry, melon, leek, garlic, onion, rice, flax, and tobacco.

The interactions between the insects (*S. exigua*) and the virus SeMNPV are highly context dependent; factors, particularly diet, can have strong impacts on virulence, transmission and host resistance or tolerance.

The host range of SeMNPV was determined in cross-infection experiments concluding in being infective only for *S. exigua* and not for other noctuid lepidopteran species.

2.3.2 Field of use envisaged

Control of *S. exigua* in horticulture and home-gardening. SPEXIT is applied by foliar spraying: tractor drawn motor sprayers and knapsack sprayer.

2.3.3 Crops or products protected or treated

SeMNPV is intended to be used in pepper, cucumber, watermelon, strawberry, lettuce, carnation and hops.

The host plant species play an important role in mediating the infectivity of the entomopathogen SeMNPV to *S. exigua*, which modified the key aspects of the insect-baculovirus interactions. In the case of the SeMNPV intended used lettuces and pepper, there is no significant difference in larvae % of mortality. Virulence and transmission are positively related among genotypes of SeMNPV.

2.3.4 Method of production and quality control

CONFIDENTIAL information, please refer to confidential document Volumen 4

2.3.5 Summary of information on the development of resistance

BVs have been used to control lepidopteran pests on 2 to 3 million hectares per year worldwide, with high specificity and low environmental impact and with only sporadic and anecdotal reports of resistance. Until now, there has been no indication of decreasing efficacy of SeMNPV against *S. exigua* larvae. Despite a considerable time of use of SeMNPV in plant protection products, no indication of decreasing efficacy of SeMNPV against *S. exigua* larvae has been reported until now.

Nevertheless, development of resistance depends on the mode of action. The more specific the action and the biochemical site, with a few or single gene(s) being involved, the more probable is development of resistance. The high specificity for the host and the mode of action make the development of resistance possible. Therefore, the risk for development of a resistance to SeMNPV in *S. exigua* populations must be considered. The development of resistance of the target organisms facilitated, in part, by their unique morphology whereby multiple infective genomes are packaged together in virus particles, which are themselves occluded within the OB. SeMNPV diversity in low-density host populations can be surprisingly high. At high host densities, when virus infection is widespread, mixing of virus could provide the opportunity for virus recombination. Thus, a key issue here is whether pathogen diversity changes at different stages in the population cycle and whether this influences virulence, and potentially changes in host resistance.

Vertical transmission may be an interesting feature to improve pest control strategies, and the establishment of covert infections in populations that may eventually trigger fatal disease in larvae causing damages in the crops. SeMNPV transgenerational transmission might reduce the number of applications of baculovirus-based insecticides, improving their effectivity in field and the risk of resistance development.

It is expected that the risk of *S. exigua* developing resistance to SPEXIT is rather low. The product is therefore assumed to be a valuable component in resistance management strategies. Furthermore, in contrast to broad spectrum insecticides, the specifically acting product SPEXIT offers the advantage that natural antagonists of *S. exigua* as well as all other species are not affected.

2.3.6 Summary of adverse effects on treated crops

Effects on the quality of plants or plant products

SPEXIT and its component achieve a high efficacy against *S. exigua*, showing no symptoms in fruits during the trials with similar data to the un-treated.

Effects on the transformation process

Treatment with SPEXIT will not have any interference on transformation processes as viruses have no metabolism of their own, it does not produce residues and neither leaves residues at harvest.

Effects on the yield of treated plants or plant products

The formulation SPEXIT and its component achieve a high efficacy against *S.exigua*, showing no symptoms during the trials with similar data and total production (yield) to the un-treated control and with clear significant differences with the challenged inoculated controls.

Phytotoxicity to target plants (including different cultivars), or to target plant products

Treatment with SPEXIT and its component resulted in no symptoms of virus infection.

2.3.7 Summary of observations on other undesirable or unintended side-effects

Impact on succeeding crops

Impact on succeeding crops was not tested in the efficacy trials. However, as the persistence in water GEP study concluded that the Plant Protection Product with related Bvs have no persistency in the leachate from plants treated with, it could be concluded that there is no risk of SeMNPV infection with this leachate to succeeding crops.

Impact on other plants, including adjacent crops

Impact on other plants including adjacent crops was not tested, as there are no indications that the plant protection product could affect adjacent crops via vapor drift.

Impact on treated plants or plant products to be used for propagation

Not relevant. The formulation SPEXIT is not for used in plants or plants product to be use for propagation, more specifically it is not intended to be use in the production of seeds, cuttings or runners for propagation.

Effects on beneficial and other non-target organisms

In the different tests and studies conducted with related BVs, no effects on the incident of other non-target organisms or environmental effects have been observed. There is no studio on beneficial and other non-target organisms performed with SeMNPV-Bv0004. This is considered a data gap.

2.3.8 Methods to prevent loss of virulence of seed stock of the microorganism

Studies on long-term storage indicate that nucleopolyhedrovirus preparations are affected by the method of extraction, purification, formulation and storage conditions. Stability in storage is improved by storing at low temperatures and away from light.

The studies testing the physical, chemical and technical properties were determined for other baculovirus products (MADEX, containing *Cydia pomonella* granulovirus (CpGV), HELICOVEX containing *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) and LITTOVIR containing *S. littoralis* nucleopolyhedrovirus). The composition of MADEX, HELICOVEX and LITTOVIR is comparable to that of SPEXIT.

MADEX, LITTOVIR and HELICOVEX are grey-brown and odorless suspension concentrates which are not explosive, oxidising or flammable. The pH is within the neutral range. No loss of efficacy is noted when MADEX, LITTOVIR or HELICOVEX is stored at – 18 °C for two years. The technical properties of MADEX, LITTOVIR and HELICOVEX indicate that no particular problems are to be expected when it is used as recommended.

Before and after storage the LD_{50} of the test items was not significantly lower than that of the reference item. Therefore, the test items are considered to be stable when stored for 42 months at 5 ºC.

The DNA of OB is highly degraded during storage at 25ºC. In contrast, DNA purified from OBs that had been stored refrigerated or frozen did not suffer degradation.

Jenkins and Grzywacz (2000) have pointed out that bacteria proliferate rapidly after death of an infected insect and that a target of 1 x 10^8 CFU/ml (aerobic conditions) for liquid formulations or 5 10^8 CFU/g for dry powders is likely to be both safe and attainable. According to Lasa et al, 2008 "Spodoptera exigua Multiple Nucleopolyhedrovirus formulations stored as an OB suspensions contained in general an average of 1.9 (±0.5) 10^8 CFU/g, composed of ~50% aerobes and ~50% anaerobes.

According to Lasa *et al*, 2008, the quantities of microbial contaminants that are present in a formulated OB suspension of a baculovirus microbial insecticide contained an average of 1.9 (±0.5) 10^8 CFU/g, composed of ~50% aerobes and ~50% anaerobes.

The SeMNPV isolate used to produce SPEXIT does not have any morphological characteristics that differ from the classical description of the species. The activity of SeMNPV stored under refrigerated or frozen conditions was comparable that of newly formulated material. SeMNPV products storage and distribution in refrigerated conditions would favour a product shelf life of over 18 months.

There is no specific storage stability study on SPEXIT product. Considering physic-chemical properties of the formulation SPEXIT over 2 Years at 5 °C is required. The microbial contaminants have to be determined before and after 2-year storage. This determination has to be performed with validated methods or with international standard methods and have to be in accordance with the threshold limits indicated in OECD 65 (Oct. 2011).**This is considered a data gap. The storage stability study was requested by the RMS and is initiated.**

2.4 FURTHER INFORMATION

2.4.1 Recommended methods and precautions concerning handling, storage, transport or fire

Handling of SeMNPV does not require special precautionary measures or protective clothing.

The usual precautions for handling chemicals should be observed:

- To avoid any direct contact with the product.
- To wash hands after contamination with the product.
- To keep (remaining) product away from waters.

(1) Storage conditions: Store in original package only	
Advises for Storage with other Products	None
Other Information on Storage	– To maintain quality: Store below 0 °C – Stored in the refrigerator (< 5 °C) for two years – Stored at -18 °C for years without any loss of activity
(2) There are no restrictions regarding transport on land or sea	
(3) In case of fire the following information and instructions are given	

Extinguishing media	– Water mist, alcohol resistant foam, carbon dioxide, dry powder
Measures unsuitable for safety reasons	– Water-jet, foam
Special hazard properties (products or vapours from thermal combustion):	– Vapours cause coughing. – At elevated temperatures (> 200 °C), there is a risk of exothermic polymerization. – At temperatures > 280 °C, acrolein may be formed.
Protective equipment	– No specific recommendations. Use protective clothing
Cleaning/disposal	– Spray remaining product dispersed in liquid manure. Small amounts can be added to compost
Other advices	– Avoid contact with oxidizing agents. Cool closed containers with water
(4) The standard protection measures for workers are adequately protecting their health in case SeMNPV containing material is released or spilled accidentally at the manufacturing facilities	
(5) The contaminated area may be cleaned by sweeping up spill, and spillage can be safely disposed of in accordance with all applicable federal, state, and local environmental regulations.	
(6) **First aid measures**	
General advice	– Change any contaminated or wetted clothing at once. If poisoning occurs contact a doctor
Skin contact	– Remove contaminated clothing. – Seek medical advice if irritation develops. – Launder clothes before reuse. – After contact with skin, wash immediately with plenty of water
Eye contact	– Rinse thoroughly with plenty of water. – Eyelids should be hold away from the eyeball to ensure thorough rinsing. – Seek medical advice if irritation develops
Ingestion	– No typical symptoms and affects known
Inhalation	– This is only possible by exposure to HOT product or to spray. – Move to fresh air, rest, half upright position, loosen clothing. – Oxygen or artificial respiration if there is difficulty in breathing. – Seek medical advice after significant exposure. – Symptomatic treatment is advised.
Other information	– None
Advice to Physician	– Symptomatic treatment is advised
Persons who may want to seek medical attention upon accidental contact to SeMNPV, should inform the physician about the identity of the virus on species level, and may show the label of the packaging as supporting information	

2.4.2 Procedures for destruction or decontamination

With regard to destruction and decontamination the following accidental release measures and general information and recommendations are given:

Decomposition/ persistence	– Decomposition is achieved by thermal combustion at 600 °C without any residues or hazardous products
Special advice for humans and environment	– Keep (remaining) product away from waters – Avoid any contact with the product – Non-hazardous to honeybees due to the selectivity of the product – Non-hazardous to relevant populations of beneficial due to selectivity of the product
Disposal/ Product	– Waste resulting from the use of the product must be disposed on site or on an approved waste disposal facility – Empty the sprayer out in the field being treated by spraying out on to a relatively pest free part of the field left unsprayed or under-dosed for the purpose – Do not exceed the maximum dose approved for the crop
Disposal/ Packages	– Recommendations: add empty packages to local waste disposal or recycling system
Cleaning Agent	– No special cleaning necessary

2.4.3 Measures in case of an accident

Specific measures in case of an accident are not required, since SeMNPV is a common BV, and this family imposes no risk for environment or human health.

SeMNPV is highly host-specific and does not cause any harm to organisms other than the target organism *S. exigua*.

For further details on first aid measures, see Vol 3, B4 on the active ingredient, section B.4.3.

2.5 ANALYTICAL METHODS

2.5.1 Methods for the analysis of the microorganism as manufactured

2.5.1.1 Methods for the identification of the microorganism

The occlusion bodies of SeMNPV are counted under the light microscope at 1000-fold magnification. The virus titre in the end-use product is adjusted to the requested content (OB/L). Morphological criteria are not suitable enough for the characterisation of the isolate, as all NPVs have a very similar morphology. The identity of the virus can be verified by molecular techniques as restriction endonuclease analysis of viral DNA (REN).

The digestion of viral DNA by different specific RENs, produce a specific restriction patterns and small genotypic variations can be identified in the restriction map between different BVs isolates.

Quantification method: Occlusion body counts.

The virus titre in a purified NPV suspension is determined by direct counting under a light microscope. Since the size of the virus occlusion bodies is at the limit of light microscopic resolution, it is necessary to use dark field or phase contrast observation and the most powerful magnification possible (40-fold lens magnification). The disadvantage of a very restricted depth of focus can be compensated to some extent by the use of a counting chamber (hemocytometer).

Genotypic characterization (Molecular identification)

The molecular identification was determined by restriction endonuclease analysis (REN). The analysis of viral DNA was performed to confirm both identity and genomic integrity of SeMNPV.

REN analysis profiles of different isolates of commercial BVs showed some genetic differences; most evident in SeUS1 (SPEXIT) that contained five submolar bands, which suggested the presence of different genotypic variants within the isolate. SeMNPV isolate SeUS1 can be differentiated by restriction endonuclease analysis from two commercial SeMNPV isolates and other four Mexican isolates.

The SeMNPV. isolate SeUS1 can be differentiated by restriction endonuclease analysis from the rest of commercial SeMNPV isolates and other four non-commercial isolates.

The different identity of SeMNPV isolate Bv-0004 and SeMNPV isolate SeUS1 is unclear. Confirmation of differentiation between isolate Bv-004 and SeUS1 need to be provided by the applicant. **A data gap is therefore identified.**

Phenotypic characterization (Biological identification)

The biological activity of the isolates in terms of mean lethal doses (LD50), mean time to death (MTD), and OBs (occlusion bodies) yield is considered necessary

The **median lethal dose (LD_{50})** is determined in second-instar *S. exigua* larvae from insect colonies. The pathogenicity of isolates is determined by the droplet-feeding method.

Pathogenicity, as **LD_{50}** based on an average ingested volume of 0.33 ml per larva in this instar.

ime–mortality response as **Mean time to death (MTD)** of the isolate is determined for the dose of approximately 27 OBs per larva per larva for second and fourth-instar *S. exigua*. The concentration is previously determined for the isolate, which is previously estimated to result in ~90% mortality.

Determination of OB yield: as mean values of weight gain (MWG) and OB yield per larva (OBY).

OBs production is determined in batches of 45 fourth-instar larvae that are starved overnight and inoculated with an OB concentration (~ 27 OBs per larvae) that resulted in ~ 90% mortality. Newly molted *S. exigua* larvae are individually weighed and allowed to drink from aqueous suspensions as described above. Larvae that consumed the inoculum are placed individually on an artificial diet and maintained at 25 ºC. The weight and molting time of individual larvae is registered daily until death to assess the influence of infection on the development of larvae and OB production. Weight measurements are taken immediately before inoculation in fourth instar larvae and after larvae are checked daily for mortality from 3 d post infection until death or pupation. When larvae become moribund and show clear signs of advanced infection, they are individually transferred to clean 1.5-ml vials. The OB collection procedure is identical to that mentioned above for virus amplification. Three replicates are performed. OBs yield is calculated using 40–45 NPV-killed larvae that are randomly selected for the isolate. OBs are process and purify as described above. Purified OBs ware resuspended in 1ml of sterile distilled water and appropriately diluted for titration by triplicate using a Neubauer chamber.

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

Either SeMNPV is of natural origin and not a mutant or genetically modified organism; a mutant can come up in following generations and differs from the parental isolate. This mutant can be less effective in the control of the target insect, and can have different biological characteristics.

Additional information should be requested from the applicant. It would be necessary to confirm there are no modification in the molecular pattern and in the biological activity of the seed stock:

- **Molecular variability need to be determine by REN analysis.**
- **Biological activity variability need to be determine compared with the reference seed stock, in terms of mean lethal doses (LD_{50}), mean time to death (MTD), and OBs (occlusion bodies) yield.**

A data gap is therefore identified.

2.5.1.3 Methods to differentiate a mutant of the microorganism from the parent wild strain

Either SeMNPV is of natural origin and not a mutant or genetically modified organism; a mutant can come up in following generations and differs from the parental isolate. This mutant can be less effective in the control of the target insect, and can have different biological characteristics. Data gap is pointed in the section.

2.5.1.4 Methods for the establishment of purity of seed stock from which batches are produced and methods to control that purity

There is no provided information. Clarification is needed regarding the production process.

Each production batch is started from the initial seed stock culture, which is maintained as frozen vials. Thus mutations in the original parent strain SeMNPV are excluded. Any spontaneous mutation occurring during production will most likely be reflected in a change of agarose gel restriction enzyme pattern and/or biological activity. Thus, any change need to be detected during identification and quality control:

- Molecular variability need to be determine by REN analysis.

- Biological activity variability need to be determine compared with the reference seed stock, in terms of mean lethal doses (LD_{50}), mean time to death (MTD), and OBs (occlusion bodies) yield.

2.5.1.5 Methods to determine the content of the microorganism in the manufactured material used for the production of formulated products and methods to show that contaminating microorganisms are controlled to an acceptable level

There is no provided information. Technical grade of MPCA is a hypothetical stage in a continuous production process of the product SPEXIT. Therefore, no information is required for the MPCA. Methods to show that contaminating microorganisms are controlled to an acceptable level for MPCP, are presented.

Quantification method for OBs counts.

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

There is no provided information. Based on the biology of the TC, there are no indications that SeMNPV have the potential to form toxins or metabolites of concern for human health or for the environment.

2.5.1.7 Methods to control the absence and to quantify (with appropriate limits of determination) the possible presence of any human and mammalian pathogen

There is no provided information.

Technical grade of MPCA is a hypothetical stage in a continuous production process of the product SPEXIT. Therefore, no information is required for the MPCA.

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

The stability of the microorganism need to be evaluated phenotypic and genotypically according to provided method

2.5.1.9 Methods to determine and quantify residues (viable or non-viable) of the active microorganism

It is also essential to evaluate the genetic stability of the virus isolate.

Technical grade of MPCA is a hypothetical stage in a continuous production process of the product SPEXIT.

2.5.2. The active microorganism(s) on and/or in crop, in foodstuffs and feeding stuffs, in animal and human body tissues and fluids, in soil, in water (including drinking water, ground water and surface water) and in air where relevant (i.e. viable residues)

There is lack of any harmful effect on men and domestic animals, without regard to the fact that the intake of spray deposits of active viruses can be excluded. Following the above justification, the description of analytical methods is limited to the microorganism itself.

Different methods are described to determine SeMNPV in water and soil. Microscopic counts of the nucleopolyhedra permit an assessment of the virus concentration. Quantitative bioassays enable calculations on the number of active (infective) viruses in the product as well as on plants, in soil and in water. The identity with the parent strain can be determined by restriction endonuclease analysis of viral DNA. The methods mentioned above are comparatively easy to handle with standard equipment and reliable enough to be used for routine inspection.

No residue definition is provided for SeMNPV for environmental matrices. Therefore, analytical methods for the determination of residues of SeMNPV in food and feed, animal tissue, soil, water, or air are not required. Methods submitted for the active microorganism are applicable for the product as well.

No residue definition is applicable for SeMNPV. Therefore, no post-registration monitoring methods are required. SeMNPV does not produce any metabolites of concern.

2.5.2.1 Relevant metabolites (especially toxins) on and/or in crop, in foodstuffs and feeding stuffs, in animal and human body tissues and fluids, in soil, in water (including drinking water, ground water and surface water) and in air where relevant (i.e. non-viable residues)

SeMNPV is not known to produce any human pathogens, no analytical methods are required.

2.5.2.3 Methods to identify any contaminating microorganisms of the preparation

See confidential Section Annex C.

2.5.2.4 Methods used to determine the storage stability and shelf life of the preparation

See confidential Section Annex C.

2.6 IMPACT ON HUMAN AND ANIMAL HEALTH

S. exigua multicapsid nucleopolyhedrovirus (SeMNPV) is a baculovirus that belongs to the family Baculoviridae, which are arthropod-specific enveloped viruses with a circular double-stranded DNA genome.

SeMNPV acts highly specific against larvae of the beet armyworm, *S. exigua*. It is supposed not to have any harmful effects on organisms not belonging to the genus *Spodoptera*. With regard to safety considerations it is important to note that SeMNPV and the whole family of Baculoviridae are naturally present in our environment.

Numerous studies have confirmed that baculoviruses present no risk for the environment. Neither SeMNPV produces nor the end-use product (Spexit) contain any chemical compound of critical toxicological, environmental, or ecotoxicological concern.

The information presented to assess the effects on human health in relation to the active substance are basically articles published between 1967 and 1992. The assays were carried out with baculoviruses other than the baculovirus notified. Since the tests were performed many years ago, most of them have not followed the GLP guidelines.

Regarding the formulation SPEXIT, no studies have been performed with this formulation. The few studies supplied, were carried out with a formulation containing as active substance a baculovirus that is not the baculovirus notified by the applicant and have been considered additional information. Furthermore, the applicant states that the formulation tested is equivalent to SPEXIT since it contains the same coformulants. Although they are not in the same concentration, the RMS considers they could be taken into account.

2.6.1 Effects having relevance to human and animal health arising from exposure to the micro-organism or to impurities, additives, contaminating micro-organisms contained in the material used for manufacturing of formulated products

2.6.1.1 Medical data and direct observations

The basic consideration on the safe use of virus for plant protection purposes should address the ability of a certain virus species or strain to infect other organisms than the target species that is intended to be controlled.

Baculoviruses have been used for biological insect control for more than 100 years. There is no evidence that these viruses have ever caused any disease process in humans or other mammals. This assessment is supported by a long and complete safety record comprising safety tests of more than 51 entomopathogenic viruses, including more than 30 baculoviruses, in mammals.

Baculoviruses, especially Granuloviruses, have a narrow host range and are strictly host-specific to certain arthropod species. This host specificity has been demonstrated *in vivo* and *in vitro* in numerous mammalian cell lines. In fact, these viruses do not replicate in vertebrate cells. An infection of cells or animals is confined to the target species because of the molecular biological mode of action of the baculovirus.

While baculoviruses may enter mammalian cells, the species-specific nature of the infection is dependent on the promoter of the baculovirus, which is active only in Lepidoptera. SeMNPV does not affect any organism except larvae of few species within the genus *Spodoptera*. Because of its nature as a virus, a toxin is not produced. Likewise, toxic metabolites or degradation products do not occur.
The applicant presented a study where human volunteers consumed *Heliothis zea* NPV over an exposure period of five days. These examinations failed to show any significant change in the general health condition of the participating individuals

Andermatt Biocontrol AG is manufacturing SeMNPV since 2006 and has not reported any incidents related to adverse health effects, abnormalities or casualties to persons engaged in production and handling of the microbial products.

It is concluded that no adverse reactions in Andermatt Biocontrol AG personnel involved in production and handling were reported as a result of exposure to SeMNPV. No cases of sensitisation were reported in the current occupational health statement. The study is considered acceptable regarding medical surveillance on manufacturing plant personnel, no adverse health effects were observed.

2.6.1.2 Sensitisation/allergenicity observations

No case of sensitization or allergenic responses or respiratory troubles of workers were observed during mass production of different baculoviruses including SeMNPV at Andermatt Biocontrol AG (Andermatt, 2006a & b). No cases of sensitisation have been reported in a current occupational health statement and no cases of sensitisation or allergic reactions have been reported in a comprehensive literature search.

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 sensitizers.

2.6.1.3 Sensitisation studies

Table 2.6.1.3. Summary of sensitisation studies

Test substance/ potency	Method Guideline Acceptability	Species Strain Sex No./dose	Dose levels	Observations	Reference
S. littoralis NPV Potency: 6×10^{10} p/g	Method of Landsteiner Sensitisation No Guideline	Pirbright-White Guinea pig Male 8/dose	6×10^{10} p/g in form of a 1% suspension in physiological saline	*S. littoralis* NPV did not provoke signs of hypersensitivity in guinea pigs. No indications of allergenic properties	B6.1.2.1/01 ([redacted] et al.1976 a)
Granupom (formulation that contains *Cydia pomonella* GV) 2.2×10^{10} granules/mL	Method of Landsteiner Dermal sensitisation No Guideline	SPF Pirbright White guinea pigs Hoe DHPK (SPFLac) Female 10/dose	2.2×10^{10} granules CpGV/mL	The formulation Granupom did not produce skin sensitisation in guinea pigs.	B6.1.2.1/02 ([redacted] 986)
Granupom (formulation that contains *Cydia pomonella* GV) 2.2×10^{10} granules/mL	Respiratory sensitisation Nose-only, aerosol inhalation	SPF Pirbright White guinea pigs Hoe DHPK (SPFLac) Female 4/dose Male 4/dose	35 mg Granupom/m3 air for 15 min	No allergenic response. No signs of irritation were observed. No changes in respiratory parametres	B6.1.2.1/03 ([redacted] 1992)

The applicant presented three sensitisation studies, two dermal and one respiratory sensitisation studies. There are not validated methods for the assessment of respiratory sensitization. Moreover, the dermal sensitization studies are not performed according to accepted methods. The toxicological studies were performed with other baculoviruses than SeMNPV. Following Regulation (EC) 283/2013, all microorganisms should be considered potential sensitizers.

2.6.1.4 Acute oral toxicity, pathogenicity and infectiveness

Table 2.6.1.4. Summary of acute oral toxicity studies

Test substance/ potency	Method Guideline Acceptability	Species Strain Sex No./dose	Dose levels	LD_{50}	Observations	Reference
Prodenia litura NPV Potency: 3×10^9 PIB /kg	Acute Oral Tolerance test No Guideline	Rat SPF Wistar Male 20/dose Female 20/dose	50 mg/kg bw	LD_{50} > 50 mg/kg bw that corresponds with 3×10^9 PIB /kg bw	No clinical signs or pathological changes	B6.1.2.2.1/01 ([redacted] et al. 1976 b)
Autographa californica nuclear polyhedrosis virus (AcNPV) Potency: 5×10^9 PIB/kg bw	Single oral administration No Guideline	Rat SPF Wistar Male 20/dose Female 20/dose	10 mL/kg bw that corresponds with 156.3 mg/kg bw	LD_{50} > 156.3 mg/kg bw that corresponds with 5×10^9 PIB/kg bw	No evidence of adverse reactions was obtained that might be indicative of pathogenicity or toxicity	B6.1.2.2.1/02 ([redacted] 1980)

The studies were performed with *Prodenia litura* NPV and *Autographa californica* NPV not the baculovirus notified.

No evidence of adverse reactions was obtained that might be indicative of toxicity. Infectivity could not be assessed because the results of virological examination were not submitted. Based on the available knowledge on baculoviruses, it is not likely that experimental AcNPV administration will result in colonisation and replication in rats or other mammals. Baculoviruses do not infect vertebrates.

Since no studies with SeMNPV are available, LD_{50} oral rat estimated for the baculoviruses tested are:

LD_{50} oral rat > 3×10^9 PIB *Prodenia litura* NPV /kg bw
LD_{50} oral rat > 5×10^9 PIB *Autographa californica* NPV /kg bw

2.6.1.5 Acute inhalation toxicity, pathogenicity and infectiveness

Table 2.6.1.5. Summary of acute inhalation toxicity studies

Test substance/ Route	Method Guideline	Species Strain Sex No./dose	Dose levels	LC_{50}	Observations	Reference
Granupom (formulation that contains *Cydia pomonella* GV) 2.2×10^{10} granules/mL	Respiratory sensitisation Nose-only, aerosol inhalation No guideline	SPF Pirbright White guinea pigs Hoe DHPK (SPFLac) Female 4/dose Male 4/dose	35 mg Granupom/m^3 air for 15 min		No evidence for sensitisation following intradermal induction and inhalative challenge was obtained	B6.1.2.1/03 ([redacted] 1992)
Mamestra brassicae NPV 1×10^{10} PIB/mL	Inhalation Test Aerosol produced by	Guinea pigs	*Mamestra brassicae* NPV 1×10^{10} PIB/mL	$LC_{50} > 1 \times 10^{10}$ PIB *Mamestra brassicae* NPV /mL	No signs of irritation in the lungs and	B.6.1.2.2.2/02 (Gröner et al 1978)

Test substance/ Route	**Method Guideline**	**Species Strain Sex No./dose**	**Dose levels**	**LC_{50}**	**Observations**	**Reference**
Laspeyresa pomonella GV 2×10^{12} granula/mL	fine spraying of 3 mL of an aqueous suspension for 5 min. No guideline		*Laspeyresa pomonella* GV 2×10^{12} granula/mL	$LC_{50} > 2 \times 10^{12}$ granula *Laspeyresa pomonella* GV /mL	respiratory passages.	
Heliothis zea NPV 1.2×10^{8} PIB/mg	Inhalation Test Aerosol spray of the virus suspension was spraying directly to the back of the mouth	Rhesus monkeys 5 male 5 female 2 of each sex: single dose 3 of each sex: 26 weekly doses	1 mg/kg that provided 1.2×10^{8} PIB	$LC_{50} > 1.2 \times 10^{8}$ PIB/kg	Body weight gains, temperature, haematology, blood chemistry, and histopathology of treated monkeys were similar to those of untreated monkeys.	B.6.1.2.2.2/03 (Ignoffo et al 1975)

One of the three studies provided by the applicant is about respiratory sensitisation and was evaluated instead of acute inhalation toxicity. In addition, the test was performed with other baculoviruses that are not the baculovirus notified. These studies are not adequate for the evaluation of acute inhalation toxicity because the doses tested are lower than the limit test dose level and very short times of exposure (5-15 min) were performed. **This should be considered a DATA GAP. Therefore, no LC50 for acute inhalation toxicity can be derived.**

2.6.1.6 Acute intraperitoneal/subcutaneous/intravenous toxicity, pathogenicity and infectiveness

Table 2.6.1.6. Summary of acute intraperitoneal/subcutaneous/intravenous toxicity studies

Test substance/ Route	**Method Guideline**	**Species Strain Sex No./dose**	**Dose levels**	**LC_{50}**	**Observations**	**Reference**
Prodenia litura NPV 6×10^{8} PIB per 10 mg dry substance	Intravenous tolerance Test No Guideline	SPF Wistar Rats (WISK-SPF 71) Male 20/dose Female 20/dose	10mg/kg	LC_{50}> 10 mg/kg that corresponds with 6×10^{8} PIB	No mortality during the study period. No severe toxic signs were recorded	B6.1.2.2.3/01 ([redacted] et al. 1976b)
Mamestra brassicae NPV 1×10^{10} PIB/mL *Laspeyresa pomonella* GV 2×10^{12} granula/mL	Single intraperitoneal injection No Guideline	Mice-NMRI 10 animals/dose	0.5 mL of 1×10^{10} PIB/mL 0.5 mL of 2×10^{12} granula/mL	LC_{50}> 5×10^{9} PIB/animal LC_{50}> 1×10^{12} granula/animal	No mortality or morbidity during the study period. Decline in the leucocyte values appeared one day after injection but this had returned to normal by the end of eight days	B.6.1.2.2.3/02 (Gröner, et. al 1978)

Test substance/ Route	**Method Guideline**	**Species Strain Sex No./dose**	**Dose levels**	**LC_{50}**	**Observations**	**Reference**
Heliothis zea NPV 1.2×10^8 PIB/mg	Single subcutaneous dose No Guideline	Rhesus Monkey Male 5/dose Female 6/dose	1 mg/kg bw	LC_{50}> 1 mg/kg bw that corresponds with 1.2×10^8 PIB/kg bw	No mortality during the study period. Infective virus, viral antibodies, or viral antigens were not found in blood drawn from treated animals.	B.6.1.2.2.3/03 (Ignoffo et al 1975)

The three studies presented to determine the intraperitoneal, subcutaneous or intravenous toxicity were carried out with other baculoviruses employing different animals (mice, monkeys and rats). From the results obtained, it can be concluded that baculoviruses did not induce symptoms in animals after injections tests.

2.6.1.7 Genotoxicity

There is some evidence that viruses that infect vertebrate cells themselves or their enzymes might exhibit mutagenic (mainly clastogenic) properties that can be detected by classical tests for gene mutations or chromosome aberrations (references cited by Döller and Gröner, 1983, Reimann and Miltenburger, 1983; Gröner, 1986). However, no such indications were found for baculoviruses that infect only invertebrate cells.

Table 2.6.1.7 Summary of genotoxicity studies

Method, guideline, deviations1 if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations /Results**	**Reference**
Sister chromatid exchange (SCE) rates Chromosomal aberrations test No guidelines No deviations	*Autographa californica* NPV (AcNPV) replicated in *Mamestra brassicae* cell culture (IZD-Mb-0503)	<u>Sister Chromatid Exchanges</u> The Indian muntjak cells were treated for 50 h with AcNPV using a dose of 120-180 $TCID_{50}$/cell ($TCID_{50}$, tissue culture infective dose). The mean number of SCEs per chromosome was 1.5. A similar result was obtained with mouse cells which had been treated with doses of 160 to 1550 $TCID_{50}$/cell. <u>Chromosomal aberrations</u> The Chinese hamster cells and human lymphocytes were treated with AcNPV at a concentration of 250 $TCID_{50}$/cell.	There was no difference in SCE-rates as compared with control cells None of the samples showed increased aberration rates after virus inoculation. Presence of AcNPV in the cytoplasm of mammalian cells but not in the nucleus and certainly not complete or partial replication. There was no adverse effect on cell proliferation, nor was a cytopathogenic effect (CPE) induced in such cultures.	B.6.1.2.3.1/01 (Reimann & Miltenburger 1983)

The applicant presented a published work where the test for clastogenicity in mammalian cells is carried out with a different baculovirus than the notified microorganism. **Baculoviruses do not affect other organism like vertebrates and not produce any metabolite therefore a new study carried out with SeMNPV should not be necessary**.

Chromosomal aberrations were not observed *in vitro* after exposure of vertebrate cells (frog) for four hours to nuclear polyhedrosis virions of *Trichoplusia ni* (McIntosh, 1975; cited by Krieg, 1976). **The applicant should have provided the article by McIntosh 1975 where chromosomal aberrations in frog cells were evaluated. The only information provided is the sentence mentioned above**.

The applicant has not presented a report where a bacterial reverse mutation test is carried out. This should be considered a data gap.

The applicant has not presented a report where the test for gene mutation in mammalian cells is carried out. The applicant states that virus lack of 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**.

2.6.1.8 Cell culture studies

Table 2.6.1.8 Summary of cell culture studies

Method, guideline, deviations1 if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Infectivity and replication of Baculoviruses in primary cells	700 virions *Heliothis zea* NPV/cell	A confluent monolayer of cells of primary African green monkey kidney, human primary embryonic kidney (HEK), human carcinoma of cervix (HeLa) and human diploid embryonic lung (W1-38) were inoculated with approx. 700 virions *Heliothis zea* NPV/cell	The virus did not develop in any of these cells. Cytopathic effects were not observed initially or after three serial passages through these cell types. All virus-inoculated cells failed to agglutinate guinea pig erythrocytes and presence of *H. zea* NPV did not interfere with the ability of a mammalian virus, i.e. Echo-11, to replicate in primate cells	B.6.1.2.4/01 (Ignoffo & Rafajko 1972)
Infectivity and replication of Baculoviruses in primary cells	*P. rapae* GV	Single oral dosis 50mg/ kg body weight to pigs, cows, lambs, chickens, rabbits, mice, birds, frogs, fish, shrimps and silkworms and inoculation the virus was inoculated into human lung cells, rabbit kidney cells and chicken embryonic cells in tissue cultures.	Neither cytopathogenic changes nor virus replication were observed under the electron microscope.	B.6.1.2.4/02 (Xuebao 1982)
Infectivity and replication of Baculoviruses in primary cells Cell culture study 1)Cell viability assay 2)Cell proliferation assay	Active non-occluded *Orgyia pseudotsugata* multicapsid nucleopolyhedrovirus (OpMNPV)	Seven cell lines from fish and one from an amphibian were exposed to active non-occluded OpMNPV. Cells from rainbow trout fry were also exposed to this virus by means of forced adsorption.	In some experiments these cells were challenged with infectious pancreatic necrosis virus, to test for viral interference. No cytopathic effects were observed in the exposed cells. . No changes occurred in growth rate, nor in the cell`s response to subculture. No increase in virus titre in culture passages was demonstrable. Exposure of rainbow trout fry cells to baculovirus failed to interfere with their susceptibility to infectious pancreatic necrosis virus. No evidence was found that the baculovirus is capable of entering into or altering the cells used in these studies	B.6.1.2.4-03 (Martignoni. 1978)
Infectivity and replication of Baculoviruses in primary cells	*Autographa californica* NPV (*Ac*NPV)	Human HeLa cells or primary human embryonic kidney cells, simian CV1 cells, hamster BHK 21 (B3) cells or *Muntiacus muntjak* cells growing in monolayer cultures were inoculated at multiplicities ranging from 0.1 to 100 plaque-forming units/cell.	AcNPV does not replicate as infectious particles. Cells did not reveal any cytopathic effects at any time after inoculation, nor was polyhedral formation observed. *Ac*NPV did not multiply in any of the cell lines studied. Viral DNA replication or transcription could not be detected. There was no evidence for the persistence of viral DNA or of fragments of viral DNA in mass cultures of mammalian cells.	B.6.1.2.4/04 (Tjia et al 1983)
Infectivity and replication of	*Autographa californica* NPV (AcNPV)	Interaction study of *Ac*NPV between (3.5×10^2 - 1.6×10^5) PFU/ml with the insect cell lines	All lines were able to adsorb and engulf virus particles. However, there was no evidence for viral	B.6.1.2.4/05 (Gröner et al 1984)

Method, guideline, deviations1 if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations /Results**	**Reference**
Baculoviruses in nonpermissive cell lines		TN368 from *T. ni* and CP169 from *C. pomonella*, and the Chinese hamster cell line CHO-K1	replication in the cell lines CP169 and CHO-K1	
Infectivity and replication of Baculoviruses in primary cells	*Autographa californica* NPV (AcNPV)	Mammalian cell cultures, i.e. a permanent cell line of human origin (Hela Ohio), one from kidney cells of monkey (Vero cells), and a primary culture of rat embryonic fibroblasts (REC) were inoculated with 50 infectious units of AcNPV per cell.	Production of typical virus inclusion bodies did not occur in any cell culture tested. None of the effects characteristic of virus infection were present. Also trials failed to re-infect insect cells with culture supernatant and cell contents from the mammalian cell test. It is concluded that after three passages no virus replication had taken place.	B.6.1.2.4/06 (Röder &Pünter1977)
Activation of endogenous C-type retroviruses.	*polyhedra and DNA of AcNPV, and polyhedra of M. brassicae NPV and L. dispar NPV*	Standard cell cultures of mouse, rat, monkey and man. Cells were treated with NPV, NPV-DNA, C-type virus-activating chemicals and chemicals alone and in combination.	In NPV-treated cell cultures no C-type retrovirus activation was detectable. In simultaneous treatments of the cells with NPVs and chemicals no potentiating effects by NPV could be detected. Virions of NPV in mammalian cell cultures upon re-isolation remained infectious in homologous insect cell cultures. No influence on growth or morphology of the treated mammalian cells was observed.	B.6.1.2.4/07 (Schmidt & Erfle 1982)

The applicant submits information from more than ten publications from the available literature, the most recent from 1990. None of the studies were carried out with SeMNPV but with other baculoviruses. In addition, some of the studies are not cell culture studies and should not have been included in this section (i.e. Döller & Gröner, 1981 and Döller et al., 1983a). Nevertheless, the in vivo tests performed to evaluate the replication of baculoviruses in vertebrates conclude that no virus replication had taken place in vertebrates (Döller & Gröner, 1981).

The studies reported that baculoviruses do not replicate in mammalian cells. Furthermore, there was no evidence for the persistence of viral DNA or of fragments of viral DNA in mass cultures of mammalian cells. **The studies carried out with cell lines can be considered acceptable but it should be taken into account that the studies were not performed with SeMNPV**.

2.6.1.9 Short term toxicity, pathogenicity and infectiveness

The applicant has presented a number of studies, published between 1975 and 1981, which were carried out with different animals, mice, rats, guinea pigs, dogs, and monkeys that were exposed to repeated doses of baculoviruses (other than SeMNPV, notified by the applicant).

Oral short-term studies

Mice

Nutrient baits were soaked in virus suspensions and offered for a period of 99 days. The total dose/animal was 3 x 10^9 polyhedra of *M. brassicae* NPV or 5 x 10^{11} granula of CpGV, respectively. No clinical signs of toxicity were detected. Autopsy did not reveal macroscopical alterations or histological changes in the examined organs and tissues (Gröner et al., 1978).

Rats

Multiple small dose 90-day exposure to *N. lecontei* NPV had no detectable, harmful effects (reviewed by Cunningham & Entwistle, 1981).

Guinea pigs

After oral administration (by intubation) of a total of 5 x 10^{11} polyhedra (*M. brassicae* NPV) over a period of 99 days, no differences in behaviour and body weight gain in comparison to controls were observed. Testing for antibodies against NPV antigens in the gel diffusion test proved negative (Gröner et al., 1978).

Dogs

Twenty-seven young adult purebred beagle dogs were fed *L. dispar* NPV at three dose levels. No changes in appearance, behaviour, and appetite were observed. Treated beagle dogs showed no important changes in haematology-, clinical biochemistry- and urinalysis values. Gross pathology and histopathology examinations indicated no abnormal findings (Lewis & Podgwaite, 1981).

Monkeys

Three male and three female young adult rhesus monkeys received 26 weekly oral doses (by gavage) of 6 x 10^8 PIB/kg bw of *Heliothis zea* NPV. In a similar test the monkeys received 26 weekly subcutaneous doses of 1.2 x 10^8 PIB/kg bw. All monkeys did not show clinical signs of toxicity or reduced body weight gains. Haematology, blood chemistry, and histopathology of treated monkeys were similar to those of untreated monkeys. Infective virus, viral antibodies, or viral antigens were not found in blood drawn from treated animals (Ignoffo et al., 1975).

Inhalatory short-term studies

Rhesus monkeys

Three male and three female young adult rhesus monkeys received 26 weekly inhalative doses of 1.2 x 10^8 PIB/kg bw of *Heliothis zea* NPV. Body weight gains, temperature, haematology, blood chemistry, and histopathology of treated monkeys were similar to those of untreated monkeys. Infective virus, viral antibodies, or viral antigens were not found in blood drawn from treated monkeys (Ignoffo et al., 1975).

There is no evidence that baculoviruses are toxic or pathogenic following various routes of (repeated) exposure.

2.6.1.10 Specific studies on toxicity, pathogenicity and infectiveness

Considering the studies presented for Tier I, baculoviruses do not cause long-term health effects, so studies on chronic toxicity, pathogenicity and infectiveness, carcinogenicity and reproductive toxicity could be waived. Nevertheless, the applicant presents information of reports where carcinogenicity and teratogenicity of baculoviruses were assessed.

Carcinogenicity: *Heliothis zea* NPV were injected subcutaneously into neonate rats and intravenously into adult rats. There was no significant change in the rate of spontaneous tumour formation in any of those tests (reviewed by Krieg, 1976). *Lymantria dispar* NPV was fed to albino rats over a period of 2 years. The tumour incidence or other microscopic lesions found were not attributable to the treatment (reviewed by Lewis & Podgwaite, 1981).

Teratogenicity: Twenty-four female mated rats were fed with *Heliothis zea* NPV during the 5th to 14th day of gestation. None of the virus-fed or control rats showed abnormalities in general physical condition, behaviour, or in body weight gain. Administration of NPV to pregnant dams did not affect number or weight of fetuses, resorption sites, or relationship between corpora lutea and implantation site.

No teratogenic or carcinogenic effects were observed for the baculoviruses tested. Because of the high degree of similarity within the family Baculoviridae, the results of studies with other baculoviruses may be considered applicable to SeMNPV. The information provided is considered acceptable.

2.6.1.11 Toxicity studies on metabolites

Viruses do not produce metabolites, as they do not have metabolism of their own. So toxic metabolites or degradation products do not occur. For a virus, potential formation of substance with antimicrobial activity or the development and spread of resistance to antibiotics of medical importance is not of concern.

2.6.2 Impact on human health arising from exposure to the micro-organisms or to impurities, additives, contaminating micro-organisms contained in the material used for manufacturing of formulated products

It can be agreed that the virus itself does not raise a concern for human health except for sensitisation because all microorganisms are considered potential sensitizers.

Table B.2.6.2-1 Overview of the available data

Study	Test material	Species	Result	References
Acute oral toxicity	*Prodenia litura* nucleopolyhedrovirus (PlNPV) 3×10^9 PIB/kg bw	Wistar Rat	No clinical signs or pathological changes. This is additional information.	[redacted] 1976b
Acute oral toxicity	*Autographa californica* nucleopolyhedrovirus (AcNPV) 0.725 - 0.790 $\times 10^9$ PIB/animal	Wistar Rat	Oral LD50 (combined) > 5×10^9 PIB AcNPV/kg bw Toxicity, pathogenicity or infectivity was not evaluated. This is additional information.	[redacted] 1980
Acute oral toxicity	*Orgyia pseudotsugata NPV* 5.8×10^{10} and 1.8×10^{11} PIB/kg bw	Rat	No systemic toxicity/pathogenicity were evaluated	Martignoni 1978
Sensitisation by inhalation	Granupom (formulation that contains *Cydia pomonella* GV) (2.2×10^{10} granules/mL)	Guinea Pig	There is not method.	[redacted] 1992
Inhalation	*Mamestra brassicae* NPV (1×10^{10} PIB/mL) *Cydia pomonella* GV (2×10^{12} granula/mL)	Guinea Pig	Not conclusive	Gröner et al. 1978
Intraperitoneal single dose	*Mamestra brassicae* NPV (1×10^{10} PIB/mL) *Cydia pomonella* GV (2×10^{12} granula/mL)	NMRI mice	No evidence of alterations in organs	Gröner et al. 1978
Clastogenicity	*Autographa californica* nucleopolyhedrovirus (AcNPV)	Chinese hamster cell line B14F28 Human lymphocytes	No chromosomal aberrations were observed	Reimann and Miltenburger,1983
Cell culture studies	*Autographa californica NPV*	Human HeLa cells	No cytopathic effects at any time after inoculation, nor was polyhedral formation observed	Tjia, 1983

2.6.3 Summary of product exposure and risk assessment

The Microbial Pest Control Product SPEXIT containing the technical active ingredient *S. exigua* multicapsid nucleopolyhedrovirus (SeMNPV) is intended to be used for treatment against the beet armyworm on pepper and leafy vegetables in the field and in greenhouses by professional users and by non-professional users.

SPEXIT is formulated as a suspension concentrate, containing 3.75×10^{12} viable granules of SeMNPV in 1 L product.

The maximum single dose rate is 0.2 L/ha, corresponding to 7.5×10^{11} OB/ha in the field and in home gardens applied as foliar spray up to 18-times per growing season with a minimum interval of 6 days depending on the number of sunny days.

Basic acute toxicity studies

The applicant has not performed any study with the formulation notified SPEXIT, the studies available were performed with a formulation called GRANUPOM that has as active ingredient a baculovirus which is not SeMNPV

The applicant stated that the co-formulants are equivalent. Although the concentrations are not the same, Granupom contains the same coformulants.

Acute oral toxicity:

The applicant did not perform studies with the formulated product SPEXIT nor with another formulation equivalent to the notified SPEXIT so this is considered a DATA GAP.

Acute inhalation toxicity**:**

The applicant has not presented studies that evaluate the acute inhalation toxicity, only information regarding respiratory sensitization for baculoviruses that are not the virus notified by the applicant that was submitted for the evaluation of the active substance. Therefore this should be considered a DATA GAP.

Acute percutaneous toxicity:

Microorganisms do not penetrate intact human skin. Hence, no study on dermal toxicity was performed with the SeMNPV nor with the formulated product SPEXIT. The applicant presented information regarding skin sensitisation test carried out with other baculoviruses (in the evaluation of the active substance). Because of the high degree of similarity within the family Baculoviridae, the results of studies with other baculoviruses may be considered applicable to SeMNPV. **Therefore, the non-submission of a test report by the applicant can be considered acceptable**.

Additional acute toxicity studies

The applicant presented studies where skin and eye irritation tests were carried out. The studies were not carried out with the formulated product SPEXIT but with a formulation called Granupom SC (CpGV). The baculovirus tested is shown not to be eye nor skin irritant. Although the study has not been conducted with SeMNPV, according to the OECD Consensus Document (ENV / JM / MONO (2002) 1), baculovirus species are extremely host-specific and only occur in arthropods. Baculoviruses are not infective for mammals and replication does not occur in mammalian cells. **Therefore, the study carried out with another baculovirus could be extrapolated to the one notified.**

Sensitisation studies

Following the Regulation (EC) 283/2013, SeMNPV and SPEXIT (formulated product) should be regarded as potential sensitizers.

2.6.3.1 Exposure and personal protective equipment (PPE)

The Microbial Pest Control Product SPEXIT containing the technical active ingredient *S. exigua* multicapsid nucleopolyhedrovirus (SeMNPV) is intended to be used for treatment against the beet armyworm on pepper and leafy vegetables in the field and in greenhouses by professional users and by non-professional users.

SPEXIT is formulated as suspension concentrate, containing 3.75×10^{12} viable granules of SeMNPV in 1 L product.

The maximum single dose rate is 0.2 L/ha, corresponding to 7.5×10^{11} OB/ha in the field and in home gardens applied as foliar spray up to 18-times per growing season with a minimum interval of 6 days depending on the number of sunny days.

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.

Risk assessment

Baculoviruses such as SeMNPV acts highly specific against larvae of the beet armwyworm, *S. exigua*, so it is supposed not to have any harmful effects on humans or other organisms not belonging to the genus *S.*. Nevertheless, standard hygienic procedures should be maintained when handling virus formulations.

No adverse effects including allergic reactions (neither by skin contact nor by inhalation) have been reported in humans who were in close contact with baculoviruses. There is no evidence of allergenicity when inhaled. Nevertheless, following the Regulation (EC) 283/2013, all microorganisms should be considered potential sensitizers.

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 baculoviruses.

Estimation of operator exposure
Since an AOEL has not been established at Community level for the active substance, there is no parameter to calculate the exposure, so the risk assessment has not been performed.

Estimation of worker exposure
Worker exposure is considered negligible as dermal exposure is not relevant for SeMNPV and inhalation exposure is considered not relevant for cultivation work or harvest.

Estimation of bystander and resident exposure
Following the above given reasons for abstaining from an estimation of operator risk assessment, this also applies with regard to bystanders.

The product is diluted in water prior to application. The product concentrate is added to an appropriate amount of water before its application using a vehicle mounted spraying equipment, a hand-held sprayer or a knapsack sprayer. There is a potential for accidental skin exposure during mixing and loading activities, therefore the use of suitable protective clothing (coveralls and gloves) is recommended. Since the product is a suspension concentrate, the generation of particles or aerosols of inhalable size is not expected during the mixing and loading. The application of SPEXIT using a hand-held sprayer may result in dermal contamination from spray drift and directly from spraying equipment; therefore the use of suitable protective clothing (coveralls and gloves) is recommended. Spray application may also generate particles of inhalable size, therefore the additional use of respiratory protective equipment is recommended.

Personal protective equipment (PPE)
As the product contains microorganisms as active substances, the use of PPE is recommended as a precautionary measure

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

2.7 RESIDUES IN OR ON TREATED PRODUCTS, FOOD AND FEED

SeMNPV is naturally occurring entopathogen virus (see Documents Vol 3 MA sections 1, 3 and 5). Viruses are not able to produce metabolites. SeMNPV is widespread in Europe. SeMNPV like all BVs is a highly host-specific virus which is not harmful to non-arthropods, including domestic animals and human. Furthermore, it is highly specific for the species *Spodoptera exigua*. It does not produce toxins or secondary metabolites of toxicological concern.

SeMNPV is of natural origin and not genetically modified. It belongs to the family of BVs which are naturally present in our environment. Their application in pest control means only a fluctuation of the virus titre in the biotope of the pest insect.

The experience that contact of BVs with man or animals does not impose any risk for their health has been confirmed by numerous acute and subacute toxicity studies performed with several different BVs (refer to MA Section 5). Tests on mammalian cell cultures as well as on mutagenicity, teratogenicity and carcinogenicity all gave negative results (Krieg, 1976). Furthermore, several acute toxicity studies with the formulation Granupom (containing 2.2 x 10^{13} granula *Cydia pomonella* granulosis virus /L) also did not show any adverse effects on mammals (see Documents Vol 3 MP Section 7).

It is unable to enter plant tissues and to infest them, and cannot multiply on plant surfaces. Multiplication can only occur after ingestion by an appropriate host, where the occlusion bodies have to be dissolved; thereby liberating the infective virions (please refer to MA Section 2 Point 2.4). This can only happen inside *S. exigua* larvae. Instead, it is rapidly inactivated on plant surfaces by UV light. Thus, it is unlikely to occur on treated food/feed stuffs in concentrations considerably higher than under natural conditions. Experimental studies have demonstrated the high sensitivity of BVs to UV light (see Documents MA Section 6 Point 6.1.1). Stable virus deposits, therefore, are not assumed.

Viruses belonging to the family Baculoviridae and their potential effects on animals and humans, when applied to food or feed, were extensively reviewed and the results were published in the EFSA Opinion on 2009, 2010, 2011, 2012, and 2017. Hence it was agreed that the family Baculoviridae is the highest taxonomic unit that should receive a QPS recommendation in the registration process. Viruses belonging to the family Baculoviridae are included in the QPS list and proposed as plant protection products under the Council Directive 91/414/16 are exempted from certain data requirements such as oral toxicity data. The OECD also concluded in 2002 that BVs were safe to use for products meant for human consumption. BVs were also classified as Risk Group 1 (RG1) agents, as they were not related to any disease of humans.

Due to this lack of any toxicity potential to mammals, residue data on SeMNPV are considered not relevant.

2.7.1 Further information required - exposure to consumers

2.7.1.1 Non-viable residues

All BVs are rapidly inactivated by the UV-portion in sunlight (Vol.3 MA B.2.2). Residues of virus deposits may therefore only persist as non-viable residues, consisting of the protein forming the occlusion body and the inactivated virion. Non-viable virus particles do not have any effect on man or the environment.

2.7.1.1 Viable residues

All BVs are rapidly inactivated by the UV-portion in sunlight (Vol.3 MA B.2.8 and Vol.3 MA B.7.1.1). Stable viable residues of virus deposits, therefore, are not assumed.

Introduction of SeMNPV Bv-0004 isolate in greenhouse (protected) and open field pepper and leafy vegetables (lettuce crops) is not expected to affect the level of natural occurrence of the virus.

Therefore, determination of residues of the components of the formulation SPEXIT isolate Bv-0004 in or on treated products, food and feed is not considered relevant.

2.8 FATE AND BEHAVIOR IN THE ENVIRONMENT

2.8.1 Persistence

The whole family *Baculoviridae* are naturally present in our environment. As BVs are closely associated with their host, occurrence of corresponding Bv is linked to the presence of its host.

The persistence of the virus is important in the soil of all crops and its incidence is related to the phenological stage of the crop and the season (highest in spring and summer).

Sublethal infections by SeMNPV are common in field populations of *S. exigua*. Such covert infections were found to persist for at least five generations. Next to baculoviruses, many *S. exigua* field populations also exhibit covert infections by other virus species. In the field, the prevalence of co-infections in wild population is likely to have an impact on the performance of a SeMNPV insecticide.

The persistence of the OBs on plant surface determine the period during which a lethal dose can be ingested by the larvae.

The reproduction, dispersal, transmission and/or survival of BVs are enhanced by manipulation of the host behaviour. This is evident from the pre-death climbing behaviour (towards elevated positions) of infected larvae (tree-top disease), which makes sense from an evolutionary point of view, since thus the virus may be spread over a larger area of plant foliage, allowing an increased virus transmission to subsequent generations of caterpillars. The tree-top disease allows an increased persistence and multiplication of SeMNPV.

2.8.1.1 Competitiveness under environmental conditions

BVs are rapidly inactivated when exposed to sunlight. The persistence of a virus-based insecticide on the surface of the crop plant strongly influences its efficacy, since with greater persistence the likelihood of the pest consuming a lethal OB dose increases. BVs are highly sensitive to UV degradation. Thereby, solar radiation was found to be the main factor affecting the OB persistence on plant surfaces (UV radiation is reduced in greenhouses as the walls filters much UV)

There is a negative correlation of the persistence with solar radiation in both, greenhouse and field trials.

Physicochemical characteristic of the crop can influence OB degradation, as exudates of some plants can rapidly inactivate OBs.

2.8.1.2 Persistence in soil

BVs can easily reach the soil, either during rain following application or after the death of an infected larva and subsequent release of inclusion bodies, followed by rainfall.

Viruses thus persisting in soil following treatments or natural epizootics provide a reservoir for initiation of epizootics in succeeding generations of host insects and is a mechanism by which a long-term effect of virus introductions may be attained. The inactivation rate of viruses not only depends on soil type and pH, but also on microbial activity.

In soil, they rapidly adsorb to soil particles and are not leached to deeper soil layers. Activity is thus maintained in the upper soil and remains accessible to further host generations, leading to a sustainable effect on the host insects.

2.8.1.3 Persistence in water

Most viruses in intact inclusion bodies are reasonably stable in aqueous suspension. However, the pH and salt concentration of water influences stability. In addition, once introduced into a water body, the viral particles are likely to deposit and are absorbed by sediments. Furthermore, there is evidence of deactivation/destruction by the UV portion of sunlight in aqueous suspensions. Moreover, it is assumed that the protein of the virus will be

completely mineralised by bacterial action in water and sediment. There are no risks of pollution of surface or groundwater expected due to the high level of retention of the viral particles by the soil.

2.8.1.4 Persistence in air

A rapid degradation of SeMNPV in air is assumed, since inactivation by sunlight is the most important factor causing loss of activity of viruses in the field environment. Moreover, SeMNPV occlusion bodies might not evaporate, since consist of several high molecular weight proteins.

2.8.2 Multiplication and virus transmission

The highly persistent virus OBs are responsible for horizontal transmission to healthy susceptible larvae that consume OB-contaminated plant material. However, when host population densities are low and conditions for horizontal transmission are unfavorable, vertical transmission, from parents to offspring, plays an important role in the survival of the virus.

Sublethal baculovirus infections have been reported in a number of lepidopteran species. For vertical transmission to occur the virus must persist in the adult host as a covert or sublethal infection which does not prevent adult reproduction. The maternally mediated transmission is double that of parental male mediate transmission.

Vertical transmission of the insect SeMNPV virus and the role of this strategy in the survival of these pathogens in natural populations was demostrated. It was confirmed SeMNPV can establish sublethal infections in larvae that survive after having consumed OBs and these infections can be dose-dependent.Vertically-transmitted infections also permit virus dispersal and the colonization of new areas of habitat through the migration of infected adult hosts.

Transmission of SeNMPV is largely horizontal and low levels of vertical transmission occurs. High levels of covert infection suggested that the virus could persist in a non-symptomatic form.

2.8.3 Mobility

Stability of BVs would not appear to be directly influence by wind. Virus can be translocated by wind, as the substrate such as soil, insect debris, or foliage is moved.

Infection (SeMNPV) causes *S. exigua* larvae to die in an elevated position. This result would improve dissemination of viral occlusion bodies over plant foliage and would increase probability of transmission to healthy conspecific larvae. The phenomenon of tree-top disease is described by different researchers. This baculovirus induced host behavioral manipulation contributes to the SeMNPV mobility. Infected *S. exigua* larvae migrate to the top of the plant prior to death. It is assumed that this behavior is adaptive for the virus, since it ensures the optimal dissemination of progeny virus onto lower foliage and enhances the visibility (of the dying larvae) for birds, spreading the virus over longer distance.

2.8.3.1 Mobility in soil

BVs can easily reach the soil, either during rain following application or after the death of an infected larva and subsequent release of inclusion bodies, followed by rainfall.

Viruses thus persisting in soil following treatments or natural epizootics provide a reservoir for initiation of epizootics in succeeding generations of host insects and is a mechanism by which a long-term effect of virus introductions may be attained. The inactivation rate of viruses not only depends on soil type and pH, but also on microbial activity.

In soil, they rapidly adsorb to soil particles and are not leached to deeper soil layers. Activity is thus maintained in the upper soil and remains accessible to further host generations, leading to a sustainable effect on the host insects.

Baculoviruses are able to leach through a column of soil. Viral activity has been found in a depth of 15 cm for the loamy sand and down to 30 cm in the sand. The exponential decrease of activity indicate a low risk of reaching deeper soil layers and therefore the groundwater. The NPV matrix protein polyhedrin is genetically and serologically closely related to the granulin, the matrix protein of GV. The good retention of baculoviruses by soil is attributed to these particular proteins envelope of the virus particles consisting of granulin for granulovirus and polyhedron for polyhedrovirus

2.8.3.2 Effects of the microorganism on drinking water analysis

There is no evidence of SeMNPV deactivation/destruction by the UV portion of sunlight in aqueous suspensions. It is assumed that the protein of the virus would be mineralised by bacterial action in water and sediment, but there is no confirmation.

There are no risks of pollution of surface or groundwater expected due to the retention of the viral particles by the soil. The level of retention of the viral particles by the soil is highly dependence on the soil structure and physicochemical characteristics of the soil particles.

Even all the evidence of the potential inactivation of the BVs, most viruses in intact inclusion bodies are reasonably stable in aqueous suspension. No information has been provided in relation to potential interferences of with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC.

A data gap is therefore identified.

2.9 SUMMARY OF EFFECTS ON NON-TARGET SPECIES

SeMNPV belongs to the genera alphabaculovirus and acts highly specific and exclusively against larvae of the beet armyworm, *S.exigua* and is not supposed to have any harmful effects on organisms not belonging to the family of *Noctuidae*. In accordance with host specify, the occurrence of SeMNPV is linked to the presence of the host, *S. exigua*. Therefore, their application in pest control means only a fluctuation of the virus titre in the biotope of the pest insect. Effect on non-target organisms and environment behaviour of SeMNPV would considered similar to related BV CpGV and the ecotoxicological studios on CpGV SC appropriate to assess whether risk might be caused by application of SeMNPV in a plant protection product.

Studies assessing the effect of the representative formulation, SPEXIT to non-target organisms are not available. In order to considered SeMNPV isolate Bv0004 as a low risk microorganism according to Commission regulation EU 2017/1432, "*Baculoviruses shall be considered as being of low risk unless at strain level they have demonstrated adverse effects on non-target insect*s" the effect of non-target insect have to be evaluated in SeMNPV isolate Bv0004. Therefore, the RMS cannot considered SeMNPV as a low risk microorganism.

2.9.1 Summary of effects on birds (and other terrestrial vertebrates)

No cases of viral toxicity or pathogenicity were observed in studies specifically designed to include pathology in avian species (chicken, turkey, pheasant, dove, mallard, sparrow and quail) using different BVs others than SeMNPV.

No specific studies at strain level on the toxicity, infectiveness or pathogenicity towards birds were submitted by the applicant. The applicant refers to general information in BV.

Even all considerations above mentioned, the RMS consider convenient to include at least one specific short-term dietary pathogenicity/toxicity study with the product SPEXIT, due to the insectivorous birds can eat contaminated insects contained the dissolved OB in the active substance treated area (worst-case assumptions). There is no information on the short-term and long-term espouse on the ingestion by the birds of *S. exigua* infected larvae by the virus from SPEXIT spraying. No acute end-points are available and no studies on birds were submitted on infectivity from Andermatt Biocontrol GmbH.

The RMS consider that a study need to confirm the reports from literature excluding adverse effects on birds.

2.9.2 Summary of effects on aquatic organisms

Considerable numbers of BV under natural conditions without any observable damage has always confronted aquatic organisms. Heavy doses of *Heliothis zea* NPV and other NPVs had been tested on at least seven different fish species and no negative effects were reported in the published literature. A study on the effects of the formulated product Granupom, containing *Cydia pomonella* GV on rainbow trout is submitted in the dossier. Neither significant mortality nor sublethal effects were observed at all concentrations below the nominal concentration level of 100 mg Granupom/L over 96 h. The LC50 value at 96 h was estimated to be 100 mg Granupom/L (> 2.04 x 10^9 OB/L at a density of 1080 g/L), the highest concentration tested. Similar results are expected with SPEXIT.

A study on the effects of the formulated product Granupom, containing *C. pomonella* GV, on *Daphnia magna* is submitted in the dossier. An acute toxicity study on *Daphnia magna* was conducted with the product Granupom containing *Cydia pomonella* GV. At 48 h Granupom did not show any toxic effects on *Daphnia magna*. The EC_{50} is greater than 100 mg/L, the NOEC is 100 mg/L (2.04 x 10^9 OB/L at a density of 1080 g/L), the highest concentration tested, with a probability of more than 99.9 %. Similar results are expected with SPEXIT.

Two studies on the effects of the formulated product Granupom, containing *C. pomonella* GV, on *Scenedesmus subspicatus* were submitted. Based on the observation made during first test, the NOEC was 100 mg/L, the EC_{50} was determined to be 100 mg/L Granulosevirus CpGV. Second study on the effects of the formulated product Granupom, containing *C. pomonella* GV on *Lemna gibba*, no effects were determined at 100 mg/L. No LOEC and EC_{50} could be determined for any growth parameter; the NOEC was 100 mg/L.

Due to the close relationship between all lepidoptera BVs and since SeMNPV containing formulation SPEXIT does not contain any ingredients of toxicological concern (see confidential data on Volume 4), information obtained for Granupom (CpGV) is also valid for SPEXIT (SeMNPV).

2.9.3 Summary of effects on plants other than algae

No member of the Baculoviridae family is known to infect plants. Thus it is assumed highly unlikely that SeMNPV would be phytotoxic or phytopathogenic.

2.9.3 Summary of effects on bees

A study on the effects of the formulated product Granupom, containing *C. pomonella* GV on *Apis mellifera* is submitted. According to the results of this study it can be assumed that the oral LD_{50}/72 h is above 3.5 x 10^7 granula/bee (> 20300 µg/bee) and the contact LD_{50}/48 h of Granupom is above 4.4 x 10^7 granula/bee (> 25500 µg/bee).

RMS conclusion:

The RMS has considered:

1- SeMNPV containing formulation SPEXIT does not contain any ingredients of toxicological concern (see confidential data on Volume 4).
2- SPEXIT and GRANUPOM have the same additive and at the same quantity according with information provided in Volume 4, Appendix 3.
3- Close relationship between all lepidopteran BVs.
4- CpGV and SeMNPV do not belong to the same group of baculoviris family (SeMNPV is an alphabaculovirus and CpGV belongs to betabaculovirus).
5- CpGV and SeMNPV have different target organisms.

Taking all above mentioned consideration together, the RMS considers the results of the studies with GRANUPOM not applicable for SeMNPV. The RMS would considered convenient to include at least one specific study with bees, due to the relevance of these species, that can be exposure to the dissolved OB in the active substance treated area

(worst-case assumptions). There is no information on the short-term and long-term espouse on the ingestion by bees of *S. exigua* virus from SPEXIT spraying. No acute end-points are available and no studies on bees were submitted on infectivity from applicant.

The RMS consider that a study need to confirm the reports from literature excluding adverse effects on bees.

2.9.4 Summary of effects on arthropods other than bees

BVs are characterised by a very high host specify, with host ranges normally comprising only few species within a genus, rarely different genera of one family. In general, the host range never exceeds the order and only rarely the family. Cross-infection of lepidopteran NPVs or GVs to insects other than Lepidoptera has never been observed The toxicity of GRANUPOM (or Granulosevirus CpGV SC) to non-target arthropods other than bees was evaluated in laboratory tests. The effects of the formulated product Granupom, containing *C. pomonella* GV, on *Aphidius rhopalosiphi*, *Typhlodromus pyri*, and *Poecilus cupreus* were submitted.

RMS conclusion:

The RMS has considered:

1- SeMNPV containing formulation SPEXIT does not contain any ingredients of toxicological concern (see confidential data on Volume 4).
2- SPEXIT and GRANUPOM have the same additive and at the same quantity according with information provided in Volume 4, Appendix 3.
3- Close relationship between all lepidopteran BVs.
4- CpGV and SeMNPV do not belong to the same group of baculoviris family (SeMNPV is an alphabaculovirus and CpGV belongs to betabaculovirus).
5- CpGV and SeMNPV have different target organisms.

Taking all above mentioned consideration together, the RMS considers the results of the studies with GRANUPOM not applicable for SeMNPV. The RMS would considered convenient to include at least one specific study with beneficial lepidopteran, due to the relevance of these species, that can be exposure to the dissolved OB in the active substance treated area (worst-case assumptions). There is no information on the short-term and long-term espouse on the ingestion by other related lepidopteran of *S. exigua* virus from SPEXIT spraying. No acute end-points are available and no studies on arthropods other than bees were submitted on infectivity from the applicant.

The RMS consider that a study need to confirm the reports from literature excluding adverse effects on beneficial arthropods other than bees.

2.9.5 Summary of effects on earthworms

The effects of the formulated product Granupom, containing *C. pomonella* GV, on earthworms were assessed in a submitted study. No mortality was observed at any Bv concentration evaluated. There were no significant difference between body weights compared to the control at all CpGV SC concentrations.

The median lethal concentration LC_{50} of CpGV SC to *Eisenia foetida* determined after 14 days exposure is shown to be greater than 1000 mg/kg of artificial soil, corresponding with 1.67×10^{10} OB/kg artificial soil (assuming a density of 1.2 mg/L). According to the results, it is confirm the innocuous effect of the BV CpGV SC on earthworms.

2.9.6 Summary of effects on soil microorganisms

The effects of the formulated product Granupom, containing *C. pomonella* GV, on soil microorganisms were assessed. The impact on soil respiration of two type of soil is considered as negligible (< 15 % deviation) even at 5.0 L/ha CpGV SC application rate. Due to the close relationship between all lepidopteran BVs and since SeMNPV containing formulation SPEXIT does not contain any ingredients of toxicological concern (see confidential data on Volume 4), information obtained for Granupom (CpGV) is considered valid for SPEXIT (SeMNPV).

2.10 SUMMARY AND EVALUATION OF ENVIRONMENTAL IMPACT

2.10.1 Distribution and fate of SPEXIT

2.10.1.1 Fate and behaviour in soil

BVs can easily reach the soil, either during rain following application or after the death of an infected larva and subsequent release of inclusion bodies, followed by rainfall.

Viruses thus persisting in soil following treatments or natural epizootics provide a reservoir for initiation of epizootics in succeeding generations of host insects and is a mechanism by which a long-term effect of virus introductions may be attained. The inactivation rate of viruses not only depends on soil type and pH, but also on microbial activity.

In soil, they rapidly adsorb to soil particles and are not leached to deeper soil layers. Activity is thus maintained in the upper soil and remains accessible to further host generations, leading to a sustainable effect on the host insects.

Baculoviruses are able to leach through a column of soil. Viral activity has been found in a depth of 15 cm for the loamy sand and down to 30 cm in the sand. The exponential decrease of activity indicate a low risk of reaching deeper soil layers and therefore the groundwater. The good retention of these viruses by soil is attributed to the particular protein envelope of the virus particles consisting of granulin for GV and polyhedrid for BV.

For the purpose of a risk assessment, the worst-case exposure scenario was presented: A foliar application in pepper (sideward application) with up to 18 applications (2 to 3 treatments per generation, 6 generations) at a dose rate of maximum 0.2 L product/ha (7.5×10^{11} OB/ha) in water volumes of 200 L/ha employed as representative uses according with GAP table.

2.10.1.2 Fate and behaviour in water

Surface water

BVs persist immobile in the soil for a long period. Accumulation can only occur following mass multiplication in the lepidopteran host. BVs have also been detected in areas where they had never been artificially applied. It has been established that the virus titre in soil decreases rapidly after field treatment and only small numbers of BVs are found in the following year.

S. exigua MNPV consists of OB which do not dissolve in water. In general, however, it may be stated that the OB will be completely mineralised by bacterial action in water and sediment. The active ingredient is destroyed, among other factors, by the action of UV light. It may thus be stated that *S. exigua* MNPV is inactivated under natural conditions, including water.

Ground water

BVs are effectively held back by sand under all tested leaching conditions, whether with de-ionised water, raw waste water or biologically purified waste water. Strong adsorption of BVs also takes place in soils with high content of organic matter. Field lysimeter trials have also confirmed that penetration of appreciable numbers of BVs to aquifers must be considered as a highly improbable event. The results show that, under the test conditions, leaching to below 15 cm does not take place in high-humus sandy soil. In low-humus soil, the virus was still biologically detectable at a depth of 18-24 cm, but not lower. Leaching experiments in glass columns revealed high absorptiveness in the top organic soil layers and very little capacity to leach to lower layers.

Drinking water

Drinking water quality is monitored by screening for microbial indicator species. Potential interference with the analytical systems for the control of the quality of drinking water according to Council Directive 98/83/EC needs to be addressed. For drinking water coliforms or *E. coli*, enterococci, and *Pseudomonas aeruginosa* are monitored. Monitoring of these bacteria is accomplished by cultivating them on appropriate media. SeMNPV as a virus is not able to proliferate in the absence of its host even all the evidence of the potential inactivation of the BVs, most viruses in intact inclusion bodies are reasonably stable in aqueous suspension. No information has been provided in

relation to potential interferences of with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC. A data gap is therefore identified.

2.10.1.3 Fate and behaviour in air

In view of the physic-chemical characteristics and the nature of the nucleopolyhedrovirus, the possibility of air contamination can be excluded.

The virus consists of a high-molecular protein and thus has no vapour pressure and is relatively unstable under photolytic conditions. Hence, volatilisation from plant surfaces and from soil can be excluded.

2.10.2 Identification of non-target species at risk and extent of their exposure

Study on the effects of the formulated product Granupom, containing *C. pomonella* GV, on non-target organisms are acceptable for the evaluation of the effect on non-target, because similar results are expected with SPEXIT. **Nevertheless there are not covered criteria 5.2.2 for the approval of low-risk active substance**.

2.10.2.1 Effects on birds

The experience that BVs present no risk for non-target species has been confirmed by numerous literature studies. SeMNPV is highly specific and only has an effect on larvae of *S. exigua*. Therefore, birds are not at risk when exposed to SeMNPV. The ingredients of the preparation SPEXIT formulated as SC are inert and no hazards to birds are expected.

The RMS consider that a study need to confirm the reports from literature excluding adverse effects on beneficial arthropods other than bees.

2.10.2.1 Effects on fish

Based on the predicted environmental concentration (PECsw), calculated as 188.36 µg SPEXIT/L (6.13 x 10^5 OB/L) previously, the margin of safety (MOS; corresponding to TER) for freshwater fish is derived from the LC_{50} value. Based on the available data the MOS value of fish was calculated. Based on the submitted data on effects on aquatic organisms and the intended use in fields and glasshouses, the calculated margin of safety values are high and it is anticipated that the potential risk posed to *S.exigua* multicapsid nucleopolyhedrovirus (SeMNPV) to fish is acceptable.

2.10.2.1 Effects on freshwater invertebrates

An acute toxicity study on *Daphnia magna* was conducted with the product Granupom containing *Cydia pomonella* GV. At 48 h Granupom did not show any toxic effects on *D. magna*. The EC_{50} is greater than 100 mg/L, the NOEC is 100 mg/L (2.04 x 109 OB/L at a density of 1080 g/L), the highest concentration tested, with a probability of more than 99.9 %. Similar results are expected with SPEXIT. Based on the predicted environmental concentration (PECsw), calculated as 188.36µg SPEXIT/L (6.13 x 10^5 OB/L) previously, margin of safety (MOS; corresponding to TER) for freshwater invertebrates is derived from the EC_{50} value. Based on the submitted data on effects on aquatic organisms and the intended use in fields and glasshouses, the calculated margin of safety values are high and it is anticipated that the potential risk posed to *S. exigua* multicapsid nucleopolyhedrovirus (SeMNPV) to daphnids is very low and acceptable.

2.10.2.3 Effects on single cell algae

An acute toxicity study on *Scenedesmus subspicatus* was conducted with the formulation CpGV SC (= Granupom). No significant effects were detected at 100 mg/L, the highest concentration tested. Therefore, the EC_{50} was estimated to be > 100 mg Granupom/L (corresponding to > 2.04 x 10^9 OB/L at a density of 1080 g/L), with a probability of 95% because no inhibitory effect was observed at this concentration level. Similar results are expected with SPEXIT. Based on the predicted environmental concentration (PEC_{sw}), calculated as 188.36 µg SPEXIT/L previously, the margin of safety (MOS; corresponding to TER) for algae is derived from the EC_{50} value. Based on the submitted data on effects on aquatic organisms and the intended use in fields and glasshouses, the calculated margin of safety values are high and it is anticipated that the potential risk posed to *S. exigua* multicapsid nucleopolyhedrovirus (SeMNPV) to algae is very low and acceptable.

2.10.2.3.1 Effects on single cell algae

Tested toxic effects of CpGV on aquatic plants based on the formulated product Granupom using the duckweed *Lemna gibba*. No effects were determined at 100 mg/L. Granupom can be classified to be not toxic against *Lemna gibba*. No LOEC and EC_{50} could be determined for any growth parameter. The NOEC can be set to 100 mg/L (2.04 x 10^9 OB/L at a density of 1080 g/L). Similar results are expected with SPEXIT. Based on the predicted environmental concentration (PEC_{sw}), calculated as 72.54 µg product/L previously, the margin of safety (MOS; corresponding to TER) for aquatic plants is derived from the EC_{50}. Based on the submitted data on effects on aquatic organisms and the intended use in fields and glasshouses, the calculated margin of safety values are high and it is anticipated that the potential risk posed to *S. exigua* multicapsid nucleopolyhedrovirus (SeMNPV) to aquatic plants is very low and acceptable.

2.10.2.3.2. Effects on terrestrial vertebrates other than fish

An acute oral toxicity study has been conducted with the *Autographa californica* NPV on SPF Wistar rats. No test substance related mortalities were observed in the study, so that the LD_{50} was estimated to be > 5 x 10^9 OB/kg b.w. It is appropriate to assume, that the LD_{50} of SeMNPV will also be above 5 x 10^9 OB/kg b.w., corresponding to 1550 mg SPEXIT/kg b.w.

2.10.2.1 Effects on bees

Acute laboratory studies on bees were conducted with the formulation Granupom containing CpGV. The oral LD_{50}/72h is >3.5× 10^7 OB/bee and the contact LD_{50}/48h of CpGV is > 4.4 × 10^7 OB/bee. With 4.4 × 10^7 OB/20 µL this is equivalent to 2.2 × 10^6 OB/µL, resulting in a test concentration of 2.2 × 10^{12} OB/L in the oral toxicity test. For the contact toxicity, an application amount of 2 µL/bee of the test substance was used in the test, equivalent to 2.2 × 10^7 OB/µL; the resulting test concentration was calculated as 2.2 × 10^{13} OB/L.

It is appropriate to assume that the corresponding values for SeMNPV will also be >3.5×10^7OB/bee for oral LD_{50}/72h and > 4.4 × 10^7 OB/bee for contact LD_{50}/48h. With a density of 1.16 kg/L and a content of 3.75 ×x 10^{12} OB/L, these contents of virus occlusion bodies correspond to 10.8 mg/bee and 13.6 mg/bee of SPEXIT, respectively.

To assess the risk to honeybees following the use of SPEXIT, the margin of safety (MOS), which is the ratio of the test concentration (in OB/L) and the maximum field concentration (OB/L), was determined. From the calculated MOS it is concluded that the use of SeMNPV even at multiple application rate imposes no risk to bees. Therefore, under conditions of field use no adverse effects on natural populations of *Apis mellifera* are expected following application of SPEXIT.

The RMS consider that a study need to confirm the reports from literature excluding adverse effects on beneficial arthropods other than bees.

2.10.2.1 Effects on arthropods other than bees

Effects of the formulation GRANUPOM on non-target arthropods other than bees have been submitted. GRANUPOM (or Granulosevirus CpGV SC) contains the same co-formulations as SPEXIT. Therefore, studies conducted with GRANUPOM (or Granulosevirus CpGV SC) are fully applicable to assess possible effects of SPEXIT on non target arthropods other than bees. Risk assessments for SPEXIT with the proposed use pattern are

provided here and are considered adequate with regard to the evaluation of effects on non-target arthropods other than bees of the formulated product.

All available data for demonstrate that SeMNPV as any other BVs and the formulated product SPEXIT are not toxic, not pathogenic or infective to non-target arthropods.

A low margin of safety is derived for the exposure to non-target arthropods after the use of SPEXIT after multiple applications according to GAP based on up to 18 applications. It is very unlikely that the same population of non-target arthropods is exposed to each application. Furthermore, it is extremely worst-case to assume a cumulative application rate as the both active microorganism and the product will not be stable on the crop due to environmental conditions.

The tested concentration in the effect studies are below the accumulated application rate used as worst case exposure scenario. However, it has to be kept in mind that no adverse effects were observed in the studies and therefore, the obtained margins of safety likely overestimate a possible risk for non-target arthropods. Literature information further demonstrates absence of infectivity, pathogenicity or toxicity of SEMNPV or any other BVs to arthropods other than the host species *S. exigua*.

To assess the risk to other arthropods following the use of SPEXIT, the margin of safety (MOS), which is the ratio of the test concentration (in OB/L) and the maximum field concentration (OB/L), was determined. According to this calculation, a risk to *Poecilus cupreus* can be excluded.

The RMS consider that a study need to confirm the reports from literature excluding adverse effects on beneficial arthropods other than bees.

2.10.2.1 Effects on earthworms

The acute toxicity of the formulation CpGV SC (= Granupom) to the earthworm *Eisenia foetida* was determined in a laboratory study. The median lethal concentration LC_{50} of Granupom to *Eisenia foetida* determined after 14 days exposure was shown to be greater than 1000 mg Granupom/kg artificial soil, which is equivalent to 3.25×10^9 viable granules/kg soil. It is appropriate to assume that the corresponding values for SeMNPV will also be 3.25×10^9 OB/kg soil. Based on the predicted environmental concentration (PEC_{soil}) calculated as 5.57 mg SPEXIT/kg soil, corresponding to 1.8×10^7 OB/kg soil previously for multiple applications, assuming as a worst case that no degradations occurs between applications, the margin of safety (MOS) for earthworms is derived from the LC_{50} value. The calculated MOS value is high, indicating an acceptable acute risk to earthworms after application of SPEXIT at the maximum recommended use rate. Literature information further demonstrates absence of infectivity, pathogenicity or toxicity of BVs to earthworms.

2.10.2.1 Effects on soil microorganisms

An assessment of the side effects of CpGV on the activity of the soil microflora was conducted using the formulation CpGV SC (= Granupom). The impact on soil respiration is considered as negligible (< 15 % deviation) even at the 10-x dosage of the highest recommended Granupom application rate (5 L/ha: 5.94×10^{14} granules/ha). Considering the similarity in formulation of the products Granupom and SPEXIT and in the included BVs CpGV and SeMNPV, these results can be transferred to SPEXIT. Thus, no adverse effects on soil microflora are expected after application of SPEXIT at a total application rate of 18 applications: 3.6 L/ha: 1.35×10^{13} OB/ha.

2.10.3 Identification of precautions necessary to minimize environmental contamination and to protect non-target species

The above risk assessment demonstrates that SPEXIT is not toxic to aquatic and terrestrial species, and considering the predicted environmental concentrations, will not be hazardous to native animal populations upon applications of SPEXIT following Good Agricultural Practice. The comparison of predicted and tolerable exposure of birds, fish, daphnids, algae, terrestrial plants, terrestrial vertebrates other than birds and earthworms complies with the limit values set by the. No effects to soil microflora are expected.

Non unacceptable impact on sensitive representative species for beneficial arthropods and bees are expected, since the hazard quotient does not reveal any unacceptable risk.

In conclusion, no hazard classification or specific labelling according to Reg. (EC) No 1272/2008 is required for SPEXIT to minimize environmental contamination and to protect non-target species.

Level 3

Spodoptera exigua multicapsid nucleopolyhedrovirus (SeMNPV)

Summary and consideration with respect to the approval criteria of Regulation (EC) No 1107/2009

Identification of data gaps, proposed conditions, risk management measures, issues that could not be finalised and critical areas of concern
Proposed decision

3.1 BACKGROUND TO THE PROPOSED DECISION

3.1.1 PROPOSAL ON ACCEPTABILITY AGAINST THE APPROVAL CRITERIA – ARTICLE 4 AND ANNEX II OF REGULATION (EC) NO 1107/2009

3.1.1.1 Article 4		Yes	No	
	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		(SeMNPV) belongs to the genus alpha -baculovirus Family Baculoviridae *<u>Environmental fate and behaviour:</u>* *SeMNPV and the whole group of baculoviruses are naturally present in the environment. The exceptional properties of all current known taxa of the virus family baculoviridae resulting in a high host specificity and the fact that baculovirus itself is harmless regarding effect to human and animal health and the environment.* *SeMNPV virus are endemic in horticultural plants in most European countries. The presence of SeMNPV isolate BV-0004 does not pose an additional risk. Baculovirus don´t replicate outside the insect cell. The virus has limited stability outside host, does hardly replicate in arthropodos other than S. exigua hosts (Lepidoptera). The baculovirus do not have a cellular structure and do not produce metabolites.* *The virus is very easy transmitted by insect-insect contact, plant-cultivation and equipment contact. Furthermore, can also be transmitted less efficiently by vertical and horizontal transmission.* *SeMNPV is no persistent in water and air when the product SPEXIT is used as an enthomopathogen virus in protected and open field sweet pepper and leafy vegetalbes, lettuce crops.* *SeMNPV is unlikely to be mobile in the environment via air. No risk was found of SeMNPV infection in water or soil.* *<u>Ecotoxicology</u>:* ***Active substances:*** - *SeMNPV isolate Bv-0004.*

				SeMNPV is naturally occurring baculovirus, they do not have metabolism of their own and are not able to produce metabolites, and therefore the risk to organisms other than plants could be excluded. ***Representative uses:*** *Control of beet armyworm S.exigua in pepper plants and leafy vegetables, lettuce crops.* *No risks have been identified as a result of use of the representative product SPEXIT.*
3.1.1.2 Submission of further information (Annex II 2.2)		**Yes**	**No**	
i)	It is considered that a complete dossier has been submitted		X	
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.	X		
3.1.1.3 Restrictions on approval (Annex II 2.3)		**Yes**	**No**	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.		X	
3.1.1.4 Criteria for the approval of an active substance (Annex II 3)				
Dossier (Annex II 3.1)		**Yes**	**No**	
i)	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).		X	*ADI, ARfD and AOEL are not necessary (and not possible to be derived based on the available data) due to the low toxicological concern related to baculoviruses.*
ii)	It is considered that the dossier contains the information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined;	X		*No risk for consumer is expected since baculoviruses like SeMNPV are ubiquitous in arthropoda and there are no documented causes of harmful effects in humans.* *Viruses are not able to produce metabolites. No MRL is required.*

	(b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.			*As regards S. exigua nuclear polyhedrosis virus no specific MLRs were set, so the default value of 0.01mg/kg laid down in article 18 (1) (b)of the regulation EC No 396/2005* *The EFSA concluded in EU No588/2014 that the substance S. exigua nuclear polyhedrosis virus is not pathogenic to humans and does not require a quantitative consumer risk assessment.*
iii)	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		
Efficacy (Annex II 3.2)		**Yes**	**No**	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		*SeMNPV is recommended for use for the control of S. exigua in greenhouse and open field pepper crops and leafy vegetables, lettuce crops.* *The mode of action of S. exigua multicapsid nucleopolyhedrovirus (SeMNPV) is specific for the target insect Spodoptera exigua.* *Larvae ingest the virus by feeding on leaf material. In the larval gut occlusion bodies dissolve under the alkaline conditions. The liberated virions infect the midgut epithelial cells. In the nucleus of infected cells new virions are produced, which may leave the cell and infect cells of heamocoel and other tissues, such as the fat body. In these tissues occlusion of the virions in polyhedra takes place. The process continues till all tissues are infected and cell-lysis occurs. The larvae usually dies after most tissues have been infected, 3 to 4 days after feeding on contaminated leaf material.* *SeMNPV provides wide spectrum protection against S. exigua in pepper cultivars and leafy vegetable crops in greenhouse trial, with 2-3 application per pest generation, up to 6 generations at infestation (preferable on early larva instar (L1 and L2) (at any BBCH of the crops).First treatment just before hatching, at a dose of 200-1600 L/ha containing at least $7.5x10^{11}$ OB/ha.*

Relevance of metabolites (Annex II 3.3)		**Yes**	**No**	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.	X		*Not applicable. Baculovirus do not produce metabolites.*
Composition (Annex II 3.4)		**Yes**	**No**	
i)	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.	X		*The content of pure virus in SeMNPV technical is set up to be at least 2.20x10^{11} OB/g. The content of SeMNPV in SPEXIT is 3.75× 10^{12} OB/L.*
ii)	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.			*Not applicable*
iii)	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted.			*Not applicable*
Methods of analysis (Annex II 3.5)		**Yes**	**No**	
i)	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.	X		*As regards S. exigua nuclear polyhedrosis virus no specific MLRs were set, so the default value of 0.01mg/kg laid down in article 18 (1) (b)of the regulation EC No 396/2005* *The EFSA concluded in EU No588/2014 that the substance S. exigua nuclear polyhedrosis virus is not pathogenic to humans and does not require a quantitative consumer risk assessment.*
ii)	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.	X		*Acceptable methods are available for the determination of virus in the MPCA and the MPCP. Adequate methods are also available to determine the presence of other microorganisms potentially pathogen. See point 2.5 in level 2.*
iii)	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		

Impact on human health (Annex II 3.6)				
Impact on human health - ADI, AOEL, ARfD (Annex II 3.6.1)		**Yes**	**No**	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.		X	*It is not possible to derived reference values based on the available data. ADI, ARfD and AOEL are not necessary due to the low toxicological concern related to baculoviruses.*
Impact on human health – proposed genotoxicity classification (Annex II 3.6.2)		**Yes**	**No**	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B**.		X	*There is a DATA GAP concerning bacterial reverse mutation test.*
Impact on human health – proposed carcinogenicity classification (Annex II 3.6.3)		**Yes**	**No**	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B**.		X	*Not relevant.*
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			*Not relevant*
Impact on human health – proposed reproductive toxicity classification (Annex II 3.6.4)		**Yes**	**No**	

i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B**.		X	*Not relevant*
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			*Not relevant*
Impact on human health – proposed endocrine disrupting properties classification (Annex II 3.6.5)		**Yes**	**No**	
i)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogenic category 2 and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties**.		X	*Not relevant*
ii)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 2 and** in addition the RMS considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**.		X	*Not relevant*
iii)	Linked to either i) or ii) immediately above. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			*Not relevant*

Fate and behaviour in the environment				
Persistent organic pollutant (POP) (Annex II 3.7.1)		**Yes**	**No**	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	*S. exigua multicapsid nucleopolyhedrovirus belongs to the family of baculoviruses. POP criteria do not apply to baculoviruses* *The criterion is not relevant for microorganisms.*
Persistent, bio accumulative and toxic substance (PBT) (Annex II 3.7.2)		**Yes**	**No**	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	*S. exigua multicapsid nucleopolyhedrovirus belongs to the family of baculoviruses. PBT criteria do not apply to baculoviruses* *The criterion is not relevant for microorganisms.*
Very persistent and very bioaccumulative substance (vPvB) (Annex II 3.7.3)		**Yes**	**No**	*The criterion is not relevant for microorganisms*
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	*S. exigua multicapsid nucleopolyhedrovirus belongs to the family of baculoviruses. PBT criteria do not apply to baculoviruses.* *The criterion is not relevant for microorganisms.*
Ecotoxicology (Annex II 3.8)		**Yes**	**No**	
i)	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.	X		*Study on the effects of the formulated product Granupom, containing C. pomonella GV, on non-target organisms are acceptable for the evaluation of the effect on non-target, because similar results are expected with SPEXIT.* ***Nevertheless there are not covered criteria 5.2.2 for the approval of low-risk active substance.*** ***5.2.2 Criteria is not been achieve with the current documents and studies on non-target organism. The applicant must include at least studies with arthropods other than bees and bees with the strain SeMNPV Bv-0004.***
ii)	It is considered that, on the basis of the assessment of Community or internationally agreed test guidelines, the substance **HAS** endocrine disrupting properties that may cause adverse effects on non-target organisms.		X	*Not relevant for virus.*
iii)	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.			*Not relevant*

iv)	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.	X		*Not relevant*
Residue definition (Annex II 3.9)		**Yes**	**No**	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.	X		*The residue definition is not requiered* *The residue definition for soil, water, sediment and air is OB by default.*
Fate and behaviour concerning groundwater (Annex II 3.10)		**Yes**	**No**	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		

3.1.2 PROPOSAL - CANDIDATE FOR SUBSTITUTION

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		X	*Not relevant for microorganisms.*

3.1.3 Proposal – Low risk active substance

Low-risk active substances		Yes	No	
	It is considered that the active substance **shall be considered of low risk**. In particular it is considered that the substance **should NOT be classified or proposed for classification** in accordance with Regulation (EC) No 1272/2008 as at least one of the following: — carcinogenic, — mutagenic, — toxic to reproduction, — sensitising chemicals, — very toxic or toxic, — explosive, — corrosive. In addition it is considered that **the substance is NOT**: — persistent (half-life in soil more than 60 days), — has a bioconcentration factor higher than 100, — is deemed to be an endocrine disrupter, or — has neurotoxic or immunotoxic effects.		X	*S. exigua* nuclear polyhedrosis virus is included in the list of active substances expected to meet the requirements of article 22 of the Regulation according to Commission notice concerning a list of potentially low-risk active substances approved for use in plant protection (2018/C 265/02). The list is established for informative purposes on the basis of information available in the dossiers and assessment reports that substantiated the approval of the substances under Directive 91/414/EEC. Based on this information, the active substances approved under this Directive were screened for their compliance with the requirements of Article 22 and specifically the criteria of Annex II point 5 to the Regulation ('low-risk criteria'). The screening was performed by the Commission with the assistance of the Working Group on Low-risk substances and products. According to Commission regulation (EU) 2017/1432 point (10) "*It should be clearly indicated that baculoviruses which are a host-specific family of viruses infecting exclusively arthropods and occurring predominantly in the insect order of Lepidoptera, are to be considered as low-risk substances as there is no scientific evidence that baculoviruses have any negative effect on animals and humans (2). A* ***baculovirus should be considered of low-risk unless at strain level it has demonstrated adverse effects on non-target insects*** There are consolidate information to support SeMNPV as a low-risk substance. Nevertheless, studies assessing the effect of the representative formulation, SPEXIT to non-target organisms are not available. **In order to considered SeMNPV isolate Bv0004 as a low-risk microorganism according to Commission regulation EU 2017/1432, the effect of non-target arthropod need to be confirmed at strain level in SeMNPV isolate Bv0004.** RMS (30/01/2019) has suggested to the applicant the convenience for inclusion further assays on non-target arthropods in order to have the evidence there is no harmful effect on related non-target organisms of the specific baculovirus on charge (SeMNPV isolate Bv-004). **The RMS consider that at least two specific studies with the**

				product SPEXIT need to be included in order to exclude adverse effects on insect others than *Spodoptera exigua*. One studio preferable would be done with honeybees. The applicant in respond to RMS requirements (08/03/2019) has considered *"further testing as unnecessary waste of testing animals and resources"* . *In accordance with Commission Regulation (EU) 2017/1432, of 7 August 2017, amending Regulation (EC) No 1107/2009 of the European Parliament and the Council concerning the placing of plant protection products on the market as regards the criteria for the approval of low-risk active substances, it is considered that an active substance consisting in a micro-organism:* *5.2.1. An active substance which is a micro-organism may be considered as being of low-risk unless at strain level it has demonstrated multiple resistance to anti-microbial used in human or veterinary medicine.* *5.2.2. Baculoviruses shall be considered as being of low-risk unless at strain level they have demonstrated adverse effects on non-target insects.'* *Multiple resistance to anti-microbial used in human or veterinary medicine is not applicable to viruses: viruses are not metabolically active and therefore cannot produce antimicrobial substances; they are not sensitive to antibiotics and therefore cannot become resistant to these substances or spread resistance.* ***Moreover, RMS would like to raise our concern about the following issues:*** ***None of the studies have been performed with the active substance. The results have been extrapolated from studies performed with other baculovirus.*** • ***Microorganisms per se are considered potential sensitizers; therefore, risk mitigation measures may be necessary.*** • ***There is a great lack of information of the active substance. Specifically, there are DATA GAP in the following sections:*** o *Sensitisation studies* o *Acute oral toxicity, pathogenicity and infectiveness* o *Acute inhalation toxicity, pathogenicity and infectiveness* o *Genotoxicity (bacterial reverse mutation test)* *Study on the effects of the formulated product Granupom, containing C. pomonella GV, on non-target organisms are acceptable for the evaluation of the effect on non-*

				*target, because similar results are expected with SPEXIT. **Nevertheless there are not covered criteria 5.2.2 for the approval of low-risk active substance.*** ***5.2.2 Criteria is not been achieve with the current documents and studies on non-target organism. The applicant must include at least studies with arthropods other than bees and bees with the strain SeMNPV Bv-0004***

3.1.4 LIST OF STUDIES TO BE GENERATED, STILL ONGOING OR AVAILABLE BUT NOT EVALUATED

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 Identity of the active substance or formulation				
Name and species description, strain characterisation				
C1.2.2 Name and specie description. The origin of the isolate should be clarified. The statement "the strain is deposited since 2006 in the German Collection of Microorganisms and Cell Cultures (DSMZ)" seems to be insufficient. Description of the deposit would be convenient to provided: original deposit date, strain designation, isolate from, country, date of original sampling, history, Genbank accession numbers, store conditions, supplier, and the method to confirmation strain specie.	All representative uses	X		
B.1.3.1 Accession number in culture collection. *According to principle uniforms described on regulation 546/2011 "The deposition of the strain at an internationally recognised culture collection shall be checked "*	All representative uses	X		

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
The preservation of the MPCAs SeMNPV in DSMZ makes the isolate searchable and accessible at once. Confirmation document regarding further descriptions of the isolate Bv-0004 need to be provide: Depositor, Origen of the isolate, Host isolation, receipt and acceptance, identification of the microorganism, scientific description and proposed taxonomic designation and stability statement. Then isolate can be traced through all publications it is mentioned in, including patent files, with its assigned number (Bv-0004).Further iinformation and confirmation document (from the German Collection of Microorganisms and Cell Cultures (DSMZ)), on BV-0004 isolate deposit is considered necessary.				
B.1.3.3 Test procedures and criteria used for identification at strain level				
C1.3 Specification of the material used for manufacturing of formulated products				
C1.3.1 Content of the microorganism The content of MPCA in the TGAI. According to data requirement on regulation 283/2009 "*1.4.1. Content of the micro-organism. The minimum and maximum content of the micro-organism in the material used for manufacturing of formulated products, must be reported.* Confirmation on Strain identity. - The document Kessler 2018a does not provide information of the standard batch, only explain	All representative uses	X		

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
the method of analysis. The company need to explain the composition of the reference batch and numbering it. - The company has not confirmed information on GLPs.				
C1.3.2 Identity and content of impurities, additives and contaminating microorganisms. The Information regarding the content of the organic impurities consist of faeces, larval tissue and residues of the artificial diet are not provided by the company in the evaluated batches (from producer companies #1 and #2). Producer companies #1 and #2 need to included impurities from rest of the insect and synthetic medium.	All representative uses	X		
C1.5.1 Method for production *According to data requirement on regulation 283/2009 "1.4.1. Information provided relates to a pilot plant production system"* - Kessler 2018c, document KMA3.4/01 slightly explain the production process. A scheme of the production process would be convenient. The production system need to be elaborate.	Relevant for all representative uses	X		
1.4.2. Identity and content of impurities, additives, contaminating micro-organisms	Relevant for all representative uses		**X**	
3.1.4.2 Biological properties of the active substance and physical, chemical and technical properties of the formulation				
3.2.5 Infectiveness, dispersal and colonisation ability. Under a persistence and dispersion point of view, It would be necessary to know the transmission strategies of SeMNPV Bv-0004 isolate.Vertical transmission genotypes are generally capable of producing a high prevalence of persistent infection in insect adults that survived an inoculum challenge during the larva stage, since horizontal transmission genotypes tended to have higher OB		X		

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
pathogenicity and faster speed of kill compared to vertical transmission genotypes. **The mechanism of virus transmission and dispersion (vertical and/or horizontal) in SeMNPV isolate Bv-0004 is unknown.** RMS recommend the development of similar studios already made in other related baculovirus, for the isolate of studio SeMNPV-Bv0004.				
Genetic stability and factors affecting it				
B.2.7. GENETIC STABILITY AND FACTORS AFFECTING IT *According to data requirement on 283/2013 2.7"information on genetic stability (e.g. mutation rate of traits related to the mode of action or uptake of exogenous genetic material) under the environmental conditions of proposed use must be provided"* Genetic stability must be evaluate in a long-term study for SeMNPV Bv-0004 isolate and under environment conditions of proposed used.	Relevant for all representative uses Relevant due to possible mutation of the isolate	X		
Environmental factor that can influence in environment survival outside the host. All information provided relate to physical, chemical and technical properties of the plant protection product are for virus MPCA in general, and some are related to BV species other than SeMNPV. According to data provided, the sun light may play an importance role in the persistence and survival of SeMNPV. The RMS recommends the evaluation of UV light in the target MPCA SeMNPV isolate Bv-0004. There is no specific storage stability studio on SPEXIT product. Considering physico-chemical properties of the formulation SPEXIT over 2	Relevant for all representative uses Relevant due to possible mutation of the isolate	X		

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
Years at 5 °C is required. This is considered a data gap.				
3.1.4.3 Data on uses and efficacy				
Effects on beneficial and other non-target organisms In the different tests and studies conducted with related BVs, no effects on the incident of other non-target organisms or environmental effects have been observed. There is no studio on beneficial and other non-target organisms performed with SeMNPV-Bv0004.	Relevant for all representative uses Relevant due to possible effect in other biological treatments	**X**		
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
-				
3.1.4.5 Methods of analysis				
Best available technology for identification SeMNPV isolate Bv-0004 phenotipic and genotypic is not fully support by the study submited. Futher information of molecular characterization of SeMNPV Bv-0004 need to be submitted. The method to identify and fully discriminate at strain level need to be verified by testing variants of SeMNPV as well as other viruses, viroid, bacterium, fungi and oomycetes. The different identity of SeMNPV isolate Bv-0004 and SeMNPV isolate SeUS1 is unclear.	Relevant for all representative uses	X		

Data gap	**Relevance in relation to representative use(s)**	**Study status**		
		No confirmation that study available or on-going	**Study on-going and anticipated date of completion**	**Study available but not peer-reviewed**
Confirmation of differentiation between isolate Bv-004 and SeUS1 need to be provided by the applicant. **B.4.1.2 Methods for providing information on possible variability of seed stock/active microorganism** Additional information should be requested from the applicant. It would be necessary to confirm there are no modification in the molecular pattern and in the biological activity of the seed stock: - Molecular variability need to be determine by REN analysis. - Biological activity variability need to be determine compared with the reference seed stock, in terms of mean lethal doses (LD_{50}), mean time to death (MTD), and OBs (occlusion bodies) yield.				
B.4.1.3 Methods to differentiate a mutant of the microorganism from the parent wild strain Data not provided by the applicant. Either SeMNPV is of natural origin and not a mutant or genetically modified organism; a mutant can come up in following generations and differs from the parental isolate. This mutant can be less effective in the control of the target insect, and can have different biological characteristics.	Relevant for all representative uses	X		
B.4.1.4 Methods for the establishment of purity of seed stock from which batches are produced and methods to control that purity There is no provided information. Clarification is needed regarding the production process.	Relevant for all representative uses	X		

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
Each production batch is started from the initial seed stock culture, which is maintained as frozen vials. Thus mutations in the original parent strain SeMNPV are excluded. Any spontaneous mutation occurring during production will most likely be reflected in a change of agarose gel restriction enzyme pattern and/or biological activity. Thus, any change need to be detected during identification and quality control: - Molecular variability need to be determine by REN analysis. - Biological activity variability need to be determine compared with the reference seed stock, in terms of mean lethal doses (LD50), mean time to death (MTD), and OBs (occlusion bodies) yield.				
B.4.1.8 Methods to determine storage stability, shelf-life of the microorganism, if appropriate The stability of the microorganism need to be evaluated phenotypic and genotypically according to provided method (B4.1.1) Genetic changes are linked to a loss in infectivity, which results in reduced fitness followed by extinction in the field.	Relevant for all representative uses	X		
Methods to prevent loss of virulence of seed stock of the microorganism There is no specific storage stability study on SPEXIT product. Considering physic-chemical properties of the formulation SPEXIT over 2 Years at 5 °C is required. The microbial contaminants have to be determined before and after 2-year storage. This determination has to be performed with validated	Relevant for all representative uses	X		

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
methods or with international standard methods and have to be in accordance with the threshold limits indicated in OECD 65 (Oct. 2011).This is considered a data gap.				
3.1.4.6 Toxicology and metabolism				
Sensitisation studies	Relevant for all representative uses	X		
Acute oral toxicity, pathogenicity and infectiveness	Relevant for all representative uses	X		
Acute inhalation toxicity, pathogenicity and infectiveness	Relevant for all representative uses	X		
Bacterial reverse mutation test	Relevant for all representative uses	X		
3.1.4.7 Residue data				
-				
3.1.4.8 Environmental fate and behaviour				
Persistence in water Even all the evidence of the potential inactivation of the BVs, most viruses in intact inclusion bodies are reasonably stable in aqueous suspension. No information has been provided in relation to potential interferences of with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC. A data gap is therefore identified.	Relevant for all representative uses	x		

Data gap	**Relevance in relation to representative use(s)**	**Study status**		
		No confirmation that study available or on-going	**Study on-going and anticipated date of completion**	**Study available but not peer-reviewed**
3.1.4.9 Ecotoxicology				
In order to achieve low risk status for the MPCA SeMNPV and its MPCP SPEXIT, *In accordance with Commission Regulation (EU) 2017/1432, of 7 August 2017, amending Regulation (EC) No 1107/2009 of the European Parliament and the Council concerning the placing of plant protection products on the market as regards the criteria for the approval of low-risk active substances, it is considered that an active substance consisting in a micro-organism: 5.2.2. Baculoviruses shall be considered as being of low-risk unless at strain level they have demonstrated adverse effects on non-target insects.'* **Ecotoxicological studiess in closed related non-target insect species would be required**	Relevant for all representative uses	X		
B.9.1 Effects on birds Specific short-term dietary pathogenicity/toxicity study with the product SPEXIT, due to the insectivorous birds can eat contaminated insects contained the dissolved OB in the active substance treated area (worst-case assumptions). There is no information on the short-term and long-term espouse on the ingestion by the birds of S. exigua infected larvae by the virus from SPEXIT spraying. No acute end-points are available and no studies on birds were submitted on infectivity from MPP Andermatt Biocontrol GmbH.	Relevant for open field use	x		

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
The RMS consider that a study need to confirm the reports from literature excluding adverse effects on birds.				

3.1.5 ISSUES THAT COULD NOT BE FINALISED

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

Area of the risk assessment that could not be finalised on the basis of the available data	Relevance in relation to representative use(s)

3.1.6 Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.
An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

Critical area of concern identified	Relevance in relation to representative use(s)
	[specify if concern relates to all or specific representative use/use scenario/product or to all uses/products]

3.1.7 Overview table of the concerns identified for each representative use considered

Note: If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.

Representative use:		**Pepper crops and leafy vegetables (Greenhouses and open field (X)[1]**
Operator risk	Risk identified	
	Assessment not finalised	
Worker risk	Risk identified	
	Assessment not finalised	
Bystander risk	Risk identified	
	Assessment not finalised	
Consumer risk	Risk identified	
	Assessment not finalised	
Risk to wild non target terrestrial vertebrates	Risk identified	
	Assessment not finalised	
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified	
	Assessment not finalised	
Risk to aquatic organisms	Risk identified	
	Assessment not finalised	
Groundwater exposure active substance	Legal parametric value breached	
	Assessment not finalised	
Groundwater exposure metabolites	Legal parametric value breached	
	Parametric value of 10 µg/L[(a)] breached	
	Assessment not finalised	
Comments/Remarks		

[1] The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	**Justification**
Identity at strain level	*The information submitted is considered to be almost sufficient, however a common approach for all SeMNPV strains would be desirable.*
Low-risk criteria for baculovirus according to	*The information submitted is considered to be NOT sufficient, since the effect on non-target organism (insects) has not been proved with the specific MPCA SeMNPV isolate Bv-0004.*
Environmental factor that can influence in environment survival outside the host.*Sunligh.*	*All information provided relate to physical, chemical and technical properties of the plant protection product are for virus MPCA in general, and some are related to BV species other than SeMNPV. According to data provided, the sun light may play an importance role in the*

Area(s) where expert consultation is considered necessary	**Justification**
	persistence and *survival of SeMNPV. The RMS recommends the evaluation of UV light in the target MPCA SeMNPV isolate Bv*-0004.
Genetic stability	*Methods to differentiate a mutant of the microorganism from the parent wild strain.* *Either SeMNPV is of natural origin and not a mutant or genetically modified organism; a mutant can come up in following generations and differs from the parental isolate. This mutant can be less effective in the control of the target insect, and can have different biological characteristics.*
Persistence in water Even all the evidence of the potential inactivation of the BVs, most viruses in intact inclusion bodies are reasonably stable in aqueous suspension	*Potential interferences of with the analytical systems for the control of the quality of drinking water provided for in Directive 98/83/EC.*

3.2 PROPOSED DECISION

[redacted]

3.3 RATIONAL FOR THE CONDITIONS AND RESTRICTIONS TO BE ASSOCIATED WITH ANY APPROVAL OR AUTHORISATION(S), AS APPROPRIATE

3.3.1 Particular conditions proposed to be take into account to manage the risks identified

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
	[redacted]

APPENDICES

Appendix 1 Guidance documents used in this assessment

OECD Working document on the evaluation of microbials for pest control (ENV/JM/MONO(2008)36)

Guideline developed within the Standing Committee on the Food Chain and Animal Health on the taxonomic level of microorganisms to be included in Annex I to Directive 91/414/EEC (SANCO/10754/2005 - rev.5)

Working Document on Microbial Contaminant Limits for Microbial Pest Control Products (SANCO/12116/2012 - Rev.0)

Guidance document for the assessment of the equivalence of technical grade active ingredients for identical microbial strains or isolates approved under regulation (EC) No 1107/2009 (SANCO/12823/2012 - rev. 4)

OECD Guidance document for single laboratory validation of quantitative analytical methods – guidance used in support of pre- and post-registration data requirements for plant protection and biocidal products (ENV/JM/MONO(2014)20)

Working document on Technical Material and Preparations: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414 (SANCO 3030/99 rev.4)

OECD guidance to the environmental safety evaluation of microbial biocontrol agents. Series on pesticides No. 67. ENV/JM/MONO (2012)1. 17 Feb 2012.

FOCUS, 1997. Soil persistence models and EU registration. SANCO/7617/VI/96.

FOCUS, 2000. FOCUS Groundwater Scenarios in the EU review of active substances. Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference SANCO/321/2000-rev.2. 202 pp, as updated by the Generic Guidance for FOCUS groundwater scenarios, version 1.1 dated April 2002.

FOCUS, 2001. FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC. Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp., as updated by the Generic Guidance for FOCUS surface water scenarios, version 1.1 dated March 2012.

EFSA guidance “Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009”

Appendix 2 Reference list